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Development and Spread of Diseases in Spring Camelina (*Camelina sativa* (L.) Grantz) when using Various Treatments

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Abstract: Spring camelina is a promising oilseed crop for the dry steppe zone of Northern Kazakhstan. However, the expansion of its sowing areas is constrained by the lack of scientific research, especially on the phytosanitary state. The article presents the results of the study of fungi of the *Alternaria* and *Fusarium* genus isolated from the affected organs of spring camelina plants and provides measures to control them in the field conditions of the region. The research aimed to identify common diseases and determine their development and distribution under different backgrounds when treated with fungicides. To identify fungal strains, the method of determination of the direct nucleotide sequence of the intergenic transcribed region was used, followed by the determination of nucleotide identity with the sequences deposited in the Gene Bank international database, as well as the construction of phylogenetic trees with nucleotide sequences. As a result of the analysis, phytopathogenic strains belonging to *Alternaria alternata*, *Alternaria tenuissima* and *Fusarium acuminatum* were identified. During field studies, the development and spread of diseases under different background conditions of fungicide treatment versus control were analyzed. Of the products used, the Pictor fungicide (active ingredients: Boscalid 200 g/l + dimoxystrobin 200 g/l) showed high efficiency, compared with the Extrasolbiofungicide (Bacillus subtilis, strain H-13). The results of the study can serve as a basis for the development of chemical and biological control methods aimed at specific pathogens.

Keywords: Spring Camelina, Alternaria Alternata, Alternaria Tenuissima, Extrasol, Fusarium Acuminatum

Introduction

Spring camelina (*Camelina sativa* (L.) Grantz) is an annual, small-seeded oilseed crop of the cruciferous family (*Brassicaceae*), originating from Asia Minor. Currently, camelina is studied and cultivated in Russia, North America, Japan, Australia, Ukraine, Mongolia, northern China, Korea and Western Europe (Guy *et al*., 2014; Fujita *et al*., 2014; Leus and Martynenko, 2018; Ghamkhar *et al*., 2010).

Camelina is considered a multi-purpose culture. Camelina oil can be used in the food industry and as a dietary oil. As a technical oil, it is used for the manufacture of drying oil, biodiesel, in medicine and perfumery. Camelina seed oil in foreign countries is used, first of all, as a source of biodiesel (Prakhova *et al*., 2018; Kurasiak-Popowska *et al*., 2018; Moser, 2010; Gesch, 2014). The agronomic value of spring camelina is explained by the fact that the culture is undemanding to temperatures, tolerates frosts up to 8-10°C during the germination period, at the same time, during the growing season it tolerates a lack of moisture and high temperatures, which allows it to be cultivated in a wide range of soils and climatic conditions (Gamayunova and Moskva, 2016; Tulkubaeva and Vasin, 2017; Ahmed *et al*., 2017; Sagirova and Vlasova, 2020).

In Kazakhstan, the first information about the cultivation of camelina dates back to the middle of the 20th century. Currently, in the Republic of Kazakhstan, camelina is grown in small areas in the North Kazakhstan, East Kazakhstan, Kostanay, Akmola regions, although there are prospects for expanding the sown areas of this

crop. Currently, three varieties of camelina are included in the State Register of Breeding Achievements Recommended for Use in the Republic of Kazakhstan, namely, Isilkulets, Ligena and Omskymestny (Omsk local) (Arinov, 2020; Izotov *et al*., 2019).

Studies by Serdyuk *et al.* (2015) and Pluzhnikova *et al.* (2016) have established that, in comparison with other cultures of the cruciferous family, this culture is resistant to diseases. Camelina has a higher resistance to harmful objects in comparison with rapeseed and mustard. In practice, this leads to significant savings in crop protection costs. The following diseases are often found on camelina crops.

Downy mildew (*Peronospora camelinae* Gäum.). The disease affects the leaves, stems and pods. Diseased plants lag in growth. A solid white mold forms on the underside of the leaf. On the stem, one can observe the same continuous tubular-shaped mold. The development of the disease is faster in cool (14-16°C) and rainy weather. The intensive development of the disease occurs mainly after the formation of pods. The disease is most dangerous when it is affected at the germination stage and can result in the death of whole plants.

Powdery mildew (*Erysiphe communis* Grev. *f. Camelinae* Jacz.) occurs on camelinae in the second half of the growing season. It appears as white fuzzy mold on the upper side of leaves, petioles, stems and pods. By the end of the growing season, dotted dark cleistocarpies 65-75 × 30-35 μm in size are formed among white mold. Plants are affected both in wet warm years and in dry ones. Plants that have lost their turgor, weakened by drought, are easily infected with powdery mildew. However, this disease on camelina is not particularly harmful and has no economic value.

