

STUDY OF THE EFFECT OF ENDOPHYTIC INSECT PATHOGEN FUNGUS ON THE DEVELOPMENT OF PROGRESSIVE MATERIAL IN TOMATO CROP

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Abstract

The present study refers to the effect of endophytic insect pathogenic fungus on the growth of tomato crops. It can colonize a wide variety of plants and can cause increased plant growth and protect against parasites (Canassa, 2019). Specifically, the fungus used is Beauveria bassiana Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) after isolation in target insects, which is considered insect pathogenic and endophytic. The aim is to investigate the effect of the insect pathogenic fungus as endophytic on the growth of plants belonging to important crop vegetables, such as tomatoes. The study is of great significance, as the evidence of the use of the fungus with this technique will be used as an innovative economic strategy for the improvement and growth of plants without additional nutrients (Tall and Meyling, 2018). It is defined as a biological treatment other than systematic human intervention since Beauveria bassiana spends all or part of its life cycle intracellularly or/and between cells forming colonies in healthy tissues of their host plants typically without causing obvious disease symptoms (Wilson, 1995).

Keywords: *endophytic fungi, Beauveria bassiana, propagating material, development*

1. INTRODUCTION

From antiquity to the present day, people's diet is significantly based on ingredients from plant materials. People started to realize that the cultivation of these plants was vital, since it offered them a variety of nutritious ingredients, while some of them even had pharmaceutical properties. The evolution of cultivation techniques happened both on national and international grounds. (Olympios, 2015).

In Greece, climate constitutes the primary factor that serves the development of a large planting material variety. The number of the planting materials originally cultivated was limited. During the last 60 years the crop areas as well as the output are notably increased. Due to the overgrowing demand for basic planting materials, the production spread during the year, while with the proper expertise were found means of protection from the weather conditions (Olympios, 1994). However, all crops require expensive nutrient supplements coming with an environmental cost. International developments are rapid and influenced by the pressure for increased food production. This leads to a need to design sustainable solutions in order to increase efficacy as well as more efficient use of resources such as nutrients. Some fungi are able to promote plant growth according to relevant studies indicating such effects, conducted under optimal conditions with plenty of materials (Tall and Meyling, 2018).

The present study investigates the effect of the endophytic insect pathogenic fungus *Beauveria Bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) on the growth of plants belonging to in the major horticultural crops of tomato. The procedure was conducted endophytically via their propagating material. The use of the fungus doesn't apply by contact using a formulation, but by the solution of known conidial concentration (10^8) and by baptizing the propagating material (seeds) of tomato for 24 hours.

2. MATERIALS AND METHODS

2.1 Materials

To carry out the work, propagating material (seeds) of tomatoes of two varieties per species (“Macedonia” and “Areti”, a total number of 80 seeds) was used (Table 1). Half seeds (40) of each propagating material for both varieties was used. Suspensions of the fungus *Beauveria bassiana* (a local strain of Achaia) were created of a specific conidia (10^8) concentration, where half of the seeds. Forty (40) of each propagating material were immersed for 24 hours for both varieties. Water was used as a control, then all seeds were planted in 30 *60 cm styrofoam seedboxes, divided into groups. From the sowing, measurements were taken twice a week until the stage of flowering of the plants. First, the germination was measured, and then the stem thickness, the height of the plant, the length of the leaf (on each leaf separately), and the width of the leaf (on each leaf separately). The plants were transplanted the fourth week after germination in individual pots based on growth, and irrigated daily.

Plant	Variety	Number of seeds	Treatment
Tomato	Macedonia	20	Control witness
Tomato	Macedonia	20	<i>Beauveria bassiana</i>
Tomato	Areti	20	Control witness
Tomato	Areti	20	<i>Beauveria bassiana</i>

