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Original article

Fungal pathogens found in tissues of herbaceous plants growing in the Yereymentau District, Akmola region

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ABSTRACT

The biocontrol programs that are currently developed focus on specific microorganisms that live in plant tissues, also known as the endophytes. This article discusses pathogenic micromycete species that infected herbaceous plants growing in the Yereymentau District, Akmola Region. Four fungi species were detected that belong to the same genus. Information about the species composition and a brief description of each fungus were provided. The seasonal behavior of rust fungi was investigated. As it turned out, rust fungi tend to go through the uredinium phase in July and to produce teliospores in September. The research population includes the most common species of rust fungi. This article was first to identify phytopathogenic fungi that attack herbaceous plants in the Yereymentau District and their seasonal behavior. The findings may contribute towards expanding the global fungal database with information about fungal diseases specific to the given region and towards combat against a variety of phytopathogenic fungi.

1. Introduction

Nowadays, there are biocontrol programs developed that tackle specific microorganisms that live in plant tissues, also known as the endophytes. Some authors postulate that fungi and various bacteria can act as endophytes, since many among them exhibit strong antagonistic activity against pathogenic microorganisms (Lugtenberg et al., 2016; Burkin and Kononenko, 2018).

Plants gain from the energy they consume to produce an endophyte biomass in the sense that the resultant micromycetes serve the plant to keep it healthy (Caro et al., 2017; Hernández-Ochoa et al., 2019).

Micromycetes are able to affect not only the entire plant but also its shoots; they can spread in the direction of the meristem growth. The endophytic fungi are not associated with specific plant organs and can develop differently depending on the plant tissue changes (Ownley et al., 2010).

To restore the diversity of micromycetes, the resistance of 182 soybean species [Glycine max (L.) Merr.] to the most common fungal diseases was investigated in laboratory and field settings (Zatybekov et al., 2018). This was the first attempt to examine the genetic diversity in soybean using microsatellite DNA markers associated with resistance to fusariosis and cercosporosis.

Information about the new types of fungi helps estimate the global number of fungi and establish whether the current level of biodiversity is maintained. Besides, the compounds produced by these species may be utilized in new applications (Huang et al., 2008). Identifying the most realistic estimate is challenging, especially if the estimates are based on the incomplete data (Thongkantha et al., 2008; Berestetskiy et al., 2018). The lack of insights regarding the composition of phytopathogenic fungi in

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herbaceous plants and their spread in the Yereymentau district is a serious gap in the scientific knowledge.

Whether a soybean seed becomes infected depends on the seed fraction. For instance, the non-calibrated soybean seeds (varieties Misulya, Mazhesta, Decabit) are influenced stronger. The prevalence rate for Fusarium, Phomopsis, and Ascochyta species ranged from 0.5 to 12% (Maui, 2016).

The gravest effects of fungal diseases were observed in strawberries. The strawberry plant (*Fragaria* × *ananassa*) is one of the most popular plants grown and consumed worldwide. Strawberries have a unique, very desirable taste and aroma and are one of the most popular edible spring and summer fruits in Turkey. The economic importance of strawberry production is increasing in Erzurum (eastern Anatolia) and Turkey. According to official statistics, the year of 2007 saw the production of 8.5 tonnes of organic strawberries in Erzurum. High yields of high-quality strawberries demand a healthy growth environment. Plant diseases are the major factors backing production in Turkey. The Erzurum's strawberry diseases include the gray mold (*Botrytis cinerea*), leaf spot (*Mycosphaerella fragariae*), and a range of soil diseases (*Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp. and *Verticillium* sp.) (Eken, 2009).

Dog rose (*Rosa canina*) that grows in the wild in eastern Anatolia (Turkey) is also cultivated as an ornamental in this country. I summer 2019, fungal diseases of dog roses were found attacking the Turkish provinces, Erzurum and Ardahan, with the incidence of 70% and 40%, respectively (Lyange et al., 2011). The infected branches and twigs were yellowish-brown in color, the inner bark was black, and the dark pycnidium-like structures were found on the necrotic bark. The infected tissues were disinfected in 2% NaOCl for 2 min and placed on media with potato dextrose agar (PDA) at 25° C. The fungus was isolated and grown in a separate pure culture. Single-spore colonies were exposed to daylight for 3 to 4 weeks to induce pycnidial development.

Conidia were hyaline, aseptate, slightly curved and measured 4. 5–6.7 × 0.9–1.1 μm in size (n = 100). Based on these morphological traits, the fungus was identified as Cytospora rosarum Greville (Rodas et al., 2008). Inocula for pathogenicity tests were conidial suspensions prepared from 21-day-old cultures, adjusted at a concentration of 2.2 × 106 conidia/ml using a haemocytometer. Inoculum was sprayed onto wounded twigs of healthy dog rose plants. Control plants were wounded and sprayed with sterile water. Inoculated and control plants were covered with plastic bags for 72 h in a glasshouse at 23 ± 2° C.

Ricania simulans causes damage to almost all plants that grow along the eastern coast of the Black Sea. The use of chemicals against this pest is prohibited in this region due to tea cultivation. In this light, there is a need for new strategies (Gokturk et al., 2018). Given the awareness on the negative effects of chemical pest control and with the increasing awareness on environmental issues, the alternative methods have been sought in the past. In this context, studies were conducted to find new methods of biological pest control.

