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

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Abstract

This study examines the potential of endophytic insect pathogenic fungi to enhance pepper crop growth. Specifically, the study focuses on the use of Beauveria bassiana Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae), an insect pathogenic and endophytic fungus isolated from target insects that has been shown to promote plant growth and protect against parasites (Canassa, 2019). The aim of this research is to investigate the effects of using this fungus as an endophytic treatment on the growth of pepper plants. This study is important as the use of this innovative technique has the potential to serve as an economic strategy for improving plant growth without the need for added nutrients (Tall and Meyling, 2018). This biological treatment differs from systematic human intervention, as Beauveria bassiana spends its life cycle within or between cells forming colonies in healthy tissues of host plants, typically without causing obvious disease symptoms (Wilson, 1995).

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

1. INTRODUCTION

Throughout history, plants have played a significant role in human nutrition and medicine, leading to the evolution of cultivation techniques both nationally and internationally (Olympios, 2015).

In Greece, the climate has eased the development of a wide variety of planting materials, which has expanded over the past 60 years due to increasing demand. However, the use of costly nutrient supplements to support crop growth has had an environmental impact. As the pressure for increased food production grows, there is a need for sustainable solutions that optimize resource use.

One promising approach is the use of fungi to promote plant growth, as demonstrated in studies conducted under optimal conditions with abundant materials (Tall and Meyling, 2018).

The present study aims to investigate the endophytic insect pathogenic fungus *Beauveria bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) and its effect on the growth of pepper plants, a major horticultural crop. The fungus was applied endophytically to the propagating material of the pepper plant by baptizing the seeds in a solution of known conidial concentration (108) for 24 hours, rather than by applying a formulation directly to the plant.

2. MATERIAL & METHODS

2.1 Material

For this study, propagating material in the form of seeds from two varieties per species of pepper ("Florinis" and "Tigani") were used, with a total of 80 seeds as detailed in Table 1. Half of the seeds (40) from each propagating material were selected for the experiment. Suspensions of the fungus *Beauveria bassiana* (a local strain of Achaia) were prepared at a specific concentration of conidia (108), with half of the seeds from each propagating material being immersed in the suspension for 24 hours. The other half of the seeds were used as a control and were immersed in water. All seeds were

then planted in 30*60 cm styrofoam seedboxes, which were divided into groups. Measurements were taken twice a week from the time of sowing until the flowering stage of the plants, including germination rate, stem thickness, plant height, leaf length (measured for each leaf separately), and leaf width (measured for each leaf separately). After four weeks of germination, the plants were transplanted into individual pots based on their growth and were irrigated daily.

Table 1. Experimental design by variety

Pepper	Florinis		
	Tigani P-13		

2.2 Methods

The experimental procedure involved the use of 40 seeds from each pepper variety ("Florinis" and "Tigani P-13"), resulting in a total of 80 seeds. Half of the seeds from each variety were immersed in a suspension having a specific concentration (108) of the fungus *Beauveria bassiana* for 24 hours, while the other half were immersed in water to serve as controls. On May 13, 2021, the seeds were planted in separate 30*60cm seedboxes, with 20 "Florinis" pepper seeds (control), 20 "Florinis" pepper seeds (with *Beauveria bassiana*), 20 "Tigani" pepper seeds (control), and 20 "Tigani" pepper seeds (with *Beauveria bassiana*). Germination of the pepper seeds was checked for two weeks, during which the thickness and height of the shoot were recorded. 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 each plant, were recorded. Transplantation was done in individual pots during the fourth week, after which measurements of the thickness, shoot height, length, and width of the leaves of each plant were taken twice a week until the plants flowered, which took two months. The plants were irrigated daily.

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, leaves were randomly collected from pepper plants using sterile scissors at 30- and 50-day intervals from the application of the insect pathogenic fungus to the propagating material (seeds) with embryos (108). Each leaf was cut into 1 cm diameter discs in a filament chamber. The samples were surface sterilized by immersing in 96% ethanol solution for one minute, 6% sodium hypochlorite solution for 5 minutes, and finally in 96% ethanol solution for 30 seconds, following the method by Luginbuhl and Muller (1980). The sterilized samples were then transferred to an SDA substrate using a sterile hook and incubated in the dark at 25 °C ± 2 and 80% humidity for 14 days (Img. 1). The conidia germination on pepper leaves was evaluated using an optical microscope (Zeiss Axionstar plus 40×). The substrate was prepared by dissolving 65g of 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 medium was sterilized in an oven for 15 minutes at 121 °C, as per the method by Chase et al. (1986).

