Inclusion of plant immunity inducers in the fruit crops protection system for the purpose of reducing the pesticide load

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Abstract. One of the new environmentally friendly areas of plant protection is the use of natural protective mechanisms in protection against phytopathogens. The purpose of this study was to evaluate the role of plant immunity inducers of various chemical nature (poly-beta-hydroxybutyric acid, arachidonic acid ethyl ester and chitosan lactate) when included in peach and apple protection systems against diseases dominating in the region of wet subtropics of Russia and Abkhazia (leaf curl and scab, respectively). Studies on peach against leaf curl were conducted on the variety ‘Redhaven’ (Sochi, Russia) in 2014-2016, and on the apple tree against scab on the variety ‘Golden Rangers’ (Gulripshi district, Abkhazia) in 2016-2019. The standard was the peach and apple tree chemical protection system adopted in the region. We studied the effectiveness of tank mixtures of plant immunity inducers (Albit®, Immunocytophite®, Ecogel®) with half dosages of pesticides used in the standard. The biological effectiveness of all mixtures with plant immunity inducers on peach exceeded the standard, and on the apple tree was at the standard level. The best results were obtained in variants with Ecogel® and Albit®. In variants with plant immunity inducers crop yields were 23-60% higher than in the control.

1. Introduction
One of the main elements of fruit crop cultivation technologies is their protection against diseases and pests. The main pest of the apple tree is Cydia pomonella L., the main diseases are apple scab (Venturia carpophila E.E. Fisher), powdery mildew (Podosphaera leucotricha (Ellis & Everh.) E.S. Salmon) and fruit rot (Monilinia fructigena Honey). The main pest of the peach is Grapholita molesta Busck., the main diseases are peach leaf curl (Taphrina deformans (Berk.) Tul.), shot hole disease (Stigmina carpophila (Lév.) MB Ellis) and fruit rot (Monilinia laxa (Aderh. & Ruhland) Honey). So, the cultivation of apple and peach is traditionally carried out using chemical pesticides that are used repeatedly during the growing season. The humid subtropics of the Black Sea coast of the Caucasus are a resort region where the use of chemical protective agents is limited or prohibited. In this regard, the issue of greening existing plant protection systems while maintaining their effectiveness, the search for new, environmentally friendly plant protection products are one of the most relevant in...
agronomy [1, 2]. Wide application of ecological plant protection systems is a good opportunity for minimizing a negative impact on the environment [3].

The introduction of plant immunity inducers into plant protection systems, the action of which is based on the activation of mechanisms of non-specific resistance, is one of the new ecologically safe areas of plant protection [4-6]. Different from the conventional biocidal agrochemicals, synthetic chemical inducers of plant immunity activate, bolster, or prime plant defense machineries rather than directly acting on the pathogens [7]. Immunity inducers provide several positive effects: increasing the immune status, enhancing growth processes, stimulating reproductive properties, etc., which ultimately leads to increased crop yields [8, 9].

Immunity inducers of various origins have proven themselves in cereals and vegetables, but only a few have been tested on wood crops, including fruit crops [8-16]. Treatment with resistance inducers or beneficial microorganisms provides long-lasting resistance for plants to a wide range of pathogens [17].

The purpose of this study was to evaluate the role of plant immunity inducers of various chemical nature (poly-beta-hydroxybutyric acid, arachidonic acid ethyl ester and chitosan lactate) when included in peach and apple protection systems against diseases dominating in the region of wet subtropics of Russia and Abkhazia (leaf curl and scab, respectively). Studies of the possibility of using immunity inducers in fruit crop protection systems in humid subtropics are being conducted for the first time.

2. Materials and methods

Studies on peach against leaf curl were carried out in the orchards of Russian Research Institute of Floriculture and Subtropical Crops (Sochi, Russia) on the variety ‘Redhaven’ in 2014-2016. Studies on the apple tree against scab were carried out in the stands of Institute of agriculture of Academy of sciences of Abkhazia (Gulripshi district, Abkhazia) in the variety ‘Golden Rangers’ in 2016-2019.