Fungi of the order Uredinales, also known as the rust fungi or rusts, cause diseases in many plants. The symptoms include spots or stripes that are usually rusty-brown in color. The rust disease of cereals and other agricultural plants has been known since ancient times.

The Kingdom Fungi contains a variety of eukaryotic organisms, including molds, yeasts, mushrooms, bracket fungi, plant rusts, smuts, and puffballs. Fungi have a complex metabolism, which differs from animals and plants. They release enzymes into the environment and absorb the breakdown products of enzyme action. Some of these enzymes are well-known allergens (Matia-González et al., 2015). The phylogenetic relationships among fungi were unclear until recently because classification was based on the sexual state morphology. Fungi lacking an obvious sexual stage were assigned to the artificial, now-obsolete category of Deuteromycetes or Fungi Imperfecti.

Rust fungi parasitize on higher vascular plants in many families. A distinctive feature of this fungal group is that it has variable life cycles. Some fungi species can complete sporulation on a single host plant (autoecious) while other fungi species need more than one host (heteroecious) (Levetin et al., 2016).

The seasonal behavior of phytopathogenic fungi depends on weather. The qualitative and quantitative alterations in humidity and temperature affect the growth of fungi and depend not only on the season but also on the time of day. Apart from that, phytopathogenic fungi depend on host plant and its growing season length.

The behavior of rust fungi varies. That is, species of the genus *Puccinia* develop faster within Ereymentau District, causing damage to many herbaceous plants. A damaged plant consequently loses fertility and slows down in developing its height.

Moisture, or the amount of precipitation, exhibits the greatest impact on the rust fungi. Unlike other fungi, these species have a three-stage development cycle (spring, summer and winter) and generate five types of spores, i.e., spermatozoa, ecociospores, uredinospores, teliospores basidiospores.

Pine needle rusts are a common finding at the Skadovsky Zvenigorod Biological Station, Moscow State University. These species normally complete a full life cycle. The seasonal behavior of this pathogen on different hosts was examined in. The rust fungus *coleosporium tussilaginis* developing on pine and other host plants. In Problems of Forest Phytopathology and Mycology: Proceedings of the X International Conference dedicated to the 80th Birthday of Vitaly Ivanovich Krutov. Karelian Research Centre of RAS, Petrozavodsk, 25–28).

Fungal diseases are among factors driving the shortage of durum wheat crops. Brown rust is a common disease of durum wheat in all growing regions. It is caused by the biotrophic pathogen *Puccinia triticina Erikss*., which also affects other *Triticum* species, the neighboring genus *Aegilops*, and cereal grasses. Iranian scholars have detected the emergence of new fungal pathogens in Zanjan: *Colletotrichum truncatum*, *Leptotrochila medicaginis*, *Phoma medicaginis*, *Rhizoctonia crocorum* on alfalfa. Seasonal dynamics and prevalence of alfalfa fungal pathogens in Zanjan province, Iran. International Journal of Plant Production. 2. 1735–6814). In 2000, the mean incidence of these pathogens varied by sampling time and field location. For instance, in 2000, it correlated negatively with mean monthly temperature and positively with monthly total rainfall, indicating that reductions in diseases were associated with higher temperature and less humidity, irrespective of fungal species.

Yereymentau District specializes in the livestock production. Herbaceous plants make up about half of the forage base in this sector.

The varying species composition of herbaceous vegetation suggests the existence of diverse phytopathogenic micromycetes. The microscopic rusts dominate among them. These fungi species affect not only the appearance of plants but also their life cycles thereby reducing their biological value and resistance to adverse environmental factors (Ospanova et al., 2018a, 2018b).

This work aims to conduct a comprehensive analysis of species diversity, seasonal behavior, and spread of phytopathogenic fungi in herbaceous plants growing in the Yereymentau District. The results may contribute towards expanding the global fungal database with information about fungal diseases specific to the given region and towards combat against a variety of phytopathogenic fungi.

2. Materials and methods

2.1. Sampling and categorization of rusts

A collection of fungal-infected herbaceous specimens was prepared between May and October within a 2014/2016 timeframe. The pathogen pathways, behavior, and the degree of plant damage were investigated. The overall views of fungal-infected were captured by camera. An MBR-3 microscope (LOMO, Russia) and an MBS-1 binocular microscope (LZOS, Russia) were used to study the sporocarps of phytopathogenic fungi, their mycelium, spores, and infected plant parts. The pathogenic fungi were investigated according to the Zhuravlev's method (1979).

The spreading behavior of rust fungi in response to temperature, humidity, and rainfall seasonality was explored. Data on weather conditions for the period from 2014 to 2018 were provided by the Center for Hydrometeorology located in Yereymentau.

The pathogenic fungi specimens were categorized into family, genus, and species groups by means of the reference book "Flora of Spore-Bearing Plants of Kazakhstan."

3. Results

The research results were summarized in the classification of phytopathogenic fungi and hosts, and a brief description of detected specimens was provided.

