

Article

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 Str^R 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 phosethyl and insecticide deltamethrin. There were used commercial formulations of the tested pesticides in this study. Pesticides were used in the recommended concentration (aluminium phosethyl—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 (10^9 CFU/mL) was plated on PA containing pesticide and streptomycin ($10 \mu g/mL$). After 48 h of cultivation at 28 °C, the number of streptomycin-resistant (Str^R) colonies was counted. Mutagenic effect of pesticides was estimated by increasing the number of Str^R colonies formed on the medium with pesticide and streptomycin in comparison with the number of Str^R colonies that grew on the medium with streptomycin only [15].

Serological methods. The belonging of R form and Str^R 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 1×10^7 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 $p \le 0.05$.

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 $p \le 0.05$; "0"—control.

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 2.5×10^8 CFU/mL (it was 97% smaller than control without pesticides), and of *P. syringae* pv. *tomato* IZ46—8.0 × 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 65% of the control and 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 68.0×10^8 CFU/mL (80% to control) and for *P. syringae* pv. *tomato* IZ46—183.5 × 10⁸ 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).



 $\Box P$. syringae pv. tomato IZ28 $\Box P$. syringae pv. tomato IZ46

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).

Pesticide	Pesticide Concentration, mg/L	Frequency of Occurrence of Str ^R Mutants of Strains, ×10 ⁻⁸	
		P. syringae pv. tomato IZ28	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
Deltamethrin	2.5	$10.0 \pm 0.2 *$	8.0 ± 0.1 *
	0.25	15.0 ± 0.3 *	12.0 ± 0.4 *
	0.025	14.0 ± 0.2 *	$15.0 \pm 0.2 *$
Control **		1.5 ± 0.1	2.0 ± 0.1

Table 1. Induction of Str^R mutations *P. syringae* pv. *tomato* for pesticides.

*—Statistically significant differences between the control and a variant of the experiment at level $p \le 0.05$; **—the number of Str^R colonies that grew on the medium with streptomycin only.

We selected colonies of spontaneous and induced by aluminium phosethyl and deltamethrin Str^R mutants of *P. syringae* pv. *tomato* IZ28 and *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^R mutants of *P. syringae* pv. *tomato* did not differ from the original strain. In the microagglutination reaction, induced and spontaneous Str^R mutants of *P. syringae* pv. *tomato* demonstrated a serological relationship with serum to the typical strain of *P. syringae* pv. *tomato* R140. All studied Str^R mutants of *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.

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

Table 2. Physiological and biochemical properties Str^R mutants of *P. syringae* pv. *tomato*.

Previously, during the study of the action of pesticides on the causative agent of wheat basal bacteriosis *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 *P. syringae* pv. *tomato* at the actions of aluminium phosethyl and deltamethrin.

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

The incidence of R form of *P. syringae* pv. *tomato* IZ28 and *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.



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

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	8×10^{-2} *	3×10^{-2} *
	0.25	1×10^{-2} *	1×10^{-2} *
	0.025	4×10^{-3} *	2×10^{-2} *
Control **		1×10^{-4}	1×10^{-4}

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

*—Statistically significant differences between the control and a variant of the experiment at level $p \le 0.05$; **—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.	R Form <i>P. syringae</i> pv. tomato IZ28 and IZ46				
	tomato IZ28 and IZ46	Spontaneous	Induced by Deltamethrin			
Gram staining	-	-	-			
Oxidase	-	-	-			
Growth on LB Broth	Uniform turbidity	Film, sediment	Film, sediment			
	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 phosethyl, 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 phosethyl and deltamethrin to induce resistance to streptomycin in the strains of *P. syringae* pv. *tomato*.

We found that aluminium phosethyl fungicide does not cause an increase in the number of Str^R 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 Str^R colonies of strains of *P. syringae* pv. *tomato*. Thus, deltamethrin induced an increase in the frequency of formation of Str^R 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* pv. *atrofaciens* [14,15].

