RESEARCHES CONCERNING THE BIOLOGICAL PARAMETERS OF FUNGUS *FUSARIUM GRAMINEARUM (*SCHW)

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Abstract: *The fungus Fusarium graminearum is one micromycetes than can produce significant crop losses, and wheat,specially. Fungus attack contributes to impairment of quality indictors of wheat seed and is present in micoflora responsible for the appearance of black-point attack at seeds of wheat.Understanding the biology of pathogens gives us the information regarding the right moment to apply control measures in order to discontinue the infection development. Our studies have been oriented toward defining the biological development parameters of fungal growth, under controlled conditions. Research has been performed in the laboratory of Mycology from Research - Development Institute for Plant Protection Bucharest and Phytopathology laboratory of the Faculty of Agriculture of the University of Agricultural Sciences and Veterinary Medicine of Bucharest. The biological material used was the phytopathogenic fungus Fusarium graminearum, isolated from the seeds of variety Capo wheat, came from" Probstdorfer Saatzucht" Romania SRL, location Modelu, Calarasi County. Infected seeds were kept wet chambers for sporulation and fungal isolation was done by direct isolation method. Fungal strain was stored on sterilized potato dextrose agar (PDA) slants, at 4^0C. The influences of some abiotic factors (temperature, relative atmospheric humidity, pH value and the influence of medium culture) were studied and the results are presented in this paper. The results showed that the optimal humidity limit was established at values over 75%. The Fusarium graminearum fungal colony development is influenced by temperature values: the lowest temperature limit for colony development was 6^0C; the optimum temperature needed for colony development was between 21-30^0C; the highest temperature level may be considered at 36^0C. The colonies grow fast on Malt-Agar and growth medium on Czapek'sDox-Yeast extract-Agar (CYA) growth medium (initial pH 6).*

Key words: *seed, fungus, biological parameters*

INTRODUCTION

The fungus *Fusarium graminearum* Schw anamorphic form of the pathogen *Gibberella zeae* (Schw) Petch produces redness of ears or Fusarium wheat. The attack of fungus produces average loss of 1-3%, but in the rich precipitation years the percentage of attacked ears exceeds over 65% in sensitive varieties (Barbulescu et al., 2002). Viorica Iacob et al, 1998 shows that in very favorable conditions the pathogen attack may cause loss of 100%.

Manifestation of the disease differs with the host vegetation stage but the worst attack occurs to herring-bone when it can be observed the bleaching of ears. At the basis of discolored little ears and also on the rachis and blade develops pinkish white mold representing the mycelium and asexual fructifications of the fungus. They extend to the whole ear which acquires a reddish tint that gives the name of disease (Cristea S., 2005). Duthie J.A et al. (1987) made research on transmission of *F. graminearum* from seed to stems of winter wheat. *F. graminearum* attack on the ears is favored by more than 90% humidity, temperature greater than 20̊ C, a high infectious task in the atmosphere and existing wheat during blossoming ripening in milk (Cristea S., 2005).

MATERIAL AND METHOD

The research conducted was aimed to determine the biological parameters of the fungus *Fusarium graminearum* in conditions 'in vitro'. The minimum, optimal and maximum thresholds were determined for temperature, humidity and pH values of the culture medium factors. It was followed the influence of these factors on the growth and sporulation of fungus.

The pathogen was isolated from the wheat caryopses Capo variety, origin "Probstdorfer Saatzucht" Romania, SRL Modelu location, Calarasi County. The isolated fungus from biological material was then pricking out on PDA medium (potato-glucose-agar).

There were performed measurements of the colony diameter and observations on the sporulation of the fungus. After 3 days was observed the formation of mycelium and the sporulation of pathogen. The minimum thresholds, optimum and maximum for the development of the fungus at different temperatures was achieved by maintaining Petri vessel inoculated with *F. graminearum* in thermostats with temperature between 2-40˚C for 14 days, period in which linear measurements were made of colonies. Atmospheric relative humidity influence on the development of fungus colonies was achieved desiccator vessels in which were made values ranging between 15% and 100% (method Tuite, 1968). Under these conditions the replicated mushrooms on PDA medium in Petri plates with a diameter of 8 cm, without lid, were introduced in desiccators where they were kept for 21 days. It was appreciated the development of colonies by measuring the colonies diameter and the presence of sporulation.

