The Effect of Pesticides on the Tomato Bacterial Speck Disease Pathogen Pseudomonas Syringae pv. Tomato

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Abstract: A significant part of the used pesticides does not reach the target organisms and, while remaining in the agrophytocenosis, influences all living organisms in it. Having a toxic and often mutagenic effect, pesticides induce morphological and physiological changes in the cells of microorganisms and are the cause of phenotypic heterogeneity of their populations. However, the effect of pesticides on phytopathogenic bacteria as non-target microorganisms remains out of the field of view for most researchers. However, the use of pesticides can lead to expansion of the diversity of existing phytopathogens and, as a consequence, complications of identification of the pathogens, loss of resistance by plants varieties, and increased harm from diseases caused by them. This study is focused on the effect of pesticides used in tomato plantations on the causative agent of bacterial speck of this crop—Pseudomonas syringae pv. tomato. The studies were carried out using the methods of classical microbiology. The mutagenic action of pesticides was recorded, taking into account the increase of the number of streptomycin resistance mutations in bacteria in the case of pesticide action. It is established that the fungicide aluminium phosethyl is characterised by a bacteriostatic effect on P. syringae pv. tomato. Deltamethrin insecticide does not affect the growth of P. syringae pv. tomato. However, there is an increase in the frequency of streptomycin resistance mutations in both studied strains of P. syringae pv. tomato after using deltamethrin. It is shown that the frequency of occurrence of R (rough colonies) forms of P. syringae pv. tomato IZ28 and IZ46 after using deltamethrin increased by 100 times when in comparison to the frequency of spontaneous morphological dissociation, or smooth-to-rough (S-R) mutation, of these bacteria. Therefore, aluminium phosethyl is characterised by moderate bacteriostatic action against P. syringae pv. tomato. Deltamethrin does not influence the growth of the pathogen of tomato speck but increases the frequency of formation of StrR mutants and R forms of phytopathogenic bacteria.

Keywords: Pseudomonas syringae pv. tomato; pesticides; aluminium phosethyl; deltamethrin; antibacterial activity; mutagenic action; morphological dissociation

1. Introduction

Tomato is affected by a number of bacterial diseases, the harmfulness of which is determined by climatic conditions and the general condition of plants [1–4]. Regarding Ukraine, the causative agent of black rot Xanthomonas vesicatoria, the causative agent of bacterial speck Pseudomonas syringae pv. tomato and bacterial canker pathogen Clavibacter michiganensis subsp. michiganensis are economically significant [5]. Changes in the populations of microorganisms are natural and can lead to the expansion
of the genetic and phenotypic diversity of existing pathogens and, as a consequence, complications with identification of the pathogen, loss of resistance by varieties, and increased harm from bacterial diseases. The use of pesticides is one of the factors that increases variability of phytopathogens [6–8].

Most pesticides used in agriculture have a wide range of effects. On the one hand, it provides the possibility of their application for the control of a wide range of pathogens and pests. On the other hand, it determines their influence on non-target organisms [9–13].

Phytopathogenic bacteria in agrophytocenoses are influenced by all factors (both biotic and abiotic) that act in these ecological niches. Pesticides are one of the most important abiotic factors of agrophytocenoses. It is known that a significant proportion of pesticides act on non-target organisms and have mutagenic activity [10]. Stress caused by abiotic factors and, in particular, by the action of pesticides, induces morphological and physiological changes in bacterial cells and phenotypic heterogeneity in the cell populations of phytopathogenic bacteria [14,15]. Scientists intensively investigated the effect of pesticides on soil microorganisms and bacteria in the plant phyllosphere [10,16]. However, the effect of pesticides on plant pathogenic bacteria as non-target microorganisms has almost completely remained outside the attention of researchers. In particular, the mutagenic activity of pesticides relative to phytopathogenic bacteria has not yet been extensively studied. Previously, we have shown that some pesticides, which are widely used in grain crops, have mutagenic activity or cause morphological dissociation of the agent of wheat basal bacteriosis P. syringae pv. atrofaciens [15].

Components of the bacterial outer membrane play an important role in adaptation of gram-negative bacteria to various selective factors. Morphological dissociation, or smooth-to-rough (S-R) mutation, is a step-by-step process that results in an irreversible change [17]. The resulting R mutants are unable to revert to their original state, and often fail to survive in a host with an intact immune system. S colonies are recognised by their moist nature and are indicators of freshly isolated wild bacterial strains. R colonies are rough, dry, granulated, and mutant types of bacteria that lack most of the surface proteins including the capsule and lipopolysaccharides. R colonies are formed by bacteria that are usually avirulent. The ability to show variations in both smooth–rough ways and from rough to smooth (R-S) colonies has also been observed in phytopathogenic bacteria. Processes of step-by-step S-R mutations are bacterial responses to changing conditions.

