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Synergistic effect of *Metarhizium flavoviride* and *Serratia marcescens* on western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae)

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Abstract

Background The western flower thrips (WFT), *Frankliniella occidentalis* Pergande **(**Thysanoptera: Thripidae**)**, is an important polyphagous pest in both greenhouses and fields. Due to its wide range of host plants and short life cycle, the pest causes overwhelming damage and has led to the destruction of many crops. The combined use of entomopathogenic microorganisms could be an important option to overcome the difficulties in controlling WFT. The virulence of thirty local entomopathogen isolates was tested on WFT, and possibilities of combined application for WFT control were investigated.

Results All isolates were virulent for both the second larval stage and the adult stage of WFT. *Serratia marcescens* Se9 was the most virulent bacterial isolate with a mortality of 54 and 69.6% against the second larval and the adult stages of WFT, respectively. The LC_{50} values of the Se9 isolate were determined to be 4×10^6 cfu/ml for the second larval stage and 6.3×10^6 cfu/ml for the adult stage. Among the fungal isolates, *Metarhizium flavoviride* As18 showed a mortality rate of 92.1 and 74.5% against the second larval and the adult stages of WFT, respectively. The LC_{50} value was determined to be 1.6×10^4 and 7.1×10^4 conidia/ml for the second larval and adult stages of WFT, respectively. The combined application of *S. marcescens* Se9 and *M. flavoviride* As18 at different concentrations generally performed better than single treatments, indicating an additive or synergistic interaction. While the single treatment with *S. marcescens* and *M. flavoviride* caused a mortality of 20.4 and 49.5%, respectively, the combined application (*S. marcescens* LC_{25}; *M. flavoviride* $100\times LC_{25}$) resulted in a mortality of 95.7% of the second larval stage. Similarly, the combined application caused 96% mortality in the adult stage, while the single treatments with *S. marcescens* and *M. flavoviride* caused 11.3 and 61.3% mortality, respectively.

Conclusion The study showed that the combined application of *S. marcescens* (LC_{25}) and *M. flavoviride* ($LC_{25}\times100$) resulted in synergism against both second larval and adult stages of WFT. This is the first study to show that the combination of *S. marcescens* and *M. flavoviride* had synergistic potential to suppress the WFT population. In future studies, these microorganisms should be formulated together as biopesticides and tested under greenhouse or field conditions.

Keywords Thrips, *Frankliniella occidentalis*, Entomopathogens, Combined effect, Synergism

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Background

The western flower thrips, *Frankliniella occidentalis* Pergande (WFT) (Thysanoptera: Thripidae), is an important destructive sucking pest of a wide range of more than 250 plant species from 65 families (Reitz 2009). They not only cause direct feeding damage to leaves, flowers and fruits, but are also the most effective vectors of tospoviruses such as tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (He et al. 2020). Biological characteristics such as polyphagous, short development times, high reproductive potential, high dispersal ability and competitiveness make the pest difficult to control (Mouden et al. 2017).

Control of WFT is mainly based on the frequent use of broad-spectrum insecticides, including organophosphates, carbamates and pyrethroids. The overuse of insecticides has led to the development of resistant populations to more than 30 active ingredients (Mavridis et al. 2023). In addition, toxicity to beneficial nontarget organisms, environmental pollution and residue problems on marketable crops limit their use (Mouden et al. 2017). For these reasons, the search for reliable biological control methods that can protect the ecological environment and effectively and continuously control the population of WFT has become an important area of research for the integrated control of WFT.

Entomopathogenic microorganisms are natural means of controlling insects' populations because they are naturally pathogenic to a range of insect pests and are derived from nature; therefore, they have little to no adverse effects on the environment. Entomopathogenic viruses and bacteria needed to be ingested or enter the host body in some way to cause the infection, but entomopathogenic fungi (EPF) directly infect through insect cuticle and do not require ingestion to cause infection (Mannino et al. 2019). This offers an advantage in the control of sap-feeding insect species with piercing-sucking mouthparts such as WFT. Several EPF, *Lecanicillium lecanii, Beauveria bassiana, Metarhizium anisopliae, M. brunneum, M. flavoviride, Neozygites parvispora* and *Isaria fumosorosea*, have been successfully used to control WFT (Skinner et al. 2012). Among them, *B. bassian*a and *M. anisopliae* are the most effective for controlling WFT (Li et al. 2021).

EPFs have been shown to control insect pests but have a relatively slow action compared to chemical insecticides, as fungal pathogens have a latent period in their host after infection (Sharma and Sharma 2021). Another potential disadvantage of EPFs is their relatively short shelf life compared to conventional chemical insecticides. To overcome this disadvantage, EPFs have been combined with adjuvants, insecticides, predatory mites or other entomopathogens. For example, Zhang et al. (2021) showed that the combined use of predatory mites *Stratiolaelaps scimitus* and granular formulation of *B. bassiana* improved control of WFT in eggplant under greenhouse conditions. Similarly, Kivett et al. (2016) showed that the combination of *M. anisopliae* and insect growth regulator azadirachtin improved control of WFT under laboratory conditions. Furthermore, Ge et al. (2020) demonstrated that *M. anisopliae* in combination with sublethal doses of conventional insecticide imidacloprid had a better control effect on WFT than the individual fungal biocontrol agent. However, synergism between EPF and bacteria against WFT has not been reported.

