Fungal diseases in pistachio trees caused by *cylindrosporum concentricum* pathogen: A case study of Uzbekistan

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Abstract. This article discusses the fungal infections of pistachios and the results of the fight against them. On a yearly basis, pistachio diseases have a significant impact on yields. Three drugs were utilized in the research. All of them are drugs recommended for use in agriculture. The drugs were used in the recommended doses and the results were shown. As a result of this research, a large part of the pistachio crop can be preserved. It is also recommended to produce a drug that has shown good results against the disease.

1. Introduction

The industry of tree nuts has an important impact on the economy of several countries and the raw materials are obtained by cultivating or extracting the nuts from forests. Tree nuts are excellent sources of nutrients and preventing diseases. In contrast, they are affected by environmental factors, the nut species, varieties or cultivar and processing temperatures. They can favor some mycotoxin fungi producers, such as, Aspergillus and Penicillium species, common in some nuts and their by-products. Mycotoxins can cause economic losses and there is objective evidence of mycotoxin toxicity to man and animals. In this context, the objective of this review is to present some of the tree nuts and peanuts most commonly associated fungi and mycotoxins [1].

The most valuable type of walnut in Uzbekistan is handon pistachio. Currently, pistachio plantations cover more than 30,000 hectares [2]. In recent years, the area under walnuts (pistachios, almonds, walnuts) has increased significantly. In particular, these crops have been expanded due to the low-irrigated or non-irrigated areas of the country. However, factors that negatively affect pistachio yields reduce yields each year. *Cylindrosporiosis* and *septoriosis* caused by fungi are common in our country. Under the influence of these diseases, pistachio leaves are stained, unable to perform their function, and the yield is reduced.

In Uzbekistan, foothills and mountain slopes cover a large area. These lands are mainly dry lands and are often used as pastures. The economic efficiency is much lower when crops are planted on those lands. It is advisable to plant pistachios on such lands.

In this regard, in recent years the area under walnuts (pistachios, almonds, and nuts) has increased significantly. In particular, these crops have been expanded at the expense of the low-irrigated or non-irrigated areas of the country.

There are a number of opportunities for further development of this sector and high yields from these crops. This requires the widespread introduction of modern intensive gardening technology [3]. An important task of this technology is to protect the crop from pests, especially diseases, which cause the
loss of a large part of the crop. Today, several fungal diseases are observed in handon pistachio plantations.

*Tsihindrosporiosis* (*cylindrosporium concentricum*) - The disease is widespread, especially in the southern region, where pistachios grow. Under conditions of high infectious load, the disease spreads very rapidly, infecting up to 90% of plants (pistachio.uz), *Septoriosis* (*septoria pistacia A.*) and others [4].

*Cylindrosporiosis*, also known as white spot, is the most common disease in pistachio plantation in Uzbekistan. Its causative agent is the fungus *Cylindrosporium concentrium*. The pathogen overwinters in the affected parts of the plant as well as in the seeds in the form of spore carriers and conidia. Conidia are firmly rooted in the mature seed coat and are also stored in tree debris, broken branches, shed leaves. With the onset of the growing season, pathogenic spores become carriers and begin secondary damage to the plant [5].

Symptoms of the disease appear on the leaves and stems of the plant. At first, light green spots appear on the surface of the leaves. Later, these spots turn dark brown and evoke a burn-like appearance. Damaged leaves change shape, dry out prematurely. Favorable conditions for the spread of the disease: +10, +150S humidity, temperature and light, rainy weather; wind, and closer planting of trees.

Damaged tree can not perform its function, productivity decreases, if timely measures are not taken to control the disease, the disease spreads to 90% of the plant [6, 7].

Today, the total area of land planted with pistachios is 30,000 hectares [2]. Resolution of the President of the Republic of Uzbekistan dated May 11, 2017 No PP-2966 "On the organization of the State Committee of Forestry of the Republic of Uzbekistan" [1], Resolution of the Cabinet of Ministers of the Republic of Uzbekistan dated January 19, 2015 No 5 This dissertation research to some extent serves as a scientific basis for the decision and the tasks in other normative legal acts.

Toxigenic fungi and mycotoxins were introduced into human food supplies about the time when mankind first began to cultivate crops and store them from one season to the next, which was about 10 000 years ago. Not all fungi associated to tree nuts are mycotoxin producers; however, many strains are often associated with dried fruit and seeds [8].

2. Method

2.1. Geographical Location

Scientific research was conducted in two regions of the Republic of Uzbekistan. The first experimental area was a 20-hectare pistachio plantation located in Gallaorol district of Jizzakh region.

The phytosanitary condition of pistachio plantations in Jizzakh region has been studied since April 2019 (Figure 1) [9]. Every year, pistachio plantations are severely damaged by fungal diseases. As a result of these diseases, pistachio yields are reduced.