Species composition. In the Yereymentau District, the following phytopathogenic fungi were found (Table 1):

– *Puccinia graminis* Pers. on the host plant *Helichrysum arenarium* L.;

– *Puccinia agropyrina Erikss* (DC) Lév. on the host plant *Eletrigia repens* L.;

– *Puccinia graminis* Pers. on the host plant *Stipa* L.;

– *Puccinia absinthii* (DC) Lev. on the host plant *Artemisia taurica*;

– Puccinia graminis Pers. on the host plant *Cirsium arvense* L.;

– *Puccinia chrysanthemi* (DC) Lev. on the host plant *Artemesia vulgaris* Krasch.;

– *Puccinia absinthii* (DC) Lév. on the host plant *Artemisia cina* O. Berg.;

– *Puccinia absinthii* (DC) Lév. on the host plant *Artemisia campestris*;

– *Puccinia graminis* Pers. on the host plant *Helictotrichon desertorum* Vill.;

– *Puccinia absinthii* (DC) Lév. on the host plant *Artemisia dracunculus* L.

Comparing fungi specimens isolated from the herbivorous plant species in the Yereymentau District, it becomes evident that the given region is a place where four rust genera of the order *Uredinales* can be found. Of these, all belong to the genus Puccinia: *Puccinia graminis* P., *Puccinia agropyrina* E., *Puccinia absinthii* (DC) L., *Puccinia chrysanthemi* (DC) L.

3.1. Fungi profiles

Over three years of research, four fungi species were detected (Figs. 1–10): *Puccinia graminis* P., *Puccinia agropyrina* E., *Puccinia absinthii* (DC) L., *Puccinia chrysanthemi* (DC) L.

3.2. Puccinia graminis

3.2.1. Scientific classification

Order: *Uredinales*
Genus: *Puccinia*
Species*: Puccinia graminis* Pers.

– ***Symptoms.*** Black-brown streaks appeared on the stems and leaves. The spore count varied between host plant species and was 15–25 in *Helichrysum arenarium* L. and *Stipa* L.; 15 in *Cirsium arvense L.*; and 35–50 in *Helictotrichon desertorum Vill.* The spore sizes were 21.3–24.4 × 8.2–8.9 μm (*Helichrysum arenarium* L.); 18.6–21.4 × 14.3–16 μm (*Stipa* L.); 28–35 × 17.6–18.8 μm (*Cirsium arvense L.*); and 22–30 × 12–15 μm (*Helictotrichon desertorum Vill.*).

– *Host Range.* Black-brown rust was found on the leaves of *Helichrysum arenarium* L. (Fig. 1), *Stipa* L. (Fig. 2), and *Cirsium arvense L.* (Fig. 3) as well as on the stems of *Helictotrichon desertorum Vill.* (Fig. 4).

– *Growth Area.* The specimens appeared near the city of Yereymentau and were found on different plants over the years: *Helichrysum arenarium* L. (on 08/12/2014), *Stipa* L. (on 06/20/15), *Cirsium arvense L.* (on 07/25/15), and *Helictotrichon desertorum Vill.* (on 08/15/15).

3.3. Puccinia agropyrina Erikss (DC) Lév.

Symptoms. Spots or specks were found on the stems (Fig. 5). The spore count was in the range from 15 to 25. The spore size was 16–32 × 16–23 μm.

– Host Plant. Eletrigia repens.

– *Growth Area.* The specimen appeared near the city of Yereymentau and was first found on 06/10/2015.

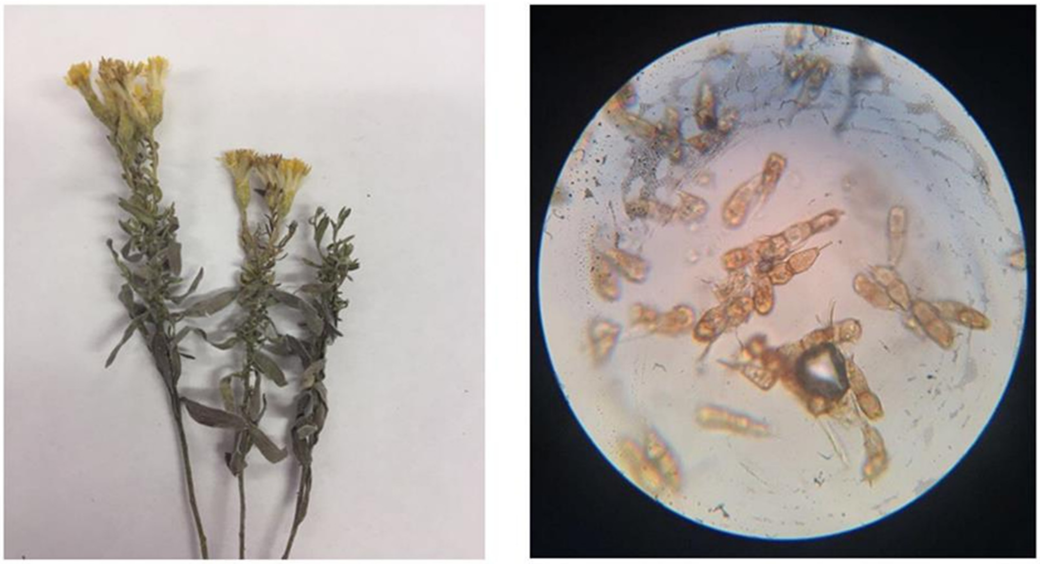
3.4. Puccinia absinthii (DC) Lév.