White rust (*Albugo candida* (Gmel: Pers.) O. Kuntze) (= *Cystopus candidus* Pers.) appears on camelina during the flowering period. It affects leaves, stems, peduncles, flowers and pods. On the leaves, pale yellow small spots 0.3-0.5 mm in diameter, single or numerous, are found and whitish shiny pads (pustules of the fungus) are formed on the underside. The same pustules cover all affected plant parts. On the stems and peduncles, they merge in the form of long strips or tubes. When the epidermis covering the pads is destroyed, a white powdery mass spills out, the zoosporangia of the causative agent of the fungus.

The defeat of the culture by various fungal diseases directly depends on climatic and weather conditions. In the laboratory and field studies carried out by many scientists, symptoms of alternariosis and fusariosis were found. These infections affect leaves, stems and pods (Serdyuk *et al*., 2015; Pluzhnikova *et al*., 2016; Gannibal, 2011; Mir *et al*., 2020).

During our research, symptoms of *Alternaria* and *Fusarium* lesions were also identified, which aroused interest and further research of these diseases. Therefore, the purpose of our research was to identify the causative agents of fungal diseases Alternaria and Fusarium and determine their development and distribution under different backgrounds when treated with fungicides. The tasks included determining the species composition of pathogens under specific soil and climatic conditions of the region, determining the development and spread of diseases in the culture's growing seasons and calculating the biological effectiveness of the drugs.

Materials and Methods

Study Period and Location

To achieve the set goal, in 2018 and 2019, field experiments (Table 1) were conducted at the experimental site of Kamenka i D LLP, located in the Sandyktau district of the Akmola region (Kazakhstan). This territory belongs to the forest-steppe zone of northern Kazakhstan. Laboratory analyzes were carried out in the laboratories of the regional state enterprise "National Center for Biotechnology" of the Ministry of Education and Science of the Republic of Kazakhstan, as well as in the specialized laboratories of the Department of Plant Protection and Quarantine of the Kazakh Agro Technical University named after S. Seifullin (Nur-Sultan, Kazakhstan).

Study Object and Material

The research object was the Isilkulets spring camelina variety, bred at the Siberian experimental station of the State Research Institution "All-Russian Oilseed Crop Research Institute" (GNU VNIIMK) named after V.N. Pustovoit, Russian Federation (RF).

The material for the research was represented by two samples of the mycelium of fungi of the *Alternaria* and *Fusarium* genus, isolated from the affected organs of spring camelina plants (leaves, stem, seeds, pod).

DNA Isolation Process

Genetic identification of fungi of the *Alternaria* and *Fusarium* genus based on the analysis of the nucleotide sequence of the Intergenic Transcribed region (ITS region) was carried out at the National Center for Biotechnology RSE of the Ministry of Education and Science of the Republic of Kazakhstan.

For DNA isolation, we used a buffer solution containing 100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, proteinase K 100 g/mL. The cultures were centrifuged, the supernatant was removed and the precipitate was transferred into sterile mortars. Liquid nitrogen was added and the mixture was triturated to a powder. Then 100 μL of the resulting suspension was transferred into a sterile 1.5 mL tube. Then 500 μL of the corresponding buffer was added. The mixture was incubated for 18 h. Further, purification was carried out

by the phenol/chloroform method. For this purpose, 750 μL of chloroform/isoamyl alcohol (24/1) was added, mixed thoroughly and centrifuged at 12,000 rpm for 10 min. The aqueous phase was transferred to a new tube and the purification was repeated with phenol/chloroform/isoamyl alcohol (24/24/1). After centrifugation, the aqueous phase was transferred into new clean tubes and DNA was precipitated with 0.6 volumes of isopropyl alcohol. It was centrifuged at 12,000 rpm for 10 min, the DNA pellet was washed once with 70% ethyl alcohol, followed by centrifugation and removal of the liquid phase. Then the precipitate was dried in air for 15 min. DNA samples were dissolved in 100 μL of a single TE buffer and stored at minus 20°C.

The DNA concentration was measured spectrophotometrically using a NanoDrop spectrophotometer at a wavelength of 260 nm.

Amplification of the ITS Region

The PCR reaction was performed using ITS 5 (5'-ggaagtaaaagtcgtaacaagg-3') and ITS 4 (5'-tcctccgcttattgatatgc-3') primers in a total volume of 30 μL. The PCR mixture contained 10 ng of DNA, 1U. of Taq DNA Polymerase (Amplechem), 0.2 mm of each dNTP, 10x* NH4 buffer (Amplechem), 10 pmol of each primer (De Vegas *et al*., 2006). The PCR amplification program included prolonged denaturation at 94°C for 5 min; 30 cycles: At 95°C for 30 sec, at 52°C for 30 sec, at 72°C for 1 min; final elongation for 7 min at 72°C. The PCR program was performed using the Engine Tetrad 2 Cycler PTC-0240 DNA amplifier (Bio-Rad).