The frequency of spontaneous morphological dissociation of strains of *P. syringae* pv. *tomato* was 1×10^{-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* 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 correction of 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* 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* pv. *tomato*. Deltamethrin insecticide does not affect the growth of *P. syringae* pv. *tomato*, but causes an increase in the frequency of formation of Str^R mutants and R forms of strains of *P. syringae* pv. *tomato*.

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References

- 1. Eichenlaub, R.; Gartemann, K.-H. The *Clavibacter michiganensis* subspecies: Molecular investigation of Gram-positive bacterial plant pathogens. *Annu. Rev. Phytopathol.* **2011**, *49*, 445–464. [CrossRef] [PubMed]
- 2. Wang, Y.; Zhang, Y.; Gao, Z.; Yang, W. Breeding for Resistance to Tomato Bacterial Diseases in China: Challenges and Prospects. *Hortic. Plant J.* **2018**, *4*, 193–207. [CrossRef]

- 3. Stall, R.E.; Jones, J.B.; Minsavage, G.V. Durability of resistance in tomato and pepper to Xanthomonads causing bacterial spot. *Annu. Rev. Phytopathol.* **2009**, *47*, 265–284. [CrossRef] [PubMed]
- 4. Singh, S.; Singh, D.R.; Kumar, K.; Birah, A. Eco-friendly management modules for bacterial wilt (*Ralstonia solanacearum*) of tomato for protected cultivation in a tropical island ecosystem. *Biol. Agric. Hortic.* **2014**, *30*, 219–227. [CrossRef]
- 5. Kolomiets, J.; Grygoryuk, I.; Butsenko, L. Bacterial diseases of tomato plants in terms of open and covered growing of Ukraine. *Ann. Agrar. Sci.* **2017**, *15*, 213–216. [CrossRef]
- Hollomon, D.W. Fungicide resistance: Facing the challenge—A review. *Plant Prot. Sci.* 2015, 51, 170–176. [CrossRef]
- 7. Corio-Costet, M.-F.; Dufour, M.-C.; Cigna, J.; Abadie, P.; Chen, W. Diversity and fitness of *Plasmopara viticola* isolates resistant to QoI fungicides. *Eur. J. Plant Pathol.* **2011**, *129*, 315–329. [CrossRef]
- 8. Karaoglanidis, G.S.; Thanassoulopoulos, C.C. Phenotypic Instability of *Cercospora beticola* Sacc. Strains Expressing Resistance to the Sterol Demethylation-Inhibiting (DMI) Fungicide Flutriafol after Cold Exposure. *J. Phytopathol.* **2002**, *150*, 692–696. [CrossRef]
- 9. Asad, M.; Lavoie, M.; Song, H.; Jin, Y.; Fu, Z.; Qian, H. Interaction of chiral herbicides with soil microorganisms, algae and vascular plants. *Sci. Total Environ.* **2017**, *580*, 1287–1299. [CrossRef]
- 10. Gu, L.; Bai, Z.; Jin, B.; Hu, Q.; Wang, H.; Zhuang, G.; Zhang, H. Assessing the impact of fungicide enostroburin application on bacterial community in wheat phyllosphere. *J. Environ. Sci.* **2010**, *22*, 134–141. [CrossRef]
- 11. Zhang, B.; Bai, Z.; Hoefel, D.; Tang, L.; Wang, X.; Li, B.; Li, Z.; Zhuang, G. The impacts of cypermethrin pesticide application on the non-target microbial community of the pepper plant phyllosphere. *Sci. Total Environ.* **2009**, 407, 1915–1922. [CrossRef] [PubMed]
