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Distribution and damage of bacterial canker on lemon bushes in the conditions of the Southern part of Azerbaijan

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SUMMARY

This paper summarises and presents the results of two-year (2020–2021) field and laboratory studies on the spread and harmfulness of bacterial canker of citrus crops (Xanthomonas citri (Hasse) Dowson.) on mid-ripening lemon cultivars (Citrus limon L.). Experimental field research conducted by us in 2020–2021. It has been established that bacterial canker (Xanthomonas citri) is found mainly on lemon bushes (Citrus limon L.). The pathogen also causes canker (Xanthomonas citri) on kinkans (Fortunella margarita (Lour) Swingle.), poncirus (Poncirus (L.) Raf.), lime (Cirtus aurantiifolia (Christm.) Swingle.). Japanese cultivars of oranges (Citrus sinensis (L.) Osbeck.) and citron (Citrus medica L.) are distinguished by significant tolerance. The most resistant are tangerines (Cirtus reticulata Blanco.) – they are almost not affected by bacterial canker (Xanthomonas citri).

We have established environmental factors that contribute to the wide spread and development of bacterial canker (Xanthomonas citri) on lemon bushes in the southern part of Azerbaijan.

The main indicators of the harmfulness of bacterial canker (Xanthomonas citri) have been identified. The potential for the death of canker-infected lemon fruits has been established. The characteristic features of external and internal signs of the disease were established. A scheme for combating bacterial canker (Xanthomonas citri) has been developed, and the optimal concentration of fungicides that retard the growth and development of pathogens has also been established. Photographs of bacterial canker (Xanthomonas citri) damage to lemon fruits are presented.

Keywords: *lemon, prevalence, harmfulness, bacterial canker, control measures*

INTRODUCTION

Lemon (*Citrus limon* L.) is a valuable subtropical fruit crop and is one of the representatives of the rue family (*Rutaceae* Juss.) (Guliyev, 1979).

Lemon fruits are characterized by high nutritional, dietary and medicinal properties. The pulp of a lemon (*Citrus limon* L.) contains: 88% water, 2% sugar, 1% pectin, 6% acid, 0.5% minerals, as well as vitamins A, B_1, B_2, C and PP (Guliyev, 2018; Guliev and Sheryshov, 1977).

Lemon essential oil (*Citrus limon* L.) is widely used in perfumery. A significant amount of it is produced in Italy, France, USA, Spain, Portugal, Brazil (Guliev, 1977).

In the former USSR, the commercial lemon crop (*Citrus limon* L.) is cultivated outdoors only in Azerbaijan and Georgia, as well as in some areas on the Black Sea coast. In other places, lemon (*Citrus limon* L.) is cultivated in greenhouses, and in Odessa, Transcarpathian regions, as well as in the Crimean peninsula of Ukraine, the states of Central Asia, Dagestan (Russian Federation), central Russia – in greenhouses or trenches.

Lemon trees (*Citrus limon* L.) have a height of 3–5 m, spreading crown, grow up to 100 years. Grafted plants begin fruiting 2–4 years after planting (Guliyev, 2000).

Lemon branches (*Citrus limon* L.) are often prickly. Has 4 growth waves starting from April; the second wave – in June; the third – August, the last – from September to December (Guliev, 2013).

Lemon (*Citrus limon* L.) is affected by many types of infectious diseases. The species composition and degree of development of some of them largely depend on the ecological and geographical conditions of the southern part of Azerbaijan.

Among the complex diseases of lemon (*Citrus limon* L.) grown in the conditions of the Lankaran-Astara geographical zone on the territory of Azerbaijan, the dominant position is occupied by bacterial canker (*Xanthomonas citri* (Hesse) Dowson.) (Guliyev and Huseynova, 2021).

