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# Control of cucumber downy mildew disease under greenhouse conditions using biocide and organic compounds *via* induction of the antioxidant defense machinery

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**Cucumber as an important vegetable crop faces a variety of abiotic and biotic stresses, especially the recently appeared fungicide-resistant strains of *Pseudoperonospora cubensis*, the causal agent of downy mildew disease. Herein, the present study aimed to evaluate the effect of *Trichoderma asperellum* strain T34 as a biological commercial product and potassium phosphite (KPHI) against *P. cubensis* and investigate the ability of these compounds to activate the plant defense system to suppress *P. cubensis* infection. In two separate experiments, the foliar applications with T34 or KPHI significantly reduced the severity of downy mildew disease and the area under the disease progress curve compared to water control. Scanning electron microscopy (SEM) of *P. cubensis* treated with T34 and KPHI showed shrunken and distorted sporangiophores and sporangia. Moreover, two tested compounds enhanced cucumber plants' growth and yield parameters under greenhouse conditions. The tested compounds protected the membrane permeability of infected cucumber leaves and significantly reduced electrolyte leakage (EL %) compared to water control. These findings were associated with activating enzymatic antioxidant enzymes (catalase, peroxidase, and polyphenol oxidase). Our findings suggest that T34 and KPHI can be environmentally safe alternatives to chemical fungicides to control downy mildew disease in cucumber and other cucurbit crops.**

**Keywords** Biological control, Cucumber, Downy mildew, *Pseudoperonospora Cubensis*

Cucumber (*Cucumis sativus* L., family *Cucubitaceae*), is economically, one of the most popular vegetable crops grown around the world both in the open fields and under greenhouse conditions[1]. Furthermore, it ranks fourth among the most popular vegetable crops in the world[2]. In 2022, the global production of cucumber reached 94,718,396.55 tons and the harvested area was 2,174,347 hectares[3]. Egypt cultivated 20,403 hectares of cucumber, yielding 23.74 tons per hectare with a total production of 484424.68 tons in the same year[3]. Cucumber plants are attacked by several phytopathogens such as fungi, fungi-like organisms, viruses, and bacteria which cause serious diseases and crop loss[4].

However, downy mildew disease, caused by the oomycetes *Pseudoperonospora cubensis*(Berkeley & Curtis) Rostov, is the most dangerous and damaging foliar disease affecting cucumber production worldwide. It can lead to yield losses of up to 100%[1,5]. Under favorable conditions of heat and humidity, the disease rapidly spreads and can devastate crops in a short time. This severe affliction not only reduces the quality and productivity of cucumber crops but also results in significant economic losses in all growing regions[6].

*P. cubensis*is an obligatory, air-borne, and biotrophic pathogen that can attack the shoot system of cucumber and other cucurbit plants from seedlings to the mature stage[7,8]. The characteristic symptoms of cucumber downy mildew include yellow spots on the upper surface of the leaves, while the lower surface displays gray, fluffy

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growths, which are the sporangiophores of the fungus[9]. Effective management of cucumber downy mildew is crucial to mitigate the damage caused by the disease and to minimize crop losses.

Generally, there are several ways to control cucumber downy mildew, such as planting resistant cultivars, rotating crops, using chemical control, biological control, and so on[9]. Chemical fungicides are the most common strategy for controlling downy mildew. However, the widespread and regular use of chemical fungicides poses several environmental and health risks to humans, animals, and non-target organisms as well as others. Moreover, the widespread application of fungicides leads to new strains of the pathogen that resist chemical fungicides[10,11]. Therefore, it is essential to search for a safe, low-cost alternative strategy that has no harmful impact on the environment, humans, or non-target organisms.

Biological control of cucumber downy mildew is one of the possible options to fully or partially reduce the use of harmful chemical fungicides. Furthermore, its low cost and environmental friendliness make it one of the most sustainable alternative strategies for controlling plant diseases. In general, biological control of cucumber downy mildew relies on different mechanisms, such as antagonism, competition, or production of secondary metabolites[11]. Several effective bioagents have been applied for controlling cucumber downy mildew, for instance, *Trichoderma* species work well as biocontrol agents against a variety of plant diseases. Different species of *Trichoderma* can control cucumber downy mildew disease such as *Trichoderma harzianum*[12].

Furthermore, the effectiveness of *Trichoderma viride* and *T. asperellum* in managing cucumber downy mildew disease was shown in another study conducted by Shoukry et al.[4]. Although many plant diseases can be effectively controlled by various *Trichoderma* species, few studies have been done on how well these species work to prevent cucumber downy mildew disease. Additionally, not enough investigations have been conducted on the physiological and biochemical mechanisms underlying their usage. On the other hand, mineral nutrients play a vital role in the life cycle of plants and are necessary for plant growth.

Plants require amounts of potassium (K), phosphate (P), and nitrogen (N) for growth and biomass production. Mineral nutrients play a critical role in the interactions between plant hosts and phytopathogens, with varying concentrations of these minerals affecting plant resistance to diseases. This can occur through several mechanisms, including influencing enzyme activity, cell wall permeability, and amino acid synthesis[13]. In regard, a previous study has shown that potassium possesses antifungal properties against *Botrytis cinerea* and *Sclerotinia sclerotiorum*, the pathogens responsible for gray mold and white mold in cucumber plants, respectively[14,15]. Additionally, potassium has been found to reduce the incidence of downy mildew in sweet basil and *Plantago ovata*[16].

The fertilization with potassium reduced the incidence of downy mildew in cucumber[17]. Moreover, potassium phosphite (KPHI) reduced the infection with *Pythium ultimum* var. *ultimum* on cucumber plants and *Phytophthora infestans*on tomato plants[18,19]. Although potassium phosphite was used in controlling several plant diseases, its relation with the host defense system and the antioxidant enzyme stimulation was not extensively studied. Herein, we investigated the potential application of *Trichoderma asperellum* strain T34 as a biological product and potassium phosphite solution against *P. cubensis*, the causal agent of downy mildew disease in cucumber plants under greenhouse conditions. In addition, the physiological and biochemical effects of these two compounds on *P. cubensis*-infected cucumber plants were investigated. Moreover, we suggest that *T. asperellum* strain T34 and KPHI are sustainable solutions to chemical fungicides for controlling plant diseases, totally or partially.

## Materials and methods

### Tested compounds

In the current study, we investigated the potential use of two commercial products to manage cucumber downy mildew disease caused by *P. cubensis* under greenhouse conditions. The first product was *Trichoderma asperellum* strain T34 12% WP, is a biological agent standardized at a concentration of $1 \times 10^9$ spores/g, and the second was Atlante, a potassium phosphite (KPHI) solution containing potassium and phosphorus. Additionally, the chemical fungicide Orondis (containing Oxathiapiprolin 2.77% and Mandipropamid 23.10%) was used as a positive control, and distilled water served as the negative control. These products were sourced from Shoura Chemical Company. The application rates for the treatments were as follows: *Trichoderma asperellum* strain T34 12% WP was applied at a rate of 0.5 g per liter of water, and Atlante (KPHI) was used at a concentration of 2.5 ml per liter of water. Orondis was applied at the recommended dose of 0.5 ml per liter of water.