The experiment included 5 variants in six-fold repetition on a peach and five-fold repetition on an apple tree (one tree – one repetition):
1. Control (water treatment, without fungicides and immunity inducers).
2. Production treatment: on peach – Delan® (dithianon) (0.7 kg/ha) – 1 decade of April, Scor® (diphenonazole) (0.2 l/ha) – 1 decade of May and I decade of June; on the apple tree – Scor® (diphenonazole) (0.2 l/ha) – 1 decade of May; Topaz® (penconazole) (0.3 l/ha) – I decade of June; Strobi® (kresoxim-methyl) (0.2 kg/ha) – I and III decades of July.
3. Albit® (poly-beta-hydroxybutyric acid) (250 ml/ha) with half doses of fungicides.
4. Immunocytophyte® (arachidonic acid ethyl ester) (0.6 g/ha) with half doses of fungicides.
5. Ecogel® (chitosan lactate) (15 l/ha) with half doses of fungicides.

Prior to the experiment, the plantings were treated once with a Bordeaux mixture (3%) during the period of bud swelling. The terms of treatment with tank mixtures of fungicides with Albit®, Immunocytophyte® and Ecogel® corresponded to the production ones. Diagnosis of diseases was carried out on the 7th day after treatment. Accounting for diseases development was carried out according to the generally accepted method [18]. Biological effectiveness is calculated using the Abbot formula.

In April 2017, during the initial development of leaf curl, peach leaves were selected for analysis of the presence of pathogen DNA in plant tissues. To isolate pathogen DNA from plant tissues, leaves were taken from three different parts of the crown. Genomic DNA was isolated using the CTAB method for nucleic acid extraction. DNA quality was checked by electrophoresis at 150 V in a 1% agarose gel prepared based on 0.5 M Tris-borate-EDTA buffer with the addition of ethidium bromide. Oligonucleotides (primers) were synthesized in the company Synthol. To detect Taphrina deformans (Berk.) Tul. DNA, we used the species-specific primer TDITS1 5'-TCTCCGGATGGGTTCAC-3' (used in conjunction with the universal fungal primer NL4) [19]. DNA concentration and purity were measured by spectrophotometric method on an IMPLEN N5 instrument [20].

All research results were statistically processed in the MSExcel.
3. Results and discussion

The positive result of the use of immunity inducers in the peach protection system is to increase the culture’s resistance to leaf curl. During the research period, a moderate degree of exposure to *Taphrina deformans* on peach leaves was observed. In all variants of the experiment the pathogenesis of leaf curl was of the same type. Visual diagnosis revealed a high degree of leaf damage by the pathogen *T. deformans*. However, in the experimental variants, the number of affected leaves was on average 5.2 times less compared to the control (table 1).

**Table 1.** The degree of peach leaf curl development (R, %) (*Taphrina deformans*) after treatment with immunity inducers, 2014-2016 (Sochi, Russia).

| Experience variant                  | Peach leaf curl development (M±m, %) |
|------------------------------------|--------------------------------------|
|                                    | 2014 | 2015 | 2016 | 2016 | 2016 | 2016 |
| Control                            |      |      |      |      |      |      |
|                                    | April | May | June | April | May | June | April | May | June | April | May | June |
|                                    | 24.8 | 21.0 | 11.8 | 19.3 | 31.2 | 14.2 | 23.1 | 20.0 | 12.6 |      |      |      |
|                                    | ±2.6 | ±2.7 | ±2.0 | ±1.8 | ±4.9 | ±2.1 | ±1.6 | ±2.8 | ±1.0 |      |      |      |
| Production treatment (Standard)    |      |      |      |      |      |      |      |      |      |      |      |      |
|                                    | 13.2 | 8.0 | 6.1 | 11.4 | 7.8 | 7.0 | 9.4 | 8.8 | 7.8 |      |      |      |
|                                    | ±2.1 | ±1.9 | ±1.5 | ±1.9 | ±1.9 | ±1.2 | ±0.8 | ±1.3 | ±1.2 |      |      |      |
| Albit® with half doses of fungicides |      |      |      |      |      |      |      |      |      |      |      |      |
|                                    | 8.7 | 6.2 | 6.3 | 5.3 | 6.2 | 6.4 | 4.8 | 8.1 | 6.5 |      |      |      |
|                                    | ±0.6 | ±0.5 | ±1.5 | ±1.0 | ±2.2 | ±1.1 | ±1.2 | ±1.3 | ±0.8 |      |      |      |
| Immunocytophyte® with half doses of fungicides |      |      |      |      |      |      |      |      |      |      |      |      |
|                                    | 7.7 | 7.6 | 6.0 | 8.4 | 8.7 | 7.4 | 8.6 | 8.0 | 8.2 |      |      |      |
|                                    | ±1.3 | ±1.2 | ±1.5 | ±0.8 | ±1.4 | ±1.1 | ±1.3 | ±0.7 | ±1.3 |      |      |      |
| Ecogel® with half doses of fungicides |      |      |      |      |      |      |      |      |      |      |      |      |
|                                    | 5.5 | 4.0 | 1.0 | 3.6 | 7.0 | 5.8 | 4.7 | 7.2 | 5.4 |      |      |      |
|                                    | ±1.5 | ±1.2 | ±0.3 | ±1.2 | ±1.2 | ±1.3 | ±0.6 | ±1.1 |      |      |      |      |
| LSD₀₅                          | 1.2 | 1.0 | 0.6 | 1.0 | 1.7 | 0.5 | 1.3 | 0.8 | 0.5 |      |      |      |