Therefore, the main objective of this study was to screen the virulence of thirty indigenous entomopathogen isolates against second larval and adult stages of WFT and to show the possibilities of the combined use of the most virulent bacterium and fungus in the biocontrol of WFT.

Methods

Rearing western flower *thrips*

WFT collected from infested greenhouses in Antalya, Turkey, were used to establish a laboratory colony. To obtain uniformly aged thrips for the experiments, synchronized rearing of WFT was performed on kidney beans (*Phaseolus vulgaris* L.) in 1-l glass jars (18 cm × 10 cm) with snap lids fitted with fine-mesh ventilation holes. Rearing jars were maintained in a climate chamber at 25 °C, 70% RH and L16: D8 h photoperiod (Price et al. 2022). The second larval stage and adults were the target used in bioassays.

Entomopathogens

Entomopathogens were obtained from the Entomopathogen Culture Collection of the Department of Biology, Karadeniz Technical University in Trabzon, Turkey. Thirty entomopathogenic microorganisms isolated and defined in previous studies and whose insecticidal properties were determined were used for the study (Table 1).

Frozen glycerol stock suspension of bacteria (100 µl) was spread on nutrient agar medium to obtain single colonies for each isolate and incubated overnight at 30 °C. A single pure colony was inoculated into 10 ml nutrient broth medium. After incubation, the culture was centrifuged at 5000 rpm for 5 min to remove the medium. The pellet was washed with sterile phosphate buffer solution (PBS) and resuspended in sterile distilled water. Then the bacterial density was measured at OD_{600} (optical density) and adjusted to 1.89 ($\approx 1.8 \times 10^9$ cfu/ml) (Ben-Dov et al. 1995). The bacterial suspensions were

Table 1 Entomopathogens used in this study

	Strain	Species	Origin	References
Bacterial isolates	Sn10	*Bacillus thuringiensis*	*Sesamia nanogrioides*	Eski et al. (2015)
	Se13	*Bacillus thuringiensis*	*Spodoptera exigua*	Eski et al. (2018)
	MnD	*Bacillus thuringiensis*	*Malacosoma neustria*	Katı et al. (2005)
	Xd3	*Bacillus thuringiensis*	*Xyleborus dispar*	Sezen et al. (2008)
	Ta1	*Bacillus thuringiensis*	*Tuta absoluta*	Eski et al. (2024)
	Ta6	*Bacillus thuringiensis*	*Tuta absoluta*	Eski et al. (2024)
	Se9	*Serratia marcescens*	*Spodoptera exigua*	Eski et al. (2018)
	Sn14	*Serratia marcescens*	*Sesamia nanogrioides*	Eski et al. (2015)
	Sn8	*Bacillus safensis*	*Sesamia nanogrioides*	Eski et al. (2015)
	Cq1	*Bacillus safensis*	*Cimbex quadrimaculatus*	Cakici et al. (2015)
	Ar2	*Bacillus polymyxa*	*Anoplus roboris*	Demir et al. (2002)
	Cq2	*Bacillus subtilis*	*Cimbex quadrimaculatus*	Cakici et al. (2015)
	Tp11	*Bacillus pumilus*	*Thaumetopoea pityocampa*	İnce et al. (2008)
	Se2	*Lysinibacillus. macroides*	*Spodoptera exigua*	Eski et al. (2018)
	Ld4	*Pseudomonas putida*	*Leptinotarsa decemlineata*	Muratoğlu et al. (2011)
Fungal isolates	As2	*Metarhizium flavoviride*	*Amphimallon solstitialis*	Biryol et al. (2020)
	As18	*Metarhizium flavoviride*	*Amphimallon solstitialis*	Biryol et al. (2020)
	KTU2	*Metarhizium brunneum*	Soil	Sevim et al. (2010b)
	Gg12	*Metarhizium brunneum*	*Gryllotalpa gryllotalpa*	Sönmez et al. (2016)
	BL5	*Metarhizium brunneum*	Soil	Eski and Gezgin (2022)
	BL23	*Metarhizium brunneum*	Soil	Eski and Gezgin (2022)
	KTU24	*Beauveria bassiana*	*Thaumetopoae pityocampa*	Sevim et al. (2010a)
	KTU57	*Beauveria bassiana*	*Rhynchites baccus*	Sevim et al. (2014)
	Hp4	*Beauveria bassiana*	*Hypera postica*	Yucel et al. (2018)
	Pa4	*Beauveria bassiana*	*Pristiphora abietina*	Biryol et al. (2021)
	Gg1	*Beauveria bassiana*	*Gryllotalpa gryllotalpa*	Sönmez et al. (2016)
	B8	*Beauveria bassiana*	Soil	Unpublished data
	Pa3	*Lecanicillium muscarium*	*Pristiphora abietina*	Biryol et al. (2021)
	KTU42	*Isaria fumosorosea*	Soil	Sevim et al. (2010b)
	KTU1	*Isaria fumosorosea*	Soil	Sevim et al. (2010b)

then serially diluted to 10^8 cfu/ml and stored at 4 °C until used in the bioassays.

