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***Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur causative agent of helminths of durum wheat**

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Abstract: Helminthosporiosis occurs wherever cereals are grown and can manifest itself in different forms, which is the reason why it has different names: brown spot, black spot, or root rot. A study on the occurrence and development of the fungus *Cochliobolus sativus* in different varieties of durum wheat was conducted in the experimental field of the FCI – Chirpan, in the period 2020/2023. Phenological observations were conducted annually and the appearance and development of helminthosporiosis during the growing season was monitored in dynamics. Potato dextrose agar (PDA) was used for primary isolations and multiplication of cultures. During the years studied, the pathogen *C. sativus* was observed in the form of numerous small, dark brown spots on the leaves of durum wheat. At first, the spots are round, and later - oblong with a yellow halo. Symptoms become visible after emergence and most often on the lower leaves. The mycelium of the fungus is septate, dark olive-brown and grows in the intercellular space of the attacked plant tissue. The ascomycete fungus *Cochliobolus sativus* has been identified in two consecutive years on the leaves of durum wheat varieties Vazhod and Railydur. For accurate diagnosis and identification, the characters with the highest taxonomic value-morphology and size of asexual sporulation were taken into account. Conidiophores emerging through the stomata, bearing conidia that are septate, straight or slightly curved, and dark olive-brown, have been observed. In the conducted study, a different level of pathogen manifestation was observed, depending on the phenophase in which the durum wheat is located.

Keywords: *Cochliobolus sativus*; durum wheat; symptoms; isolate; diagnosis

INTRODUCTION

Helminthosporiosis occurs wherever cereals are grown and can manifest itself in different forms, which is why it has different names: brown spots, black spot or root rot, can develop throughout the growing season. The fungus *Cochliobolus sativus* has been found to split into races that have different attitudes towards the hosts of this pathogen. The hosts of helminthosporiosis are over 100 plant species. These are primarily wheat, barley, rye, oats and a number of grasses from the genera *Agropyrum*, *Bromus*, *Setaria*, etc. Infection occurs by conidia, as the sexual form is extremely rare in natural conditions. *C. sativus* is a hemibiotroph. The biotrophic phase is short and consists

of the formation of appressoria, with the help of which direct penetration of the infectious hyphae through the cuticle occurs (Kumar et al., 2001). During the necrotrophic phase, mycelial entry into the mesophyll tissue occurs and cell death occurs in the affected plant parts. Pathogenicity is associated with the formation of toxins (Ludwig, 1957; Nilsson et al., 1993; Bach and Kimati, 1999). It attacks a wide range of hosts, but mainly wheat and barley (Kumar et al., 2002). It is the causative agent of several different diseases, the most important of which are leaf spot (helminthosporiosis, spot blotch) and root rot (common root rot). It manifests itself on the leaves in the form of brown necrotic spots. Significant losses in wheat, reaching up to 50%, have been found

in countries with hot and humid climates in Africa (Kenya, Sudan, South Africa, Tanzania), South Asia (India, Indonesia, Thailand, Bangladesh, Nepal), South America (Argentina, Brazil), North America (Indiana, Kansas, Minnesota, Montana, North and South Dakota), Australia and New Zealand (Villareal et al., 1995; Kumar et al., 2002; Acharya et al., 2011). It is also found in European territory (Austria, Belgium, Germany, Italy). The optimal temperature for infection and disease development is 28°C.

After sequencing three isolates of *C. sativus* originating from Australia, McDonald et al (2015) found that one of them contained a gene almost identical to ToxA, described in *Parasitagonospora nodorum* and *Pirenophora tritici repentis*. Further analysis revealed that ToxA was present in 30% of Australian isolates, although this gene is predominant in the *C. sativus* population, in resistance selection, the susceptible gene Tsn1 should be eliminated from wheat varieties in disease-affected areas (Figueroa et al., 2018).

Sources of resilience have been identified (Kumar et al., 2009; 2010). Resistance to helminthosporiosis is a quantitative trait and is controlled by the additive action of more than two genes (Joshi et al., 2004). Mapping of resistance loci confirms the involvement of many genes in the control of this trait (Singh et al., 2016).

The aim of the present study is to isolate, identify, and monitor the occurrence, development, and intensity of helminthosporiosis caused by *Cochliobolus sativus* in different durum wheat varieties. The study also aims to determine the phenological phases in which the disease manifests most noticeably, to perform morphological identification and diagnosis of the pathogen under field and laboratory conditions, and to provide a scientific basis for sustainable disease management through appropriate agrotechnical, breeding, and phytosanitary measures.

