Toxin-forming properties of Siberian isolates of the genus *Fusarium* fungi

N S Kozulina¹, A V Vasilenko¹, A A Vasilenko² and Zh N Shmeleva²

¹ Krasnoyarsk Scientific-Research Institute of Agriculture – separate subdivision of FRC KSC SB RAS, 66, Svobodny Avenue, Krasnoyarsk, 660041, Russian Federation
² FSBEI HE Krasnoyarsk State Agrarian University, 90, Mira Avenue, Krasnoyarsk, 660049, Russian Federation

E-mail: wasilenkoaa@ya.ru

**Abstract.** The results of laboratory studies on the toxin-forming genus *Fusarium* fungi are presented in the article. Fungal toxins are metabolites of various chemical nature, usually non-protein, often secondary, having more or less specific pathological effects on the human body, higher animals, plants and microorganisms. The toxins of the genus *Fusarium* fungi affecting cereal plants are well known. However, the ability to toxin formation varies among different populations of species in this genus. The high degree of cereal infection rate in Siberia with soil phytopathogens can be the reason for their low yield, as well as pose the danger when using cereals affected by toxin-forming fungi in the food industry and in the animal feed preparation. Therefore, the study of toxins produced by Siberian strains of the genus *Fusarium* fungi is a necessary step for certification of the seed fund in Siberia. The analysis of the obtained materials shows that the most toxic are the metabolites of *Fusarium* strains 2, 3, 4, 5, T13m, Z2-04, T2ec, J1kc, B-7.1(b), B-1.2, B-3.2(C), B-6.1, B-4.2, since less than 25% of barley seeds germinate when they were processed with these metabolites. The metabolites of j7ec, B-7.1(K) strains are not toxic to barley seeds.

1. Introduction

The genus *Fusarium* fungi produce toxic compounds that are dangerous to humans, animals and plants [1; 2]. Phytoxins, suppressing the vital activity of plant cells in small concentrations, are determinants of many plant diseases, in particular fusarioses, and contribute to soil fatigue due to the accumulation of toxic substances [3; 4; 5].

The role of toxins in pathogenesis is diverse: some may function as pathogenicity factors, others as virulence factors. Pathogenicity is understood here as the ability to cause disease. Virulence is defined as the degree of the pathogen activity. The factors responsible for pathogenicity are necessary for the occurrence of the disease. The factors responsible for virulence have properties that determine the severity of the disease, but are not themselves necessary for the disease onset.

Phytopathogenic micromycetes are characterized by specialization – the ability to parasitize a particular plant, organs and tissues. Specialization can be narrow and broad; species and varietal; phylogenetic and ontogenetic.

Thus, some *Fusarium* species consist of several forms that differ in phytotoxicity, pathogenicity and aggressiveness. This is probably due to their genetic isolation from each other. The manifestation of a high degree of toxicity is not always correlated with high pathogenicity and aggressiveness. Isolates that
cause an infectious process and are non-pathogenic were identified.

Harmful effects of root rot pathogens of the genus *Fusarium* fungi are determined not only by dangerous toxins. In some cases, a large number of unidentified substances – products of life (culture fluid or growth substrate) were more dangerous for plants and animals than purified toxins. The study of phytotoxins contributes to understanding the mechanisms of pathogenesis and is aimed at finding plant protection products that could selectively affect certain stages of these substances biosynthesis [5; 6].

The purpose of the research is to study the ability of toxins formation by Siberian strains of *Fusarium* fungi on spring barley seeds.

2. Research methodology
Phytopathological and microscopic methods were used during the research [7; 8; 9; 10].

In order to determine the phytopoxticity fungi were grown on a liquid medium of the following composition (g·l⁻¹): sucrose – 40; glycerine – 10; (NH₄)₂H₂PO₄ -1; K₂HPO₄ -3; MgSO₄ -2; NaCl - 5.

The results were processed by calculating primary statistical indicators for qualitative characteristics [11; 12; 13], using the computer software Stat Soft 6.0.

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3. Research results
The study of phytotoxicity of fourteen isolates of the genus *Fusarium* fungi showed that the toxic effect on the seeds of spring barley of the “Acha” variety was almost identical. Table 1 shows the degree of phytotoxicity of Siberian isolates of the genus *Fusarium* fungi in relation to barley seeds.