![Figure 1. Experimental area, 2019. Jizzakh region of Republic of Uzbekistan](image-url)
2.2. Diagnosis

To diagnose the disease, 100 leaves and fruits as well as 10 leaf blades and young twigs are examined without being removed from 4 sides of the selected pistachio tree at the time of counting. The degree of damage is taken into account on each leaf, fruit, leaf blade and young twigs on the following scales (see Table 1).

Table 1. Determination the infection on pistachio by scales

| Scale 1 - to determine the infestation of pistachio leaves with cylindrosporiosis, septoria | Points | Description |
|---|---|---|
| 0 | no damage | leaves have 1-5 small, barely visible spots |
| 1 | spots occupy up to 10% of the leaf surface | |
| 2 | spots occupy 10-25 percent of the leaf surface | |
| 3 | spots occupy 26-50 percent of the leaf surface | |
| 4 | Spots occupy more than 50 percent of the leaf surface | |

| Scale 2 - to determine the contamination of pistachio fruit with fungal diseases |
| Points | Description |
|---|---|
| 0 | no damage | fruit has 1-3 small, barely visible spots |
| 1 | spots covered up to 5% of the fruit surface | |
| 2 | spots covered 5-25 percent of the fruit surface | |
| 3 | spots covered 26-50 percent of the fruit surface | |
| 4 | The spots covered more than 50 percent of the fruit surface | |

| Scale 3 - to determine the infestation of pistachio leaf handle and young (green) twigs with fungal diseases |
| Points | Description |
|---|---|
| 0 | no damage | There are 0.1-1.5 small, barely visible spots |
| 1 | leaf handles and young (green) branches damaged up to 5 percent | |
| 2 | leaf handles and young (green) branches are damaged up to 25 percent | |
| 3 | more than 25 percent of leaf handles and young (green) branches are damaged | |
| 4 | N/A | |

2.3. The problem formulation

The development of the disease is determined by the following formula [10]:

\[ R = \frac{\Sigma (a \cdot b) \cdot 100\%}{N \cdot K} \]  

where,

- \( R \) - % of disease progression,
- \( \Sigma (a \cdot b) \) - the sum of the product of the affected organs in points,
- \( a \) - the number of infected plants,
- \( N \) - the total number of observed plant members,
- \( K \) - the highest score on the scale.

The biological effectiveness of fungicides used against the disease is calculated by the following formula:
\[ T = \left( R_n - \frac{R_t}{R_n} \right) \times 100 \quad (2) \]

where,

- \( T \) - biological efficiency, \( \% \),
- \( R_n \) - controlled disease progression, \( \% \),
- \( R_t \) - experimental disease progression, \( \% \).

The effect of various fungicides against pathogenic fungi is studied on the basis of the method VNIIXSZR:

The fungicides being tested are weighed and dissolved in acetone, then 3 cm³ of the drug is measured with pipette and placed into a flask at 40 °C in nurturing artificial condition. The flask is shaken and poured into petri dishes and left for 24 hours. After 24 hours, the fungi tested with a planting needle are inoculated into 3 places of the treated artificial nutrient condition in a petri dish and placed in a thermostat at 24 °C. After 3 days, the growth of fungi is taken into account.

3. Results and Discussions

In Uzbekistan, pistachios mainly face with cylindrosporiosis and septoriosis. *Cylindrosporium pistaceae* (Desm) Vassil fungus stimulates *Cylindrosporium* disease. In this case, the leaves and fruits of pistachios are damaged. As the spots grow, they cover the entire leaf blade. The spots are covered with black perforated pads (mushroom fruit). In the flesh of the fruit small spots first appear and then expand and cover almost the entire flesh of the fruit. The disease begins to appear in the first half of May, and then the percentage of damaged trees and the rate of damage increase sharply. In early July, the illness is observed widely [11].

The disease is widespread, especially in the southern region, where pistachios grow. Under conditions where the infectious load is strong, the disease spreads very rapidly, infecting up to 90% of plants [12].

The damage caused by the disease is huge. When the damage is severe, the leaves first turn yellow, bend and fall off, disrupting the tree’s assimilation process, slowing growth and reducing yields. When the fruit is damaged early, the kernel in it does not form till the end; the strongly damaged fruit dries and sheds [13].

The source of infection is mainly damaged leaves and fruits, in which the infection persists throughout the winter [14, 15].

![Figure 2. Samples from plantation](image_url)
| Calculated leaves | Evaluation of 100 damaged leaves on a scale |
|------------------|------------------------------------------|
| 1 2 1 3 3 2 1 4 2 1 3 |
| 2 3 2 4 1 0 0.1 1 3 3 2 |
| 3 4 3 3 2 2 1 0.1 1 2 3 |
| 4 3 1 2 4 4 3 2 3 1 2 |
| 5 3 1 4 1 2 1 3 0 2 1 |
| 6 4 4 2 2 3 3 1 0 1 2 |
| 7 2 1 3 3 4 2 3 0.1 4 3 |
| 8 3 2 4 3 2 1 2 4 0 3 |
| 9 2 2 3 2 0.1 4 3 2 1 1 |
| 10 3 3 4 0.1 3 0 2 3 2 2 |

