International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 8, Issue 6 -2021

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.06.012

A Study on Identification of Peashrub (*Caragana arborescens.*Lam) Powdery Mildew Fungi in Green Plantations in the Capital City of Mongolia

Tsend ITGEL[1] Ph.D.
Associate Professor, Laboratory of Forest Protection, Plant protection institute, Mongolia, Ulaanbaatar Zaisan -210153

Gantumur GANZUL[2]
Ph.D Student., Laboratory of Forest protection, Plant protection institute, Ulaanbaatar, Mongolia

Janchiv TEMUUJIN[2]
Ph.D. Student., Laboratory of Biotechnology, Plant protection institute, Ulaanbaatar, Mongolia

*Corresponding author: *itgel4200@yahoo.com/itgel4200@gmail.com*

Abstract

This study is aimed at determining powdery mildew prevalence and disease severity index of Siberian peashrub (*Caragana arborescens*) in the woody-bush plantations in the capital city Ulaanbaatar of Mongolia. The shrubs were assessed for powdery mildew disease incidence and disease severity index. Also, we intended to identify the pathogenic fungi species. The survey was carried out in four different parks and one alley in Ulaanbaatar city. The leaf samples were collected from the diseased pea-shrubs from those places. The identification of fungi at the genus level was carried out by using macroscopic and microscopic examinations depending on the fruiting body-cleistothecia shape, appendages, asci, ascospores, and arrangement of ascospores. For the molecular identification of the isolated fungi at the species level, the extracted fungal DNA was amplified by PCR using specific internal transcribed spacer primer (ITS1/ITS4). The PCR products were sequenced and compared with the other related sequences in GenBank (NCBI). The fungus species were identified as *Erysiphe palczewskii*. The results showed that the *Erysiphe palczewskii* is the most abundant pathogen fungus obtained from all the locations.

Keywords: Siberian pea-shrub; *Caragana arborescens*; green plantation; powdery mildew; *Erysiphe palczewskii*; Mongolia;

1. Introduction

In the green plantations of Ulaanbaatar city are popular broad-leafed trees, bushes such as - poplar (Populus sp)elm (Ulmus sp), hawthorn (Crataegus sp), sweetbrier rose (Rose sp), Siberian peash-rub (Caragana arborescens), and conifer trees like pine (Pinus sp),spruce (Picea sp), larch (Larix sp).

From those plants, pea-shrub is widespread in urban green space.

Caragana arborescens Lam is known for its tolerance of many environmental conditions including droughts, temperatures to -38°C, infertile soils, sunny sites, high winds, alkaline soils, and saline conditions (Henderson, D.C and Chapman R, 2006)[7].

The Siberian peash-rub is a native species in Siberia, northern and central Mongolia, growing primarily around river banks, rocky landscapes, and on the outskirts of coniferous forests. They are remarkably resistant to droughts, grow well in relative lack of sunshine and nutrient-poor soil. These qualities make them a popular species in urban forestry all over Mongolia. Furthermore, their strong root systems help protect the soil from erosion and as a species in the Fabaceae family, are good at nitrogen fixation, enriching the poor urban soil. Another benefit lies in their ability to harbor predators such as lacewings and parasitic wasps, contributing to the conservation of biodiversity in urban environments (Jamyandorj Kh 2020)[17].

Caragana arborescens, commonly called Siberian pea-tree, is a large, rounded, broadleaf deciduous shrub, multi-stemmed, erect, oval, 2-5 m tall, slightly less spread, but can be grown as a tree. Leaves alternate, pinnately compound, 4-8 cm long, with 8-12 leaflets, each 1-2.5 cm long, obovate to elliptic-oblong, apex rounded, and mucronate (abrupt tip), pubescent when young, bright green. Flowers pea-like, yellow, 1.5-2 cm long, on stalks (pedicels) 1.5-2.5 cm long. Fruit pod, 3.5-6,5 cm long, 3,5-5,0 cm width, with 6 reddish-brown, oblong to spherical seeds, 2.5-4 0 mm in diameter; pods open with a popping sound when ripe (Jamyandorj Kh 2020)[17].

On the other hand, *C. arborescens* is susceptible to powdery mildew,and its species are found in Asia, Europe, Canada, the USA, and Russia (Vajna, 2006; Aleš Lebeda et al., 2008a,b; Tomoshevich, 2015)[1,13, 15]. Powdery mildew severely affects the leaves and shoots of C. arborescent (Vajna, 2006)[13].

