Honeybees

Biology, Behavior and Benefits

Danièle Dreesen
Editor

Insects and Other
Terrestrial Arthropods
Biology, Chemistry
and Behavior

NOVA

INSECTS AND OTHER TERRESTRIAL ARTHROPODS:
BIOLOGY, CHEMISTRY AND BEHAVIOR

HONEYBEES

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CONTENTS

Preface **vii**

Chapter 1 The Genetic Structure of Dark European Honey Bee Population in the Ural **1**
Rustem A. Ilyasov, Aleksandr V. Poskryakov and Alexei G. Nikolenko

Chapter 2 Herbal Dietary Supplement with Antifungal Effect for Increasing the Productivity of Honey Bee Colonies **15**
Rustem A. Ilyasov and Rashit G. Farkhutdinov

Chapter 3 Alternatives for Controlling Pathogens and Parasites to Improve Honeybee Health **23**
Natalia Damiani, Martín Pablo Porrini, Martín Javier Eguaras and Liesel Brenda Gende

Chapter 4 Bee Defensin-1 Seasonal Quantitative Variability in Honeys and Its Role in Bee Health Protection **53**
Ivana Valachova and Juraj Majtan

Chapter 5 Nutritional and Medicinal Properties of the Honeybee Larvae **71**
Mitsuhiro Aoki, Shinobu Fukushima, Akio Ohkuma and Tomoki Tatefuji

Chapter 6 Regulation of Honeybee Foraging in Response to Food-Source Profitability **91**
Ken-ichi Harano and Masaki Hayashi

Chapter 7 In Which Categories of Scholarly and Professional Journals Are Honeybee-Related Research Articles Most Likely to Be Found? How Has This Changed Between the 1945-2005 *vs.* 2006-2015 Timespans? **107**
Tony Stankus

Index **125**

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Chapter 2

HERBAL DIETARY SUPPLEMENT WITH ANTIFUNGAL EFFECT FOR INCREASING THE PRODUCTIVITY OF HONEY BEE COLONIES

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ABSTRACT

The advantages of using for feeding the honey bees by sugar syrup with ethanol extract from 15 medicinal plants were presented in the article. This extract has immunomodulatory effect on honey bees and has antifungal activity against pathogenic fungus *Ascosphaera apis*. Feeding by sugar syrup with the extract was led to a stability of wintering, was increased a productivity of honey and eggs in comparison with the control group. Antifungal activity of the extract against ascosphaerosis was similar with activity of the nystatin. Immunomodulatory effect of the extract on honey bees was similar with effect of *Rhodiola rosea*.

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Keywords: Honey bee, dark European bee, *Ascosphaera apis*, *Apis mellifera mellifera*, immunity, ascosphaerosis, immunomodulatory effect, ethanol extract of medicinal plants, antifungal activity, productivity of the honey

INTRODUCTION

Ascosphaerosis or chalkbrood is an infectious disease of larvae of honey bees caused by parasitic fungus *Ascosphaera apis*. *Ascosphaera apis* infects the brood of honey bees in 3-6 days age through feeding pollen and nectar with spores (Gilliam et al. 1988; Aronstein et al. 2010; Tuktarova, Farkhutdinov 2013). Ascosphaerosis kills all larvae, disrupts changes of generations in colony and reduces the total number of worker bees (Eguaras et al. 2005).

Current trend of spreading ascosphaerosis on the apiaries is very dangerous. Now ascosphaerosis has spread worldwide. For the first time ascosphaerosis was registered on apiaries in 1985 in the Republic of Bashkortostan (Tuktarova, Farkhutdinov 2013). About 14% of honey bee colonies were infected by ascosphaerosis in the Republic of Bashkortostan in current time.

Numerous stressful factors of the environment decreased the immunity of honey bee colonies (Alaux et al. 2010). The humidity and bad nutrition of honey bees in hives lead to increase the incidence of ascosphaerosis (Alaux et al. 2010; Evans, Spivak 2010; Sandrock et al. 2014). The treatment of bee's colonies with ineffective drugs against ascospaerosis leads to growing of resistance *A.apis* to many other drugs (Barnett et al. 2007, Sandrock et al. 2014). Current beekeepers try to avoid treatment bees with chemical drugs and begin to using treatment with natural drugs as extracts of medicinal plants. The natural drugs have activity against different pathogens and do not suppress the immunity of honey bees.

The products of beekeeping without chemical drugs become safe for human (Strachecka et al. 2014). Therefore, using for treatment of bees presented in this article the extract of 15 medicinal plants allow for beekeepers to increase the immunity of honey bees and to preserve the purity of the products of beekeeping.