Frozen glycerol stock suspension of fungi (100 µl) was grown for 3 days at 25 °C by spreading on Saboraud Dextrose Agar (SDA) medium. A single colony was inoculated onto fresh SDA medium and sporulated for 2 weeks at 25 °C. The conidia were harvested by adding 10 ml of sterile distilled water containing 0.01% Tween 80 to the sporulating fungi and scraping the conidia from the agar surface with a sterile cell spreader. The resulting suspension was vortexed for 1 min to homogenize it. The suspension was then filtered through a double layer of sterile cheesecloth into 50-ml sterile Falcon tubes to remove mycelium and agar pieces. The conidial concentration was determined with the Neubauer haemocytometer under the light microscope and adjusted to concentration of 10^7 conidia/ml.

Screening test

The efficacy of the entomopathogens was tested separately against the second larval stage and the adult WFT under laboratory conditions. The tests were carried out according to IRAC test method No. 10. Bean leaves were placed in plastic boxes (15×15 cm), which were disinfected with sodium hypochlorite (1%). Moist cotton was used to prevent the leaves from drying out. Thirty second instar larvae were transferred to the leaves in the boxes using a suction tube. 1 ml of the prepared bacterial (10^8 cfu/ml) and fungal (10^7 conidia/ml) suspensions was sprayed onto the leaves using a mini hand sprayer. Sterile water was used as control for bacteria and sterile water with 0.01% Tween80 for fungi. Experiments were performed according to a completely randomized experimental design with 4 replicates, where each box was scored as a single plot and replicated 2 times. Bioassays were performed in a climate chamber

with a temperature of 26 °C, 70% relative humidity and a 16/8 photoperiod (light: dark). The bioassays were also performed with the adult stage described above. Mortality was monitored daily for 5 days, followed by rate corrections according to the Abbott formula (Abbott 1925). Data were then subjected to analysis of variance (ANOVA), followed by Tukey's HSD multiple comparison test using SPSS statistical software to assess differences between treatments. In addition, the estimates of the median lethal time (LT_{50}) and their confidence limits were calculated using probit analysis (Finney 1971).

Concentration response test

Concentration experiments were carried out with 3 bacteria and 3 fungi that had the lowest LT_{50} value and the highest insecticidal effect on the larvae and adults of the pest. Six different concentrations of microorganisms were obtained by tenfold serial dilution of each stock suspension. The bacterial isolates *S. marcescens* Se9, *B. safensis* Cq1 and *B. thuringiensis* Sn10 were prepared at concentrations ranging from 10^8 to 10^3 cfu/ml, and fungal isolates *M. flavoviride* As18, *B. bassiana* Hp4 and *L. muscarium* Pa3 at concentrations ranging from 10^8 to 10^3 conidia/ml. The bioassays were then performed as indicated in the screening tests. The median lethal concentrations (LC_{50}) of the microorganisms were calculated for the larvae and adult stages of WFT using probit analysis in the statistical software SPSS.

Determination of Synergism

Metarhizium flavoviride As18 and *S. marcescens* Se9, which had the lowest LC_{50} value, were used to investigate the synergistic effect of the isolates on WFT. The combinations of isolates were prepared at different concentrations (Table 2) and tested separately for the second larval stage and the adult stage of WFT as described in the screening test. The co-toxicity factors were calculated using the following equation:

$$CTF = (Oc - Oe)/Oe \times 100.$$

where Oc is the mortality caused by the combination application and Oe is the sum of the mortality rates of the isolates making up the combination alone. Values > 20 represent synergistic effect, $-20 \leq$ values ≤ 20 represent additive effect and values < −20 represent combinations that are antagonistic (Ma et al. 2008).

Results

As a result of the screening tests performed with entomopathogenic bacteria, it was found that all bacteria are pathogenic for the larvae and adults of WFT, but their virulence was different. The highest virulence on the second larval stage at a concentration of 10^8 cfu/ml was observed with the isolates *S. marcescens* Se9 (54%) and *B. safensis* Cq1 (51%). The other isolates caused less than 50% mortality (F = 108.13; df = 14; $p < 0.05$) (Fig. 1). The median lethal time (LT_{50}) for bacterial isolates with a concentration of 10^8 cfu/ml on the second larval stage of WFT was determined by probit analysis, and the lowest LT_{50} values were 4.67 (*S. marcescens* Se9), 4.79 (*B. safensis* Cq1) and 4.87 (*B. thuringiensis* Sn10) days (Table 3). The LC_{50} values of these isolates for the second larval stage were determined to be 4×10^6, 3.9×10^6 and 8.3×10^7 cfu/ml, respectively (Table 4). These isolates showed highest virulence also on the adult stage. *S. marcescens* Se9, *B. safensis* Cq1 and *B. thuringiensis* Sn10 caused 69.54,

Table 2 Median lethal time (LT_{50}) of bacterial isolates at a concentration of 10^8 cfu/ml for the second larval and adult stages of WFT

