MELLIFERA RESEARCH ARTICLE

Preliminary Trials on The Efficacy of Propolis in The Control of The*Varroa destructor* (Mesostigmata:Varroidae) Ectoparasite of The Honey Bee *Apis mellifera intermissa* (Hymenoptera: Apidae)

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Received: 04th March, 2021; accepted:18th April, 2021; published:15th July, 2021

A B S T R A C T

Honey bee colonies, *Apis mellifera intermissa* in particular, are affected by *Varroa destructor* mites which threaten their existence. Several chemical treatments are used, but the parasite has become resistant to them. In recent years, plant origin substances have been used as a natural alternative means for combating the parasite. In this study, the acaricidal effect of propolis is evaluated in the laboratory. Seven Varroa mites were placed on filter paper (3 ×3cm) in petri dishes. Subsequently, the filter paper was impregnated with a dose of 0.2 ml (200 µl) of the propolis and ethanol solution. Four repetitions were carried out with a control without treatment. The results showed that propolis extract with 70% ethanol (EEP) is highly toxic against Varroa. The mortality rates of the Varroa mite were 40% to 100% after 30 and 120 minutes of exposure, respectively. On the other hand, treating mites with 100 mg of propolis powder showed mortality rates of 20% to 100% after 12 to 24 hours of exposure. Finally, when bees were exposed to EEP or propolis powder, they were not affected by the treatment.

Keywords: *Apis mellifera, Varroa destructor,* Parasite, Propolis, control, toxicity

Introduction

Propolis is a resinous, gummy substance with a viscous consistency collected by bees on certain parts (buds and bark) of plants, mainly trees [1]. It contains almost 50% resin and vegetable balm, 30% wax, 10% essence and aromatic oils, 5% pollen

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and 5% organic debris [2]. Propolis is rich in biochemical constituents, comprising mainly a mixture of flavonoid polyphenols, aglycon flavonoids and phenolic acid [3].

Propolis is used as a biocide by bees; it is responsible for the low incidence of bacteria and mold inside the hive. Bees may use it to coat the entrance and frames and to prevent drafts from entering the hive. It is also used to enbalm killed enemies if they are too large to be removed by worker bees from the hive [4] .

Propolis also plays an essential role in apitherapy. Indeed, it is known for its antimicrobial ([5][6], anti-inflammatory [7], antiviral [8][9] and antifungal characteristics [10]. However, a few studies have determined the insecticidal and acaricidal action of propolis [11, 12].

This study aims at determining the role of propolis in the biological control of the *V.destructor* mite. Hence, *V. destructor* is considered a formidable parasite in the collapse of *A.mellifera* bee colonies world wide.

Materials and Methods

Harvesting Propolis

The harvest of propolis is carried out during spring of 2018 on bee colonies at the educational apiary of the vocational training center of Tizi Ouzou (Algeria). With a Mediterranean climate and a temperate sub-humid bioclimatic stage.

The harvest of propolis is carried out by two methods:

- scraping hive and the frames: The obtained propolisis cleaned fromthe debris stuck to it (bees, wood, wax cover, Varroa etc.), weighed and put in boxes.

- using a particular grid called a "propolis grid"which is a food-grade plastic grid made frommany small interstices. It is placed on the body of the hive under the frame cover. Once these interstices are closed with propolis, the grid is removed and placed in a refrigerator. When cold, the propolis becomes brittle, and the twisting of the grid will detach the small pieces.

Preparation of Propolis and Ethanol Solutions

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For the preparation of the propolis solution, the protocol by Strehlet al. (1994) [13] was followed:

- The harvested propoliswas first heated in a saucepan filled with water to remove the debris (wax, pollen, etc.) stuck to it.

- The mixture of propolis and water was filtered with a colander. The propolis was stored in the freezer at -4°C for about two days and then crunched into a fine powder.

- This powder was added to ethanol in a ratio of 1 g of propolis to 10 ml of solvent.

- The mixture was left for maceration for a week, with frequent shaking.

- After maceration, the mixture was heated in a water bath at 70°C for 30 minutes then filtered. The resulting liquid extract was called Ethanolic Propolis Extract (EEP), stored in the refrigerator for later use.

Varroa Sampling

Mites were collected from infested colonies. A sample of 100 cells of capped brood was opened using forceps. The nymphs were removed to collect the present Varroaeither at the bottom of alveolus or stuck on the nymph as shown in Figure 1.