The aim of this work was to determine the effect of pesticides recommended for use in tomato plantations in Ukraine on the causative agent of bacterial speck of this crop—Pseudomonas syringae pv. tomato.

2. Materials and Methods

**Bacterial strains.** In this work, the strains of P. syringae pv. tomato IZ28 and P. syringae pv. tomato IZ46, which were isolated in the Zaporizhia region of Ukraine from the tomato varieties Alamina rozhevyi F1 and Krystal F1 affected with bacteriosis, respectively, were investigated. The strains are highly aggressive on tomato [5]. The strains are stored in the collection of microorganisms of the Department of Phytopathogenic Bacteria of the Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine.

**Pesticides.** In this study, we used pesticides recommended for use in tomatoes plantings [18]: fungicide aluminium phosphethyl and insecticide deltamethrin. There were used commercial formulations of the tested pesticides in this study. Pesticides were used in the recommended concentration (aluminium phosphethyl—40 mg/L, deltamethrin—0.25 mg/L), as well as 10 times higher and 10 times lower concentrations.

**Influence of pesticides on the growth and morphology of colonies of phytopathogenic bacteria.** To determine the effect of the pesticides on P. syringae pv. tomato LB Broth (Lennox) was used. The studied compounds were introduced into the LB medium, into which phytopathogenic bacteria had been already inoculated. After 48 h of cultivation at 28 °C, several series of 10-time dilutions were plated on potato agar (PA) [18] to count the number of bacterial cells (CFU/mL) and determine the
ability of pesticides to induce morphological dissociation in phytopathogenic bacteria. Morphology and structure of bacterial colonies were studied in 48–72 h after plating on PA. The size of the colonies, their shape, structure and consistency, surface, profile, edges, colour were characterised. Physiological and biochemical properties of bacteria were studied by well-known methods [19].

Detection of streptomycin resistance mutations in phytopathogenic bacteria. The mutagenic activity of pesticides against phytopathogenic bacteria was recorded as an increase in the number of streptomycin resistance mutations during cultivation on the medium containing the pesticide. For this purpose, 0.1 mL of bacterial cell suspension (109 CFU/mL) was plated on PA containing pesticide and streptomycin (10 µg/mL). After 48 h of cultivation at 28 °C, the number of streptomycin-resistant (StrR) colonies was counted. Mutagenic effect of pesticides was estimated by increasing the number of StrR colonies formed on the medium with pesticide and streptomycin in comparison with the number of StrR colonies that grew on the medium with streptomycin only [15].

Serological methods. The belonging of R form and StrR mutants to P. syringae was established by serological methods. For the microagglutination reaction on a glass slide, a drop of antiserum at the dilution of 1:10 or 1:20 and a drop of saline were applied. Into each drop, a small amount of bacteria was inserted and mix to form a barely visible turbidity. In 1–2 min, in the case of a positive reaction, the gluing of bacteria into conglomerates was detected. Only in the control drop, the uniform suspension of bacteria without gluing was observed [19].

Hypersensitivity reaction on tobacco leaves. The ability of S and R forms of P. syringae to induce the hypersensitivity reaction was determined using the method of injection-infiltrations on the leaves of Nicotiana tabacum [19]. To do this, a suspension of cells of two-day cultures of the studied bacterial strains with a concentration of 1 × 107 CFU/mL was introduced under the leaf epidermis. The suspension of cells was prepared on sterile tap water. As a negative control, under the epidermis of the leaf, sterile tap water was introduced. The presence of necrosis was observed in a day.

Statistica. Statistical processing of the research results was performed using the STATISTICA v. 6.0 application software package, which, according to the Student t-test, were statistically significant at the significance level p ≤ 0.05.

3. Results

To determine the toxic effect of pesticides on the pathogen of tomato bacterial speck, they were introduced into the liquid culture medium LB Broth, on which P. syringae pv. tomato strains were incubated for 48 h. We found that the fungicide aluminium phosethyl is toxic to strains P. syringae pv. tomato IZ28 and P. syringae pv. tomato IZ46 (Figure 1).