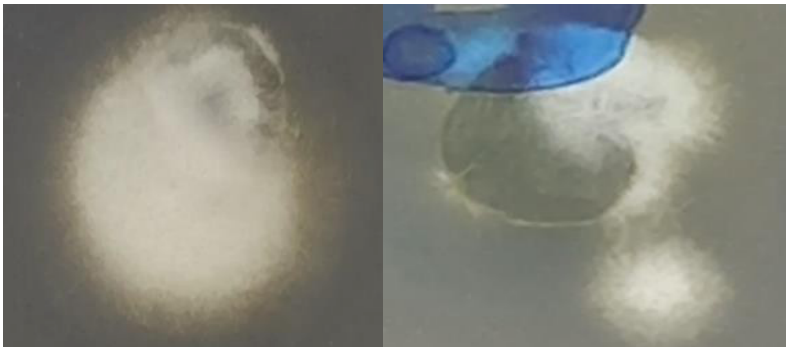


Image 1. Re-isolation of the insect pathogenic fungus from pepper leaves on SDA substrate at 25^{O}C

3. RESULTS

3.1 Measurements of pepper varieties

3.1.1 Average height and thickness of pepper shoots varieties "Florinis" and "Tigani-P-13"

In the results, it was seen that the average height of the control group for the "Florinis" variety (Img. 2) was higher (24.3 cm) than that of the group treated with the fungus (21.3 cm). The average thickness of the pepper shoots in the "Florinis" variety with fungus treatment was slightly higher (0.58 cm) than that of the control group (0.51 cm).

For the "Tigani P-13" variety (Img. 3), the average height of the plants with fungus treatment was lower (26.05 cm) than that of the control group (19.65 cm). In the "Florinis" variety, the average thickness of the pepper shoots with fungus treatment was higher (0.59 cm) than that of the control group (0.56 cm).