Table 1. Experimental design by variety

2.2 Methods

For the experimental procedure, for each application (control, *Beauveria bassiana*) 40 tomato seeds from each variety (“Macedonia”, “Areti”) were used, having a total of 80 seeds. The 20 seeds from each tomato variety (“Macedonia”, “Areti”) were immersed in a suspension of a specific conidia concentration (10^8) with the fungus *Beauveria bassiana* for 24 hours so that the seeds become the hosts of the fungus. The remaining 20 seeds of each tomato variety (“Macedonia”, “Areti”) were immersed in water to witness the experiment. Subsequently (13/05/21), the tomato seeds were planted separately in 30*60cm strainer seedboxes, 20 “Macedonia” tomato seeds (control), 20 “Macedonia” tomato seeds (with *B. bassiana*), 20 tomato seeds “Areti” (control), and 20 “Areti” tomato seeds (with *B. bassiana*). The germination of the seeds of the tomato varieties “Macedonia”, and “Areti” (control and *B. bassiana*) was checked for the next 2 weeks. During the second week, the first recording of the thickness and height of the shoot was made from each plant that sprouted of the varieties “Macedonia” and “Areti” (control and *B. bassiana*). At the end of the second week, the thickness and height of the shoot as well as the length and width of the leaves of the varieties “Macedonia”, and “Areti” (control and *B. bassiana*) were recorded. During the fourth week, transplanting was done in individual pots, and then the thickness, shoot height, length, and width of leaves of every plant were recorded. At the end of the transplant and in the context of plant growth, all the data were measured twice a week. The experimental process lasted 2 months until the time of flowering of the plants. Daily irrigation was carried out.

2.2.1 Isolation of the insect pathogenic fungus from the leaves on the SDA substrate

To investigate the presence of insect pathogenic fungus in the endophytic stage, random leaves were collected from tomato and pepper plants with sterile scissors, at intervals of 30 and 50 days from the application of the insect pathogenic fungus to the propagating material (seeds) with embryos (10^8). Each sheet was then cut into 1 cm diameter discs in a filament chamber. Samples were sterilized by surface immersion in 96% ethanol solution for one minute, 6% sodium hypochlorite solution for 5 minutes, and finally in 96% ethanol solution for 30 seconds (Luginbuhl and Muller 1980). They were then transferred to an SDA substrate using a sterile hook and incubated in the dark at $25^{\circ}\text{C} \pm 2$ and

80% humidity for 14 days (Img. 1). The germination of conidia on tomato leaves was assessed using an optical microscope (Zeiss Axionstar plus 40×). For the preparation of the substrate were used 65g SDA (Sigma-Aldrich, USA) with 0.01% streptomycin (0.05 mg/ml to prevent bacterial contamination) in 1000 ml of cold sterile water, which was heated to boiling point to dissolve the medium completely. The material was sterilized in an oven for 15 minutes at 121 ° C (Chase et al. 1986).

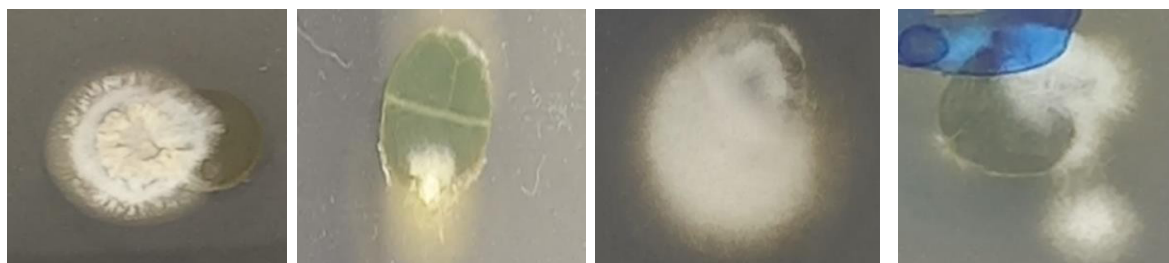


Image 1. Re-isolation of the insect pathogenic fungus from tomato and pepper leaves on SDA substrate at 25°C.

3. RESULTS

3.1 Measurements of tomato varieties

3.1.1 Average height and thickness of tomato shoots varieties Macedonia and Areti

The average height of the control witness (35.5 cm) in the variety “Macedonia” (Img. 2) is higher than the one with the fungus intervention (29.4 cm). The measurement of the tomato shoots’ thickness in variety the “Macedonia” in the average number with the fungus intervention is 0.65 cm, slightly higher than the control witness (0.62cm) .

Variety “Areti” (Img. 3) has an average height of plants with the fungus intervention (27.90 cm) which is lower than the control witness (33.7 cm). In tomato “Areti”, the average measurement of the tomato shoots thickness with the fungus intervention seems higher, with a final measurement at 0.7 cm instead of the witness’ 0.5 cm.



Image 2. Plants “Macedonia” control / *B. bassiana*.