![Figure 1. Effect of aluminium phosethyl on the growth of P. syringae pv. tomato: *—Statistically significant differences between the control and a variant of the experiment at level p ≤ 0.05; “0”—control.](image-url)
After 48 h of the cultivation of phytopathogenic bacteria with aluminium phosethyl at the concentration of 400 mg/L, the number of cells of *P. syringae pv. tomato* IZ28 was $2.5 \times 10^8$ CFU/mL (it was 97% smaller than control without pesticides), and of *P. syringae pv. tomato* IZ46—$8.0 \times 10^8$ CFU/mL (it was 96% smaller than control without pesticides) (Figure 1). Aluminium phosethyl was toxic to *P. syringae* pv. *tomato* IZ46 at the concentration of 40 mg/L too. At an aluminium phosethyl concentration of 40.0 mg/L in 48 h of cultivation, the number of cells of *P. syringae pv. tomato* IZ28 was 65% of the control and of *P. syringae pv. tomato* IZ46—51%. At an aluminium phosethyl concentration of 4.0 mg/L, the number of cells of *P. syringae pv. tomato* IZ28 was 85% of the control, and of *P. syringae pv. tomato* IZ46—88%. Thus, the fungicide aluminium phosethyl is characterised by antibacterial activity against pathogen of tomato bacterial speck *P. syringae pv. tomato*. However, the antibacterial effect is significant only at the concentration of 400 mg/L, which is 10 times higher than recommended for plant treatment.

Earlier, studying the effect of a wide range of fungicides on phytopathogenic bacteria, we found that the aluminium phosethyl fungicide (800 g/kg) is characterised by antibacterial action only in the concentration, which is 10 times higher than the recommended dose [20].

The insecticide deltamethrin was characterised by low toxicity against both strains of *P. syringae pv. tomato* (Figure 2). The number of cells in the culture fluid after 48 h of cultivation with the introduction of deltamethrin at the concentration of 2.5 mg/L was for *P. syringae pv. tomato* IZ28 68.0 to $10^8$ CFU/mL (80% to control) and for *P. syringae pv. tomato* IZ46—183.5 to $10^8$ CFU/mL (81% to control). When using deltamethrin in the culture medium at the concentrations of 0.25 and 0.025 mg/L, the number of the cells of both strains of *P. syringae pv. tomato* almost did not differ from pesticide-free control (Figure 2).

![Figure 2](image_url)

**Figure 2.** Effect of deltamethrin on the growth of *P. syringae pv. tomato*: “0”—control.

Therefore, we found that deltamethrin is characterised by low toxicity against the pathogen of tomato bacterial speck *P. syringae pv. tomato*.

The next stage of the work was to determine the mutagenic activity of pesticides used for treating tomato plantings against phytopathogenic bacteria.

In terms of the actions of the aluminium phosethyl fungicide at the concentration of 400.0 mg/L for *P. syringae pv. tomato* IZ28 and *P. syringae pv. tomato* IZ46, the appearance of Str$^R$ colonies was not observed, which in our opinion is due to the significant toxicity of aluminium phosethyl in this concentration (Table 1).

In studies with lower concentrations of aluminium phosethyl, the frequency of Str$^R$ colonies of *P. syringae pv. tomato* was no difference from the frequency of spontaneous Str$^R$ colonies of these strains.

Therefore, we determined that aluminium phosethyl did not cause an increase in the frequency of occurrence of Str$^R$ mutants of strains of *P. syringae pv. tomato* (Table 1).
Table 1. Induction of Str\textsuperscript{R} mutations \textit{P. syringae pv. tomato} for pesticides.

| Pesticide            | Pesticide Concentration, mg/L | Frequency of Occurrence of Str\textsuperscript{R} Mutants of Strains, ×10\textsuperscript{−8} |
|----------------------|-------------------------------|-----------------------------------------------|
|                      |                               | \textit{P. syringae pv. tomato IZ28} | \textit{P. syringae pv. tomato IZ46} |
| Aluminium phosethyl  | 400.0                         | –                               | –                               |
|                      | 40.0                          | 1.0±0.02                        | 1.0±0.07                        |
|                      | 4.0                           | 1.0±0.1                         | 2.0±0.1                         |
|                      | 2.5                           | 10.0±0.2*                      | 8.0±0.1*                       |
| Deltamethrin         | 0.25                          | 15.0±0.3*                      | 12.0±0.4*                      |
|                      | 0.025                         | 14.0±0.2*                      | 15.0±0.2*                      |
| Control **           |                               | 1.5±0.1                        | 2.0±0.1                        |

\*—Statistically significant differences between the control and a variant of the experiment at level \(p \leq 0.05\); **—the number of Str\textsuperscript{R} colonies that grew on the medium with streptomycin only.