MATERIAL AND METHODS

The study was conducted on the colonies of the dark European bees *Apis millifera millifera* L. which inhabited in classical hives with 12 frameworks in the Republic of Bashkortostan. We have researched the effect of feeding the honey bees with sugar syrup with an ethanol extract of medicinal plants on the biological features of colonies in comparison with the control group without feeding with extract.

The ethanol extract was obtained from 15 species of the medicinal plants: grass of *Veronica longifolia*, leaves of *Betula pendula*, grass of *Filipendula ulmaria*, flowers of *Calendula officinalis*, needles of *Picea abies* or *Abies sibirica*, grass of *Echinacea purpurea*, leaves of *Eucalypti viminalis*, grass of *Equisetum arvensis*, flowers of *Helichrysum arenarium*, grass of *Melissa officinalis*, grass of *Thymus serpyllum*, bark of *Populus tremula*, grass of *Chelidonium majus*, garlic *Allium sativum* and thallus of moss *Lichen islandicus*.

The extraction of medicinal plants was prepared by maceration in 40% ethanol during 14 days at room temperature. The syrup with 40% ethanol extract of *Rhodiola rosea* was taken for the comparative analysis (Shafikova, Farkhutdinov 2013). The 40% ethanol extract was evaporated to 20% concentration before mixing with syrup. The bees from control group were fed syrup with 20% ethanol only.

The chemical composition of extract of plants was determined on liquid chromatograph mass spectrometer LCMS-2010EV “Shimadzu” (Japan).

The effect of extract of medicinal plants on the biological features of honey bee colonies was evaluated during autumn. Investigation was performed according to standard veterinary procedures (Foley et al. 2014) on 15 colonies of honey bees which were separated into 3 groups (5 colonies in each group). The feeding of honey bee colonies the syrup with extract was held at the end of August. Each colony was fed 1 liter syrup with extract two times at interval 7 days.

The immunomodulatory effect of the extract on honey bee colonies was researched in comparison with immunomodulatory effect of extract of *Rhodiola rosea*. The colonies from first group were fed with sugar syrup mixed with 20% of extract of *Rhodiola rosea*; from second group - with 20% of extract of medicinal plants; from third group (control) – with 20% of ethanol. The evaluation of immunomodulatory effect was made every 10 days in 2 month.

The antifungal activity of the extract on honey bee colonies was researched in comparison with antifungal activity of solution of nystatin (0.5 g was dissolved in liter of 40% ethanol). The colonies from first group were fed with sugar syrup mixed with 20% of nistatin; from second group - with 20% of extract of medicinal plants; from third group (control) – with 20% of ethanol. The evaluation of antifungal activity was made every 10 days in 2 month.

The activities of ferments of honey bees such as catalase (EC 1.11.1.6) from the rectal glands, peroxidase (EC 1.1.11.7) from the gut and invertase (EC 3.2.1.26) from the hypopharyngeal glands were determined according to the published protocol (Radwan et al. 1984; Persano et al. 1990; Allen et al. 1991; Roderick, 2000; Vorlova, Pridal 2002; Weirich 2002; Babacan, Rand 2005; Al-Sherif et al. 2012; Shafikova, Farkhutdinov 2013).

RESULTS

The mass spectrometric analysis has shown the composition of 40% ethanol extract of medicinal plants: menthol - 50%, xanthoxol - 50%, 2.4-dyhydroxycinnamic acid – 23.45%, herniarin/psoralen – 5.05%, pulegone – 4.45%, eugenol – 3.95%, thymol - 3,95%, xanthoxol – 3.45%, p-coumaric acid/eugenol – 3.1%, imperatorin – 3.05%, 3-nitrocinnamic acid – 2.15%, geraniol/citral – 2.10%, 2.4-diacetoxycinnamic acid - 2%, geraniol/citral – 1.6%, khellin – 1.45%, anethole – 1.45%.

The inhibition of growth zone of *Ascosphaera apis* was measured in 3 days on agar in the Petri dish. The growth zone of *Ascosphaera apis* had 12.2 mm in agar with 20% the extract of medicinal plants (extract of plants in 40% ethanol) and had 10.2 mm in agar with 20% nystatin solution (0.5 g of nystatin was dissolved in liter of 40% ethanol). Thus antifungal activity of the extract of medicinal plants was higher than antifungal activity of nystatin solution.

The activities of ferments of honey bees such as catalase from the rectal glands, peroxidase from the gut and invertase from the hypopharyngeal glands determine the immunity and quality of preparation of bee colonies to wintering.The activities of the ferments catalase, peroxidase and invertase of honey bees were determined in November. The activity of catalase of honey bees was higher by 88% in the group which was fed syrup with extract of *Rhodiola rosea* than in the control group. The activity of peroxidase of honey bees was higher by 9% in group which was fed syrup with extract of medicinal plants and was higher by 16% in group which was fed syrup with extract of *Rhodiola rosea* than in control group. The activity of invertase of honey bees

was higher by 32% in group which was fed syrup with extract of medicinal plants and was higher by 40% in group which was fed syrup with extract of *Rhodiola rosea* than in control group (Table 1).