– *Symptoms.* Numerous spots or specks appeared on the stems. The spore count varied between host plant species and was 15–25 in *Artemisia taurica;* 9–15 in *Artemisia cina* O.Berg.; 50–70 in *Artemisia campestris*; and 35–50 in *Artemisia dracunculus* L. The spore sizes were 16–32 × 16–23 μm (*Artemisia taurica* and *Artemisia dracunculus*) and 18.5–23.5 × 9–11 μm (*Artemisia cina* O.Berg. and *Artemisia campestris*).

– ***Host Range.*** Spots and specks were found on the leaves of *Artemisia taurica* (Fig. 6) and *Artemisia campestris* (Fig. 7) as well as on the stems of *Artemisia cina* O.Berg. (Fig. 8) and *Artemisia dracunculus* L. (Fig. 9).

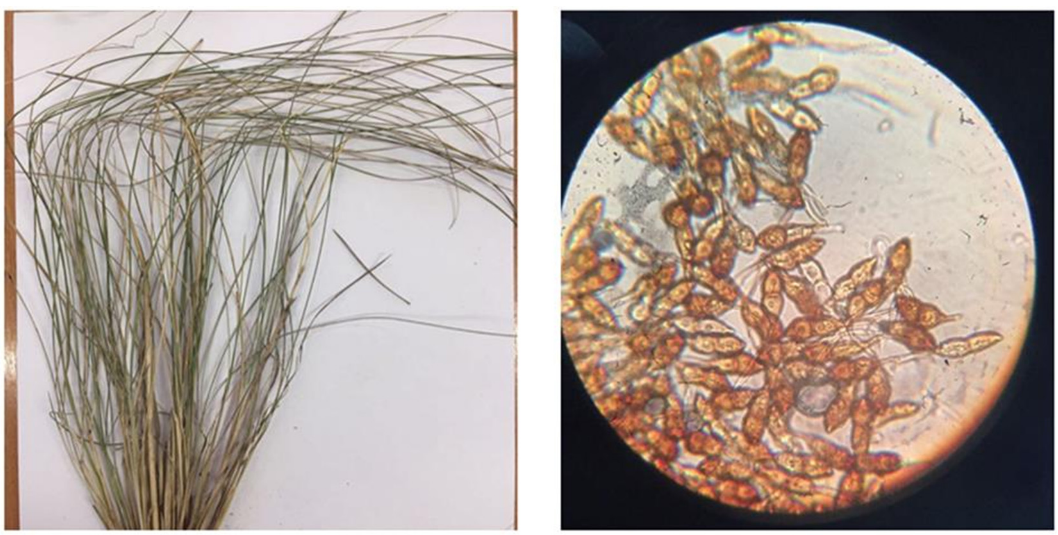
Table 1
The Species Composition of Phytopathogenic Fungi and Their Hosts.

Order	Species	Host Plant	No. of Species per District
Uredinales	*Puccinia graminis P.*	*Helichrysum arenarium L.* *Stipa* spp. *Cirsium arvense L.* *Helictotrichon desertorum V.*	1
	Puccinia agropyrina E.	*Eletrigia repens L.*	1
	Puccinia absinthii (DC) *L.*	*Artemisia taurica L.* *Artemisia cina O.* *Artemisia campestris L.* *Artemisia dracunculus L.*	1
	Puccinia chrysanthemi (DC) *L.*	*Artemesia vulgaris* K.	1



a) b)

Fig. 1. Leaves (a) of *Helichrysum arenarium* L. infected with telia (b) of *Puccinia graminis* Pers.



a) b)

Fig. 2. Leaves (a) of *Stipa* L. infected with telia (b) of *Puccinia graminis* Pers.

– *Growth Area.* The specimens appeared near the city of Yereymentau and were found on different plants over the years: *Artemisia taurica* (on 07/25/2015); *Artemisia cina* O.Berg. (on 08/15/2015); *Artemisia campestris* (on 08/15/2015); and *Artemisia dracunculus* L. (on 08/15/2015).

3.5. *Puccinia chrysanthemi (DC) Lév.*

– *Symptoms.* Uredinia appeared in linear rows on the stem. The spore count was in the range from 35 to 50. The spore size was 34.7–38.8 $\times$ 18.9–20.6 μm (Fig. 10).

– **Host Plant.** Artemesia vulgaris.

– *Growth Area.* The specimen appeared near the city of Yereymentau and was first found on 08/15/2015.

This study examined the seasonal behavior of the most commonly detected rusts. In July, rust fungi were in their uredinium state and the production of teliospores began in September.

During the research period, the temperature conditions were favorable for the fungus *Puccinia graminis* to grow. Although, the indicators of fungal growth were poor in 2014 due to the insufficient rainfall. The year 2015/2016, however, was a period of intensive growth.

