SUMMARY

of the doctoral thesis entitled:

RESEARCH ON WHEAT CONTAMINATION WITH SPECIES OF *FUSARIUM* UNDER THE CONDITIONS OF CLIMATE CHANGES

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Wheat is the most widespread crop worldwide and it represents the major raw material for food industry; it is equally of an essential interest for the agricultural sector. Fusariosis is a disease that affects cereals when temperature and humidity are high, therefore specialist research and the identification of methods to combat it are required in the context of increasingly unfavorable climate changes.

The doctoral thesis entitled "**RESEARCH ON WHEAT CONTAMINATION WITH SPECIES OF *FUSARIUM* UNDER THE CONDITIONS OF CLIMATE CHANGES**" comprises two parts totaling 5 chapters. The first part consists of the current state of research on *Fusarium* contamination of wheat crops, and the second part includes our own research.

CHAPTER I addresses issues from the specialized literature and represents Part I of the thesis. It is entitled "CURRENT STAGE OF KNOWLEDGE CONCERNING WHEAT FUSARIOSIS", and in the pages of this chapter, the elements of pathology, ecology and management of *Fusarium* species are presented, joined by suggestive images. Also, in the same chapter, information is added regarding the mycotoxins associated with *Fusarium* species.

Part II of the thesis details in 4 chapters the framework in which the *in-vivo* research has been conducted concerning the contamination of wheat with species of *Fusarium* (CHAPTER II), as well as the *in-vitro* research on the contamination of wheat caryopsis with species of *Fusarium* (CHAPTER III), followed by research on the selection of low doses of fungicides to combat *Fusarium* fungi (CHAPTER IV) and the use of microbial strains to inhibit the development of phytopathogenic fungi (CHAPTER V).

In CHAPTER II of the thesis, entitled "*IN-VIVO* RESEARCH ON WHEAT CONTAMINATION WITH SPECIES OF *FUSARIUM*", the two locations where the research took place are presented: Unirea commune, Brăila county, and Topolovaţu Mare commune, Timiş county, and the climatic conditions specific to the research period, 2017-2020, in which 4 varieties of the *Triticum aestivum species* (common autumn wheat) are analyzed. The experience followed two factors, in three repetitions, and it was organized in the two different locations from the pedoclimatic point of view. Among the four varieties of wheat that have been studied, the most sensitive to fusariosis attack was the Miranda variety, and the most resistant was the Glosa variety, followed by the Apache and PG101 varieties. When treated with fungicides, the best results in limiting the attack were observed in the Glosa variety, followed by the Apache and PG101 varieties. The average productions obtained in the two experimental fields were between 4854 kg/ha (the Miranda variety, untreated) and 7187 kg/ha (the Glosa variety, treated with the usual dose), in Unirea - Brăila county, and between 4602 kg/ha (the Miranda variety, untreated) and 7513 kg/ha (the Apache variety, treated with the usual dose), in Topolovăţu Mare - Timiş county.

The correlations between the doses of fungicides applied and the productions achieved in the varieties studied in the two experimental fields were highly negative (the lower the dose, the higher the number of spikes attacked by fusariosis).

CHAPTER III, entitled "*IN-VITRO* RESEARCH ON THE CONTAMINATION OF WHEAT CARYOPSES WITH SPECIES OF *FUSARIUM*", presents the material and methods by which the potential fungal contaminants were detected and isolated from and on the wheat caryopses harvested from the two climatic areas between 2017-2020, of the previously mentioned varieties. Two semi-selective culture media were used for *Fusarium* species: PPA medium and MGA medium, both supplemented with antibiotics. It was found that the morphology of the colonies varied both from one isolate to another, in the case of cultivation on the same substrate, and depending on the nutrient substrate, in the case of the same strain cultivated on different nutrient media. Thus, MGA medium proved to be more effective for the isolation of *Fusarium* sp. strains, with fewer contaminants than PPA. To identify the contaminants isolated from the wheat caryopses, both classic microbiological methods (highlighting some microscopic characters of the fungal isolates) and methods based on a series of biochemical characteristics (BIOLOG system) or molecular methods (PCR with species-specific primers) were used.

This chapter resulted in the detection, isolation and biochemical and molecular identification of 9 strains belonging to this genus. The isolated strains were characterized in terms of morphology, growth rate and pigmentation. For the 9 isolates obtained from the plant material, the results were different: among the analyzed strains, only two were classified as *F. graminearum* species (strains E25A F1-10/ Sp2 and E28A 2-1/ Sp7+Sp8 P2 B), and two as *F. culmorum* (strains marked E25A F1-10/ Sp3 and E28A F2-1/ Sp7+Sp8 P2 A).