Isolates	LT_{50} (FL, %95) (days)	Slope ± *SE*	LT_{95}	*df*	X^2
Sn10	4.87 (4.52 –5.43)*	0.8 ± 0.055	7.01	3	6.62
	4.73 (4.58 –4.91)	1.0 ± 0.051	6.91	3	2.61
Se13	6.13 (5.74 –6.72)	0.6 ± 0.073	8.74	3	2.07
	6.06 (5.69 –6.61)	0.7 ± 0.072	8.65	3	2.66
MnD	5.51 (5.26 –5.85)	0.7 ± 0.064	7.88	3	3.88
	5.55 (5.29 –5.90)	0.6 ± 0.061	8.02	3	4.39
Xd3	5.53 (5.29 –5.87)	0.7 ± 0.068	7.80	3	2.39
	5.70 (5.41 –6.10)	0.6 ± 0.062	8.24	3	4.16
Ta1	6.33 (5.88 –7.13)	1.0 ± 0.114	8.53	3	0.91
	6.36 (5.90 –7.16)	1.0 ± 0.103	8.70	3	0.54
Ta6	6.11 (5.73 –6.75)	0.9 ± 0.117	8.14	3	0.50
	7.07 (6.39 –8.26)	0.5 ± 0.073	10.3	3	3.11
Se9	4.67 (4.50 –4.86)	0.8 ± 0.044	7.10	3	2.88
	4.35 (4.23 –4.47)	1.3 ± 0.053	6.18	3	3.68
Sn14	5.01 (4.84 –5.21)	1.0 ± 0.061	7.06	3	3.18
	5.90 (5.22 –7.55)	0.6 ± 0.059	8.71	3	6.31
Sn8	5.67 (5.41 –6.06)	0.7 ± 0.076	7.90	3	1.50
	5.97 (5.61 –6.48)	0.6 ± 0.061	8.75	3	5.11
Cq1	4.79 (4.65 –4.96)	1.0 ± 0.058	6.78	3	4.46
	4.50 (4.23 –4.86)	1.0 ± 0.055	6.38	3	6.22
Ar2	6.41 (5.93 –7.34)	0.6 ± 0.124	8.66	3	3.23
	5.91 (5.57 –4.40)	0.6 ± 0.062	8.63	3	5.05
Cq2	5.64 (5.38 –6.01)	0.7 ± 0.078	7.80	3	1.00
	5.86 (5.53 –6.33)	0.6 ± 0.061	8.57	3	5.02
Tp11	5.68 (5.42 –6.07)	1.0 ± 0.086	7.75	3	1.85
	6.19 (5.42 –8.18)	0.6 ± 0.061	9.14	3	5.63
Se2	5.77 (5.49 –6.19)	1.0 ± 0.095	7.78	3	1.28
	6.12 (5.73 –6.70)	0.6 ± 0.064	8.94	3	4.20
Ld4	6.31 (5.86 –7.10)	1.0 ± 0.118	8.46	3	0.57
	6.40 (5.91 –7.38)	1.0 ± 0.140	8.47	3	1.80

* For each isolate, the LT_{50} value for larvae is given in the first row and for adults in the second row

SE Standard error, *df* degree of freedom, X^2 Chi-square

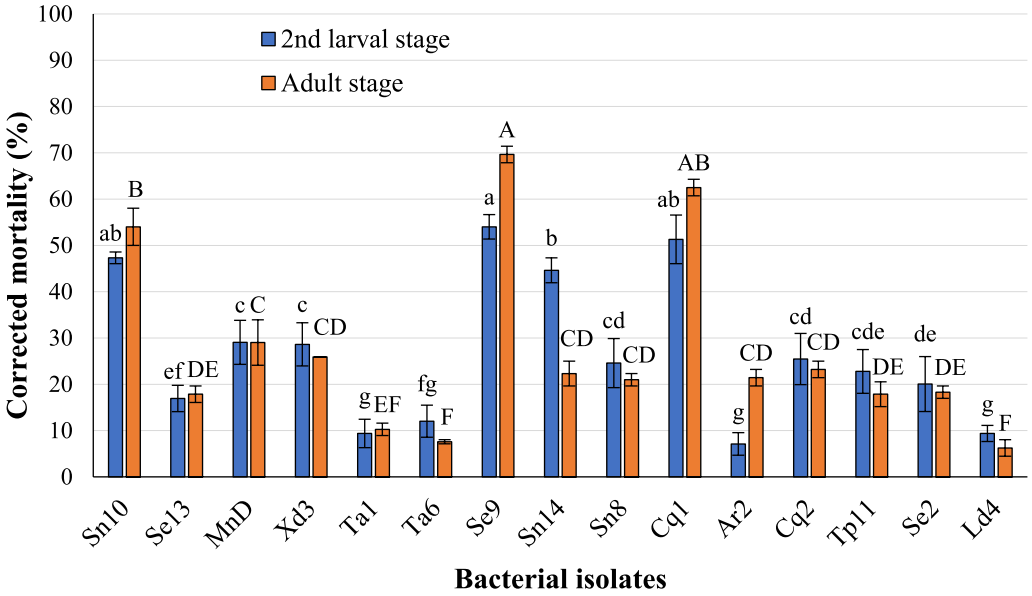

Fig. 1 Virulence of bacterial isolates on the second larval and adult stages of WFT at a concentration of 10^8 cfu/ml. The bars show the means of mortality rates obtained from bioassays with four replicates, corrected according to the Abbott formula. The error bars show the standard deviation between the mean values. The different lowercase and uppercase letters indicate statistical differences (ANOVA, Tukey's HSD test, $p < 0.05$)

Table 3 Median lethal concentration (LC_{50}) of bacterial isolates for the second larval and adult stages of WFT