### Greenhouse experiments

Two greenhouse experiments (9 m × 40 m) were conducted during the spring season of 2024 at the Vegetable Diseases Research Department, Plant Pathology Research Institute (PPRI), Agricultural Research Center (ARC), Egypt, located in Kafr El-Sheikh Governorate (31.0748°N, 30.9401°E). These experiments aimed to evaluate the effect of *Trichoderma asperellum* strain T34 (T34) and potassium phosphite (KPHI) on cucumber plants naturally infected with *P. cubensis*. The cucumber seeds used were from the American hybrid variety B33, imported through the Al-Mohandas Seed Company. After 21 days, the seedlings were transplanted into ARC greenhouses, with rows and plants spaced 50 cm apart. Approximately 45 days post-transplantation, when the cucumber plants began to show symptoms of downy mildew, they were treated with the tested compounds. Four foliar spray treatments were applied at four-day intervals. The experiments were completely randomized, with 12 biological replicates for each treatment to ensure statistical reliability. Greenhouse conditions were carefully controlled to promote pathogen development and plant growth, maintaining high relative humidity (around 90%) and optimal temperatures between 20 °C and 25 °C. Plants were grown in loamy soil, and a drip irrigation system ensured consistent and uniform irrigation, maintaining optimal soil moisture levels throughout the experiment. A balanced fertilizer is applied according to recommended agricultural practices. For pest control,

Lambda 5%, containing the active ingredient lambda-cyhalothrin, was used. All experimental procedures were conducted with the approval of the Agricultural Research Center, ensuring compliance with institutional guidelines and ethical standards.

## Disease measurements

Disease severity (DS%) was assessed every 4 days using a nine-point (0–9) scale to determine the percentage of leaf area covered by lesions, as described by Call et al. (2012)[20]. Ten plants were randomly selected for each replicate, and disease severity was rated as follows: (0 = 0%, 1 = 1–5%, 2 = 6–10%, 3 = 11–20%, 4 = 21–30%, 5 = 31–50%, 6 = 51–65%, 7 = 66–80%, 8 = 81–99%, and 9 = 100%). The disease severity percentage was calculated using the following formula:

$$\text{Disease severity } \% = \frac{\Sigma\ (\text{n} \times \text{v})}{9\ \text{N}} \times 100$$

Where: n = number of infected leaves in each category; v = numerical value assigned to each category; N = total number of infected leaves.

Moreover, the Area under the Disease Progress Curve (AUDPC) for each replicate was calculated following the method suggested by Pandey et al. (1989)[21], using the formula:

$$\text{AUDPC} = \text{D}[1/2(\text{Y}_1 + \text{Y}_\text{K}) + (Y_2 + Y_3 + \cdots + Y_{\text{k}-1})]$$

Where: D = time interval between assessments; $Y_1$ = disease severity at the first assessment; $Y_k$ = disease severity at the final assessment; $Y_2$, $Y_3$,… $Y_{k-1}$ = disease severity at intermediate assessments.

## Vegetative growth, photosynthetic pigments, and yield parameters

Plant height (cm) was recorded at 10, 20, 30, and 40 days post-treatment (dpt). Additionally, the number of fruits per plant and the fruit yield (kg per plant) at sale size were recorded at the end of the experiment. Furthermore, the percentage increase in fruit yield over the control was calculated to evaluate treatment efficacy. Photosynthetic pigments, including chlorophyll a and chlorophyll b, were measured using spectrophotometric methods as described by Moran and Porath (1980)[22]. Briefly, 1000 mg of fresh cucumber leaves were cut into small pieces and immersed overnight in 5 mL of N, N-dimethylformamide at 4 °C. The solution was then filtered through Whatman 47 mm GF/C filter paper. The absorbance of the filtrate was measured against a blank of N, N-dimethylformamide at wavelengths of 663 nm for chlorophyll a and 647 nm for chlorophyll b. The chlorophyll content was reported as mg $g^{-1}$ fresh weight (mg $g^{-1}$FW) using the following formulas according to Moran and Porath[22].

$$Chl.a = 12.76A663 - 2.79A647(mg/gFW).$$

$$Chl.b = 20.76A647 - 4.62A663(mg/gFW).$$

## Scanning electron microscopy (SEM) observation

Scanning electron microscopy (SEM) was performed to study the effect of T34 and KPHI, in addition to both controls on sporangia and sporangiophores of *P. cubensis*. Cucumber leaves infected with downy mildew were collected 24 h post-treatment (hpt). The method for processing cucumber leaves was based on Harley and Fergusen[23]. Sections of tissue measuring about 4 $mm^2$ were fixed for 24 h at 4°C in 0.2 M phosphate buffer (pH 7.2) containing 3% glutaraldehyde. These were then exposed for one hour at 25°C to osmium tetroxide (1% $OsO_4$). Samples were dried to the critical point after being passed through acetone concentrations that increased in concentration. SEM photographs were performed by using JEOL-JSM-5200 LV at the electronic microscope unit, Faculty of Agriculture, Mansoura University.

## Antioxidant enzymes activity

Cucumber leaves were collected at 0, 24-, 48-, and 72-hpt to assess the activity of three antioxidant enzymes including catalase (CAT), guaiacol-dependent peroxidases (POX), and polyphenol oxidase (PPO). The activity of three selected antioxidant enzymes was colorimetrically assessed at 25°C by a UV-160 spectrophotometer (Shimadzu, Japan). Briefly, approximately 500 mg of freshly homogenized leaves were extracted using 3 mL of Tris buffer (50 mM, pH 7.8) that contained 7.5% polyvinylpyrrolidone (PVP) and 0.001 M ethylenediaminetetraacetic acid (EDTA-$Na_2$). Subsequently, the mixture was centrifuged at 12,000 rpm for 20 min at 4°C according to[24].

## Catalase activity

The CAT enzyme activity was conducted using the methodology of Aebi (1984)[25]with minor modifications by[26]. The mixture of reaction was 75 µL of pure enzyme extract, 150 µL of 269 mM $H_2O_2$ solution, and 3 mL of 0.1 mM sodium phosphate buffer (pH 6.5). The extinction coefficient of $H_2O_2$ was 0.040 $mM^{-1}$ $cm^{-1}$, and the CAT activity was determined by observing the decomposing process of $H_2O_2$ at 240 nm in a quartz cuvette.

## Peroxidase activity

Moreover, the activity of the POX enzyme was determined using the method mentioned by Harrach et al.[27] with minor modifications as described by El-Nagar et al.[24]. Briefly, a mixture of 100 µL of guaiacol, 100 µL of 12 mM $H_2O_2$, and 2.2 mL of 100 mM sodium phosphate buffer (pH 6.0) was added to approximately 100 µL of crude

enzyme extract. Using an extinction value of 26.6 $mM^{-1}$ $cm^{-1}$, the development of conjugate was recorded by an increase in absorbance at 436 nm.

### Polyphenol oxidase activity

The activity of the PPO enzyme was determined according to the method mentioned by[28]. The reaction mixture included 3 mL of buffered catechol solution (0.01 M), newly made in 0. 1 M phosphate buffer (pH 6.0). To start the reaction, 100 µL of crude enzyme extract was added. PPO activity was calculated by recording the changes in the absorbance at 495 nm every 30 s for three minutes.

