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Original Article

Research Regarding *Alternaria brassicae* Fungus on Mustard: Biology, Morphology and Control

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Abstract

In Romania *Alternaria* black spot of mustard (*Brassica alba* L), incited by *Alternaria brassicae*. *Alternaria brassicae* fungus can be manifesting on every plant organ in every stage of mustard development. The study must be done in order to establish its needs regarding certain some abiotic factors such as: temperature, and pH value. To change the pH, were used sodium hydroxide solution or hydrochloric acid for each variant. Research has focused development of the fungus *Alternaria brassicae* on different culture mediums and also the importance of plastic and energy sources for the growth of *A. brassicae* fungus. Mustard plants are attacked by conidia of *A. brassicae* (Berk.) Sacc. It causes damping off if infection occurs in younger plants and severe leaf spot symptoms on infections of older plants. After appearance of the first symptoms of disease, stringent fungicide spray program is an effective way to reduce losses. For controlling *Alternaria brassicae* infection some fungicides were used. *Alternaria* black spot disease caused by *Alternaria brassicae* in mustard was studied in two crop seasons, 2012 and 2013 in Malu city, county of Ialomița. At Malu city, epidemics of the disease was recorded for both seasons. Among cultivar were more susceptible than Alex. Infected seeds with spores on the seed coat or mycelium under the seed coat are the main means of distribution for these pathogens.

Keywords: abiotic factors, *Alternaria brassicae*, culture medium, fungicides, morfology, mustard.

1. Introduction

Alternaria brassicae (Berk.) Sacc. [6] is a plant pathogen able to infect most *Brassica* species including mustard. *Alternaria* black spot is an important disease of mustard around the world [1]. *Alternaria brassicae* is a dominant invasive species. *Alternaria brassicae* causes lesions on mustard leaves which have necrotic centers surrounded by chlorotic areas (Fig. 1.).

Figure 1. Leaf of mustard with *Alternaria* black spot The lesions consist of necrotic areas surrounded by chlorotic areas

This leads to reduction in photosynthetic area, defoliation and accelerated senescence.

The pathogen synthesizes abscisic acid which would aid in the accelerated senescence [2]. Severe

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infection causes substantial yield loss as a result of early defoliation, flower-bud abortion, premature ripening, siliquae dehiscence and seed shriveling [7]. The abiotic factors play an important role during the fungus development and pathogenicity. The effect of environmental factors, temperature (28-32°C), relative humidity (>80%) and alternative light/darkness are good parameters for the disease development and its epidemics [8].

The study must be done in order to establish its needs regarding certain abiotic factors such as: temperature and pH value. The study is necessary in order to get to know the moment when the first infections occur [5].

2. Material and Method

Seeds used were free from infection of *Alternaria* species. The sick biological material of mustard, which was formed from leaves with specific disease symptoms. In laboratory conditions, phytopathogenic fungus *Alternaria brassicae* was grown on artificial agar media with PGA (potato-glucose-agar).

Alternaria brassicae fungus was placed on PGA culture medium, in Petri recipients of 8 cm in diameter, and then each of them was placed in thermostates at temperatures of 2-38°C. The experiments were carried out in factorial Randomized Complete Block Design with 4 replications.

The factorial combination was 2 cultivars x 3 treatments. Were tested two mustard cultivars (Alex and Amog) approved in Romania under conditions of natural contamination with *Alternaria brassicae*. We analyzed cultivars reactions to *Alternaria* black spot, recording frequency, intensity, attack degree, and the possibility of crop protection by treatments applied during the growing season.

Each cultivar had two plots in each replication, one was fungicides-treated and the other untreated. Three fungicides: Caramba Turbo SL (metconazole-30 g/l + mepiquat chloride 210 g/L), Pictor SC (boscalid 200 g/L + dimoxystrobin 200 g/L), and Matiz EW (tebuconazole 250 g/L) applied in different schemes were tested at the recommended dose from CODEX [3].

First treatment was made at growth stage BBCH 31 and the second treatment was applied at growth stage BBCH 59 [4].

For calculating the efficacy was taken into account attack degrees of variants with and without treatments.

Morphological parameters were analyzed to the optical microscope.

3. Results and Discussions

We have identified on siliques and leaves straight or slightly curved conidia, obclavate, pale olivaceous brown, 55-72 x 12-24 µm (Fig. 2.) with transverse and vertical septa. On PGA culture medium, the *Alternaria brassicae* fungus develop fast-growing thick colonies which are white-gray, with silky aspect and dark-grey reverse.

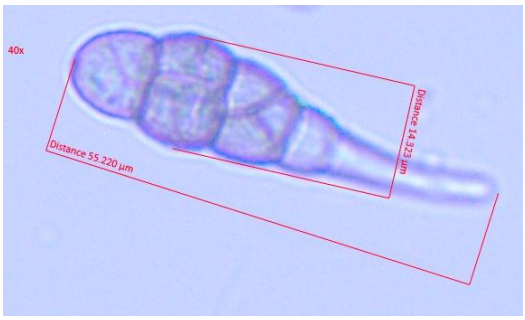

Figure 2. Mature conidia of *Alternaria brassicae*

pH values of the substrate on which the fungus grows influences the growth and fructification. At pH values of 4-7 *A. brassicae* fungus forms a good vegetative mass (Fig. 3) and numerous conidia.