The PCR products were purified by the enzymatic method using Exonuclease I (Fermentas) and alkaline phosphatase (Shrimp Alkaline Phosphatase, Fermentas) (Zhang *et al*., 2002). The sequencing reaction was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions, followed by separation of the fragments on an automatic genetic analyzer 3730x l DNA Analyzer (Applied Biosystems).

The phylogenetic analysis was performed using the Mega 5 software. The ClustalW algorithm was used to align the nucleotide sequences; the phylogenetic tree was constructed using the Neighbor-Joining (N-J) method.

Electrophoresis of amplified products was carried out in 1.5% agarose gel in 1x TAE buffer.

Gel loading: 1.5 grams of agarose, 8 μL of ethidium bromide were added to 100 mL of 1x TAE buffer. Electrophoresis took place under the influence of 120 V for 40 min.

Determining the Development and Spread of Diseases

During the growing season of spring camelina in the fields, observation of the development of diseases was carried out. The symptoms of the disease were described and plants with signs of infection with fungi of the *Fusarium* and *Alternaria* genus were selected (Fig. 1). Diseases were counted in the following phenological phases of the spring camelina development: Rosette, stemming, flowering, green pod, yellow-green pod.

In the field, when accounting for diseases, two indicators are determined: The distribution or number of affected plants in crops and the development or degree of damage to organs. Disease spread (P) is set according to the formula (1):

$$P = \frac{n \times 100}{N} \quad (1)$$

where, P is the prevalence of the disease, %; n is the number of organs with signs of the disease in the sample; N is the total number of analyzed organs in the sample.

The degree of development (R) of the disease or the average affection of individual organs in percentage is determined by the formula (2):

$$R = \frac{\sum ab}{NK} \quad (2)$$

where R is the degree of development of the disease, %; $\sum ab$ is the sum of the products of the number of diseased organs by the corresponding score or percentage of damage to leaves, stems, seeds and pods; N is the total number of analyzed organs in samples; K is the highest score on the scale. To obtain more accurate results, special scales are used that characterize the intensity of the development of a particular disease.

In our studies, the biological effectiveness of fungicides was determined in the "rosette" phase of the culture. The biological effectiveness of fungicides is determined by the modified Abbott formula, comparing the spread of the disease before and after treatment (3):

$$C = \frac{100(P - p)}{p} \quad (3)$$

where, C is the biological effectiveness of the fungicide, %.

P = The prevalence of the disease in the control variant

P = The prevalence of the disease in the experimental variant

After identification and determination of spring camelina diseases on the experimental site of Kamenka and D LLP, field studies were carried out to identify effective products to control them. The actions of the Pictor fungicide with the active ingredient boscalid 200 g/l + dimoxystrobin 200 g/l and the Extrasolbiofungicide with active microorganisms Bacillus subtilis, strain H-13, were studied in comparison with the variant without treatment (the control sample). These products are registered on the territory of the Republic of Kazakhstan and are used on such oilseed crops as sunflower, winter and spring rapeseed to control diseases such as gray rot, white rot, alternariosis, Fusariosis. These products have been tested on camelina for the first time and the results of this research can be valuable for scientists and industrialists who cultivate or research this crop.

All variants of the experiment were placed sequentially in 3 replicates. Plot size 4.4 m × 20 m = 88 m^2 (Table 1). Sowing date: May 20. The seeding rate equals 6 million viable seeds per hectare. Seeding depth: 3-4 cm. The previous crop 1 was wheat after fallow; in the spring, the soil was harrowed with the BIG-3 harrow with the physical ripeness of the soil by 4 cm. Sowing was carried out with SZS-2.1 seeders with a row spacing of 21 cm. Harvesting was carried out with conventional grain harvesters, depending on the ripening conditions of the camelina. In 2018, camelina was harvested separately and in 2019 by direct harvesting at the stage of full ripeness of all heads.

Soil and climatic conditions during the growing season of culture

"Kamenka and D" LLP is located in the central zone of the Akmola region. The amount of precipitation is 250-280 mm. The growing season ranges from 110 to 120 days. The climate of the zone is sharply continental, dry, with hot summers and cold winters. The daily and annual temperature ranges are very large. Spring and autumn are poorly expressed. There are many sunny days; the amount of solar heat received by the earth in summer is almost as great as in the tropics. Cloudiness is negligible. Annual precipitation decreases from north to south, with a maximum in July and a minimum in February. Snow cover lasts an average of 150 days.

