Research on the identification and incidence of *Alternaria alternata* attack on the tomato crop

SUMMARY OF THE DOCTORAL THESIS

PhD student **Antonia Luminița FLOREA**

Scientific coordinator **Prof. univ. dr. Vasile Constantin FLORIAN**

1. INTRODUCTION

Tomatoes are some of the most appreciated and cultivated vegetables. The main components that determine the quality of tomato fruits are: the content in sugars, protides and other essential amino acids. The main therapeutic properties are: detoxification, inhibition of cancer, reduction of blood pressure and prevents hypertension and infarction (INDREA et al., 2012)

The environment itself influences crop plants that can be beneficial to normal plant development or induce various sensitivities of the cultivated plant species. Between the environment and the plant there is a complex and permanent interdependence relationship.

Among the biotic factors that influence the production and quality of tomatoes the most known are the phytopathogens, which produce various symptoms on all organs of the plant. Early blight is produced by the species of the genus *Alternaria* (INDREA et al., 2012; GANNIBAL, 2015; LAWRENCE et al., 2016), it is one of the most well-known diseases generically named alternariosis (ASLAM et al., 2020; RABIEI et al., 2022). In addition to the species of *Alternaria*, there is a wide range of pathogens that can cause significant damage in tomato culture (QIN et al., 2022). Among them we mention: Tomato ringspot virus, Cucumber mosaic virus, *Xanthomonas campestris* pv. *vitiscarnosae* - Bacterial staining of the tomatoes, *Clavibacter michiganensis* subsp. *Michiganensis* - Bacterial withering of tomatoes, *Pythium debaryanum*, *Rhizoctonia solani*, *Phytophthora nicotianae* var. *parasitica* - Falling and rotting of the seedlings, *Phytophthora infestans* - late blight, *Leveillula taurica* - Tomato flouring, *Boeremia lycopersici* - Rotting of tomato stems, *Septoria lycopersici*- Tomato septoria leaf spot, *Botrytis cinerea* - Gray rot of tomatoes, *Verticillium dahliae* - Verticillium wilt, *Fulvia fulva*- Brown staining of tomato leaves, *Neocosmospora solani, Fusarium oxysporum* - Fusarium withering (BOBEȘ, 1983; OROIAN et al., 2006; MEENA et al., 2022).

2. STRUCTURE OF THE DOCTORAL THESIS

Doctoral thesis: Research on the identification and incidence of *Alternaria alternata* attack on tomato culture, comprises 141 pages having a number of 8 chapters, 93 figures and 39 tables. The work is structured in two parts. The first part includes bibliographic documentation on the issues discussed. It consists of two chapters and comprises 44 pages (31,2% of the total work) and 39 figures. The second part is the personal contribution that extends over 97 pages, includes 6 chapters and contains the research from the experimental years 2019-2021. The bibliography includes both local and foreign scientific papers, it lists 240 sources, of which 201 citations from publications and 39 web citations.

Current state of knowledge:

Chapter 1. The history and actuality of the theme - this chapter includes the presentation of the history of the genus *Alternaria* and the current classification of species within this genus.

Chapter 2. Characteristics of the genus *Alternaria*-this chapter refers to the description of the main morphological characters of the species of the genus *Alternaria* and the main species attacking the tomato culture.

Personal contribution:

Chapter 3 The objectives pursued -relate the main objectives proposed for scientific research

Chapter 4 The peculiarities of the experimental environment - in this chapter we find described the climatic conditions in the experimental areas

Chapter 5 Material and method - is found experimental protocol, sampling mode and statistical-mathematical methods used

Chapter 6 Results and discussions - this section contains the results obtained in the experimental years at tomato culture

Chapter 7 Conclusions and recommendations- in this chapter are the main conclusions and recommendations based on the objectives pursued**.**

Chapter 8 Originality and innovative contributions of the thesis- are the innovative ideas of personal contribution in the case of the debated theme.

3. THE PURPOSE AND THE OBJECTIVES OF RESEARCH

The main purpose of this work was to identify tomato cultivation resistant to the attack of alternariosis

In order to achieve the goal, the following main objectives have been set:

Morphological characterization of *Alternaria* spores

The behaviour of various tomato cultivar to artificial infections with *Alternaria alternata* in protected areas;

The behaviour of the various tomato cultivars to natural and artificial infections with *Alternaria alternata* in the open field;

The determination of tomato production obtained in the three experimental years

4. MATERIALS AND METHODS

The biological material used was fruits, leaves and stems infected with *Alternaria* sp. from tomatoes purchased from the markets, from the University Botanical Garden and other sources. Five varieties of tomatoes were subjected to the experiment: Perun, San Marzano, Marmande, Romus, Bajaja.