In 2014, the maximum level of leaf curl development was 24.8%, in 2015 – 31.2%, in 2016 – 23.1%. The inclusion of immunoinductors in the treatment of peach entailed a positive effect, which was expressed in containing the disease development. A significant protective effect of fungicides in half doses was established together with Albit® and Ecogel®. The degree of peach leaves damage by leaf curl in 2014 in the experimental variants using Ecogel® was lower than the result obtained in the standard by 58%, and in the experimental variants using Albit® – by 34%. A similar pattern was established in 2015-2016. The obtained data indicate the significant role of nonspecific immunity induced by Albit® and Ecogel® in the fight against peach leaf curl.

The protective properties of immunity inducers in the fight against the development and spread of peach leaf curl are reflected in the dynamics of the biological effectiveness during the period of maximum development of disease (third decade of April) (figure 1).

In the standard, the biological effectiveness was in the range of 40-60% depending on the year of study. In all experimental variants after the first treatment of peach with tank mixtures of immunity inducers with fungicides, the level of biological effectiveness during the period of maximum leaf curl development (third decade of April) exceeded the values obtained in the standard variant. Variants with Ecogel® and Albit® had higher biological effectiveness which is a consequence of protective effect growth with the combined influence of the immunity inducer and fungicide. The variant with Immunocytophyte® had a smaller effect, but in this case, the biological effectiveness was higher than in the standard. In June, when leaf curl development naturally decreased, the biological effectiveness of variants with immunity inducers was at the standard level.

It was noted in the third year of application the biological effectiveness of tank mixtures with immunity inducers increased.

The use of immunity inducers not only reduced the degree of leaf curl development (obviously, due to increased plant resistance) but also suppressed the pathogen development. In the case of intense leaf curl damage, a high amount of *T. deformans* DNA was found in leaf tissues – 303.5 ng/g (leaves with
symptoms of the disease). The results of PCR detection of *T. deformans* in asymptomatic peach leaves showed the presence of pathogen DNA in all samples studied. In the asymptomatic leaves in the control variant, the phytopathogen DNA content was lower than in the leaves with symptoms and amounted to 170.4 ng/g. After treatment with immunity inducers in asymptomatic leaves, an even lower phytopathogen content was recorded – 86.2 and 84.1 ng/g of tissue in variants with Albit® and Ecogel®, respectively. The obtained data suggest that the use of immunity inducers contributed to the activation of protective mechanisms that impede the direct development of *T. deformans* in peach leaves. Apparently, the leaf curl symptoms begin to appear after reaching a certain level of accumulation and development of the pathogen in the plant leaves.

![Figure 1](image_url)

Figure 1. Biological effectiveness of immunity inducers in tank mixtures with fungicides in the fight against *Taphrina deformans* in 2014-2016 (April).

The use of Albit® and Ecogel® in a tank mixture with fungicides also showed high profitability – 133.9 and 87.0%, respectively (profitability of the production treatment was 73.3%) (table 2). An increase in profitability was noted due to increased yields and lower costs for drugs used in the proposed protection systems.

