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Biocontrol Potential and Mitigation of Abiotic Stress Effects of *Meyerozyma guilliermondii* on Cucumber (*Cucumis sativus* L.)

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Abstract: This study aims to evaluate the biocontrol potential of *Meyerozyma guilliermondii* (CECT13190), an endophytic yeast, and its role in mitigating the adverse effects of abiotic and biotic stress in cucumber plants. The relevance of this study lies in addressing the threat of *Fusarium* wilt, a major fungal disease that impacts cucumber crop productivity, as well as the exacerbation of food scarcity caused by climate change-induced abiotic stress factors such as high temperatures and drought. The study was conducted in a greenhouse environment where *Cucumis sativus* seedlings were exposed to biotic (*F. oxysporum* inoculation) and abiotic stress conditions (heat and water deficit). The impact of *M. guilliermondii* on treated plants' physiology, growth, development, and flowering was assessed. The study confirmed the biocontrol activity of *M. guilliermondii* against *F. oxysporum* and highlighted its positive effects as a plant growth promoter. It enhanced overall plant health, activated natural defense mechanisms against *F. oxysporum*, and alleviated the detrimental impacts of abiotic stress. Notably, *M. guilliermondii* also induced early flowering in cucumber plants. This research underscores the potential of *M. guilliermondii* as a biocontrol agent for managing *Fusarium* wilt, enhancing stress tolerance, promoting early flowering, and offering promising prospects for sustainable crop production amidst fungal diseases and climate change-induced stressors. The findings emphasize the importance of utilizing *M. guilliermondii* to improve cucumber crop productivity and address food scarcity challenges.

Keywords: biocontrol agent; *Fusarium* wilt; plant physiology; sustainable crop production; climatic change

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1. Introduction

Cucumber (*Cucumis sativus* L.) cultivation is widespread, with annual global production exceeding 80 million tons [1]. In Spain, the total production in 2022 was 770,000 tons, making it the largest producer in the European Union [2]. *Fusarium* wilt, caused by the fungal pathogen *Fusarium oxysporum f. sp. cucumerinum*, is a devastating plant disease that affects various crop species, including cucumber. This fungal infection leads to wilting, stunted growth, vascular damage, and eventually, plant death [3]. *Fusarium* wilt poses a significant threat to global crop industries as it affects some of the most important crop plants, including cucumbers, leading to substantial economic losses and food scarcity. This pathogen has been identified in cucumber-producing regions worldwide and has been described as a significant economic threat to global cucumber growers [4]. The pathogen can persist in the soil for long periods, making it difficult to manage through conventional methods, such as crop rotation, application of chemical fungicides, and breeding resistant crops [3,5]. Therefore, exploring alternative strategies, such as biocontrol [6,7], is crucial for the effective management of *Fusarium* wilt and ensuring sustainable crop production.

Furthermore, while chemical control plays a crucial role in preventing food losses and addressing food security concerns, its adverse effects have led to an increasing demand for healthier food alternatives. To decrease or eliminate the reliance on synthetic fungicides

in agriculture while ensuring economically sustainable fruit protection against pathogens, it is essential to substitute these products with tailored strategies. This approach should consider specific factors such as the pathosystem involved, the type of Biological Control Agent (BCA) used, and the prevailing environmental conditions [8].

Abiotic stress, including high temperatures and drought, significantly restricts plant growth, development, and agricultural productivity. These stressors disrupt physiological processes, resulting in decreased crop yields and posing a threat to global food security. Climate change exacerbates the impact of abiotic stress on plants, with rising temperatures, altered rainfall patterns, and extreme weather events compounding the challenges faced by agricultural systems worldwide. Plant adaptation and resilience are vital for sustaining agricultural production and meeting the food needs of a growing global population [9]. High temperatures impair photosynthesis and nutrient uptake and increases oxidative stress.

The consequences of abiotic stress on crop productivity are extensive, including crop failures, yield losses, and reduced nutritional quality, affecting food availability, affordability, and nutritional diversity. Vulnerable regions dependent on agriculture are particularly susceptible, exacerbating social and economic disparities. An increase in temperature of about 3–4 °C could lead to a yield reduction of 15–35% in Africa and Asia and 25–35% in the Middle East [10].

Addressing abiotic stress is crucial for sustainable agriculture and global food security. Exploring beneficial microorganisms, like *Meyerozyma guilliermondii* [11–14], offers the potential to mitigate abiotic stress effects, enhance stress tolerance, and improve crop performance. Most plants serve as ecological hosts for diverse communities of fungal endophytes that reside within plant tissues without causing harm. On the contrary, these microorganisms establish mutualistic relationships with plant organisms, providing them with various benefits such as withstanding biotic and abiotic stresses (excessive temperature, drought, or salinity), enhancing nutrient acquisition, and increasing yield [15]. Understanding stress-alleviating mechanisms can guide the development of innovative strategies to enhance plant resilience, optimize resource use, and ensure sustainable food production in the face of changing environmental conditions.

Photosynthetic pigments and superoxide are considered reliable indicators of stress in plants due to their close association with fundamental physiological processes and susceptibility to stress-induced disruptions [16,17]. Photosynthetic pigments, such as chlorophylls and carotenoids, play a crucial role in capturing light energy and facilitating photosynthesis. During stress conditions, the quantity and composition of these pigments can be altered, leading to a decrease in photosynthetic efficiency. Changes in photosynthetic pigments can serve as early indicators of stress, reflecting the plant's response to adverse environmental conditions.

This research aims to provide insights into the biocontrol potential of *Meyerozyma guilliermondii* and its ability to alleviate the negative impacts of both biotic and abiotic stress on cucumber plants, contributing to the development of sustainable disease management strategies and improved crop resilience.

2. Materials and Methods

2.1. Source of Meyerozyma guilliermondii

The selected endophytic yeast *Meyerozyma guilliermondii* CECT13190 (Patent ES 2 792 777 A1) was isolated from tissue samples of *Vitis vinifera* plants that had been previously infected with *Fusarium equiseti* [18] but showed no symptoms of the disease. The ability of this yeast to establish a mutualistic relationship with *Vitis vinifera* and exhibit resistance against *Fusarium vascular* wilt disease [19] prompted its selection for further investigation as a potential biocontrol agent (BCA) against other *Fusarium* species as *Fusarium oxysporum f. sp. cucumerinum* in cucumber plants.

2.2. Experimental Design

This study followed an experimental design (Figure 1) to assess the efficacy of *Meyerozyma guilliermondii* CECT13190, a biocontrol agent, in mitigating biotic and abiotic stresses.