The experiment was conducted:

1. Phytopathology laboratory within USAMV Cluj-Napoca, pathogen determinations were made according to the morphological characteristics of the spores in order to identify the species of *Alternaria* from the biological material taken, it was carried out the isolation of the pathogen on the culture media, the preparation of the inoculum to perform infections in the experimental field and other determinations of the fruit

2. In the phytotron, the necessary seedlings were produced for both experiments inside the greenhouse and for field experiences, artificial infections were carried out on tomato varieties.

3. The experimental stage in the field was carried out in Cluj and Turda, Perun, San Marzano, Marmande, Romus, Bajaja varieties were subjected to natural and artificial infections, inoculum was applied when tomatoes reached phenophase 6-7. The number of spores applied to the plant was approximately 3000.

The main observations and determinations in the field and phytotron were: attack frequency (F%), the intensity of the attack (I%), the degree of attack (G.A.%)

5. RESULTS AND DISCUSSION

Results on the characteristics of *Alternaria* spores

Following the measurements and observations made at *Alternaria* spores, in the four experimental years, according to the data obtained and the literature, two species of *Alternaria* genus have been identified (*A. alternata* and *A. solani*). Identification of *Alternaria alternata* species was carried out by following several criteria: length, width and diameter of conidia, but also by the number of septa.

In the experimental years, the species *Alternaria alternata* was present, with the following morphological characteristics: 20-60µ length, width 8-12µ, and, between 3-11 transverse septa and 1-2 longitudinal septa and the conidia had the dark brown colour. *Alternaria* conidia are formed on chained conidiophores.

The limits of the length and width of the *Alternaria alternata* conidia were in accordance with the values offered by the scientific literature. All of the measurements made in 2019 including the median and the average, fall within the above-mentioned limits.

Results regarding the behaviour of various tomato cultivars to artificial infections with *Alternaria alternata* in protected areas

Following the observations made in the phytotron the most resistant varieties were Bajaja and Perun, with the lowest degree of attack (16.06% and 18.20%), tomato varieties with large fruits (Marmande and Romus) showed higher sensitivity to artificial infections with *Alternaria alternata* (31.67% and 43.36%) with very significant positive differences from the witness. As for the evolution of the disease, the average degree of attack of alternariosis increases with the advance in vegetation of plants and the number of observations. Compared to the attack degree recorded at the first observation, the attack rates were higher in the other two observations (27.42% and 48.33%), the

highest value of the degree of attack of alternariosis was recorded at the last observation (48.33%), and the lowest degree of attack was observed at all varieties at the first observation.

At this time, the Perun variety has the lowest degree of attack (2.53%). Compared to the first observation at the next two readings, the degree of attack increases in all varieties, based on of the evolution in vegetation and the conditions of artificial infection.

A clearer picture of the sensitivity of the varieties shows us the classification of the Duncan Test. From this it can be seen that the most sensitive variety was Romus, with the highest degree of attack at 90 days after infection (72.92%), with the highest degree of attack, followed by Marmande and San Marzano (55.00% and 52.92% respectively). In the conditions of the artificial infection in phytotron, the Bajaja variety has the best behaviour, with the lowest degree of attack, in the last interval of infection (26.25%).

Results regarding the behaviour of various tomato cultivars to natural and artificial infections with *Alternaria alternata* in Cluj (2020)

In 2020, in the experimental field of the Faculty of Agriculture, placed in the Agrobotanical Garden was established an experience in which five tomato cultivars were tested, under conditions of natural and artificial infection with *Alternaria alternata*, in order to prepare the biological material and the inoculum for the future research. From the interpretation of the data, obtained in 2020, on the degree of attack we can see that there are differences between the varieties taken in the study especially to the type of infection. Thus, an increase in the degree of attack was in the case of hight pressure of infection with 14.4 % compared to natural infection. As expected, the artificial infection leads to an increase of the degree of attack on all cultivars.

Results on the behaviour of various tomato cultivars in natural and artificial infections with *Alternaria alternata* in Turda (2021-2022)

In the case of the frequency of the attack in 2021, the values were higher in conditions of artificial infection compared to natural infection, at all varieties taken in the study at the first observations. Reported at the influence of climatic conditions we note that the year 2021 was favourable to the attack of *Alternaria alternata*. Against the background of climatic conditions in 2021, under natural conditions of infection the variety with the lowest frequency of the attack was Marmande (60%), and in 2022 was the Perun variety with 43.3 % in conditions of natural infection and with the lowest frequency (60%) in conditions of artificial infection. At the second observation at the vast majority of varieties and in both experimental years the frequency of attack reached 100%. Thus, under natural infection conditions *Alternaria alternata* can cause significant damage to tomato crops, compromising practically the plants in their entirety 60-90 days after the first infections.