The main part of the territory belongs to the denudation-accumulative type of relief. These are the watershed plains of the Zhabai and Zhylandinka rivers, dissected by a younger network, with the presence of hollows, lakes and depressions. In general, the relief of the land-use area is convenient for mechanized soil cultivation. The soil cover on the territory of the economy is mainly represented by ordinary chernozem (humus - 3.0-5.1%, nitrogen (0-40 cm) - 30.80-49.9 mg/kg, phosphorus (0-20 cm) - 11.0-26.4 mg/kg, potassium - 389-500 mg/kg).

According to the Balkashino weather station in the Akmola region, the spring of 2018 was humid and protracted, which contributed to the development and spread of diseases. In the 3rd decade of May, 147% of precipitation fell relative to the average annual norm. The precipitation was extremely unstable. In contrast to dry July, August was rainy. In general, during the growing season, precipitation fell significantly more than the average annual values. In 2019, during the period of growth and development of spring camelina, 154 mm of atmospheric precipitation fell, this figure turned out to be 23.5 mm lower than the average long-term data. The month of May turned out to be arid; 14 mm of precipitation fell in the 1st and 2nd decades of the month. In general, only 17 mm of precipitation fell in May; this indicator turned out to be lower than the average annual by 21.5 mm. The lack of moisture also affected the germination of the mushroom. The seedlings were not friendly. The beginning of the germination phase was recorded on June 4. It lasted until June 12. In June and July, precipitation fell, respectively, 40 and 55 mm; these indicators slightly differed from the long-term average by 1.5 and 2.5 mm. The main amount of precipitation fell in the 3rd decade of July - 35.0 mm and the end of August – 30.0 mm (Fig. 2). In 2018, during the sowing and germination of field crops, the average monthly temperature turned out to be 2-4°C lower than the long-term indicators, which led to an extension of the period from seed germination to the emergence of seedlings. Temperatures in June were below normal. In the 1st-2nd decades of July, elevated temperatures had a favorable effect on the growth and development of plants. However, in the 3rd decade, warm weather was replaced by cool. In August, after a short heat in the first decade of August, the average daily air temperature was below normal (by 1-3°C). The average monthly air temperatures in May and June in 2019 were 0.3 and 2.9ºC below the long-term average. The temperature regime of the period of growth and development of camelina plants was uniform and favorable. In the 2nd-3rd decades of July, elevated air temperatures had a beneficial effect on the growth and development of camelina. In August, the long-term average monthly air temperature indicators were at the same level as the average annual indicators (Fig. 3).

The large amount of precipitation in summer after a spring drought had a positive effect on the development and spread of diseases.

Fig. 1: Organs (leaves (A), pods (B), stems and seeds (C)) of camelina with signs of infection with Alternaria fungi

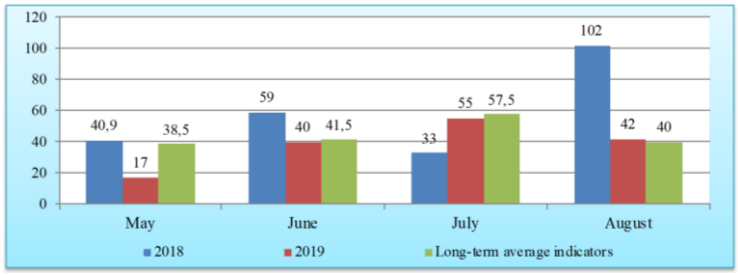

Fig. 2: Average monthly precipitation for 2018-2019 in comparison with long-term average indicators, mm (according to the data of the Balkashino meteorological station, the Sandyktau district)

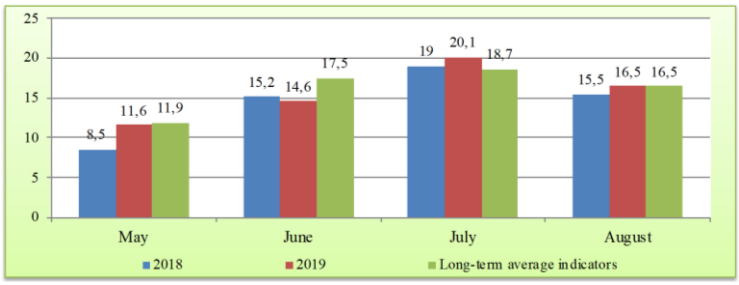

Fig. 3: Average monthly air temperature for 2018-2019 in comparison with long-term average indicators, ºC (according to the data of the Balkashino meteorological station, the Sandyktau district)

