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**RESEARCH** **Open Access**

# Simultaneous use of *Beauveria bassiana* and *Bacillus subtilis*-based biopesticides contributed to dual control of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) and tomato powdery mildew without antagonistic interactions

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**Abstract**

**Background** Implementing pest and disease control techniques that have low environmental impact is important for sustainable agriculture. Microbial biopesticides are an effective approach due to their low environmental impact and low risk of resistance development. Because it is not usually possible to control multiple pests and diseases with a single microbial biopesticide, it is essential to investigate the potential for combining microbial biopesticides with varying control spectrums effectively. Many biopesticides have antimicrobial activity and may therefore interact negatively in combination.

**Results** This study demonstrated that a mixture of *Beauveria bassiana* and *Bacillus subtilis* formulations proved potential for simultaneous control of greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) and tomato powdery mildew (*Oidium neolycopersici*). Three greenhouse experiments were conducted to assess the efficacy of mixed and single-use treatments. A laboratory experiment comparing the insecticidal effect of each treatment was also conducted. In all greenhouse experiments, the combined treatment controlled the greenhouse whitefly (78.9–88.3%) and tomato powdery mildew (47.2–81.0%) compared to untreated controls, which was as well as each treatment alone. In some greenhouse and laboratory experiments, the mixed treatment showed an approximately 1.32 to 1.78 times higher insecticidal effect compared to single-use treatments. Regarding the control efficacy against the pest and disease, negative effects of microbial agents on each other were not observed.

**Conclusions** These results demonstrated the effectiveness of concurrent use of two microbial pesticides examined on dual control of pest and disease and showed potential for improved control of certain pests. The knowledge of this work could suggest the possibility of more environmentally friendly pest control systems with the use of microbial pesticides.

**Keywords** Microbial biopesticide, *Beauveria bassiana*, *Bacillus subtilis*, Greenhouse whitefly, *Trialeurodes vaporariorum*, Fungal–bacterial interaction

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## Background

Use of crop production technologies that have a low environmental impact is important for sustainable agriculture (Greenberg et al. 2012). Biopesticides offer an effective complementary approach in integrated pest management due to their low environmental impact and low risk of resistance development (Gurulingappa et al. 2011). Use of biopesticides should be optimized for effective pest control. Because biopesticides are living microorganisms, they must be handled differently from chemical pesticides in many ways (Brownbridge and Buitenhuis 2019); they require suitable physicochemical conditions (Abbaszadeh et al. 2011). Like chemical pesticides, however, it is difficult to control multiple pest species in a cultivation environment by using only a single biopesticide. Therefore, it is important to explore the possibility of simultaneous control of pests and diseases through the combined use of multiple biopesticides that have different control spectra.

Tomato production is adversely affected by various pests and diseases, such as the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) and tomato powdery mildew (*Oidium neolycopersici*) (Sekine et al. 2022). Because they usually occur together, it would be ideal to find a biocontrol method that could simultaneously control both pests. Microbial biopesticides registered to control these pests included *Beauveria bassiana* and *Bacillus subtilis*, in Japan. *B. bassiana* is a well-known entomopathogenic fungus that is anamorphic of *Cordyceps bassiana* in the family Clavicipitaceae (Sung et al. 2007). It produces proteolytic enzymes and toxins and exhibits insecticidal effects (Zimmermann 2007). BotaniGard WP®, a wettable powder formulation that contains *B. bassiana* (strain GHA), as its active ingredient, was registered for using against whiteflies on tomato, and it has been reported to grow both endophytically and epiphytically on tomato (Nishi et al. 2021). *B. subtilis* is the most studied species in the genus, *Bacillus* (Kovács 2019). It is ubiquitous in the environment (Wu et al. 2021) and has been found as both an endophyte and an epiphyte (Wang et al. 2018). *B. subtilis* is known to synthesize numerous secondary metabolites that inhibit other microorganisms (Mnif and Ghribi 2015). Batistar WP®, a wettable powder formulation containing *B. subtilis* (strain Y1336), as its active ingredient, was registered for using against tomato powdery mildew and gray mold (*Botrytis cinerea*). Because *B. bassiana* and *B. subtilis* both exhibit antimicrobial properties, they may interact antagonistically under certain conditions. Indeed, microbial interference has been observed, when *B. bassiana* and *B. subtilis* were co-cultivated on agar plates (Toledo et al. 2011). However, interference may not be observed under the relatively nutrient-poor conditions on plant leaf surfaces. If these microbial biopesticides do not excessively interfere with each other under actual cultivation conditions, simultaneous control of a wide range of pests and diseases including whiteflies and powdery mildew could be possible.