Canker disease of citrus fruits (*Citrus* L.) was first discovered in 1827 in India (Kalyuzhny, 1985). Currently, the disease has spread widely in Central America, Argentina, USA (Louisiana, Florida, Texas), Morocco, Mozambique, Madagascar, Congo, South Africa, Afghanistan, India, Indochina, China, Japan, Sri Lanka, Malay Peninsula, Malay Archipelago and the Philippines, New Zealand, northern Australia, the Hawaiian Islands, etc. Previously, in the former Soviet Union (until 1985), bacterial canker of citrus crops (*Xanthomonas citri*) was absent. For the first time, this disease was discovered in 2020 by Professor F. Guliyev and doctoral student L. Huseynova on lemon bushes in the southern geographical zone of Azerbaijan (Lankaran region) (*Figure 1*).

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Figure 1: **Professor F. Guliyev at the lemon nursery**

The question arises: what is canker?

Canker is a disease of cultivated and wild plants, characterized by excessive, abnormal growth of trunks, branches, roots, less often other organs, leading to the formation of growths and tumors. The causative agents of plant cankers in most cases are fungi and bacteria. Canker-type diseases also include those that are characterized by the appearance of difficult-to-heal or non-healing ulcers on plants (Pospelov et al., 1973).

Symptoms of the disease appear on the leaves, leaf petioles, shoots, branches and fruits of the lemon (*Citrus limon* L.) (Jalagonia, 1979).

Initially, small greasy or watery spots appear on the leaves, more often from the underside, which then translucent. Over time, the spots acquire a yellowish-brown color, denser than the surrounding tissue, their middle grows and rises, forming a small tubercle. Further growth of the tissue leads to rupture of the epidermis of the leaf and the emergence of a tumor of a spongy structure. The tumor tissue also ruptures and a fossa with raised edges forms in its center. A light yellow halo is observed around the tumor. Old spots darken and harden. They are round at first, later they take on an irregular shape. Their size and color depend on the sensitivity of the cultivars and environmental conditions (*Figure 2*).

Figure 2: **Bacterial canker on lemon leaves**

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Similar spots form on the petioles, causing the death of the leaves.

Convex areas (up to 15 cm) of chestnut-brown color appear on the bark of shoots and branches. When girdling the shoots and branches, the latter die off.

It should be noted that at first small spots appear on the fruits of the lemon (*Figure 3–4–5*). And then the spots gradually increase in size and turn into a cankerous ulcer.

Figure 3–4–5: **The first stage of bacterial canker on lemon fruits**

On fruits, canker growths are the same in structure as on leaves, but do not have a light halo around the growth, and crater-shaped depressions are better expressed (*Figure 6–7–8*). The growths often merge, forming a crust, and crack as the fetus grows (*Figure 9–10*). Only the peel is affected. Most diseased fruits fall before ripening.

Xanthomonas citri (Hesse) Dowson. penetrates the plant through wounds, but can also through stomata. Bacteria fill the intercellular spaces, causing hypertrophy of the tissues of the host plant. The development of cancer depends on temperature and humidity. The disease is exacerbated by rainfall and rising temperatures. Seedlings and young plants are more susceptible to the disease than mature trees.