**Table 1.** Toxicity of metabolites of Siberian isolates of the genus *Fusarium* fungi on spring barley seeds of the “Acha” variety.

| Sort                     | Isolate | Laboratory germination, % | Infection rate, % | Toxicity degree |
|--------------------------|---------|----------------------------|-------------------|----------------|
| *Fusarium sporotrichioides* | Z2.04   | Xₐverage = 0.2, d = 0.9    | Xₐverage = 5.6, d = 7.8 | Highly toxic |
| *Fusarium chlamydosporum* | J7cc    | Xₐverage = 2.6, d = 6.0    | Xₐverage = 14.6, d = 8.3 | Highly toxic |
| J1kc                     | B-1.2   | Xₐverage = 0.5, d = 2.7    | Xₐverage = 13.9, d = 9.1 | Highly toxic |
| *Fusarium oxysporum*     | J1kc    | Xₐverage = 0.2, d = 1.3    | Xₐverage = 5.3, d = 8.1 | Highly toxic |
| B-7.1(b)                 | 1.1     | Xₐverage = 2.2, d = 6.9    | Xₐverage = 8.8, d = 8.8 | Highly toxic |
| *Fusarium heterosporum*  | B-6.1   | Xₐverage = 0.2, d = 0.9    | Xₐverage = 2.25, d = 5.6 | Highly toxic |
| 4                        | 0.5     | Xₐverage = 2.2, d = 4.0    | Xₐverage = 6.4, d = 8.1 | Highly toxic |
| 5                        | 0       | Xₐverage = 0, d = 2.1      | Xₐverage = 5.1, d = 8.6 | Medium toxic |
| *Fusarium sambucinum*    | B-7.1(k)| Xₐverage = 4.5, d = 7.6    | Xₐverage = 17.3, d = 8.6 | Highly toxic |
| *Fusarium semitectum*    | B-3.2(c)| Xₐverage = 0.4, d = 1.4    | Xₐverage = 12.2, d = 9.4 | Highly toxic |
| *Fusarium moniliforme*   | T13m    | Xₐverage = 0.75, d = 3.4   | Xₐverage = 12.4, d = 9.9 | Highly toxic |
| T2ec                     | 1.25    | Xₐverage = 3.5, d = 6.8    | Xₐverage = 8.8, d = 8.8 | Highly toxic |
| Control: sterile water   | 9.25    | Xₐverage = 9.6, d = 1.25   | Xₐverage = 9.9, d = 9.9 | Non toxic   |

The toxicity degree was calculated in relation to sterile water, if less than 25% of the seeds germinated after being treated with fungi toxins, then the isolate metabolites were toxic.

It was found that the degree of the culture fluid phytopoxticity of the isolates varied: the average degree of toxicity is characteristic for isolate *F. sambucinum* Bb-7.1(K) – laboratory seed germination.
is 4.5% of the control; a high degree is noted for all other studied isolates – laboratory seed germination is in the range from 0% to 2.6% of the control. The highest degree of phytotoxicity was observed in the isolate 5 F. sambucinum – 0% of germinating seeds (figure 1).

Figure 1. Sterile water (control) and the effect of metabolites of 5 F. sambucinum isolate on the germination of spring barley seeds, respectively.

Sorts of F. sporotrichioides, F. moniliforme, F. heterosporum, F. oxysporum, F. chlamydosporum, F. sambucinum and F. semitectum are a group of highly toxic species that inhibit the germination of spring barley seeds of the “Acha” variety, and it should be considered that the population of these species in Siberia is phytotoxic. No non-toxic isolates were found among the studied ones, which indicates the high competitiveness and adaptability in comparison with other species.

The lowest infection rate of seeds by fusariosis was 1.25% when treated with sterile water. The highest infection of seeds by fusariosis was when treated with metabolites of isolates 2, 3 F. sporotrichioides, B-1.2, J7cc F. chlamydosporum, B-7.1(K) F. sambucinum, T13m F. moniliforme and B-3.2(c) F. semitectum, the average infection rate of seeds was higher than 12.2%. It is also observed that isolates 4 and 5 of F. sambucinum and isolate B-6.1 of F. heterosporum are highly phytotoxic for barley seeds and have low aggressiveness (they cause an infectious process on average from 2.1% to 4%).

As a result of statistical processing by one-factor dispersion analysis, it was found that the metabolites of isolates of the genus Fusarium fungi have a significant effect on the germination of spring barley seeds. In addition, metabolites of Fusarium fungi isolates have a significant effect on the infection rate of spring barley seeds. The results are reliable, since F is greater than F critical, the differences are proven at a confidence level of 0.99.

The estimation of dispersion homogeneity for isolates 3 F. sporotrichioides, J7cc F. chlamydosporum, B-7.1(b) F. heterosporum, B-7.1 (K) F. sambucinum and T2cc F. moniliforme showed that the level of difference between the variances is minimal and equal, and the hypothesis of equality of variances can be accepted. The variation in germination is 10.4%, 11.1%, 11.1%, 18.2% and 28.6%, respectively (confidence levels are 0.89, 0.89, 0.89, 0.82, and 0.71, respectively). And the estimation of uniformity of dispersions for all other Siberian isolates of the genus Fusarium fungi showed that the variation on the basis of germination is from 0% to 1.1%. Thus, it is possible to accept the hypothesis of difference of dispersions (the confidence level is from 0.98 to 1.0).