### Electrolyte Leakage (EL %)

Electrolyte Leakage (EL% ), was measured at 72 hpt by the methods of Whitlow et al.[29], and[30]. Briefly, 20 discs (1cm2) of cucumber leaves were placed into individual flasks filled with 25 mL of deionized water (Milli-Q 50, Millipore, Bedford, MA, USA). Flasks were rotated for twenty hours at room temperature to encourage electrolyte leakage from damaged tissues. Using an Acromet AR20 electrical conductivity meter, the initial electrical conductivity of each flask was noted. Subsequently, flasks were submerged for one hour at 80∘C in a hot water bath to promote cell rupture. After the flasks were shaken for 20 h at 21∘C on the Innova 2100 platform shaker, each flask's final conductivity was noted. The EL % was measured using the following formula:

$$EL\% = (Initial\ conductivity)/(Final\ conductivity) \times 100$$

### Statistical analyses

All experiments were done using a completely randomized design with twelve replicates for each treatment. The analysis of variance technique (ANOVA) was used to statistically examine all obtained results. Subsequently, the Tukey–Kramer honestly significant difference test (Tukey HSD, $p \leq 0.05$) as a post hoc analysis for pairwise comparisons.

## Results

### Effect of T34 and KPHI on disease symptoms and disease development of downy mildew

The results from both experiments demonstrated that treatment with *Trichoderma asperellum* strain T34 (T34) and potassium phosphite (KPHI) effectively reduced downy mildew symptoms on cucumber leaves compared to the water control at 8 days post-treatment (dpt) (Fig. 1A). T34 and KPHI significantly reduced disease severity (%) and the area under the disease progress curve (AUDPC) for cucumber downy mildew in both Experiment I and Experiment II (Fig. 1B–E). Among the treatments, T34 proved to be the most effective. T34-treated plants recorded the lowest disease severity percentages at 8, 12, and 16 dpt, with values of 13.93 ± 0.94%, 21.77 ± 1.69%, and 32.23 ± 0.56%, respectively (Fig. 1B). Additionally, T34 achieved the lowest AUDPC value of 235.67 ± 11.39 (Fig. 1D) in Experiment I, followed closely by KPHI. The outcomes of Experiment II mirrored those of Experiment I, consistently showing the effectiveness of T34 and KPHI in controlling cucumber downy mildew (Fig. 1C and E).

### T34 and KPHI improve the plant height and photosynthetic pigments profile of infected cucumber plants

Cucumber plants showed a significant increase in height (cm) when treated with *Trichoderma asperellum* strain T34 (T34) and potassium phosphite (KPHI) compared to the water-treated control in both experiments (Fig. 2A and B). At 30 and 40 days post-treatment (dpt), plants treated with T34 recorded the highest heights of 177.59 ± 5.21 cm and 195.44 ± 2.58 cm, respectively. This was followed by KPHI-treated plants, which reached 158.29 ± 8.55 cm and 174.61 ± 4.47 cm in experiment I (Fig. 2A). Similar results were observed in experiment II, where T34 again resulted in the highest plant heights of 193.59 ± 3.81 cm and 206.45 ± 2.81 cm (Fig. 2B), with KPHI treatment coming in second. In experiment I, the foliar application of T34 and KPHI significantly increased chlorophyll a (Fig. 2C) and chlorophyll b (Fig. 2D) contents compared to the water-treated plants. Notably, T34 treatment resulted in the highest chlorophyll a and chlorophyll b levels at 72 h post-treatment (hpt), measuring 4.23 ± 0.38 mg/g FW and 1.87 ± 0.08 mg/g FW, respectively. KPHI treatments also elevated chlorophyll levels to 3.53 ± 0.12 mg/g FW for chlorophyll a and 1.51 ± 0.11 mg/g FW for chlorophyll b (Fig. 2C and D). Additionally, Orondis fungicide treatment improved chlorophyll a and b levels at 72 hpt, recording 3.97 ± 0.18 mg/g FW and 2.33 ± 0.20 mg/g FW, respectively (Fig. 2C and D). Similarly, in experiment II, chlorophyll a and b contents in cucumber leaves increased significantly as a result of T34 and KPHI treatments (Fig. 2E and F).

### Effect of tested compounds on yield parameters

Figure 3 demonstrates the effects of the tested compounds on the yield parameters of cucumber plants grown under greenhouse conditions. The assessed parameters include the number of fruits, the weight of the fruits, and the percentage increase compared to the control treatment. These results are presented for both experiment I (Fig. 3A-C) and experiment II (Fig. 3D-F). In experiment I the exogenous application of commercial products, T34 and KPHI, significantly increased the number and weight of fruits per plant in comparison to the water control plants (Fig. 3A and B respectively). T34-treated cucumber plants yielded a considerably higher number (44 ± 2.02) and weight of fruits (3.43 ± 0.21 kg $plant^{-1}$) than the water control group. Without a difference, KPHI treatments ranked second (41 ± 1.20) and (3.13 ± 0.09 kg $plant^{-1}$). The number of cucumber fruits in the fungicide-treated plants was 42.33, which means there were no significant differences between T34 and KPHI (Fig. 3A-C). Experiment II corroborated these findings, consistently demonstrating that both T34 and KPHI treatments significantly increased the number and weight of fruits when compared to the control (Fig. 3D-F).

