



# Interactions between *Penicillium mallochii* and the endoparasitoid *Venturia canescens* Grav. (Hymenoptera: Ichneumonidae), and implications for combined biocontrol of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae)

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## Abstract

The application of biological control agents in combination can result in synergistic, neutral or antagonistic interactions, depending on the concentrations of the agents, the timing of the application and the host species. *Ephestia kuehniella* is a prominent storage pest, and *Venturia canescens* is recognised as a significant biological control agent for *E. kuehniella*. *Penicillium mallochii* is a recently identified species with an impact on insects. This study presents the first information on the interaction between *P. mallochii* and the parasitoid *V. canescens*, and the combined biological control of *E. kuehniella* using these two different control agents. The aim of the study is to evaluate the direct effect of *P. mallochii* conidia and ethanol extract applications on *E. kuehniella*, and the fungus-parasitoid-host interaction in three stages. The study investigates the impact of *V. canescens* on infected host larvae, the effect of fungal infection on parasitized host larvae, and the interaction between fungus and adult parasitoids. The results indicate that *P. mallochii* had a negligible effect on the developmental parameters of *E. kuehniella*, but it did reduce the total number of eggs. In the interaction studies, it was observed that the adult emergence time increased in all scenarios; however, it was determined that the adult parasitoid longevity was extended only in the parasitization stage after the dose application and shortened in the other two stages. Effective time management and the application of correct interaction scenarios are important to prevent antagonistic interactions in interaction scenarios.

**Keywords** *Ephestia kuehniella* · *Venturia canescens* · *Penicillium mallochii* · Interaction · Combined biocontrol

## Introduction

In communities of biological control agents, interactions within the group are common, including those between parasitoids and entomopathogenic fungi (EPF) (Mesquita and Lacey 2001; Rashki et al. 2009). Interactions between entomopathogens and other biological control agents can be categorised as synergistic, neutral, or antagonistic, depending on the specific biological control agents, their concentrations, application timing, and host species (Beline 2018).

Synergistic interactions result in a higher mortality rate of the pest population than the total individual mortality caused by each agent separately. Neutral interactions occur when natural enemies do not interact, resulting in a total mortality rate that is equivalent to the sum of individual deaths caused by each agent. Antagonistic interactions arise when the total mortality rate is lower than if one of the natural enemies acted alone (Straub et al. 2008; Rashki et al. 2009).

Research into the interactions between EPF and other natural enemies (parasitoids and predators) is a key area of research in this field (Roy et al. 2006; Ormond et al. 2011; Martins et al. 2014; Bayissa et al. 2016). In bio-control, the use of integrated EPF with a parasitoid has been shown to enhance the effectiveness of pest control. In this context, while the microbial control agent can protect the crop, parasitoids keep the pest population below a damage threshold (Baverstock et al. 2010). However, EPF with a broader host

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range can infect non-target insects, including parasitoids, leading to direct harmful effects on this biological control agent (Martins et al. 2014). Consequently, it is imperative to comprehensively assess the potential lethal or non-lethal consequences of entomopathogens on the behavioural and biological parameters of parasitoids (Mesquita and Lacey 2001; Kim et al. 2005; Rashki et al. 2009; Aiuchi et al. 2012; Emami et al. 2013).

*Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is a cosmopolitan storage pest that has been shown to cause significant damage in industrial flour mills and has spread worldwide through international trade (Jacob and Cox 1977; Ayvaz and Karabörklü 2008). *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) is a solitary, koinobiont endoparasitoid. It is a general parasitoid attacking lepidopteran larvae from the families Noctuidae, Tortricidae, Gelechiidae, Tineidae, and Pyralidae, including harmful insects in stored products (Gomes 2021). *Penicillium mallochii* Rivera, Urb & Seifert (Eurotiales: Aspergillaceae), a little-known fungal species, has been isolated from the caterpillars of *Rothschildia lebeau* (Lepidoptera: Saturniidae) and *Citheronia lobesis* (Lepidoptera: Saturniidae) in Costa Rica (Rivera et al. 2012). The study by Güner et al. (2025) was the first to report the pathogenicity of *P. mallochii* on *Cadra cautella* Walker (Lepidoptera: Pyralidae).

Although there is significant evidence in the literature supporting that *V. canescens* is a promising candidate for the biological control of *E. kuehniella* (Özkan et al. 2004; Roberts et al. 2004; Eliopoulos 2006), no studies have been found regarding the insecticidal activity of *P. mallochii* on *E. kuehniella*. This research aims to evaluate the direct impact of *P. mallochii* infection on *E. kuehniella* and the fungus-parasitoid interaction in three stages. The study investigates the influence of *V. canescens* on the infected host larvae, the effect of fungal infection on parasitized host larvae, and the interaction of the fungus with adult parasitoids.

## Materials & methods

### Fungus

The strain used in this study was isolated from beech bark in Balıkesir, Türkiye, in 2017. The strain was identified through molecular methods at Adıyaman University (TR) (NCBI Gen bank accession number: OR856720). It is stored with the code CB-16 (*P. mallochii*) in the Fungal Herbarium of Balıkesir University Microbiology Research Laboratory. For the preparation of the conidial suspension and metabolite production, the fungus was cultured on Malt Extract Agar (MEA) in the dark at 28 °C for 14 days. After the incubation period and the completion of colony development,

10 mL of sterile water containing 0.01% Tween 20 was added to the Petri dishes to prepare the conidial suspension. The spores were then carefully scraped (Fancelli et al. 2013; Güner et al. 2025). The conidial count in the suspension was determined to be  $1 \times 10^9$  conidia/mL using a Thoma slide. The procedure described by Velmurugan et al. (2010) and Bouhri et al. (2020) was followed for the production of fungal ethanol extract. The obtained extract was lyophilized using a freeze dryer (CHRIST ALPHA 1–2 LD). The extract doses of *P. mallochii*, 0.5 g of, was dissolved in 10 mL of Phosphate-buffered saline (PBS) to create the main stock solution with a concentration of 50 mg/mL. All stock solutions were stored in a deep freeze at –20 °C. To prepare 10 mL (10 mg/mL concentration) solution, 2 mL of stock solution was taken and 8 mL of PBS was added. Therefore, the final solution concentrations were 10 mg/mL (Güner et al. 2025).

### Insect culture

Colonies of *E. kuehniella* and *V. canescens* were reared and maintained at Balıkesir University Department of Biology, Faculty of Science and Letters, Türkiye. The insect cultures were maintained in the laboratory under conditions of  $25 \pm 1$  °C temperature,  $65 \pm 5\%$  relative humidity, and a 12:12-h (L:D) photoperiod. The laboratory temperature was regulated using a 9000 BTU air conditioner and a thermostat-controlled radiator. Temperature and humidity values in the laboratory were continuously monitored using a TFA 30.5013 digital indoor-outdoor thermos-hydrometer and a maximum-minimum thermometer.

### *E. kuehniella* colony

The source of laboratory stocks and successive cultures of *E. kuehniella* moths was a core culture established in Animal Physiology Research Laboratory, containing larvae, pupae, and adults. Every day (excluding weekends), male and female adults were collected from these cultures and placed in glass jars of various volumes containing food. The jar openings were sealed with cloth to facilitate air circulation. To feed the flour moths, a mixture consisting of 40% wheat flour, 20% corn flour, 20% barley flour, and 20% fine bran was used. Third-instar *E. kuehniella* larvae ( $25 \pm 5$  mg) were used in the study (Güner 2024).

