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Ecological mite control agents, particularly against *Varroa destructor*

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Abstract: *Varroa destructor* is an external parasitic mite that attacks honey bees *Apis cerana* and *Apis mellifera*. *Varroa* mites can be controlled through commercially available miticides as well as non-chemical means. Our research work has been conducted on plants extracts reducing the mite population. We have obtained promising results for extracts of yew and walnut.

Keywords: *Varroa* mites, extract of yew, extract of walnut

INTRODUCTION

Varroa destructor is an external parasitic mite that attacks honey bees *Apis cerana* and *Apis mellifera.* The disease caused by the mites is called varroatosis.

Varroa destructor can only replicate in a honey bee colony. It attaches to the body of the bee and weakens the bee by sucking hemolymph. A significant mite infestation can lead to the death of a honey bee colony, usually in the late autumn through early spring.

Varroa mites can be treated with commercially available miticides. Miticides must be applied carefully to minimize the contamination of honey that might be consumed by humans and they may cause the development of resistance in the mites [1].

V. destructor can also be controlled by non-chemical means. Most of these

means do not eliminate the mites completely and usually are supported by chemical treatment. Our group conducts research on plant extracts reducing the mite population. We have obtained promising results for extracts from yew and walnut.

MATERIALS AND METHODS

Isolation of an extract from yew-tree

The yew twigs with needles (0.5 kg) were crushed and ethyl acetate (1 l) was poured over. The material was placed in ultrasonic bath and extracted by shaking for 30 min. After filtration, the solution was concentrated under reduced pressure. We obtained about 60 g of dry residue which was extracted with 1 l of hexane. The filtrate was concentrated and 10 g of the residue **(1)** was received.

Part of the residue insoluble in hexane was dissolved in ethyl acetate/ dichloromethane 4:1 (300 ml). The filtrate was concentrated under low pressure. 13 g of residue **(2)** was obtained.

Separation of yew-tree extract by column chromatography

Each 3 g sample of **(1)** and **(2)** was concentrated with silica gel for chromatography. Chromatography column was packed with 120 g of silica gel. The samples were placed in the column and eluted with solvents in order: hexane, hexane-ethyl acetate (10:1; 4:1; 1:1), ethyl acetate, methanol.

Each of the obtained colorful fractions, about 100 ml, was collected separately and concentrated under reduced pressure. Contents of the fractions were analyzed by TLC (Merck, Silica gel 60 F 254). The TLC plates were developed in a chamber with a mobile phase − chloroform:methanol 7:1. As a result we could observed in a visible light, a lot of colorful and interconnected spots what indicates presence of many substances in each fraction. At this stage of research the identification of compounds wasn't possible.

Subsequently, from isolated fractions of extract **(1)** and **(2),** we selected some of them and examined their activity to reduce the mite population. The results are in Table 1.

Isolation of walnut extract

Leaves of walnut (0.5 kg) were collected in May, cut into small pieces and treated with ethyl acetate (1 l) for three days. All material was shaken in ultrasonic bath for 15 min. The solvent was evaporated under low pressure at 30 °C to produce a dry residue (80 g). The remained residue was dissolved in

acetonitrile/ethyl acetate 4:1 (300 ml). The filtrate was concentrated under low pressure. 22 g of residue was obtained.

Separation of walnut extract by column chromatography

3 g of each sample was concentrated with silica gel. Column was packed with 120 g silica gel for chromatography. The samples were placed in the column and eluted with solvents in order: hexane, hexane:ethyl acetate (8:1; 6:1; 4:1; 2:1), ethyl acetate, methanol.

The colorful fractions (orange, yellow, green, brown) of about 50 ml were collected and solvents were removed under reduced pressure. The fractions were applied onto a silica gel TLC plate (Merck, silica gel 60 F 254) and developed with hexane:dichloromethane:acetonitrile (5:3:1). A juglone standard was run on each plate (juglone is an active substance occurring in the walnut leaves [2]). Presence of many substances in each fraction was revealed by TLC chromatography. The spots were colorful, numerous and interconnected – identification of individual compounds wasn't possible.