The fruiting bodies (uredinia) of the fungus *Puccinia agropyrina Erikss* (DC) Lév. appeared yellowish on the bottom of the steam in July. The September 2014/2015saw the formation of dark-brown teliospores in the uredinia at 48% relative humidity. In 2016, the humidity was even higher, up to 54%, triggering an outbreak of said spores. The optimum temperature for the development of urediniospores ranges from 20 to 25 °C. The average temperature for the

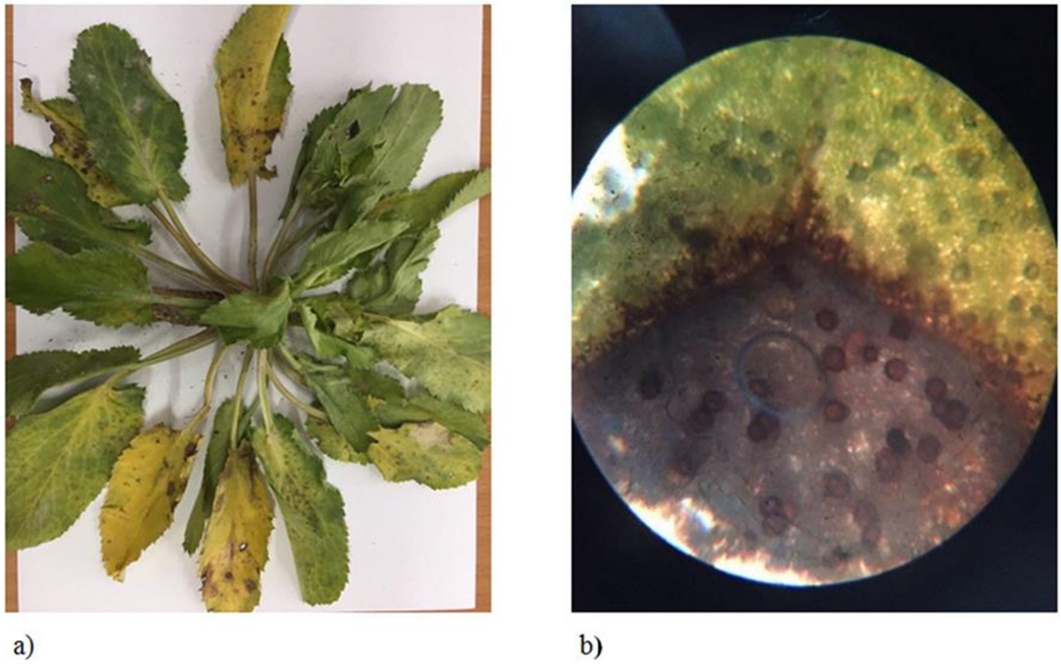


Fig. 3. Leaves (a) of *Cirsium arvense* L. infected with telia (b) of *Puccinia graminis* Pers.