Figure 6–7–8: **Crater-shaped cankerous ulcers on lemon fruits**

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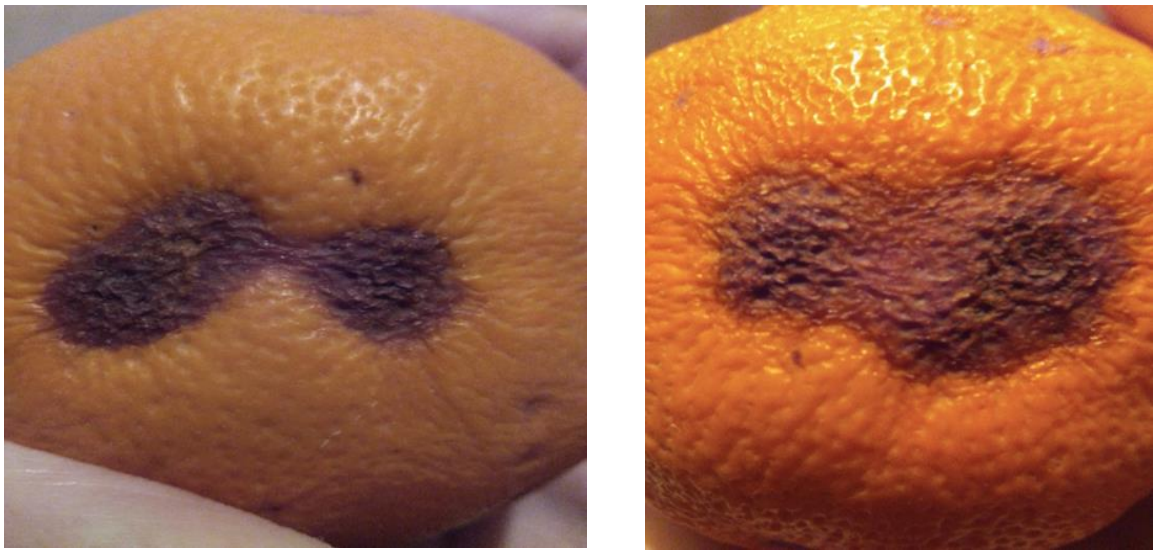
Figure 9–10: **Confluent cankerous ulcers on the same lemon fruit (beginning and after)**



Purpose and objective of research

The purpose of this work was to study the characteristics of the spread, development and harmfulness of the bacterial canker of the lemon (*Xanthomonas citri*), as well as to develop effective and environmentally balanced sets of methods for protecting against the disease.

To achieve this goal, it was supposed to solve the following tasks:

- To study the distribution, development and harmfulness of bacterial canker of lemon (*Xanthomonas citri*) in the conditions of the southern part of Azerbaijan;
- To identify the main environmental factors contributing to the widespread spread of bacterial lemon canker (*Xanthomonas citri*) in the southern part of the country;
- To study the biological characteristics of the causative agent of bacterial canker of the lemon - a fungus (*Xanthomonas citri*);
- To assess the resistance of local cultivars of lemon («Lankaran Local» and «Lankaran Meyer») to bacterial canker (*Xanthomonas citri*);
- Determination of the effectiveness of modern plant protection products and the timing of their use in the fight against bacterial canker of lemon (*Xanthomonas citri*) in the southern part of Azerbaijan;
- Establish the cost-effectiveness of the recommended protective measures.

MATERIALS AND RESEARCH METHODS

To determine the spread and development of the causative agent of bacterial canker of the lemon (*Xanthomonas citri*) in 2020–2021. conducted route surveys of industrial plantations of lemon (*Citrus limon* L.) located in the southern part (Lankaran-Astara geographical zone) of Azerbaijan. The experiments were laid against the background of the agrotechnical cultivation of lemon (*Citrus limon* L.) recommended for this zone. Field experiments were laid in 5 variants of 3-fold repetition. Observations and records of the prevalence and development of bacterial canker of the lemon (*Xanthomonas citri*) were carried out systematically throughout the entire vegetation, according to the methods generally accepted in phytopathology (Chumakov et al., 1974; Khokhryakov et al., 2003). The harmfulness of the bacterial canker of lemon (*Xanthomonas citri*) was studied on susceptible cultivars «Lankaran Meyer» and «Lankaran Local» (*Figure 11–12*).

Figure 11: **Cultivar «Lankaran Meyer»**

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Figure 12: **Cultivar «Lankaran Local»**

Microscopic analysis of plants selected by us during route surveys in 2020–2021 revealed a bacterium, *Xanthomonas citri* (Hasse) Dowson. belonging to the *Xanthomonadaceae* family.

