ISSN: 1220-3173; E-ISSN: 2457-7618

THE EFFICIENCY OF A PHYTOTHERAPEUTIC ASSOCIATION TO COMBAT NOSEMOSIS IN HONEY BEES IN ROMANIA

EFICIENȚA UNEI ASOCIERI FITOTERAPEUTICE PENTRU COMBATEREA NOSEMOZEI ALBINELOR ÎN ROMÂNIA

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ABSTRACT | REZUMAT

Honey bees (*Apis mellifera*) are essential to maintaining ecosystems, contributing to the stability of biodiversity through pollination. Today it is known that the failure of pollination leads irremediably to the loss of plant cultures and, as a consequence, will induce food security concerns. Honey bees can be affected by various factors, one of these being *Nosema spp.,* a specific microsporidian to adult bees and, a threat to bee populations around the world. The present study evaluated the therapeutic efficacy of a new conceived phytotherapeutic product in the bee nosemosis (Protofil®) from a pharmaco-clinical point of view. In this aim, the results of the treatment revealed a good efficacy, recommending the treatments with Protofil, which brings with it the benefits of organic honey, without residues, and without undesirable effects.

Keywords: *Apis mellifera, Nosema spp.,* Phytotherapy, Protofil

Albinele (*Apis mellifera*) sunt esențiale pentru menținerea ecosistemelor, contribuind la stabilitatea biodiversității prin polenizare. Astăzi se știe că eșecul polenizării duce la pierderea iremediabilă a culturilor de plante, și în consecință, va induce probleme de securitate alimentară. Albinele pot fi afectate de diverși factori, unul dintre aceștia fiind *Nosema spp.,* microsporidie specifică albinelor adulte și o amenințare pentru populațiile de albine din întreaga lume. In prezentul studiu, a fost analizată eficiența terapeutică a unui produs fitoterapeutic nou în nosemoza albinelor (Protofil®), din punct de vedere farmaco-clinic. În acest scop, s-a observat că rezultatele tratamentului au relevat o eficacitate bună, ceea ce recomandă tratamentul cu Protofil, care aduce cu el beneficiile mierii ecologice, fără reziduuri și fără alte efecte nedorite.

Cuvinte cheie: *Apis mellifera, Nosema spp.,* Fitoterapie, Protofil

Honey bees are essential for maintaining the ecosystems, contributing to the stability of biodiversity through pollination, which is a vital factor for a wide range of wild crops and plants. Today it is known that the failure of pollination will lead irremediably to the loss of plant cultures and, as a consequence, will induce food security concerns. Statistics have been published to support the value of the pollinators, including the fact that 75% of the crops are pollinated by insects and that 57 species (mainly bees and only two vertebrates) are the principal pollinators for 107 plant cultures around the world (1, 7, 11).

Honey bees (*Apis mellifera*) can be affected by a multitude of diseases, most often conditioned by favorable factors. The main incriminated factors are weak families, prolonged wintering, lack of cleaning flights, food additives, excessive humidity in the hive, etc. Under these circumstances, nosemosis, which is a specific microsporidian to adult bees and a major threat, can be most commonly found in bee populations around the world, in Europe and Romania as well (3, 4, 9, 13, 17, 18, 19).

In Europe, the drug weapon against nosemosis was circumscribed to a few active substances and, finally, to a single product, the Fumagillin (Fumidil), an antibiotic obtained from cultures of *Aspergillus fumigatus.* Unfortunately, although an efficient product, due to the risk of residues, EMA has excluded this product from use in Europe in February 2016 (8, 10, 14, 15, 16).

Herbal medicine can be a possible alternative to combat nosemosis, therefore, we can use plants with anti-microsporidian activity, such as *Hypericum perforatum* flowers, *Achillea millefolium* flowers, *Mentha piperita* leaves, *Matricaria chamomilla* flowers, *Ocimum basilicum* leaves, and flowers, etc. (12, 20)

In the present study, were analyzed from a phar-

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maco-clinical point of view the efficacy of *Protofil®,* an approved association in Romania, composed of basil, thyme, yarrow and, respectively, dandelion, in an episode of nosemosis in South of Romania.

MATERIALS AND METHODS

The place of researches

The study was carried out in Dolj County, Romania, within a private apiary between March 2018 and October 2018. In the apiary, nosemosis was initially suspected and then identified in the Laboratory of Parasitology from the Directorate of Veterinary Sanitary and Food Safety (DVSFS), Dolj County, Romania.