Although powdery mildew is very prevalent in Siberian pea-shrubs in Mongolia, there have not been any scientific studies regarding the topic. So we intended to assess disease incidence and determine pathogenic species of this disease.

2. Materials and Methods

This research was carried out in the woody-bush plantations of Ulaanbaatar city. We had chosen four parks &one alley. The coordination of the places is shown in table 1.

Table 1. Disease recording and sample collecting places in Ulaanbaatar city

№	Park name	The coordinate of the places
1	“Asashōryū” park	47^0 55’12.05”N 106^0 54’51.06”E
2	“Uchral’s” park	47^0 55’18.3”N 106^0 54’54.5”E
3	“Bell” park	47^0 55’02.62”N 106^0 55’04.15”E
4	“Turkish-Mongolian friendship” park	47^0 55’04.45”N 106^0 55’19.13”E
5	“Baga toiruu” alley	47^0 55’11.44”N 106^0 55’25.88”E

2.2. Data collection on the field

Data were collected on disease incidence and disease severity index. Disease incidence was determined based on the symptoms of the infected plants. The proportion of diseased plants was estimated by formula (Waller J.M 2002)[12]as follows:

$$P\% = n * 100/N$$

Where:

P%= Disease incidence;
n = number of infected plant units;
N = total number (healthy and infected)of plant units assessed

The disease severity index described the damage caused by the diseases on plants' leaves.

A modified 0–4 visual scale of Chumakov (1974)[16] based on disease symptoms, was used to score the infected plants as follows:

0: No disease symptoms;
1: Powder on 10% of leaf surface; (1point)
2: Powder on 11-25 % of leaf surface; (2point)
3: Powder on 26-50 % of leaf surface;(3point)
4: Powder on 51% of leaf surface; (4point)

Disease Severity (%) = nxv/4N x100;

Where,

(n)= Number of plants in each category,
(v) = Numericalvalues of symptoms category.
(N)= Total number of plants,
(4) = Maximum numericalvalue of symptom category.

2.3. Collection of Samples

The plant leaves samples from diseased Siberian pea shrubs were collected from the Ulaanbaatar city parks in August 2020 (Table 1). The leafsamples were collected into small sterilized plastic bags andbrought to the laboratory for further studies.

2.4 Macroscopic and Microscopic Examination of Isolated Fungi

The fungal morphology was studied macroscopically by observing the fruiting body, appendages, and asci, ascospores features (color, shape, size, and) by a compound microscope with a digital camera.

A small portion of mycelium was subjected to a few drops of sterilized water on a sterile glass slide. A coverslip was placed on it. The fungal preparation was observed under low and high power magnification of the microscope (Olympus Light Microscope).

2.5. Molecular Identification of Fungal Species:

2.5.1 Materials

Materials used in this experiment for the test were collected directly from the 3 sites pea shrub tree (Uchral's park", "Asashōryū",s park, "Turkish-Mongolian friendship" park). Three parts *Erysiphe sp.* samples were prepared. These samples were visible on the distribution of pathogens, air-dried, and stored at at $+4^0$Cin the refrigerator.

Genetics and Molecular Research 14 (4): 18546-18551 (2015)

Methods

Reagents

Reagents required for the CTAB method included DNA extraction buffer (50 mMTris-HCl, pH 8.0, 0.7 mM NaCl, 10 mM EDTA, pH 8.0, 24:1 chloroform/isoamyl alcohol, isopropanol, hexa-nol, and 0.1% Tris, 0.002% EDTA).

2.5.2 Reagents

Reagents required for the CTAB method included DNA extraction buffer (50 mMTris-HCl, pH 8.0, 0.7 mM NaCl, 10 mM EDTA, pH 8.0, 24:1 chloroform/isoamyl alcohol, isopropanol, hexa-nol, and 0.1% Tris, 0.002% EDTA).

2.5.3. DNA extraction and PCR amplification

From whole plant leaves were covered with pathogenic fungi we brushed down to separate pathogenic fungi. Powdery mildew DNA was extracted using the CTAB method [4]. In the process of extracting DNA from the infected plant tissue with the CTAB method, we aimed to select the samples with a large lesion and multiple pathogens.

Three parts plant pathogen DNA samples were extracted and used as a template. ITS primers were used for PCR amplification. The total volume of the PCR amplification system was 50 µL, including 25µL MasterMix (Thermofisher), 1µL each primer, 2µL (15-20 ng) DNA template, and 22µL ddH2O. The reaction conditions were 95°C for 5 min, 95°C for 30 s, 56°C for 30 min, and 72°C for 30sec for 35 cycles, followed by 72°C for 10 min. PCR products were detected on a 1.5%agarose gel.