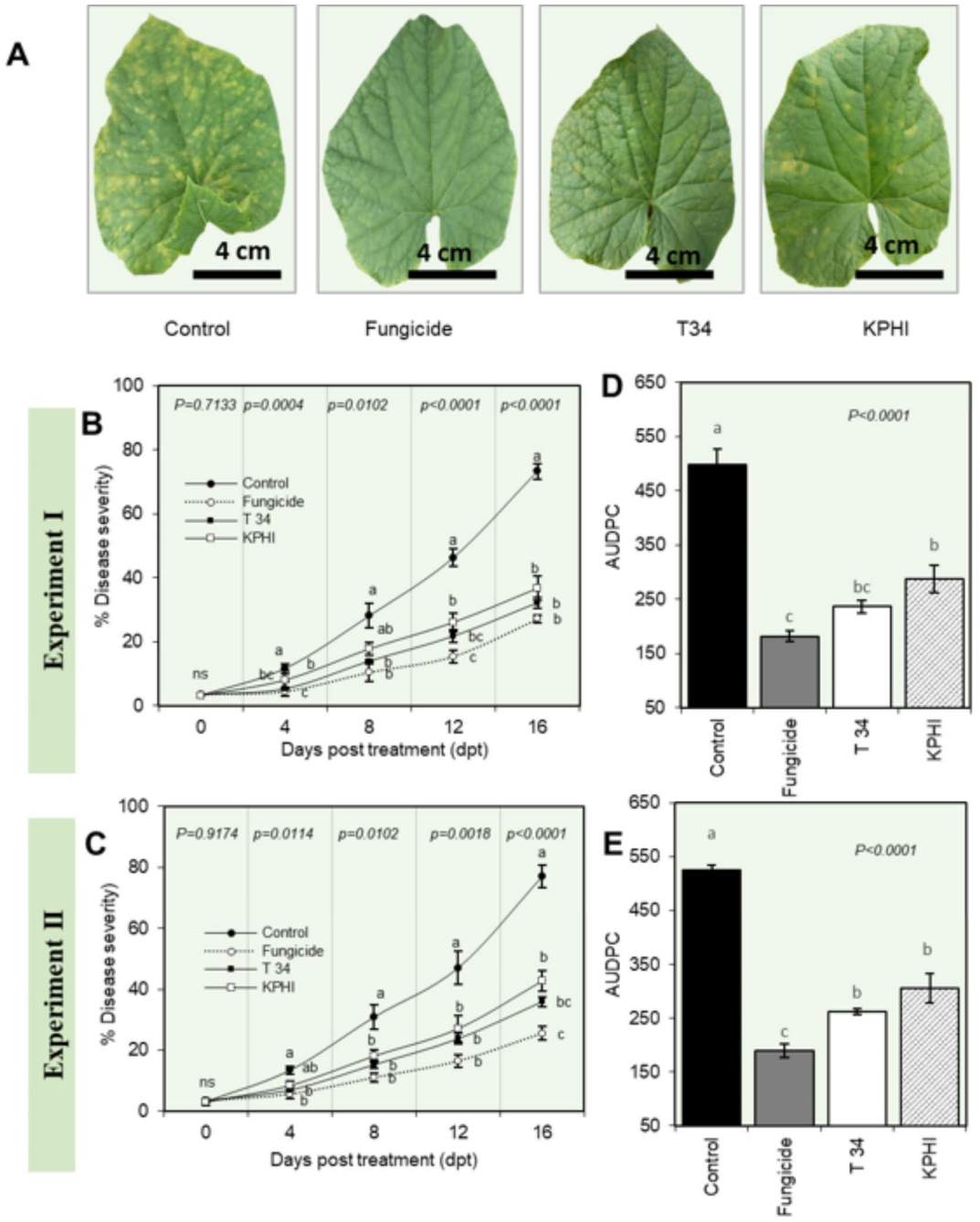


**Fig. 1**. Effect of two tested compounds, T34 and KPHI, on the development of cucumber downy mildew disease caused by *P. cubensis* under greenhouse conditions. (A) Disease symptoms of cucumber downy mildew at 8 days post-treatment. (B,C) The disease severity of downy mildew disease on cucumber plants after the treatment with T34 and KPHI in experiment I and experiment II respectively. (D,E) The area under disease progress curve (AUDPC) of downy mildew disease on cucumber plants after the treatment with T34 and KPHI in experiment I and experiment II respectively. Values show the means ± standard errors. Different letters denote statistical differences among treatments according to Tukey's HSD test ($p < 0.05$).

## Scanning Electron Microscopy (SEM)

To explore the mechanisms involved in the suppression of sporangiophores and sporangia of *P. cubensis*, the causal agent of cucumber downy mildew, by treatments with *Trichoderma asperellum* strain T34 (T34), potassium phosphite (KPHI), and the fungicide Orondis, the morphological changes in the hyphae of *P. cubensis* treated with these compounds were examined using Scanning Electron Microscopy (SEM) at 24 h post-treatment (hpt). In the SEM observations, the sporangia and sporangiophores of *P. cubensis* in the control treatment appeared regular and normal (Fig. 4A). However, the sporangia and sporangiophores of *P. cubensis* treated with T34,

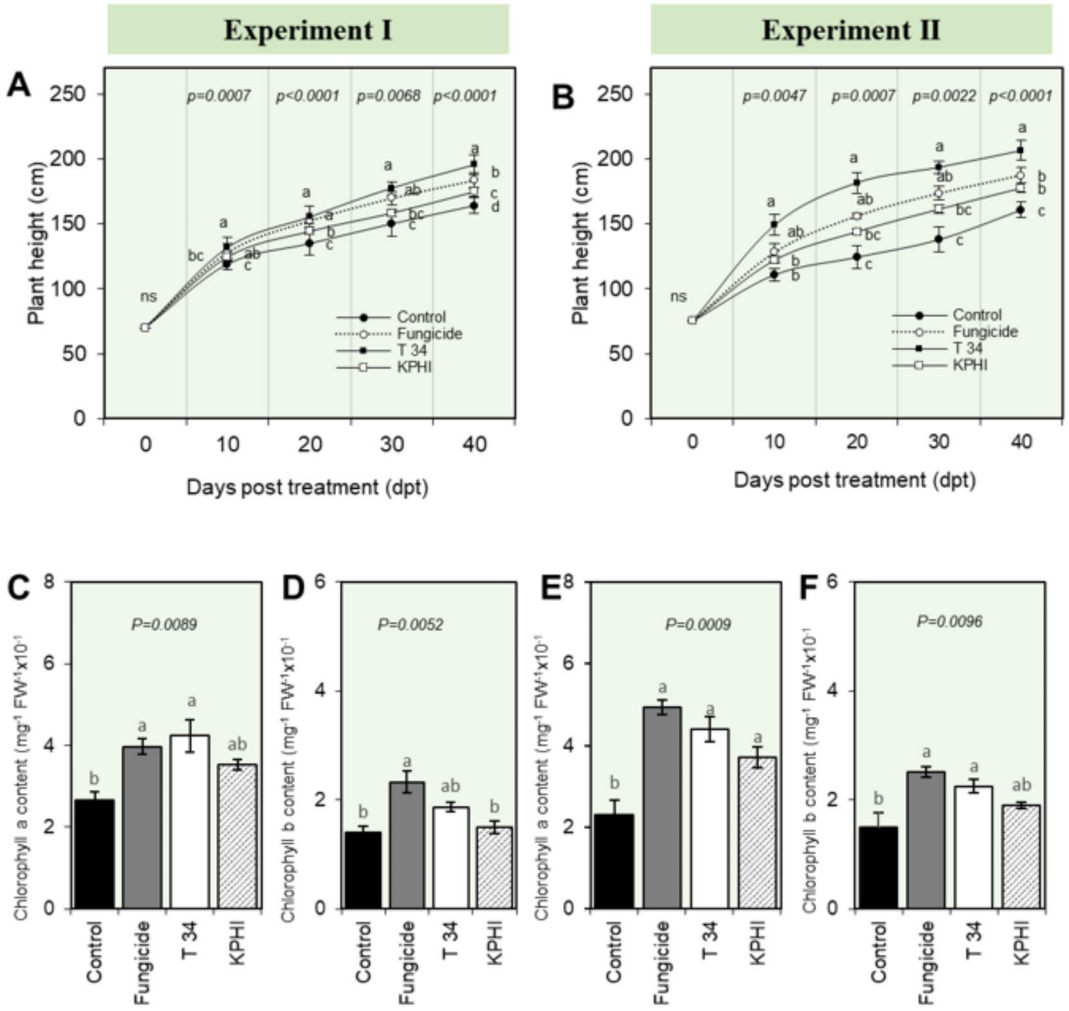


**Fig. 2**. . Effect of two tested compounds, T34 and KPHI, on the plant height (cm) and photosynthetic pigments of cucumber plants infected with *P. cubensis* under greenhouse conditions. (A, B) Plant height (cm) in experiment I and experiment II respectively. (C, D) Chlorophyll a and Chlorophyll b in experiment I respectively. (E, F) Chlorophyll a and Chlorophyll b in experiment II respectively. Values show the means ± standard errors. Different letters denote statistical differences among treatments according to Tukey's HSD test ($p < 0.05$).

KPHI, and Orondis exhibited considerable damage. These included turgor loss of sporangia, cell deformation, irregular shrinkage, and cellular collapse (Fig. 4B-D).

