Alternaria helianthicola RAO AND RAJ., A NEW PARASITE ON SUNFLOWER IN YUGOSLAVIA

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Summary

Alternaria helianthicola, Rao and Raj. was first determined as a parasite on sunflower in Yugoslavia in 1988.

Disease symptoms are manifested on young sunflower cotyledons and leaves, in the form of small-size chlorotic spots, which have necrotic and brown centres.

On sunflower seeds and in pure culture on PDA, the fungus produces light-grey, medium-profuse mycelial film. Examined under the microscope, the young mycelium is hyaline. The old mycelium is light brown and brown, which depends on temperature and the nutrient medium, divided with septa and has multicellular conidia and conidiophores.

The dimension of conidia is 34.44 x 11.15 μm on average, with 1-5 longitudinal septa and 1-3 transversal septa.

On PDA, temperature between 0 and 5°C were minimum for fungus development, maximum ones were between 40 and 45°C, and optimum was around 25°C.

At an optimum temperature (25°C), and on seven nutrient media, the best media for the development of the mycelium was on acid-synthetic medium (ASM). The best medium for the fructification was carrot. For sunflower, this fungus is pathogenic less than *Alternaria helianthi, ALternaria helianthinficiens and Phomopsis helianthi.*

This fungus overwinters on infected harvest residues in field and on infected seeds in storehouses.

Introduction

A number of parasitic fungi, systematically classified in several species, are the causal agents of spots on sunflower. However, the spot disease are most often caused by fungi from *Alternaria* genus, namely, *Alternaria alternata, Alternaria zinniae, Alternaria helianthi* (Aćimović, 1969) and *Alternaria helianthinficiens* (Aćimović, Lačok, 1991), *Alternaria helianthicola* Rao and Raj. and *Alternaria protenta* sp. nov.

(Simmons, 1986). Investigating the causal agents of spots on sunflower, we often isolated fungi from *Alternaria* genus in our isolates, which were determined previously as *Alternaria* sp. or *Alternaria* spp. (Aćimović, 1988). Using the most current literature, we determined *Alternaria helianthinficiens* Simmons, Walcz and Roberts and *Alternaria helianthicola* Rao and Rajagopalan (Simmons, 1986, Rao and Rajagopalan, 1977). *Alternaria helianthinficiens* was first determined as the causal agent of new sunflower disease in Yugoslavia by Aćimović and Lačok in 1991. We shall describe in this paper the disease symptoms and some characteristics of *Alternaria helianthicola*, the new causal fungi of spots on sunflower in Yugoslavia.

Symptoms and conventional name of the disease

Infected seeds are the main source of the disease. When testing germination of sunflower seeds in laboratory, the seeds had poor germination and several species of the genus *Alternaria* spp., of which *Alternaria helianthicola* was most significant. Strongly infected seeds have poor germination and light grey mycelial film develops on seed coat surface (Figure 1). The fungus penetrate through seed coat to external part of endosperm, causing irregular, dark brown spots (Figure 2). The fungus penetrate through endosperm to germ and injure it, causing poor seed germination. Weakly infected seeds germinate, but black, large spots, which are the disease symptoms, occurred on root, stem and cotyledons of the germ. Later on, characteristic grey, medium profuse film occurred on the spots and the germ decays before the secondary roots are formed. Due to the fungus effect, there comes to torsion and deformation of individual parts of root and stem (Figure 3).

In field, the first disease symptoms occurred on cotyledons in the form of black spots, immediately after sprouting. From the cotyledons, the infection progresses towards the stem, primary leaves, and, later on, towards the head where it infects the flower and the seeds. At the beginning of the infection, small, chlorotic spots occur on the infected plant parts. As the fungus spreads into the leaf, stem and head tissue, the spots are brown with yellow external margins and reach the size of 0.5 to 1 cm in diameter. Small, brown, angular, slowly progressing spots, which do not cause economically important leaf wilting, are characteristic for this fungus. According to our results, this fungus is harmful on seed quality since it decreases germination rate and damages the germ. However, it does not occur on the seeds off all cultivated hybrids equally. For most hybrids, this fungus is not economically important. However, the fungus attacks strongly the head and seed of some hybrids decreasing significantly germination percentage and destroying germs.