For the determination of the pH environmental values influence it was used PDA environment in which was changed pH value, with values between 3-11. Colonies diameter was measured every 3 days and it was watched the sporulation.

RESULTS AND DISCUSSIONS

The temperature parameter has a decisive role in achieving of infections and pathogen development. The data of table 1 shows that the minimum temperature of formation the colonies was 6° C. At this temperature value, however, the sporulation was absent. The temperature of 12° C and 16 °C leads to a better development of colonies and has been recorded the presence of conidiums in small numbers on the surface mycelium.

At 20°C the colonies were sporulated well. The optimum temperature needed to develop colonies is between 21 °C and 30 °C, when was registered a colony diameter of 70 mm, smooth, dense, dark carmine, with red reverse appearance. Sporulation was very good, the number of conidiums being numerous (fig.1).

After 32° C the colonies development was weaker, also the number of formats conidiums.

The maximum threshold of temperature can be considered at the temperature of 36°C. Formed colonies have a frail appearance and sporulation did not occur.

Table 1

Influence of temperature on fungus colonies development
Fusarium graminearum

TºC/days	2	4	6	8	10	12	14	Observation after 14 days
	Colonies diameter in mm							
2	0	0	0	0	0	0	0	0 0
4	0	0	0	0	0	0	0	0 0
6	0	0	0	0	0	0		0 0
8	0	0	2	8	10	10	12	Mv+ 0
10	0	0	4	13	15	27	24	Mv + Sp +
12	0	0	8	17	21	23	30	Mv + Sp+
14	0	0	10	20	30	30	33	Mv ++ Sp +
16	0	0	10	20	24	32	38	Mv ++ Sp +
18	0	0	11	20	35	46	58	Mv ++ Sp +
20	0	0	35	43	57	67	70	Mv ++ + Sp ++
22	0	0	36	54	60	67	70	Mv++ + Sp +++
24	0	0	38	57	60	68	70	Mv + ++ Sp +++
26	0	0	42	58	70	70	70	Mv ++ + Sp+++
28	0	0	30	40	70	70	70	Mv+++ Sp +++
30	0	0	35	45	70	70	70	Mv + ++ Sp +++
32	0	0	32	36	45	59	68	Mv +++ Sp+++
34	0	0	10	25	30	30	32	Mv + Sp +
36	0	0	10	12	18	20	22	Mv + Sp 0
38	0	0	0	0	0	0	0	Mv 0
40	0	0	0	0	0	0	0	The growth stops

mv± = very poor vegetative mass
mv.+ = poor vegetative mass
mv ++ = good vegetative mass
mv +++ = very good vegetative mass
Sp+++ = abundant sporulation

0= fungus did not sporulated
Sp ± = very poor sporulation
Sp+ = poor sporulation
Sp ++ = good sporulation

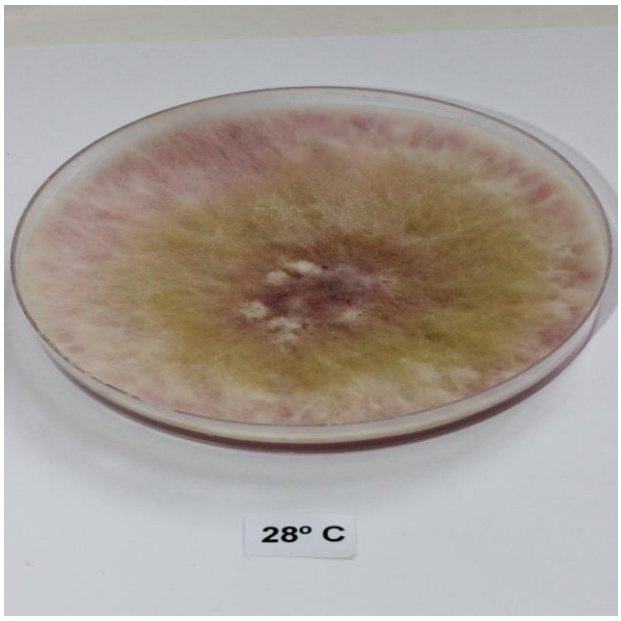

Fig. 1 - *F. graminearum* fungus development at 28°C, under controlled conditions

