Research Article

Effect of abiotic stressors on T-2-producing environmental isolates of *Fusarium sporotrichioides*

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ABSTRACT

Aim: External factors have a diverse effect on the growth and development of fungi, as well as on their toxin formation. The objective of this study was to evaluate the effect of temperature and humidity on the toxin formation of *Fusarium sporotrichioides* isolates from different climatic geographic regions of Africa and Eurasia. **Materials and Method:** The T-2 toxin was identified using RIDASCREEN T-2 ELISA test system. A total of 26 isolates were identified using mycological methods. It was established that 2 isolates from Mari El (Central Russia), 3 from Kazakhstan, and 4 from Zambia are producers of T-2 mycotoxin. **Result and Discussion:** Abiotic factors affect the growth of fungi but depending on the region of origin of the fungal isolate, and there was a variation in optimal temperature conditions. For isolates from samples obtained from Kazakhstan and Mari El, the optimum temperature was 25°C, and from Zambia, it was 30-35°C. It was established that the temperature difference may be the provoking factor of the intensification of toxin formation. **Conclusion:** During mycological monitoring studies, it is necessary to take into account the climatogeographic features of the region that affect the growth and development of microscopic fungi and the processes of toxin formation, and the stressors can have a provoking effect on the formation of mycotoxins by fungi of the genus Fusarium.

KEY WORDS: Humidity, Microscopic fungi, Temperature, Toxin formation

INTRODUCTION

Fusarium fungi can be found in any part of a plant, moreover, from 50% to 100% of cases of grain infection can be atypical, with no visible signs of damage.[1] Mycotoxins produced by different species of *Fusarium* fungi have traditionally been associated with grains grown in temperate climates, as these fungi need lower temperatures for their growth and mycotoxin production.[2,3]

One of the representatives of a large group of trichothecene mycotoxins is the T-2 toxin, which has the strongest toxic effect.[4] The main producer of T-2 toxin is *Fusarium sporotrichioides*. The most intensive accumulation of toxins is observed at high humidity and low temperature. The accumulation of T-2 toxin in natural conditions is associated mainly with late-harvest and field overwintered grain. Trichothecene mycotoxins are characterized by a significant immunodepressant effect, which is manifested by a decrease in the chemotaxis and phagocytosis of various neutrophils and macrophages.[5]

External factors have a diverse effect on the growth and development of fungi, as well as on their toxin formation.[6-8] The indication of *Fusarium* fungi and their experimental studies should be carried out taking into account the environmental factors that may affect both the phenotypic indicators[9,10] and the molecular rearrangements of the fungi and their toxins.[11]

The objective of this study was to evaluate the effect of temperature and humidity on the toxin formation of *F. sporotrichioides* isolates obtained from different climatogeographic regions.

MATERIALS AND METHODS

Primary isolation of fungi from samples of grain, soils, and stems of plants from different climatogeographic regions (Mari El, Kazakhstan, Zambia) was carried

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out by their inoculation on Czapek medium. The composition of the medium (in g) is as follows: Glucose - 15, $NaNO_3$ - 2, KH_2PO_4 - 1, $MgSO_4$ - 0.5, KCl - 0.5, agar-agar - 15, and 1 L of water. Before inoculation, Czapek's nutrient agar was melted in a water bath, cooled up to 45-50°C to suppress the accompanying bacterial flora, and added with antibiotics (penicillin - 50,000 units and streptomycin - 100,000 units per 1 L of medium). The prepared agar in liquid form was poured into sterile Petri dishes and allowed to solidify on a horizontal surface. To isolate the fungi from the grain, the seeds were placed into Petri dishes on the medium surface, 10 pieces of each, so that they do not come into contact with each other. To isolate the fungi from the soil, 1.0 g of soil was taken, placed in a flask with 100 mL of a sterile 0.1% surfactant solution (with Tween-80) in distilled water, and disintegrated the sample by shaking it on a shaker for 15-20 min. This suspension No.1 was used to prepare serial dilutions: 1:1,000, 1:10,000. To isolate the fungi from the stems of the plants, the test stems were cut with sterile scissors into pieces of about 2 cm in length. The cut stems were transferred with sterile tweezers to the surface of Czapek's agar. Up to 10 pieces of stems were spread out in the medium in Petri dishes so that they do not come in contact with each other. Petri dishes with inoculants were placed in a thermostat wrapped in sterile paper and kept at a temperature of 22-25°C for 7-10 days. Growth and sporulation were observed on day 3, and identification was performed on days 5-7.