There has been found no conventional name of the disease in literature. It is

usually known as "leaf spot disease". Our proposal for the disease, which corresponds to the disease symptoms is: "Small brown spot disease".

Material and Methods

The fields sown with different sunflower hybrids were monitored regularly in the course of vegetation, in Yugoslavia. The infected samples of sunflower were inspected macroscopically and microscopically in the laboratory. The pure cultures of pathogens were isolated from the infected parts of sunflower with the aim to identify disease agents and to test their pathogenicity.

In last five years, we have regularly obtained in pure culture, from small size brown spots on leaf and infected seed, one fungus which had been identified as *Alternaria helianthicola* in 1988. Its pathogenicity was tested on sunflower plants in field and in greenhouse. Our research work was extended from the disease symptoms to morphology and biology of the fungus.

Results

Fungus development in pure culture

The fungus isolates were obtained from the samples of infected leaves and seeds taken from different sunflower genotypes and from identical but taken from different locations. No differences were found between different fungus isolates. Pure cultures of the fungus were isolated and studied on the PDA. We shall briefly describe the mycelium, conidium and conidiophores which are produced by the fungus in the pure culture.

Mycelium

The fungus cultured on the PDA produced grey-white, airy, medium profuse and medium consistent mycelium at all temperatures. Examined under the microscope, it is hyaline at low temperatures and light brown at high temperatures (Figure 4). Under the microscope, two types of mycelial parts could be observed, the old one which is brown and young marginal part which is light brown. The mycelium is divided with perceptible, diagonal septa and branched with acute or obtuse angles (Figure 5).

Conidia and conidiophores

Conidia and conidiophores develop at the tips of young mycelium (hyphae). When the mycelium reaches a certain size, it branches to side hyphae which produce 2-3 cells, then stop to grow, bulge at the tips, get round and become dark brown (Figure 5). Later on, conidiophores occur on bottom part, divided with 1-3 septa. At the upper part, conidia are divided with transversal and longitudinal septa. Old conidia are irregularly pear-shaped and multicellular, while the young ones are unicellular and two-cellular. Conidia are different in colore, depending on maturity. Old conidia and conidiophores are brown (Figure 6).

The dimension of conidia is 34.44 x 11.5 μm, with 1-5 longitudinal and 1-3 transversal septa.

The size of conidia given by Simmons (1986) are larger than in our case, but he reported that their size varied strongly.

Conidia germinate in a drop of water, producing hyphae. They are produced on nutrient medium, but after a few sowing, mycelium lost the power of reproducing conidia.

Effect of some ecological factors on the development of the fungus

There are no literature data on this fungus and this area of study. In our investigation, we tested the effect of temperature and some nutrient media on the development of the fungus.

Effect of temperature

We tested the effect of temperature on the fungus grown on PDA. Identical portions of the pure culture of the fungus were placed in Petri dishes 90 mm in diameter, each with approximately 10 ccm of the nutrient medium. After that, the Petri dishes were put in a polythermostat at 5, 10, 15, 20, 25, 30 and 35°C. The development of the fungus was monitored at certain time intervals for 30 days. During this period, mycelial growth and the occurrence of reproductive organs (conidia) were monitored. Table 1 presents the results obtained.

Tab. 1 EFFECT OF TEMPERATURE ON MYCELIAL GROWTH (mm) AND FRUCTIFICATION OF *Alternaria helianthicola*

Period in days	**Temperature** (in °C)								
	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C
3	-	-	-	7,40	12,00	12,00	9,20	2,50	-
6	-	-	10,60	24,20	35,75	30,20	14,60	4,25	-
8	-	1,80	21,80	38,20	52,00	36,60	32,50	7,75	-
10	-	6,20	31,00	51,80	68,00	54,60	32,50	7,75	-
11	0,80	9,00	41,40	62,60	90,00	77,20	38,25	7,75	-
13	2,60	14,20	53,40	79,20	90,00	75,20	48,75	8,75	-
18	6,80	22,20	78,00	90,00	90,00	90,00	90,00	8,75	-
24	10,80	33,40	90,00	90,00	90,00	90,00	90,00	8,75	-
30	13,20	37,40	90,00	90,00	90,00	90,00	90,00	9,50	-
Occurrence of conidia	-	-	-	+	+++	+	-	-	-