This study assessed the effect of simultaneous use of these two microbial biopesticides on greenhouse whitefly and tomato powdery mildew, as well as the amount of the microbial agents present on tomato leaf surfaces, in greenhouse environments. In total, three greenhouse experiments were conducted, employing different cultivation methods and varying biopesticide application methods. In addition, a laboratory experiment was conducted to assess the insecticidal effects of concurrent use of these biopesticides.

## Methods

### Greenhouse experiments

All greenhouse experiments were conducted at the Miyagi Prefectural Agricultural and Horticultural Research Center using tomato variety 'SR Saifuku' (Kaneko Seeds, Takasaki, Japan). Four treatment areas were set up inside each greenhouse: no biopesticide (control), *B. bassiana* formulation, BotaniGard WP® (Bb), *B. subtilis* formulation, Batistar WP® (Bs), and both biopesticide formulations (Bb + Bs). Temperature and humidity data in the greenhouse were collected by using a data logger (TR-72wf, T&D Corporation, Nagano, Japan).

#### *1. Experiment 1: High-bench culture sprayed with a diluted microbial biopesticide*

The experiment was conducted from June to September 2022 in a greenhouse (8 m × 30 m). Each tomato seedling, sown on June 22, was transplanted into its own planter (23 cm wide × 64 cm long × 18 cm deep) on August 5. The planters were placed on a bench (50 cm high), and the plants were watered twice a week. Both *B. bassiana* and *B. subtilis* formulations were prepared at a 1000-fold dilution (*B. subtilis*, $1.0 \times 10^6$ spores/ml; *B. bassiana*, $4.4 \times 10^7$ conidia/ml) and sprayed at a rate of 300 l/1000 $m^2$ by using a backpack sprayer. Spraying was conducted on August 17, 24 and 31, 2022.

In each treatment, 24 plants were selected for the pest and disease survey (total = 96; Additional file 1: Fig. S1A). The height of each plant was visually divided into three equal parts and two compound leaves were randomly chosen from each of the upper, middle, and low parts (total = six compound leaves per plant) and the number of whitefly nymphs were counted. The proportion of leaflets infected by powdery mildew on four compound leaves in the middle and low parts of each plant were also calculated.

To analyze the abundance of the two microbial agents on tomato leaf surfaces, six plants were selected from each treatment, and a subset of the plants was surveyed for pests and disease (Additional file 1: Fig. S1A) and cut a 1-cm-diameter leaf disk from the middle part of each plant at least, 7 days after the most recent biopesticide application. Leaf disk samples were put in individual 1.5-ml plastic tubes and transported to the laboratory in a cooler box. Sterile distilled water (1 ml) was added to each tube and vortexed for 30 s. Aliquots (20 µl) from each tube were inoculated onto the surface of agar plates and spread evenly with a Conrage stick. To isolate *B. bassiana*, a potato dextrose agar medium, containing 30 ppm streptomycin, was used and incubated at 25 °C for 3 weeks before counting colony-forming units. Colonies of *B. bassiana* were identified by their characteristic dense white mycelia (Chang et al. 2021). To isolate *B. subtilis*, nutrient agar medium (Nissui, Tokyo, Japan) was used and incubated at 40 °C for 3 weeks before counting colony-forming units. Colonies of *B. subtilis* (Y1336) were identified by their distinctive and prominent "beanstalk-like" structures (Bridier et al. 2011).

