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THE INFLUENCE OF SEVERAL ABIOTIC FACTORS ON *FUSARIUM* SPP. BIOLOGY

Roxana DUDOIU[1,2], Stelica CRISTEA[2], Daria POPA[1], Carmen LUPU[1], Maria OPREA[1]

[1]Research Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Bldv, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: roxyanna_21@yahoo.com

Abstract

Wheat (Triticum aestivum L.), one of the most widely grown winter cereal crop in Romania, is grown on approximately 2 million ha. Fusarium species affect yield and grain quality because of mycotoxins production. Fusarium spp. is one the most frequently pathogenic species of wheat and understanding its biology provides information regarding the optimal timing to implement specific control measures in order to stop the infection process of the disease.
The occurrence and development of toxigenic fungi affects stored products causing quality depreciation, products aggregation and also toxins and allergens production. Micromycetes development on stored cereal seeds is favoured by temperature, light and atmospheric moisture present in storage units, and their fluctuations in time.
The aim of our research was to determine the influence of some biological parameters (temperature, pH, light and culture media) on the vegetative fungal growth and development, under controlled conditions. The biological material consisted in one strain of Fusarium spp., isolated from caryopses of wheat, from samples taken from a storage unit in Paulesti, Prahova County. It was established that the fungus develops in a large scale of pH, forming specific colonies between low-acid and high alkaline values (pH 4-10). Optimal temperature values were between 20 and 28 Cº, with a 6º C minimum and no growth above 36 Cº. Very good sporulation and vegetative growth was obtained under continuous light conditions.

Key words: *wheat, biologic parameters, Fusarium spp.*

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the most important crops cultivated in Romania due to its favorable growth conditions.
Wheat (*Triticum* spp.) (Donner et al., 2000) is a cereal grain, originally from the Levant region of the Near East and Ethiopian Highlands but now cultivated worldwide. In 2010, world production of wheat was 651 million tons, making it the third most-produced cereal after maize (844 million tons) and rice (672 million tons). However, pathogens that contaminate wheat may survive for extended periods (Berghofer et al., 2003; Cabanas et al., 2008; Gashgari et al., 2010). Wheat was also found to be contaminated in variable amounts by potentially toxigenic fungi including *Aspergillus, Alternaria* and *Fusarium* (Halt, 1998; Tournas and Katsoudas, 2008). These fungi are present in soil and plant material, cause the decay of stored grain and food (Herrman, 2002).
Fungal growth, especially *Aspergillus flavus* and *Fusarium* spp. in wheat, facilitated by hot and humid conditions, poses a major risk through production of mycotoxins (Radoi et al, 2011). Infection of grains in the field by fungi could result in the production of mycotoxins during cultivation, harvesting, storage, transport and processing.
In order to maintain high quality wheat for both short- and long-term storage, grain seeds must be protected from weather, growth of microorganisms, and pests (Cristea et al., 2008, Mali et al., 2015, Berca et al., 2015, Berca and Cristea, 2015).
The most important species of fungi and mycotoxins that could contaminate maize grains are *Aspergillus flavus* and aflatoxins, *Fusarium verticillioides*, *F. proliferatum* and fumonisins and *F. graminearum* and *F. tricinctum* and trichothecenes and zearalenone (Ittu et al., 2010).
Despite decades of intense research, the moulds infection is still a major challenge for scientists

(Munkvold, 2003). Micromycetes' development on stored grains is conditioned by temperature and atmospheric humidity present in stored areas and by its fluctuations in time (Cristea et al., 2004, Mardare et al., 2015). Toxigenic moulds are present, due to various climatic factors, in different stages of food and feed production, including crop growth, harvesting, transport, storage and handling (Beyer et al., 2006).The most common genera of fungi identified in stored maize grains are *Aspergillus, Penicillium* and *Fusarium*. (Cristea, 2005, Pana et al., 2014, Mardare et al., 2014).
Our research was focused on establishing the influence of several biologic parameters on the fungi's growth and development.

MATERIALS AND METHODS

Studies on the influence of abiotic factors, such as temperature, pH value, light and culture medium on the growth and sporulation of *Fusarium* spp. strain, were performed in laboratory conditions. The biological material consisted in wheat caryopses from Glosa cultivar, taken from a storage unit in Paulesti, Prahova County. The grains were placed in a wet room, where the mycelium formation was observed after 3 days. The *Fusarium* spp. strain was obtained by classic isolation technique using Ulster method (Hulea et al., 1969, Raicu et al., 1978) and successive sub-culturing in Petri dishes of 10mm diameter with potato-dextrose-agar growth medium, incubated at 24ºC (Radu et al., 2011) .
The temperature influence on the growth and sporulation of *Fusarium* spp. was monitored between 2ºC and 40ºC, in order to establish the minimum, optimal and maximum growth value. Mycelia disks of 5 mm diameter were inoculated in Petri dishes with PDA medium, incubated in thermostats, at temperatures between 2ºC and 40ºC, and colonies were observed and measured at a 3 days interval for a period of 15 days.
The influence of pH values on *Fusarium* spp. biology was determined using PDA culture medium with modified pH values using sodium hydroxide or hydrochloric acid solutions. The fungus was inoculated on medium with pH values from 3 to 11. Colonies diameters were measured at a 3 days interval, observing also the sporulation process, during 12 days.
It was also observed the fungus reaction to light by incubating mycelia disks on PDA medium at continuous light, continuous darkness, light/dark alternating for 8h/16h and 12h/12h.
In order to establish which nutrient substrate is most favorable for the growth and sporulation of *Fusarium* spp. were chosen different media: potato-dextrose-agar, malt-agar (semi-synthetic medium), Czapex Dox agar (synthetic medium) and natural media like wheat seeds, rice seeds, barley seed (Constantinescu, 1974).