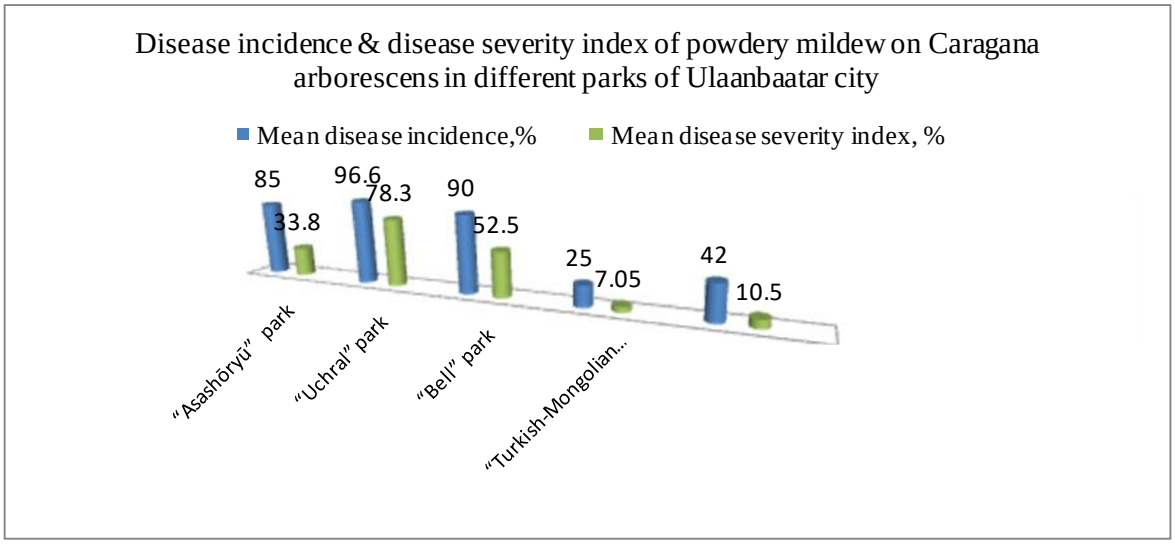
2.5.4. Sequencing and Analysis

The PCR products were sent for sequencing to the Macrogen Genome Research center (Seoul, Korea).
The obtained sequences were compared with the other related sequences using BLAST search in GenBank (NCBI).

3. Results

3.1. Powdery mildew incidence & disease severity index on Caragana arborescens

In the middle of August, 2020, powdery mildew incidence and disease severity index were recorded in each observation separately at each 5 spot in all parks which were involved in survey (Graphic 2).



By our survey were established that disease incidence and disease severity index are high (by 96.6% and 78.3 %, respectively) in "Uchral's" park when "Turkish-Mongolian friendship's" park disease incidence is estimated at 25.0%, severity index is 7.05%

3.2. Disease symptom

We have collected leaf samples of infected pea shrubs and identified disease by symptoms first. White grey sporulating mycelium covered both the upper and undersides of *Caragana arborescens* leaves. On both sides of leaves cleistothecia (chasmothecia) were also found (Photo 1& 2).

Photo 1. Diseased by powdery mildew peashrub (*Caragana arborescens*) branch Taken by Tsend.Itgel Ulaanbaatar. 2020

Photo 2. Leaf samples of *Caragana arborescens* affected by powdery mildew. Taken by Tsend.Itgel Ulaanbaatar. 2020

Fungi

In this study, the isolated fungi were examined based on microscopic and morphological characteristics (Photo 2.A, 2.B, 2. C)

The fruiting body- cleistothecia of the fungi is dark brown color, sphere, convex, has 6-13 appendages which is straight, hyaline, 216.68-232.13-µmlong,the tip is dichotomouslybranched (Photo 3.A).

Photo 3.A. Cleistotecia

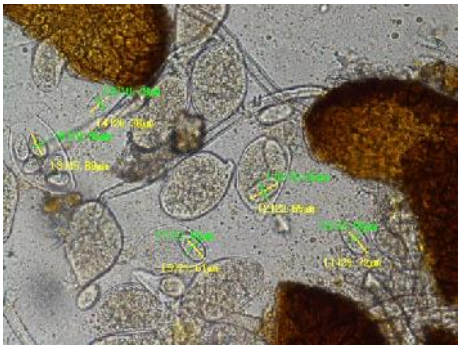


Photo 3.B Asci

Photo 3.C. Asci with ascospores

Taken by Tsend.I 2020

The results of the measurement are shown in table 2.

