

# Biocontrol effect of *Fomes fomentarius* mushroom against *Varroa destructor*

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Received 13.12.2024

Accepted 24.03.2025

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### Summary

*Varroa destructor* is the most important pest of western honeybees. In recent decades, numerous methods have been developed to control this mite. The most common and effective method is the use of chemicals. However, they pose different threats to bees and their products. Therefore, non-chemical methods should also be developed. This study investigated the efficacy of different formulations of the fungus *Fomes fomentarius* in controlling the *Varroa* mite. The results showed that the efficacy of smoke, dribble, and gel treatments was 63.80%, 69.17%, and 72.13%, respectively. These treatment methods had no negative effects on the number of sealed brood areas and bee-covered frames ( $P > 0.001$ ). Burning the fungus with the beekeeper's smoker led to a rapid death of mites on adult bees. The gel treatment had an extended-release effect. This mushroom and its formulations are recommended for mite control. The efficacy of various mushrooms against *Nosema ceranae* and deformed wing virus has been investigated by other researchers. However, as there is no source in the literature for the use of fungi to control the *Varroa* mite, we believe this study is the first.

**Keywords:** apiculture, control, honeybees, mortality, *Varroa*

Beekeeping is an agricultural activity that can be practiced in many countries around the world. The most common and densest pollinator in the world is the honeybee. However, it has been reported that the density of pollinator insects has declined in recent years (44). Climate change, environmental pollution, destruction of natural areas, uncontrolled use of pesticides in crop production, and diseases and pests are cited as causes of the decline in bee population (21, 44). Bee diseases and pests can be categorized into parasitic infestations (37), viral infections (16), bacterial and fungal diseases (8), as well as other threats, such as predators and environmental stressors (36). It is known that the most damaging parasite for honeybees (*Apis mellifera* L.) worldwide (38) is the *Varroa* mite (*Varroa destructor* (4)). Although various control methods have been developed against this mite, there is still no fully effective control method. The improper use of chemical acaricides, including incorrect dosages or deviations from recommended application procedures, can lead to resistant *Varroa* strains, pesticide residues in bee products, and negative effects on bee health (19). Current data suggest that pesticide residues, particu-

larly acaricides, can accumulate in bee bread, propolis, and beeswax at concentrations exceeding 1,000 µg/kg. Tau-fluvalinate, coumaphos, chlorfenvinphos, chlorpyrifos, and amitraz are acaricides commonly detected in hive products. Estimates indicate that coumaphos and chlorfenvinphos may accumulate in beeswax at levels posing potential health risks to comb honey consumers. However, pesticide residues do not appear to transfer to royal jelly, likely due to the filtering activity of nurse bees during secretion (50). In order to minimize these issues, studies on organic and biological control methods should be intensified.

Scientists are increasingly interested in mushrooms because of their medicinal properties. In particular, mushrooms belonging to the genus *Agaricus* are a focus of attention because of their components, such as glucans, mannan, and lentinan (51). The natural polysaccharides contained in *Fomes fomentarius* are responsible for its antimicrobial activity. Studies conducted with humans and animal models confirm the efficacy of β-glucan in combating various infectious diseases (14). Some extracts of *F. fomentarius* contain carbohydrates in the form of beta-glucans and

small amounts of alpha-glucans (29), which showed antimicrobial activities (54). According to one study,  $\beta$ -glucans showed antimicrobial activity in insects through the activation of the Toll and Imd signaling pathways (24). Diets containing 0.5% and 2%  $\beta$ -glucan inhibited deformed wing virus replication (33). The use of  $\beta$ -glucans can also be used to strengthen the innate immune defense against *Varroa* mites and other common pathogens of the honey bee (35).