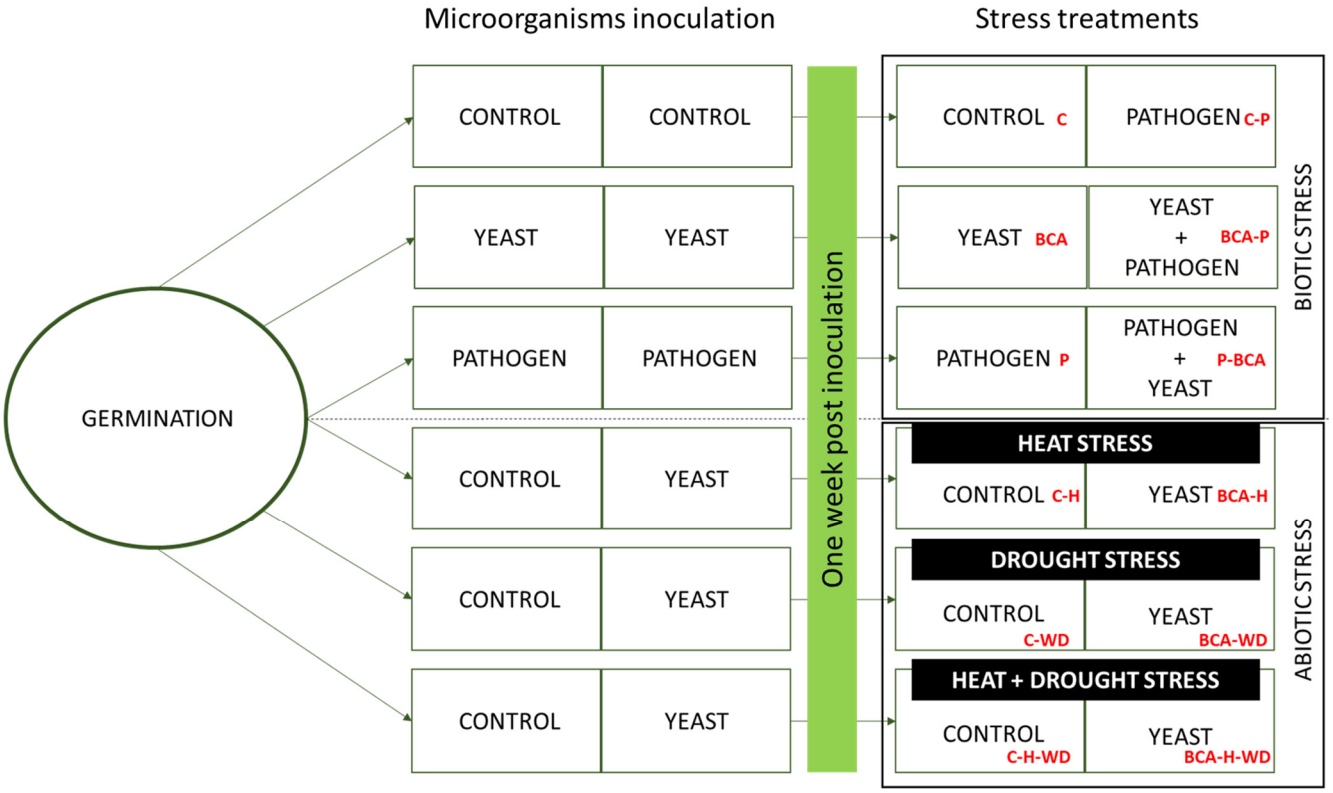

Figure 1. Experimental design of biotic and abiotic stress treatments in combination with the inoculation with the biocontrol agent *Meyerozyma guilliermondii* CECT13190. The diagram illustrates the arrangement of seedling trays in three greenhouses for biotic stress treatments (control (C), biocontrol agent (BCA), and pathogen (P)) and three greenhouses for abiotic stress treatments (heat (H), water deficit (WD), and combined heat and water deficit). Each seedling's greenhouse represents a specific treatment group, as described in the Section 2.

Twenty-four randomly selected germinated cucumber seedlings from the Petri dish were transplanted into each cell plant tray. A total of 12 cell plant trays were planted, comprising 288 seedlings in total. The trays were paired up and placed in three greenhouses for biotic stress treatment and three greenhouses for abiotic stress treatment. The experiment was replicated two times.

2.2.1. Biotic Stress Experiment

First Round of Inoculations—each seedling greenhouse had a diverse treatment:

- Control Greenhouse (C);
- Greenhouse with Biocontrol Agent (BCA);
- Greenhouse with Pathogen (P).

Second Round of Cross-Inoculations—within each greenhouse, the two trays were separated, and the following cross-inoculations were performed:

- C + P;
- BCA + P;
- P + BCA.

The other pair of each tray was reinoculated with the same treatment as the first inoculation.

2.2.2. Abiotic Stress Experiment

First, the BCA was inoculated in three trays. Subsequently, each inoculated tray was paired with an un-inoculated tray, and the pairs were subjected to different treatments: one pair to heat treatment (H), one pair to water deficit (WD), and the remaining pair to the combined treatment (water deficit + heat; WD + H), each pair within the same seedling greenhouse.

2.3. Seed Sterilization and Germination:

Cucumber seeds were aseptically sterilized by soaking them in a 50 mL Falcon tube containing 70% ethanol. After that, the seeds were gently agitated for 30 s. The ethanol was discarded, and the seeds were immersed in a 2.5% sodium hypochlorite solution (active chlorine) to cover the seeds completely. The solution was gently agitated for 15 min. After the exposure to sodium hypochlorite, the bleach solution was discarded, and the seeds were rinsed three times with sterile distilled water for 10 min each.

Sterilized Petri dishes (15 cm in diameter) were prepared by lining them with two layers of sterile filter paper. Sterile distilled water was added to saturate the filter paper. Thirty seeds were sown in each Petri dish, arranged in a rectangular pattern (5×6). The plates were kept in darkness in a growth chamber at 22 ± 2 °C for four days until germination of the seeds. The germination of the seeds was assessed by observing the emergence of the radicle.

2.4. Planting

Twenty-four randomly selected germinated cucumber seedlings from each Petri dish were transplanted into each cell plant tray; each cell has a volume of 21 cm^3. The plant trays were irrigated with 40 mL of sterile water and then placed in a growth chamber at 22 ± 2 °C with a 16 h light/8 h dark photoperiod for two weeks.

2.5. Inoculation

Three separate greenhouses were used for the experiment: one seedling greenhouse was inoculated with the pathogenic fungus, another was inoculated with the endophytic yeast, and the remaining greenhouse served as the control (Figure 1).

Inoculation with the yeast was performed when cucumber plants had developed true leaves. A volume of 0.5 mL of yeast suspension (concentration of 10^8 CFU/mL) was added to the germination trays' cells in two plant trays. After two weeks, the germination trays were divided, with one tray inoculated with yeast (BCA) and the other serving as a control (C). The plants were irrigated with 30 mL of sterile water.

To ensure reproducibility and consistency in experimental conditions, the *F. oxysporum* mycelium was cultivated on Potato Dextrose Agar (PDA) plates until it covered the surface completely. This mycelial culture was then homogenized in 100 mL of sterile water, resulting in a uniform solution. This solution was applied through irrigation at a rate of 2 mL per plant.

The control agent was prepared similarly, using Potato Dextrose Broth (PDB) cultivated for 24 h to achieve a concentration of 10^7–10^9 CFU/mL. Both the pathogenic fungus and the control agent were applied using the same method to maintain consistency across treatments.

After 7 days, each group was subdivided into two identical groups of 24 plants, which were then separated into individual greenhouses. For each original treatment group (Control, Pathogen, and Antagonist), half of the plants were maintained under the same conditions, while the other half were inoculated with a different species of microorganism. This created three new treatment groups: antagonist + pathogen (BCA + P), control + pathogen (P), and pathogen + antagonist (P + BCA).

Abiotic Stress Treatments

The plant trays designated for heat stress (H) and combined heat and water stress (H-WD) were placed in a greenhouse with a radiant heating system, maintaining an internal temperature of 40 °C. The remaining two plant trays for water deficit stress (WD) treatments were kept in the growth chamber. Watering was suspended for the water stress treatments (WD and H-WD), while the other plants were irrigated to maintain a 1 cm depth of water in the plant trays. After one week, 2 mL of a 10% Hoagland solution was added to each plant cell.