Table 1: Experiment arrangement

Variant	Products	Replications I	II	III
Control	-	1	4	7
Biological background	Extrasol (Bacillus subtilis, strain H-13), consumption rate - 2 l/ha	2	5	8
Chemical background	Pictor (boscalid 200 g/L + dimoxystrobin 200 g/L) 40% c.c., consumption rate - 0.4 kg/ha	3	6	9

Results

Identification of the Spread Diseases Pathogens

During the phyto examination, isolates of the *Fusarium* and *Alternaria* fungi were obtained from parts of the root, stem, leaf and pod. Isolation and cultivation of pure cultures of the fungus were carried out on Potato Sucrose Agar (PSA). Fungal colonies that had appeared on the fragments were examined under a microscope.

Two *Alternaria* species were previously identified*: A. alternata*, *A. tenuissima* and *Fusarium acuminatum.*

Characteristics of Cultural and Morphological Features the Spread Diseases Pathogens

Alternaria alternata has a dark gray color, the shape of the colonies is round, with a rhizoid edge, the edge of the colonies is wavy, the diameter of the colonies is 89 × 90 mm on the 7th day, the conidia are branched, the number of conidia in the chain is 3-6; the conidia have an ovoid, clavate shape.

Alternaria tenuissima has an olive-gray or dark gray color, the shape of the colonies has a rhizoid edge, the

edge of the colonies is smooth, the diameter of the colonies on the 7th day is 86 × 84 mm, the conidia are not branched and the number of conidia in the chain is 5-11; the conidia have a cylindric, clavate shape (Fig. 4).

Fusarium is the causative agent of rot and wilting of plants of many families. The first signs of wilting of spring camelina plants were noted from the flowering phase to the green pod phase.

As a result of research, it has established that *Fusarium* disease of spring camelina occurs annually. *Fusarium Acuminatum* is the causative agent of rot and wilting of plants of many families. It is found on leaves, stems and pods. Aerial mycelium on potato sucrose agar, potato agar is white, the substrate is purple. The sizes of macroconidia on PSA on days 15-17: With 3 partitions 29-56 × 3.0-4.0 μ, with 4 partitions 32-56 × 3.0-4.0 μ (Fig. 5).

The identification of two strains of fungi was carried out by the method of determining the direct nucleotide sequence of the ITS region, followed by determining the nucleotide identity with the sequences deposited in the Gene Bank international database, as well as constructing phylogenetic trees with nucleotide sequences.

The DNA of phytopathogenic fungi of the genus *Alternaria* and *Fusarium* was isolated. The electrophoretic analysis confirmed the presence of this genus of fungi. For the sequencing reaction, we used the following pair of primers: ITS 5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3'). After PCR amplification, electrophoresis was performed to confirm the presence of amplicons. The result is shown in Fig. 6.

As seen from Fig. 6, a specific band is present in all wells, which indicates the presence of a PCR product.

PCR products were purified from unbound primers by the enzymatic method using Exonuclease I (Fermentas) and alkaline phosphatase (Shrimp Alkaline Phosphatase, Fermentas).

The nucleotide sequences of the ITS region of the identified fungi were analyzed and combined into a common sequence in the SeqMan software (DNA Star). After that, the terminal fragments were removed (nucleotide sequences of primers, fragments with a low-quality index). The obtained sequences were identified in GeneBank using the BLAST algorithm (Clarridge, 2004; Shevtsov *et al*., 2010).

Additionally, phylogenetic trees were constructed with sequences deposited in the GeneBank international database (Gomzhina and Gannibal, 2021).

Figure 7 shows that the nucleotide sequence of the ITS region of sample 1 is located on the same branch with representatives of *Alternaria alternata* and *Alternaria tenuissima*. The nucleotide sequence of sample 3 is located on the same branch with representatives of *Fusarium acuminatum*. Taking i nto account the maximum percentage of coincidence of the analyzed sequence in the international database using the BLAST algorithm, as well as a result of phylogenetic analysis, it was established that sample 3 belonged to *Fusarium acuminatum*. Due to the genetic proximity of these species, the species of the analyzed samples cannot be determined using the ITS region. Other methods of analysis are required.

The results of the identification of 2 strains in the ITS region are shown in Table 2.

As a result of the molecular genetic typing method, we identified phytopathogenic strains 1 and 3 belonging to *Alternaria alternata*/*Alternaria tenuissima* and *Fusarium acuminatum*. The results of the genetic identification of phytopathogenic fungi can be used as molecular biological characteristics of strains.