### *V. canescens* colony

The source of laboratory stocks and successive cultures of *V. canescens* was the adult individuals present in Animal Physiology Research Laboratory. For rearing the parasitoids, ten adult parasitoids (4–5 days old) were transferred

into 0.5 L transparent plastic containers containing approximately 100 g of sterilized diet and around 100 final instar *E. kuehniella* larvae. The parasitoids were fed daily with cotton balls soaked in a 50% diluted floral honey solution. After 24 h of parasitization, the parasitoids were removed from the containers to prevent possible superparasitism. To ensure adequate air circulation within the containers, an equal number of holes were made on the sides using push pins (Özkan 1999; Güner 2024).

### Effect of *P. mallochii* on *E. kuehniella* larvae

Three independent replicates were conducted on separate dates for each treatment group—conidial suspensions, ethanol extract, and control—using 10 healthy third-instar *E. kuehniella* larvae per replicate, resulting in a total of 30 larvae per treatment. For the conidial treatment groups, concentrations prepared in PBS at  $10^5$ ,  $10^6$ ,  $10^7$ ,  $10^8$ , and  $10^9$  conidia/mL were used, whereas ethanol extract was applied at concentrations of 10, 5, 2, 1, 0.5, and 0.1 mg/mL. PBS was administered to the control groups. The concentrations used in the study were prepared by diluting the main stock solutions, and 2  $\mu$ L of each solution was applied via topical application along the dorsal midline of larvae from the prothorax using a micropipette. After treatment, each larva was transferred individually into sterile Petri dishes lined with filter paper and maintained at  $26 \pm 2$  °C, 60% relative humidity, under a 12:12-h (L:D) photoperiod. Larval mortality data were used to calculate LD50, LD60, LD70, LD80, and LD90 values via probit analysis using SPSS software. Additionally, the effects of varying doses of conidial suspensions and ethanol extract on larval and pupal stages, adult emergence time, adult longevity, weight and fecundity of *E. kuehniella* were monitored daily.

### Efficiency of *V. canescens*, *P. mallochii* and their combination on *E. kuehniella*

#### Larvae first exposed to the parasitoid and then treated with the fungus.

The aim of this experiment was to evaluate the development of newly emerged parasitoids after exposing host larvae first to the parasitoid *V. canescens* and then to the fungus *P. mallochii*. Each larva was exposed to a 1-day-old *V. canescens* for 24 h. After 24 h, the parasitoids were removed from the dish, and the potentially parasitized larvae were treated with different conidia and metabolite doses of *P. mallochii* after 1, 4, or 8 days. PBS-treated larvae were used for the control group. The experimental and control groups were placed in plastic petri dishes (60  $\times$  15 mm) in an incubator at  $25 \pm 1$  °C temperature,  $65 \pm 5\%$  relative humidity, and a 12:12-h (L:

D) photoperiod. The emergence numbers of parasitoids and the biological development (adult emergence time and adult longevity) of the parasitoid were monitored. All experiments related to parasitoid individuals were repeated three times at different time intervals.

#### Larvae first treated with fungus and then exposed to the parasitoid

The aim of this experiment was to evaluate the development of newly emerged parasitoids after exposing larval hosts first to the fungus *P. mallochii* and then to the parasitoid *V. canescens*. The larvae were topically treated with conidia and metabolite doses of *P. mallochii*. In this scenario, larvae treated with PBS were used as the control group. Subsequently, the larvae were parasitized by a 1-day-old *V. canescens* at different time intervals (after 1, 4, or 8 days). After 24 h, the parasitoids were removed from the dish, and the potentially parasitized experimental and control groups were placed in plastic petri dishes (60  $\times$  15 mm) in an incubator at  $25 \pm 1$  °C temperature,  $65 \pm 5\%$  relative humidity, and a 12:12-h (L:D) photoperiod. The emergence numbers of parasitoids and the biological development (adult emergence time and adult longevity) of the parasitoid were monitored.

#### The parasitoid first treated with fungus and then the larva is exposed to the parasitoid

This stage of the experiment was conducted to observe the parasitic behavior of the parasitoids and to monitor the developmental biology of newly emerged parasitoids because of direct application of *P. mallochii* to adult parasitoids. Parasitoids exposed to different concentrations of ethanol extract and different concentrations of conidial suspension were brought together with untreated larvae, and parasitoid behavior (cleaning, host searching, host contact, host penetration, host avoidance, non-host searching, and host evasion) was monitored. Parasitized larvae were transferred to plastic petri dishes (60  $\times$  15 mm), placed in an incubator at  $25 \pm 1$  °C with a relative humidity of  $65 \pm 5\%$ , and subjected to a 12:12-h (L:D) photoperiod, while the number of parasitoid emergences and the biological development (adult emergence time and adult longevity) of the parasitoid were monitored. All experiments were repeated three times at different time intervals.

### Statistical analyses

One-Way Analysis of Variance (ANOVA) test was applied for each set of data obtained from different doses of *P. mallochii* extract and conidial suspension applied to *E. kuehniella*. Two-Way Analysis of Variance (ANOVA) and

**Table 1** Mortality of *E. kuehniella* larvae after exposure to different concentrations of *P. mallochii* conidial suspension and ethanol extract

Treatment	Cons./dose	Number of exposed larvae	Number of dead larvae	Mortality rate (%)
Conidial suspension concentrations (Conidia/mL)	Control	30	0	0
	10 <sup>5</sup>	30	11	36.66
	10 <sup>6</sup>	30	13	43.33
	10 <sup>7</sup>	30	22	73.33
	10 <sup>8</sup>	30	25	83.33
	10 <sup>9</sup>	30	26	86.66
Ethanol extract concentrations (mg/mL)	Control	30	0	0
	0,1	30	8	26.66
	0,5	30	16	53.33
	1	30	17	56.66
	2	30	18	60
	5	30	20	66.66
	10	30	28	93.33

**Table 2** LD values of *P. mallochii* conidial suspension and ethanol extract applied to the larvae of *E. kuehniella*

Treatment	N <sup>a</sup>	X <sup>2</sup> (df)	Slope ± SE	Lethal concentrations (conidia/mL/larvae) <sup>b</sup> LD (%95 CL)
Conidial suspension	180	1.715(3)	6.414 ± 1.279	LD <sub>50</sub> = 10 <sup>5.861</sup> (10 <sup>5.171</sup> –10 <sup>6.353</sup> )
				LD <sub>60</sub> = 10 <sup>6.420</sup> (10 <sup>5.850</sup> –10 <sup>6.690</sup> )
				LD <sub>70</sub> = 10 <sup>7.076</sup> (10 <sup>6.539</sup> –10 <sup>7.835</sup> )
				LD <sub>80</sub> = 10 <sup>7.929</sup> (10 <sup>7.273</sup> –10 <sup>9.217</sup> )
				LD <sub>90</sub> = 10 <sup>9.286</sup> (10 <sup>8.266</sup> –10 <sup>11.774</sup> )
				LD <sub>99</sub> = 10 <sup>13.512</sup> (10 <sup>10.945</sup> –10 <sup>21.553</sup> )
Ethanol extract	210	4.760(4)	0.796 ± 0.158	Lethal concentrations (mg/mL/larvae) <sup>b</sup>
				LD <sub>50</sub> = 0.606 (0.272–1.064)
				LD <sub>60</sub> = 1.260 (0.693–2.351)
				LD <sub>70</sub> = 2.758 (1.556–6.646)
				LD <sub>80</sub> = 6.901 (3.426–26.257)
				LD <sub>90</sub> = 24.618 (9.227–195.804)
LD <sub>99</sub> = 504.717 (86.173–26,048.346)				