Table.2 Somecharacteristicsof the teleomorph stage *Erysiphe palczewskii*

Cleistotecia		Asci			Ascospores		
Diameter (µm)	Appendages lenth (µm)	Width (µm)	Lenth (µm)	L / W ratio	Width (µm)	Lenth (µm)	L / W ratio
Mean ±SD (min-max)		Mean ±SD (min-max)			Mean ±SD (min-max)		
213.04 (284.17-115.89) SD±80.59	216.67 (232.13-216.68) SD±13.79	33.86 (29.29-39.36) SD±3.28	53.84 (48.78-58.68) SD±2.79	1.59 (1.46-1.63) SD±0.08	11.3 (8.85-13.65) SD±1.25	23.14 (15.8-25.81) SD±3.1	2.05 (1.61-2.34) SD±0.22

As shows Tabl.2 Cleistotecia's diameter is 115.89-284.04 µm, appendages 216.68–232.13 µm long.asci 29.29–39.36 µm wide, 48.78–58.68 µm long short-stalked, shape index 1.46-1.63.

The fungi produce 3–8 asci (Photo 2.B) with 3–7 ellipsoid-ovoid ascospores (Photo 2.C) 15.80–25.81 µm long and 8.85–13.65 µm wide, shape index hesitatesbetween 1.61–2.34.

3.4. Molecular identification

The evolutionary history was inferred using the Neighbor-Joining method [10]. The optimal tree with the sum of branch length = 0.02909722 is shown. The evolutionary distances were computed using the Tamura-Nei method [11]and are in the units of the number of base substitutions per site. This analysis involved 5 nucleotide sequences (MEGA X). The obtained three sequences were compared with the other related sequences using BLAST search in GenBank (NCBI) [8] which were 98% identical to *Erysiphe palczewskii* .

The phylogenetic trees of pathogen *Erysiphe palczewskii* show Figure. 1.