Image 3. Plants “Areti” control / *B. bassiana*

3.1.2 Leaf length of tomato varieties “Macedonia” and “Areti”

The average length of the leaves of tomato varieties “Macedonia” and “Areti”. The length of the leaves ranged from 5 to 6 leaves for each plant, whose length was separately measured per date.

Based on the comparison of measurements on all dates as well as in the final measurement, it appears that the average length of the leaves “Macedonia” (control) (Fig. 1) excels numerically as well as in the number of leaves with a total of 6 leaves in correlation to the plants with the fungus (*B. bassiana*).

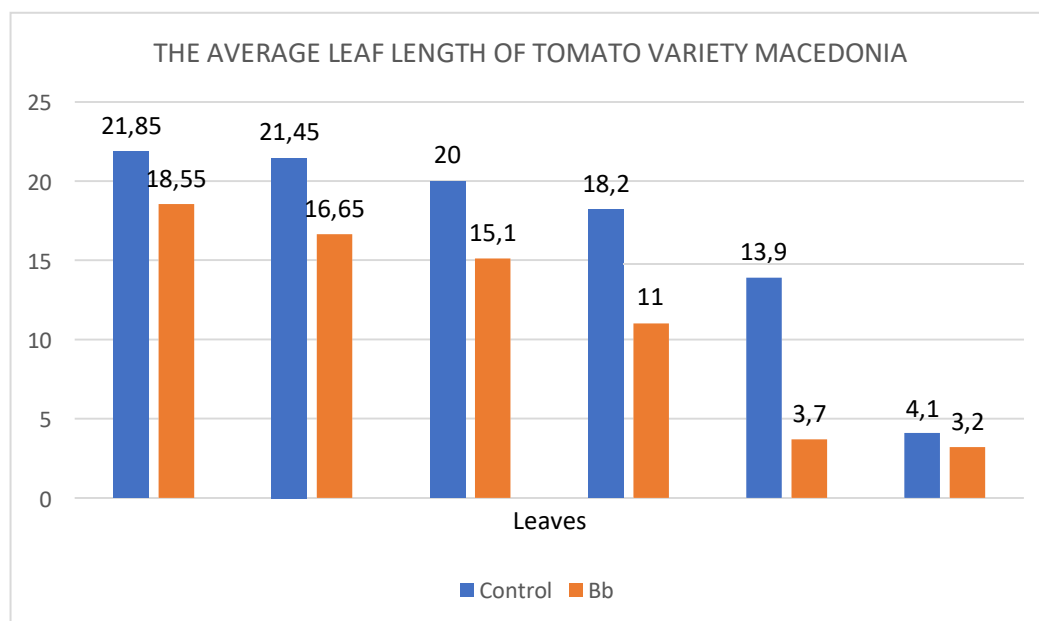


Fig. 1. Average length of the leaves “Macedonia”

Based on the comparison of measurements, it seems that the average length of the leaves of the “Areti” variety (*B. bassianna*) is higher than the average length of the leaves of the “Areti” variety (control) in leaves 1 and 2 (Fig. 2). However, in all leaves, there is a difference with the “Areti” plants (control) having 5 leaves in correlation to the “Areti” plants (*B. bassianna*) having 6 leaves.