<sup>a</sup>Total number of insects used for the bioassay

<sup>b</sup>Values are displayed with the lower and upper confidence limits

One-Way Analysis of Variance (ANOVA) were conducted to evaluate the time-dependent effects of applying different doses of *P. mallochii* extract and conidial suspension to *V. canescens*. Tukey's Honestly Significant Difference (HSD)

**Table 3** Changes in larval and pupal duration, and adult emergence time of *E. kuehniella*

	Dose	Larval stage (d) $\bar{x} \pm SE^*$	Pupal stage (d) $\bar{x} \pm SE^*$	Adult emergence time (d) $\bar{x} \pm SE^*$
Conidial Suspension conidia/mL	Control	4.90 ± 0.312a	10.20 ± 0.309ab	17.93 ± 0.460a
	10 <sup>5</sup>	4.74 ± 0.396a	9.68 ± 0.612ab	16.74 ± 0.709a
	10 <sup>6</sup>	3.47 ± 0.229a	11.53 ± 0.412a	16.71 ± 0.391a
	10 <sup>7</sup>	5.38 ± 0.844a	7.88 ± 0.766b	16.50 ± 1.350a
	10 <sup>8</sup>	4.60 ± 0.400a	9.40 ± 0.812ab	16.00 ± 0.949a
	10 <sup>9</sup>	3.25 ± 0.250a	10.75 ± 1.250ab	16.25 ± 1.493a
Ethanol Extract mg/mL	Control	4.67 ± 0.375a	8.87 ± 0.361a	16.53 ± 0.686a
	0.1	4.86 ± 0.636a	9.00 ± 0.400a	16.82 ± 0.802a
	0.5	6.07 ± 0.929a	9.86 ± 0.455a	18.07 ± 0.788a
	1	5.85 ± 0.619a	9.54 ± 0.489a	17.54 ± 0.821a
	2	4.25 ± 0.329a	9.17 ± 0.345a	15.17 ± 0.441ab
	5	3.60 ± 0.618a	8.30 ± 0.539a	14.80 ± 0.772ab
	10	2.50 ± 1.5a	7.50 ± 0.500a	11.50 ± 0.500b

\*Within the same column, values sharing the same letter (a–b) are not significantly different ( $P > 0.05$ ; Tukey's HSD test)

Test was used for post hoc analysis of significant differences between means (SPSS 18.0., Chicago, IL). A significance level of 0.05 was considered in the evaluations.

## Results

### Effects of *P. mallochii* on *E. kuehniella* larvae

The application of *P. mallochii* conidial suspension and ethanol extract to *E. kuehniella* larvae resulted in a dose-dependent mortality rate. No dead larvae were observed in the control groups, while high mortality rates were observed at the highest doses (Table 1). The application test of the ethanol extract yielded an LD<sub>50</sub> of 0.606 mg/mL (95% confidence interval, 0.272–1.064 mg/mL) and the conidial suspension. Conversely, the conidial suspension exhibited an LD<sub>50</sub> of 10<sup>5.861</sup> conidia/mL (95% confidence interval, 10<sup>5.171</sup>–10<sup>6.353</sup> conidia/mL) (Table 2).

The effects of varying concentrations of ethanol extract and conidial suspension on larval, pupal stages and adult emergence time were presented in Table 3. The results of the conidial suspension ( $F = 2.844$ ;  $df = 5, 77$ ;  $p = 0.021$ ) and ethanol extract ( $F = 1.818$ ;  $df = 6, 96$ ;  $p = 0.104$ ) applications revealed that the duration of the larval period was not significantly different from the control. In the pupal period, a decrease was observed in conidia groups at the 10<sup>7</sup> conidia/mL dose compared to the control ( $F = 3.925$ ;  $df = 5, 77$ ;  $p = 0.003$ ), while no difference was observed in ethanol extract doses ( $F = 1.252$ ;  $df = 6, 96$ ;  $p = 0.287$ ). When adult

emergence time was examined, no difference was observed in the conidial dose ( $F=1.069$ ;  $df=5,77$ ;  $p=0.384$ ), while a decrease was observed only at the highest dose (10 mg/ml) compared to the control in the ethanol extract application ( $F=2.406$ ;  $df=6,96$ ;  $p=0.033$ ).

The effects of varying concentrations of ethanol extract and conidial suspension on adult longevity, weight and fertility were presented in Table 4. While no significant differences were observed between doses in adult longevity after ethanol extract application compared to the control group ( $F=1.550$ ;  $df=6, 96$ ;  $p=0.170$ ), an increase was observed at the  $10^9$  conidia/mL and a decline was observed at the  $10^7$  conidia/mL dose in conidial applications compared to the control group ( $F=9.236$ ;  $df=5, 77$ ;  $p=0.000$ ). No significant difference was observed in weight data, conidia ( $F=2.209$ ;  $df=5, 77$ ;  $p=0.062$ ) and ethanol extract ( $F=2.116$ ;  $df=6, 96$ ;  $p=0.058$ ) applications. A significant decrease in the total egg number was observed in all experimental groups compared to the control in conidia ( $F=45.744$ ;  $df=5, 44$ ;  $p=0.000$ ) and ethanol extract ( $F=55.793$ ;  $df=6, 50$ ;  $p=0.000$ ) applications.

### Efficiency of *V. canescens*, *P. mallochii* and Their Combination on *E. kuehniella*

#### Larvae first exposed to the parasitoid and then treated with the fungus

*P. mallochii* different metabolite and conidial concentrations were applied to *E. kuehniella* larvae on days 1, 4, and 8 after parasitization. The results showed that as the dose concentration increased after parasitization, the number of adult

**Table 4** Changes in adult longevity, weight, and number of eggs of *E. kuehniella*

	Dose	Adult longevity (d)	Weight (mg)	Total egg number
		$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$
Conidial Suspension conidia/mL	Control	9.67±0.603ab	9.32±0.239a	121.67±8.496a
	$10^5$	8.00±0.426ab	9.41±0.205a	34.40±8.861b
	$10^6$	11.59±1.546a	10.14±0.174a	10.80±2.778b
	$10^7$	4.25±1.098b	8.71±0.461a	2.00±1.839b
	$10^8$	11.80±1.908a	9.72±0.486a	0b
	$10^9$	18.75±0.479c	9.70±0.571a	29.75±13.425b
Ethanol Extract mg/ mL	Control	10.67±0.647a	11.89±673a	145.19±8.507a
	0.1	10.14±0.471a	9.90±425a	17.27±4.804b
	0.5	8.57±0.817a	10.07±861a	7.14±2.832b
	1	9.08±0.820a	11.60±940a	22.00±4.555b
	2	8.42±1.069a	9.55±529a	2.00±1.528b
	5	9.90±0.586a	9.62±547a	5.25±2.056b
	10	7.50±0.500a	8.65±550a	4.00±1.000b