Artificial infection has caused damage by high attack frequency values since the first reading. It can be observed that by increasing the pressure of infection the frequency of attack increases by 20%.

Regarding the intensity of the attack of *Alternaria alternata* under artificial infection conditions throughout the experimental period they had higher percentage values than in the natural infection case. During the vegetation period the situation changes, so the intensity of the attack on all varieties increases in both conditions of infection, especially in conditions of artificial infection, thus, in 2021, the varieties with the highest attack intensity values of up to 36.80% are San Marzano and Romus, respectively, at the opposite end in 2022 the same varieties have the lowest percentage being between 14.17 % and 15.83%. An important observation is that the intensity of the attack, at all varieties, is significantly influenced by climatic conditions. The recorded values, there is a significantly higher level of intensity in 2021 than in 2022. When we comparing the last and first observation, significant differences are found both between varieties and in response to the method of infection. Overall, the first observation showed an increase in intensity of 3-15% in the second observation (natural infection) and 5-18% in the case of artificial infection.

The degree of attack gives us an overview of the dynamics of the presence of the pathogen, since it is calculated based on the values of the frequency and intensity of the attack, expressed in percentage. This index reinforces the fact that climatic conditions have a very significant impact on the dispersion of the pathogen on the cultivated surface in both types of infection. The data recorded shows that the year 2021 was more favourable to the *Alternaria alternata* infection, compared to 2022, applying the same culture technology.

Tomato production results obtained in Cluj in the experimental field (2020)

- **Results on the number of fruits per plant**

The number of fruits per plant is an important indicator in determining the yields. In the climatic conditions of 2020, the average number of fruits per plant was between 3.42 and 7.95, with the most fruit in the Bajaja variety. The type of infection significantly influenced the production of fruit on the plant, under conditions of artificial infection their number was lower in all varieties taken in the study.

- **Results on fruit weight**

Next to the number of fruits formed on a plant, their weight helps us in estimating tomato yields per plot and subsequently per hectare. The average fruit weight of the varieties under study ranged from 35.18g to 64.48g in large-fruited varieties and between 6.18g - 8.89g in cherry tomato varieties. The type of infection influenced the weight of the formed fruits, under conditions of artificial infection, the weight of the fruit decreased (28.91g) compared to the average weight of the fruits coming from naturally infected plants (32.63g), but the differences were not statistically assured.

- **Results on production**

Under the experimental field conditions in Cluj, the production per hectare ranged from 6.9 to 7.7 t/ha for cherry-type tomato varieties and between 15.6 -36.6 t/ha to large-fruited tomato varieties, the largest production is registered to the Marmande

variety. Under conditions of artificial infection, the production is significantly lower and the average production losses are approaching 10 tons.

Tomato production results from the Turda experiences (2021-2022)

- **Results on the number of fruits per plant**

In the climatic conditions of 2021-2022 the average number of fruits per plant was between 5.94 and 60.13 with the most fruit was recorded at the Bajaja variety and the smallest at the Marmande variety. The type of infection significantly influenced the production of fruit on the plant, so in artificial infection the average number of fruits was lower (14.90) and with very significant negative difference from the witness (23.97) (natural infection). In the triple interaction between experimental factors, it is noted that the number of fruits formed varied depending on the variety studied, the type of infection, the climatic conditions of the experimental years.

- **Results on fruit weight**

The average weight of the fruits formed on the plants, under field conditions, at the Marmande variety had fruits with an average weight of 104.2 g and for cherry tomato varieties had the lowest average weight of the fruit. Based on the climatic conditions of the two experimental years and under different conditions of infection, the average weight of the fruit had different values. According to data in 2021 the average weight was less than 50 g, with a higher value under conditions of artificial infection (49,28). In 2022 the weight of the fruit was high, over 50g, but at high infection pressure the average weight was lower (53.79). It can be stated that in the conditions of a year less favourable the manifestation of the pathogen on the weight of the fruit is higher. Correlating with the low number of fruits it can be found that the weight of the fruit is not significantly influenced by the pressure of infection.

- **Results on production**

As regards tomato production, we can see that the variety Marmande and Bajaja had the highest yields per hectare 78.7t and 77.3t, and the smallest production is Perun (26.01 t/ha). Climate conditions influenced production, compared to the average of experience in 2022 tomatoes had more favourable conditions, the output obtained was higher than the previous year (67.1t/ha). The type of infection negatively influenced the production of tomatoes. From the data obtained it is noted that under conditions of high infection pressure tomato production was lower by 25 t/ha compared to natural infection.