After the morphological identification of the causative agent of bacterial canker (*Xanthomonas citri*), studies were carried out to study the prevalence and development of them in the southern regions of the republic. When establishing the prevalence and development of lemon canker (*Xanthomonas citri*) in the farms of Lankaran, Astara and Massali regions, lemon plantations were examined in 2–3 plots. 50 bushes were counted diagonally in each plot. All available trunks in the bush were examined and the infection was determined according to the five-point scale developed by us (Chumakov et al., 1974; Peresypkin, 1969; Zhuravlev and Sokolov, 1969:

0 point – healthy;
1 point – darkening of the root collar by 25%;
2 points – darkening over 25%, peeling and exfoliation of the bark in the area of the root neck;
3 points – darkening over 50%, peeling and flaking of the bark up to the branches;
4 points – darkening over 50%, peeling and peeling of the bark affects the branches, the leaves turn yellow;
5 points – darkening over 50%, peeling and peeling of the bark cover all the branches, they dry out, the leaves fall off.

The intensity of damage to lemon leaves (*Citrus limon* L.) by bacterial canker (*Xanthomonas citri*) was assessed on a scale:

0 point – there is no lesion;
1 point – from 1 to 10% of the leaf surface is affected;
2 points – from 11 to 25% of the leaf surface is affected;
3 points – from 25 to 50% of the leaf surface is affected;
4 points – more than 50% of the leaf surface is affected.

Accounting for the development of bacterial canker (*Xanthomonas citri*) on lemon fruits (*Citrus limon* L.) was carried out using the following scale:

0 point – fruits are healthy;
1 point – single very small, barely noticeable spots without cracks on the fruits;
2 points – single spots (1–3), clearly visible, 0.5–1.0 cm in diameter, without cracks or with slight cracks;
3 point – a significant number of clearly visible spots, 0.5–1.0 cm in diameter, without cracks or with light cracks;
4 points – spots are numerous, difficult to count, with deep cracks, 1 cm in diameter or more.

According to the accounting data, the spread (P,%) and development (R,%) of the disease were calculated.

The development of the disease **(R,%)** was determined by the formula (1) (Khokhryakov et al., 2003):

$$R=(\sum(nxb))/Nxn \qquad (1)$$

where **Σ(n×b)** is the sum of the products of the number of diseased plants **(n)** and the corresponding damage score **(b)**, **N** is the total number of registered plants, pcs., **K** is the highest damage score of the registration scale for converting the disease development score into a percentage category.

The prevalence of the disease **(P)**, expressed as a percentage, was calculated by formula (2) (Khokhryakov et al., 2003):

$$P=n/Nx100 \qquad (2)$$

where **n** is the number of diseased plants in samples, pcs., **N** is the total number of registered plants in samples, pcs.

Biological efficiency **(BE)**, expressed as a percentage, was calculated by formula (3) (Dyakov, 2012):

$$BE=(Mk-Mo)/Mkx100 \qquad (3)$$

where **Mk** is an indicator of the development of the disease in control (protective measures were not taken); **Mo** is an indicator of the development of the disease in the experiment (with protective measures).

Along with field experiments, we also carried out laboratory experiments.

All microscopic studies were performed on fresh plant material. For analysis, we took tissues with the initial stage of the development of the disease, since in the later stages they are often colonized by saprotrophic microorganisms, which cannot be distinguished from phytopathogenic ones under a microscope.

Plant organs (leaves, fruits, shoots, etc.) were first carefully washed in running water, then, at the border between diseased tissue and healthy tissue, small pieces were taken with a sterile scalpel and placed on clean glass slides. A small amount of sterile water was added and the tissue was minced with a sharp scalpel. A few minutes later, the bacterium *Xanthomonas citri* (Hasse) Dowson. begins to diffuse from plant tissue into water. The preparation was covered with a cover slip and observed under a microscope. As already noted, at the site of one of the sections, one can observe the release of bacteria from the affected tissue. Sometimes it is enough to crush plant tissue in a drop of water in order

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to establish the presence of bacteria in the material under examination under a microscope.

We supplemented the microscopic examination with differential Stoughton staining.