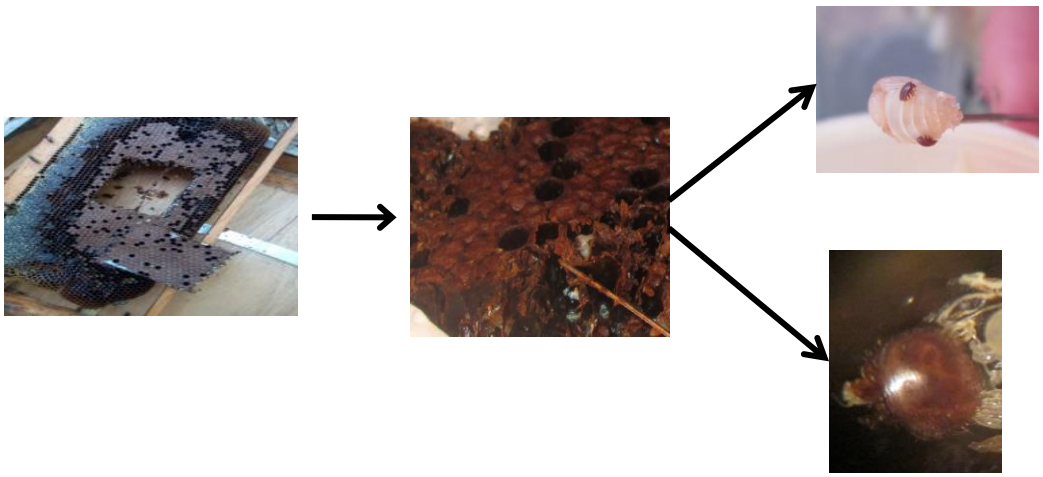

Figure 1. Removal of Varroa from the brood

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Application of the EEP Solution

Against Varroa: To test the effect of propolis on Varroa, the method of Garedew et al. (2002) [14] was adopted as follows:

Seven Varroamites were placed on filter paper (3 ×3cm) in petri dishes. Subsequently, the filter paper was impregnated with a dose of 0.2 ml (200 µl) of the EEP solution. Four repetitions were carried out with a control without treatment.

Varroa mites were left in contact with the EEP solution for 30 seconds. Nymphs of bees, which served as food for the mites, were introduced into the Petri dishes.

The mites' behavior was observed under a magnifying glass for 10, 30 and 60 minutes and every hour after treatment. The test was carried out at ambient temperature of approximately 22 to 25°C, and the treated mites were incubated at 28 ± 1 ° C, and 60% RH.

Each mite was classified as mobile or an inactive mite showing no movement of the legs or the rest of the body [15]. If a mite has remained inactive after 8 hours from the start of treatments, it is considered dead.

Against bees: To determine the effect of propolis on bees, each container's filter paper was impregnated with 0.2 ml (200 µl) of the ethanolic propolis extract (EEP) solution. Four repetitions were carried out with an untreated control. The results were read after 10, 30 and 60 minutes of exposure and every hour after treatment.For each exposure time and each repetition, the dead bees were counted under the EEP solution's effect.

Application of PropolisPowder

Against Varroa: Seven Varroa mites were placed in Petri dishes. Each Varroawas directly sprinkled with approximately 500mg of propolispowder. Parasite mortality was monitored in the same way mentioned before with the EEP solution.

Against bees: 50 bees were placed in crates. The bees were directly sprinkled with about 500mg of propolis powder. The bee mortality monitoring was carried out in the same way as mentioned before with the EEP solution.

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Results and Discussion

Effect of Propolis on Varroa

The average rates of mortality of Varroa mite observed as a function of the duration of exposure with the solution of ethanolic extract of propolis and propolis powder were presented in Figure2.

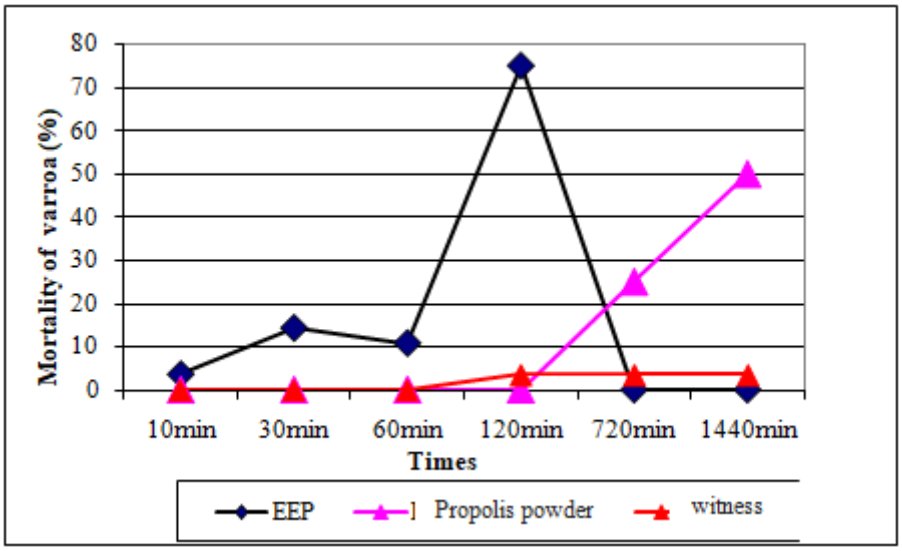

Figure 2. Evolution of Varroa mortality according to the type of treatment.