MATERIALS AND METHODS

Cochliobolus sativus (S. Ito & Kurib.) Drechsler ex Dastur (= *Bipolaris sorokiniana*

(Sacc.) Shoemaker), is an ascomycete fungus belonging to the group of foliar pathogens of wheat. A study on the occurrence and development of the fungus *Cochliobolus sativus* in different varieties of durum wheat was conducted in the experimental field of the FCI – Chirpan, in the period 2020/2023. The study included durum wheat varieties that are achievements of breeding programs in our country and abroad. The Bulgarian varieties “Progres” and “Vazhod” were obtained as a result of mutational and mutational-hybridization selection (Yanev et al., 2000; Yanev, 2006). A success of modern durum wheat breeding are the newer varieties “Reyadur”, “Ramilidur”, “Saya” and “Predel”. “Predel” is the first variety of the Institute of FCI - Chirpan, carrying a marker gene for high grain quality (Dechev et al., 2010). In recent years, and especially after Bulgaria’s entry into the EU, free import of seeds of foreign varieties has begun, which necessitates their testing in the conditions of our country. This study includes 6 foreign varieties: “Auradur”, “Pescadou”, “Saragolla”, “Yukon”, “GK Bétadur”, “Meridiano”, two from Italy and one each from Austria, Hungary, France and Germany.

Systematic phenological observations were conducted and the appearance and development of spots during the growing season was monitored in dynamics. After visual assessment of the size and shape of the spots in field conditions, samples were collected for observation of reproductive structures of the causative agents, isolation and further diagnosis. Potato dextrose agar (PDA) was used for primary isolations and multiplication of cultures. Isolation of the pathogen from infected leaf tissue was performed after the collected infectious material was placed in paper bags and, after 1-week drying at room temperature, stored in a refrigerator at 4-5 °C. Isolations were made directly from asexual structures of the fungal pathogen - pycnidia.

Identification of the pathogen *Cochliobolus sativus* and description of the symptoms caused by it. The main method for determining the fungal pathogen causing helminthosporiosis in cereals is comparative-morphological. Symptoms are described on naturally infected durum wheat

plants. Identification was made through direct observation of the pathogen's structures formed on plant tissue and during cultivation in a nutrient medium, and was based on a combination of morphological and cultural characteristics. The characters with the highest taxonomic value were taken into account: morphology and dimensions of asexual sporulation, colony morphology. Microscopy of temporary preparations was performed.

RESULTS AND DISCUSSION

During the survey of the areas with durum wheat, the following manifestation of helminthosporiosis was identified. In 2020 and 2021, leaf manifestations were observed in the varieties "Vazhod" and "Railidur", while no manifestations of the pathogen were observed in the foreign varieties. In the indicated varieties, the manifestation of the pathogen *C. sativus* is observed in the form of numerous small, dark brown spots on the leaves of durum wheat. At first, the spots are round, and later - oblong with a yellow halo. The center of older spots is often lighter. As the disease progresses, the spots merge and cover a large part of the leaf surface (Fig. 1). Symptoms become noticeable after emergence and most often on the lower leaves. The mycelium of the fungus is septate, dark olive-brown and grows in the intercellular space of the attacked plant tissue. Conidiophores emerge through the stomata

singly or in groups. They are septate, straight, dark olive-brown, measuring 50-150 x 6-8 μm . Conidia are septate, straight or slightly curved, dark olive-brown, ovoid to oblong, measuring 30-70 x 12-25 μm (Fig. 2). The sexual morph was not established during the studied period.

Conidiophores withstand variable temperatures and air humidity. The study found that the spores germinate at a temperature of 25°C and the mycelium grows at an optimal temperature of 28°C. The manifestation of the disease in 2020 and 2021 was favored by suitable air temperature, higher humidity, denser sowing of wheat, and damage from other diseases, including the leaf spot complex. The pathogen persists in seeds or in post-harvest residues. During the experiment, preservation of spores on the seeds or preservation of mycelium under the seed coat was observed. In the spring, not only perithecia, but also conidiospores form on the stubble. The pathogen has also been observed overwintering on wild hosts such as sedge, oat, etc.

Data from field and laboratory observations confirm that the pathogen successfully infects various varieties of durum wheat, with the most susceptible varieties during the observed period being the "Vazhod" and "Railidur" varieties. The morphological characteristics and cultivation of the fungus on PDA medium prove the presence of the pathogen and assist in the accurate diagnosis of the disease.

The results obtained confirm that *Cochliobolus sativus* is a highly adapted phytopathogen with



Figure 1. Symptoms caused by *Cochliobolus sativus* on a leaf of durum wheat /variety Vzhod/

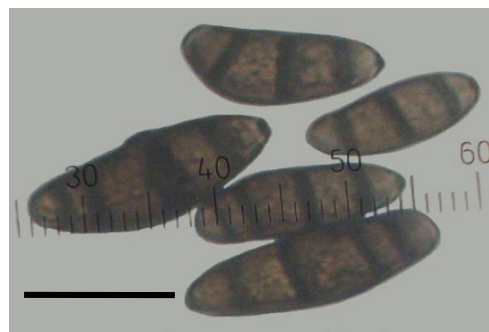


Figure 2. Conidia of the asexual morph of *Cochliobolus sativus*; scale bar = 20 μm

clearly expressed symptoms under suitable climatic conditions. The observed leaf lesions correspond to those described in other studies, confirming the identity of the pathogen. Its isolation in the varieties “Vazhod” and “Railidur” in two consecutive years is an indicator of varietal susceptibility, which emphasizes the need to include the disease for monitoring in breeding programs for resistance.