We selected colonies of spontaneous and induced by aluminium phosethyl and deltamethrin Str\textsuperscript{R} mutants of \textit{P. syringae pv. tomato IZ28} and \textit{P. syringae pv. tomato IZ46}, and identified their morphological, cultural, serological properties and ability to induce hypersensitivity reactions on tobacco leaves (HR) (Table 2). According to morphological and cultural properties, induced and spontaneous Str\textsuperscript{R} mutants of \textit{P. syringae pv. tomato} did not differ from the original strain. In the microagglutination reaction, induced and spontaneous Str\textsuperscript{R} mutants of \textit{P. syringae pv. tomato} demonstrated a serological relationship with serum to the typical strain of \textit{P. syringae pv. tomato R140}. All studied Str\textsuperscript{R} mutants of \textit{P. syringae pv. tomato} induced HR on tobacco leaves that evidenced in favour of the maintenance of virulence that was inherent to the original strains.

Table 2. Physiological and biochemical properties Str\textsuperscript{R} mutants of \textit{P. syringae pv. tomato}.

| Test                          | Str\textsuperscript{S} (Original Form) | Str\textsuperscript{R} Mutants: \textit{P. syringae pv. tomato IZ28} and IZ46 |
|-------------------------------|----------------------------------------|-------------------------------------------------------------------|
|                               | \textit{P. syringae pv. tomato IZ28}   | \textit{P. syringae pv. tomato IZ28 and IZ46}                     |
|                               | IZ46                                   | Induced by Aluminium Phosethyl | Induced by Deltamethrin |
| Gram staining                 | -                                      | -                      | -                      |
| Oxidase                       | -                                      | -                      | -                      |
| Growth on LB Broth Fermentation: | Uniform turbidity | Uniform turbidity | Uniform turbidity |
| glucose, mannose, arabinose, sorbitol, inositol | +                       | +                      | +                      |
| glucose (anaerobic), lactose, maltose | -                                   | -                      | -                      |
| HR on tobacco                 | +                                      | +                      | +                      |
| Microagglutination reaction with antiserum in dilution: | 1:20 | + | + |

Previously, during the study of the action of pesticides on the causative agent of wheat basal bacteriosis \textit{P. syringae pv. atrofaciens}, we observed morphological dissociation of phytopathogen with the appearance of R forms in variants with the addition of pesticides [14]. Therefore, we have studied the morphological characteristics of \textit{P. syringae pv. tomato} at the actions of aluminium phosethyl and deltamethrin.

For the strain \textit{P. syringae pv. atrofaciens} UCM B-1011, the incidence of spontaneous R forms was \(5 \times 10^{-3}\) [14]. For the strains of the pathogen of tomato speck \textit{P. syringae pv. tomato IZ28} and \textit{P. syringae pv. tomato IZ46}, the appearance of spontaneous R forms (control) was rare—the frequency of R forms did not exceed \(1 \times 10^{-4}\) (Figure 3, Table 3).

Under the action of aluminium phosethyl, there was no increase in the frequency of occurrence of the R form of \textit{P. syringae pv. tomato}. At the same time, in terms of the actions of the insecticide deltamethrin, there was observed the appearance of a large number of mutated colonies increased in size—matte, flat with jagged edges (Figure 3), which we have identified as R forms of \textit{P. syringae pv. tomato}.

The incidence of R form of \textit{P. syringae pv. tomato IZ28} and \textit{P. syringae pv. tomato IZ46} in terms of the actions of deltamethrin increased by 100 times in comparison with the frequency of spontaneous
morphological dissociation of these bacteria (Table 3). At the same time, morphological dissociation of
the bacteria was observed both at the action of deltamethrin at a concentration of 0.25 mg/L, and at a
concentration of 0.025 mg/L of this insecticide.

![Image of bacteria with R and S forms]

**Figure 3.** S and R forms colonies *P. syringae pv. tomato* IZ28 under the action of deltamethrin.

**Table 3.** Morphological dissociation of *P. syringae pv. tomato* for deltamethrin action.

| Pesticide       | Pesticide Concentration, mg/L | Frequency of Occurrence of R Forms of Strains |
|-----------------|-------------------------------|----------------------------------------------|
|                 |                               | *P. syringae pv. tomato IZ28* | *P. syringae pv. tomato IZ46* |
| Deltamethrin    | 2.5                           | $8 \times 10^{-2}$ *            | $3 \times 10^{-2}$ *            |
|                 | 0.25                          | $1 \times 10^{-2}$ *            | $1 \times 10^{-2}$ *            |
|                 | 0.025                         | $4 \times 10^{-3}$ *            | $2 \times 10^{-2}$ *            |
| Control **      |                               | $1 \times 10^{-4}$              | $1 \times 10^{-4}$              |

*—Statistically significant differences between the control and a variant of the experiment at level $p \leq 0.05$; **—the number of spontaneous R colonies that grew on the medium without pesticides.