2.6. Measurement of Physiological Parameters and Plant Growth Analysis

2.6.1. Phenology and Morphology

The developmental stage of the plants was assessed according to the extended version of the BBCH scale [20], which allows the determination of the phenological stage in major crop species.

Leaf area was calculated from photographs of the leaves using contrast techniques with ImageJ® v 8.1.0 software [21]. Each cucumber plant was carefully extracted from the germination tray cell to measure the length of the aerial part and the length of the main root, and the root-to-shoot ratio was calculated.

The transition from vegetative to reproductive growth was determined by the appearance of floral buds. At the end of the experiment, the number of flowers on each cucumber plant was counted.

2.6.2. Measurement of Oxidative Stress

The presence of superoxide radicals was assessed using the NBT (nitro blue tetrazolium) method, which is based on the formation of formazan crystals through the NBT reduction mediated by superoxide radicals. The protocol described by Monteoliva et al. (2019) [22] was followed, and the percentage of NBT inhibition was transformed into a direct measure of SOD enzyme activity.

Leaves with formazan deposits were photographed, and the relative area of formazan to the total leaf area was quantified using ImageJ® software.

2.6.3. Determination of Photosynthetic Pigments (Chlorophylls and Carotenoids)

Total chlorophyll and carotenoid contents were determined from lyophilized aerial parts of each cucumber plant, following the protocol described by López-Hidalgo et al. (2021) [23]. The concentration of three photosynthetic pigments, namely Chlorophyll A, Chlorophyll B, and Carotenoids, was evaluated. To determine the pigment concentrations, the following formulas (Welburn, 1994) [24] were used:

$$\text{Chlorophyll A } (\mu g\ mL^{-1}) = 12.7\ A\ (663) - 2.69\ A\ (645)$$

$$\text{Chlorophyll B } (\mu g\ mL^{-1}) = 22.9\ A\ (645) - 4.86\ A\ (663)$$

$$\text{Carotenoids } (\mu g\ mL^{-1}) = (1000 * A\ (470) - 1.43 * \text{Chlorophyll A} - 97.63 * \text{Chlorophyll B})/209$$

2.7. Symptoms Analysis

To assess the disease incidence in *C. sativus* plants, direct observation of symptoms related to *Fusarium* wilt was conducted. The darkening lesions in the xylem vessels due to *Fusarium* wilt typically appear as dark spots in the plant's vascular tissues. These affected areas exhibit abnormal coloring, ranging from brown to black, within the xylem conduits. The inner neck region of each plant was individually inspected to identify signs of rot or necrosis that may have affected the xylem vessels. The necrotic areas were further examined in detail at 40× magnification using a binocular microscope.

The symptoms were studied by classifying the plants into three categories: asymptomatic (no visible signs of lesions), symptomatic (presence of dark spots in the vascular system), and dead plants.

2.8. Dual Plate Confrontation

To determine the effectiveness of the biocontrol agent in reducing pathogen growth, dual plate confrontations were conducted using a PDA (Merck, Germany) medium. The potential antagonist was inoculated in the middle of the plate, creating a line along the agar surface. At a distance of 2.5 cm, a circular disk measuring 1 cm in diameter and colonized by *F. oxysporum* was placed. As a control, plates containing only the *Fusarium* disk were prepared in the same position. The plates were incubated at 25 ± 2 °C for 7 days. The pathogenic fungus's radial growth towards the plate's center was evaluated daily for one week. By comparing with the controls, the percentage of radial growth inhibition of the pathogen (PICR) caused by the antagonistic yeast was determined. The PICR was calculated according to the formula (Ezziyyani et al., 2004) [25]:

$$PICR = (R1 - R2)/R1 \times 100$$

where R1 is the radial growth of the pathogen in the control plate, and R2 is the radial growth of the pathogen in the confrontation plate.

2.9. Statistical Analysis

In this study, each experimental unit consisted of 288 plants, and the experiment was replicated two times, with three replicates for each treatment group, totaling 48 plants per treatment. The statistical analysis was performed using the STATISTICA® software. A one-way analysis of variance (ANOVA) was conducted to compare the means of the different treatment variables. Duncan's test was applied to display the grouping and differences among treatments.

3. Results

3.1. Plant Growth and Development

Significant variations in leaf area were observed among *C. sativus* seedlings exposed to diverse treatments, particularly those subjected to abiotic stress conditions. Early treatment with the yeast endophyte (BCA and BCA-P) resulted in notably higher leaf area values compared to other conditions, accompanied by a maximal total leaf count. In contrast, plants exclusively inoculated with the pathogen during the initial phase (P) exhibited the lowest leaf area and leaf number values. This trend was particularly pronounced in plants facing abiotic stress, such as water deficit (C-WD) and high temperature (C-H), where leaf area and leaf number were notably affected, showcasing the impact of these stressors on plant morphology (Figure 2).

To explore the potential of *Meyerozyma guilliermondii* in mitigating abiotic stress-induced morphological effects, we evaluated the root-to-shoot length ratio (Table 1). The presence of the yeast correlated with increased shoot length under heat-water deficit stress and enhanced root length in cucumber plants facing combined heat and water deficit stress. Despite variations in individual root and shoot lengths across treatments, the ratio between them remained consistent and did not exhibit significant differences. This suggests a uniform response to abiotic stress, irrespective of the specific treatment, providing valuable insights into the yeast's resilience-promoting effects on cucumber plants under adverse environmental conditions.