After 10 minutes of exposure to EEP, 3% of the Varroa was recordeddead. This mortality rose to 75% after 120 minutes of exposure. With the propolis powder, the parasites did not die until after 12 hours of exposure, with a rate of 25%. At 1440 minutes of contact with the treatment, the mortality of the Varroamite reached 50%. In the control batch, low mortality was observed (3.57%) after 120 minutes.

Effect of Propolis on Bees

The average rates of mortality in bees recorded according to the duration of exposure with the ethanolic extract solution of propolis and propolis powder were presented in Figure 3. After 720 minutes of exposure to EEP and propolis powder, the bee mortality rates were meager.

During this study, the acaricidal efficacy of propolis was tested against the parasite *V. destructor*. This bee product was used in two forms: as a liquid solution which is the ethanolic extract (EEP), and as a solid, which is propolis powder. The results showed that the EEP was very effective due to the total mortality of 100% after 120 minutes of exposure. Indeed, the

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harmful effect of this extract was quickly observed by the Varroa throughout the exposureduration, the mortality rate of the Varroawent from 3.57% to 75%.

On the other hand, propolis powder had a slow effect on Varroa because it was only at 720 minutes of exposure; the first mortality rate of 25% was recorded. The mortality rate only reached 50% after 1440 minutes of contact. According to Garedew et al. (2002) [14], the extraction of propolis in 70% ethanol made it possible to obtain and release most of the biologically hydrophobic active components. Furthermore, they suggested that contact with propolis solutions could lead tothe rigidity of the cuticle of mites, facilitating the entry of the active compounds present in propolis.

The death percentage of Varroa mites by EEP varied between 60.5% and 90% after 30 seconds of exposure in different regions of Argentina, and they assumed that the acaricidal activity of propolis extracts was due to the various bioactive components and the phenolic constituents present in the propolis [12] .

Popova and al. (2014) [16] suggested that propolis's acaricidal effect against Varroa mites resulted from its highly variable chemical composition, which largely depends on the plant harvested.

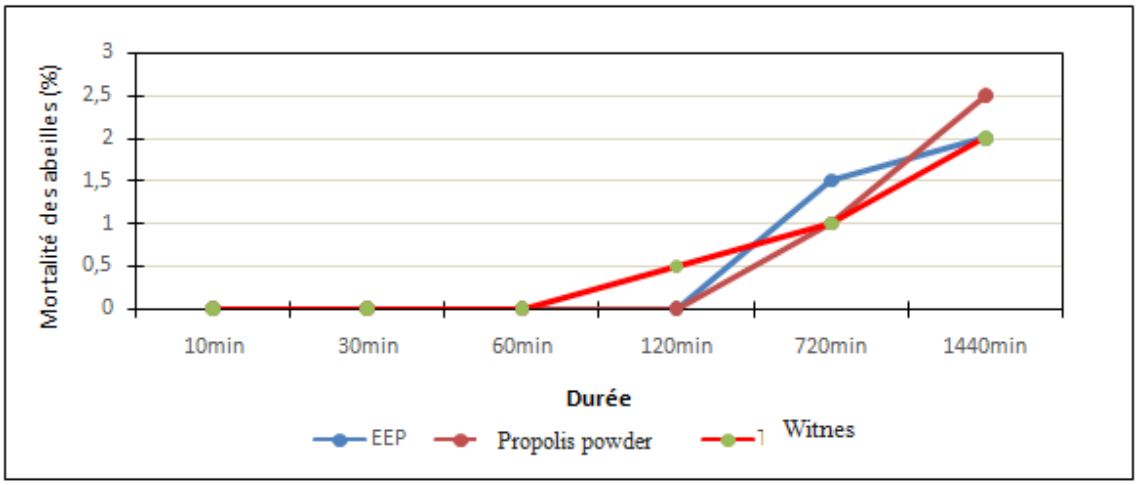

Figure 3. Evolution of bee mortality according to the type of treatment

The biological activity of propolis against several microorganisms was also demonstrated by Burdock (1998) [17]. Other studies have shown the inhibitory effect of propolis on Gram

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+, Gram- strains [18,19], and anaerobic bacteria [20, 21]. This effect depends on the investigated strain, the propolis's origin, and the used solvent [22]. Besides, propolis has antifungal [23,24], antiviral, antiprotozoal and antiparasitic properties due to its composition of polyphenols and flavonoids [25, 26]. Another work has reported that a thin-layer chromatography screening showed pinocembrin, caffeic acid, kaempferol, phenethylcaffeate, chrysin and galangin in Iranian propolis. The total flavonoids and phenolics are 7.3% and 36% respectively, which suggests that Iranian propolis's intense antimicrobial activity may be due to high levels of phenol and flavonoid compounds.