Previous studies have reported that the mushroom species *Fomes fomentarius*, *Ganoderma applanatum*, *G. resinaceum*, and *Trametes versicolor* exhibit strong antiviral properties against two bee viruses, deformed wing virus (DWV) and Lake Sinai virus (LSV) (48). Extracts from *Agaricus bisporus* have been found to provide a protective effect against *Nosema ceranae* in bees (17). The lethal effect of the *Metarhizium* fungus on *Varroa* mites has also been established (23). Additionally, it has been reported that extracts from *F. fomentarius* can be digested by bees at a rate of 1% (v/v) without causing harm (40). Furthermore, certain mushrooms, including *Xerocomus* spp., *Sepedonium* spp., and *Hypomyces chrysospermus*, have been found to attract bees (49). It has been observed that local beekeepers burn tinder fungus in the beekeeper's smoker and apply the smoke in the hives to control *Varroa* mites. This study aimed to evaluate the potential of tinder fungus (*Fomes fomentarius*) as a biological control agent against the honey bee pest *Varroa destructor*. Specifically, we investigated the effectiveness of its three different application methods – dribble, smoking, and gel – while hypothesizing that these formulations could reduce mite infestations without harming bee health.

## Material and methods

**Study site and honeybee colonies.** The study was conducted on the campus of Muş Alparslan University, Turkey (38°44'50" N 41°26'19" E) in the fall of 2024 (from September 6 to October 6). The Muş province has a harsh continental climate due to its altitude (1308 m) and distance from the sea. The temperature varies between -29°C and +37°C. During the study, the average values of pressure (hPa), humidity (%), wind speed (m/sec), and temperature (°C) were 867.7, 33.5, 0.9, and 18.9, respectively (5). A total of 20 honey bee colonies (5 colonies per group in 3 treatment groups + 1 control group) were used in the study. The colonies were of a modern type in the Langstroth structure with a capacity of 10 wooden frames. The bottom board of the colonies was a model with a grid-drawer suitable for counting falling mites. All colonies included in the study had 10 frames (combs) covered by bees. The sealed brood area (cm<sup>2</sup>) was approximately the same in all treatment groups. The average brood area ranged from 365 cm<sup>2</sup> to 396 cm<sup>2</sup>. The queens of the colonies were of the Caucasian race (*A. mellifera caucasica*) and were born in 2022. All queen bees were sisters from the same breeding colony. They mated naturally in June.

The study period (September-October) and the area in which the study was carried out were relatively dry in terms of the bees' nutrient supply. In the vicinity of the hives, there were no agricultural activities or other materials that could harm the bees.

***Fomes fomentarius* treatment.** Gel and aqueous preparations were prepared according to a method described by Gedik et al. as follows (13): The aqueous extract used for the formulation was obtained by extracting 5 g of mushroom powder in 100 mL of purified water at 80°C in a water bath (Daihan Scientific, WB-22, Korea) for 30 min. The extract was then filtered through Whatman grade 1 filter paper, and 2% carbomer 940 was added to the filtrate. Triethanolamine was added and mixed to obtain a gel consistency. The formulation was kept at 25 ± 1°C for 48 hours to observe any phase separation.

Clarity, pH, and viscosity analyses were performed to characterize the gel. All experiments were repeated four times. Clarity was assessed visually against a dark background and classified as cloudy (+), clear (++), or very clear (++++). The pH measurement was performed with a digital pH meter (Mettler Toledo S 220, Switzerland) and averaged over four measurements. Viscosity analysis was performed with a vibro-viscometer (AND, SV-10, Japan) at 32 ± 2°C and a frequency of 50 Hz. For a spreading test, 0.5 g of the gel was spread on a circle with a diameter of 2 cm pre-marked on a glass plate. Another glass plate weighing 0.5 kg was then placed on top and left for 5 minutes. The diameter of the gel after spreading was measured.

The mushrooms were collected from beeches and cut up. A total of 100 g of mushrooms was burned in beekeeper's smoking machines, and the smoke was applied 10 times through the entrance of the beehive.

The liquid extract was mixed with drinking water at 1% (v/w), and 5 ml was dropped between the frames. The water was purchased on the market and was suitable for human consumption. It was not analyzed.

