

**FROM [WWW.APIMONDIA.ORG](http://WWW.APIMONDIA.ORG)****Control of nosemosis - the treatment with “Protofil”**

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**ABSTRACT:**

The incidence of nosemosis remains at high level in Romania.

Taking into consideration intrinsic and extrinsic factors which determine the infection level of the adult bees with protozoan *Nosema apis* spores, treatment method with **Protofil** has been tested with regard to his efficacy in control of nosemosis, without pollution for hive products.

**Protofil** is a natural product obtains by hydro alcoholic extraction. Through the substances obtain from different plants, by his vitamins and microelements that it contains, the product prevents the development cycle of *Nosema apis*, inhibits the intestinal pathogen flora and stimulate the digest enzymatic secretion of bees and larvae.

This test had been carried out on 270 colonies supervised for 5 years, between 1998 – 2002.

The positive diagnosis, the intensity determination and the estimation of the parasitism's spreading had been achieved on the basis of the exams carried out in the laboratory.

**Key words:** *Apis mellifera* / nosemosis / natural treatment / Protofil

**INTRODUCTION:**

The *Nosema apis* (Zander) microsporidia is a parasite of the adult bees, which invades the epithelial cells of the middle part of the intestine, especially in the rear section, leading to their destruction (Manual of Standards for Diagnostic Test and Vaccines, 3rd edition, 1996). The disease exists all over the world and the examination of the bees contributes to the prevention of the infection spreading around the healthy colonies. The specie suspicious to getting sick is *Apis mellifera*, but other species in the *Apis* genus also. According to the OIE statistics, the parasite is spread in 43% of the countries having bee colonies (Office International des des Epizooties, 2002).

The disease has an enzootic character and it usually evolves clinically at the end of winter – beginning of spring, being favored by a complex of intrinsic and extrinsic factors that intercondition with each other. Among the intrinsic factors there are: the existence of weak strength colonies in the apiary and the colony age too, because only the adults get infected; among the individuals in a colony the queens and worker bees especially and the incidence is smaller among the drones. Among the extrinsic factors we have to mention: the climate, for example the sudden variations of temperature, the long and cold winters, the inappropriate hygiene of the beehive, some associated diseases such as amoebiasis and the storage in the hive on winter period of the black honey, because of its alkaloids contain (Bailey L., 1981).

In Romania the Nosemosis evolves both under a chronic and unapparent form and acute with visible signs causing the depopulation of the colony and therefore the death of the bees.

The seriousness of the disease is due to the parasite virulence, which acts mechanically, inflammatory and irritatively over the epithelium, middle section of the intestine and toxic actions (Photo 1). The toxins act on the nervous way and cause failure in fly, motion, being followed by paralysis and death.

The **primary infection source** is represented by the sick adult bees, the queens and drones that eliminate the parasite spores through the contaminated diarrhea excrements, honey and pollen.



The **secondary infection sources** are represented by:

- the contaminated diarrhea excrements, splashed on the combs, combs, in the honey, pollen, on the beehive walls, etc.
- the excrements of various parasite living in the beehive, such as *Galleria melonella*, *Achroe grisella*, *Braula coeca*;
- the biologic material and the inventory objects coming from the contaminated beehives;
- the water sources near the contaminate beehives.

The **infection** is made by be ingestion of spores together with food or during performing works in the beehive (Popa A., 1965, Root Al., 1990).

After the spore ingestion, they reach the intestinal epithelium, where the sporal micropile penetrates the peritrophic membrane of the intestine. The first spores loose the micropile and get into the cytoplasm of the epithelial cells where they replicate. The self-infection can be at the same time a new infection. In a short while, the spores multiply and reach a high number. The infected bees cannot fly and can contain up to 500 million spores (Manual of Standards for Diagnosis Test and Vaccines, 4<sup>th</sup> edition, 2000).

The spores have a variable viability. In the feces they can last up to 2 years and in honey or inside the dead bees up to one year. In most of the cases the combs are infected too. The spores can be destroyed by exposure to temperatures of at least 60°C for 15 minutes. The acetic acid vapors of at least 60% inactivate the spores of any kind, the exposure time depending on the concentration of the solution and vary from several hours for low concentrations to several minutes for high concentrations. Such procedures come under the jurisdiction of national veterinary control authorities with protocols that vary from country to country. (Forsgren E., Fries I., 2003).

The data that were presented demonstrated the resistance of the sporal stage of *Nosema apis*, so in this way, the parasitoid action of some substances with therapeutic proprieties on this level it is impossible (Haque M.A., Canning E.U. 1995, Pohl F. 1993).

In Romania and in the other European countries, nosemosis has an increased incidence due to the number of cases and its spreading. The incidence of this endo-parasitosis represents the main cause of the depopulation registered within the bee colonies and it is shows by overall statistic data, which refer to the disease dynamics in Romania during the monitoring of the beehives treated with Protofil (table1).