The estimation of homogeneity of dispersions for isolates 3 F. sporotrichioides and B-1.2 F. chlamydosporum showed that the variation in infection rate is 2.8% and 3.3%, respectively, and it is
possible to accept the hypothesis of variance differences (at the confidence level of 0.97 and 0.96, respectively). And the estimation of uniformity of dispersions for all other Siberian isolates of the genus *Fusarium* fungi showed that the variation on the basis of infection rate is from 6.9% to 37.9% and it is possible to accept the hypothesis of equality of dispersions (at the confidence level from 0.63 to 0.94).

The calculation of the Student’s criterion for evaluating the reliability of results on the effect of metabolites of *Fusarium* fungi on the germination of spring barley seeds of the “Acha” variety showed that the differences between the control (water) and the metabolites of isolates are reliable in all variants (at the confidence level of 0.99) (table 2).

**Table 2.** The value of the Student’s criterion ($t_o$) when evaluating the effect of metabolites on the germination of spring barley seeds.

| Sort                  | Isolate   | $S_x$ | $t$-statistics | t critical double-type | $P(T<=t)$ double-type |
|-----------------------|-----------|-------|----------------|------------------------|-----------------------|
| *Fusarium sporotrichioides* | 2         | 1.24  | 5.95*          | 0.01                   |
|                       | 3         | 2.90  | 7.22           | 4.39*10^-6             |
|                       | Z2-04     | 0.33  | 6.54           | 0.01                   |
| *Fusarium chlamydosporum* | J7cc     | 2.99  | 5.79           | 4.68*10^-5             |
|                       | B-1.2     | 0.45  | 6.29           | 0.01                   |
| *Fusarium oxysporum* | J1kc      | 0.15  | 6.57           | 0.01                   |
|                       | F. heterosporum | B-7.1(b) | 2.99 | 7.09 | 5.39*10^-6 |
|                       | F. moniliforme | T13m | 0.56  | 6.09 | 0.01 |
|                       | T2ec      | 5.29  | 5.76           | 4.93*10^-5             |

Note: * - value ($t_o$) at the confidence level of 0.05 is 2.2

The calculation of the Student’s criterion for evaluating the reliability of the results on the effect of *Fusarium* fungi metabolites on the infection rate of spring barley seeds of “Acha” variety showed that the differences between the control (water) and the metabolites of 2 and 3 isolates of *F. sporotrichioides*, J7cc and B-1.2 *F. chlamydosporum*, *F. oxysporum* J1kc, B-7.1(b), *F. heterosporum*, B-7.1(K), *F. sambucinum*, B-3.2(C), *F. semitectum*, T13m and T2ec *F. moniliforme* are reliable (at the confidence level of 0.99). The differences between the control (water) and the metabolites of the isolates Z2-04 *F. sporotrichioides*, B-6.1 *F. heterosporum*, 4 and 5 *F. sambucinum* are not reliable (at the confidence level of 0.95, 0.78, 0.69 and 0.44, respectively) (table 3).

**Table 3.** The value of the Student’s criterion ($t_o$) when evaluating the effect of metabolites on the germination of spring barley seeds.

| Sort                  | Isolate   | $S_x$ | $t$-statistics | t critical double-type | $P(T<=t)$ double-type |
|-----------------------|-----------|-------|----------------|------------------------|-----------------------|
| *Fusarium sporotrichioides* | 2         | 23.78 | 6.51*          | 1.38*10^-5             |
|                       | 3         | 47.17 | 7.43           | 3.18*10^-6             |
|                       | Z2-04     | 14.81 | 2.13           | 0.05                   |
| *Fusarium chlamydosporum* | J7cc     | 8.81  | 8.32           | 8.56*10^-7             |
|                       | B-1.2     | 42.08 | 6.03           | 3.06*10^-5             |
| *Fusarium oxysporum* | J1kc      | 6.24  | 2.96           | 0.01                   |
|                       | B-7.1(b)  | 3.17  | 5.43           | 2.14                   | 8.8*10^-5             |
The degree of internal infection rate of spring barley seeds increases under the influence of metabolites of the genus *Fusarium* fungi. Thus, for further research, strains showing a high and medium degree of phytotoxicity against spring barley seeds were selected. It was found that the metabolites of isolates of the genus *Fusarium* fungi have a significant effect on the germination of spring barley seeds. The analysis of the obtained materials shows that the most toxic are the metabolites of *Fusarium* strains 2, 3, 4, 5, T13m, Z2-04, T2ec, J1kc, B-7.1(b), B-1.2, B-3.2(C), B-6.1, B-4.2, since less than 25% of the seeds germinated when they were processed with these metabolites. The metabolites of j7ec, B-7.1(K) strains are not toxic to barley seeds.

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