A total of 1 g of the gel was used in each colony of this group. It was randomly applied to a brood frame. The colonies were treated five times at six-day intervals. The control colonies were not treated.

$$\text{Treatment Efficacy (TE, \%)} = \frac{\text{Initial Mite Count} - \text{Final Mite Count}}{\text{Initial Mite Count}} \times 100$$

***Varroa* mite infestation measurement.** The *Varroa* infestation rate for adult bees was determined by icing sugar methods before and at the end of the study (10, 20). The numbers of bees and *Varroa* mites were used to calculate the *V. destructor* percent infestation of each colony with the formula below (10). The *Varroa* infestation rate (%) was determined one day before the first treatment, and colonies with approximately equal infestation rates were included in the treatment. The reinfestation rate was determined fifteen days and thirty days after the first treatment. The daily mite fall in debris was monitored to determine an indicative efficacy of the treatment during the treatment periods. The total number of mites falling on the bottom board from all colonies in the group (including the control group) was recorded on three consecutive days after each treatment.

$$VIAB (\%) = \frac{TVMAB}{TNAB} \times 100$$

where: VIAB = *Varroa destructor* percent infestation of adult bees, TVMAB = total number of Varroa mites on adult bees, TNAB = total number of adult bees.

**Measurement of the strength and brood area of the bee colonies.** It was examined whether the treatment methods had a negative effect on adult bees and brood production. The number of frames covered by bees and the sealed brood area (cm<sup>2</sup>) were determined at an interval of 15 days according to the Puchta method (2). Since the sealed brood area (cm<sup>2</sup>) was elliptical, the area of the ellipse was measured using the equation

$$S (\text{cm}^2) = \pi \times \frac{A}{2} \times \frac{a}{2}$$

where: S (cm<sup>2</sup>) = sealed brood area, A (cm) = length of the major axis, a (cm) = length of the minor axis, π = 3.14.

**Statistical analyses.** The data normality assumption was checked with the Kolmogorov-Smirnov test, and the distribution of the variables was found to be normal (p > 0.05). In repeated measures, the data were analyzed with a mixed analysis of variance. Sphericity was examined with Mauchly's sphericity test. When the sphericity assumption was met (p > 0.05), the results from the sphericity-assumed test were used. In cases where the sphericity assumption was violated (p < 0.05), the epsilon (ε) value was examined. The Greenhouse-Geisser correction was applied when ε < 0.75, while the Huynh-Feldt correction was used when ε > 0.75. Post hoc comparisons between groups were conducted using Duncan's and Bonferroni tests. Descriptive statistics are presented as means and standard errors. All statistical analyses were performed using the SPSS software package.

## Results and discussion

**Mushroom treatment effectiveness.** Analysis of variance regarding the percent mortality of Varroa mites in colonies treated with different forms of mushroom showed that the treatment method (p < 0.001), time (p < 0.001), and dose (p < 0.001) were significant. Similarly, the interaction between time and treatment (p < 0.001) showed significant differences. Among the three different treatment methods, the largest effect on average mite infestation was found for the gel treatment. In the untreated group (control), infestation increased by 43.23% (Tab. 1).

**Sealed brood.** It was found that the treatment methods had no negative effect on the brood production of the colonies (p > 0.001). The effect of time on brood production was statistically significant (p < 0.001). However, since the study was conducted in the fall, the decrease in the sealed brood rate is normal. For this reason, the interaction between treatment and time was statistically significant (p < 0.001). The largest brood area was observed in the dropping treatment and control groups (Tab. 2).

**Colony strength.** It was found that the treatment methods did not cause adult bees to die or leave the colony. It was statistically confirmed that the treatments had no negative effect on the number of bee-covered frames (p > 0.001). Similarly, the interaction between treatment and time was insignificant (p > 0.001). According to Table 3, the reason for the statistical significance of time is directly related to the dates when the study was conducted.

Tab. 1. Mite infestation of adult bees during the treatment (%) (mean ± SEM)