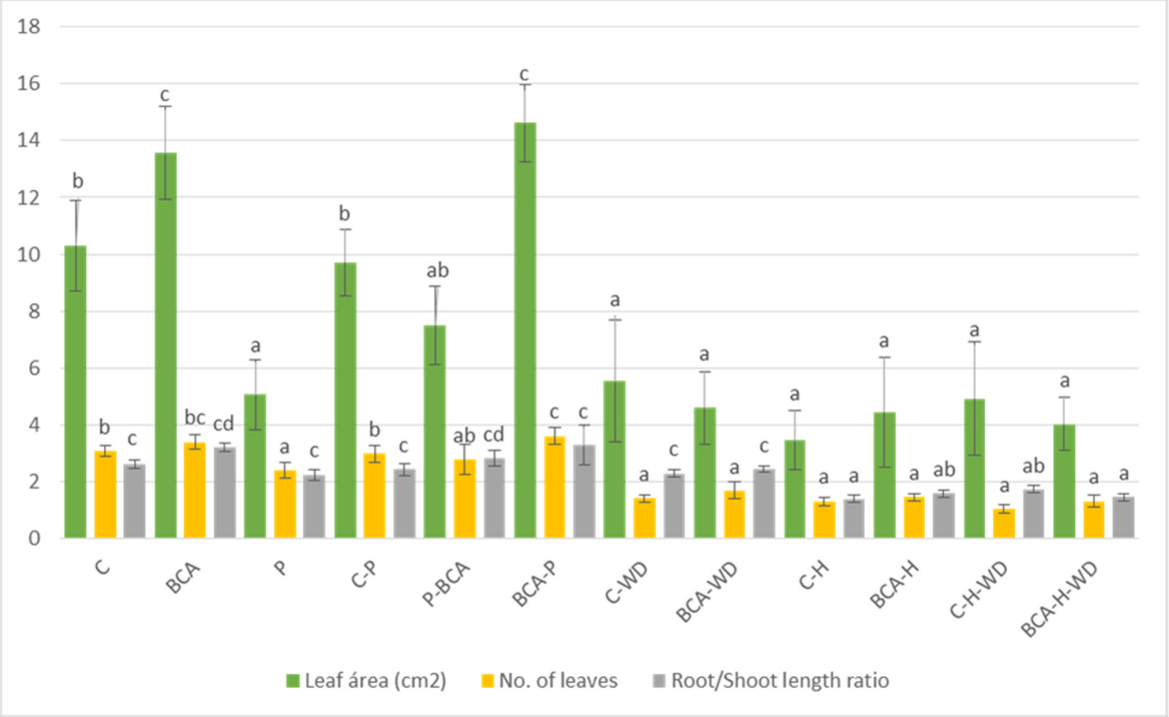

Figure 2. Means and Standard Deviations of leaf area (cm^2), number of leaves, and root–shoot length ratio in cucumber plantlets subjected to biotic and abiotic stress treatments. Biotic treatments included inoculation with *M. guilliermondii* (BCA) and *F. oxysporum* (P), and those subjected to a second round of inoculation: control + *F. oxysporum* (C-P), *M. guilliermondii* + *F. oxysporum* (BCA-P) and *F. oxysporum* + *M. guilliermondii* (P-BCA). Abiotic treatments included heat stress (H) and water deficit (WD) and combinations of them. Different letters correspond to statistically different groups (Duncan test, $p \leq 0.05$). The study involved a total of n = 48 plantlets for each treatment.

Table 1. Means and Standard Deviations of Chlorophyll b, Carotenoids, and Superoxide Dismutase (SOD) in cucumber plantlets subjected to biotic and abiotic stress treatments. Biotic Treatments included inoculation with *M. guilliermondii* (BCA) and *F. oxysporum* (P), and those subjected to a second round of inoculation: control + *F. oxysporum* (C-P), *M. guilliermondii* + *F. oxysporum* (BCA-P) and *F. oxysporum* + *M. guilliermondii* (P-BCA). Abiotic treatments included heat stress (H) and water deficit (WD), as well as combinations of them. Different letters correspond to statistically different groups (Duncan's test, $p \leq 0.05$). The study involved a total of n = 48 plantlets for each treatment.

Treatment	Chlb mg/g Dry Weight	SD	Carotenoids mg/g Dry Weight	SD	SOD U/mg Protein	SD
C	6.38 ^{c}	0.41	3.13 ^{d}	0.19	97 ^{e}	8.06
BCA	6.15 ^{bc}	0.70	3.03 ^{d}	0.34	70.10 ^{c}	6.64
P	4.84 ^{b}	0.63	2.44 ^{cd}	0.26	57.73 ^{b}	10.02
C-P	7.36 ^{d}	0.21	3.58 ^{de}	0.29	68.04 ^{c}	7.59
BCA-P	7.83 ^{d}	0.51	3.80 ^{e}	0.25	79.38 ^{f}	6.15
P-BCA	6.93 ^{cd}	0.58	3.39 ^{de}	0.28	67.36 ^{c}	9.60
C-WD	3.70 ^{b}	0.50	1.13 ^{b}	0.12	54.94 ^{a}	8.65
BCA-WD	2.35 ^{a}	0.06	0.62 ^{a}	0.13	49.48 ^{ab}	10.85
C-H	5.85 ^{bc}	0.74	1.06 ^{ab}	0.36	79.38 ^{d}	8.06
BCA-H	5.36 ^{c}	0.35	2.94 ^{d}	0.31	51.54 ^{b}	6.64
C-H-WD	3.35 ^{b}	0.22	1.46 ^{b}	0.06	45.96 ^{ab}	10.02
BCA-H-WD	5.76 ^{bc}	0.38	2.29 ^{c}	0.28	44.32 ^{a}	7.59

Cucumis sativus plants subjected to *M. guilliermondii* inoculation and water deficit stress exhibited an early flowering phenotype (Figure 3). The Duncan multiple range test was applied to evaluate the mean number of flowers in response to different biotic and abiotic stress treatments ($p \leq 0.05$). Significant variations were identified between the groups (Figure 3D), suggesting distinct effects on flower production between treatments associated with biotic stress (P) and abiotic stress (WD, H). The results obtained for *M. guilliermondii* treated plants under water deficit stress showed significantly higher flowering compared to the control plants (9.46 $\pm$ 0.9 in BCA-WD and 3.08 $\pm$ 0.6 in C-WD), highlighting their unique impact on the floral response. These results underscore the differential influence of biotic and abiotic stressors on flower development in the experimental setup.

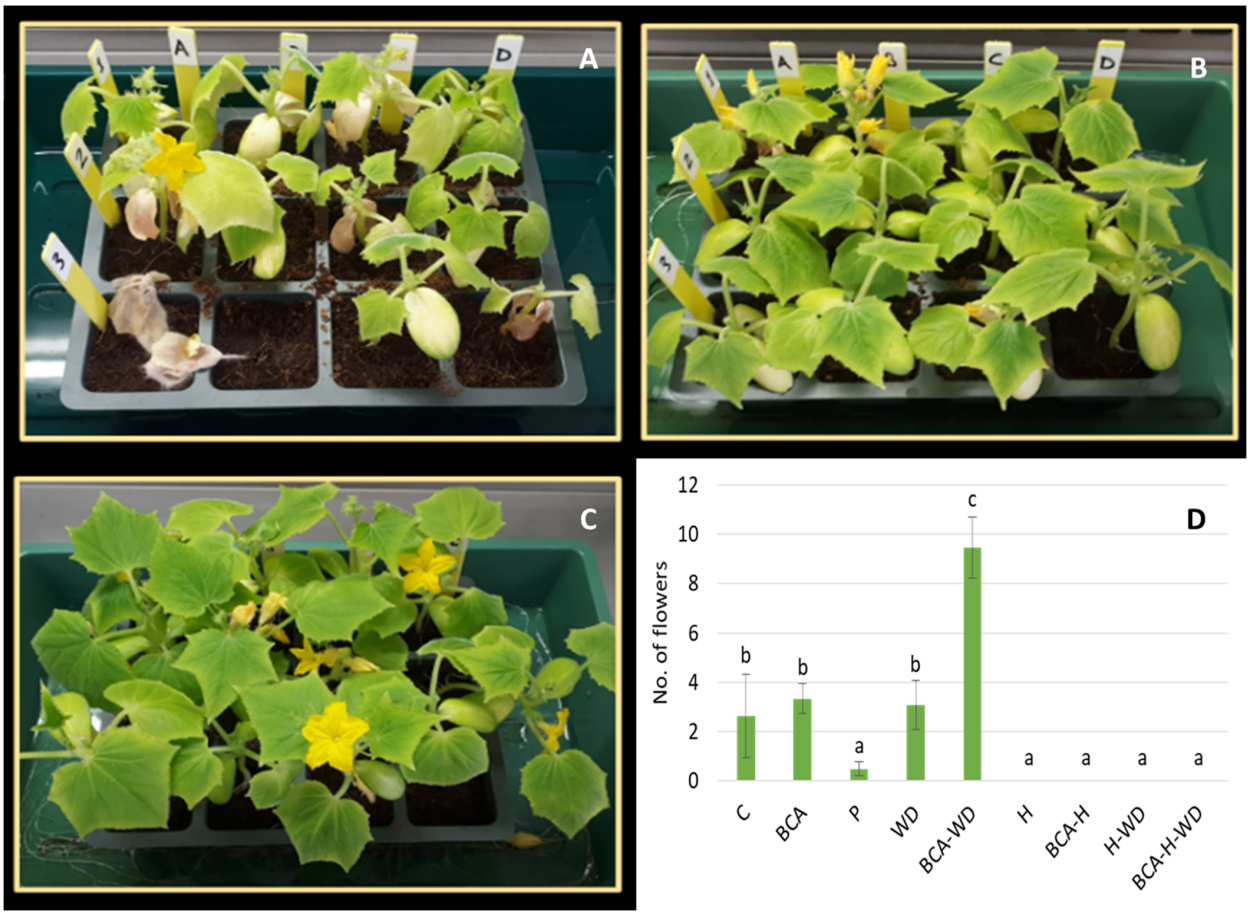

Figure 3. Flowering in cucumber plants growing under diverse conditions: (**A**) *F. oxysporum* inoculated (P); (**B**) Control; (**C**) *M. guilliermondii* inoculated (BCA). The histogram (**D**) represents the number of flowers in the plants from each treatment (n = 48); control (C), biocontrol agent (BCA), pathogen (P), heat (H), water deficit (WD), and combined treatments. Different letters accompanying the data in the figure denote statistically distinct groups ($p \leq 0.05$) via Duncan's test.