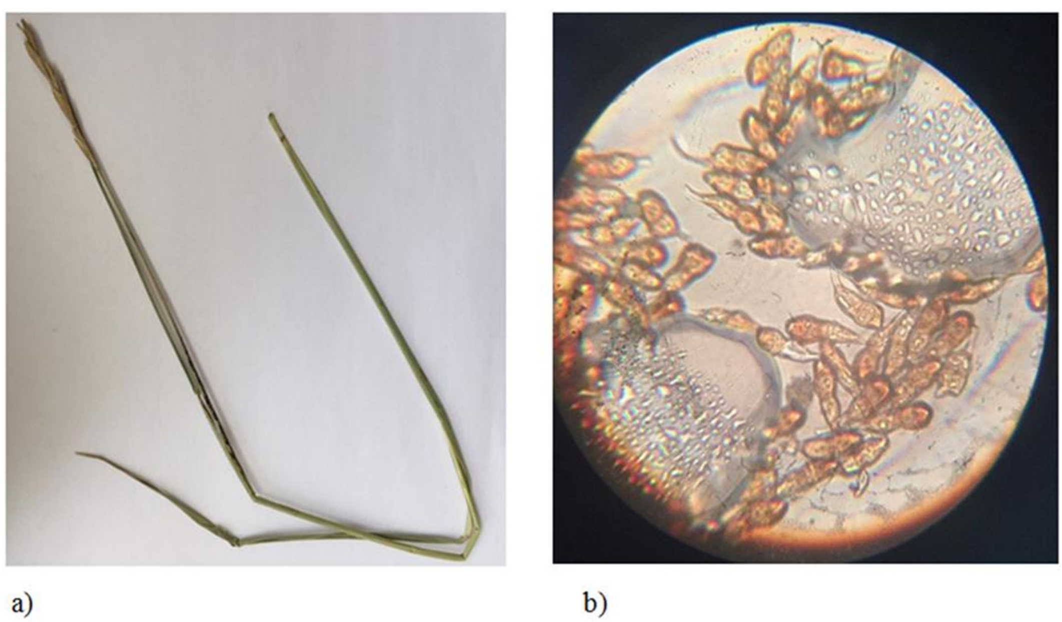


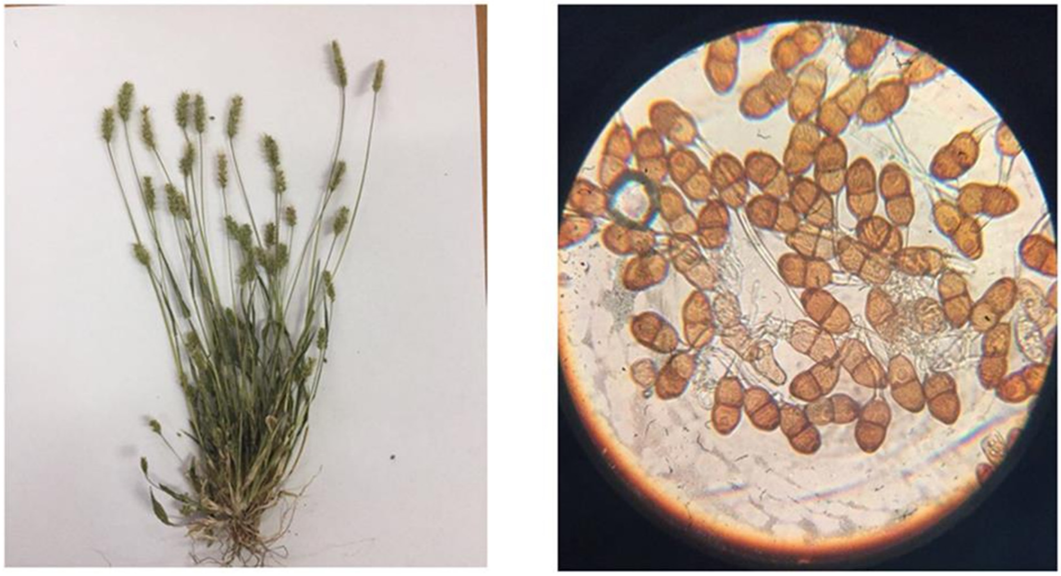
Fig. 4. Steam (a) of *Helictotrichon desertorum* Vill infected with telia (b) of *Puccinia graminis* Pers.

month of July in 2014/2016 was 20.6° C, 21° C, and 20.9° C, respectively. These temperature conditions were favorable for the *Puccinia agropyrina Erikss* (DC) Lév. to produce urediniospores, Yet, during the observation period, the incidence of fungal diseases caused by this particular pathogen was moderate due to insufficient rainfall in 2014.

Puccinia graminis (Pers.) P. Karst exists in its uredinial stage between July and August. The annual prevalence rate of the given rust is depicted in Fig. 11. Its optimum temperature is 16 to 22°. According to weather records for the period 2014/2016, the mean temperature in June/July 2014 was 17.8 and 20.8 °C and precipitation was 10.1 mm. In years 2015 and 2016, the mean temperature during the same period was 11 °C and precipitation varied from 6 to 19.8 mm. This means that although the temperature was favorable for the fungus during the period, the amount of precipitation in 2014 was insufficient. For this reason, the spread of rust fungi was slow in 2014 and rapid in 2015/2016.

4. Discussion

The present finding is that phytopathogenic fungi in the Yereymentau District represent four species of the order *Uredinales*. Inflorescences and fruits typically have the smallest amount of



a) b)

Fig. 5. Leaves (a) of *Eletrigia repens* infected with telia (b) of *Puccinia agropyrina Erikss* (DC.) Lév.

a) b)

Fig. 6. Leaves (a) of *Artemisia taurica* infected with telia (b) of *Puccinia absinthii (DC) Lév.*

micromycetes. Previously, individual micromycetes demonstrated an antagonistic potential against the following phytopathogens: *Fusarium graminearum*, *Cladosporium* sp., *Phytophtora infestans* and *Botrytis cinerea*. Strains with a wide, narrow and limited spectrum of action have not been identified (Ignatova and Brazhnikova, 2014).

Tozlu et al. (2018) studied the emergence of negative effects of chemicals on humans and environment. With an increasing awareness on environmental problems, alternative methods were sought to combat fungal diseases. In this context, successful results were obtained by conducting studies on the suppression of pathogens by fungi that can be used in biological control. Tozlu tested the impact of 2 *Trichoderma harzianum* specimens isolated from *Aesculus hippocastanum* and *Pinus sylvestris* on *Fusarium oxysporum* (ET 21, ET 32, ET 34, ET 46, ET 53, ET 55, ET56) obtained from potato and pepper; *Alternaria alternata* (ET 9, ET 42) obtained from apples and tomatoes; *Sclerotinia sclerotiorum* (ET 30, ET 48) and *Fusarium solani* (ET 45, ET 50) obtained from cucumber; and on *Geotrichum Candidum* (ET 13) obtained from carrots. The ET 4 bioagent isolates exhibited a strong effect on G. *candidum* and little effect on F. *oxysporum*, S. *sclerotiorum*, F. *solani* and A. *alternata*. The ET 14 bioagent isolates exhibited a strong effect on A. *alternata* and had little impact to other specimens. If the control against these pathogens, the employability of T. *harzianum* must be tested in the field setting, and environmentally friendly drugs may be prepared that will make an important contribution to agriculture (Tozlu et al., 2018).