To isolate the fungi into pure cultures, the direct transfer method was used: A small piece of mycelium or part of the colony was carefully sampled by the mycological hook and placed on the surface of the medium. The species of the fungi of the genus *Fusarium* was determined by spore cultivation. A study of the various properties of fungal species was carried out on monosporous isolates: A suspension of conidia was prepared in sterile water, and the spore concentration was counted under a microscope at low magnification and adjusted to a concentration of 10-30 spores in a drop. A drop of the suspension was applied to the surface of the agar medium with a growth stopper (2×10^{-4} Triton X-100) so that 10-20 spores were introduced into the dish. A glass spatula was used to distribute the suspension on the surface of the medium. After 3-5 days, a separately growing colony of fungus was sampled. Under a microscope, the dishes were viewed in the transmitted light from the lower side, and the sprouted, separately located conidia of the fungus were marked with a marker. The species of fungi were determined using the microscopic fungi indicator.[12]

Identification of T-2 toxin was carried out in the mycelium with the medium at the fungal isolation stage and in the rice substrate for determining the effect of temperature and humidity using RIDASCREEN T-2 ELISA test system.

RESULTS

Determination of Isolate Toxicity

The conducted mycological studies identified 26 isolates of *F. sporotrichioides*. At the same time, the isolates of *F. sporotrichioides* species were present in samples from all regions - 7 from Mari El, 8 from Kazakhstan, and 11 from Zambia. Toxicity was determined by the biotesting method using *Paramecium caudatum* to detect toxic isolates. The fungal colonies were removed from the agar surface, placed in test tubes, ground to a mushy state, poured in distilled water at a ratio of 1:1 by volume, stirred, and left at 4-8°C for 24 h. Further, two drops of extract from the fungal culture were applied to a slide with a Pasteur pipette, and one drop of medium with protozoa *P. caudatum* was added. We recorded the time of the beginning of the experiment and observed the behavior of paramecia under a microscope (lens ×10). If within 3-5 min no death of protozoa comes, the slide was placed in a Petri dish on a filter paper circle moistened with water to prevent the droplets from drying out. Evaluation of the result of the bioassay was given by the reaction of the death of paramecium during 60 min. The results of studying the toxicity of the obtained isolates are shown in Table 1.

It was found that 2 isolates from Mari El, 3 from Kazakhstan, and 4 from Zambia are toxic and produce T-2 mycotoxin. Isolates of *F. sporotrichioides* species that showed the maximum toxic reaction to the *P. caudatum* test culture and produced T-2 mycotoxin were sampled for subsequent studies (one from each region).

Influence of Temperature and Humidity on the Contamination of the Grain by Micromycetes at Artificial Contamination

The experiment was carried out using rice grain artificially contaminated with three isolates of the fungus *F. sporotrichioides*. The scheme included preparation of the inoculum (3 isolates of fungus *F. sporotrichioides* obtained by inoculating a clean culture, were incubated at 26°C for 12 days, and inoculated on a sterile grain substrate rice) and subsequent cultivation under various temperature and humidity conditions. The inoculum was a homogeneous suspension of fungal cells in 0.01% Tween-80 aqueous solution (about 20,000 diaspores/mL). Fungi were cultivated for 30 days, with subsequent inoculation on media and counting of colony-forming units (CFU/g of substrate).

The results of the study of the effect of various temperature and humidity conditions on the growth

and accumulation of the mycelial mass of the *F. sporotrichioides* isolates selected from samples from Mari El, Kazakhstan, and Zambia are shown in Table 2.

It was established that *F. sporotrichioides* isolate obtained from Kazakhstan samples showed the maximum fungal mass and sporulation at a substrate moisture content of 40% and temperature of 25°C. A similar situation was observed for the isolate from the samples of Mari El. We can also note a certain feature - the isolate from the samples of Mari El (the northernmost region of the studied) showed that, even at a temperature of 15-20°C, the growth of the mycelium is less than at 25°C but higher than in other isolates. The substrate contaminated with an isolate from the soil of Zambia shows high contamination with spores at 30-35°C.

Table 1: *F. sporotrichioides* isolates from different regions and their toxicity

Study objects	Number of *F. sporotrichioides* isolates		Toxic isolates, %
	Total, units	Toxic, units	
Mari El			
Soil, feed	7	2	28.5
Kazakhstan			
Feed	8	3	37.5
Zambia			
Soil	11	4	36.3

F. sporotrichioides: Fusarium sporotrichioides

The Influence of the Temperature Stress Factor on the Toxin Formation in Micromycetes

The scheme included: Preparation of an inoculum (3 isolates of *F. sporotrichioides*), inoculation on a sterile grain substrate (rice) with a moisture content of 35%, and stationary culture with a periodic temperature change (stress factor) for 4 weeks in three modes: I - 3 days at 28°C, 11 days at 25°C, and 14 days at 4°C; II - 3 days at 28°C, 11 days at 25°C, and 14 days at 18°C; and III - only at 25°C. The results of determining the influence of the temperature stress factor on the toxic production by micromycetes are shown in Table 3.