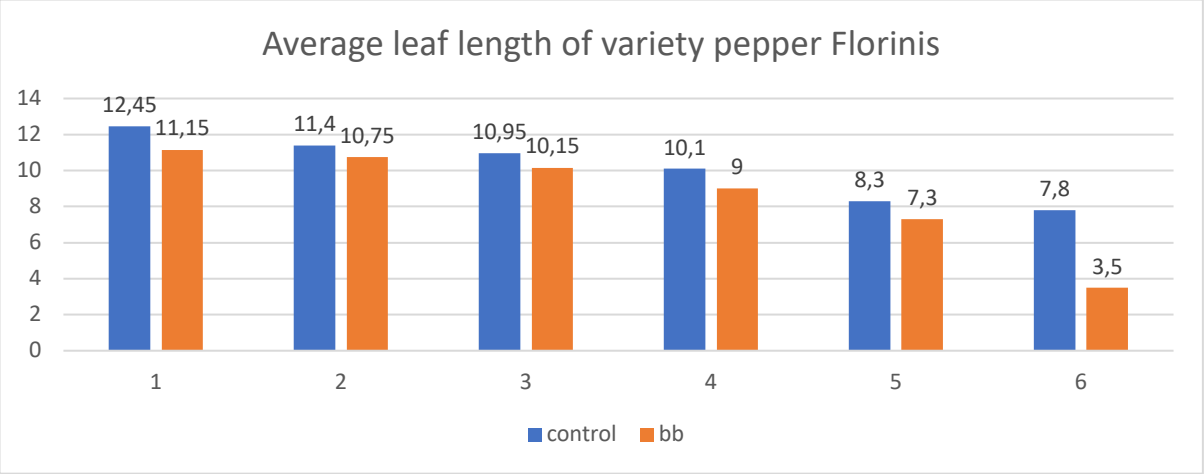
Control / *B. bassiana*

Image 2. Plants "Florinis" variety **Image 3.** Plants "Tigani P-13" variety

3.1.2 Leaf length of pepper varieties

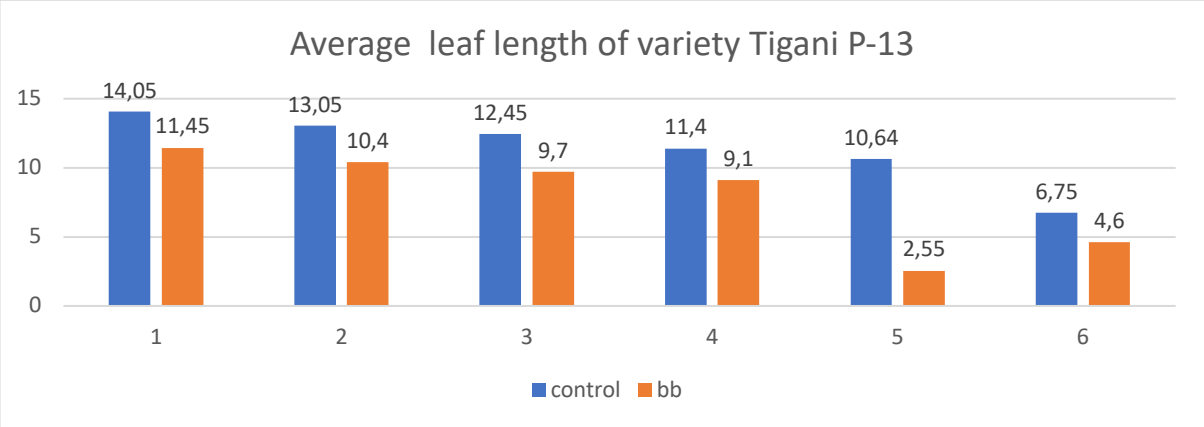
The average length of leaves, which was measured separately for each plant on all dates, ranged from 5 to 6 leaves. After comparing the measurements on all dates and the final measurement, it was found that the average length of leaves in the control group of the "Florinis" variety (Fig. 1) was higher both numerically and in the number of leaves, with a total of 6 leaves, compared to the plants with the *Beauveria bassiana* fungus.

Figure 1. Average leaf length of variety pepper Florinis



After comparing the measurements, it appears that in leaves 1 and 2 (Fig. 2), the average length of the leaves of the "Tigani P-13" variety (Beauveria bassiana) is higher than the average length of the leaves of the "Tigani P-13" variety (control). However, when considering all leaves, there is a difference between the two groups, with the "Tigani P-13" plants (control) having 5 leaves compared to the "Tigani P-13" plants (Beauveria bassiana) having 6 leaves.

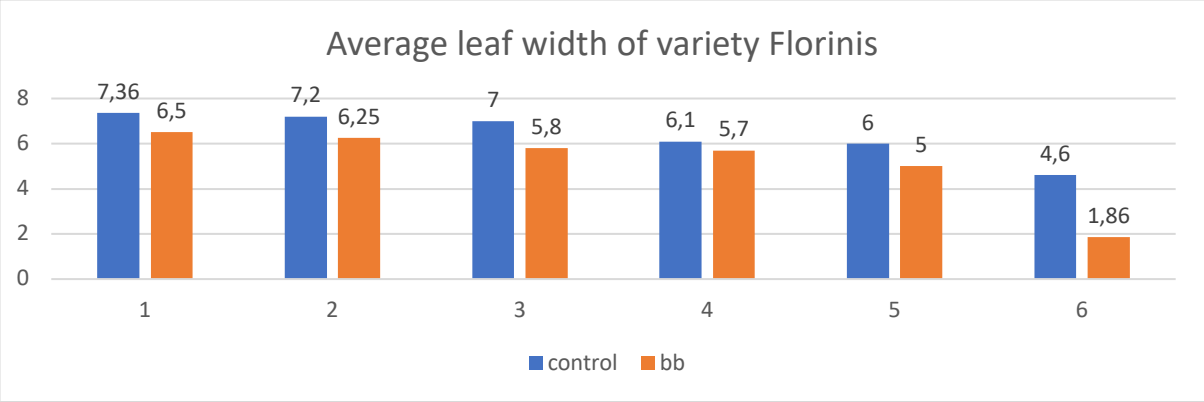
Figure 2. Average leaf length of variety Tigani P-13