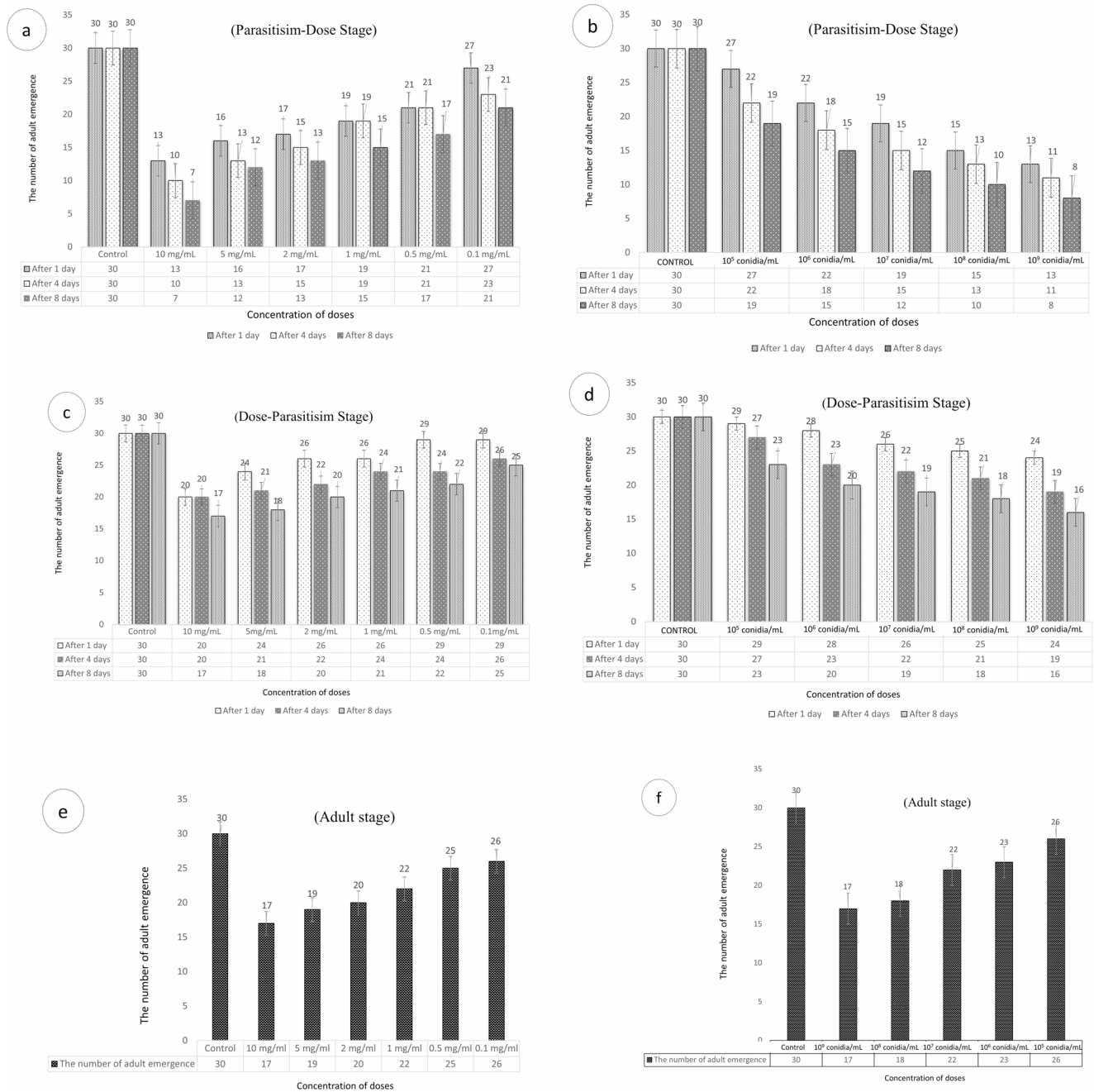
\*Within the same column, values sharing the same letter (a–c) are not significantly different ( $P>0.05$ ; Tukey's HSD test)

emergences decreased Data revealed that groups receiving the dose on the 1. day after parasitism were less affected, whereas application on the 8. day post-parasitism had the greatest impact on the parasitoid emergence rate (Fig. 1a, b).

After parasitization, the reduction in the adult emergence time observed in the lowest dose of  $10^5$  conidia/mL compared to the control group was significant ( $F=36.271$ ;  $df=5, 174$ ;  $p=0.000$ ), whereas the increases observed at the other doses ( $10^7$ ,  $10^8$ , and  $10^9$  conidia/mL) were also significant. When the data for dose application 4 days after parasitization were examined, the increases at doses of  $10^7$ ,  $10^8$ , and  $10^9$  conidia/mL compared to the control were found to be significant ( $F=71.506$ ;  $df=5, 174$ ;  $p=0.000$ ). For dose application 8 days after parasitization, the increases at doses of  $10^6$ ,  $10^7$ ,  $10^8$ , and  $10^9$  conidia/mL compared to the control were significant ( $F=110.484$ ;  $df=5, 174$ ;  $p=0.000$ ). In the ethanol extract application, there was no statistically significant difference between the doses compared to the control group 1 day after parasitization ( $F=7.212$ ;  $df=6, 154$ ;  $p=0.000$ ). However, 4 days after parasitization, fluctuations in all doses except 5 mg/mL compared to the control were found to be significant ( $F=35.399$ ;  $df=6, 136$ ;  $p=0.000$ ). When the data for 8 days after parasitization were examined, the increases at doses of 0.1 and 10 mg/mL and the decrease at the dose of 5 mg/mL compared to the control were statistically significant ( $F=30.210$ ;  $df=6, 116$ ;  $p=0.000$ ) (Table 5).

According to the two-way ANOVA results, the pre-adult development duration of *V. canescens* in the extract application was dependent on both the experimental groups ( $P=0.000$ ) and time ( $P=0.000$ ), and the interaction between the experimental and control groups and the dose amount was influenced by time ( $P=0.000$ ) (Table 9). Similarly, in the conidial suspension application, the pre-adult development duration of *V. canescens* was dependent on both the experimental groups ( $P=0.000$ ) and time ( $P=0.000$ ), and the interaction between the experimental and control groups and the dose amount was influenced by time ( $P=0.000$ ) (Table 9).

When the effect of ethanol extract application at different concentrations on the adult longevity of *V. canescens* was examined, the decreases observed in all doses compared to the control were significant 1 day after parasitization ( $F=19.442$ ;  $df=6, 154$ ;  $p=0.000$ ), 4 days after parasitization ( $F=14.557$ ;  $df=6, 136$ ;  $p=0.000$ ), and 8 days after parasitization ( $F=19.778$ ;  $df=6, 116$ ;  $p=0.000$ ). Similarly, in the conidial suspension application, the decreases observed in all doses compared to the control were significant 1 day after parasitization ( $F=258.356$ ;  $df=5, 174$ ;  $p=0.000$ ), 4 days after parasitization ( $F=519.688$ ;  $df=5, 174$ ;  $p=0.000$ ), and 8 days after parasitization ( $F=566.624$ ;  $df=5, 174$ ;  $p=0.000$ ) (Table 6).



**Fig. 1** Number of adults emerged: **a** parasitism–dose stage (ethanol extract); **b** parasitism–dose stage (conidia); **c** dose–parasitism stage (ethanol extract); **d** dose–parasitism stage (conidia); **e** adult stage (ethanol extract); and **f** adult stage (conidia)

According to the two-way ANOVA results, the adult longevity of *V. canescens* in the extract application was dependent on the experimental groups ( $P=0.000$ ) but not on time ( $P=0.205$ ). The interaction between the experimental and control groups and the dose amounts was influenced by time ( $P=0.000$ ). In the conidial suspension application, the adult longevity was dependent on both the experimental groups ( $P=0.000$ ) and time ( $P=0.000$ ), and the interaction between

the experimental and control groups and the dose amounts was influenced by time ( $P=0.000$ ) (Table 9).