The nucleotide sequence of the pathogen of the *Alternaria* genus (identified culture 1) is located on the same phylogenetic branch with *Alternaria alternata* and *Alternaria tenuissima*, the percentage of identity of the *Alternaria* genus between them amounts to 99.7%. As seen in Fig. 7, identified culture 3 is phylogenetically related to *Fusarium acuminatum* (100%).

Development and Spread of Identified Diseases, Depending on the Treatment with Fungicides

The phytosanitary state of crops at any level of agricultural technology is in a certain dependence on the climatic conditions of the area and the weather, which is established in the period from germination to seed ripening. In this case, moisture availability and thermal conditions have a particularly noticeable effect.

The meteorological conditions in 2018-2019 differed both from the average long-term conditions and among themselves, which made it possible to comprehensively evaluate the effectiveness of different products against diseases. The weather conditions had a significant impact on the phytosanitary situation in the crops of spring camelina.

In our studies, the weather conditions in 2018 were favorable for the development of fungi of the *Fusarium* and *Alternaria* genus. A decrease in temperature, good soil moisture in the third decade of May and June, as well as abundant rainfall in the first and second decade of August, contributed to the diseases of spring camelina plants. The fungicidal and biofungicidal treatment of crops in the rosette phase against *Fusarium* and *Alternaria* significantly stopped the development and spread of diseases and restrained them until the green pod phase. This was facilitated by the low amount of precipitation with the temperature regime at the level of the average annual value. In early August, there were heavy rains (102 mm) and the dynamics of development and distribution changed sharply upward (*Fusarium*: R = 14.3-21.5% and P = 27.5-86.4%; *Alternaria*: R = 14.3-20.6% and P = 25.2-84.3%). Compared with the control variant, a significant decrease in the development and spread of diseases was observed in the variant with the use of the Pictor fungicide.

In 2019, during the growing seasons of the crop, a low amount of precipitation fell and a high-temperature background was observed, which led to a slow increase in diseases. The development and spread of the diseases were lower in all phases of growth and development of the crop compared to 2018. Besides, compared with the control variant, a significant reduction in the development and spread of diseases was observed in the variant with the use of the Pictor fungicide (Table 3 and 4).

Biological Effectiveness of the Use of Fungicides against Diseases

The biological effectiveness of the use of fungicides is the result of their use in the field, which is expressed by indicators of a decrease in the spread of the disease or the degree of damage to treated plants (%).

In our studies, when determining the biological effectiveness of fungicides, we took into account the degree of spread of diseases after 5 days, 10 days and 15 days in comparison with the values of the control variant.

As can be seen from the results, the Pictor fungicide was distinguished by the maximum biological effectiveness in 2018 and 2019 in the experiment for all calculation periods (for fusariosis: 71.5-86.4%; for alternariosis: 77.5-84.7%), which is higher than the values demonstrated by the Extrasolbiofungicide by 12.1-6% and 15.6-10% respectively. It was noted that in humid 2018, the effectiveness of products against alternariosis was higher than for fusariosis. In dry 2019, on the contrary, the effectiveness of drugs against fusariosis was higher than for alternariosis (Table 5 and 6).

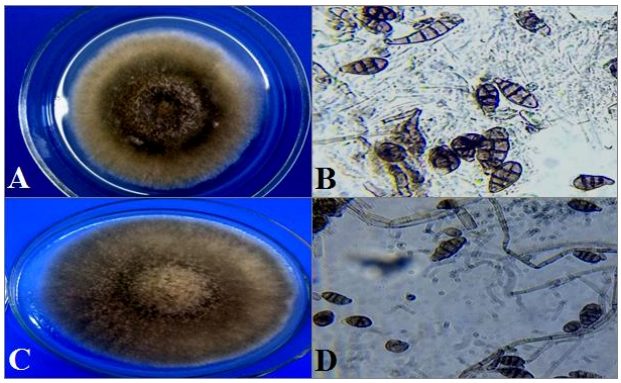

Fig. 4: Alternaria alternate (A, B); Alternaria tenuissima (C, D) - the color of colonies on PSA (A, C) and conidiophores appearing on the surface of agar (B, D)

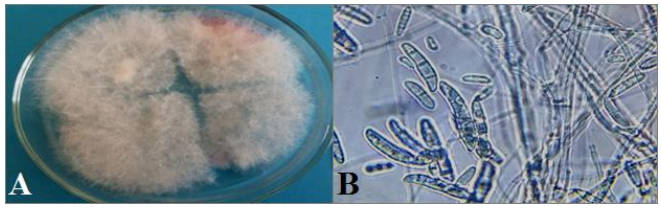

Fig. 5: *Fusarium acuminatum* - the color of colonies on PSA (A) and conidiophores appearing on the surface of agar (B)