## T34 and KPHI enhance the antioxidant enzyme activities

The activities of three antioxidant enzymes including, catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO), were assessed at 0, 24, 48, and 72 h post-treatment (hpt) with the tested compounds (Fig. 5). Overall, the enzymatic activities of CAT, POX, and PPO were notably higher following the exogenous application of T34, KPHI, and fungicide compared to the water control treatment in both experiment I and experiment II (Fig. 5A). In experiment I, CAT activity significantly increased 24 hpt with T34, reaching $50.94 \pm 2.29$ µm $H_2O_2$ $g^{-1}$ FW $min^{-1}$. In contrast, it took 48 h post-treatment for the fungicide to achieve its peak CAT activity of $61.06 \pm 1.47$ µm $H_2O_2$ $g^{-1}$ FW $min^{-1}$, followed by KPHI with $43.20 \pm 2.17$ µm $H_2O_2$ $g^{-1}$ FW $min^{-1}$ at the same time point (Fig. 5A). POX activity rapidly increased 24 hpt with the fungicide, recording the highest value of $43.67 \pm 3.38 \times 10^{-3}$ µm Tetraguaiacol $g^{-1}$ FW $min^{-1}$ before decreasing. However, T34 achieved the highest POX activity at 48 hpt (Fig. 5B). Although PPO activity gradually increased and peaked at 72 hpt across all treatments, there were no significant differences between the tested compounds and the fungicide (Fig. 5C). In experiment II, the results for POX and PPO activities were consistent with those of experiment I (Fig. 5E and F). However, for CAT activity, KPHI replaced the fungicide and recorded the highest peak at 48 hpt (Fig. 5D).

## T34 and KPHI reduce electrolyte leakage (%)

Generally, the cell membrane permeability of cucumber leaves increased due to downy mildew infection in experiment I and experiment II (Fig. 6A and B). However, foliar application with commercial products (T34

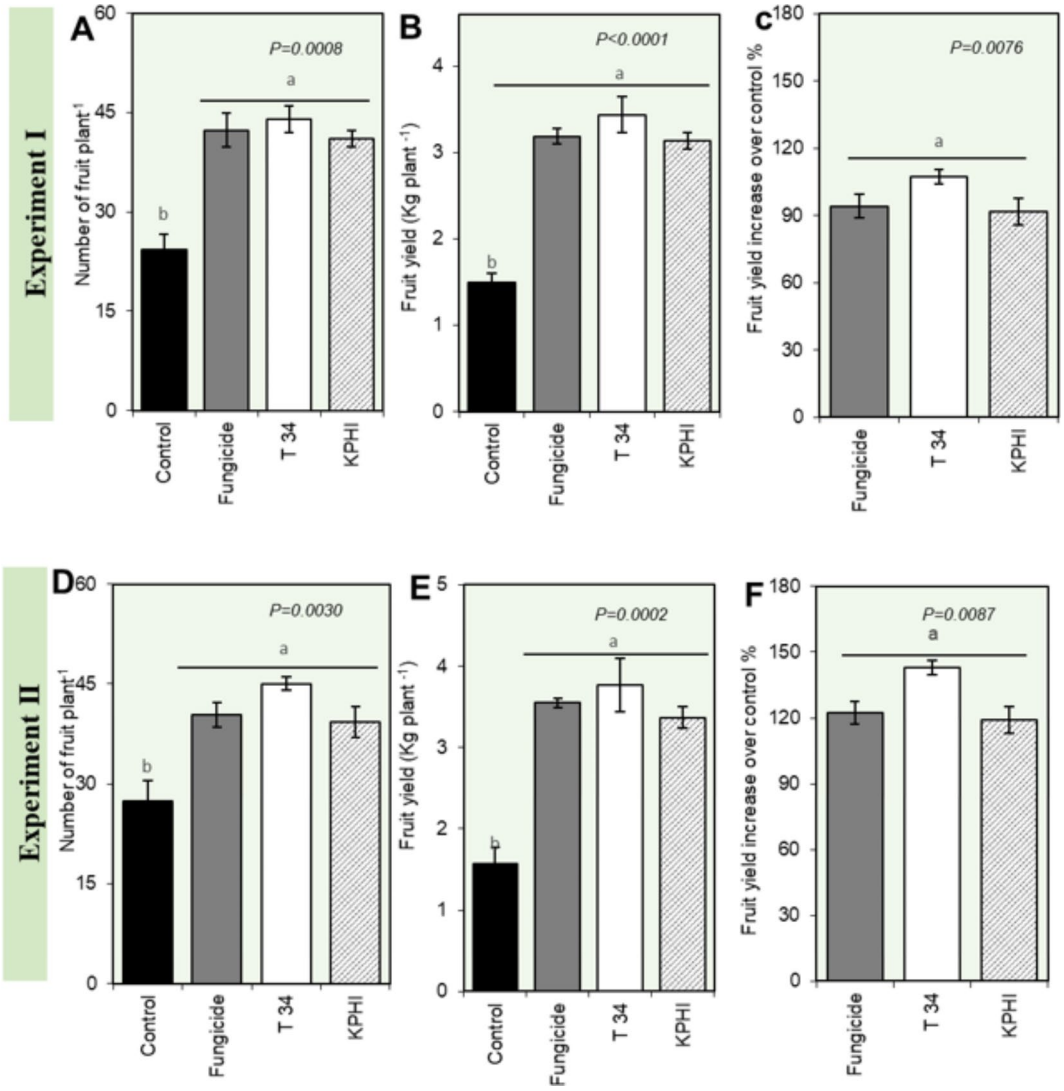


**Fig. 3**. Effect of two tested compounds, T34 and KPHI, on the yield parameters of cucumber plants infected with *P. cubensis* under greenhouse conditions. (A-C) Number of fruit plant$^{-1}$, Fruit yield Kg plant$^{-1}$ , and % Fruit yield increase over control respectively, in experiment I. (D-F) Number of fruit plant$^{-1}$, Fruit yield Kg plant$^{-1}$ , and% Fruit yield increase over control respectively in experiment II. Values show the means ± standard errors. Different letters denote statistical differences among treatments according to Tukey's HSD test ($p < 0.05$).

and KPHI) and chemical fungicide significantly reduced the cell membrane permeability of cucumber leaves, compared with the non-treated infected plants, at 72 hpt (Fig. 6A and B). Briefly, Orodnis fungicide was the best treatment that protected cell membranes followed by T34, and KPHI (32.33 ± 2.66, 39.67 ± 1.45, 42.67 ± 2.33, 34.33 ± 0.67,40.00 ± 4.51 and 44.33 ± 1.77 μ s/cm$^2$ in experiment I and experiment II respectively (Fig. 6A and B). It is worth noting that the treatment with a biological commercial product (T34) was comparable to Orodnis fungicide with no significant difference between them suggesting similar effectiveness (Fig. 6A and B).

## Discussion

Cucumber downy mildew, caused by *P. cubensis*, is one of the most significant threats to cucumber plants and other cucurbit crops, resulting in substantial yield losses worldwide. Traditionally, chemical fungicides have been used to control this disease. However, their high costs and environmental concerns often make them uneconomical. Additionally, *P. cubensis*adapts quickly, leading to the emergence of new strains that are resistant to these chemical treatments[7,31,32]. As a result, chemical fungicides may become ineffective against these new strains[33], prompting farmers to use multiple fungicides to achieve effective disease control[34]. Given these challenges, exploring safer and more environmentally friendly alternatives for managing cucumber downy mildew is essential. Biocide compounds and non-traditional commercial products present promising strategies for combating this disease while reducing environmental impact.