Insect stage	Isolates	LC_{50} (FL, %95) (cfu/ml)	Slope ± *SE*	LC_{95}	*df*	X^2
Larvae	Se9	4.0×10^6 (1.0×10^6 – 3.0×10^7) b	0.5 ± 0.022	4.18×10^{11}	4	15.55
	Sn10	8.3×10^7 (3.2×10^7 – 2.8×10^8) a	0.28 ± 0.02	2.96×10^{14}	4	6.317
	Cq1	3.9×10^6 (8.1×10^5 – 4.4×10^7) b	0.4 ± 0.022	4.81×10^{11}	4	20.01
Adult	Se9	6.3×10^6 (2.8×10^6 – 1.7×10^7) a	0.24 ± 0.02	2.65×10^{14}	4	3.763
	Sn10	8.2×10^7 (3.1×10^7 – 2.9×10^8) c	0.25 ± 0.02	5.59×10^{14}	4	4.351
	Cq1	1.9×10^7 (4.5×10^6 – 1.9×10^8) b	0.7 ± 0.022	1.93×10^{13}	4	9.159

SE Standard error, *df* degree of freedom, X^2 Chi-square

62.5 and 54% mortality, respectively, on adult stage (Fig. 1). Virulence of the other isolates ranged from 6.25 to 29 (F = 122.89; df = 14; $p < 0.05$). The lowest LT_{50} value for adult stage was obtained with *S. marcescens* Se9, *B. safensis* Cq1 and *B. thuringiensis* Sn10 isolates at 4.35, 4.5 and 4.73 days, respectively (Table 3). The LC_{50} values of these isolates are given in Table 4.

All fungi tested were pathogenic to the second larval stage of WFT. The virulence of the fungal isolates ranged from 8.7 to 92.1% (F = 144.4; df: 14; $p < 0.05$) (Fig. 2). Among the isolates, the virulence of *M. flavoviride* As18, *L. muscarium* Pa3 and *B. bassiana* Hp4 was 92.1, 85.1 and 86.1%, respectively. The LT_{50} values of the isolates for the second larval stage were 3.37 days for *M. flavoviride* As18, 3.68 days for *B. bassiana* Hp4 and 3.87 days for *L. muscarium* Pa3 (Table 4). As in the second larval stage, the isolates with the highest virulence on adult stage were *M. flavoviride* As18 (74.5%), *B. bassiana* Hp4 (70.5%) and *L. muscarium* Pa3 (69.2%) (Fig. 2). The LT_{50} values at a concentration of 10^7 conidia/ml for *M. flavoviride* As18, *B. bassiana* Hp4 and *L. muscarium* Pa3 were determined to be 4, 4.19 and 4.23 days, respectively (Table 4). The LC_{50} values of these isolates for the second larval stage and adults are shown in Table 5.

Binary combinations of *S. marcescens* Se9 and *M. flavoviride* As18 were used to determine the synergistic effect, using the co-toxicity factor (CTF) as a criterion. The CTF values for combination 3 were + 36.92, indicating a strong synergy on the second larval stage. The other combinations with CTF values < + 20 showed an additive or antagonistic effect (Table 6). Moreover, combination 2 and 3 showed a synergistic effect on the adult stage of WFT with CTF values of + 22.49 and + 32.38, respectively. Combination 7 showed an antagonistic effect. The other combinations with CTF

Table 4 Median lethal time (LT_{50}) of fungal isolates at a concentration of 10^7 conidia/ml for the second larval and adult stages of WFT

Isolates	LT_{50} (FL, %95) (days)	Slope ± *SE*	LT_{95}	*df*	X^2
As2	7.00 (6.35 –8.05)*	0.5 ± 0.055	10.77	3	2.59
	7.80 (6.87 –9.50)	0.4 ± 0.061	11.95	3	2.32
As18	3.37 (3.06 –3.59)	1.0 ± 0.043	5.17	3	7.27
	4.00 (3.88 –4.14)	1.0 ± 0.040	6.27	3	1.25
KTU2	4.32 (4.19 –4.46)	1.0 ± 0.046	6.43	3	2.50
	4.65 (4.50 –4.83)	1.0 ± 0.048	6.90	3	5.25
Gg12	4.66 (4.49 –4.85)	0.8 ± 0.043	7.16	3	3.06
	4.96 (4.77 –5.18)	0.8 ± 0.049	7.39	3	2.76
B5	5.73 (5.06 –7.23)	0.6 ± 0.047	8.90	3	6.63
	6.44 (5.95 –7.17)	0.5 ± 0.052	9.90	3	2.32
B23	4.40 (4.28 –4.54)	0.8 ± 0.050	6.39	3	0.83
	4.74 (4.59 –4.92)	1.0 ± 0.051	6.93	3	0.77
KTU24	5.08 (4.87 –5.33)	0.8 ± 0.048	7.65	3	3.82
	5.31 (5.09 –5.60)	0.8 ± 0.055	7.77	3	2.30
KTU57	5.31 (4.88 –6.10)	0.7 ± 0.058	7.67	3	5.59
	5.72 (5.43 –6.13)	0.6 ± 0.066	8.20	3	3.39
Hp4	3.68 (3.57 –3.78)	1.2 ± 0.045	5.49	3	1.90
	4.19 (4.07 –4.32)	1.0 ± 0.046	6.20	3	2.79
Pa4	4.16 (4.05 –4.29)	1.0 ± 0.046	6.17	3	1.76
	4.28 (4.16 –4.42)	1.0 ± 0.047	6.33	3	1.34
Gg1	4.76 (4.62 –4.93)	1.0 ± 0.057	6.75	3	0.29
	4.83 (4.69 –5.00)	0.9 ± 0.061	6.78	3	5.07
B8	4.84 (4.68 –5.03)	1.0 ± 0.052	7.05	3	0.75
	5.11 (4.93 –5.33)	1.0 ± 0.063	7.18	3	1.18
Pa3	3.87 (3.62 –4.15)	1.1 ± 0.047	5.66	3	6.65
	4.23 (4.12 –4.36)	1.0 ± 0.047	6.23	3	3.10
KTU42	5.58 (5.32 –5.93)	0.8 ± 0.064	7.97	3	0.72
	5.82 (5.52 –6.27)	1.0 ± 0.083	8.03	3	1.25
KTU1	5.96 (5.62 –6.46)	0.8 ± 0.070	8.50	3	0.60
	6.42 (5.94 –7.22)	0.7 ± 0.094	8.88	3	0.62