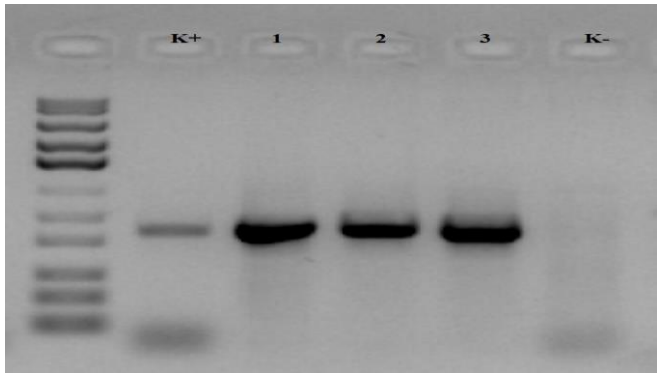

Fig. 6: Electrophoretogram of PCR amplification products of the ITS region. 1,3 - samples (numbering according to bps). (M) - molecular weight marker (100 - 10,000 bp, from 100-1,000 step 100 bp, Fermentas), (K-) negative control sample; (K +) positive control sample

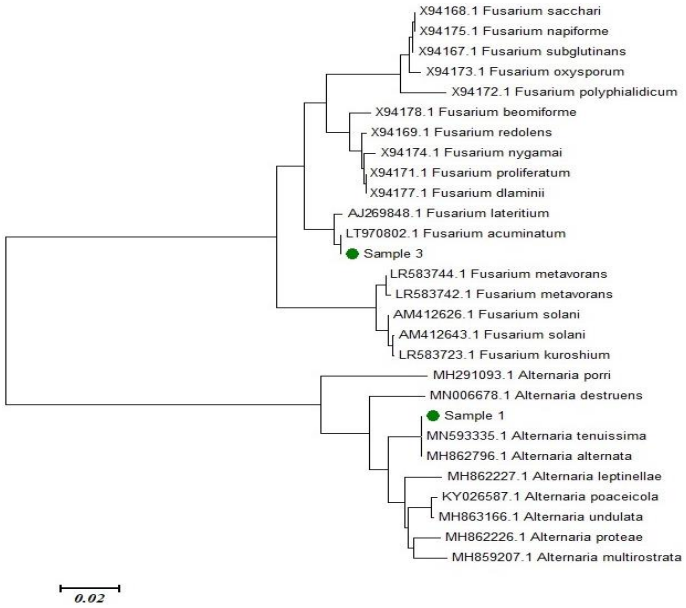

Fig. 7: Phylogenetic tree built on the basis of the analysis of a fragment of the ITS region of the alternaria and *Fusarium genus*

Table 2: Summary results of identification in the ITS region

Name	Identification result	Note
1	*Alternaria alternata*/*Alternaria tenuissima*	Determined based on the phylogenetic tree constructed by the N-J algorithm
3	*Fusarium acuminatum*	Determined based on the phylogenetic tree constructed by the N-J algorithm

Table 3: Development and distribution of fusariosis during the growing season

	Development phase							
	Rosette		Stemming		Flowering		Green pod	
Variant	R, %	P, %	R, %	P, %	R, %	P, %	R, %	P, %
2018								
Control background	12.0	28.0	15.9	46.6	17.9	58.3	21.5	86.4
Biological background	7.6	17.0	8.0	18.9	8.6	23.5	16.3	34.6
Chemical background	6.8	12.4	7.3	13.3	7.9	17.7	14.9	27.5
2019								
Control background	7.6	16.5	8.3	26.5	8.9	32.6	9.3	36.2
Biological background	2.0	4.3	2.6	5.2	3.0	6.3	3.8	10.3
Chemical background	1.3	2.8	1.5	3.6	2.3	4.7	2.7	8.6

P - the prevalence of the disease, %; R - the degree of development of the disease, %

Table 4: Development and distribution of alternariosis during the growing season

	Development phase							
	Rosette		Stemming		Flowering		Green pod	
Variant	R, %	P, %	R, %	P, %	R, %	P, %	R, %	P, %
2018								
Control background	12.0	30.0	15.6	48.9	17.4	57.9	20.6	84.3
Biological background	6.6	16.6	7.8	18.6	8.5	20.6	15.7	33.5
Chemical background	4.0	10.0	5.3	11.0	6.2	13.3	14.3	25.2
2019								
Control background	8.0	19.9	8.9	30.0	9.3	37.3	10.0	42.6
Biological background	2.6	6.5	2.9	7.6	3.6	8.4	4.7	11.9
Chemical background	1.4	3.5	1.8	4.6	2.5	5.9	3.2	9.5