Legend:

- \- no occurrence
- \+ weak occurrence
- +++ strong occurrence

The results in Table 1 show that after 3 days the mycelial growth was registered at 20, 25, 30, 35 and 40°C. After 6 days, mycelial growth was registered at 15°C, after 8 days at 10°C, after 11 days at 5°C. The mycelium developed quickly at 25°C, when the maximum diameter of 90mm was achieved after 11 days. Relatively intensive mycelial growth was registered at 20, 30, 35 and 40°C. However, first conidia were registered after 8 days at 25°C, after 13 days at 30°C and after 18 days at 20°C. It can be concluded that in field, plants can be infected with mycelium at 5 to 40°C, with conidia at 15 to 20°C and 30 to 35°C, which are the temperatures favourable for fungus fructification.

Effect of nutrient medium on fungal development

Seven different media were used in this investigation: water agar, bean agar, prune agar, onion agar, carrot agar, PDA and acid-synthetic agar. The optimum temperature for fungus development was 25°C. Additional practices were performed according to the same method described above. Table 2 shows the results obtained.

It is evident from the results in the Table 2 that two groups of media are significant. The prune, onion, carrot and acid-synthetic media belong to the first group, on which mycelium developed intensively. Mycelium growth was weak on water, bean, and potato-dextrose agars, which belong to the second group. The occurrence of conidia was detected on four media: prune, carrot, PDA and acid-synthetic agar. No fructification occurred on other media.

Pathogenicity and characteristics of the fungus

Pathogenicity of the fungus was tested in the field on disease-free plants of sunflower hybrids NS-H-26 and NS-H-45. The plants were inoculated at the beginning of flowering. Mycelia were injected in the medium part of the stem. The fungi used for the inoculation were *Alternaria helianthicola, Althernaria helianthinficiens* and *Phomopsis helianthi.* Ten plants of NS-H-26 and NS-H-45 were inoculated with each fungus. External disease symptoms were monitored on all plants and infected stems were dissected after 7, 14 and 30 days to observe the distribution of the fungi in sunflower tissue.

The incubation period of the three tested fungi was three days. However, the rate of spreading of the three fungi in plant tissue was different. It was fastest in *Phomopsis helianthi,* then in *Al. helianthinficiens,* and slowest in *Al. helianthicola,* indicating this fungus as low pathogenic. However, the strong seed infection with this fungus decreases seed germination for 50 - 90%, so that

Tab. 2 Effect of nutritive medium on the growth of mycelium (in mm) and fructification of *Alternaria helianthicola* at 25°C

Period in days	NATURAL MEDIA						ARTIFICIAL MEDIA
	Water agar	Bean medium	Prune medium	Onion medium	Carrot medium	PDA	ASM*
3	-	6,00	6,00	7,60	5,80	4,80	8,20
5	-	13,80	18,80	20,60	18,20	14,80	20,00
8	-	35,80	37,80	42,60	34,40	29,60	42,60
10	5,60	45,00	49,40	55,60	51,80	37,20	52,40
17	10,40	73,20	**90,00**	85,80	85,00	63,40	**90,00**
19	11,60	74,60	90,00	**90,00**	**90,00**	69,00	90,00
28	12,20	79,60	90,00	90,00	90,00	74,20	90,00
Occurrence of conidia	-	-	+	-	+	+	+

* ASM - acid-synthetic medium

Legend:

- \- no occurrence
- \+ weak occurrence

this fungus has been considered the most pathogenic of sunflower. This phenomenon demands a serious investigation in the future. Mitigating circumstance is that this fungus, according to our knowledge, attacks strongly the seed of only a few sunflower genotypes.