Treatment	BT	FAT	MAT	$\bar{x}$	TE %	T	TM	T * TM Int.
Smoking	18.04 ± 0.96 <sup>b</sup>	10.76 ± 1.18 <sup>c</sup>	6.54 ± 0.58 <sup>d</sup>	11.78 ± 1.37 <sup>B</sup>	63.80	< 0.001	< 0.001	< 0.001
Dribble	17.44 ± 2.06 <sup>b</sup>	10.22 ± 1.60 <sup>c</sup>	5.46 ± 0.86 <sup>d</sup>	11.04 ± 1.57 <sup>B</sup>	69.17			
Gel	17.68 ± 1.27 <sup>b</sup>	10.54 ± 0.81 <sup>c</sup>	4.72 ± 0.67 <sup>d</sup>	10.98 ± 1.51 <sup>B</sup>	72.13			
Control	17.30 ± 1.30 <sup>b</sup>	20.78 ± 1.18 <sup>b</sup>	24.42 ± 1.04 <sup>a</sup>	20.83 ± 1.00 <sup>A</sup>	–			
$\bar{x}$	17.62 ± 0.67 <sup>A</sup>	13.08 ± 1.17 <sup>B</sup>	10.29 ± 1.91 <sup>C</sup>	13.66 ± 0.86	43.23			

Explanations: a, b, c, d – means followed by different letters in the same column are different (p < 0.001); A, B, C – means followed by different letters in the same row and column are different (p < 0.001); SEM – standard error mean; BT – before treatment; FAT – fifteen days after treatment; MAT – one month after treatment;  $\bar{x}$  – average; TE – treatment efficacy; T – treatment; TM – time; Int. – interaction

Tab. 2. Sealed brood area of colonies before and after treatment (cm<sup>2</sup>/colony) (mean ± SEM)

Treatment	BT	MAT	$\bar{x}$	T	TM	T * TM Int.
Smoking	377.80 ± 13.74	339.55 ± 9.90	358.68 ± 10.22	0.384	< 0.001	0.643
Dribble	396.08 ± 13.70	352.16 ± 20.52	374.12 ± 13.74			
Gel	365.00 ± 14.55	312.57 ± 7.82	338.78 ± 11.70			
Control	374.03 ± 17.01	337.43 ± 16.44	355.73 ± 12.71			
$\bar{x}$	378.23 ± 7.27 <sup>A</sup>	335.43 ± 7.46 <sup>B</sup>	356.82 ± 6.18			

Explanations: A, B – means followed by different letters in the same row are different (p < 0.001); SEM – standard error mean; BT – before treatment; MAT – a month after treatment;  $\bar{x}$  – average; T – treatment; TM – time; Int. – interaction

Tab. 3. Strength of colonies before and after treatment (frame/colony) (mean  $\pm$  SEM)

Treatment	BT	MAT	$\bar{x}$	T	TM	T * TM Int.
Smoking	9.00 $\pm$ 0.01 <sup>a</sup>	8.20 $\pm$ 0.20 <sup>bc</sup>	8.60 $\pm$ 0.16 <sup>A</sup>	0.049	< 0.001	0.030
Dribble	8.60 $\pm$ 0.25 <sup>ab</sup>	7.80 $\pm$ 0.20 <sup>cd</sup>	8.20 $\pm$ 0.20 <sup>AB</sup>			
Gel	8.40 $\pm$ 0.25 <sup>ab</sup>	7.60 $\pm$ 0.24 <sup>de</sup>	8.00 $\pm$ 0.21 <sup>B</sup>			
Control	9.00 $\pm$ 0.01 <sup>a</sup>	7.20 $\pm$ 0.20 <sup>e</sup>	8.10 $\pm$ 0.31 <sup>B</sup>			
$\bar{x}$	8.75 $\pm$ 0.10 <sup>A</sup>	7.70 $\pm$ 0.13 <sup>B</sup>	8.23 $\pm$ 0.73			

Explanations: a, b, c, d, e – means followed by different letters in the same column are different ( $p < 0.001$ ); A, B – means followed by different letter in the same row and column are different ( $p < 0.001$ ); SEM – standard error mean; BT – before treatment; MAT – a month after treatment;  $\bar{x}$  – average; T – treatment; TM – time; Int. – interaction