P - the prevalence of the disease, %; R - the degree of development of the disease, %

Table 5: Biological effectiveness of drugs against fusariosis

	Distribution (P) of fusariosis after fungicidal treatment in the rosette phase, %			Biological effectiveness, %		
Variant	After 5 days	After 10 days	After 15 days	After 5 days	After 10 days	After 15 days
2018						
Control background	33.0	39.3	46.6	-	-	-
Biological background	17.6	18.3	18.9	46.6	53.4	59.4
Chemical background	12.6	12.9	13.3	61.8	67.1	71.5
2019						
Control background	19.7	22.9	26.5	-	-	-
Biological background	4.6	4.9	5.2	76.6	78.6	80.4
Chemical background	3.0	3.3	3.6	84.7	85.6	86.4

Table 6: Biological effectiveness of drugs against alternariosis

	Distribution of alternariosis after fungicidal treatment in the rosette phase, %			Biological effectiveness, %		
Variant	After 5 days	After 10 days	After 15 days	After 5 days	After 10 days	After 15 days
2018						
Control background	35.3	41.6	48.9	-	-	-
Biological background	17.2	17.9	18.6	51.3	56.9	61.9
Chemical background	10.3	10.6	11.0	70.8	74.5	77.5
2019						
Control background	23.2	26.4	30.0	-	-	-
Biological background	6.9	7.3	7.6	70.3	72.3	74.7
Chemical background	3.9	4.2	4.6	83.1	84.1	84.7

Discussion

According to research results (Murphy, 2016; Ergönül and Özbek, 2020), spring camelina has many positive qualities (early ripeness, drought resistance, resistance to some pests and diseases) that make it unique among oilseeds. This is the main reason why it aroused great interest in the world as an alternative to oilseeds (Ghamkhar *et al*., 2010; Prakhova *et al*., 2018).

Spring camelina is considered a disease-resistant crop (Schuster and Friedt, 1995) or a crop rarely affected by diseases (Makowski and Lehmann, 1995). In our experiments, the degree of development and spread of diseases was significantly influenced by weather conditions during the growing seasons of the crop. Thus, a decrease in temperature, good soil moisture in the third 10-day period of May and June, as well as heavy rainfall in the first and second 10-day periods of August, 2018 contributed to the defeat of fungal diseases by spring camelina. In the control variants in the "green pod" phase, the development and spread of *Fusarium* blight was R = 21.5%, P = 86.4% and *Alternaria* – R = 20.6%, P = 84.3%. Many scientists (Séguin-Swartz *et al*., 2009) argue that spring camelina is resistant to Alternaria and that it is mainly affected by such diseases as cruciferous keels, white rust and downy mildew. However, other studies (Gannibal, 2011; Mir *et al*., 2020) show that fungi of the genus Alternaria, under favorable conditions with a high degree of development and distribution, pose a threat to the future harvest and its quality.

Protective measures against the above-mentioned diseases should be aimed at preventing and reducing the number of harmful organisms. Their ecological basis is formed by agrotechnical methods, with the help of which conditions are created for good growth and development of plants, contributing to a decrease in their infestation by various harmful organisms (Prakhova *et al*., 2018). The chemical method is used in combination with agrotechnical methods and only when it is not possible to reduce the number of harmful objects to a level below the harmfulness threshold. In our research, along with the chemical method, we applied the biological method.

The Extrasol biological product tested by us, along with high environmental safety, had a high efficiency (in 2018 - 59.4-61.9% and in 2019 - 74.7-80.4%), which allows us to recommend it for use in industrial applications and further research. Since the advantage of biologized systems is to increase their environmental safety by reducing the chemical load on crops, at a lower cost due to the replacement of expensive chemical agents with biological ones, in reducing the stress phytotoxicity of fungicides.

Conclusion

As a result of studies, pathogenic fungi *Alternaria alternata*/*Alternaria tenuissima*, *Fusarium acuminatum*, causing diseases of fungi of the *Alternaria* and *Fusarium* genus, were identified from the affected organs of spring camelina. In addition to the prevailing weather conditions in the region, the degree of development and spread of

these diseases was significantly influenced by treatment with fungicides. The use of chemical and biological fungicides stopped further development and spread of diseases and showed high biological effectiveness.

Author's Contributions

All authors equally contributed in this study.

Ethics

This article is original and contains unpublished material. The authors declare that are no ethical issues and no conflict of interest that may arise after the publication of this manuscript.

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