* For each isolate, the LT_{50} value for larvae is given in the first row and for adults in the second row

SE Standard error, *df* degree of freedom, X^2 Chi-square

values between −20 and 20 showed additive effects on the adult stage of WFT (Table 7).

Discussion

Natural enemies in an agroecosystem play a significant role in keeping pests from reaching economic threshold level. Insect-pathogenic microorganisms as pesticides not only suppress pest populations but also ensure sustainable agriculture as they are host-specific, leave no toxic residues, have no phytotoxic effects, are harmless to humans and provide self-sustaining pest control.

Results of the screening test showed that thirty different indigenous entomopathogenic microorganisms isolated from different sources were pathogenic to both the second larval stage and adult of WFT. However, the results also showed that there were differences in virulence within genera, species and isolates. Apart from two bacterial isolates, the virulence did not exceed 30%. Since entomopathogenic bacteria need to be digested by the insects to be effective, their use in the control of sucking pests such as WFT is not common. However, there are studies that show they can be effective. Helyer and Brobyn (1992) tested the commercial product Bactospeine garden, containing *B. thuringiensis*, against WFT larvae and observed a mortality of 87%. Similarly, Bilbo et al. (2020) indicated that two commercial bioinsecticides, Venerate (*Burkholderia* strain A396) and Grandevo (*Chromobacterium subtsugae* strain PRAA4-1), significantly reduced thrips populations in a commercial staked tomato field. In the present study, *S. marcescens* Se9 and *B. safensis* Cq1 isolates caused 54 and 51.3% mortality on the second larval stage, respectively. These isolates also showed 69.6 and 62.5% mortality rates on adult of WFT. Pathogenicity of *S. marcescens* has been reported in various agricultural pests such as *Helicoverpa armigera* (Mohan et al. 2011), *Bemisia tabaci* (Karut et al. 2020), *Heliothis virescens* (Sikorowski et al. 2001), *Bombyx mori* (Tao et al. 2022), *Anoplophora glabripennis* (Deng et al. 2008), *Spodoptera exigua* (Eski et al. 2018) and *Rhynchophorus ferrugineus* (Zhang et al. 2011). However, this study is the first report on the pathogenicity of *S. marcescens* against WFT. Its virulence depends on its extracellular hydrolytic enzymes, including chitinases, proteases and nucleases as well as toxins with hemolytic and cytotoxic activities (Tao et al. 2022). In these reports, virulence of *S. marcescens* isolates ranged between 50 and 100%, suggesting the existence of host species-specific interactions between these virulence factors and the insects' innate immune system.

Similarly, the fungal isolates tested were found to be pathogenic for the WFT and differed in their virulence. Among the isolates, the virulence of *M. flavoviride* As18, *L. muscarium* Pa3 and *B. bassiana* Hp4 on second larval stage was 92.1, 85.1 and 86.1%, respectively, and the differences between their virulence were non-statistically significant ($p > 0.05$). There are many studies on the use of fungi in the control of WFT as they do not need to be eaten by insects to be effective and can initiate infection directly from the cuticle. These studies have also shown that the virulence of different species and even different strains of the same species may vary (Kim et al. 2020). Sengonca et al. (2006) investigated the virulence of two different strains of *M. flavoviride* to first instar larvae of WFT and reported that *M. flavoviride* strain 5744 was more pathogenic than *M. flavoviride* strain 1164. In the present study, *M. flavoviride* As18 caused 92% mortality,