### *2. Experiment 2: Soil culture sprayed with a diluted microbial biopesticide*

The experiment was conducted from January 13 to April 14, 2022, in two greenhouses (5 m × 10 m). Tomato seedlings were sown on October 20, 2021, and transplanted on December 7, 2021. The plants were arranged in a two-row zigzag pattern with 40 cm among plants and 20 cm between rows. Plants were irrigated when the soil surface became dry, as needed. Spraying of the microbial biopesticides was performed as in Experiment (1) and conducted on January 13, 22, 30 and February 3.

From each treatment, 18 plants were selected for the pest and disease survey (total = 72; Additional file 1: Fig. S1B). Two compound leaves were randomly chosen from each of the upper, middle, and lower parts (total = six compound leaves per plant) to count the number of whitefly nymphs. The ratio of leaflets infected by powdery mildew on four compound leaves in the middle and lower parts of each plant was counted. To assess the abundance of the microbial agents on tomato leaf surfaces, 1-cm-diameter leaf disks were collected from six plants from each treatment (Additional file 1: Fig. S1B). Detection of microbial agent colonies was performed as described for Experiment 1.

### *3. Experiment 3: Soil culture dusted with microbial biopesticides*

The experiment was conducted from July 13 to August 19, 2022, in two greenhouses (5 m × 10 m). Seedlings sown on May 13 were transplanted on July 7, 2022. Plant arrangement and irrigation were the same as in Experiment (2). Microbial biopesticides were applied via dusting at the recommended volume (*B. bassiana* formulation, 300 g/1000 $m^2$; *B. subtilis* formulation, 450 g/1000 $m^2$) at a rate of approximately 2.0 g/sec using an electric hand blower (UB142D, Makita, Tokyo, Japan), on July 13, 22, and August 5, 2022. To prevent contamination, treatment areas were separated by a polyolefin-based film.

For 18 plants in each treatment (Additional file 1: Fig. S1C), two compound leaves were randomly selected from each of upper, middle, and lower parts (total = six compound leaves per plant) and counted the number of whitefly nymphs. The infection rate of tomato powdery mildew was assessed on August 10. For each plant, 20 compound leaves from the middle part of each plant were checked for symptoms of tomato powdery mildew. In this experiment, abundance of microbial agents on tomato leaf surfaces was not recorded.

### Laboratory experiments

Eighty adult females of greenhouse whitefly were collected from pumpkins grown at the research center and allowed them to lay eggs on two kidney bean plants, each with two primary leaves, for 24 h. After removing the adults, the plants were grown for 7 days at 25 °C, 50% RH, and under a 16 L: 8 D photoperiod. It was confirmed that the whitefly had developed into second-instar nymphs, and 34 leaf disks (approximately 2 $cm^2$) were made, with each disk hosting 4 to 31 nymphs. After counting the number of nymphs on the leaf disks, the leaf disks were immersed in the following solutions for 30 s: tap water (control, $N = 9$), *B. bassiana* (Bb, $N = 8$), *B. subtilis* (Bs, $N = 9$), and a mixture of *B. bassiana* and *B. subtilis* (Bb + Bs, $N = 8$). The leaf disks were incubated for 7 days at 25 °C, 50–70% RH, and incubated under a 16 L: 8 D photoperiod for 9 days before the number of surviving and dead nymphs on the leaf disks were counted and the insecticidal effects of each treatment were calculated. Nymphs with discolored bodies or visible fungal mycelial growth were considered dead.