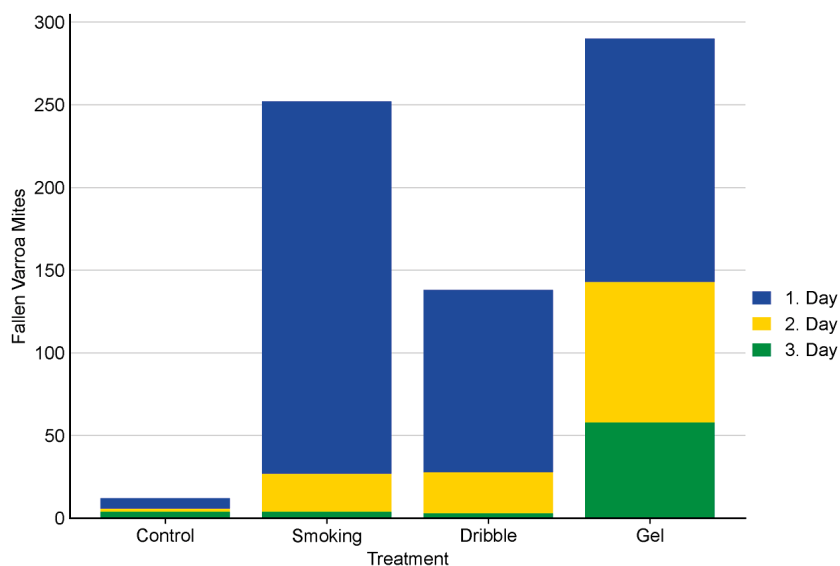


Fig. 1. Daily count of Varroa mites falling on the bottom board of the hive

**Fallen mites.** The numbers of mites that fell on the bottom board in the control and treatment groups on three consecutive days after application were compared. The smoking method caused the fastest reduction in the number of mites on adult bees on the first day after application. We assumed that the gel formulation, which increased adhesion to the surface and kept the contents more stable than the extract or smoke, would produce the effect for a longer period of time. The gel treatment was an extended-release and long-acting method. Compared with the other treatment methods, the dribble method resulted in the lowest reduction of *V. destructor* mites, which indicates its limited effectiveness in controlling mite infestations (Fig. 1).

*Varroa destructor* is one of the most important parasites that has a devastating impact on the beekeeping industry and threatens honey bee populations worldwide. As far as the natural control methods are concerned, beekeepers may choose among different products available on the market, but they often find it difficult to select one that is effective, harmless, and easy to use. The aim of this study was to evaluate the effectiveness of environmentally friendly practices in Varroa mite control and to provide sustainable solutions that both protect bee health and maintain ecological balance. The results suggest that natural methods

and biological control strategies can be both effective and more environmentally friendly alternatives to chemical treatment. The trial was performed in the fall, which is the period when acaricide treatments are carried out in the Muş province, Türkiye.

The highest acaricidal efficacy (72.13%) was achieved with the long-term administration of the *F. fomentarius* gel. In our experiment, the smoking method showed a lower acaricide efficacy (63.80%). It can be assumed that the efficacy of acaricidal treatments increases when they are carried out over longer periods of time and more frequently. Further studies should be conducted to confirm this hypothesis. During the summer, up to 90% of the mite population can remain within the brood (42). Our study confirms this, as brood activity was present in the colonies, and the Varroa mite infestation rate in adult bees increased to 43.23% in the untreated control group. Interestingly, Fries et al. (12) and Rosenkranz et al. (43) independently reported that untreated colonies with an adult bee infestation rate of more than 30% in the summer were unlikely to survive the following winter. Complete mite control was not achieved, as brood activity continued during the one-month treatment period. The infestation rate increased especially in the untreated control group as a result of mite reproduction in the capped brood. To fully evaluate the effectiveness of the methods, tests should be carried out during the brood-free period.

Ensuring biosafety and food safety is becoming increasingly important in the control of bee diseases. Recently, the saprophytic (harmless to humans and other mammals) and entomopathogenic (harmful to insects) properties of fungi in nature have begun to be discovered. Stamets et al. (48) reported that the antiviral effects of *F. fomentarius* and *Ganoderma applanatum* were significant depending on the dose. This aligns with our findings, where different mushroom-based treatments significantly increased the mortality of *Varroa destructor* mites. Among the formulations tested, the gel form exhibited the highest efficacy, resulting in the largest reduction in mite

infestation, while the infestation rate in the untreated control group increased by 43.23%. This suggests that the bioactive compounds in these fungi, including  $\beta$ -glucans and phenolic compounds, may contribute to the observed effects by enhancing immune responses or directly affecting mite survival.