In the conducted study, a different level of manifestation of the pathogen was observed, depending on the phenophase in which the durum wheat is located, Table 1.

In the initial phase of development of durum wheat BBCH 21 – 25 – brotherhood, no visible symptoms of helminthosporiosis were observed. This suggests that the pathogen *Cochliobolus sativus* may be present latently in tissues or soil without causing visible damage. The lack of symptoms may also be due to unfavorable conditions for the development of the pathogen (e.g. dry and cool spring). In this phase, 0% infection is observed: no visible symptoms.

Early onset of symptoms was observed in the BBCH 30 – 31 phase – beginning of spindle formation, in the presence of a wet and warm spring, the first, albeit single, spots were registered on the lower leaves. This indicates that the pathogen

begins active development already in the vegetative stage, especially under favorable climatic conditions. The appearance of symptoms at this early stage is an indicator of high inoculation potential or susceptibility of the varieties. In the beginning of the spindle phase, 2% of the affected leaf sheath is observed.

The first distinct visual symptoms and well-defined symptomatology were observed during BBCH 37–39 grading, when round to elliptical spots with a brown center appeared. This is a critical moment, as it is during this phase that the photosynthetic activity of plants is most intense and damage to the foliage can have a significant impact on yield. In the emergence phase, 10% of the affected leaf area is observed.

During the flowering phase BBCH 51 – 59, an intensive spread of the disease was observed. The spots expand, a characteristic yellow halo appears – a typical sign of a necrotizing fungal infection. In this phase, the pathogen enters a phase of maximum activity and the infection progresses rapidly. In the flowering phase, 35% of the leaf area is affected. A sharp increase in intensity is observed, with the spots merging and affecting a large part of the foliage. This is the time of the most intensive development of the pathogen. The disease significantly reduces photosynthetic activity.

Table 1. Occurrence of symptoms of helminthosporiosis depending on the phenological phase of durum wheat (varieties “Vazhod” and “Railidur”) - data from the Institute of FCI- Chirpan (2020–2023)

Phenological phase (BBCH)	Observed symptoms of helminthosporiosis	% leaf area affected	
		Variety Vazhod	Variety Railidur
21–25	No symptoms observed. Infection still latent	0%	0%
30–31	Very faint, single spots on the lower leaves	2%	2%
37–39	First visible spots appear on the lower leaves. Round to elliptical spots	10%	10%
51–59	The spots expand, yellow halos appear. Increased development of the pathogen	35%	40%
71–75	The spots merge, covering a large part of the foliage. Intensive infection	65%	65%
83–87	The spots dry up, necrosis appears. Symptoms by grade are not noted	50%	50%

In the grain filling phase of BBCH 71 – 75, the symptoms cover a significant part of the leaf surface, with merging of the spots observed. This significantly reduces the area for photosynthesis, which can compromise grain filling and lead to reduced yield. This phase is particularly critical in sensitive varieties, as plants cannot compensate for losses in metabolic activity. At the beginning of grain filling, 65% of the leaf area is affected. This is the peak of infection. The spots cover most of the leaves.

In the BBCH 83 – 87 ripening phenophase, partial drying and necrosis of the spots is observed. The pathogen gradually loses activity, and the symptoms do not spread to the clusters. This indicates that the main damage has already occurred, and further development of the disease is limited by the maturation of the crop and the depletion of nutritional resources. In the ripening phase, 50% of the leaf area is affected. The symptoms are beginning to stabilize, some of the spots have necrotized, and the development of a new infection is limited. Losses have already occurred, but there is no further spread of the disease across the clusters.

Tracking the dynamics of the development of symptoms of helminthosporiosis (%) during the growing season shows that the most intensive development of the fungus *Cochliobolus sativus* is in the period between earing and grain filling, when the disease progresses rapidly. Humidity and temperature during this period determine the strength of the infection. For sensitive varieties such as “Vazhod” and “Railidur”, it is important to start monitoring already in the spindle phase in order to react before the symptoms appear on a mass scale.

CONCLUSION

The ascomycete fungus *Cochliobolus sativus* has been identified in two consecutive years on the leaves of durum wheat varieties “Vahod” and “Railidur”. For accurate diagnosis and identification, the characters with the highest taxonomic value - morphology and size of asexual sporu-

lation, were taken into account. Conidiophores were observed emerging through the stomata, bearing conidia that were septate, straight or slightly curved, and dark olive-brown. The sexual morph was not established during the studied period.

The first symptoms appear already in the spindle phase under suitable conditions, the mass development of the disease begins in the leafing phase. The flowering and grain filling phases are critical, as this is when the pathogen causes the most serious damage. The varieties “Vazhod” and “Railidur” exhibit symptoms in dynamics with the corresponding phases, which confirms their sensitivity and emphasizes the need for selection of resistant genotypes.

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