### Data analysis

Statistical analysis was performed by using R software v. 4.2.1 (R Core Team 2022). The effect of biopesticide treatments in greenhouse experiments on the abundance of whitefly, the proportion of leaflets infected by powdery mildew, or the abundance of each microbial agent were evaluated by using generalized linear mixed models constructed by using the "lme4" package (Bates et al. 2011). The following were used as response variables: the number of whitefly nymphs in all stages assuming a negative binomial distribution, the proportion of leaflets infected by tomato powdery mildew assuming a binomial distribution, and the abundance of each microbial agent on leaf surfaces assuming a Gaussian distribution. Treatments were treated as fixed effects and dates and plant IDs were treated as random effects. In addition, planter strip IDs were included as random effects in Experiment 1, while greenhouse IDs were treated as random effects in Experiments 2 and 3. Chi-square ($\chi^2$) and $P$-values for the fixed effects were calculated by the Wald test using the "Anova" function in the "car" package (Fox et al. 2012). To compare the number of whiteflies, the proportion of leaflets infected with powdery mildew, or the abundance of each microbial agent among four treatments, Tukey's HSD test ($P < 0.05$) was conducted with the function "glht" in the "multcomp" package (Hothorn et al. 2008). In the laboratory experiment, the mortality of whitefly was compared among treatments by using a generalized linear model with a binomial distribution, followed by Tukey's HSD test using the "lme4" and "multcomp" packages. In the model, mortality was used as the response variable and treatments were used as the explanatory variable.

## Results

In all greenhouse experiments, the use of microbial biopesticide significantly affected the density of whitefly nymphs (likelihood ratio test, Experiment 1: $\chi^2 = 31.58$, $P < 0.001$; Experiment 2: $\chi^2 = 61.63$, $P < 0.001$; Experiment 3: $\chi^2 = 74.06$, $P < 0.001$). Microbial biopesticide significantly affected tomato powdery mildew compared to controls in Experiments 1 and 3 (likelihood ratio test, Experiment 1: $\chi^2 = 16.33$, $P < 0.001$; Experiment 2: $\chi^2 = 1.73$, $P = 0.631$; Experiment 3: $\chi^2 = 143.02$, $P < 0.001$). Temperature and humidity conditions for the greenhouse experiments are shown in Additional file 3: Fig. S3.

The Bb + Bs treatment simultaneously suppressed both whitefly and powdery mildew compared to controls in Experiments 1 and 3 ($P < 0.001$, Tukey's test, Figs. 1A, C, 2A, C). In Experiment 2, whitefly density was lower in the Bb + Bs treatment area than in the control area ($P < 0.001$, Tukey's test, Fig. 1B), but non-significant effect was detected for tomato powdery mildew in any treatment due to its overall low density (Fig. 2B).

Application of Bb or Bs alone suppressed whitefly density in all greenhouse experiments ($P < 0.05$, Tukey's test, Fig. 1). Moreover, in the high-bench culture experiment, application of Bb suppressed powdery mildew ($P < 0.01$, Tukey's test, Fig. 1A). In both experiments, no pests or diseases other than whitefly or tomato powdery mildew were observed.