Table 1. The activity of ferments catalase, peroxidase and invertase of honey bee colonies which were fed syrup with ethanol extract of medicinal plant and *Rodiola rosea* in comparison with control

Feeding by syrup with	Activity of catalase, u/ml	Activity of peroxidase, µg of oxidized OPD / g×min	Activity of invertase, conv. units/hour
extract of *Rhodiola rosea*	8.20	0.48	3.5
extract of medicinal plants	6.90	0.45	3.3
ethanol (control)	4.36	0.41	2.5

The assessment of the incidence of ascosphaerosis of honey bees was performed by counting the number of infected larvae in each colony. The incidence of ascosphaerosis had not differed between groups in the beginning of the assessment. The incidence of ascosphaerosis was decreased by 29% in the group which was fed syrup with nystatin, was decreased by 6% in the group which was fed syrup with extract of medicinal plants and was increased by 16% in the control group in 10 days (Table 2).

Table 2. The incidence of ascosphaerosis of honey bee colonies which were fed by syrup with ethanol extract of medicinal plant and nystatin in comparison with control

Feeding by syrup with	The number of infected larvae pcs. per 10 cm^2			
	in beginning	in 10 days	in 20 days	in 30 days
dissolved nystatin	39±4	28±4	15±5	4±1
extract of medicinal plants	34±5	32±3	18±4	3±1
ethanol (control)	36±5	42±4	49±5	44±3

The incidence of ascosphaerosis was decreased by 62% in the group which was fed syrup with nystatin, was decreased by 48% in the group which was fed syrup with extract of medicinal plants and was increased by 36% in the control group in 20 days. The incidence of ascosphaerosis was not detected

in the both groups and was higher by 22% than in beginning in the control group in 30 days (Table 2).

The assessment productivity of the honey was performed by weighing the amount of produced honey in each group in comparison with control. The assessment productivity of the egg was performed by counting the number of capped brood in each group in comparison with control (Table 3).

Table 3. The productivity of the honey and eggs of honey bee colonies which were fed by syrup with ethanol extract of medicinal plant and nystatin in comparison with control

Feeding by syrup with	Number of capped brood, x1000 cells		Amount of produced honey, kg	
	05 may	25 august	05 may	25 august
dissolved nystatin	138.4±9.6	103.2±5	4.57±0.6	26.3±0.9
extract of medicinal plants	143.6±4.7	115.2±6	4.38±0.7	28.1±0.5
ethanol (control)	139.8±3.6	91.2±4.4	4.62±0.4	12.6±0.4

The average productivity of the honey and eggs had not differed significantly in spring. The average productivity of honey was 26 kg in group which was fed syrup with nystatin, was 28 kg in group which was fed syrup with extract of medicinal plants and was 12 kg in control group in autumn.

The average productivity of eggs which assessed by counting of capped brood was 103 000 in group which was fed syrup with nystatin, was 115 000 in group which was fed syrup with extract of medicinal plants and was 91 000 in control group in autumn. Thus, highest average productivity of eggs was in group which was fed syrup with extract of medicinal plants (Table 3).

DISCUSSION

This research showed the opportunity of using the feeding syrup with ethanol extract from 15 medicinal plants of honey bees for treatment the ascosphaerosis and stimulation their immunity.

The colonies of the first group which were fed syrup with extract of medicinal plants were cured faster than colonies of the control group. Thus the ethanol extract of plants has immunomodulatory effect for honey bees and antifungal activity against pathogenic fungus *Ascosphaera apis*. The increase of activity of catalase, peroxidase and invertase ferments of honey bees

prepares them to successful wintering and improves them digestive system to long winter period. The feeding sugar syrup with an ethanol extract of medicinal plants be able to lead honey bee colonies to a stability of wintering, an increasing of productivity of the honey and eggs.

The antifungal effect of the ethanol extract of medicinal plants against *Ascosphaera apis* is similar with antifungal effect of the nystatin. The immunomodulatory effect of the ethanol extract of medicinal plants for honey bee colonies is similar with immunomodulatory effect of the extract of *Rhodiola rosea*.

The impact of ethanol extract of medicinal plants on honey bee colonies interests the beekeepers since the treatment with extract provides quick recovery the honey bee colonies from ascosphaerosis and increases all biological characteristics and productivity of the honey.

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