3.1.3 Width of pepper leaves for varieties Florinis and Tigani P-13

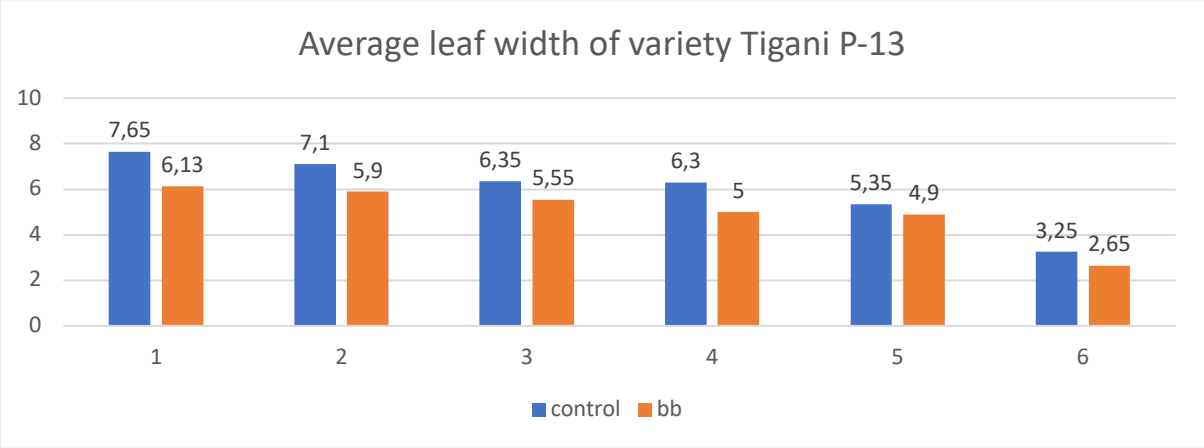
Based on the comparison of the measurements on all dates, the average width of the leaves of the "Florinis" pepper variety (control) without intervention is superior compared to the average width of "Florinis" (Beauveria bassiana) with the intervention of the fungus (Fig.3).

Figure 3. Average leaf width of variety Florinis



Based on the comparison of the measurements on all dates the measurement of the average leaf width of pepper "Tigani P-13" (control) without intervention is greater in all leaves of "Tigani P-13" (Beauveria bassiana) plants with fungus intervention (Fig. 4).

Figure 4. Average leaf width of variety Tigani P-13



3.2 Summary analysis of results and display of pepper

The results from the “Florinis” and “Tigani P-13” pepper varieties show that the application of the fungus had a positive effect on the thickness of the plant stem. However, in terms of other measured characteristics such as plant height, leaf length, and width, the application of the fungus did not show a significant effect phenotypically. This information is displayed through diagrams and tables for the Florinis and Tigani P-13 varieties.

4. DISCUSSION

The study conducted at Patras, Greece aimed to investigate the effect of the endophytic insect pathogenic fungus *Beauveria bassiana* Balsamo on the growth of pepper plants via their propagating material, under specific experimental conditions. In contrast to earlier findings, which showed no significant differences in plant height (Tefera and Vidal 2009) with the application of *Beauveria bassiana* on leaves, seeds, or soil, the current study used a solution of known conidial concentration (108) to baptize the pepper seeds for 24 hours.

While there have been numerous studies on endophytic insect pathogenic fungi, only a limited number of them have been conducted. As a result, it is widely believed that there are still many unexplored endophytes due to the limited research in this field. The existing literature supplies insight into the colonization of endophytic insect pathogenic fungi in various host plants and their effects. Therefore, it is imperative to discover and evaluate new insect pathogenic endophytic strains to further advance our knowledge in this area.

5. CONCLUSION

In conclusion, the cultivation of pepper has experienced a significant increase in both crop areas and output in the last 60 years. Despite the development of protection methods against weather conditions and the expansion of production throughout the year, nutrient supplements are still needed, which can be expensive and have an adverse impact on the environment. To meet the demands for sustainable and efficient solutions for increasing food production, some insect pathogenic fungi have been identified as promoters of plant growth. However, earlier studies have shown that these effects were achieved under optimal conditions with abundant materials (Tall and Meyling, 2018).

Therefore, it is crucial to investigate several aspects, including the use of insect pathogenic fungi as endophytes to colonize plants at the seed stage, as some fungal isolates may be successful as endophytes depending on biotic and abiotic factors. Additionally, it is important to note that endophytes can supply benefits to plants, as demonstrated by Mantzoukas and Eliopoulos (2020).

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