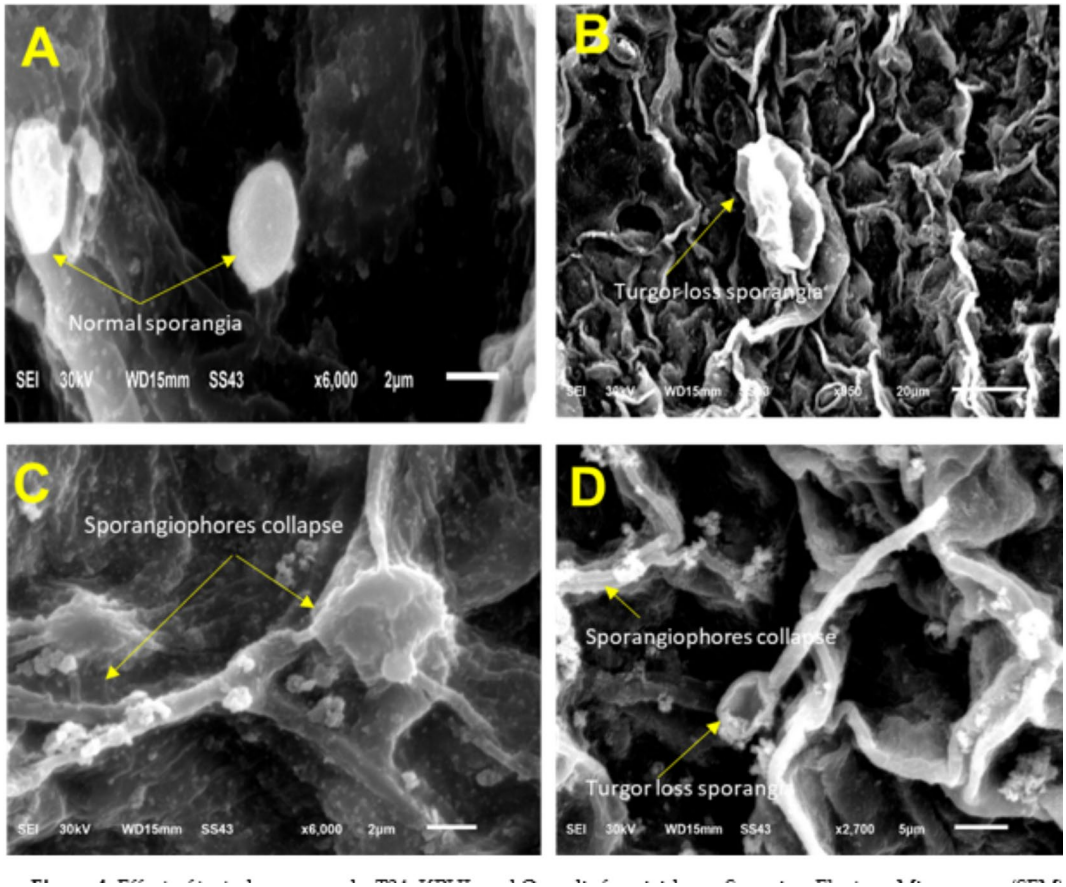


**Fig. 4**. Effect of tested compounds, T34, KPHI, and Orondis fungicide, on Scanning Electron Microscopy (SEM) micrograph of *P. cubensis*. (A) control (water treatment), (B) Orondis fungicide, (C) *Trichoderma asperellum* strain T34, and Potassium phosphite KPHI.

In the current study, the effects of two commercial products *T. asperellum* strain T34, as a biocide compound, and Atlante as a potassium phosphite solution KPHI were evaluated under greenhouse conditions against downy mildew disease of cucumber. Present results showed that T34 and KPHI reduced the severity of cucumber downy mildew disease under greenhouse conditions. Previous studies support our findings about the potential role of different *Trichoderma* species including, *T. viride*, *T. asperellum*, and *T. harzianum*against downy mildew disease under open fields or greenhouse conditions[4,12].

Recent research has extensively explored the potential of various *Trichoderma* species in controlling foliar plant diseases, including downy mildew and others. For instance, *T. harzianum* and *T. viride*have demonstrated significant success as biological control agents against squash powdery mildew, producing remarkable results[35]. However, there has been limited investigation into the antagonistic effects of *Trichoderma asperellum* strain T34 as a biocidal agent against *P. cubensis*. Further research is warranted to fully understand the capabilities and applications of this strain in plant disease management. The antifungal properties of *Trichoderma*species are attributed to several highly effective mechanisms. These include their rapid growth on various substrates, strong tolerance to abiotic stress, quick colonization after inoculation, and ability to outcompete pathogens for nutrients and space[36]. Moreover, the application of potassium and phosphorus, whether through fertilizers or foliar sprays, has demonstrated a significant reduction in the incidence of various plant diseases. For instance, potassium phosphite has been shown to markedly decrease the severity of late blight in tomatoes[18].

Mineral nutrients play a crucial role in the interactions between plant hosts and phytopathogens, influencing plant resistance through various biochemical and physiological pathways[13]. Furthermore, our study's SEM investigations revealed that *Trichoderma asperellum* (T34) caused significant damage to the sporangiophores and sporangia of *P. cubensis*. Similarly, potassium phosphite (KPHI) induced sporangial damage, further supporting its role in disease suppression. The reduction in cucumber downy mildew severity observed in our experiments can be attributed to the direct effects of T34 and KPHI on the pathogen, as well as their ability to reduce the colonization of leaf tissues. These findings underscore the potential of biocontrol agents and mineral nutrients as effective alternatives to chemical fungicides in managing plant diseases.

Additionally, in the present study, the application of the *Trichoderma asperellum* strain T34 (T34) and potassium phosphite (KPHI, trade name Atlante) resulted in a significant increase in photosynthetic pigments, specifically chlorophyll a and b. This enhancement in pigment levels can be attributed to the direct effects of these compounds on the pathogen *P. cubensis*, as well as a reduction in the size of disease spots on the surface of