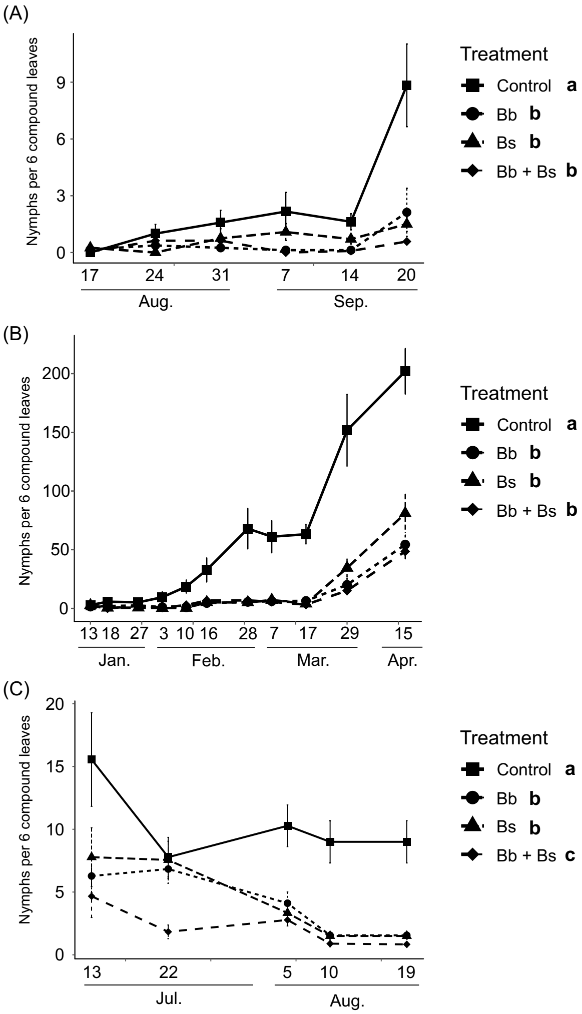

**Fig. 1** Number of greenhouse whitefly nymphs in **A** high-bench culture sprayed with diluted microbial formulation, **B** soil culture sprayed with diluted microbial formulation, and **C** soil culture dusted with microbial formulation. Different letters indicated a significant difference among treatments (Tukey's test, $P < 0.05$). Error bars represented the mean ± standard error Control, no biopesticides; Bb, *Beauveria bassiana*, Bs, *Bacillus subtilis*; Bb + Bs, *B. bassiana* and *B. subtilis*

No consistent trends were observed in the amount of microbial agents present on tomato leaves, and results varied among experiments. Leaf levels of biopesticides were not lower in the Bb + Bs treatment compared to the single-use treatment. In Experiment (1), *B. bassiana* abundance was higher in the Bb + Bs treatment area ($P < 0.05$, Tukey's test; Additional file 2: Fig. S2A). On the other hand, *B. subtilis* showed similar trends in both the Bb + Bs and Bs treatment areas ($P = 0.200$, Tukey's test; Additional file 2: Fig. S2B). In Experiment (2), abundances of both *B. bassiana* and *B. subtilis* did not differ significantly between the Bb + Bs and single-use treatment areas ($P > 0.05$, Tukey's test; Additional file 2: Fig. S2C, 4D), whereas biopesticide densities in the single-use treatment area were higher than in the control area (Tukey's test, Bb, $P = 0.029$, Additional