The appearance of R forms is associated with rearrangements in the cell wall elements or
polysaccharide capsule, with a change or complete absence of enzymes of biosynthesis of these cell
structures as a result of mutation [21,22]. Such mutations are considered to be pleiotropic, because in
addition to changes in the morphology of colonies, there is a decrease in virulence and change of the
serological reaction of mutant forms [23].

It was found that according to physiological and biochemical properties, all R dissociants are not
different from each other and from the initial S form of *P. syringae pv. tomato* (Table 3). They were
oxidase negative, used glucose, mannose, arabinose, sorbitol, and inositol as sole source of nutrition,
and did not consume anaerobically glucose, lactose and maltose (Table 4).

**Table 4.** Physiological and biochemical properties of S and R forms *P. syringae pv. tomato*.

| Test                           | S Form *P. syringae pv. tomato* IZ28 and IZ46 | R Form *P. syringae pv. tomato* IZ28 and IZ46 |
|--------------------------------|------------------------------------------------|----------------------------------------------|
|                                | Spontaneous | Induced by Deltamethrin |
| Gram staining                  | -           | -                         |
| Oxidase                        | -           | -                         |
| Growth on LB Broth             | Uniform turbidity | Film, sediment |
|                                | Fermentation: | Fermentation: |
| glucose, mannose, arabinose,   | +           | +                         |
| sorbitol, inositol             | -           | -                         |
| glucose (anaerobic), lactose,  | -           | -                         |
| maltose                        | +           | +                         |
| HR on tobacco                  | +           | +                         |
|                                | Microagglutination reaction with antiserum in dilution: | + |
|                                | 1:20        | +                         |
In terms of cultivation in LB Broth, S-forms of *P. syringae* pv. *tomato* IZ28 and *P. syringae* pv. *tomato* IZ46 gave homogeneous growth, and R dissociants formed film and sediment. Such differences in the nature of growth in broth were observed in the study of morphological dissociants of *P. syringae* pv. *atrofaciens* UCM B-1011 induced by insecticide Alpha Super with the active substance alpha-cypermethrin [15].

4. Discussion

Without denying the economic benefits in the nearest future, scientists around the world are increasingly thinking about remote environmental and medical problems caused by the excessive use of pesticides [13,24,25]. This is evidenced by a huge number of studies of toxic, genotoxic, mutagenic, teratogenic effects of pesticides. However, there are still many gaps in the study of the effects of pesticides on non-target organisms. In particular, it concerns the influence of pesticides on phytopathogenic bacteria as non-target organisms.

We have found that most pesticides in recommended concentrations have no significant effect on the growth of phytopathogenic bacteria but can show mutagenic activity against them [20]. However, data on this effect of pesticides on bacteria are limited and therefore studies on this issue do not lose their relevance.

To carry out research, we have chosen the pathogen of tomato speck (the most used vegetable crop in Ukraine) because of its wide distribution in our country [5] and because *P. syringae* are well known as epiphytes. Constantly being in the phyllosphere, bacteria of the *P. syringae* type are influenced by all substances that are used for the treatment of tomato plantings. We identified that aluminium phosethyl at the concentration of 400 mg/L and 40.0 mg/L is characterised by toxic effect on the pathogen of tomato bacterial speck *P. syringae* pv. *tomato*. Deltamethrin at the concentration of 2.5 mg/L, 0.25 mg/L and 0.025 mg/L was characterised by low toxicity for both strains of *P. syringae* pv. *tomato*.

According to the literature data, deltamethrin insecticides have the genotoxic effects [26–28]. In these studies, it was shown that deltamethrin at the doses of 50, 100, 200 mg/kg/bw significantly increased the formation of micronuclei in erythrocytes and splenocytes of mice at the 48th hour after application. The greatest dose (200 mg/kg) was identified to have the greatest number of micronuclei, and its difference from the control groups was found to be statistically significant (p < 0.001). According to these results, it was determined that in acute toxic doses, deltamethrin showed genetic toxicity in somatic cells of mice and provided a slight and statistically insignificant induction in lower doses [27]. It has been established that using the commercial formulation of deltamethrin (Decis 25) cause the increase of micronuclei (MN) frequencies in *T. rendalli* at doses of 1.0 and 5.0 mg/kg, and in mice there was no MN induction [28].