Other researchers have recently demonstrated the potential therapeutic effects of *F. fomentarius*, identifying compounds with potent antiviral activity against human immunodeficiency virus HIV-1 and antimicrobial properties against *Candida albicans* and *Helicobacter pylori* (14, 45). In our study, the presence of bioactive components in the fungal extracts may have played a role in reducing mite loads, which supports the hypothesis that certain fungal metabolites contribute to antimicrobial and antiviral defenses in honey bee colonies. The results of Zhao et al. (54) showed weak antimicrobial activity in phenyl-ethanediol isolated from the fruiting bodies of *F. fomentarius*. However, the overall effectiveness of the mushroom-based treatments in our study suggests that the mite reduction was due to a combination of multiple bioactive compounds, rather than a single isolated metabolite.

In addition, this extract contains carbohydrates in the form of  $\beta$ -glucans with minor amounts of  $\alpha$ -glucans (29). The presence of polysaccharides in the polar extracts was considered very important, as a previous study by Senyuk et al. (45) had shown that a water-soluble melanin-glucan complex (containing 80% melanins and 20%  $\beta$ -glucans) completely inhibited the growth of *C. albicans* (45). In our study, although we did not specifically analyze the immune response at the individual bee level, the significant reduction in mite infestation, particularly in colonies treated with the gel formulation, suggests an immunomodulatory effect that contributes to the suppression of mite populations. Another study has shown that  $\beta$ -glucans have antimicrobial activity in insects via activation of the Toll and Imd signaling pathways (24). This mechanism may partially explain the impact of fungal treatments on mite reduction in the present study, as  $\beta$ -glucans are known to enhance immune defenses in insects, potentially increasing the resilience of honey bees against parasitic stressors.

The use of  $\beta$ -glucan as a dietary supplement has also been shown to improve the immune defense of honey bees (33). In our study, although the treatment methods did not negatively affect brood production or colony strength, the reduction in mite infestation suggests a possible link between fungal compounds and improved colony health. An increase in phenol oxidase activity (PO) was observed in *Apis mellifera* fed a diet containing varying concentrations of 1,3-1,6  $\beta$ -glucan (18). Although our study did not directly measure PO activity, the significant reduction in mite loads following treatment suggests that fungal components may have influenced immune-related responses in bees,

indirectly contributing to improved colony resilience. It has been found that the increased PO activity of older honey bee workers compensates for the natural loss of hemocytes (34). This is particularly relevant, given that our results showed no negative effects of treatments on colony strength, indicating that fungal-based interventions did not impose additional stress on the bees.

The PO cascade plays a role in immune defense against viruses, as shown by previous studies on mosquitoes and *Pimpla turionellae* (9, 41). In addition, 0.5% and 2%  $\beta$ -glucan diets have also been associated with an inhibitory effect on deformed wing virus (DWV) replication (33). Since *Varroa destructor* is a known vector of DWV, our results suggest that reducing mite loads through fungal treatments could have secondary benefits in decreasing viral transmission within the colony. This is particularly important, since DWV infections are among major contributors to colony losses worldwide.

The upregulated immune response genes of *V. destructor* identified by Kunc et al. (32) included apidaecin 1, abaecin, hymenoptaecin, defensin 1, RISC-loading complex subunit TARBP2, dicer, argonaute-2, peptidoglycan recognition protein S2, and beta-1,3 glucan binding protein. The use of  $\beta$ -glucans can also enhance innate immune defenses against pathogens, such as *Varroa* and other common honeybee pathogens, as  $\beta$ -glucans effectively support the immune system against them (35). In our study, the significant reduction in mite infestation following treatment, particularly with the gel formulation, aligns with previous findings that  $\beta$ -glucan-based treatments can modulate immune responses and improve host resistance to parasitic infections.