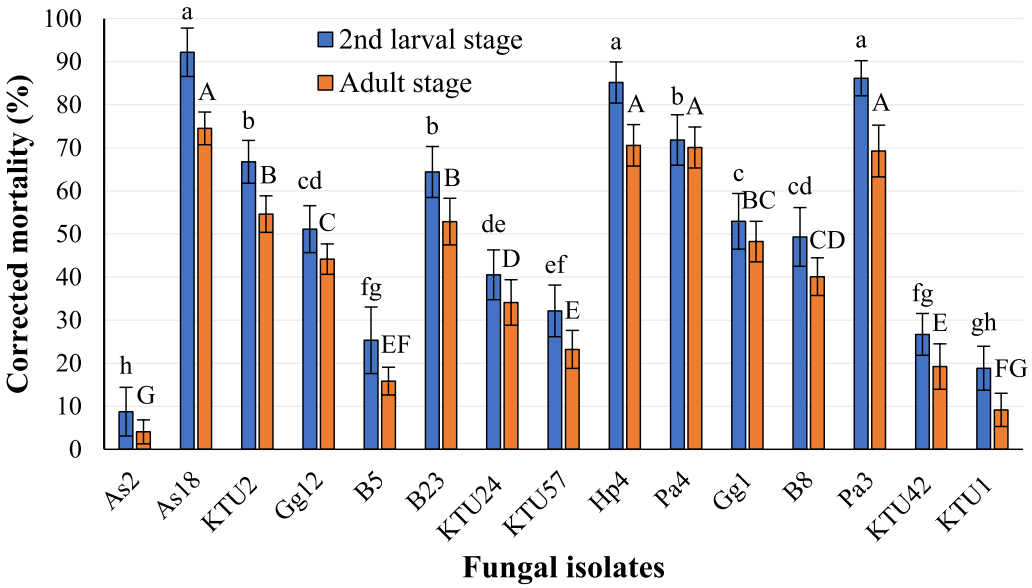

Fig. 2 Virulence of fungal isolates on the second larval and adult stages of WFT at a concentration of 10^7 cfu/ml. The bars show the means of mortality rates obtained from bioassays with four replicates, corrected according to the Abbott formula. The error bars show the standard deviation between the mean values, different lowercase and uppercase letters indicate statistical differences (ANOVA, Tukey's HSD test, $p < 0.05$)

Table 5 Median lethal concentration (LC_{50}) of fungal isolates for the second larval and adult stages of WFT

Insect stage	Isolates	LC_{50} (FL, %95) (conidia/ml)	Slope ± *SE*	LC_{95}	*df*	X^2
Larvae	As18	1.6×10^4 (6.0×10^3–3.7×10^4) a	0.6 ± 0.026	5.50×10^7	4	8.903
	Hp5	4.1×10^4 (1.7×10^4–8.6×10^4) b	0.5 ± 0.024	2.40×10^8	4	7.120
	Pa3	4.6×10^4 (1.0×10^4–1.5×10^5) b	0.5 ± 0.023	3.50×10^8	4	18.29
Adults	As18	7.1×10^4 (2.7×10^4–1.6×10^5) a	0.5 ± 0.023	1.20×10^9	4	7.779
	Hp5	2.4×10^5 (1.5×10^5–3.9×10^5) b	0.5 ± 0.022	1.12×10^{10}	4	4.871
	Pa3	8.0×10^5 (5.1×10^5–1.2×10^6) c	0.4 ± 0.022	2.74×10^{10}	4	5.607

SE Standard error, *df* degree of freedom, X^2 Chi-square

while *M. flavoviride* As2 caused only 8% mortality on second instar larvae. The susceptibility of WFT to EPF varied with the developmental stages. Sengonca et al. (2006) found that the LC_{50} value of *B. bassiana* strain 4591 was 3.55×10^4 conidia/ml for first larval stage and 1.32×10^6 for adult stage. Similarly, the LC_{50} value of *M. flavoviride* As18 was lower for second larval stage than for adult. It can be assumed that these differences in susceptibility at different stages of development are due to the thickness of the cuticle or metamorphosis. In addition, an increasing amount of antifungal substances on the cuticle may inhibit spore germination and penetration (Eski and Gezgin 2022). On the other hand, the pathogenicity of EPF depends on the ability of their enzymatic equipment, which consists of lipases, proteases and chitinases that degrade the insect's integument (Mondal et al. 2016; Shin et al. 2020). However, a variety of factors such as water, ions, fatty acids and nutrients on the surface of the cuticle influence spore germination (Liu et al. 2023). In addition, the microorganisms in the gut could also play a crucial role in the development and ecology of the host's defenses against fungal pathogens. Zhou et al. (2023) showed that internal microorganisms of *F. occidentalis* were involved in the infection process of *Lecanicillium* sp. and that disruption of the internal microbial balance leads to recognizable sublethal effects. Therefore, the differences in virulence could be explained by many factors that influence the infection process.

The use of two different biological control agents against the pest may increase virulence or accelerate the infection process as they act independently on different points of host susceptibility. Mantzoukas et al. (2013) suggested that when fungi and bacteria are applied simultaneously, their interactions have synergistic effects on insect mortality as both agents act independently, but it depends on the particular combinations of pathogens and host species. In the present study, the combined infections with

Table 6 Interactions between *S. marcescens* Se9 (*Sm*-Se9) and *M. flavoviride* (*Mf*-As18) against second larval stage of WFT

Combination	Concentration		Observed mortality (%)	Expected mortality (%)	Co-toxicity factor	Interaction type
	Sm-Se9 (cfu/ml)	*Mf*-As18 (conidia/ml)				
1	LC_{25}	LC_{25}	47.63	43.32	9.94	Additive
2		$10\times LC_{25}$	63.67	56.35	12.99	Additive
3		$100\times LC_{25}$	95.79	69.96	36.92	Synergistic
4	$10\times LC_{25}$	LC_{25}	51.37	57.23	−10.12	Additive
5		$10\times LC_{25}$	71.42	70.26	1.65	Additive
6		$100\times LC_{25}$	85.26	83.87	1.65	Additive
7	$100\times LC_{25}$	LC_{25}	47.76	69.31	−31.09	Antagonistic
8		$10\times LC_{25}$	64.70	82.34	−21.42	Antagonistic
9		$100\times LC_{25}$	75.89	95.95	−20.90	Antagonistic
10	LC_{25}	0	20.46	–		
11	$10\times LC_{25}$	0	34.37	–		
12	$100\times LC_{25}$	0	46.45	–		
13	0	LC_{25}	22.86	–		
14	0	$10\times LC_{25}$	35.89	–		
15	0	$100\times LC_{25}$	49.50	–		