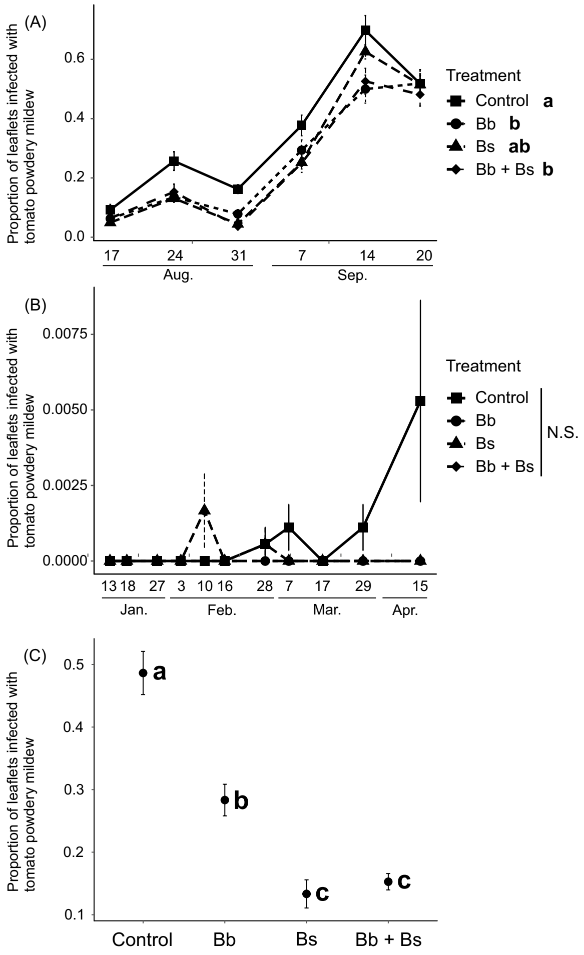

**Fig. 2** Infection rate of tomato powdery mildew in **A** high-bench culture sprayed with diluted microbial formulation, **B** soil culture sprayed with diluted microbial formulation, and **C** soil culture dusted with microbial formulation. In **C**, the survey was conducted on August 10, 2022. Different letters indicated a significant difference among treatments (Tukey's test, $P < 0.05$). Error bars represented the mean ± standard error Control, no biopesticides; Bb, *Beauveria bassiana*, Bs, *Bacillus subtilis*; Bb + Bs, *B. bassiana* and *B. subtilis*

file 2: Fig. S2C; Bs, $P = 0.025$, Additional file 2: Fig. S2D).

In the laboratory experiment, treatment with either Bb or Bb + Bs significantly increased whitefly mortality (likelihood ratio test, $\chi^2 = 116.98$, $P < 0.001$). The Bb + Bs treatment had the highest insecticidal effect against whitefly nymphs, followed by the Bb treatment ($P < 0.001$, Tukey's test; Fig. 3). No insecticidal effect was observed in the Bs treatment. Results in all experiments are summarized in Table 1.

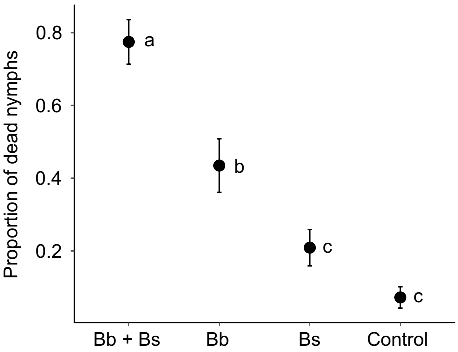

**Fig. 3** Mortality of second-instar nymphs of greenhouse whitefly 9 days after immersion for 30 s in the following solutions: tap water (control, $N = 9$), *Beauveria bassiana* formulation diluted 1000-fold (Bb, $N = 8$), *Bacillus subtilis* formulation diluted 1000-fold (Bs, $N = 9$), and a mixture of both biopesticide formulations diluted 1000-fold (Bb + Bs, $N = 8$)

## Discussion

The results suggested that combining microbial pesticides showed a potential for simultaneous control of both pest and disease. Applying *B. bassiana* and *B. subtilis* in combination did not reduce their suppression of whitefly and tomato powdery mildew in greenhouse experiments, notwithstanding variations in microbial agent abundance on tomato plant leaves. The variation in microbial abundance could be attributed to differences in temperature and humidity within the greenhouse, as these factors significantly influenced their growth and proliferation. *B. bassiana* and *B. subtilis* have been reported to negatively affect each other's growth on nutrient-rich agar media (Toledo et al. 2011). On leaf surfaces, there are likely to be fewer available resources, such as mono- and disaccharides (Mercier and Lindow 2000), compared to agar media and therefore slower growth, so direct interactions between the two microbes may be less likely to occur. Previous studies have shown that resource availability affected the relationship among microorganisms (Boddy 2000). Thus, even if microbial agents interfered with each other on nutrient-rich medium, combined application may not have adverse effects on pest and disease control in the actual cultivation environment.

The Bb + Bs treatment had a stronger insecticidal effect against whitefly nymphs than the Bb alone treatment in the laboratory as well in the greenhouse experiment with dusting. Several previous studies have reported that the pathogenic effect of entomopathogenic fungi was enhanced, when they are used in combination with other insecticidal factors. For example, when *Metarhizium robertsii* fungus was applied with the insecticide, avermectin, it inhibited the function of gut detoxification enzymes, enhancing the effect of the insecticide (Kryukov et al.