3.2. Photosynthetic Pigments Content

To investigate the impact of the yeast on *C. sativus* photosynthesis, the total chlorophyll, and carotenoid concentrations were quantified in both, the abiotic stress experiment and the biocontrol experiment (Table 1).

In the biocontrol experiment, notable differences in chlorophyll b and carotenoid levels were observed in the analysis of photosynthetic pigments in leaf samples (Table 1). Plants inoculated with *F. oxysporum* from the beginning (P) exhibited the lowest levels of chlorophyll b, with a reduction of up to 40% compared to the control. Conversely, treatments infected with the pathogen in a later round displayed higher concentrations of chlorophyll b.

Regarding carotenoids, treatments inoculated with the BCA (BCA and BCA-P) showed higher carotenoid levels than the other treatments (Table 1). However, the treatment infected with the pathogenic fungus (P) exhibited a significantly lower carotenoid concentration compared to the treatment where the BCA was inoculated before *F. oxysporum* infection (BCA-P), but it was not significantly different from the P-BCA treatment.

In the abiotic stress experiment, significant differences (Duncan test, $p \leq 0.05$) were observed only in chlorophyll b content between yeast-treated cucumber plants and control plants under water deficit conditions (Table 1). However, concerning carotenoids, yeast-treated (BCA) plants exhibited lower concentrations compared to the control in the water deficit treatment and the opposite trend under heat stress.

These findings suggest that the impact of the yeast on photosynthetic pigments is context-dependent.

3.3. Oxidative Stress

The levels of superoxide dismutase (SOD) enzyme activity exhibited significant differences (Duncan test, $p \leq 0.05$) among treatments, as detailed in Table 1.

Plants inoculated with the pathogen (P) displayed lower enzyme activity levels compared to those inoculated with the biocontrol agent early on (BCA and BCA-P). Similarly, the preventive biocontrol treatment with the antagonist (BCA-P) demonstrated higher SOD activity values than the two infectious treatments where the endophytic yeast was not employed (P and C-P). Furthermore, the curative treatment, where the antagonist was inoculated after infection (P-BCA), also exhibited significant differences in enzymatic activity compared to the treatment exclusively inoculated with *F. oxysporum* (P) (Table 1).

Concerning abiotic stress treatments, no significant differences were observed for *M. guilliermondii* inoculations in the cases of water deficit (WD) and combined heat and water deficit (WD + H). However, diverse effects were noted for the heat treatment (H), where the inoculation with *M. guilliermondii* resulted in reduced SOD enzymatic activity.

The qualitative results indicated a higher stained area (due to formazan deposits) in the leaves of control plants compared to those inoculated with the yeast in all treatments. This increased stained surface area suggests a higher concentration of superoxide anion in the leaves and, consequently, a more pronounced impact of the stress treatment on those plants. It could be inferred that the yeast effectively reduces the generated concentration of superoxide anion and demonstrates its capability to mitigate abiotic stress (Figure 4).