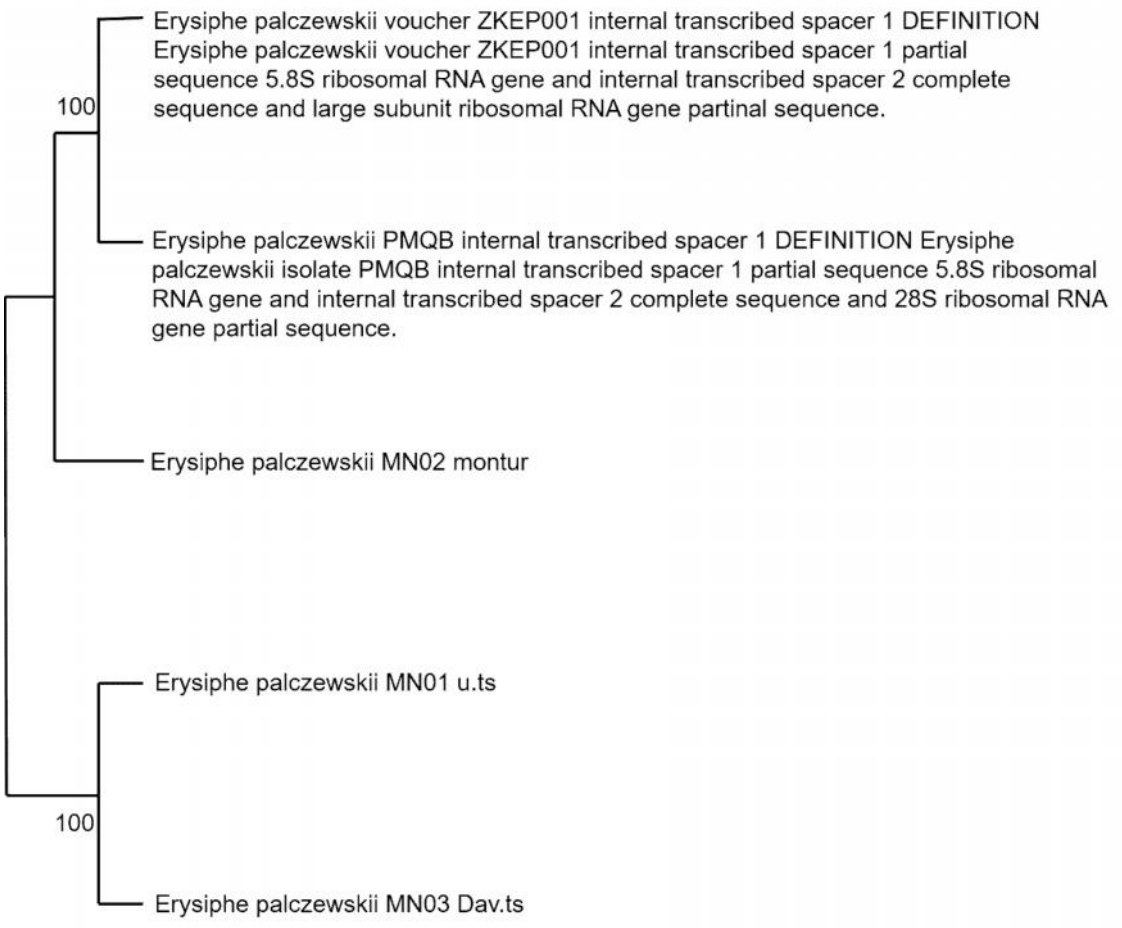


Figure. 1. Phylogenetic trees of pathogen Erysiphe palczewskii

4. Discussion

We determined the prevalence and disease development index of pea-shrubs powdery mildew in 4 parks and 1 alley in Ulaanbaatar, took leaf samples from diseased trees, and diagnosed them with external signs. During the analysis, the diameter of the body-cleistote of the fungal seed, ascus, and ascospore was measured and photographed.

Many scientists have studied pea-shrubs powdery mildew, including diseases of green shrubs in urban areas. For example;

The researcher Ainash K. Ospanova (2006) had revealed 60 species of pathogenic fungi from 21 species of trees belonging to 12 genera and 15 species of bushes from 9 genera in Kazakhstan [2]. Among those fungi on the *Caragana arborescens*were recorded 2 species- *Leveillula taurica f. caraganae* and *Trichocladia caraganae* which causes powdery mildew.

In Alaska Glawe, D. A., and Laursen, G. A. (2005) recorded *Microsphaera* (*Erysiphe*) *palczewskii* Jacz on ornamental species *Caragana arborescens* Lam and gave information on the morphology and taxonomy of this species [5].By research in Alaska was confirmed that chasmothecia of the fungi were dark brown to black, convex, 100-125 (-155) µm in diameter; dichotomously-branched appendages- (95-)155-270 (-325) µm in length. Ascites measuring (54.5-) 56-63 (-64.5) × 31.5-43.5 (45) µm long. Asci contained 6 to 8 ovoid, pale yellowish ascospores - (15.5-) 16-22 (-24) × 10-14.5 µm [5]. C Nischwitz, G Newcombeet.al. according to a study conducted in Americashort-stalked asci averaged 67 (± 7.1) µm × 37 (± 5.2) µm, and the ascospores were 21 (± 2.0) µm × 13 (± 0.8) µm[9].

Makarova T.A., Makarov P.N. et. al. according to a study conducted in the northern part of the Tyumen region of Russia, the diameter of the cleistotetcia of the species *Erysiphe palzewskii* is 0.135 to 0.240 mm[14]; According to a study by the Czech Republic,

Aleš Lebeda, Barbora Mieslerová, Michaela Sedlářová and Miloš Pejchal (2008), the fluctuations ranged from 67.5–112.5 µm, while ours ranged from 115.89 to 284.17 µm, close to the results of these researchers[1].

E. palczewskii is origin from Asia, however in recent years the pathogen has been introduced into several European countries and has now become one of the most widespread powdery mildews in eastern Europe (Braun, 1995; Heluta & Minter, 1998)[3, 6].

In the European part of Russia, the spread of the disease has increased due to the widespread cultivation of yellow acacia for decorative purposes in the green areas of Barnaul, Tomsk, Novosibirsk and Krasnoyarsk [15] C. Nischwitz, G. Newcombe (2003)indicated that, more recently the fungus has also been introduced into North America ([9] .

We performed identified pathogens at the species level. PCR analysis of leaf samples from pea-shrub trees in three parks (Asashūryū's, Uchral's and Turkish-Mongolian friendship's) revealed that the pathogen was all the same *Erysiphe palczewskii*.