- 12. Zhang, M.; Xu, Z.; Teng, Y.; Christie, P.; Wang, J.; Ren, W.; Luo, Y.; Li, Z. Non-target effects of repeated chlorothalonil application on soil nitrogen cycling: The key functional gene study. *Sci. Total Environ.* **2016**, *543*, 636–643. [CrossRef] [PubMed]
- 13. Bull, S.; Fletcher, K.; Boobis, A.R.; Battershill, J.M. Evidence for genotoxicity of pesticides in pesticide applicators: A review. *Mutagenesis* **2006**, *21*, 93–103. [CrossRef] [PubMed]
- 14. Buletsa, N.; Butsenko, L.; Pasichnyk, L.; Patyka, V. Physiology of growth *Pseudomonas syringae* pv. *atrofaciens* for the effects of pesticides. *Mikrobiolohichnyi Zhurnal* **2016**, *78*, 52–60. [CrossRef]
- 15. Butsenko, L.; Pasichnyk, L.; Buletsa, N.; Patyka, V. Effect of insecticide Alpha Super on phytopathogenic bacteria *Pseudomonas syringae* of agrophytocenosis of wheat. *Bull. Agric. Sci.* **2017**, *3*, 18–22. [CrossRef]
- 16. Lukáčová, M.; Barák, I.; Kazár, J. Role of structural variations of polysaccharide antigens in the pathogenicity of Gram-negative bacteria. *Clin. Microbiol. Infect.* **2008**, *14*, 200–206. [CrossRef]
- 17. Sułowicz, S.; Cycoń, M.; Piotrowska-Seget, Z. Non-target impact of fungicide tetraconazole on microbial communities in soils with different agricultural management. *Ecotoxicology* **2016**, 25, 1047–1060. [CrossRef]
- 18. State Register of Pesticides and Agrochemicals Permitted for Use in Ukraine. (In Ukr.). Available online: https://data.gov.ua/dataset/389ddb5a-ac73-44bb-9252-f899e4a97588 (accessed on 27 February 2020).
- Dankevych, L.A.; Gnatiuk, T.T.; Huliaieva, H.B.; Tokovenko, I.P.; Kalinichenko, A.V. Express diagnostics of phytopathogenic bacteria and phytoplasmas in agrophytocenosis. In *Guidelines*; Suszanowich, D., Patyka, V., Eds.; Wyd-wo I Drukarnia Swietego Krzyza: Opole, Poland, 2019. Available online: https://www.researchgate.net/publication/335692227_EXPRESS_DIAGNOSTICS_OF_ PHYTOPATHOGENIC_BACTERIA_AND_PHYTOPLASMAS_IN_AGROPHYTOCENOSES (accessed on 10 November 2019).
- 20. Patyka, V.; Buletsa, N.; Pasichnyk, L.; Zhitkevich, N.; Kalinichenko, A.; Gnatiuk, T.; Butsenko, L. Specifics of pesticides effects on the phytopathogenic bacteria. *Ecol. Chem. Eng. S* **2016**, *23*, 311–331. [CrossRef]
- 21. Van den Broek, D.; Bloemberg, G.V.; Lugtenberg, B. The role of phenotypic variation in rhizosphere *Pseudomonas* bacteria. *Environ. Microbiol.* **2005**, *7*, 1686–1697. [CrossRef]
- 22. Esson, D.; Mather, A.E.; Scanlan, E.; Gupta, S.; De Vries, S.P.W.; Bailey, D.; Harris, S.R.; McKinley, T.J.; Méric, G.; Berry, S.K.; et al. Genomic variations leading to alterations in cell morphology of *Campylobacter* spp. *Sci. Rep.* **2016**, *6*, 38303. [CrossRef]
- 23. Seaton, S.C.; Silby, M.W.; Levy, S.B. Pleiotropic effects of GacA on *Pseudomonas fluorescens* Pf0-1 in vitro and in soil. *Appl. Environ. Microbiol.* **2013**, *79*, 5405–5410. [CrossRef] [PubMed]
- 24. Wong, C.S. Environmental fate processes and biochemical transformations of chiral emerging organic pollutants. *Anal. Bioanal. Chem.* **2006**, *386*, 544–558. [CrossRef] [PubMed]