As a result, it was found that the maximum toxin formation was observed in the isolate from the samples of Kazakhstan, recorded in the first culture mode where the culture stage was at 4°C - 13.24 mg/kg of the rice substrate. The maximum toxin formation in the isolate from the Mari El samples was recorded in the second culture regimen, where the culture stage was at 18°C. The lowest toxicity was observed in the isolate from Zambia samples, observed at 25°C. It should be noted that the maximum toxicity in the isolate from Zambia coincides with the maximum growth of the mycelium, but in isolates, from the samples of Kazakhstan and Mari El, these factors did not coincide, and the temperature difference can be considered a provoking factor.

Table 2: Contamination of rice grain with micromycetes during artificial inoculation of fungal isolates and inoculation for 30 days under different temperature and humidity conditions (CFU/g)

Humidity, %	Temperature, °C					
	10	15	20	25	30	35
F. sporotrichioides (Kazakhstan)						
10	2.4×10^3	2.3×10^3	2.5×10^3	3.1×10^3	2.4×10^3	2.0×10^3
20	2.8×10^3	3.0×10^3	6.2×10^5	8.9×10^6	6.6×10^4	7.4×10^3
40	18.1×10^3	11.3×10^5	4.9×10^9	8.2×10^{12}	1.7×10^7	4.3×10^4
F. sporotrichioides (Zambia)						
10	2.0×10^3	2.1×10^3	2.8×10^3	3.1×10^4	1.4×10^4	2.0×10^3
20	2.1×10^3	2.4×10^3	4.1×10^6	5.0×10^7	2.6×10^9	2.1×10^8
40	3.7×10^3	8.4×10^4	19.5×10^9	3.1×10^{14}	1.1×10^{17}	5.2×10^{11}
F. sporotrichioides (Mari El)						
10	2.2×10^3	2.4×10^3	2.3×10^3	2.9×10^3	2.1×10^3	2.2×10^3
20	2.6×10^3	3.3×10^3	14.8×10^5	7.5×10^6	5.2×10^3	2.7×10^3
40	14.7×10^4	3.1×10^9	3.7×10^{10}	4.2×10^{13}	2.5×10^7	4.1×10^4

F. sporotrichioides: Fusarium sporotrichioides, CFU: Colony-forming units

Table 3: Influence of temperature stress factors on the formation of T-2 toxin by *F. sporotrichioides* fungus isolates (mg/kg)

Fungal isolate	Culture mode		
	I 3 days at 28°C, 11 days at 25°C, 14 days at 4°C	II 3 days at 28°C, 11 days at 25°C, 14 days at 18°C	III 28 days at 25°C
F. sporotrichioides (Kazakhstan)	13.24	8.82	1.44
F. sporotrichioides (Mari El)	1.23	1.76	0.34
F. sporotrichioides (Zambia)	0.13	1.74	3.27

F. sporotrichioides: Fusarium sporotrichioides

DISCUSSION

The toxic formation in fungi *F. sporotrichioides* depends not only on their strain but also on the region from which they were isolated.[3,9,11] This was confirmed by our studies - biotesting on the *P. caudatum* test culture revealed the amount of toxin-forming isolates amounting to 28.8-37.5%. Abiotic factors affect the growth of fungi.[13,14] However, depending on the region of origin of *F. sporotrichioides* fungus isolate, the optimal temperature and humidity conditions vary. The optimal humidity for all isolates studied was 40%. The optimum temperature for isolates from the samples from Kazakhstan and Mari El was 25°C, and for isolates from Zambia samples, it was 30-35°C, which is probably due to the climatogeographic features of the region. A provoking factor of intensification of toxin formation may be a temperature drop, which is confirmed by experiments with isolates from samples of Kazakhstan and Mari El. However, this was not observed in isolates from Zambia samples.

CONCLUSION

During mycological monitoring studies, it is necessary to take into account the climatogeographic features of the region that affect the growth and development of microscopic fungi and the processes of toxin formation. Stress factors and sudden temperature changes, in particular, can have a provoking effect on the formation of mycotoxins in fungi of the genus *Fusarium*.

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