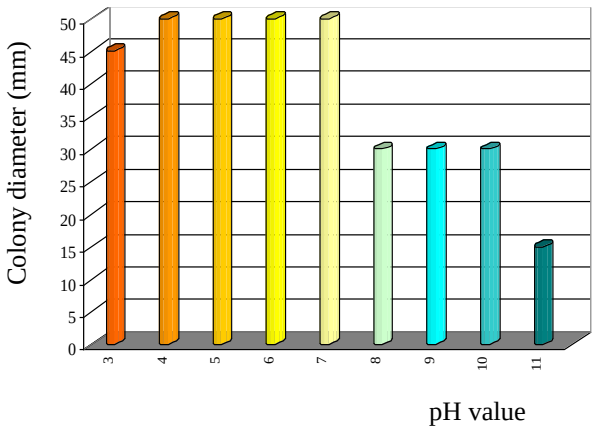

Figure 3. Dynamics development of the *Alternaria brassicae* fungus depending on the pH value of culture medium

Temperature and relative air humidity are important factors in the appearance of the first symptoms.

After artificial infection was provided throughout the period of the experiment high atmospheric humidity by keeping infected organs in wet rooms. On infected leaves incubation period of the *A. brassicae* fungus was 14 days at 2°C and 4°C.

With increasing temperature, the incubation period decreased to 7 days between 8°C and 14°C, then to 6 days between 18°C and 28°C. Then, as the temperature rises infections were stopped (Table 1). The fungus can overwinter on susceptible weeds or crop debris and on seed plants.

Table 1. The incubation period of the *Alternaria brassicae* fungus in mustard leaves infected

t°C	2	4	6	8	10	14	16	20	24	26	28	30	32	34	36	38
days	14	14	14	14	12	11	9	7	6	6	6	9	12	12	12	14

Regarding the *Alternaria brassicae* cultivation on different culture mediums: the fungus develop very good on natural mediums; mediums containing oats, wheat, barley led to a great growth in vegetative mass and abundant conidia formation. Culture mediums semisynthetic PGA and malt 2% favored the formation of colonies with specific morphological aspect and a very good fructification. Synthetic mediums Leonian and Czapek have curbed vegetative development of the fungus, and fructifications were absent. Plastic and energy sources are essential for *A. brassicae* colony development because the basics required for feeding are carbon and nitrogen.

The fungus readily metabolized inorganic nitrogen compounds as potassium nitrate and less from ammonium nitrate and ammonium phosphate. This demonstrates that the presence of potassium nitrate ground administered favoring infection caused by the *A. brassicae* fungus.

The same happens in the case of urea fertilizer, of which nitrogen is easily metabolized by the fungus.

The fungus metabolized very well carbon from monosaccharides: glucose, dextrose, levulose, maltose, mannose, trehalose, arabinose, mannitol, levulose and ribose. The scoring attack for *A. brassicae* has a particular importance for the mustard in establishing the need for chemical treatments during the vegetation season.

The data presented in Table 2 show the results on the efficacy of some fungicides to prevent and combat *A. brassicae*. In the variants where two treatments have been applied the efficacy values were double compared to variant with one treatment.

Table 2. Efficacy of some fungicides used during the growing season (years 2012 and 2013) to prevent and combat *Altelnaria* black spot to mustard

The fungicide	Cultivars	The year			
		2012		2013	
		Attack degree (%)	Efficacy (%)	Attack degree (%)	Efficacy (%)
Caramba Turbo	Alex	6.25	30.6	10.5	44.0
(treatment at the stem elongation)	Amog	9.0	30.8	13.25	44.8
Caramba Turbo at stem	Alex	2.5	72.2	5.0	73.3
elongation / Pictor at yellow bud	Amog	3.0	76.9	6.5	72.9
Matiz at stem elongation / Pictor	Alex	2.5	72.2	5.25	72.0
at yellow bud	Amog	3.5	73.1	6.75	71.8
Untreated control	Alex	9.0	-	18.75	-
	Amog	13.0	-	24.0	-

4. Conclusions

The abiotic factors are important factors in the *Alternaria brassicae* fungus evolution. For *A. brassicae* optimum development was recorder on the substrates with weak acid pH to neutral (5-7). Metabolism of monosaccharides by the *A. brassicae* fungus demonstrated pathogenicity action of the pathogen on green organs (leaves and siliques). Applying the two treatments had effectively reduced disease in the sprayed plots and and the effectiveness of treatments ranged 71.8% (2013) and 76.9% (2012). Chemical control must be integrated in an agricultural management practices.

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