RESULTS AND DISCUSSIONS

The temperature is a key factor in the development of infections and for the pathogen occurrence and growth. As shown in table 1, the *Fusarium* spp. strain has a minimum growth point at 4ºC, with weak vegetation mass, without sporulation. Between 12ºC and 18ºC, it can be observed an increased vegetation mass, with good sporulation of the fungus. The optimal temperature for growth and sporulation of this isolate is situated between 20ºC and 28ºC, when the fungus presented colonies of 80 mm diameter and abundant sporulation, after 15 days of observation (Figures 1 and 2). After 30ºC the fungus declines in development, sporulation is weaker. The maximum growth temperature is 36ºC, the colony diameter barely reached 35 mm, and sporulation is absent. After 38ºC, the fungal growth is completely inhibited (Figure 3).

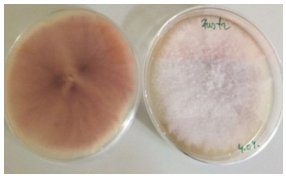

Figure 1 *Fusarium* spp. on PDA medium

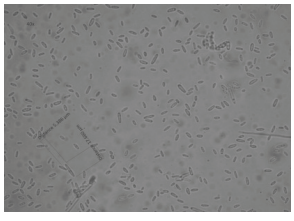

Figure 2 *Fusarium* spp. micro conidia

Table 1. The influence of temperature upon *Fusarium* spp. colony growth and development

T°C/days	3 days	6 days	9 days	12 days	15 days	Observations after 15 days	
	Colony diameter in mm						
2^0	0	0	0	0	0	Vm 0	0
4^0	0	1	4	5	5	Vm ±	0
8^0	0	5	9	15	17	Vm+	Sp+
12^0	2	8	16	23	27	Vm+	Sp+
14^0	3	18	26	38	44	Vm++	Sp+
16^0	8	20	30	34	39	Vm++	Sp++
18^0	13	24	40	47	57	Vm++	Sp+ +
20^0	13	26	38	57	80	Vm+++	Sp+++
22^o	22	34	45	68	80	Vm+++	Sp+++
24^0	23	36	46	70	80	Vm+++	Sp+++
26^0	24	45	70	73	80	Vm+++	Sp+++
28^0	23	50	64	73	80	Vm+++	Sp+++
30^0	11	48	58	68	70	Vm+++	Sp+++
32^0	8	21	28	32	35	Vm++	Sp++
36^0	2	4	6	7	7	Vm+	Sp+
38^0	0	0	0	0	0	0	0
40^0	0	0	0	0	0	0	0

Legend: Vm± = very poor vegetative mass, Vm+ = poor vegetative mass, Vm++ = good vegetative mass, Vm+++ = very good vegetative mass, Sp+ = poor sporulation, Sp++ = good sporulation, Sp+++ = abundant sporulation, Sp± = very poor sporulation, 0 = fungus did not grow/sporulate.