Pyrethroids, including allethrin, bioallethrin, deltamethrin, and esbiothrin possessed weakly mutagenic potential with base-pair substitution in the Ames test. They also slightly induced DNA damage when assessed by the comet assay [29].

For the fungicide aluminium phosphethyl, no genotoxic effect was found [30,31].

Another danger of pesticide use is the phenomenon of cross-resistance to pesticides and antibiotics [32]. According to scientists, resistance to pesticides contributes to the development of antibiotic resistance [33]. In our opinion, such antibiotic resistance can be a consequence of the mutagenic action of pesticides. Therefore, we studied the ability of aluminium phosphethyl and deltamethrin to induce resistance to streptomycin in the strains of *P. syringae* pv. *tomato*.

We found that aluminium phosphethyl fungicide does not cause an increase in the number of StrR colonies of strains of *P. syringae* pv. *tomato* on medium with streptomycin, while in terms of action of the insecticide deltamethrin, there was observed an increase in the number of StrR colonies of strains of *P. syringae* pv. *tomato*. Thus, deltamethrin induced an increase in the frequency of formation of StrR mutants of *P. syringae* pv. *tomato*. Similar activity was characteristic for another pyrethroid alpha-cypermethrin against *P. syringae* pv. *atrofaciens* [15].
Another activity of pesticides, in particular insecticides based on alpha-cypermethrin and chlorpyrifos + cypermethrin and herbicides based on tribenuron-methyl+trifensulfuron-methyl, was the ability to induce morphological dissociation with the appearance of R forms of the agent of wheat basal bacteriosis \( P. syringae \) \textit{pv. atrofaciens} [14,15].

The frequency of spontaneous morphological dissociation of strains of \( P. syringae \) \textit{pv. tomato} was \( 1 \times 10^{-4} \), which coincides with the data on this phenomenon [15]. In the case of cultivation with deltamethrin, this figure grew to hundreds of times. However, aluminium phosethyl does not cause increased morphological dissociation of strains of \( P. syringae \) \textit{pv. tomato}.

Xenobiotics are a stress factor, adaptation to which may be accompanied by changes in certain properties of microorganisms. Adaptive reactions to the action of pesticides are manifested in a variety of adaptation to biochemical and physiological processes, which, accordingly, ensure their continued existence under the conditions of such anthropogenic load. Dissociation is one of the variants of adaptive changes of bacteria and is due to the restructuring of the surface structures of cells.

The formation of S and R forms has been studied a lot for the causative agents of human infectious diseases. It was found that the S and R forms of \( Mycobacterium abscessus \) are characterised by differences in the nature of interaction with the immune cells of the macro-organism, which necessitates the search for drugs and strategies that are aimed both at the destruction of the intracellular population of the pathogen, and to prevent the formation of extracellular structures that allow R forms of \( M. abscessus \) to avoid phagocytosis [34].

Phytopathogenic \( P. syringae \) are also characterised by natural variability of the population with splitting into different morphotypes [14,15]. It is believed that in \( P. syringae \), spontaneous loss of plasmids (90 MDa) leads to loss of virulence and changes in the morphology of colonies. We have not revealed any differences in serological activity and ability to induce HR of S and R forms of \( P. syringae \) \textit{pv. tomato}.

The value of dissociation is in obtaining selective advantages by bacteria that ensure their existence in the human body or in the external environment [35–37]. It is known that S-forms are more resistant to phagocytosis. R forms, in turn, are more resistant to environmental factors.

Consequently, the phenomenon of dissociation contributes to the heterogeneity of the bacterial population, increases its stability, and expands the boundaries of species survival. Thus, dissociants differ not only morphologically but may have differences in pathogenic, virulent, and biochemical properties. The formation of R forms of bacteria also complicates microbiological diagnosis of diseases caused by them [38,39].

5. Conclusions

Therefore, it is established that aluminium phosethyl is characterised by a moderate bacteriostatic activity against \( P. syringae \) \textit{pv. tomato}. Deltamethrin insecticide does not affect the growth of \( P. syringae \) \textit{pv. tomato}, but causes an increase in the frequency of formation of \( \text{Str}^R \) mutants and R forms of strains of \( P. syringae \) \textit{pv. tomato}.

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