Overwintering and infection source

In the field, the fungus overwinters in the form of mycelia on infected plant parts. In spring, the fungus conidia occur at the temperature of 15°C and infect the young plants.

Infected seeds are another source of the disease. When inspecting the health condition of sunflower seed, conidia and mycelia of the fungus were regularly found. The rate of seed contamination ranged from severe to slight or no infection at all. Contaminated seeds transmit the infection on the field and plants.

Discussion

Alternaria helianthicola Rao and Raj. was first described as sunflower parasite in India in 1977. So far, the fungus remained practically unknown in other sunflower producing countries. The real importance of the fungus remains unknown. Our results represent a small contribution to better knowledge of this fungus as a parasite present on sunflower in Europe. We hope that our results will motivate phytopathologists to investigate this fungus and determine its economical importance for sunflower production.

Conclusion

Alternaria helianthicola Rao and Raj. was first determined in Yugoslavia in 1988.

Disease symptoms are manifested on young sunflower leaves in the form of small chlorotic spots, with necrotic and brown central part.

On sunflower seed and in pure culture on PDA, the fungus produces light grey, medium profuse mycelial film. Under the microscope, the mycelium is divided with septa, light-brown to brown, and has multicellular conidia and conidiophores.

The dimensions of conidia are 34.44 - 11.15 μm, with 1-5 longitudinal and 1-3 and transversal septa.

Temperatures between 0 and 5°C are minimum for the fungus development on PDA, maximum ones are between 40 and 45°C while the optimum is around 25°C.

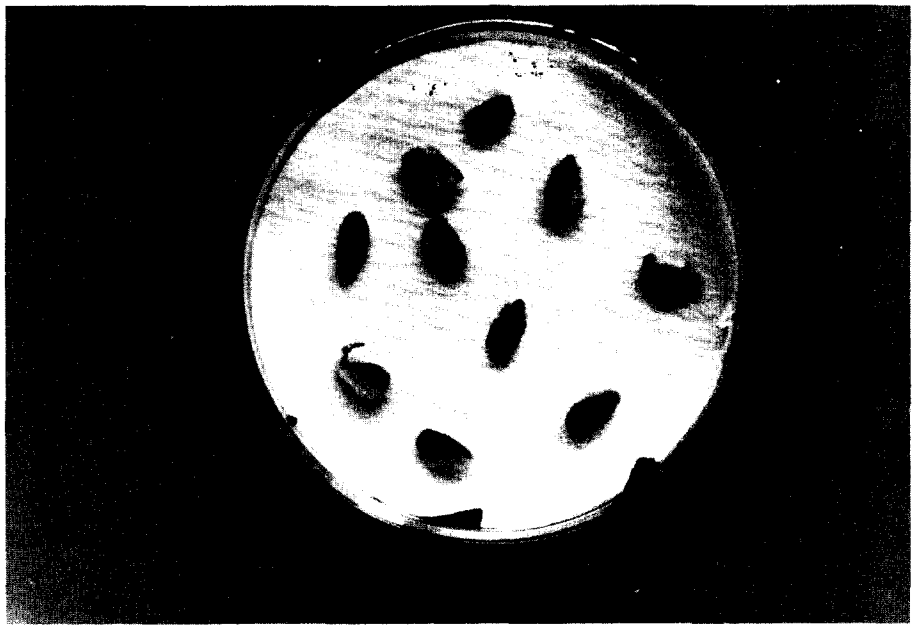

Fig. 1 *A. helianthicola.* Sunflower seeds infected by the fungus with characteristic mycelial film on seed coat.