5. Conclusion

Although extensive research has been conducted in Mongolia on diseases of legumes, potatoes, and vegetables, such as wheat, green plantation shrubs, including yellow acacia, have not been studied before. A novelty of the research is the study of the prevalence of yellow acacia, one of the main shrubs in Ulaanbaatar, and the identification and registration of pathogenic fungi at the Genbank (Accession numbers are MW940955-MW940957).

We believe that this study will be important for the further development of greenspace diseases research in urban areas of Mongolia.

Acknowledgments

The authors would like to thank Plant protection institute for providing all the requirements for this work. Also thanks to researcher Purevdorj Baatarjargal of Veterinary research institute for the technical support.

References

In English:

[1] Aleš Lebeda, Barbora Mieslerová, Michaela Sedlářová and Miloš Pejchal, 2008 Occurrence of Anamorphic and Teleomorphic Stage of *Erysiphe palczewskii* (syn. *Microsphaera palczewskii*) on *Caragana arborescens* in the Czech Republic and Austria and its Morphological Characterisation *Plant Protect. Sci. Vol. 44, No. 2: 41–48*

[2] Ainash K. OspanovaAnalyses of Taxonomy and Distribution of Fungi Species in Woody-Bush Plantations of the Pavlodar Region, Kazakhstan. *Mongolian Journal of Biological Sciences 2006 Vol. 4(2): 79-83*

[3] Braun U, 1995. The Powdery Mildews (Erysiphales) of Europe. Gustav Fischer-Verlag, Jena, Germany.

[4] Doyle, J.J. and Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19, 11– 15.

[5] Glawe,A.and Gary A. Laursen,2005. First Report of Powdery Mildew on *Caragana arborescens* and *Caragana grandiflora* in Alaska caused by *Microsphaera (Erysiphe) palczewskii*, Plant Management Network.

[6] Heluta VP, Minter DW, 1998. *Microsphaera palczewskii*. IMI Descriptions of Fungi and Bacteria 138, Sheet 1375.

[7] Henderson, D.C. and R. Chapman. 2006. *Caragana arborescens* invasion in Elk Island National Park, Canada. Nat. Areas J. 26: 261-266.

[8] Liu D, Coloe S, Baird R and Pedersen J. 2000. Application of PCR to the identification of dermatophyte fungi. J Med Microbiol., 49:493-497.

[9] Nischwitz, C. and G. Newcombe, G. 2003. First Report of Powdery Mildew (*Microsphaera palczewskii*) on Siberian Pea Tree (*Caragana arborescens*) in North America. *Plant Disease* 87, 451.

[10] Saitou N. and Nei M, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

[11] Tamura K., Nei M., and Kumar S, 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

[12] Teng P.S and James W.C Disease and yield losses Waller J.M.; Linne´ J.M.; Waller S.J Plant pathologist's pocketbook 3rd Edition, page 25-27

[13] Vajna, L. 2006. First report of powdery mildew on *Caragana arborescens* in Hungary caused by *Erysiphe palczewskii*. Plant Pathol. 55: 8

***In Russian*:**

[14] Makarova T.A., Makarov P.N Porajaemost, karagani drevovidnoi muchnistoi rosoi iperspectivi borbi c nei v Khantii-Mansiiskom avtonovnom okruge //Bilogicheskie nauki/4. Resursovedenie i introductsiya rastenii

[15] Tomoshevich M.A Formirovanie pathokomplecsov drevesnikh rastenii pri introductsii v Sibirii *//Dissertatsia na soiscanie uchenoi stepeni doctora, biologicheskikh nauk , Novosibirsk , 2015*

[16] Chumakov A. I., Minkevich I.I., Vlasov YU.I., Gavrilova E.A Osnovnie methodi phitopathologicheskikh issledovanii Moskva Kolos, 1974. p– 192 .

In Mongolian:

.

[17] Jamyandorj Kh & others Em, tsetserlegiin urgamal, Ulaanbaatar 2020

Access this Article in Online

Website: www.ijarbs.com

Subject: Plant Pathology

Quick Response Code

DOI:10.22192/ijarbs.2021.08.06.012

How to cite this article:
Tsend ITGEL , Gantumur GANZUL, Janchiv TEMUUJIN. (2021). A Study on Identification of Peashrub (*Caragana arborescens*.Lam) Powdery Mildew Fungi in Green Plantations in the Capital City of Mongolia. Int. J. Adv. Res. Biol. Sci. 8(6): 85-92.
DOI: http://dx.doi.org/10.22192/ijarbs.2021.08.06.012