On relative atmospheric humidity influence on the development of fungus colonies *F.graminearum* was observed that for the values of 15%, the colonies were not developed. To atmospheric humidity over 36.8% the formed mycelium was lax and the conidiums have not been formed. At values of 66 -72% formed colonies have had a felt soft, white aspect and fungus did not sporulated. From the values above 75.6% it was observed the formation of conidiums (table 2) With the increase of atmospheric relative humidity values the colonies development was very good, dense vegetative mass, felt, deep red color and the sporulation was sometimes abundant (fig. 2).

Table 2

Influence of atmospheric relative humidity
on the development of fungus colonies *Fusarium graminearum*

Atmospheric relative humidity RH%	Colonies diameter after 12 days	Observations
15	0	Colonies does not formed
36,8	0	Poor growth
43	35	mv± Sp 0
56	42	mv± Sp 0
66	70	mv + Sp 0
72	70	mv + Sp 0
75,6	70	mv ++ Sp+
78.6	70	Mv++ Sp++
82,9	70	Mv+++ Sp+++
88.5	70	Mv+++Sp+++
90	70	Mv+++ Sp+++
92,7	70	Mv+++ Sp+++
96.1	70	Mv+++ Sp+++
98,5	70	Mv+++ Sp+++
99	70	Mv+++ Sp+++

mv± = very poor vegetative mass
mv.+ = poor vegetative mass
mv ++ = good vegetative mass
mv +++ = very good vegetative mass
Sp+++ = abundant sporulation

0= fungus did not sporulated
Sp ± = very poor sporulation
Sp+ = poor sporulation
Sp ++ = good sporulation

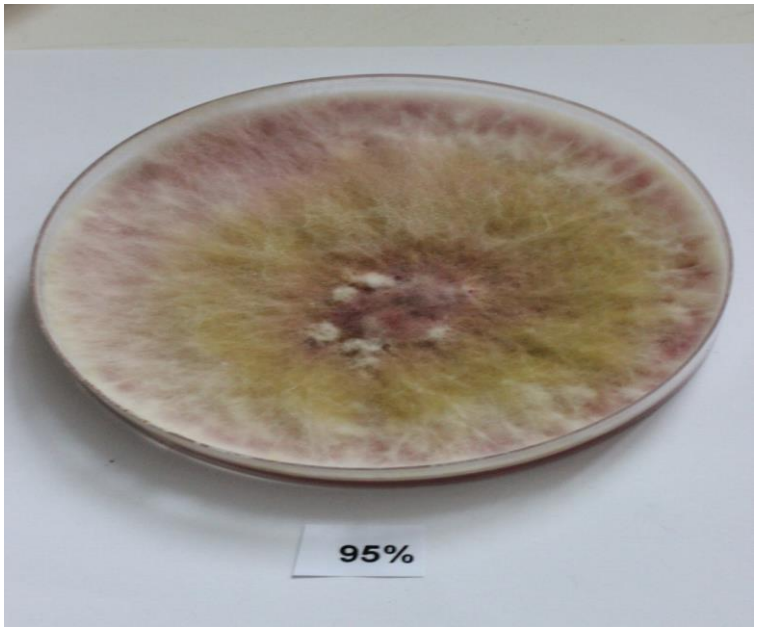

Fig. 2 - *F. graminearum* fungus development at humidity of 95%, under controlled conditions

Our research have had in view the influence of different pH values of the culture medium on growth and development of the monitored pathogen in laboratory conditions. After the evaluation of results for the experiments regarding the influence of pH values was noted that there are a wide array of development on substrates with pH values from acid to strong alkaline, the colonies forming a good vegetative mass with the conidiums appearance at pH values of 3, the optimal ranging between 4 and 7 (table 3). Once with the environment alkalinisation the *F. graminearum* fungus colonies were poorly vegetative developed, but sporulation was good (fig. 3).