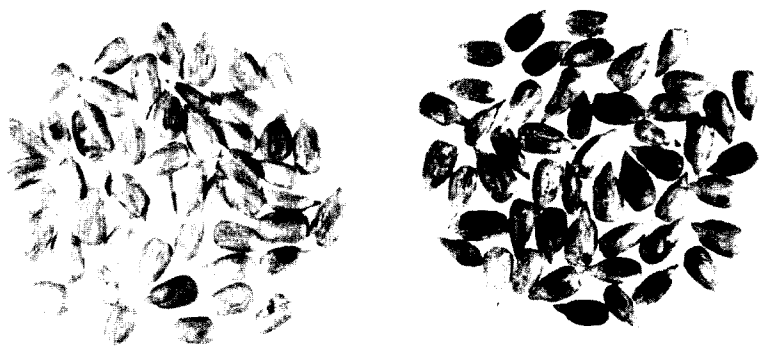

Fig. 2 *A. helianthicola.* External part of endosperm infected with the fungus (left) and uninfected endosperm (right)

Fig. 3 *A. helianthicola.* The germ obtained from the infected seed: black spots, characteristic mycelial film and torsion

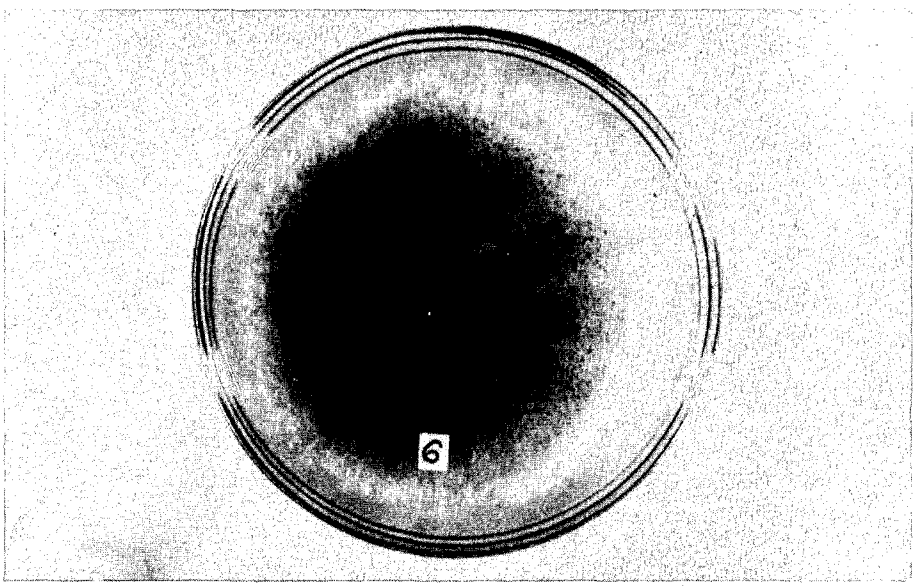

Fig. 4 *A. helianthicola.* Pure culture of the fungus grown on PDA at 25°C for 10 days

Fig. 5 *A. helianthicola.* A view of the mycelium under the microscope.

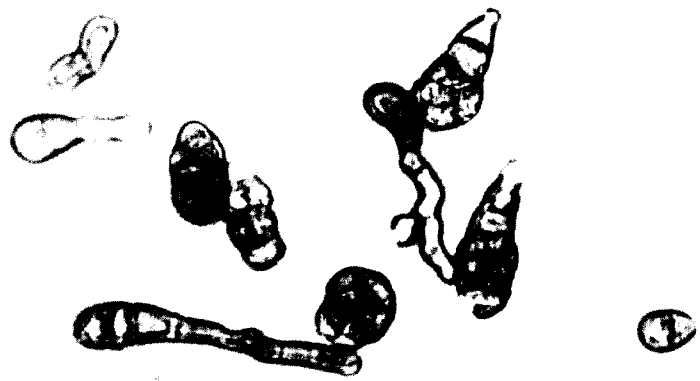

Fig. 6 *A. helianthicola.* A view of conidia and conidiophores

The fungus fructifies at the temperature from 20 to 30°C, while the optimum is around 25°C.

The effect of seven different media on the fungus was tested at optimum temperature of 25°C. The best medium for the development of the mycelium was acid-synthetic medium (ASM), and for the fructification carrot agar. This fungus is less pathogenic than *Alternaria helianthi, Alternaria helianthinficiens and Phomopsis helianthi.*

The fungus overwinters on infected harvest residues in field and on infected seeds in store.

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