On heavily infested orchards of lemon (*Citrus limon* L.), the first spraying with fungicides was carried out before the start of the growing season, the second - during the period of fruit formation. In the field, the effectiveness of the applied fungicides was studied under conditions of a natural infectious background. For this, 5–10 lemon bushes were analyzed, evenly distributed throughout the garden. 25 leaves were examined from 4 sides, assessing the degree of damage according to the generally accepted 4-point scale. Based on the obtained data, the prevalence and development of the disease were calculated according to the formulas generally accepted in phytopathology. To identify the causative agent of the disease, the determinant Khokhryakov et al. was used (2003). Statistical analysis of the obtained results was carried out in accordance with the recommendations of Dospekhov (1985).

A field experiment to assess the biological effectiveness of the use of chemicals against bacterial canker (*Xanthomonas citri*) was also established in the lemon orchards of the Lankaran region. The following fungicides were used in the studies: Koruval 50 WP (Iprodione 500 g kg^{-1}), Komore 50 WP (Diphenactonazole 150 g l^{-1} + Propiconazole 150 g l^{-1}), Alirin B (Bacillus subtilis strain B-10 VIZR, titer not less than 109 CFU l^{-1}). ml), Abiga-Peak, VS (Copper oxychloride 400 g l^{-1}). Biological efficacy was determined by the reduction in the development of bacterial canker (*Xanthomonas citri*) on the treated variants relative to the control.

RESULTS AND DISCUSSION

In the conditions of the humid subtropics of the southern part of Azerbaijan, in terms of its negative economic significance, the bacterial canker of citrus (*Xanthomonas citri*) is one of the most harmful diseases of lemon bushes (*Citrus limon* L.).

In order to identify the prevalence of this disease in the southern regions of the republic (Masally, Lankaran, Astara), we conducted route and stationary surveys on lemon plantations in this geographical region.

Among the cultivated cultivars of lemon, there are both susceptible and resistant to bacterial canker (*Xanthomonas citri*). In the conditions of the southern part of Azerbaijan, the «Lankaran Meyer» lemon cultivar, as well as hybrids obtained from cultivars susceptible to this disease, are especially affected. Cultivar «Lankaran Local» is relatively resistant to this disease (*Table 1*).

Table 1: **Distribution and development of bacterial canker of lemon (*Xanthomonas citri* (Hasse) Dowson) in the conditions of the southern part of Azerbaijan (2020–2021)**

Southern regions of the country	Lemon cultivars	2020 year			2021 year		
		Accounting date	P, %	R, %	Accounting date	P, %	R, %
Masally	«Lankaran Meyer»	5.IX	24.0	10.4	3.X	28.1	13.4
	«Lankaran Local»	5.IX	12.6	6.1	3.X	17.0	9.5
Lankaran	«Lankaran Meyer»	12.IX	31.4	17.3	14.X	37.8	19.3
	«Lankaran Local»	12.IX	18.2	9.2	14.X	24.8	10.5
Astara	«Lankaran Meyer»	25.IX	27.8	13.2	21.X	31.9	15.1
	«Lankaran Local»	25.IX	13.0	6.6	21.X	23.0	9.4
Average	«Lankaran Meyer»	-	83.2	40.9	-	97.8	47.8
	«Lankaran Local»	-	43.8	21.9	-	64.8	29.4

Note: P is the prevalence of the disease, %; **R** is the intensity of development, %

The table shows that the bacterial canker of the lemon (*Xanthomonas citri*) occurs in all surveyed areas and to a fairly high degree. Thus, in 2020, the spread of bacterial canker of lemon (*Xanthomonas citri*) in the case of the «Lankaran Meyer» cultivar ranged from 24.0 to 31.4%; and for the «Local Lankaran» cultivar, respectively, from 12.6% to 18.2%. In 2021, the prevalence of the «Lankaran Meyer» cultivar is from 28.1 to 37.8%; and for the «Local Lankaran» cultivar, respectively, it ranged from 17.0 to 24.8%.

As can be seen from the table, the smallest spread of bacterial canker of the lemon (*Xanthomonas citri*) was noted in 2020, the largest in 2021.