**Larvae first treated with fungus and then exposed to the parasitoid**

*E. kuehniella* larvae were parasitized on days 1, 4, and 8 following the application of different metabolite and conidial concentrations of *P. mallochii*. Adult emergence decreased

**Table 5** The effect of *P. mallochii* on the adult emergence time of *V. canescens* (Parasitism-dose stage)

	Dose	Adult emergence time (d)		
		After 1 day	After 4 days	After 8 days
		$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$
Conidial Suspension conidia/mL	Control	24.67±0.154ax	24.67±0.154ax	24.67±0.154ax
	10 <sup>5</sup>	23.30±0.250bx	24.33±0.227ay	25.03±0.131ay
	10 <sup>6</sup>	24.30±0.199abx	25.10±0.154ay	26.23±0.190bz
	10 <sup>7</sup>	25.83±0.267cx	26.03±0.122bx	27.10±0.200by
	10 <sup>8</sup>	26.87±0.252cdx	27.27±0.219cx	28.07±0.203cy
	10 <sup>9</sup>	27.37±0.38dx	29.37±0.379dy	31.00±0.182dz
Ethanol Extract, mg/mL	Control	24.50±0.150abcx	24.50±0.150ax	24.50±0.150ax
	0.1	23.89±0.187abx	26.46±0.318cy	26.77±0.294by
	0.5	23.92±0.169abx	26.32±0.250cy	25.53±0.274aby
	1	24.75±0.298bcx	27.24±0.206cy	24.39±0.397ax
	2	23.41±0.284axy	23.24±0.265bx	24.47±0.403ay
	5	25.50±0.574cx	24.87±0.256ax	22.71±0.370cy
	10	25.39±0.282cx	23.00±0.447by	30.86±1.243dz

\*Values within the same column (a–d) and row (x–z) sharing the same letter are not significantly different (P>0.05; Tukey’s HSD test)

**Table 6** The effect of *P. mallochii* on the adult longevity of *V. canescens* (Parasitism-dose stage)

	Dose	Adult Longevity(d)		
		After 1 day	After 4 days	After 8 days
		$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$
Conidial Suspension conidia/mL	Control	13.40±0.265ax	13.40±0.265ax	13.40±0.265ax
	10 <sup>5</sup>	11.03±0.217bx	8.83±0.118by	8.20±0.121bz
	10 <sup>6</sup>	9.23±0.190cx	7.03±0.182cy	7.03±0.148cy
	10 <sup>7</sup>	7.43±0.157dx	5.73±0.117dy	5.33±0.154dy
	10 <sup>8</sup>	5.87±0.171ex	3.17±0.699ey	3.13±0.157ey
	10 <sup>9</sup>	3.70±0.284fx	2.50±0.196ey	1.70±0.167fz
Ethanol Extract, mg/mL	Control	7.30±0.221ax	7.30±0.221ax	7.30±0.221ax
	0.1	5.52±0.802bcx	5.54±0.199bx	6.00±0.208bx
	0.5	6.08±0.584bx	5.52±0.272bx	5.53±0.307bx
	1	4.30±0.979dx	4.86±0.278bxy	5.33±0.198bcy
	2	5.82±1.220bcx	5.29±0.114bx	4.29±0.223cdy
	5	5.70±0.801bcx	5.20±0.145bx	5.43±0.173bcx
	10	4.94±1.589cdx	4.91±0.392bx	4.14±0.634dx

\*Values within the same column (a–d) and row (x–z) sharing the same letter are not significantly different (P>0.05; Tukey’s HSD test)

with increasing dose concentration and exposure duration. Among the parasitized groups, larvae dosed 1 day prior showed virtually no reduction in adult emergence at the lowest concentrations (0.1 mg/mL and 10<sup>5</sup> conidia/mL) (Fig. 1c, d).

During the adult emergence time of the parasitoid, one day after the extract dose application, the increase in parasitism at 0.1 mg/mL and the decrease at 10 mg/mL compared to the control were significant (F=15.804; df=6, 203; p=0.000). Similarly, when the data were examined four days after the dose application, the increase at 0.1 mg/mL and the decreases at 2, 5, and 10 mg/mL compared to the control were found to be significant (F=44.596; df=6,

203; p=0.000). When the data were examined eight days after the dose application, the increase at 0.1 mg/mL and the decreases at 1, 2, 5, and 10 mg/mL compared to the control were significant (F=86.447; df=6, 203; p=0.000). One day after the conidia dose application, the increases in parasitism at 10<sup>7</sup>, 10<sup>8</sup>, and 10<sup>9</sup> conidia/mL compared to the control were significant (F=102.507; df=5, 174; p=0.000). Similarly, four days after the same dose application, the increases in parasitism at 10<sup>7</sup>, 10<sup>8</sup>, and 10<sup>9</sup> conidia/mL compared to the control were also significant (F=37.349; df=5, 174; p=0.000). However, eight days after the dose application, only the increases at 10<sup>8</sup> and 10<sup>9</sup> conidia/mL compared to the control were significant (F=13.304; df=5, 174; p=0.000) (Table 7).

According to the results of two-way ANOVA, the adult emergence time of *V. canescens* was dependent on both the experimental groups (P=0.000) and time (P=0.000), and the interaction between the experimental and control groups and the dose amounts was influenced by time (P=0.000). Similarly, in the conidia application, the pre-adult development duration was dependent on both the experimental groups (P=0.000) and time (P=0.000), and the interaction between the experimental and control groups and the dose amounts was influenced by time (P=0.000) (Table 9).

One day after the dose application, the decreases in adult longevity observed at 1, 2, 5, and 10 mg/mL compared to the control were significant (F=51.952; df=6, 203; p=0.000). Four days after the dose application, the decreases in adult longevity at 0.5, 1, 2, 5, and 10 mg/mL compared to the control were significant (F=46.911; df=6, 203; p=0.000). Additionally, eight days after the dose application, the increases in adult longevity at 0.1, 0.5, 1, and 2 mg/mL and the decrease at 10 mg/mL compared to the control were significant (F=58.147; df=6, 203; p=0.000). In the groups

**Table 7** The effect of *P. mallochii* on the adult emergence time of *V. canescens* (Dose-parasitism stage)

		Adult emergence time (d)		
		After 1 day	after 4 days	after 8 days
Dose		$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$
Conidial Suspension conidia/mL	Control	24.60±0.149ax	24.60±0.149ax	24.60±0.149ax
	10 <sup>5</sup>	25.13±0.190ax	25.00±0.275abx	24.60±0.163ax
	10 <sup>6</sup>	25.27±0.209ax	25.10±0.205abx	24.60±0.195ax
	10 <sup>7</sup>	26.60±0.309bx	25.73±0.191bcy	25.10±0.251aby
	10 <sup>8</sup>	26.90±0.323bx	26.70±0.326cx	26.47±0.261cx
	10 <sup>9</sup>	31.50±0.270cx	28.97±0.373dy	25.73±0.225bcz
Ethanol Extract, mg/mL	Control	24.57±0.141ax	24.57±0.141ax	24.57±0.141ax
	0,1	25.53±0.213bx	25.40±0.223bx	25.67±0.200bx
	0,5	24.27±0.126ax	24.530.157ax	24.60±0.163ax
	1	24.53±0.266ax	24.10±0.130acx	23.10±0.162cy
	2	24.43±0.207ax	23.63±0.195cx	22.63±0.367cy
	5	24.43±0.114ax	22.27±0.95dy	21.10±0.111dz
	10	22.93±0.225cx	22.77±0.171dx	20.33±0.121dy