We selected several fractions which were isolated from extract of the leaves and juglone standard (97%) and examined their activity against the mite. The results are in Table 2.

RESULTS AND DISCUSSION

Table 1. Efficacy of the yew-tree extract against *Varroa* mite

Extract	Concentration [%]	Mortality rate per 24 h [%]	Mortality rate per 48 h [%]	Mortality rate per 72 h [%]
Residue 1	**2.1**	**100**	**100**	**100**
1/1	1.1	40	40	40
1/2	1.4	30	30	40
1/3	2.0	7.5	30	37.3
2/1	0.2	0	10	10
2/2	1.6	2.5	2.5	12.5
2/3	3.1	27	30	33
2/3	0.7	0	10	20
2/4	2.3	25	25	25
2/5	2.7	27	27	33

Fractions received from the extract **(1)** are marked as (1/1), (1/2), (1/3) and from the extract **(2)** as (2/1), (2/2), (2/3), (2/4), (2/5), (2/6).

The extract of yew (residue 1 in Table 1) has high miticidal activity. Our team patented the use of yew extract for control of *V. destructor* [3]. The active extract was isolated by column chromatography, but after separation to fractions we have observed decreasing activity to reduce the mite population (extracts 1/1, 1/2, 1/3 in Table 1). The fractions obtained by column chromatography of the residue **(2)** insoluble in hexane have lower activity than the previous fractions. We have tried to find a dominating compound with miticidal properties. For this purpose we tried to isolate it by column chromatography or semi-preparative HPLC but all attempts made so far have failed.

Table 2. Efficacy of the walnut extract against *Varroa* mite

Extract	Concentration [%]	Mortality rate per 24 h [%]	Mortality rate per 48 h [%]	Mortality rate per 72 h [%]
1	**0.97**	**100**	**100**	**100**
2	0.63	10	15	20
3	0.13	10	12.5	17.5
4	0.07	0	3	7
5	1.17	3	3	10
6	0.94	23	27	27
7	0.34	0	5	30
Juglon standard	19.5	0	0	10

One fraction of walnut extract has high miticidal activity (extract 1 in Table 2). Our team patented the use of walnut extract to control *V. destructor* [4]. We supposed that juglone affected activity against *Varroa* mite, but biological test did not confirm it.

Juglone occurs naturally in the leaves, roots, husks, and bark of plants in the Juglandaceae family, particularly the black walnut (*Juglans nigra*) [5], and is toxic or growth-stunting to many types of plants [6]. Juglone is an example of allelopathic compound, a substance that is synthesized in one type of plants and affects the growth of another ones [7]. Juglone exerts this effect by inhibiting certain enzymes needed for metabolic function. It is highly toxic to many insect herbivores [8]. However, a juglone standard, against our expectation, hasn't any special activity to reduce the mite population (juglone std in Table 2).

The fraction without juglon (extract 4 in Table 2) and bottom fraction with juglone (extract 5 in Table 2) have very weak activity. In our future tests we will focus on particular fractions. We will try to isolate individual compounds and identify them.

CONCLUSION

The mite *Varroa destructor* is a major problem for beekeeping worldwide. It can be controlled efficiently with a variety of miticides. Ecological controls do not eliminate the mites completely, but are intended to reduce the mite population to a manageable level. The plant extracts having activity to reduce the mite population do not negatively affect the honey quality. Our team have an idea to convert yew needle extract and walnut leaf extract to ecological miticides. Biological tests confirmed a possibility of using the listed plants extracts. We endeavor to isolate active compounds having properties against *Varroa destructor*. An interesting observation is that the extract of yew have a greater activity than the fractions after column chromatography. This can suggest an occurrence of the synergy. We noticed also that active substance described in literature occurring in the walnut, juglone, isn't miticide but other substances affect the *Varroa* mite properties.

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