- 25. Maltby, L.; Brock, T.; van den Brink, P. Fungicide Risk Assessment for Aquatic Ecosystems: Importance of Interspecific Variation, Toxic Mode of Action, and Exposure Regime. *Environ. Sci. Technol.* **2009**, *43*, 7556–7563. [CrossRef] [PubMed]
- 26. Ono, T.; Norimatsu, M.; Yoshimura, H. Induction of chromosome aberrations by pyrimethamine in cultured Chinese hamster (CHL) cells. *Mutat. Res. Lett.* **1994**, 323, 197–201. [CrossRef]
- 27. Ozkan, O.; Ustuner, O. Investigations about genotoxicity of deltamethrin. *Kafkas Univ. Vet Fak Derg.* **2012**, *18*, 69–74. [CrossRef]
- 28. Grisolia, C. A comparison between mouse and fish micronucleus test using cyclophosphamide, mitomycin C and various pesticides. *Mutat. Res.* **2002**, *518*, 145–150. [CrossRef]
- 29. Oztas, E.; Ulus, B.; Özhan, G. In Vitro Investigation on the Toxic Potentials of Commonly Used Synthetic Pyrethroids, Especially Esbiothrin. *Appl. In Vitro Toxicol.* **2015**, *1*, 302–307. [CrossRef]
- 30. Quest, J.A.; Hamernik, K.L.; Engler, R.; Burnam, W.L.; Fenner-Crisp, P.A. Evaluation of the carcinogenic potential of pesticides. 3. Aliette. *Regul. Toxicol. Pharmacol.* **1991**, *14*, 3–11. [CrossRef]
- 31. Fosetyl. EU Pesticides Database. Available online: https://ec.europa.eu/food/plant/pesticides/eu-pesticidesdatabase/public/?event=activesubstance.detail&language=EN&selectedID=1419 (accessed on 10 November 2019).
- 32. Curutiu, C.; Lazar, V.; Chifiriuc, M. Pesicides and antimicrobial resistance: From environmental compartments to animal and human infections. In *New Pesticides and Soil Sensors*; Grumezescu, A.M., Ed.; Academic Press: Bucharest, Romania, 2017; pp. 373–392. [CrossRef]
- 33. Rangasamy, K.; Athiappan, M.; Devarajan, N.; Parray, J.A. Emergence of multi drug resistance among soil bacteria exposing to insecticides. *Microb. Pathog.* **2017**, *105*, 153–165. [CrossRef]
- Viljoen, A.; Herrmann, J.-L.; Onajole, O.; Stec, J.; Kozikowski, A.; Kremer, L. Controlling extra- and intramacrophagic *Mycobacterium abscessus* by targeting mycolic acid transport. *Front. Cell. Infect. Microbiol.* 2017, 7, 388. [CrossRef]
- 35. Drenkard, E.; Ausubel, F.M. Pseudomonas biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature* **2002**, *416*, 740–743. [CrossRef] [PubMed]
- 36. Kirisits, M.J.; Prost, L.; Starkey, M.; Parsek, M.R. Characterization of colony morphology variants isolated from *Pseudomonas aeruginosa* biofilms. *Appl. Environ. Microbiol.* **2005**, *71*, 4809–4821. [CrossRef] [PubMed]
- Malone, J.; Jaeger, T.; Spangler, C.; Ritz, D.; Spang, A.; Arrieumerlou, C.; Kaever, V.; Landmann, R.; Jenal, U. YfiBNR mediates cyclic di-GMP dependent small colony variant formation and persistence in Pseudomonas aeruginosa. *PLoS Pathog.* 2010, *6*, e1000804. [CrossRef] [PubMed]
- 38. Clark, A. The Occupational Opportunist: An Update on Erysipelothrix rhusiopathiae Infection, Disease Pathogenesis, and Microbiology. *Clin. Microbiol. Newsl.* **2015**, *37*, 143–151. [CrossRef]
- Dankevych, L.; Leonova, N.; Dragovoz, I.; Patyka, V.; Kalinichenko, A.; Włodarczyk, P.; Włodarczyk, B. The synthesis of plant growth stimulators by phytopathogenic bacteria as factor of pathogenicity. *Appl. Ecol. Environ. Res.* 2018, *16*, 1581–1593. [CrossRef]



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