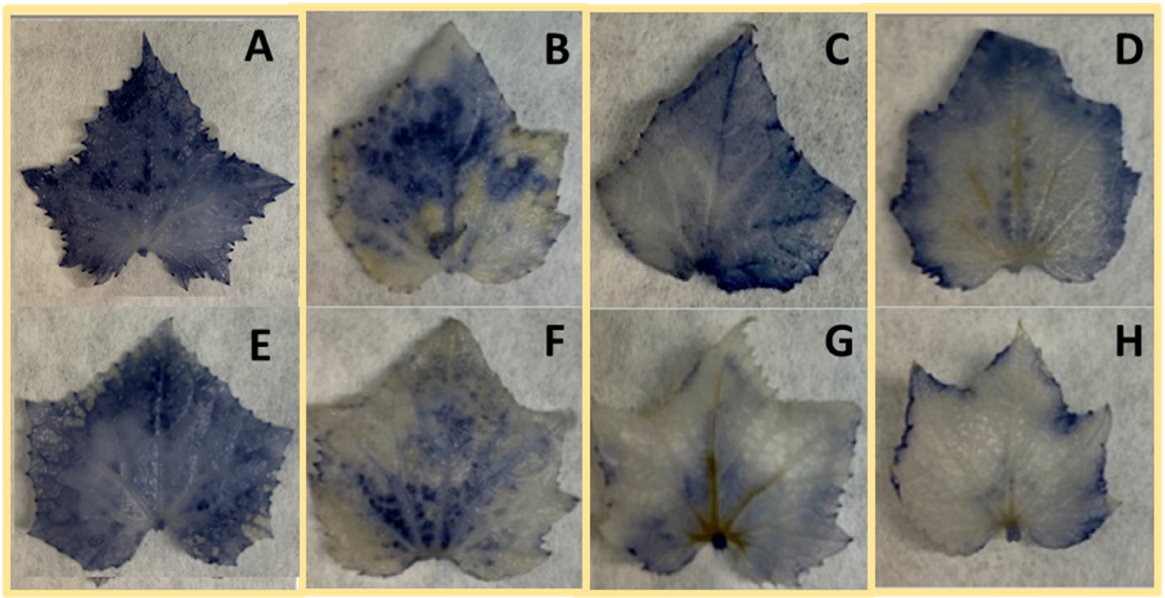

Figure 4. *Cont.*

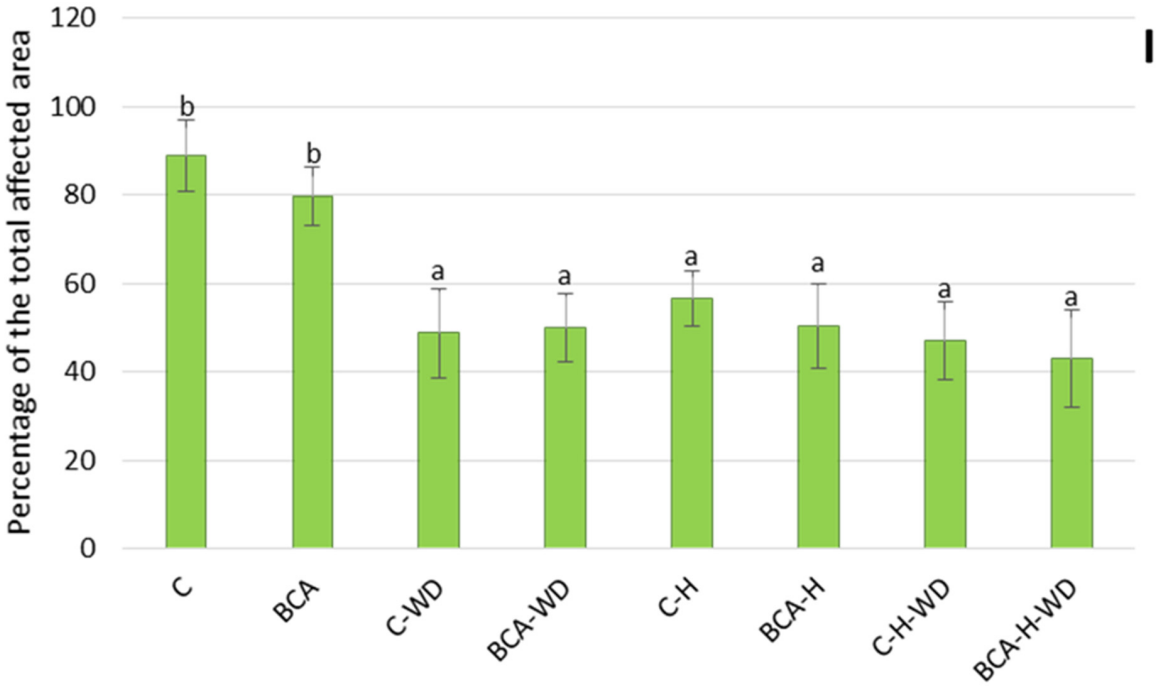

Figure 4. Representative NBT-stained leaves of cucumber plants of each abiotic stress treatment were non-inoculated (**A**–**D**) and inoculated with *M. guilliermondii* (**E**–**H**). (**A**)—Control; (**E**)—BCA; (**B**,**F**)—Water deficit; (**C**,**G**)—Heat stress; (**D**,**H**)—Combined heat and water deficit. The histogram (**I**) represents the quantitative analysis of the formazan deposit area in the leaves for the plants from each treatment (n = 48) control (C), biocontrol agent (BCA), pathogen (P), heat (H), water deficit (WD), and combined treatments. Different letters accompanying the data in the figure denote statistically distinct groups ($p \leq 0.05$) via Duncan's test.

3.4. Symptoms Analysis

Considerable differences were observed in the incidence of disease symptoms among the different treatments. Seedlings treated exclusively with the endophytic yeast or reserved as controls showed no characteristic symptoms of *Fusarium* wilt. On the other hand, all other treatments that involved inoculation with the pathogenic fungus exhibited varying degrees of disease symptoms (Figure 5).

The most severe impact on plants was observed when *F. oxysporum* was applied at its earliest stage, resulting in the highest percentage of mortality among all treatments (12.5%). The curative treatment with the biocontrol yeast slightly improved the symptom incidence compared to the previous case, reducing mortality (8.3%) and increasing the percentage of healthy plants (50%). However, the most significant reduction in *Fusarium* wilt symptoms incidence was observed in the preventive treatment, where the antagonist was initially inoculated, followed by *F. oxysporum*, compared to the treatment subjected to pathogen inoculation only in the second round.

These findings indicate that the early application of the antagonist yeast provides better disease control and significantly reduces symptom incidence compared to treatments relying solely on late pathogen inoculation. This highlights the potential of preventive biocontrol strategies in managing *Fusarium* wilt in *C. sativus*.

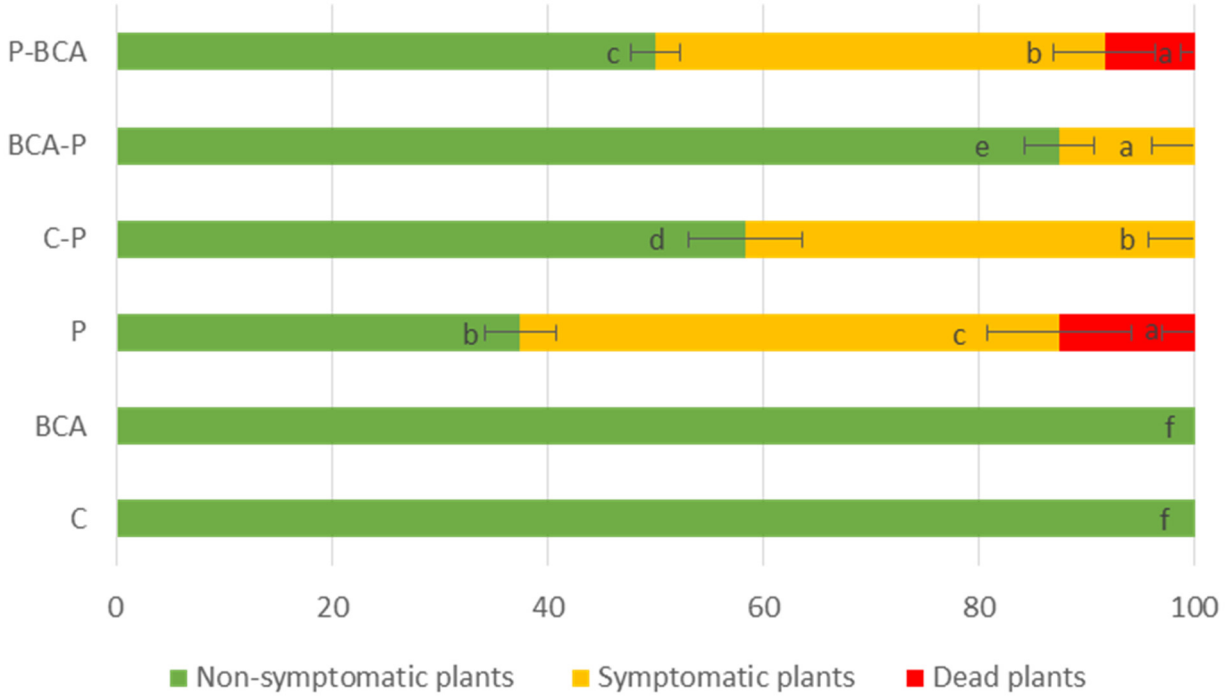

Figure 5. Incidence of symptoms of *Fusarium* wilt at the end of the experiment among the different treatments carried out. It is possible to distinguish between the treatments only inoculated in the first round of application: Control (C), *M. guilliermondii* (BCA), and *F. oxysporum* (P), and those subjected to a second round: C-P, BCA-P, and P-BCA. Different letters accompanying the data in the figure denote statistically distinct groups ($p \leq 0.05$) via Duncan's test.

3.5. In Vitro Evaluation of Meyerozyma guilliermondii Activity against F. oxysporum

Significant inhibition of radial growth of the pathogenic fungus was observed starting from the third day of growth, as indicated by the percentage of inhibition of radial growth (PICR) compared to the control (Figure 6). The PICR values remained relatively stable between days three and five, ranging around 17–18% inhibition. However, on the sixth and seventh day, a peak in growth suppression was observed, reaching a maximum PICR of 27.15% compared to the control plate.