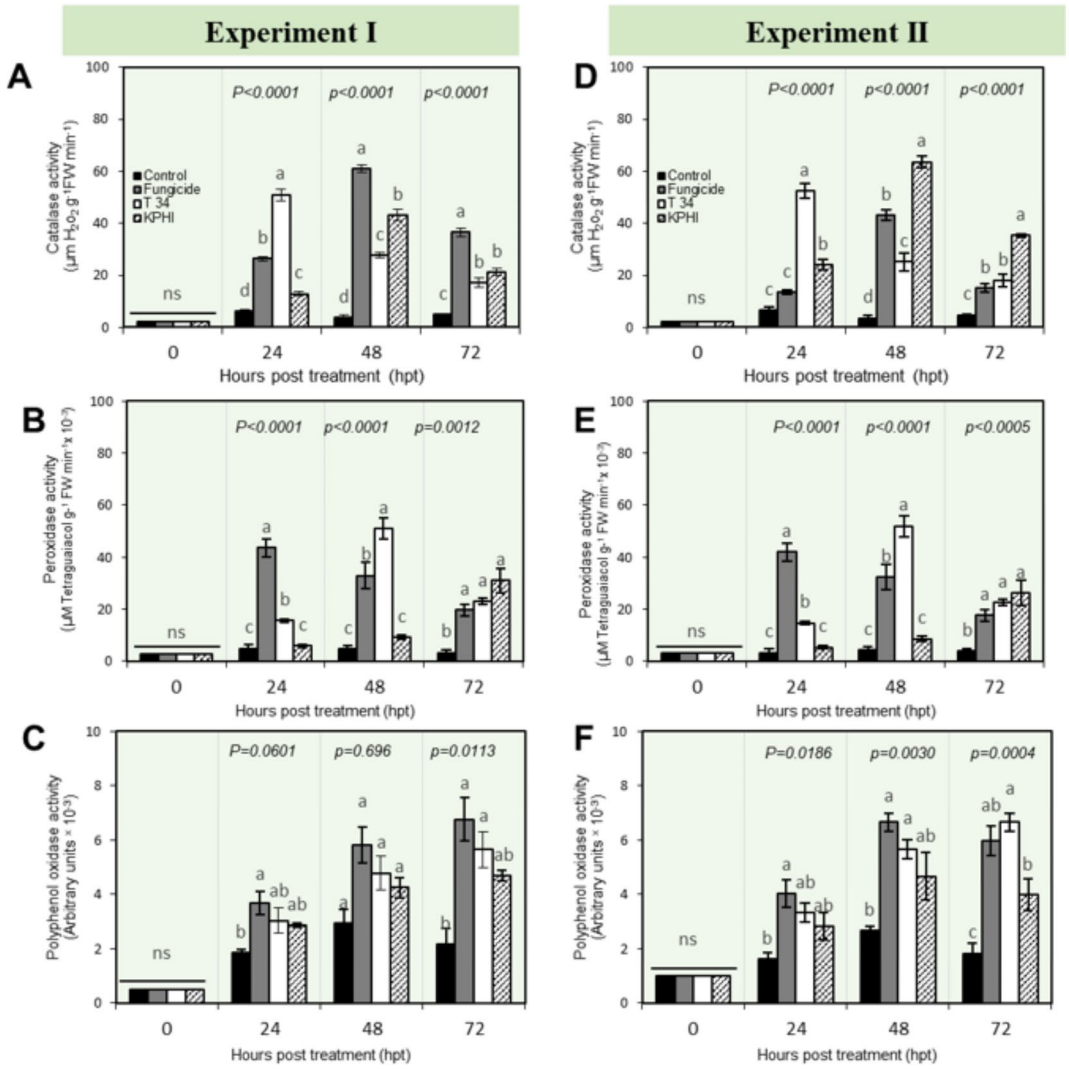


**Fig. 5**. Effect of two tested compounds, T34 and KPHI, on the antioxidant defense-related enzymes in *P. cubensis*-infected leaves under greenhouse conditions (A) catalase activity (µm $H_2O_2$ $g^{-1}$FW $min^{-1}$). (B) Peroxidase activity (µm Tetraguaiacol $g^{-1}$FW $min^{-1}$), and (C) polyphenol oxidase activity (Arbitrary unit) in experiment I. (D) catalase activity (µm $H_2O_2$ $g^{-1}$FW $min^{-1}$). (E) Peroxidase activity (µm Tetraguaiacol $g^{-1}$FW $min^{-1}$), and (F) polyphenol oxidase activity (Arbitrary unit) in experiment II.Values show the means ± standard errors. Different letters denote statistical differences among treatments according to Tukey's HSD test ($p < 0.05$).

cucumber leaves. Furthermore, the substances studied demonstrated a pronounced growth-stimulating effect, as indicated by the increased plant height observed in the treated plants. This suggests that the compounds do not have any toxic effects on the plants and instead promote healthy growth. *Trichoderma* species are well-known for their ability to stimulate plant growth through various mechanisms. They produce plant growth regulators and vitamins, which are crucial for enhancing plant development. Additionally, *Trichoderma*facilitates nutrient absorption, thereby improving overall plant health and productivity[37].

Moreover, a previous study conducted by Mohamed et al.[38], found that treatment of potato plants with T34 significantly increased the content of potato leaves with indole-3-acetic acid IAA and stimulated the growth parameters. Together, the growth trails of cucumber plants might be increased due to the high content of IAA, however, more research is required to better understand the reason for these findings. In addition, treatment of cucumber plants with potassium phosphite reduced the disease severity of *Pythium ultimum* var. *ultimum*and stimulated vegetative measurements (fresh and dry weight of shoot system and roots)[19].

Reactive oxygen species (ROS) are produced by plants as a defense mechanism against biotic stresses, including pathogen attacks. It is believed that ROS generation is one of the initial plant responses to such stresses[41,42]. However, the overproduction of ROS can have detrimental effects on cellular processes, leading to the oxidation of carbohydrates, proteins, and lipids. To mitigate the accumulation of ROS, plants employ various mechanisms, with antioxidant enzymes such as catalase, peroxidase, ascorbate peroxidase, and superoxide dismutase playing crucial roles[43].

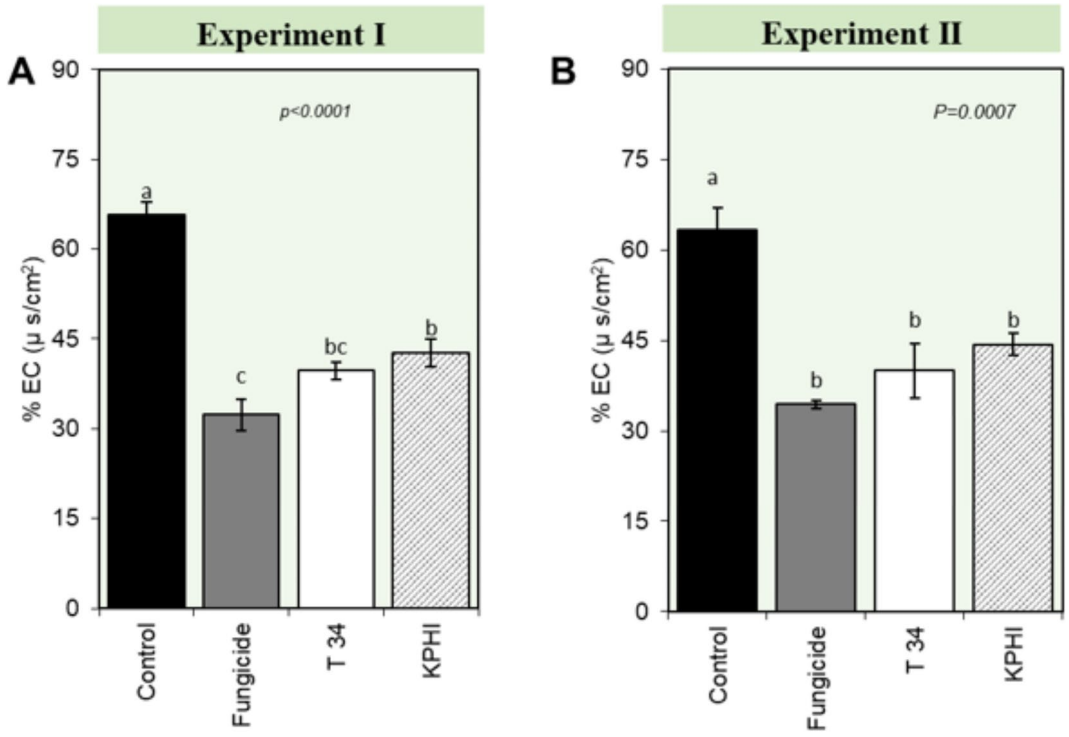


**Fig. 6**. Effect of two tested compounds, T34 and KPHI, on the electrolyte leakage of cucumber-infected leaves. (A) in experiment I(B) in experiment II.Values show the means ± standard errors. Different letters denote statistical differences among treatments according to Tukey's HSD test ($p < 0.05$).