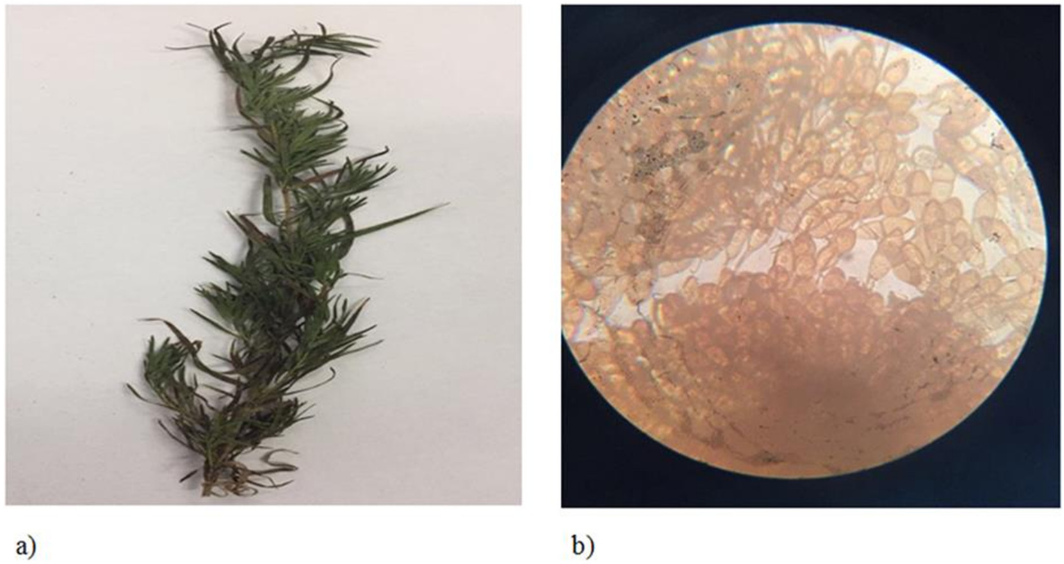


Fig. 7. Leaves (a) of *Artemisia campestris* infected with telia (b) of *Puccinia absinthii (DC) Lév.*

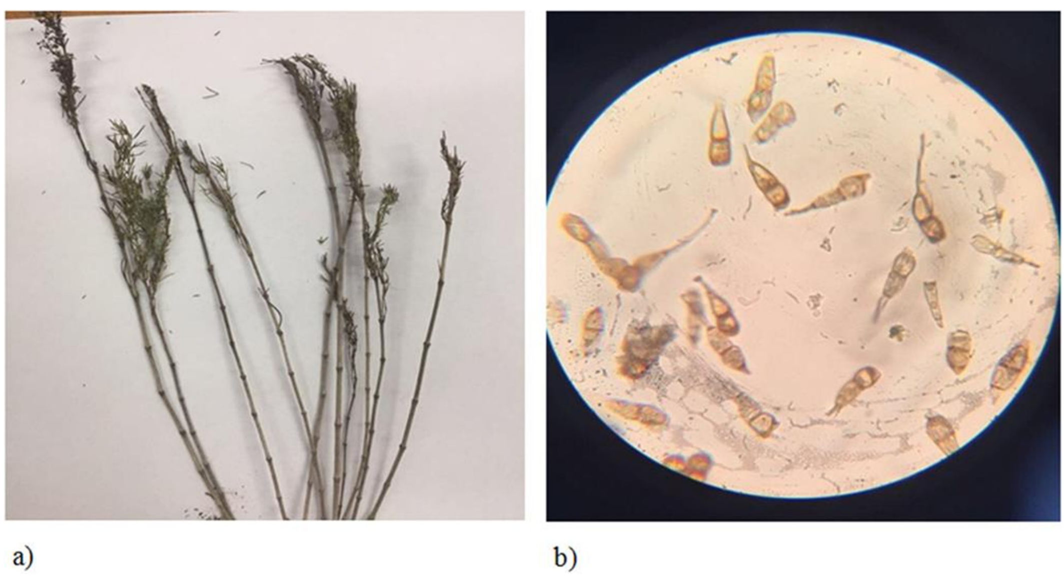


Fig. 8. Steam (a) of *Artemisia cina* O. Berg. infected with telia (b) of *Puccinia absinthii (DC) Lév.*

Temperature and humidity play a key role in driving the spread of the wheat stem rust (Prank et al., 2019). These particular factors were responsible for the spread of virulent phenotypes across the Russian Federation and neighboring countries in 2001–2005. The weather factor is crucial for heading and ripening phases. Hence, the vast majority of Russian farmers view meteorological conditions of the first summer months (June and July) as strategically important. A 10-year study of weather conditions during this critical period revealed two opposite regimes: dry and humid. The period from 1996 to 2000 was characterized by decreased relative humidity (45–50%) and high daily mean temperatures (22–25 °C). Summer 2002 was also dry with a mean relative humidity of 30–35% and a mean temperature of 17–20 °C.

The importance of this study can be put as follows. Micromycetes are a group of microorganisms that has a broad range of antagonist species. Their properties, including the secretion of extracellular enzymes, amino acids, polysaccharides, phytohormones, and phosphates, as well as the ability to dissolve other strains, allows for the employment in plant protection and probiotic production (Sazanova et al., 2019).