To explain this, we analyzed the meteorological conditions of these years and found that the most favorable conditions for the development of the disease were in 2021. This year was characterized by abundant rainfall and suitable temperatures for the development of the disease.

Thus, observations have shown that the spread and development of bacterial canker of the lemon (*Xanthomonas citri*) depends on temperature and humidity. Since, the disease is aggravated by precipitation and an increase in temperature.

It should be noted that small cankerous spots initially have a dark violet color (*Figure 13*).

According to our observations, these small spots expand with time and turn pale (*Figure 14*). After some time, these spots expand even more, darken and take on the appearance of a thick, rough, dark brown ulcer (*Figure 15–16*).

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Figure 13: **Small spots on dark violet color**

Figure 14: **While expanding spots become pale**

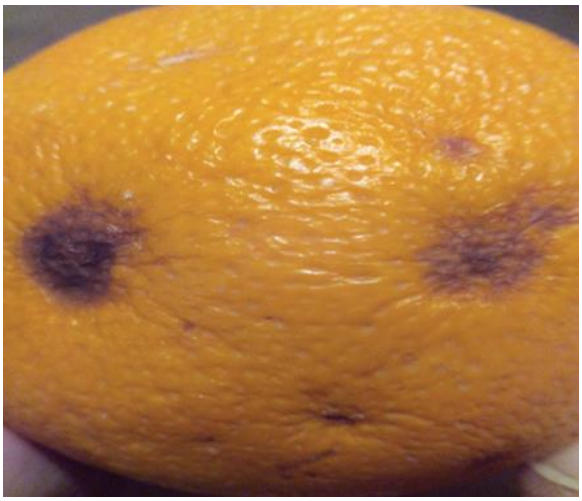

Figure 15–16: **Thick, rough, dark brown cankerous ulcers**

The causative agent of citrus canker is *Xanthomonas citri* (Hasse) Dowson. This is a mobile short bacterium with one polar flagellum, 0.5–0.75x1.5–2.0 microns in size. Rods are single or in chains, do not form spores, aerobic, gram-negative, have capsules. Colonies on meat-peptone agar are straw-yellow to amber-yellow, round, slightly raised, shiny with whole edges. On agar and boiled potatoes, the pathogen produces yellowish colonies around which a narrow white zone appears. Subsequently, it disappears, and the surface of the potato slice is covered with a thick yellow slimy coating.

Bacteria coagulate milk, liquefy gelatin, do not restore nitrates, decompose starch, form ammonia, do not form indole, acid, nor gas on dextrose, galactose, glycerin, lactose, levulose and sucrose. The optimum growth temperature of bacteria is 20–30 °C, the maximum temperature is 35 °C, at a temperature of 49–52 °C they die.

Protection of lemon against bacterial canker (*Xanthomonas citri*) is an essential element in the technology of its cultivation. Against the bacterial canker of lemon (*Xanthomonas citri*), we paid some attention to the development of a chemical method of struggle. For this purpose, the following fungicides were tested: Koruval 50 WP (Iprodione 500 g kg^{-1}), Komore 50 WP (Diphenactonazole 150 g l^{-1} + Propiconazole 150 g l^{-1}), Alirin - B (*Bacillus subtilis* strain B-10 VIZR, titer not less than 109 CFU ml^{-1}), Abiga-Peak BC (Copper oxychloride 400 g l^{-1}) (*Table 2*).

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Table 2: **Influence of fungicides on the spread and development of bacterial canker of lemon (*Xanthomonas citri* (Hasse) Dowson) in the conditions of the southern part of Azerbaijan**