The aqueous extract of *F. fomentarius* consists mainly of phenols, such as melanins, lignins, and humic acids (29). Humic acid may prevent *V. destructor* and other bee diseases. In our study, the long-lasting efficacy of the gel treatment suggests that sustained exposure to fungal compounds may provide extended protection against mites. Humic acids eliminate *Nosema* spp., which is a digestive disorder, and *V. destructor* infestation, as they increase physiological activity in the stomach and intestines of bees (52). Given that our results demonstrated a significant reduction in mite populations without negative effects on colony health, the potential for fungal extracts to contribute to overall colony resilience warrants further investigation. Han et al (23) reported that *Metarhizium brunneum* was effective in controlling *V. destructor* and that this effect was similar to that of the 2.8% oxalic acid dribble method. A study by James (28) also confirms these results. Similarly, entomopathogenic fungi, such as *Metarhizium anisopliae* (90%), *Beauveria bassiana* (90%), and *Clonostachys rosea* (60%), have been reported to significantly reduce mite infestation in adult bees (22). In addition, *V. destructor* has been reported

to be sensitive to *Metarhizium anisopliae*, *Beauveria bassiana*, *Verticillium lecanii*, and *Hirsutella* species (46). In particular, *M. anisopliae* and *B. bassiana* have been reported to cause 97% and 100% mortality of mites, respectively (46). The isolate of *Metarhizium anisopliae* var. *anisopliae* BIPESCO 5 was also found to be more than 60% effective in controlling *V. destructor* (11). Our results also show that fungal treatments are effective in the control of the Varroa mite. They were found to cause a significant increase in Varroa mortality, and this effect was directly related to the treatment method, duration, and dose ( $p < 0.001$ ). Comparison of the three different treatment methods shows that the gel had the strongest effect on the average mite infestation rate. It was observed that mite infestation increased by 43.23% in the untreated control group. The gel formulation maintained its effect for a longer time by ensuring that the content adhered better to the surface and remained stable for a long time. On the other hand, the dribble method proved least effective in Varroa mite control. Fernandez Ferrari et al. (11) reported that the application of *Metarhizium anisopliae* had no negative effect on colony strength and growth, and that the colony size after application was 3016.5 cm<sup>2</sup>. Similarly, our results show that the fungal treatments did not have a negative effect on the capped brood area or the number of bee-covered frames ( $p > 0.001$ ). Yücel (53) reported the average capped brood area to be 2343 cm<sup>2</sup> and the number of frames occupied by bees to be 5.10. These values are consistent with the capped brood area and colony size data in our study. In our study, no significant difference was found between the different treatments in terms of the average capped brood area, while the time factor was statistically significant ( $p < 0.001$ ). Han et al. (23) reported a large decrease in the number of Varroa mites until the 9<sup>th</sup> day following fungus treatment, after which the effect of treatment weakened. Kanga et al. (30), and Piralı-Kheirabadi et al. (39) reported that treatment with *Metarhizium anisopliae* caused the number of mites to decrease by an average of 2.01 and 13.1 mites per day, respectively. Bakar et al. (7) reported that an average of 13.2 mites fell to the hive floor within twelve hours. Pietropaoli and Formato (38) reported that, although the number of mites in the control group decreased by 25.5%, the treatment reduced the mite count by as much as 60.2%. In another study conducted with the same breed of bees as those used in our study, the average number of mites that fell naturally in the colonies was 2.8 per day (6).

The results of this study demonstrate that *F. fomentarius* is a promising and safe natural product for the control of *V. destructor*. This fungus is effective against pathogens such as Varroa, probably because of the beta-glucan, humic acid and polyphenols it contains, although specific concentrations in the different forms of the fungus need to be further validated. These com-

pounds have been reported by other studies, but further investigation is required to confirm their presence in the *F. fomentarius* samples used in this study. The fungus can be collected by beekeepers in the wild and used without significant problems by the fumigation method for the control of Varroa mites. Given the ease of use and feasibility of the treatments, the gel and smoking methods are particularly practical for beekeepers. In conclusion, our study found that the smoking, dribble, and gel treatments all showed promising results, with the gel treatment being most efficacious in terms of acaricide activity and honey bee tolerance under field conditions. Further research is required to fully develop and optimize natural acaricides for Varroa mite control. Since additional data is needed to refine the performance of *F. fomentarius*, future studies should focus on researching the most effective methods of its application and improving the understanding of its acaricidal properties.

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