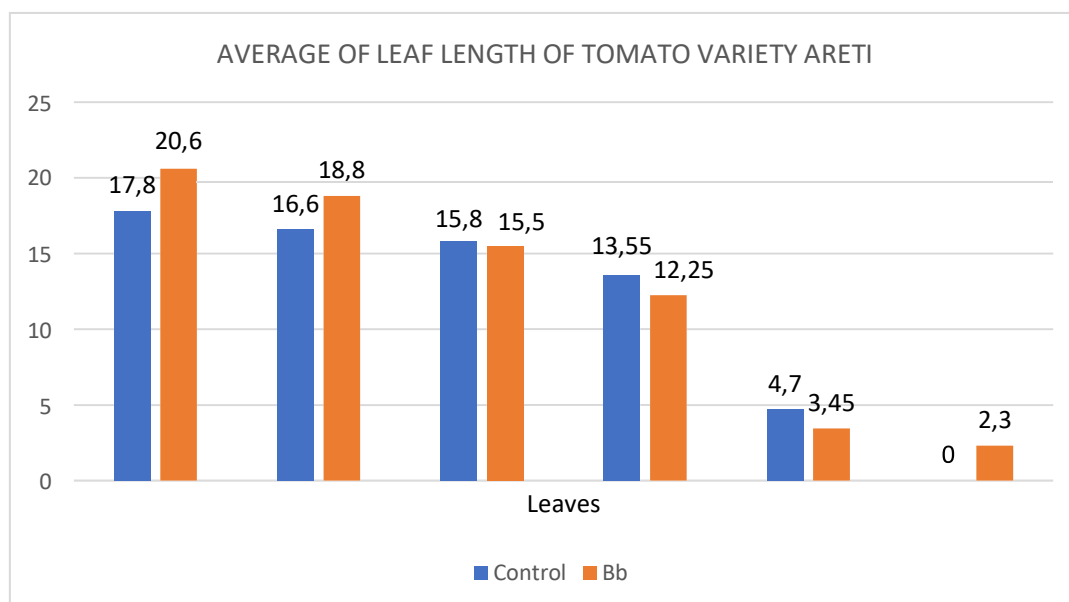


Fig. 2. Average length of the leaves “Areti”

3.1.3 Width of tomato leaves for varieties Macedonia and Areti

The average width of the leaves for varieties “Macedonia” and “Areti” ranged from 5 to 6 leaves for each plant, whose width was separately measured per date.

Based on the comparison of measurements it appears that the average width of the tomato leaves

“Macedonia” (control) (Fig. 3) excels in correlation to the plants with the fungus (*B. bassiana*) (besides leaf number 6) with an almost 5 cm difference in leaf number 2 and 3, while in leaf number 4 we see an increase at almost 8 cm.

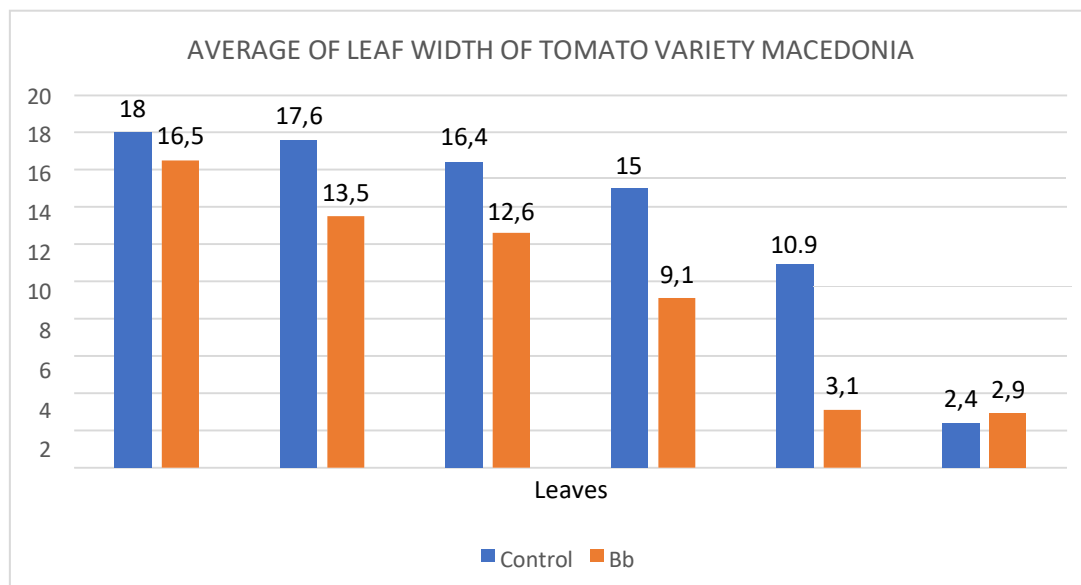


Fig. 3. Average width of the tomato leaves “Macedonia”

Based on the comparison of measurements, it seems that the average width of the leaves of the “Areti” variety (*B. bassiana*) is larger than the average width of the leaves of the “Areti” variety (control) in all leaves, besides the 5th (Fig. 4). However, there is a difference in the number of leaves with plants “Areti” (control) having 5 leaves in comparison with plants “Areti” (*B. bassiana*) having 6 leaves.