Totally 10 host plants were identified, including 4 hosts of the fungus *Puccinia graminis* (*Helichrysum arenarium* L., *Stipa* L, *Cirsium arvense L.*, *Helictotrichon desertorum Vill);* 4 hosts of the fungus *Puccinia absinthii* (*Cirsium arvense L, Artemisia cina, Artemisia campestris, Artemisia dracunculus)*; 1 host of the fungus *Puccinia agropyrina* (*Eletrigia repens.*); and 1 host of the fungus *Puccinia chrysanthemi*. This article was first to identify phytopathogenic fungi that attack herbaceous plants in the Yereymentau District and their seasonal behavior. These data are essential for the creation of information database on common

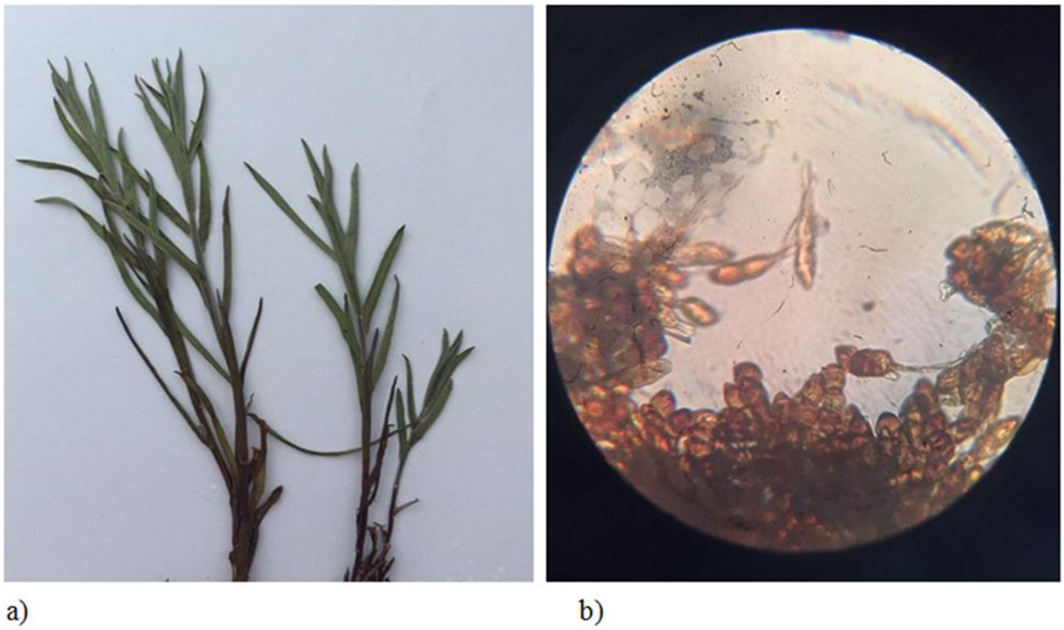


Fig. 9. Steam (a) of *Artemisia dracunculus* L. infected with telia (b) of *Puccinia absinthii (DC) Lév*.

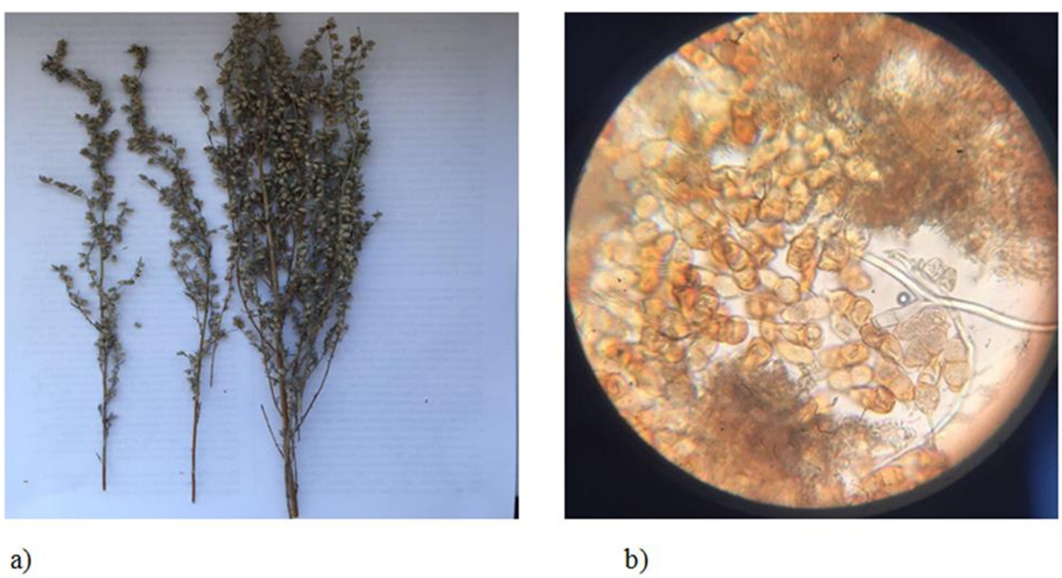


Fig. 10. Steam (a) of *Artemesia vulgaris* infected with telia (b) of *Puccinia chrysanthemi* (DC) Lév.

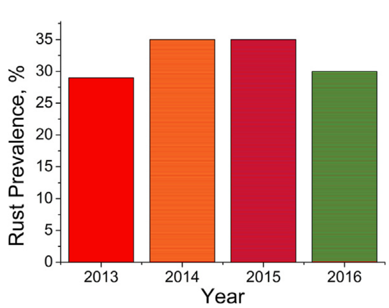


Fig. 11. Puccinia graminis Pers within Yereymentau District: Rust Prevalence by Years.

herbaceous plant diseases and may be applied to cope with these fungal diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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