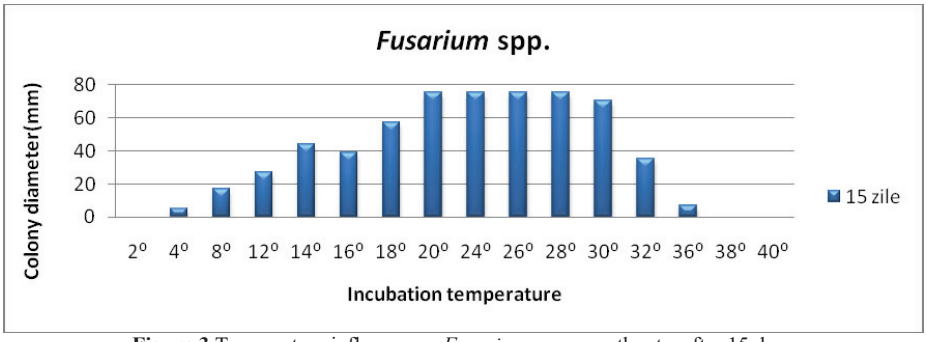

Figure 3. Temperature influence on *Fusarium spp.* growth rate, after 15 days

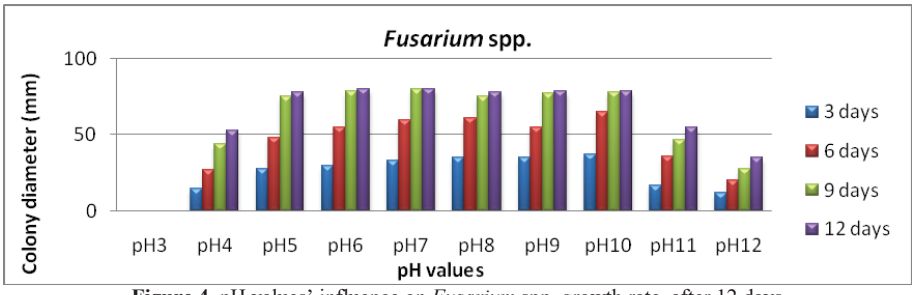

Figure 4. pH values' influence on *Fusarium* spp. growth rate, after 12 days

Regarding the influence of pH value on the development of *Fusarium* spp. fungus, after 12 days on evaluating the results of experiments it was observed that there is a wide range of pH

values substrates development, from strong acid up to high alkaline (Figures 4 and 5).

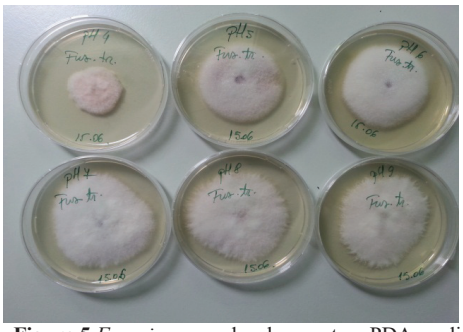

Figure 5.*Fusarium* spp. development on PDA medium with different pH values (4-9)

At pH value 4, the colonies had a good vegetative mass and conidia were observed. Optimal pH values are between 5 and 9, with very good vegetative mass and sporulation. On high alkaline culture medium, the fungus grew less vegetative, but sporulation was good.

In terms of the light influence towards the development of *Fusarium* spp., as it can be observed in table 3, the fungal colonies have developed very well in light exposure. On permanent light or alternatively light/darkness exposure, the vegetative mass of colonies was rich, velvety mycelium and sporulation was abundant. When kept into darkness during the whole observation period, the fungus formed colonies with very little vegetative mass, and conidia were rare on mycelium surface.

Table 2. Light exposure influence on fungus growth

Lightness	***Colony development***
24 hours lightness	Rich vegetative mass, velvety mycelium, abundant sporulation
Alternative lightness/darkness	Rich vegetative mass, velvety mycelium, white-pink color, abundant sporulation
Light/Darkness alternance (12h/12h)	Rich vegetative mass, felt appearance mycelium, white-pink color, rich sporulation
Light/Darkness alternance (8h/16h)	Good vegetative mass, weak sporulation
Continuous darkness	Good vegetative mass, weak sporulation

Regarding the influence of the culture substrate on the pathogen's growth the data presented in table 4, show that nutritive medium has an impact on *Fusarium* spp. growth and sporulation. The fungus grew and developed preferentially on natural mediums (wheat, rice, barley), but also had a very good vegetative mass and sporulation on semi-synthetic medium Potato-dextrose-agar, the colony diameter having reached maxim value of 80 mm. On malt-agar medium and Czapek synthetic medium the vegetation and sporulation were good.

The observations regarding the culture mediums' influence highlights that the pathogen shows plasticity on the natural growth medium.

Table 3. The influence of different culture media on *Fusarium* spp. development

Culture medium		***Observations of vegetative mass and sporulation***
Natural substrates	Barley seeds	Very good vegetative mass; good sporulation
	Wheat seeds	Very good vegetative mass; abundant sporulation
	Rice seeds	Weak vegetative mass; good sporulation
Semi-synthetic media	PDA	Very good vegetative mass; abundant sporulation
	Malt 2%	Good vegetative mass; good sporulation
Synthetic medium	Czapek Dox	Good vegetative mass; good sporulation

CONCLUSIONS

The optimal growth and development temperature for *Fusarium* spp. isolate is between 20ºC and 28ºC, with a minimum value of 4ºC. After 38ºC, the fungus does not grow.

Continuous light exposer, followed by the variant with light/darkness (12h/12h) enhanced the best growth and development of *Fusarium* spp. strain.

The pH reaction substrate was optimal for values between 5.0 and 9.0.

The most favourable culture substrate was Potato-dextrose-agar (semi-synthetic medium), with very good mycelia development and abundant sporulation, followed by the natural substrates wheat and barley. A good development of *Fusarium* spp. was determined also by Czapex Dox and Malt 2% media.

Studies regarding fungus biological parameters play a decisional role in forecasting and warning on disease induced by fungus *Fusarium* spp.

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