Phytotherapeutic product - efficacy study

Bee-keeping unit

The apiary from this study was composed of 100 hives, which are horizontally patterned in the warm bed and built of larch wood with 18 frames. In this beekeeping unit, the seasonal harvesting visit is carried out according to the rotation of the vegetal crops during the vegetation period (e.g. rape, acacia, lime, sunflower, field and forest flowers, etc.), from March to the beginning of October.

In the spring of 2018, in March, nosemosis was suspected and confirmed in the Laboratory of Parasitology from the Directorate of Veterinary Sanitary and Food Safety (DVSFS) Dolj, examinations revealing the presence of microsporidiosis.

The main changes and clinical signs observed in the apiary were:

· the exits from the winter were hard, and the stability of families disrupted;

· low incidence of juvenile breeding;

· presence of numerous dead bees;

· high agitation of the bees;

· spots of brown diarrhea on the combs, hive, and near the apiary as a result of injury to the intestinal tissue, by primary disease and overlapping the secondary infections, which are specific to this disease;

· infected bees often defecated inside the hive;

·infected bees tend to skip over the breeding stage, thus their life span is considerably reduced.

Diagnosis of nosemosis has been established based on clinical signs on samples of 50 live / moribund / dead bees collected, being differentially excluded other entities like amoebiasis, acute intoxications and, viral paralysis of bees. The anatomopathological examination of the middle gut, revealed at infected bees a medium-sized intestine, with a matte-whitish color, and without transversal striations.

Optical microscopy, with the objective of the ×40, revealed the *Nosema spp*. spores that ranged from 4 to 6 µm (being a phytotherapeutic efficiency study the differential diagnosis through RT-PCR method was not necessary).

Treatment

The treatment for this study was conducted with an original veterinary product (*Protofil*®), which is a phytotherapeutic association produced by the Institute for Research and Development for Beekeeping S.A., from Bucharest. By its composition in vitamins and microelements, this association stimulates the digestive secretion of bees, favoring food digestibility, so inhibits the realization of the life cycle of *Nosema spp.* It has also been observed that this product stimulates the egg production of the queen bees, so implicitly increases the population of young working bees.

The product (Protofil) is a brownish liquid with an aromatic odor and flavor, designed to combat Nosema spp., and a significant advantage is that it has no contraindications (without intoxication or side effects) to honey bees.

The field study

In this study, a number of 15 honey bee families were chosen from the apiary, the criterion being the relative uniformity of families in terms of honey density and production.

The bee families were divided into three lots of five bee families (*Apis mellifera*) thus:

a) Treatment 1 (T1),

b) Treatment 2 (T2),

c) Control group (C).

The two treatment groups, T1 and T2, were treated according to the scheme shown in Table 1, and the Control group received only 1:1 sugar syrup.

From each family, bees were sampled, before and after treatments, and were kept in alcohol at 60° until microscopy.

The Table 1 shows the scheme for estimating the number of bees, productions, and treatments carried out between 1 March and 1 November 2018.

In this period pastoral honey harvest was carried out between March and April in rape cultures; May - acacia; June - lime; July - August - sunflower; September - October - field and forest flowers; the honey being gathered after each culture.

The treatments with Protofil was made at the bees when exiting for flight in March, June, and September. At the T1 group was administrated 20 ml / 1 liter of syrup and 500 ml/hive, respectively, for the T2 group was administrated 30 ml / 1 liter of syrup and 500 ml/hive.

The evaluation of the number of bees from the family was done by the weighing method, knowing that: 10 honey bees ≈ 1 g, by weighing the beehive and lowering the weight of the frame and the hive box. In the studied unit, the average weight of a bee family, calculated according to this method, was 70 kg (including box and frames) and approximately 7,900-8,000 g / bee family. Extrapolation to the number of bees/g shows that in a family live around 80,000 individuals.

Table 1

The experimental scheme of the study

Months of study							
March	***April***	***May***	***June***	***July***	***August***	***September***	***October***
Weighing Hives **no. 1**			Weighing Hives **no. 2**			Weighing Hives **no. 3**	
Rape		***Acacia***	***Lime tree***	***Sunflower***		***Polyfloral***	
Control Syrup 1:1	Harvest honey **no.1**	Harvest honey **no.2**	Harvest honey **no.3**		Harvest honey **no.4**		Harvest honey **no.5**
Admin. no.1			***Admin. no. 2***			***Admin. no.3***	
T1 / 20 ml / 1 l syrup / 500 / stup			**T1** / 20 ml / 1 l syrup / 500 / stup			**T1** / 20 ml / 1 l syrup / 500 / stup	
T2 / 30 ml / 1 l syrup / 500 / hive			**T2** / 30 ml / 1 l syrup / 500 / hive			**T2** / 30 ml / 1 l syrup / 500 / hive	