**Table 2.** The economic efficiency of using immunity inducers in the system for protecting peach from leaf curls (Sochi, ‘Redhaven’ variety, average for 2014-2016).

| Experience variant                                      | Productivity, t/ha | Direct costs, ruble/ha | Conditionally net income, ruble/ha | Profit, ruble/ha | Profitability, % |
|---------------------------------------------------------|--------------------|------------------------|------------------------------------|-----------------|-----------------|
| Control                                                 | 4.0                | 143995                 | 4200                               | 160000          | 16005           | 11.1            |
| Production treatment (Standard)                         | 7.0                | 161565                 | 10837                              | 280000          | 118435          | 73.3            |
| Albit® with half doses of fungicides                    | 9.9                | 169316                 | 8018                               | 396000          | 226684          | 133.9           |
| Immunocytophyte® with half doses of fungicides          | 7.2                | 160132                 | 8674                               | 288000          | 127868          | 80.0            |
| Ecogel® with half doses of fungicides                   | 7.5                | 160801                 | 8250                               | 300000          | 139199          | 87.0            |
The study of the immunostimulating properties of Albit®, Immunocytophyte® and Ecogel® on apple trees was carried out in years characterized by different virulence of the scab pathogen (Venturia inaequalis (Cooke) G. Winter). The highest degree of scab development was observed in 2016; in subsequent years it was significantly lower, dropping to a minimum in 2019 (table 3).

**Table 3.** The degree of development (R, %) of the scab on apple leaves after treatment with immunity inducers, 2016-2019 (Gurypshi district, Abkhazia, ‘Golden Rangers’ variety).

| Experience variant | Development of the scab on apple leaves (M±m, %) |
|--------------------|-----------------------------------------------|
|                    | I decade of May | I decade of June | I decade of July | III decade of July |
| **2016**           |                |                |                |                   |
| Control            | 11.6±2.9       | 9.0±3.3        | 15.0±1.6       | 22.0±1.5          |
| Production treatment (Standard) | 2.6±0.8       | 3.8±1.9        | 6.0±1.5        | 7.0±0.7           |
| Albit® with half doses of fungicides | 3.8±2.1       | 4.0±1.5        | 4.4±0.5        | 7.6±5.1           |
| Immunocytophyte® with half doses of fungicides | 2.6±1.3       | 4.8±1.9        | 6.4±3.2        | 9.8±1.9           |
| Ecogel® with half doses of fungicides | 2.8±1.3       | 4.0±0.7        | 6.0±1.4        | 7.8±2.2           |
| **LSD**<sub>0.05</sub> | 0.7 | 0.9 | 0.7 | 0.7 |
| **2017**           |                |                |                |                   |
| Control            | 4.0±0.7        | 6.2±0.4        | 6.8±0.8        | 8.8±0.8           |
| Production treatment (Standard) | 1.8±0.8       | 3.2±0.4        | 3.6±0.5        | 3.2±0.8           |
| Albit® with half doses of fungicides | 2.0±0.7       | 2.4±0.5        | 3.8±0.4        | 3.2±1.3           |
| Immunocytophyte® with half doses of fungicides | 2.4±0.8       | 3.2±0.8        | 4.0±0.7        | 3.6±0.5           |
| Ecogel® with half doses of fungicides | 1.8±0.4       | 2.8±0.8        | 3.2±0.4        | 3.0±1.2           |
| **LSD**<sub>0.05</sub> | 0.3 | 0.4 | 0.5 | 0.6 |
| **2018**           |                |                |                |                   |
| Control            | 5.6±0.4        | 7.8±0.8        | 10.4±1.1       | 14.0±1.1          |
| Production treatment (Standard) | 1.2±0.4       | 1.8±0.8        | 4.0±0.7        | 4.0±0.8           |
| Albit® with half doses of fungicides | 1.4±0.5       | 2.4±0.8        | 5.6±0.5        | 5.2±0.7           |
| Immunocytophyte® with half doses of fungicides | 1.4±0.7       | 2.6±1.1        | 4.8±1.0        | 4.2±0.7           |
| Ecogel® with half doses of fungicides | 1.0±0.4       | 2.6±0.7        | 5.2±0.8        | 4.6±0.7           |
| **LSD**<sub>0.05</sub> | 0.4 | 0.5 | 0.8 | 1.0 |
| **2019**           |                |                |                |                   |
| Control            | 2.6±1.1        | 6.4±1.3        | 6.2±1.3        | 5.8±0.8           |
| Production treatment (Standard) | 1.6±0.6       | 2.6±0.5        | 2.0±1.0        | 1.8±0.8           |
| Albit® with half doses of fungicides | 1.4±0.6       | 3.4±1.1        | 3.6±0.8        | 3.2±1.3           |
| Immunocytophyte® with half doses of fungicides | 1.2±0.4       | 3.8±1.3        | 2.0±0.7        | 1.6±0.5           |
| Ecogel® with half doses of fungicides | 1.4±0.5       | 3.6±0.8        | 2.4±1.1        | 2.6±0.8           |
| **LSD**<sub>0.05</sub> | 0.2 | 0.5 | 0.4 | 0.4 |
In all the years of the experiment (2016-2019), the use of fungicides in the variant of production treatment restrained the intensity of scab development and reduced its negative impact. In the variants of the experiment with the use of plant immunity inducers, the degree of protective action was similar, with no significant differences from the variant of production treatment. An important advantage of these variants was a halving of the consumption of chemical fungicides.