**Table 1** Suppression effects of treated biopesticides in controlling greenhouse whitefly, *Trialeurodes vaporariorum* and tomato powdery mildew, *Oidium neolycopersici* at different conditions and periods

| Experiments | Conditions | Survey periods | Treatments | Suppression effect compared to control area (%) | |
|---|---|---|---|---|---|
| | | | | Greenhouse whitefly | Tomato powdery mildew |
| Experiment 1 | High-bench cultivation | June to September, 2022 | Bb + Bs | 88.3 | 47.2 |
| | | | Bb | 82.2 | 42.9 |
| | | | Bs | 67.2 | 35 |
| Experiment 2 | Soil cultivation | January to April, 2022 | Bb + Bs | 88.1 | – |
| | | | Bb | 86.1 | – |
| | | | Bs | 87.8 | – |
| Experiment 3 | Soil cultivation | July to August, 2022 | Bb + Bs | 78.9 | 81 |
| | | | Bb | 59.8 | 58.4 |
| | | | Bs | 58.3 | 83.8 |
| Laboratory experiments | Laboratory | – | Bb + Bs | 73.4 | – |
| | | | Bb | 41.2 | – |
| | | | Bs | 15.2 | – |

Suppression effect was calculated from the estimates in generalized liner model (GLM) or generalized liner mixed model (GLMM). The values are based on differences in the assumed distributions within the statistical model: proportion of individuals for greenhouse whitefly (Experiments 1–3), and odds ratio for greenhouse whitefly (laboratory experiment) and tomato powdery mildew (Experiments 1 and 3)

Bb, *Beauveria bassiana*, Bs, *Bacillus subtilis*; Bb + Bs, *B. bassiana* and *B. subtilis*

2021), and application of two entomopathogenic fungi had a synergistic effect for control of locusts, which was occurred by reducing the density of beneficial gut bacteria (Tan et al. 2021). The simultaneous application of *B. bassiana* and *B. subtilis* might have affected the gut microbial community and/or enzyme activity of whitefly, leading to increased mortality. Another possible factor is secondary metabolites produced in plant tissues. Tomato plants simultaneously treated with *B. bassiana* and *B. subtilis* showed induced systemic resistance (Prabhukarthikeyan et al. 2017), and inoculating tomato plants with *B. subtilis* increased secondary metabolites such as phenols that have a negative effect on the growth of insect pests, retarding whitefly *B. tabaci* development (Valenzuela-Soto et al. 2010). Here, growth-inhibiting secondary metabolites in plant tissues induced by *B. subtilis* might have extended the larval development period, increasing the likelihood of infection by *B. bassiana*. Although synergistic effects on whitefly were not detected in Experiments (1 and 2) (Fig. 1A, 1B), this may be because the whitefly density was almost zero in both Bb + Bs and Bb treatment areas. Because preventative use of microbial biopesticides, before the occurrence of pests and diseases, was recommended (Kumar and Singh 2014), application started, when whitefly density was almost zero. In Experiment (3), which was initiated under conditions of high initial whitefly density, the Bb + Bs treatment had a significantly stronger insecticidal effect than either the Bb or Bs treatment alone. This result suggested that specific combinations of microbial biopesticides could be used to control whitefly, even after pest numbers were high.

In all greenhouse experiments, Bs treatment significantly suppressed greenhouse whitefly. On the other hand, Bs treatment had non-insecticidal effect in the laboratory experiment, suggesting that indirect factors contributed to the decrease in whitefly density in greenhouse experiments. Several studies have reported that the whiteflies *B. tabaci* and *T. vaporariorum* avoid tomato plants that have been inoculated with microbes that enhanced the production of secondary metabolites (Moyo et al. 2021; Wei et al. 2020). Because inoculation of tomato plants with *B. subtilis* caused induced systemic resistance (Prabhukarthikeyan et al. 2017), the suppression of whitefly observed in all greenhouse experiments might be due to inoculated plants' ability to repel pests.