Experience options (drugs and their active substance)	Dosage, l ha^{-1}; kg ha^{-1}	After the third spraying					
		2020 year			2021 year		
		P, %	R, %	BE, %	P, %	R, %	BE, %
Koruval 50 WP (Iprodione 500 g kg^{-1})	0,5 kg ha^{-1}	22.2	13.8	64.4	17.0	8.3	79.0
Komore 50 WP (Diphenactonazole 150 g l^{-1} + Propiconazole 150 g l^{-1})	0,5 l ha^{-1}	20.1	11.5	70.4	16.8	7.4	81.2
Alirin B (*Bacillus subtilis* strain B-10 VIZR, titer not less than 109 CFU ml^{-1})	1 tablet per litres	25.0	15.9	59.0	21.1	12.3	69.0
Abiga-Peak, BC (Copper oxychloride 400 g l^{-1})	5 kg ha^{-1}	26.7	16.2	58.2	24.4	14.1	64.1
Control (without chemical treatment)	–	65.5	38.8	–	66.6	39.3	–

The results of the statistical analysis

Note: P- is the prevalence of the disease, %; **R** – intensity of development, %; **BE** – biological efficiency, %

As can be seen from the table, chemical measures give a good effect; the spread and development of the disease is reduced.

Spraying was carried out in 3 terms. Field studies conducted by us found that good results were obtained in the variant where the spraying was carried out with the fungicide Komore 50 WP (Diphenactonazole 150 g l^{-1} + Propiconazole 150 g l^{-1}), and Koruval 50 WP (Iprodion 500 g kg^{-1}) turned out to be the best in terms of the effectiveness of fungicides. Experimental field studies have established that in 2021 the biological effectiveness of the fungicides Komorée 50 WP and Koruval 50 WP in protecting lemon from bacterial canker (*Xanthomonas citri*) was 81.2–79.0%, respectively.

The annually increasing range of chemicals, including systemic fungicides, their high efficiency and speed of action have created the prerequisites for their large-scale use. This leads to negative consequences, one of which is the development of resistance. To prevent this process, the alternation of systemic and contact preparations is very important.

CONCLUSIONS

Citrus growing is one of the leading branches of agriculture in the subtropical zone of the southern part of Azerbaijan.

One of the most common and harmful diseases of lemon is bacterial canker (*Xanthomonas citri*). According to the two-year assessment of the phytosanitary state of lemon plantations in the southern part of the country, the presence of bacterial canker (*Xanthomonas citri*) was detected, despite the fungicidal protection against the disease during the growing season of lemon. The highest prevalence of bacterial canker (*Xanthomonas citri*) was noted in 2021 (before chemical treatment of lemon plantations).

The harmfulness of bacterial canker (*Xanthomonas citri*) is very high. Since the disease sharply weakens assimilation and enhances transpiration, causes premature death of leaves, death of shoots and branches and contributes to the mass abscission of green fruits. Thus, with severe damage, the yield of lemon bushes is significantly reduced.

Lemon bacterial canker (*Xanthomonas citri*) degrades product quality. Affected fruits do not meet the requirements of the standard. Fruits of medium size (25–35 mm) are more severely infected, while the bacteria infect only the peel and do not penetrate into the pulp of the fruit.

The optimum temperature for the development of bacteria is +20–30 ºC; maximum +35 ºC; at +49 ºC they die. Infected plant residues must be decomposed. But, bacteria persist in tumors until they are decomposed.

Depending on the resistance of cultivars and external conditions, the incubation period of the disease lasts from 2 days to several months. Infection occurs in places of damage or in the stomata in the presence of a drop of water.

According to the results of a two-year (2020–2021) study of the effectiveness of fungicides (Koruval 50 WP (Iprodione 500 g kg^{-1}), Komore 50 WP (Diphenactonazole 150 g l^{-1} + Propiconazole 150 g l^{-1})), Alirin B (Bacillus subtilis B strain) -10 VIZR, titer not less than 109 CFU ml^{-1}), Abiga-Peak, VS (copper oxychloride 400 g l^{-1}) it was found that their 3-fold use in the lemon defense system during the most vulnerable periods for infection reduces the development of bacterial canker (*Xanthomonas citri*) up to 50% in the general result of the application of all fungicides.

As a result of a two-year (2020–2021) study of the effectiveness of fungicides, it was found that good results were obtained in the variant where spraying was carried out with the fungicide Komore 50 WP. And the rest of the fungicides we tested required level.

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