In the current study, the foliar application of the two tested products significantly enhanced the antioxidant enzymatic activities of catalase, peroxidase, and polyphenol oxidase. Our findings suggest that treatments with *Trichoderma asperellum* strain T34 and potassium phosphite (KPHI) reduce oxidative stress in cucumber plants caused by *P. cubensis*infection. For instance, treating cucumber plants with potassium phosphite at a concentration of 4 g/L markedly increased the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT), thereby mitigating the oxidative damage caused by ROS overproduction[19].

A similar study demonstrated that *Trichoderma asperellum* strain T34 enhances the activities of antioxidant enzymes in potato plants under bacterial infection caused by *Ralstonia solanacearum*[38]. Consequently, it is hypothesized that exogenous applications of T34 or potassium phosphite can trigger and activate defense genes in cucumber plants against *P. cubensis* infection by stimulating the antioxidant defense system and improving overall plant health.

Electrolyte leakage (EL) is an indicator of cell membrane permeability[39]. The membrane permeability of cucumber leaves infected with downy mildew disease was significantly increased; however, the tested treatments (T34 and KPHI) reduced the Electrolyte leakage. In general, the permeability of plant cell membranes increases upon pathogen attack, particularly by obligate parasites such as fungi that cause powdery mildew, rust diseases, and downy mildew. This increase in permeability can be attributed to the pathogen's reliance on metabolic compounds from host cells[40]. In the current study, the observed increase in electrolyte leakage is likely due to the infection by *P. cubensis*, an obligate pathogen that exploits the host's metabolic resources. The reduction in electrolyte leakage observed with T34 and KPHI treatments suggests that these agents help maintain cell membrane integrity, thereby mitigating the damaging effects of the pathogen and enhancing overall plant health. Successful control strategies against phytopathogens will result from an understanding of the mode of action of a chemical molecule or biocontrol agent.

## Conclusions

Our findings are summarized in Fig. 7. T34 and potassium phosphite KPHI significantly reduced the development of cucumber downy mildew disease under greenhouse conditions. Moreover, the tested compounds protected the membrane permeability and minimized the electrolyte leakage %, not only that but also promoted the growth and yield parameters of cucumber plants. This may be related to the activation of antioxidant defense mechanisms (catalase, peroxidase, and polyphenol oxidase). However, further studies are required to better understand the physiological, biochemical, and molecular mechanisms involved in the fungistatic activity of the T34 and KPHI against *P. cubensis*, the causal agent of cucumber downy mildew disease. Our findings suggest that T34 and potassium phosphite KPHI might be environmentally acceptable options to reduce the utilization of chemical fungicides entirely or partially for the safer production of cucumber under infection with *P. cubensis*.

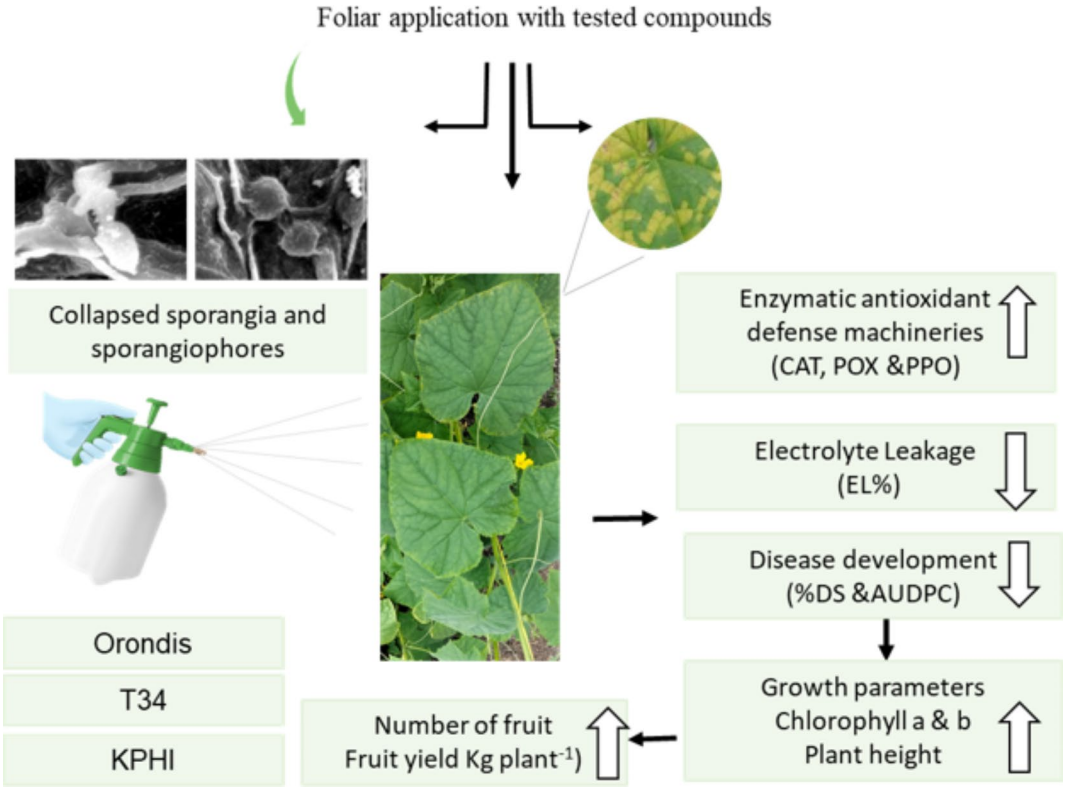


**Fig. 7**. A schematic description of the possible role of *Trichoderma asperellum* strainT34 and potassium phosphite solution KPHI against *P. cubensis*, the causal agent of cucumber downy mildew disease. Briefly, the foliar application with T34 and KPHI reduce the disease development of cucumber downy mildew disease under greenhouse conditions. This might be due to the activation of enzymatic antioxidant mechanisms (CAT, POX, and PPO) of infected cucumber plants. In addition, decrease the % electrolyte leakage and collapse the sporangiophores of *P. cubensis*. Finally, T34 and KPHI might promote the growth of *P. cubensis*-infected cucumber plants and enhance their yield parameters because of reduced disease severity. Elevated levels are indicated by an up arrow and lowered levels by a down arrow. Go to the main text for more information.

## Data availability

All data and materials are available for other researchers through the corresponding author: asmaa.elnagar@agr.tanta.edu.eg.

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## Author contributions

Conceptualization, A.E.-N., A.A. and R.A.; methodology, A.E.-N. and A.A.; software, A.E.-N.; validation, Y.S.A.M., A.H.M., A.A., R.A. and A.E.-N.; formal analysis, A.E-N.; investigation, A.A. and A.R.; resources, Y.S.A.M., A.H.M. and R.A.; data curation, A.E.-N, A.A. and R.A.; writing—original draft preparation, A.A. and A.E.-N.; writing—review and editing, R.A. and A.A.; visualization, A.E-N.; supervision, A.E-N, and A.A.; project administration, A.A., R.A. and A.E.-N.; funding acquisition, Y.S.A.M. and A.H.M. All authors have read and agreed to the published version of the manuscript.

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## Declarations

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Not applicable. The author of this manuscript is the author guide that can be found on the journal's website. Furthermore, all authors have given their OK, and this work has never been published before.

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Every author stated that there are no problems with the journal's policies.

### Competing interests
The authors declare no competing interests.

### Note
In the current study, all experiments complied with relevant institutional, national, and international guidelines and legislation.

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