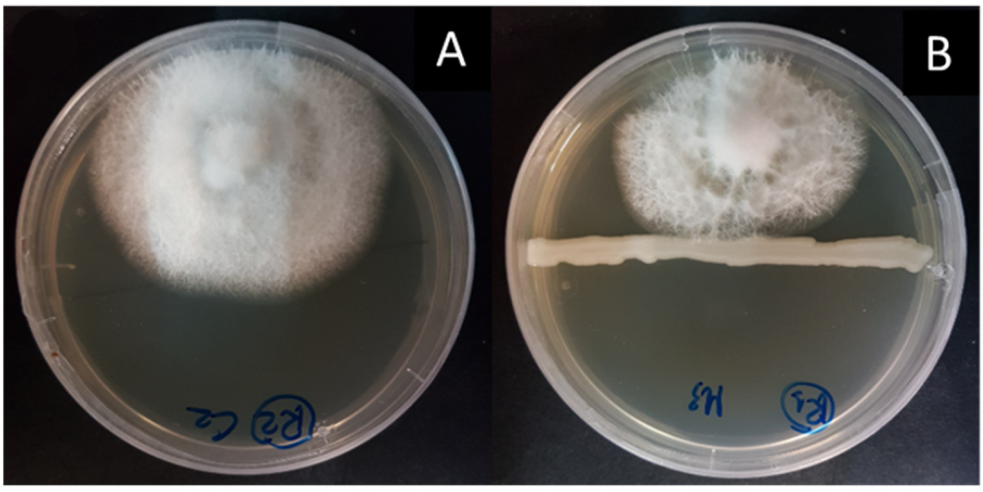

Figure 6. *Cont.*

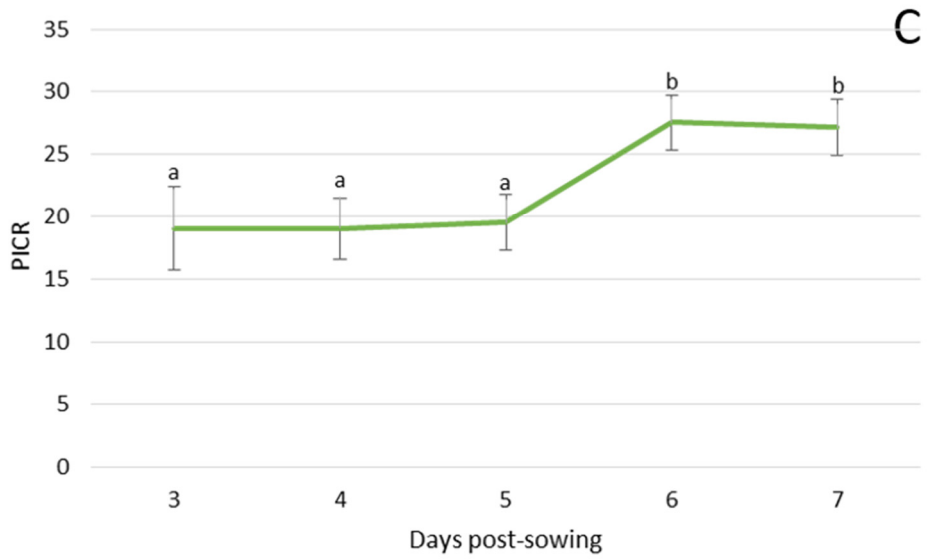

Figure 6. Dual confrontation on PDA medium between *M. guilliermondii* and *F. oxysporum* at 4 days post-sowing: (**A**) control; (**B**) the growth line of *M. guilliermondii* hinders the advancement of the pathogen. (**C**) Temporal variation in PICR (Percentage of radial growth inhibition of the pathogen) as a function of days post-sowing. Different letters accompanying the data in the figure denote statistically distinct groups ($p \leq 0.05$) via Duncan's test.

These findings demonstrate the effectiveness of the biocontrol agent in inhibiting the growth of the pathogenic fungus over time. The gradual increase in PICR indicates a progressive inhibition of the pathogen's radial growth, with a notable impact observed after the seventh day. These results highlight the potential of the tested antagonist in providing effective control against the pathogen and suggest its promising application as a biocontrol agent for managing fungal diseases in *C. sativus*.

4. Discussion

Approximately 30% of global crop production is lost due to pests and pathogens [26]. Climate change further intensifies this threat by altering the distribution and behavior of crop pests and pathogens. Therefore, it is not an overstatement to declare that ensuring crop health and global food security are the foremost challenges of the present century [27].

Although combatting plant diseases is a priority in ensuring the food security of millions of people, the drawbacks associated with the use of chemical products for disease control have led to the need for alternative biocontrol methods [28]. In this context, the use of beneficial microorganisms as control agents is considered one of the most promising approaches to adopting a more sustainable and rational crop management model [29,30].

Climate change is a significant obstacle that hampers plant growth and development on a global scale. It adversely affects growth, disrupts photosynthesis, and diminishes the physiological responses of plants. The fluctuations in the Earth's climate have garnered widespread attention among researchers, as these shifts have detrimental impacts on agriculture, leading to reduced crop productivity and compromised food security [31].

In the case of cucumber *Fusarium* wilt, the limitations associated with the use of chemical products for its control and eradication highlight the importance of focusing technological development on these new biocontrol methods involving symbiotic relationships with beneficial microorganisms [3,32].

In this study, cucumber plants were subjected to different stress treatments, and differences in the plant responses were observed depending on the nature of the treatment and the presence or absence of an endophyte.

The analysis of plant growth and development revealed significant differences among the treatments. Plants treated early with the yeast endophyte exhibited enhanced leaf area

and increased leaf number compared to the control and other treatments. In contrast, plants solely inoculated with the pathogen showed reduced leaf area and leaf number, indicating the detrimental impact of *F. oxysporum* on plant growth. The growth of fungal hyphae through the xylem ultimately results in the blockage of the vessels, causing a water deficit that hinders plant growth [33]. These results are consistent with previous studies that have reported the beneficial effects of endophytic fungi (nonclavicipitaceous endophytes) [34] and bacteria (i.e., *Burkholderia phytofirmans* [35]) on plant growth and development by promoting nutrient uptake and enhancing plant defense mechanisms.

The transition from the vegetative to reproductive growth phase, namely flowering, is a critical event in the life of a plant. Stress also regulates flowering, and various stress factors can induce or accelerate flowering or inhibit or delay it in a wide range of plant species [36]. Our results demonstrate that *M. guilliermondii* inoculation and water deficit stress led to an early flowering phenotype in *C. sativus* plants. This suggests that these factors play a significant role in regulating the timing of flowering in cucumber plants. Previous research has shown that stress conditions can have a profound impact on flowering time in various plant species. In response to adverse environmental conditions, plants may accelerate their reproductive development as a survival strategy. It is believed that the early onset of flowering allows plants to complete their life cycle and produce offspring before the stress conditions become more severe or detrimental [37].

The observed early flowering phenotype could be attributed to several factors. First, the presence of the beneficial yeast *M. guilliermondii* may have enhanced the overall physiological resilience of the plants, enabling them to cope with the combined stressors and initiate flowering earlier. It is known that certain beneficial microorganisms can stimulate plant growth and development, as well as enhance stress tolerance. This phenomenon has been studied in other plants as well, such as the medicinal plant *Coleus forskohlii*, which harbored the endophytic fungus *Phytophthora indica* and exhibited early flowering promoted by the microorganism. It appears that *P. indica* is capable of positively regulating flowering-related genes [38]. First, regarding thermal stress, the absence of early flowering could be attributed to the yeast present in the plants synthesizing indole-3-acetic acid (IAA). This adaptive strategy stimulates greater vegetative growth in cucumber plants [39].

Second, water deficit stress is known to be a significant environmental cue that influences flowering time. In *Arabidopsis*, water deficits have been shown to promote flowering under long-day conditions but delay it under short-day conditions [40]. The interaction between *M. guilliermondii* and these stressors may have further modulated the flowering response, resulting in the observed early flowering phenotype.