Table 2

Analysis of infestation degree with *Nosema spp.*

Symbol	Interpretation
+++	*Numerous spores that occupy the entire microscopic field and in each microscopic field = massive infection;*
++	*One or more spores in each microscopic field = moderate infection*
+	*The presence of one or more spores, but not in each microscopic field = poor infection*
0	*absence of spores = no infection*

Hive nr.	1			2			3			4			5		
Month	March	June	Sept.	March	June	Sept.	March	June	Sept.	March	June	Sept.	March	June	Sept.
T1 (g.)	19.5	10.8	5.6	19.6	7.9	6.7	21.6	14.8	10.5	18.7	10.3	5.9	17.8	10.1	7.8
Category	+++	++	+	++	+	0	+++	++	+	+++	+	+	+++	+	0
T2 (g.)	20.6	9.8	1.5	17.6	11.0	2.4	19.6	9.0	1.7	18.0	9.1	3.0	28.1	5.6	2.1
Category	+++	+	+	+++	+	0	++	0	0	++	+	0	++	0	0
C (g.)	21.4	20.0	24.8	20.8	20.7	25.8	19.6	17.9	18.9	19.6	21.8	25.2	23.1	19.4	19.8
Category	++	++	++	+++	++	++	++	+++	+	++	+++	+++	++	++	++

RESULTS AND DISCUSSIONS

Phytotherapeutic efficacy field assay

The bee guts were individually examined (macroscopic and microscopic) for determining the level of infection with *Nosema spp*., before and after treatment, by the laboratory of parasitology from DSVSA - Dolj. The microscopic examination of the preparations (objective of × 40) is a qualitative method, and we use for that just dead or in agony bees.

The bee infestation levels were established according to the scheme shown in Table 2.

Table 3 shows the relationship between weights/ hive/ bee families/ mortality/ weighing data for the studied groups, and Table 4 shows the correlation between honey harvesting data and the groups under the study. From the results' analysis it is merged that the proper and timely use of phytotherapy with Protofil will reduce losses to the apiary. Observing the development of families after treatments, the production of honey in correlation with the efficiency of the studied product, we ascertained the efficacy of a dose of 30 ml /1 liter of syrup at an administered dose of 500 ml/ hive. Table 5 shows the values of the principal production indicators: family weight (which gives data on the viability and health of bees) and honey/family production. The correlation between weights / beehive / bee families / weighing data for the studied lots, as well as the efficacy outcome of the treatments, revealed a very good efficacy for the T2 group and good for T1. The mortality values compared on studied groups, as well as the honey production / categories / total quantity, are confirming the judiciousness of introducing treatments with *Protofil* to the honey bees, which brings with it the benefits of a finished product, without residues, thus a net increase in quality.

Following undesirable, toxic, or secondary effects on the phytotherapeutic product, we did not see any of these effects, at 30 ml / 1 liter of syrup, at a dose of 500 ml / hive.

Table 3

Weights / hive / family of bees in the study

Group	1						2						3						4						5					
T1 (g.)	March		June		Sept.		March		June		Sept.		March		June		Sept.		March		June		Sept		March		June		Sept.	
	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F
	70.6	5.6	75.2	7.2	75.8	7.4	69.7	5.7	71.8	6.8	73.1	7.5	71.1	6.1	72.8	6.9	73.7	7.4	68.9	5.9	71.5	6.6	75.1	7.4	69.6	6.0	71.8	6.7	75.0	7.8
	M%		M%		M%		M%		M%		M%		M%		M%		M%		M %		M %		M %		M %		M %		M %	
	19.5		10.8		5.6		19.6		7.9		6.7		21.6		14.8		10.5		18.7		10.3		5.9		17.8		10.1		7.8	
T2 (g.)	71.3	6.0	73.6	7.8	74.1	7.8	74.5	7.9	77.1	8.1	79.7	8.2	68.9	6.7	73.2	7.1	77.9	7.8	70.1	6.2	72.5	6.9	76.1	7.6	71.0	7.2	74.5	7.9	80.1	8.0
	M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%	
	20.6		9.8		1.5		17.6		11.0		2.4		19.6		9,0		1.7		18.0		9.1		3.0		28.1		5.6		2.1	
C (g.)	70.9	5.9	72.8	6.5	73.1	6.6	68.3	6.0	69.9	6.2	61.5	6.1	65.1	6.0	66,1	6,4	68.3	6.9	68.9	6.0	69.4	6.1	69.9	6.0	70.4	6.8	71.3	6.9	70.0	6.8
	M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%		M%	
	21.4		20.0		24.8		20.8		20.7		25.8		19.6		17.9		18.9		19.6		21.8		25.2		23.1		19.4		19.8	