6. CONCLUSIONS AND RECOMMENDATIONS

- **Conclusions on the characteristics of *Alternaria* spores**

According to the observations and measurements made, the species *Alternaria alternata* was present, showing small brown-cafe conidia forming chained on conidiophores and *Alternaria solani* has also been identified

- **Conclusions on the behaviour of various tomato cultivars to artificial infections with *Alternaria alternata* in protected areas**

Varieties taken in the study behaved differently at the attack of alternariosis, the most resistant varieties were Bajaja and Perun, with the lowest degree of attack (16.06% and 18.20%). In all varieties studied the degree of attack of *Alternaria alternata* was the lowest at the time of the first observation. The most sensitive variety was Romus, with the highest degree of attack at the 90-day interval from the time of infection (72.92%), followed by the Marmande (55.00%). At the last observation under conditions of artificial infection in the phytotron, the Bajaja variety behaved best, with the lowest value of the degree of attack (26.25%).

- **Conclusions on the behaviour of various tomato cultivars to natural and artificial infections with *Alternaria alternata* in Cluj (2020)**

In the experimental year, large-fruited varieties did not show significant differences in terms of the degree of attack however, compared to the average experience, the Romus variety showed the lowest degree of attack (8.87%) compared to the varieties studied. The type of infection influences the degree of attack of a pathogen, under conditions of high pressure of infection reaches a value up to 17%, with a very significant positive difference from the naturally infected variant (4.76%). With the advance in vegetation the degree of attack on large-fruited tomato varieties increases, clearly observing the differences between the varieties. The high pressure of infection leads to an increase of the degree of attack in all varieties.

- **Conclusions on the behaviour of various tomato cultivars to natural and artificial infections with *Alternaria alternata* in Turda (2021-2022)**

In 2021the attack frequency values were higher in conditions of artificial infection compared to natural infection. In all varieties studied at the time of the first observations, the climatic conditions of 2021 favoured the attack of *Alternaria alternata*, the frequency of the attack values were higher this year in most varieties, the in both conditions of infection. The intensity of the attack of *Alternaria alternata*, under conditions of artificial infection, during the entire experimental period had higher percentage values than the natural infection.

- **Conclusions on tomato production obtained in the experiences in Cluj (2020)**

In the climatic conditions of 2020, the average number of fruits per plant was between 3.42 and 7.95, with the most fruit at the Bajaja variety. The type of infection significantly influenced the production of fruit on the plant, in conditions of artificial infection their production was lower. The average fruit weight of the varieties under study ranged from 35.18 g to 64.48 g in large fruit varieties and between 6.18g - 8.89g in cherry tomato varieties. Under the experimental field conditions in Cluj, the production per hectare ranged from 6.9 to 7.7 t/ha for cherry tomato varieties and 15.6 -36.6 t/ha to large fruits tomatoes.

- **Conclusions on tomato production from Turda experiences (2021-2022)**

In the climatic conditions of 2021-2022 the average number of fruits per plant was between 5.94 and 60.13. The type of infection significantly influenced the production of fruit on the plant, so in artificial infection the average number of fruits was lower (14.90) compared to the fruits formed on the plant, under conditions of natural infection (23,97). As for the weight of the fruits on the plants the Marmande variety had the highest weight, in the both experimental years and in the two conditions of experimentation. The climatic conditions influenced the production, in 2022 the production obtained was higher than the previous year (67,1t/ha). Based on the climatic conditions of the two experimental years under different conditions of infection the production varied. In 2021 and 2022 there were very significant negative differences in production for artificial infections related to those from natural infections with 20.2 t/ha and 30 t/ha respectively in 2022.

- **Recommendations**

Following the study carried out in the doctoral thesis, we recommend the cultivation, both in the field and in protected space the varieties that are resistant to *Alternaria* attack, but also varieties adapted to the climatic conditions of the specific area. Although the varieties were created with some resistance to diseases, the varieties should be retested under current conditions, because the populations of the pathogens can form new physiological races causing significant damage in tomato crops. We also recommend continuing the research related to alternariosis on the tomatoes, since several species of the same genus may be involved in the occurrence of the same disease. According to the data presented in the study, we can recommend the cultivation in the Cluj area of the Marmande variety as the most productive and if you want to grow Cherry tomatoes then we can recommend the Bajaja variety as the most productive and tolerant of the attack of *Alternaria Alternata*.

7. ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The thesis touches the current issues regarding taxonomic classification of all species of this genus intertwined with morphological identification of species of interest of tomato culture. Taking into account that is a less approached topic in Romania in this paper, two species of *Alternaria* have been identified in the pedo-climatic conditions of the Transylvanian plateau. Species of the genus *Alternaria* can cause significant damage in tomato culture in the conditions of rising summer temperatures.

The thesis can also have an international character, because the varieties taken in experience are of foreign origin and grown throughout the European continent, and this study tested the adaptability of these varieties in the conditions of our country.