\*Values within the same column (a–d) and row (x–z) sharing the same letter are not significantly different ( $P > 0.05$ ; Tukey's HSD test)

**Table 8** The effect of *P. mallochii* on the adult longevity of *V. canescens* (Dose-Parasitism stage)

		Adult longevity(d)		
		After 1 day	After 4 days	After 8 days
Dose		$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$
Conidial Suspension conidia/mL	Control	13.83±0.343ax	13.83±0.343ax	13.83±0.343ax
	10 <sup>5</sup>	15.80±0.217bx	15.97±0.305bx	17.70±0.329by
	10 <sup>6</sup>	11.07±0.325cx	12.90±0.408ay	14.03±0.320ay
	10 <sup>7</sup>	7.60±0.201dx	8.13±0.218cx	10.33±0.323cy
	10 <sup>8</sup>	4.33±0.221ex	4.90±0.205dx	7.13±0.213dy
	10 <sup>9</sup>	3.77±0.270ex	4.43±0.313dx	5.37±0.169ey
Ethanol extract, mg/mL	Control	12.77±0.317ax	12.77±0.317ax	12.77±0.317ax
	0,1	13.07±0.321ax	12.40±0.373abx	18.53±0.367by
	0,5	12.10±0.369abx	11.47±0.248bx	17.70±0.329by
	1	11.37±0.394bx	10.23±0.238cy	16.93±0.328bz
	2	11.03±0.206bx	9.07±0.166dy	15.13±0.596cz
	5	9.63±0.297cx	8.80±0.139dx	12.37±0.347ay
	10	6.53±0.243dx	8.53±0.238dy	10.70±0.390dz

\*Values within the same column (a–d) and row (x–z) sharing the same letter are not significantly different ( $P > 0.05$ ; Tukey's HSD test)

where conidia were applied, one day after the dose application, the increase in adult longevity at 10<sup>5</sup> conidia/mL and the decreases at 10<sup>6</sup>, 10<sup>7</sup>, 10<sup>8</sup>, and 10<sup>9</sup> conidia/mL compared to the control were found to be significant ( $F = 344.342$ ;  $df = 5, 174$ ;  $p = 0.000$ ). Four days after the dose application, the increase in adult longevity at 10<sup>5</sup> conidia/mL and the decreases at 10<sup>7</sup>, 10<sup>8</sup>, and 10<sup>9</sup> conidia/mL compared to the control were significant ( $F = 253.277$ ;  $df = 5, 174$ ;  $p = 0.000$ ). Similarly, eight days after the dose application, the increase in adult life span at 10<sup>5</sup> conidia/mL and the decreases at 10<sup>7</sup>, 10<sup>8</sup>, and 10<sup>9</sup> conidia/mL compared to the control were significant ( $F = 256.629$ ;  $df = 5, 174$ ;  $p = 0.000$ ) (Table 8).

According to the results of two-way ANOVA, the adult longevity of *V. canescens* was dependent on both the experimental groups ( $P = 0.000$ ) and time ( $P = 0.000$ ), and the interaction between the experimental and control groups

and the dose amounts was influenced by time ( $P = 0.000$ ). In the conidia application, the adult longevity was also dependent on both the experimental groups ( $P = 0.000$ ) and time ( $P = 0.000$ ), and the interaction between the experimental and control groups and the dose amounts was influenced by time ( $P = 0.000$ ) (Table 9).

#### The parasitoid first treated with fungus and then the larva is exposed to the parasitoid

In the adult stage, the parasitoid emergence rate was also observed to decrease with increasing dose (Fig. 1e, f). The effect of extract and conidia application at different concentrations on the adult emergence time in *V. canescens* were given in Table 10. The reductions at 0.5 and 1 mg/mL compared to the control and the increases at 5 and 10 mg/

**Table 9** Two Way Anova table showing the effects of different experimental groups (control and dosed), time and their interactions on *V. canescens*

		Source	df	MS	F	P	r <sup>2</sup>		
Larvae first exposed to the parasitoid and then treated with the fungus	Adult emergence time	Conidia	Experimental groups	5	323.302	192.375	0.000	0.68	
			Time	2	119.572	71.149	0.000		
			Experimental groups × time	10	11.201	6.665	0.000		
		Extract	Experimental groups	6	41.550	23.327	0.000	0.54	
			Time	2	40.600	22.794	0.000		
			Experimental groups × time	12	47.72	26.792	0.000		
		Adult longevity	Conidia	Experimental groups	5	1353.816	1235.620	0.000	0.92
				Time	2	203.585	185.811	0.000	
				Experimental groups × time	10	10.534	9.614	0.000	
	Extract		Experimental groups	6	54.704	47.533	0.000	0.44	
			Time	2	1.828	1.589	0.205		
			Experimental groups × time	12	3.67	3.194	0.000		
	Larvae first treated with fungus and then exposed to the parasitoid	Adult emergence time	Conidia	Experimental groups	5	218.476	123.078	0.000	0.62
				Time	2	99.517	56.063	0.000	
				Experimental groups × time	10	35.092	19.769	0.000	
Extract			Experimental groups	6	131.147	121.757	0.000	0.64	
			Time	2	82.297	76.404	0.000		
			Experimental groups × time	12	18.193	16.891	0.000		
Adult longevity			Conidia	Experimental groups	5	2084.966	832.095	0.000	0.89
				Time	2	188.313	75.154	0.000	
				Experimental groups × time	10	12.046	4.808	0.000	
		Extract	Experimental groups	6	392.131	123.251	0.000	0.74	
			Time	2	1233.433	387.683	0.000		
			Experimental groups × time	12	61.219	19.242	0.000		
			Error	609	3.182				

*df*degrees of freedom, *MS*mean squares, *F*F-test value, *P*significance level, *r*<sup>2</sup>coefficient of determination

mL in the extract were significant ( $F=72.965$ ;  $df=6, 203$ ;  $p=0.000$ ). In the conidial suspension dose application, the increases at doses of  $10^8$  and  $10^9$  conidia/mL compared to the control were significant ( $F=54.344$ ;  $df=5, 174$ ;  $p=0.000$ ). When examining the data on the effect on adult longevity, it was found that the reductions at all doses compared to the control were statistically significant ( $F=47.806$ ;  $df=6, 203$ ;  $p=0.000$ ). As the extract doses increased, the adult longevity was significantly reduced. After the conidial suspension application, the reductions in adult longevity at all doses compared to the control were also significant ( $F=80.282$ ;  $df=5, 174$ ;  $p=0.000$ ).