An abiotic or biotic stress situation, such as infection by *F. oxysporum*, induces the production of a high number of reactive oxygen species (ROS) within plant tissues [41]. These metabolites are highly damaging to the cell, so to prevent oxidative damage, their levels must be kept under control at all times through the combined action of antioxidant molecules and enzymes [42]. When an imbalance occurs in the homeostatic levels of ROS, they act as signaling molecules, mediating stress response processes and inducing plant defenses against pathogen attack. An example of this response mechanism is the increase in the activity of antioxidant enzymes such as superoxide dismutase (SOD), thereby compensating for the rise in ROS, such as the superoxide ion (O_2^-).

The effect of the treatment with the biocontrol agent can be seen in the increased levels of SOD in the leaves compared to the values of the treatments where the yeast was not applied. This suggests that the inoculation with the beneficial microorganism is capable of activating the plant's defenses through an increase in the activity of its defensive enzymes, preparing it for possible stress situations. Other authors have already demonstrated the ability of symbiotic microorganisms (plant growth promoting rhizobacteria; PGPR) to regulate the production of antioxidant enzymes, improving plant resistance against stress [43], and *M. guilliermondii* also contributes to mitigating oxidative stress in *V. vinifera* plants [19]. The staining analysis of superoxide anion further supported the yeast's potential role in reducing oxidative stress. The observed higher stained area in control plants

compared to yeast-treated plants indicated a higher concentration of superoxide anion and a greater impact of stress treatment on non-inoculated plants. The overall trends indicate that the presence of the yeast endophyte positively influences the plant's antioxidant defense system and mitigates the effects of stress.

The characterization of photosynthetic pigments revealed significant differences in the levels of chlorophyll b and carotenoids among the treatments. Plants inoculated with *F. oxysporum* from the beginning showed a significant reduction in chlorophyll b levels compared to the control, indicating the negative impact of the pathogen on chlorophyll biosynthesis. However, treatments infected with the pathogen in a later round exhibited higher levels of chlorophyll b, suggesting a potential compensatory response or different mechanisms of action at different stages of infection. These findings are consistent with previous studies that have reported alterations in chlorophyll levels as a response to pathogen infection [44].

Regarding carotenoids, plants inoculated with the yeast endophyte at the beginning of the experiment showed comparable levels to the control. However, the treatment infected with the pathogenic fungus in the second round of inoculation exhibited significantly lower carotenoid concentrations compared to the preventive treatment, where the endophytic fungus was inoculated before *F. oxysporum* infection. Interestingly, the application of the antagonist after the infection did not result in significant variations in carotenoid concentration compared to the treatment solely inoculated with *F. oxysporum*.

These observations suggest that the timing of yeast inoculation about pathogen infection plays a crucial role in influencing carotenoid accumulation in cucumber plants. It is noteworthy because carotenoids are the main component of chloroplast in plants, protecting plants from photooxidation under stress [45]. In general, yeast does not have a substantial positive impact on chlorophyll b content in cucumber plants under the tested abiotic conditions. However, in the determination of carotenoids, the results showed a higher concentration of carotenoids in yeast-treated cucumber plants compared to the control plants, with the exception of water deficit treatment. These findings align with the observations of chlorophyll content, suggesting that the yeast may not alter the effects of water stress on photosynthesis in cucumber plants. Further investigations are needed to elucidate the specific mechanisms underlying the yeast's influence on photosynthetic pigments and its potential implications for plant performance under stress conditions.

Finally, when evaluating the results of the dual confrontation on plates, it was confirmed that the yeast also has an antagonistic capability against *F. oxysporum*. The evaluation of disease symptoms revealed significant differences in symptom incidence among the treatments. Plants treated exclusively with the yeast endophyte or kept as controls did not exhibit any characteristic symptoms of *Fusarium* wilt, indicating the potential of the yeast to prevent disease development. On the other hand, treatments involving inoculation with the pathogenic fungus displayed varying degrees of disease symptoms. The most severe impact was observed when *F. oxysporum* was applied at its earliest stage, resulting in the highest percentage of mortality among all treatments. This highlights the importance of early intervention and preventive measures in managing *Fusarium* wilt in *C. sativus*. *Meyerozyma guilliermondii* has been shown to release various beneficial metabolites that can enhance plant defense mechanisms. These metabolites may include antifungal compounds, growth-promoting substances, and signaling molecules that activate plant immune responses. *M. guilliermondii* can enhance the plant's inherent defense mechanisms by modulating gene expression. For example, the interaction between *M. guilliermondii* and *Vitis vinifera* leads to the upregulation of several pathogenesis-related (PR) genes, such as thaumatin-like protein (*VviTL1*), β-1,3-glucosidase (*Vcgns1*), and chitinase (*Vcchit1b*). The increased activity of β-1,3-glucanase and chitinase observed in grape plants treated with *M. guilliermondii* indicates that the yeast can induce the production of enzymes critical for fungal pathogen resistance [19].

Beneficial microorganisms, like *M. guilliermondii* yeast, positively influence plant growth, development, and flowering while mitigating abiotic and biotic stress. They acti-

vate plant defenses, regulate antioxidant enzymes, and influence photosynthetic pigments. Additionally, the yeast displays antagonistic capability against *F. oxysporum*. These findings highlight the potential of symbiotic relationships with beneficial microorganisms for sustainable crop management and disease control.

5. Conclusions

Our study highlights the significant role of *M. guilliermondii* yeast in enhancing plant resilience and productivity of cucumber under various stress conditions. The yeast endophyte significantly improved vegetative growth, as evidenced by increased leaf area and number, even in the presence of the pathogen *F. oxysporum*. Additionally, the combined stress of yeast inoculation and water deficit led to early flowering in cucumber plants, indicating these factors' potential to regulate reproductive development and enable plants to complete their life cycle under adverse conditions. The yeast also activated plant defense mechanisms by increasing antioxidant enzyme levels, such as superoxide dismutase (SOD), which are vital for mitigating oxidative stress.

Moreover, *M. guilliermondii* influenced the regulation of photosynthetic pigments, maintaining higher carotenoid concentrations in stressed plants, thereby protecting them from photooxidation. Importantly, the yeast demonstrated antagonistic properties against *F. oxysporum*, significantly reducing disease symptoms and plant mortality, particularly when applied early. These findings underscore the potential of *M. guilliermondii* in sustainable crop management, offering an effective alternative to chemical treatments for stress alleviation and aligning with the goals of enhancing global food security and environmental sustainability. Future research needs to delve deeper into the specific mechanisms of oxidative stress mitigation by *M. guilliermondii*, as this is crucial for plant resilience and provides a comprehensive defense against both abiotic and biotic stresses.

6. Patents

This study has resulted in the following patent: Strain of *Meyerozyma guilliermondii*, Composition, and Methods to Promote Plant Growth and Activate Plant Defenses (Patent CECT13190).

Author Contributions: Conceptualization, A.G.-G., D.B.R.-C., S.I.S. and B.P.L.; Formal analysis, D.B.R.-C. and S.I.S.; Funding acquisition, A.G.-G. and B.P.L.; Investigation, D.B.R.-C. and S.I.S.; Methodology, A.G.-G., D.B.R.-C., S.I.S. and B.P.L.; Project administration, B.P.L.; Resources, A.G.-G. and B.P.L.; Supervision, A.G.-G.; Validation, A.G.-G. and B.P.L.; Visualization, D.B.R.-C., S.I.S. and B.P.L.; Writing—original draft, A.G.-G.; Writing—review and editing, A.G.-G. and B.P.L. All authors have read and agreed to the published version of the manuscript.

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