CHAPTER IV, entitled "RESEARCH ON THE SELECTION OF REDUCED DOSES OF FUNGICIDES TO COMBAT *FUSARIUM* FUNGI" was carried out as a result of the current European context, in which there is a demand to reduce the amount of pesticides used in agriculture. *In vitro* research pursued the efficacy of three commercial fungicides applied at low doses in inhibiting the mycelial growth of six strains belonging to the *F. culmorum* and *F. graminearum* species. Thus, the *F. culmorum* strains tested proved sensitive in the presence of reduced doses in the case of the products CF2-F (triadimenol + spiroxamine + tebuconazole) and CF3-FP (prothioconazole, spiroxamine and tebuconazole), compared to the product CF1-NP (trifloxystrobin and prothioconazole). Results obtained during the experiments, after 5, 7 and 10 days of incubation, showed that the *Fusarium graminearum* Fg96 strain was the most tolerant to all the pesticides used. The strain Fg96 showed mycelial growth in all variants treated with pesticide, both at the low dose and at the recommended application rate, not being inhibited in the presence of the three tested products, regardless of the dose used.

The last chapter, CHAPTER V, entitled "*IN-VITRO* RESEARCH ON THE USE OF MICROBIAL STRAINS TO INHIBIT THE DEVELOPMENT OF SOME PATHOGENIC FUNGI", highlights the possibility of reducing mycotoxin contamination of wheat crops with biological plant protection products.

The conducted experiments pursued the highlighting of the antifungal activity against *Fusarium sp.* of some microbial strains, the highlighting of the biostimulation effect of the development of wheat plants by the beneficial microbial strains, but also the isolation and characterization of new bacterial strains from the genus *Streptomyces* with anti-*Fusarium* properties. Six bacterial strains were used, resulting mainly from the collection of the Faculty of Biotechnology within USAMV Bucharest, as well as a strain of *Trichoderma pseudokonigii*. To these were added four strains belonging to the *Streptomyces* genus, isolated from the soil during the research.

The efficacy of two strains of *B. amyloliquefaciens* and of the *T. pseudokonigii Td85* strain in inhibiting the mycelial growth of *F. graminearum 183* isolate was achieved. This potential was not seen in the case of *Bacillus endophyticus 1T2* strain. The action mechanism of these strains was highlighted at the level of mycelial hyphae of the test pathogen, which showed specific changes. In the case of the *Trichoderma pseudokonigii Td85* isolate, the microscopic examination also certified the mechanism of mycoparasitism. All tested *B. amyloliquefaciens* strains were also effective in inhibiting the mycelial growth of strains of *Fusarium sp.* isolated during the research, with efficacies between 61.9% and 84.4%, the highest efficacy being recorded by the BPA strain.

In order to test the potential antagonist, 60 new bacterial strains from the *Streptomyces* genus were isolated from soil and compost, over 78% having inhibitory action on some pathogenic isolates: *Alternaria* spp., *F. graminearum, F.culmorum, R. solani, F.oxysporum* and *S. bataticola*. Four isolates (SS16, S15, Str S1 and Str S4) were characterized as effective against all test pathogens, highlighting the action mechanism: hyphal lysis (due to the action of a complex of lytic enzymes) and mycoparasitism. The four strains of *Streptomyces sp.* had the ability to produce amylases, proteases, CMC-ases, chitinases and lipases and to solubilize the phosphate.

As regards the germination process, it was noted that most microorganism treatments applied to wheat seeds caused a delay in germination compared to the untreated control. Exceptions were the *A. brassilense Sp7* and *B. amyloliquefaciens* BW strains that accelerated the germination process by one day. All applied microorganisms stimulated shoot and root growth in length, biomass amount, both fresh weight and dry weight. Both indices of seed vigor were improved upon application of microbial treatments. The best results from a practical point of view were achieved with *A. brassilense Sp7* and *B. amyloliquefaciens* BW strains, which determined an increase in seed vigor of over 50%; this represents an increase in productivity and the quality of the achieved productions.

As regards novelty aspects, in the framework of the thesis, a series of strains of filamentous fungi from the *Fusarium* genus were identified by microbiological and molecular methods, isolated from the plant material, similar to the *F. crookwellense, F. chlamydosporium* or *F. tricinctum* isolated from the plant material, similar to the species F. crookwellense, F. chlamydosporium or F. tricinctum, were identified, along with *F.graminearum* and *F.culmorum*, which suggests a change in the types of *Fusarium* species that usually contaminate wheat in Romania, possibly due to the climatic conditions of recent years. Also, following the experiments, four bacterial strains from the *Streptomyces sp* genus were selected. With pronounced antifungal activity, two of which, namely strains SS16 and Str S4, are able not only to inhibit the development of target fungi, but also cause a reduction in the level of ZEA produced by mycotoxigenic fungi. In order to prevent the contamination of wheat crops with mycotoxins, in addition to the genetic improvement of wheat varieties in terms of their resistance to the attack of pathogenic agents, a series of other integrated measures are recommended, which include agricultural technologies through the technological links of culture, the application of fungicides, molecular control and biological control