This conclusion is also confirmed by the results of the biological effectiveness of using tank mixtures with immunity inducers (figure 2).

![Figure 2](image_url)

**Figure 2.** Biological effectiveness of immunity inducers in tank mixtures with fungicides in the fight against *Venturia inaequalis* on ‘Golden Rangers’ apple in 2016-2019 (Gulrypshi district, Abkhazia, June).

In 2018 the positive effect of immunity inducers use was showed most significantly. This may be due to the cumulative effect of the drug in this group. In 2019, the biological effectiveness is lower than in previous years of the study. The reason may be, on the one hand, a very low degree of development of the apple scab in this year. On the other hand, immunity inducers, when used for several years, can lead to a decrease in effectiveness due to the depletion of the internal resources of the plant. Therefore, a decrease in biological effectiveness may signal the need to take a break in the use of these drugs.

Immunocytophyte® showed the lowest efficiency for the entire observation period. This situation can be explained by the fact that Immunocytophyte® activates the jasmonate pathway for the formation of non-specific induced immunity, while Albit® and Ecogel® activate the salicylate pathway. Perhaps this fact explains the lack of Immunocytophyte® effectiveness in countering pathogens of the biotrophic nutrition type, which include the causative agent of scab *V. inaequalis* [1].

In addition to the protective effect against the dominant in the region pathogens, the use of plant immunity inducers leads to an increase in apple yield. The yield in the control variant was 8.122 ± 0.399 kg/tree in 2017, 8.911 ± 0.378 kg/tree in 2018, and 10.126 ± 0.387 kg/tree in 2019. Application of Albit® with half doses of fungicides increased the yield of ‘Golden Rangers’ apple trees by 71.1-142.7% compared to the control variant (figure 3). Immunocytophyte® and Ecogel® also led to a significant increase in yield compared to the control and production treatment variant. The maximum values of the yield increase were obtained in the third year (2018) of the experiment. During the four years of the experiment, the frequency of fruiting was not noted. Obviously, this is due to the fact that
the plants are young and during the research period they were just increasing their productivity, reaching full fruiting.

![Figure 3](image_url) The effect of treatments with fungicides and immunity inducers on the yield of apple trees, ‘Golden Rangers’ variety (Gulripshi district, Abkhazia, 2017-2019).

Thus, during the study period a stable result was achieved in the experimental variants using Albit® and Ecogel® with half doses of fungicides, indicating their high inducing activity, which was higher or at the level of accepted production processing.

4. Conclusion
The results obtained are consistent with the literature that the protective action of immunity inducers is based on the activation of natural plant resistance mechanisms that block the development of pathogens [1, 4, 6, 9]. The salicylate pathway of nonspecific induced immunity formation, which activates the use of Albit® and Ecogel®, provides resistance to the development of pathogens of apple scab (V. inaequalis) and peach leaf curl (T. deformans), characterized by biotrophic nutrition type. This explains the unexpressed protective properties of the Immunocytophyte®, which activates the jasmonate pathway, whose role in the fight against biotrophic pathogens is ineffective [1].

The presence of T. deformans DNA in asymptomatic peach leaves was established which confirms the presence of a latent period of the disease. Treatment of peach with immunity inducers leads to a decrease in the amount of T. deformans DNA in asymptomatic leaves.

Thus, the evidence-based use of plant immunity inducers in the cultivation technologies of fruit crops is justified. Immunity inducers show high biological effectiveness against dominant pathogens when they are included in peach and apple tree protection systems in humid subtropics of the Black Sea coast of the Caucasus. The use of plant immunity inducers in fruit crops protection allows halving the application doses of chemical fungicides, while maintaining biological effectiveness at the level of the production protection system adopted in the region. This fact is a strong argument in favor of the inclusion of immunity inducers in the fruit crops protection system in resort regions and not only in them. The use of immunity inducers in protection systems stably, for 3-4 years, increases the yield of the studied fruit crops.

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