## Discussion

Parasitoid wasps, which play an important role in the biological control of harmful insects, make up a large portion of the Hymenoptera order. For parasitoids to be effectively used in the biological control of harmful insects, the host-parasitoid relationship must be well understood (Beckage and Gelman 2004). Various studies have demonstrated that fungal biological control agents and parasitoids/predators can coexist and effectively control different pest species. However, some parasitoids have been reported to experience adverse effects on their adult or larval life and other biological development parameters due to EPF (Nielsen et al. 2005; Mas et al. 2019). This study presents initial information on the interaction between *P. mallochii* and the

**Table 10** The effect of *P. mallochii* on the adult emergence time and adult longevity of *V. canescens* (Adult stage)

	Dose	Adult emergence time (d)	Adult longevity (d)
		$\bar{x} \pm SE^*$	$\bar{x} \pm SE^*$
Conidial Suspension conidia/mL	Control	24.67±0.130a	10.10±0.312a
	10 <sup>5</sup>	24.63±0.131a	8.20±0.327b
	10 <sup>6</sup>	24.73±0.209a	7.07±0.235c
	10 <sup>7</sup>	24.83±0.250a	6.27±0.295c
	10 <sup>8</sup>	25.83±0.136b	5.00±0.209d
	10 <sup>9</sup>	28.03±0.200c	3.30±0.193e
Ethanol Extract mg/mL	Control	24.67±0.130ab	9.37±0.357a
	0.1	24.00±0.173ac	8.03±0.131b
	0.5	22.47±0.093d	7.77±0.196bc
	1	23.27±0.106c	7.07±0.209 cd
	2	25.30±0.199b	6.23±0.177d
	5	26.33±0.100e	7.33±0.154bc
	10	26.43±0.317e	4.80±0.147e

\*Within the same column, values sharing the same letter (a–e) are not significantly different ( $P > 0.05$ ; Tukey's HSD test)

parasitoid *V. canescens*, as well as the combined biological control of *E. kuehniella* using these two different control agents.

Prior to the interaction studies, the LD50 values were ascertained in order to determine the lethal effects of *P. mallochii* conidial suspension and ethanol extract on *E. kuehniella*. The LD50 value was determined as 10<sup>5.861</sup> conidia/mL in *E. kuehniella* larvae when treated with conidial suspension via a topical application method, whereas the LD50 values of larvae treated with ethanol extract were determined as 0.606 mg/mL. In the present study, it was determined that the mortality rates in *E. kuehniella* were 36.66% at the lowest concentration and 86.66% at the highest concentration, depending on the conidia concentration. Furthermore, the findings of this study indicated that the mortality rate in *E. kuehniella* varied between 26.6% and 93.33% at the lowest (0.1 mg/mL) and highest dose (10 mg/mL), respectively, depending on the concentrations of ethanol extract obtained from *P. mallochii*. Güner et al. (2025) showed that *P. mallochii* conidial suspension and ethanol extract caused dose-dependent mortality on *C. cautella*. As a result, the mortality caused by conidial suspension doses/ethanol extract in larvae was observed between 16.6% and 60%/26.6% and 63.3%. The mortality rates of *P. mallochii* on *C. cautella* and *E. kuehniella* have been shown to be parallel in both our previous and current studies. Following the application of *P. mallochii* extract and conidial suspension, the emergence of adult individuals during the monitoring of developmental biology parameters enabled the determination of biological characteristics from larva to adult. While the application of different conidial suspension and extract concentrations to the last stage larvae in the experiments did not cause a significant difference in biological development

parameters, the most significant change was observed in the total egg number data. It can be posited that an increase in conidial concentration results in a decline in the number of eggs, concurrently engendering an escalation in pathogenicity by allocating pivotal host resources, such as energy, to the pathogen (Roy et al. 2006). Alterations in post-infection reproduction parameters consequent to the utilisation of fungi as biocontrol agents culminate in a diminution of insect populations. In addition to the effects of *P. mallochii* conidial suspension and ethanol extract on the developmental parameters of *E. kuehniella*, the decrease in egg numbers can be attributed to the invasive effect of mycelia on the insect and the toxic effect of its metabolites.

In interaction studies, the impact of fungal agents on the adult emergence time of parasitoids and their longevity was examined under various interaction scenarios. The larval stage of parasitoids developing within or outside the host exhibit a significant correlation with the timing of fungal application (Rashki et al. 2009). The interaction between parasitoid larval stages and the entomopathogenic fungus may also be associated with the secondary compound production of the most advanced parasitoid larval stages; this primarily prevents the colonization of the entomopathogen through the synthesis of substances (Kanost 1999; Li et al. 2012). It inhibits enzymatic breakdown and, consequently, affects the virulence of the entomopathogen (Leger 1995). Additionally, the entomopathogen can jeopardize the quality of the host (due to feeding or physiological changes) (Brooks 1993), and consequently, it can affect the biological parameters of the parasitoid, especially females (considered more susceptible than males in competition with a specific entomopathogen), because the growth rate of females is higher than that of males (Sequeira and Mackauer 1992; Rashki et al. 2009), and this also influences the development of offspring. In the present study, it was determined that changes in certain parameters related to the biology and behaviour of *V. canescens* were contingent on the time interval of application of extract and conidia doses of *P. mallochii*.

In current study, *P. mallochii* different metabolite and conidial concentrations were applied to *E. kuehniella* larvae on days 1, 4, and 8 after parasitization. Our results showed that conidia and ethanol extract concentrations sublethal to parasitized *E. kuehniella* larvae did not negatively affect the emergence of *V. canescens* adults. However, it was determined that as the dose concentration increased, the number of adult parasitoid emergence decreased. According to the study conducted by Corbet and Rotheram (1965), *V. canescens* goes through the egg stage on the 1st day after parasitization, the 1st larval stage on the 4th day after parasitization, and the 3rd larval stage on the 8th day after parasitization. In this stage, it was observed that the survival

rate of *V. canescens* in the egg and larval stages decreased, with the larval stage being more affected than the egg stage. Additionally, the number of parasitoid emergences was more affected on the 8th day according to the different application times. It has been determined that when *Heliothis zea* (Boddie, 1850) (Lepidoptera: Noctuidae) larvae were infected with the fungus *Nomuraea rileyi* (Farl.) Samson, 1974 (Hypocreales: Clavicipitaceae) first day after parasitization, the development of the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae) was inhibited (King and Bell 1978). The decrease in parasitization rate depending on the application time suggests that *V. canescens* larvae may have higher sensitivity to the germination, penetration, and colonization of *P. mallochii* conidia during their larval stages. This relationship between the first larval stage and EPF has also been observed by Kim et al. (2005); Rashki et al. (2009), and Emami et al. (2013). Parasitoids release a fungistatic substance into the host's hemolymph, which prevents mycosis development and ensures normal parasitoid development and adult emergence. This fungistatic substance is formed in connection with the time interval between parasitism and fungal infection (Emami et al. 2013). In this study, it was observed that the adult emergence time of *V. canescens* increased towards higher doses in all three-time intervals after conidial applications. The results indicated that sub-lethal conidial and metabolite concentrations for parasitized *E. kuehniella* larvae did not negatively affect the emergence of *V. canescens* adults, but changes in emergence times were observed. Our results were shown that treating *P. mallochii* after the parasitoid oviposition, 1 day or more later, allows the parasitoid to complete its development, becoming advantageous over the pathogen. This is consistent with the findings of Rohlf's (2008), who used *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) (host), *Asobara tabida* (Hymenoptera: Braconidae) (parasitoid), and *Aspergillus niger* Tiegh. (Eurotiales: Aspergillaceae) (fungus) in a study on fungus, parasitoid, and host interactions. The interaction assessment revealed that the production density of *A. niger* conidiospores was negatively affected by the presence of *D. melanogaster* larvae. In our study, it was determined that the application of doses after parasitism shortened the adult longevity at all doses and time intervals. When combining applications of *P. mallochii* and *V. canescens*, the shortening of the parasitoid's longevity during the post-parasitism dose application stage indicates that this interaction is antagonistic.