Scores on a scale: 0 0.1 1 2 3 4 Total
The number of leaves in points: 5 5 19 28 29 14 100

| Scores on a scale: 0 0.1 1 2 3 4 Total |
|------------------|------------------------------------------|
| The number of leaves in points: 47 26 10 10 7 0 100 |

| Type of Treatment: Topaz |
|--------------------------|
| 1 1 0 2 0 0 0.1 1 2 0 1 |
| 2 0 3 0.1 0 1 0 0 3 1 0.1 |
| 3 0.1 0 1 0 1 2 0 0.1 0.1 1 |
| 4 0 4 0 0 3 0.1 0 0 2 0 |
| 5 1 0 1 0 0 0.1 2 0 0 3 |
| 6 0 0.1 0 1 2 0.1 0 2 0.1 0 |
| 7 0 1 1 0.1 0.1 1 3 0 1 2 |
| 8 0 1 0 2 0.1 0 0 1 0 0 |
| 9 2 0.1 1 0 2 1 0 0.1 0 2 |
| 10 0 1 0 0 0.1 0 1 2 0.1 4 |
Septoria pistaceae A is the main ingredient of Septoria that causes disease to appear, and then the spots appear on the surface. The first signs of the disease appeared in mid-May on a pistachio plantation. There were spots on the leaves and samples were taken from them (Figure 2). These spots are caused by cylinrosporiosis. Chemicals against this disease Skor 0.1 - 0.2 litre, Topaz 0.2 - 0.3 litre, and biological drug Sporagin (Bacillus subtilis) 4 - 6 litre are used moderately.

According to the Table 2, in the control variant, disease progression was 54.65%. In the Skor-treated variant, disease progression was 13.4%. The biological efficiency was 73.3%. In the variant sprayed with Topaz fungicide, the disease developed by 17.9% and the efficiency was 64.2%. In the variant treated with the biological drug Sporagin, the efficiency was 68.7% and the disease developed to 15.7%.

As well as, the Table 2 shows that the degree of damage per 100 leaves on pistachio trees in 4 variants was assessed on a scale. The leaves were rated on a scale of 0 to 4. Based on these data, the scores are calculated by putting the formula and the development of the disease is determined, and the results are shown in Table 3:

\[
P = \frac{(4 \times 5) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1)}{4 \times 100} = 54.6\% - \text{the degree of disease progression in the control variant.}
\]

\[
P = \frac{(4 \times 7) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1)}{4 \times 100} = 13.4\% - \text{the degree of disease progression in the variant of Skor.}
\]

\[
P = \frac{(4 \times 1) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1)}{4 \times 100} = 17.95\% - \text{the degree of disease progression in the variant of Topaz.}
\]

\[
P = \frac{(4 \times 6) + (4 \times 1) + (4 \times 1) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1) + (4 \times 0) + (4 \times 1)}{4 \times 100} = 15.7\% - \text{the degree of disease progression in the variant of Sporagin.}
\]
Table 3. The spread and development of the disease

| Ingredients’ name | Spraying rate per litre | Disease spread, % | Disease development % | Biological efficiency, % |
|-------------------|-------------------------|-------------------|-----------------------|-------------------------|
| Control           | -                       | 50.25             | 54.6                  | -                       |
| Skor              | 0.25                    | 50.25             | 13.4                  | 73.3                    |
| Topaz             | 0.2-0.3                 | 50.25             | 17.95                 | 64.2                    |
| Sporagin          | 4-6                     | 50.25             | 15.7                  | 68.7                    |

The second experimental area is a 70-hectare pistachio plantation in the Josh district of Samarkand region. Research there began in April in 2019. The phytosanitary condition of the experimental site was checked. The first symptoms of the disease began to appear in early May (Figure 3). At first, small red spots appeared on the leaves of the pistachio, and then these spots turned brown and covered the entire leaf surface (Figure 4). Measures to combat *cylindrosporiosis* began in May.

![Figure 3. The beginning of *cylindrosporiosis* in handon pistachio in the experimental field of Samarkand region](image)

![Figure 4. Strong rate of infection with *cylindrosporiosis*](image)

In the fight against *cylindrosporiosis* in this experimental field, 3 types of drugs were used: Skor, Topaz and Sporagin.
When the phytosanitary situation was studied in the Josh department, the development of the disease in early May was 60.0%. After administration of the drug, the disease progression in the control variant reached 62.75%. In the skor-treated variant, the disease was detected in 16.3% and the biological efficacy was 72.8%. In the variant in which topaz was used, the disease progression was 20.4% and the biological efficacy was 66.0. In the variant in which sporagin was used, disease progression reached 18.6%. The biological efficiency was 69.0%.

4. Conclusions
The results of a year-long study showed that the main disease in the pistachio plantation was cylindrosporiosis, four drugs were tested against it. The results from it were fungicide Skor, which had a good effect against the disease. The biological efficiency of the drug was 73.3%. Research will continue and the most effective drugs will be tested and selected. Optimal control measures will be developed for production (Table 5).

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