The antimicrobial activities of propolis have been the subject of several studies against different bacteria [27, 28], yeasts [29, 30],and parasites [31]. In vitro, propolis can act directly on microorganisms, and *in vivo*, it can stimulate the immune system by activating the mechanisms involved in the destruction of microorganisms.

Bastos et al. (2008) [32]. reported that extracts of propolis from different regions of Brazil inhibit the growth of *Paenibacillus* bacteria. This activity depends much more on the botanical origin of the propolis than on the applied concentration. These results were confirmed by Wilson et al. (2015) [33]who tested propolis samples collected from 12 different regions of the United States of America against bee pathogens: *Paenibacillus larvae* and *Ascosphaeraapis.*

The extraction methods of propolis can also influence its activity due to the various compounds' solubility properties [34]. The most widely used solvents in bioassays are ethanol and water. However, ethanolic extract shows more significant antimicrobial activity than water extracts or volatile compounds. It has all water and extractable ethanol, which are biologically active components. Also, the ethanolic extract contains several bioactive components that have not been found in other solvents.

In addition, Antunez et al. (2008) [35] studies on the virtue of propolis showed its antimicrobial effect on American foulbrood. These authors also revealed that bees tolerated high concentrations of ethanolic extract of propolis administered orally with the addition of syrup.

Drescher et al. (2017) [36] reported that propolis appears to be active against a range of honey bee pests and pathogens and can be considered an immune defense mechanism in the colony. Furthermore,

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they revealed that the propolis used in its natural form, deposited massively on the frames inside the hive, has no impact on *V. destructor* but the infestation of bees by the virus of deformed wings (DWV) and the sacciform brood virus (SBV) decreased considerably. This indicates that propolis is likely to play an essential role in maintaining the health of bee colonies.

Conclusion

The evaluation of propolis's acaricidal effect was used in two forms: an ethanolic extract and propolis powder, which provided impressive preliminary results for this study. In this study, the ethanolic extract was effective and caused the death of 100% of Varroa after 120 minutes of exposure. Propoliswas proved to be promising in the control of *V. destructor*and an accessible way of control. This beehive product that bees collect to embalming intruder in the hive or plug any existing void is a product that any beekeeper can easily acquire from his/her apiary.

Bal Arısı *Apis mellifera intermissa* (Hymenoptera: Apidae) Ektoparaziti Varroa destructor (Mesostigmata:Varroidae) Kontrolünde Propolisin Etkinliği Üzerine Ön Denemeler

Öz: *Apis mellifera intermissa* başta olmak üzere bal arısı kolonileri varlıklarını tehdit eden *Varroa destructor* akarlarından etkilenir. Birkaç kimyasal işlem kullanılmaktadır, ancak parazit bunlara karşı dirençli hale gelmiştir. Son yıllarda bitki kökenli maddeler parazitle mücadelede doğal bir alternatif araç olarak kullanılmaktadır. Bu çalışmada propolisin akarisit etkisi laboratuvar ortamında değerlendirilmiştir. Petri kaplarına filtre kağıdı (3x3cm) üzerine yedi Varroa akarı yerleştirilmiştir. Ardından, filtre kağıdına 0.2 ml (200 ul) propolis ve etanol solüsyonu emdirilmiştir. Tedavi uygulanmadan kontrol ile dört tekrar yapılmıştır. Sonuçlar, %70 etanol (EEP) içeren propolis ekstraktının Varroa'ya karşı

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oldukça toksik olduğunu göstermiştir. Varroa akarının ölüm oranları, sırasıyla 30 ve 120 dakikalık muameleden sonra %40 ila %100 olmuştur. Öte yandan, akarların 100 mg propolis tozu ile tedavi edilmesi, 12 ila 24 saatlik muameleden sonra %20 ila %100 arasında ölüm oranları göstermiştir. Son olarak, arılar EEP veya propolis tozuna maruz bırakıldıklarında etkilenmemişlerdir.

Anahtar Kelimeler: *Apis mellifera*, *Varroa destructor*, Parazit, Propolis, kontrol, toksisite

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