Table 3

The influence of pH values on the development of fungus *Fusarium graminearum*

pH values	Colonies diameter (φmm)
3	45 (Mv ++, Sp+++)
4	70 (Mv +++, Sp+++)
5	70 (Mv +++, Sp+++)
6	70 (Mv +++, Sp+++)
7	70 (Mv +++, Sp+++)

8	70 (Mv +++, Sp++)
9	70 (Mv ++, Sp+)
10	70 (Mv +, Sp+)
11	55 (Mv +, Sp0)
12	35 (Mv +, Sp0)

Mv ++ = good vegetative mass Sp ++ = good sporulation
Mv +++ = very good vegetative mass Sp+++ = abundant sporulation

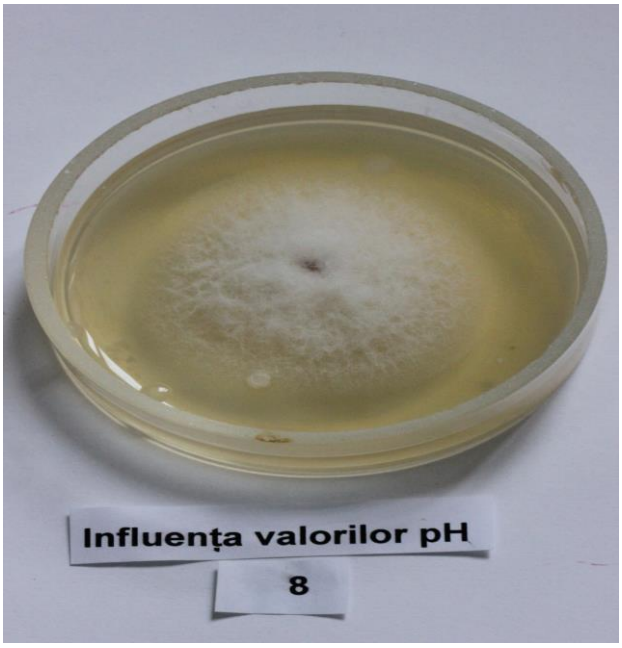

Fig. 3 - *F. graminearum* fungus development at pH=8, under controlled conditions.

Regarding the culture media influence, the data from table 4 show that *F. graminearum* fungus preferentially developed on natural culture media (barley, rice and wheat). On semisynthetic media (PDA and malt 2%), the fungus has developed very good vegetative and sporulation was very good. On Czapek medium the pathogen formed a very good vegetative mass, wit a good sporulation (fig .4).

Table 4

Influence of culture media on the development of fungus *F. graminearum*

Culture media	Fungus behavior
Natural media	
Barley seeds	Very good vegetative mass, good sporulation
Wheat seeds	Very good vegetative mass, good sporulation
Rice seeds	Poor vegetative mass, abundant sporulation
Semisynthetic media	
PDA	Very good vegetative mass, good sporulation
Malt 2%	Very good vegetative mass, good sporulation
Synthetic media	
CzapekDox	Very good vegetative mass, good sporulation

Fig. 4 - *F. graminearum* fungus colony development and reverse

CONCLUSIONS

The optimum temperature for development of the *F. graminearum* fungus in controlled conditions is between 21-30˚ C, with minimum of 6˚C to maximum threshold of 36˚C .

Relative atmospheric humidity greater than 75% determine a very good development of colonies, dense vegetative mass and abundant sporulation.

Under 'in vitro' the initial reaction of pH substrate is optimal for pathogen *Fusarium graminearum* at values between 4.0 and 7.0.

Natural culture media were the fungus developed were very good (barley, rice, wheat), when also the sporulation was abundant, especially on rice environment. Also, fungus

had a good development on PDA semisynthetic media and 2% Malt and on Czapek-Dox synthetic environment.

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