In the high-bench culture experiment (i.e., Experiment 1), the Bb + Bs and Bb treatments suppressed powdery mildew. However, by the end of the experiment, powdery mildew became widespread, and its density was similar to that in the untreated areas. *B. subtilis* suppressed plant pathogenic fungi by promoting plant growth, inducing resistance, and depriving pathogens of resources and habitats (Wang et al. 2018). To achieve these suppression effects, it was speculated that the biopesticide bacteria must infect the plant before the pathogen proliferation.

In the high-bench culture experiment, powdery mildew infection was confirmed at the start of the survey, which may have preempted the potential suppression effect of *B. subtilis*. If application is delayed, as it was here, it should be combined with other control technologies. In recent years, the effectiveness of *B. bassiana* GHA in suppressing powdery mildew of several vegetable crops, including tomato, was reported (Iida et al. 2023). The mechanism for this involved inducing the accumulation of salicylic acid in the plant, which triggered hypersensitive response (HR)-like cell death in epidermal cells and inhibited further fungal mycelial invasion. This mechanism was different from *B. subtilis* suppression mechanism, which may explain why only the Bb + Bs and Bb treatments in Experiment (1) detected the suppression of tomato powdery mildew.

In the future, clarifying the mechanism behind the enhanced insecticidal effect observed with the combined application of *B. bassiana* and *B. subtilis* on whiteflies could help optimize pest and disease control. Comprehensive analysis, such as high-throughput sequencing and assessing the production of secondary metabolites, would be necessary to deepen the understanding of these aspects, thereby contributing to the promotion of sustainable agricultural production and reduction of environmental impact.

## Conclusion

The research presented here robustly demonstrated the effectiveness of simultaneous use of *B. bassiana* and *B. subtilis*-based biopesticides for dual control of whitefly and tomato powdery mildew. Additionally, it was found that this approach enhanced the insecticidal effects against the whitefly *T. vaporariorum*. The study revealed that these two microbial agents coexisted on the leaf surface without excluding each other. These results suggested a potential for more effective biological control of pests and diseases in cultivation environments using microbial pesticides.

### Abbreviations

| | |
|---|---|
| Bb | *Beauveria bassiana* Formulation, BotaniGard WP® |
| Bs | *Bacillus subtilis* Formulation, Batistar WP® |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-024-00782-8.

**Additional file 1: Fig. S1** Tomato plant positions and treatment area arrangement in the three greenhouse experiments (A) high-bench culture sprayed with diluted microbial formulation, (B) soil culture sprayed with diluted microbial formulation, and (C) soil culture dusted with microbial formulation Control, no biopesticides; Bb, *Beauveria bassiana*, Bs, *Bacillus subtilis*; Bb + Bs, B. *bassiana* and B. *subtilis*.

**Additional file 2: Fig. S2** Abundance of Beauveria bassiana and Bacillus subtilis on tomato leaf surfaces in high-bench culture sprayed with diluted (A) B. bassiana and (B) B. subtilis and soil culture with diluted (C) B. bassiana (D) B. subtilis. Different letters indicate a significant difference among treatments (Tukey's test, $P < 0.05$). Error bars represent the mean ± standard error Control, no biopesticides; Bs, *Bacillus subtilis*; Bb + Bs, B. *bassiana* and B. *subtilis*.

**Additional file 3: Fig. S3** Temporal change of temperature (solid line with plots, left Y-axis) and humidity (dotted line, right Y-axis) in the three greenhouse experiments: high-bench culture experiment with applying diluted microbial formulation (A), soil culture experiment with applying diluted microbial formulation (B), and soil culture experiment with dusting microbial formulation (C).

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### Author contributions

All authors contributed to the study conception and design. YK, TO, TS, and SK conducted the pest and disease abundance survey and subsequent analysis. YK wrote the first draft, and all authors commented on and approved the final version of the manuscript.

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### Availability of data and materials

The data supporting this study are not publicly available. Readers are welcome to contact the corresponding author for further information regarding the data used in this research.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing Interests

The authors declare no conflict of interest.

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