Table 7 Interactions between *S. marcescens* Se9 (*Sm*-Se9) and *M. flavoviride* (*Mf*-As18) against adult stage of WFT

Combination	Concentration		Observed mortality (%)	Expected mortality (%)	Co-toxicity factor	Interaction type
	Sm-Se9 (cfu/ml)	*Mf*-As18 (conidia/ml)				
1	LC_{25}	LC_{25}	43.89	36.78	19.33	Additive
2		$10\times LC_{25}$	76.79	62.69	22.49	Synergistic
3		$100\times LC_{25}$	96.26	72.71	32.38	Synergistic
4	$10\times LC_{25}$	LC_{25}	47.64	52.67	−9.55	Additive
5		$10\times LC_{25}$	73.66	78.58	−6.26	Additive
6		$100\times LC_{25}$	91.07	88.60	2.78	Additive
7	$100\times LC_{25}$	LC_{25}	48.21	62.29	−22.6	Antagonistic
8		$10\times LC_{25}$	70.78	88.20	−19.75	Additive
9		$100\times LC_{25}$	80.80	98.22	−17.73	Additive
10	LC_{25}	0	11.34	–		
11	$10\times LC_{25}$	0	27.23	–		
12	$100\times LC_{25}$	0	36.85	–		
13	0	LC_{25}	25.44	–		
14	0	$10\times LC_{25}$	51.35	–		
15	0	$100\times LC_{25}$	61.37	–		

different concentrations of *M. flavoviride* As18 and *S. marcescens* Se9 generally led to additive and in one case to synergistic interactions. The synergistic or additive effect in the infection of insects with bacterial–fungal mixtures can probably be attributed to two main reasons. Firstly, the intestinal disturbances and general intoxication caused by bacteria interfere with insect feeding, delay their growth and prolong the inter-molt period. The delayed growth and molting may assist the infection may increase the susceptibility of the larvae to bacterial infections.

The combined use of fungi and predators (Zhang et al. 2021), nematodes and predators (Ebssa et al. 2006) and entomopathogens with conventional insecticides (Ge et al. 2020) has been reported to significantly reduce WFT populations. However, the combined use of entomopathogenic microorganisms is limited. The combined treatment of *S. carpocapsae* Nemastar and

M. anisopliae ICIPE-69 on soil stages of WFT resulted in lower emergence of adults and synergistic response compared to single treatment (Otieno et al. 2016). To our knowledge, this is the first study to show that the combined use of local *M. flavoviride* and *S. marcescens* isolates can control WFT through a synergistic effect. On the other hand, synergistic effects between EPF and bacteria have also been observed in other insect pests. For example, Beris and Korkas (2021) reported that a combination treatment of *B. bassiana* and *B. thuringiensis* caused significantly higher mortality on the larvae of the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae), than a single treatment when applied at the same time. In contrast, Ma et al. (2008) reported that an additive effect on mortality was observed when the Asiatic corn borer, *Ostrinia furnacalis* (Lepidoptera: Crambidae) was exposed to a combination of *B. bassiana* (10^7 conidia/ml) and *B. thuringiensis* toxin Cry1Ac (0.2 µg/ml). However, in the same study, combinations of sublethal concentrations of Cry1Ac and *B. bassiana* resulted in antagonism. In the present study, although additive and synergistic effects were generally observed with the combination of *M. flavoviride* and *S. marcescens*, antagonistic effects were observed in combinations using 100 times the LC_{25} value of *S. marcescens*. Competing factors between the control agents can also lead to antagonistic effects. The pigment prodigiosin, which is produced by some *Serratia* species, has been reported to have an antifungal effect (Jimtha et al. 2017). This could explain the antagonistic effect that occurs in combinations using high concentrations of *S. marcescens* Se9. Similarly, Deng et al. (2022) reported that the Japanese pine sawyer, *Monochamus alternatus* associated *Serratia* species showed a strong inhibitory effect against *B. bassiana* by reducing the germination and growth of the fungal conidia. This clearly shows the importance of the concentrations of the agents used in the combinations.

Conclusion

This is the first study to show that the combined use of the *M. flavoviride* and *S. marcescens* has a synergistic effect on the second larval stage and adult of WFT. The combination of fungi and bacteria may be promising for the development of combination preparations that cause a high mortality rate of WFT. In further studies, *M. flavoviride* As18 and *S. marcescens* Se9 should be formulated as a biopesticide to overcome the adverse effects of the environment such as UV radiation and temperature, and efficacy of biopesticide should be tested under greenhouse or field conditions to validate the results.

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Author contributions
MK, MG and AE performed the field studies. MK, DBE, AE and ID designed and performed the study. DBE and AE took part in writing original draft. ID and MG involved in review and editing. All authors read and approved the final manuscript.

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Declarations

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Competing interests
The authors declare that they have no competing interests

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