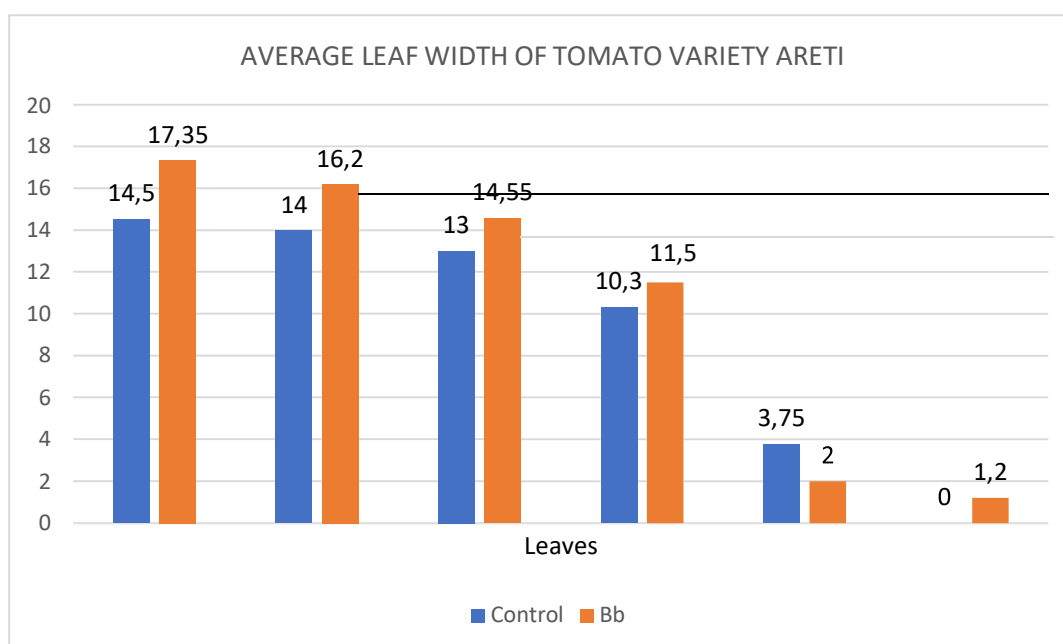


Fig. 4. Average width of the tomato leaves “Areti”

3.2 Summary analysis of results and display of tomato

According to the diagrams and tables in the variety “Macedonia”, the use of the fungus showed a positive effect on plant growth, in terms of plant stem thickness while all other measured characteristics (plant height, length, and width of leaves) did not show a similar effect, something that is phenotypically ascertained.

According to the diagrams and tables in the variety “Areti”, the application of the fungus showed a positive effect on the growth of plants, in terms of the thickness of the plant stem, while in all other measured characteristics (plant height, length, and width of leaves) did not or partially show a similar effect, something that is phenotypically ascertained.

4. DISCUSSION

As already mentioned by Tefera και Vidal (2009), the application of *Beauveria Bassiana* in leaves, seeds or soil didn't lead to major differences in plants' height.

The present study targeted the investigation of the effect of the endophytic insect pathogenic fungus *Beauveria Bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) on the growth of plants belonging to the major horticultural crops of tomato via their propagating material on very specified conditions for the needs of Thesis experiment at Patras, Greece. The use of the fungus didn't apply by contact using a formulation, but rather by the solution of known conidial concentration (10^8) and by baptizing the propagating material (seeds) of tomato for 24 hours.

Despite the numerous studies of endophytic insect pathogenic fungi, only a few of them have been studied. It is a strong belief that there are still a lot of unexplored endophytes due to the limited research in this field.

In the currently available literature, an understanding is made of the colonization of endophytic insect pathogenic fungi into different host plants and their effects.

Therefore, there is an urgent need to discover and evaluate the insect pathogenic endophytic strains.

5. CONCLUSION

During the last 60 years, the cultivation of tomatoes both in crop areas and in output has increased significantly. Due to the increasing demand for basic vegetables, the production expanded throughout the year and with the proper expertise, were developed means of protection against the weather (Olympios, 1994). However, all crops require nutrient supplements that are expensive and affect the environment. In addition, international development for the rapid increase of food production requires sustainable solutions in order to increase efficacy as well as to excel in the use of resources such as nutrients. Some insect pathogenic fungi can promote plant growth, but according to relevant studies, those effects have been conducted under optimal conditions with plenty of materials (Tall and Meyling, 2018).

To this end, it is crucial that some aspects were investigated. The insect pathogenic fungus of our thesis as endophyte used to colonize the plant at the stage of seed, some fungal isolates are more or less successful as endophytes than others depending on biotic and abiotic factors, and endophytes benefit from plants. (Mantzoukas and Eliopoulos 2020).

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