*Legend: **S** = hive (measured in kg.); **F** = bee family (kg.); **M** = mortality (expressed in g); T1 = 20 ml / L treatment; T2 = 30 ml / L treatment; C = control (placebo)*

Table 4

The correlation between the honey production data and the groups under study

Group	Cat.			1			2			3			4			5		
T1	Treat.	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3
	Month	April	June	Oct.	April	June	Oct.	April	June	Oct.	April	June	Oct.	April	June	Oct.	April	June	Oct.
	Kg. honey	18.5	2.7	4.5	17.4	2.6	4.0	19.4	3.0	4.4	20.7	3.0	4.4	18.8	2.9	4.7			
	Month	May	August	-	May	August	-	May	August	-	May	August	-	May	August	-			
	Kg. honey	7.2	27.0	-	7.0	26.9	-	7.1	26.7	-	7.6	-	-	7.7	28.4	-			
T2	Treat.	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3
	Month	April	June	Oct.	April	June	Oct.	Apr.	Jun	Oct	Apr.	Jun	Oct	Apr.	June	Oct.			
	Kg. honey	20.3	3,4	5,8	21.1	4.3	4,7	22.3	3.8	5.9	22.8	4.0	5.7	20.9	4.8	5.6			
	Month	May	August	-	May	August	-	May	August	-	May	August	-	May	August	-			
	Kg. honey	8.9	31.0	-	9.1	30.5	-	9.3	30.8	-	9.5	31.8	-	9.7	30.6	-			
C	Treat.	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3	Adm.1	Adm.2	Adm.3
	Month	April	June	Oct.	April	June	Oct.	April	June	Oct.	Apr.	Jun	Oct.	April	June	Oct.			
	Kg. honey	14.0	2.0	3.1	15.1	2.3	2.9	13.8	2.1	3.0	14.3	2.2	2.8	13.9	2.4	2.6			
	Month	May	August	-	May	August	-	May	Aug	-	May	August	-	May	August	-			
	Kg. honey	5.3	21.3	-	5.1	20.5	-	5.9	20.6	-	5.1	21.5	-	5.8	19.8	-			

The therapy in nosemosis is quite limited today, and when the colonies are severely affected, the principal recommendation, from an economic point of view, is to be destroyed, although losses are considerable. The control of infected beehives is made mainly by applying defensive measures since the treatment with antibiotics for infected colonies of bees is now forbidden in the European countries.

However, in literature, are attempts to combat nosemosis, like a new phytotherapeutic product. In this aim, our collective try to be part of the cause.

Thymol was among the first natural structures studied in the beehive infection (2, 5, 6).

For example, it was evaluated the potential of various phytocompounds used for the control of nosemosis in honey bees, like, for example thymol, vetiver essential oil, resveratrol, and lysozyme. Thymol and resveratrol are considered to be useful for the control in nosemosis, the results of this study showing that, when bees are fed, especially with thymol and resveratrol, the infection rate became considerably decreased, and bees survived longer (12).

In another study, *Nosema ceranae* infection was stopped with oxalic acid syrup, in a laboratory and also in a field study, being considered as an good alternative management strategy in nosemosis (16).

Table 5

Analysis of the parameters of viability and production of honey / hives

Group	Total production of honey /period /mean	Total family weight /group /mean	Total weight per hive /group /mean
T1 kg.)	274.6 (54.92)	372.7 (74.54)	37.5 (7.50)
T2 kg.)	356.6 (71.32)	387.9 (77.58)	39.4 (7.88)
C (kg.)	227.4 (45.48)	342.80 (68.56)	32.40 (6.48)

A comparative research developed for three years, studied the activity of fumagillin and thymol in *Nosema apis* infection in a large number of honey bee colonies. The results are confirming our investigation in the phytotherapy efficiency, bee health, honey yield and, quality, underlining the importance of alternative treatment in bees (20).

CONCLUSION

The results of the efficacy study in nosemosis revealed a very good efficacy for the T2 group, and good for T1, this study recommending *Protofil* as a therapeutic choice in attempts to combat nosemosis.

The mortality values compared on studied groups, as well as the honey production / categories / total quantity, confirming the judiciousness of recommending treatments with *Protofil* at honey bees in Romania, which brings with it the benefits of a product without residue, consequently a net increase in quality, and without side effects.

Acknowledgments:

This work was conducted in the frame of the research project *"Ensuring excellence in RDI activity within USAMVBT"* code 35PFE, submitted in competition Program 1 - Development of the national research and development system, Subprogram 1.2. Institutional performance - Institutional development projects - Projects for financing excellence in CDI.

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