Simultaneous use of fungi and parasitoids may be detrimental to parasitoids when female parasitoids search for hosts in areas contaminated with fungus conidia. Once fungal infection begins, fungus development generally prevails over parasitoid development. This is known as the priority effect in the simultaneous application of natural enemies

(Abbas 2020). To observe this effect, the second stage of interaction studies in our study involved bringing together surviving larvae with adult *V. canescens* after applying *P. mallochii* metabolite and conidia concentrations to *E. kuehniella* larvae. When parasitism was conducted after dose application, it was observed that as the dose concentration and the time after dose application increased (from day 1 to day 8), the emergence of adult parasitoids decreased. When aphids were infected with the *Pandora neoaphidis* fungus and then parasitized by the parasitoid *Aphidius rhopalosiphii* De Stefani-Perez, 1902 (Hymenoptera: Braconidae), it was observed that the parasitoid could not complete its development in less than 4 days (Powell et al. 1986). Furthermore, some parasitoids can distinguish between infected and uninfected hosts, and they do not parasitize infected hosts (Abbas 2020). Non-lethal effects on parasitoids developing in infected hosts are also possible (El-Sufty Potrich et al. 2017). The adult emergence time of *V. canescens* increased towards higher conidial doses in all three-time intervals after application. After metabolite dose applications, it was observed that the adult emergence time was shortened at the highest dose in all three days compared to the control, while it was prolonged at the lowest dose. In groups where parasitization was performed on the 8th day after dose application, it was determined that the pre-adult development period started to decrease from the 1 mg/mL dose. When pupa parasitized by *Anastrepha obliqua* Macquart (Diptera: Tephritidae) with *Coptera haywardi* Loiacono (Hymenoptera: Diapriidae) were treated with dry conidia of *Beauveria bassiana* (Bals.-Criv.) Vuill., 1912 (Hypocreales; Cordycipitaceae), it was shown that the parasitoid did not harm the egg, larva, and pupa development and did not reduce the egg yield of parasitoid females (Martinez-Barrera et al. 2019). In our study, *V. canescens* adults emerging from *E. kuehniella* larvae exposed to *P. mallochii* infection showed a decrease in longevity at high conidia and metabolite doses compared to parasitoid adults emerging from uninfected larvae (control group), while the lowest conidia and metabolite doses resulted in an extension of this period. In biological control studies, the combined application of *P. mallochii* with *V. canescens* showed that the post-parasitization stage of the parasitoid's lifespan was shortened at high doses, indicating an antagonistic interaction, while the longevity was prolonged at low conidia ( $10^5$  conidia/mL) and metabolite doses (0.1 mg/mL), indicating a synergistic interaction compared to the control. In contrast to our study, exposure of parasitoids *Aphelinus asychis* (Hymenoptera: Aphelinidae) developing in *Diuraphis noxia* (Kurdjumov, 1913) (Hemiptera: Aphididae) aphids to *Isaria fumosorosea* Wize, 1904 (Hypocreales: Cordycipitaceae) at 24, 48, or 72 h after exposure showed no effect on the lifespan (Mesquita and Lacey 2001). Interaction studies between *Paecilomyces*

*fumosoroseus* (Hypocreales: Cordycipitaceae) and the parasitoid *A. asychis* on the Russian wheat aphid (*D. noxia*) showed that parasitoid females spent less time laying eggs on aphids treated with *P. fumosoroseus* ( $5.2 \times 10^4$  conidia/cm<sup>2</sup>) after 1 h exposure for 4 to 5 days (Mesquita and Lacey 2001). In another study, the interaction between the *B. bassiana* and the aphid parasitoid *Diaeretiella rapae* (M'Intosh, 1855) (Hymenoptera: Braconidae) was evaluated under laboratory conditions. Aphids were sprayed with *B. bassiana* and then exposed to parasitoids at 0, 24, and 48 h. The best time range for the combined application of these two biological control agents was determined to be within the 0–24 h period, and the combination was found to be effective in controlling aphids (Martins et al. 2014). Parasitoids and fungi can have negative effects on each other inside the host, but outside the host, parasitoids can help fungi spread to new host populations. Therefore, it is necessary to examine both directions of this interaction to determine the net effect of the interaction between these species (Hochberg 1991a, b). The results of our experiments showed that the application timing of shared hosts could affect the outcome of intra-host interactions between parasitoids and fungi.

In the third stage of our interaction scenarios, adult parasitoids were subjected to conidia and metabolite dose applications, and the effect on parasitizing hosts was evaluated. It was observed that parasitization rates decreased and the number of emerging parasitoids decreased after high-dose applications. When applied at a concentration of  $10^7$  conidia/mL, *B. bassiana* and *Metarhizium anisopliae* (Metschn.) Sorokin, 1883 (Hypocreales: Clavicipitaceae) killed 48% and 40% of *Cephalonomia stephanoderis* Betrem, 1961 (Hymenoptera: Bethyilidae) adults, the parasitoid of the coffee bean borer *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae), respectively (Aristizabal et al. 1995). At this stage, it was determined that the pre-adult development period of adult parasitoids emerging from treated larvae increased with dose escalation in conidial suspension applications. Additionally, in metabolite dose applications, it was found that the period first shortened and then lengthened with increasing dose. When *V. canescens* was directly infected with *P. mallochii*'s conidia and metabolite, it was determined that parasitization rates decreased, and the longevity of new parasitoids emerging from *E. kuehniella* was shortened. In our study, similar to other research, the direct exposure of *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae) to dry conidia of *M. anisopliae* ICIP 20 was found to reduce the percentage of parasitism and the lifespan of the parasitoid (Mama Sambo et al. 2022). Nozad-Bonab et al. (2021) found that infecting *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) with *M. anisopliae* reduced the lifespan of the parasitoid. Similarly, Presa-Parra et al. (2021) reported that

*Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae) infected with *M. anisopliae* had a much shorter lifespan compared to controls. Our study demonstrated that fungal doses applied to parasitoid adults resulted in different death rates.

There have been no reports of infections caused by fungal species on *V. canescens* adults in the field to date, and our study, although limited, is the first laboratory study. Generally, the impact of fungi on the development of parasitoids or predators applied to the host depends on the time interval between infection and parasitism. Field studies show the success of combinations of fungi and parasitoids in controlling insect pests. With proper timing, parasitoids or predators can be combined with fungi for the biological control of insects in the field (Abbas 2020). In conclusion, and from the perspective of Integrated Pest Management (IPM), our results demonstrated the simultaneous application of *V. canescens* and *P. mallochii* on *E. kuehniella* has both positive and negative effects. A critical step in this process is the selection of doses that are 50% lethal to the target pest species of the fungus, while exhibiting low toxicity (3–5%) to the parasitoid. It is vital that these doses do not exert a detrimental influence on the parasitization behaviour and life span of the parasitoid. In the Dose-Parasitism stage,  $10^5$  conidia/mL in conidial suspension and 0.1 or 0.5 mg/mL in ethanol extract applications are considered to be applicable and effective doses in biological control studies of *P. mallochii*. Effective time management and the implementation of the correct interaction scenarios are important to prevent antagonistic interactions in this use. Therefore, further studies should be conducted in more realistic environments (such as greenhouse and field conditions) to better understand this relationship.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

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