**Table 1:** Incidence of nosemosis in Romania between 1998 – 2002 (Source: the IDAH Department of Epidemiology)

YEAR	Total no. of bee colonies	Total no. of colonies with nosemosis	OBSERVATIONS* % colonies affected by B-454
1998	370 471	11456	3,10%
1999	450 075	691	0,15%
2000	524 342	1153	0,22%
2001	576 456	790	0,14%
2002	586 429	7340	1,25%

\*The percentage includes only the bee colonies destroyed in full by the nosemosis.

From the epidemiologic records we can observe the lingering evolution of clinical nosemosis over many years in the same beehives until their full destruction.

In most of the cases the disease evolves chronically, with a non-specific symptomatology with acutizations in certain periods. Over the last years, due to the unilateral use of fumagiline, of apiarists' failure to apply the mandatory hygiene actions and the way of understanding and treating the disease, the level of infection increased and together with it the number of declared acute nosemosis centers of infection in various areas throughout the country.

## MATERIALS AND METHODS

The effectiveness of the treatments for controlling the nosemosis after applying the **Protofil** product has been assessed. Two beehives belonging to the Institute of Research – Development for Apiculture Bucharest have been monitored and the beehives I and II were included in the study, for a period of 5 years (1998-2002).

The beehives are under sanitary – veterinary supervision and the general health condition of the colonies is verified twice a year through clinical tests in the beehives and through lab analyses, first of all for the diseases in the O.I.E. B list. In all beehives, besides the nosemosis evolution there was also a low infection with the *Varroa destructor*, maintained at a low level through treatments with Mavrirol and Varachet.

The clinical testing has been performed on a total number of 270 bee colonies. For establishing the health condition of the colonies in the monitored beehives samples have been taken and they were analyzed in the laboratory following the method in the OIE Manual of Standards – 3<sup>rd</sup> edition. 10% of each beehive has been considered to be control colonies.

**Table 2:** Records of monitored colonies

No. of monitored beehive	Total no. of colonies	Colonies to which Protofil has been administered	Control colonies	Observations
I	120	108	12	-
II	150	135	15	-
TOTAL	270	243	27	-

Both of the beehives are permanently located in the southern part of the country, in Câmpia Română (the Romanian Plain) and benefits from the main harvests: spontaneous flora, acacia and lime tree, therefore the microclimate conditions are similar. When the selection of beehives was performed, the similarity of the bee colonies development level, the food supply and the level of infection with *Nosema apis* have been taken into account. The colonies are set on 5-7-9 frames with bee in vertical beehives (Dadant type model), with mobile plate and multi-level possibilities (**Photo 2**).



**Photo 2: Apiary II – General view (2002)**

The establishment of the **Protofil** product effect and therefore its efficiency has been calculated depending on the decrease of the level of infection with *Nosema apis* in the monitored colonies.

The efficiency of **Protofil** in controlling the nosemosis has been achieved only by maintaining the bee colonies under optimal conditions of rearing, feeding and hygiene.

**Protofil** is a medicine obtained exclusively from plant extracts and is destined to control nosemosis, to stimulate the development of the bee families and in cases of intoxication (Bogdan I, Muresan E, Malaiu A, Nuclean V., Sabau A, 1986).

## WAYS OF ACTION

**Protofil**, through the extractive substances obtained from plants, the vitamins and microelements it contains, stimulates the digestive enzymatic secretion of bees and larvae, leading to a high level of digestibility of food, it inhibits the intestinal pathogen flora and hinders – in a large part – the achievement of the evolution cycle of *Nosema apis* ((Bogdan I, Muresan E, Malaiu A, Nuclean V., Sabau A, 1986) The queens intensify egg laying, increases the population of beehives and their production.

The product composition includes essential oils containing the cyclic and aliphatic hydrocarbons, sesquiterpene, triterpene, phenolic compounds, oleanolic acid, flavonic compounds, microelements and vitamins, especially the B group and ethyl alcohol.

The product is a total alcoholic extract with alcohol of 96°; the plant species are part of the Romanian spontaneous flora and are represented by: *Taraxacum officinalis* (dandelion),

*Thymus vulgaris* (savory), *Achillea millefolium* (milfoil) and *Ocimum basilicum* (basle). Supplementary, **Protofil** contains the B complex vitamin solution. Because of these characteristics, **Protofil** can penetrate all sporal membranes and stop in this way the subsequent development of the parasite.

The organoleptic product is a yellow-brownish-greenish liquid, with an aromatic specific smell and a characteristic bitterish taste.

The product is delivered in plastic bottles of 500 g and 1 kg (Photo 3).

The product is flammable and has a 2 year-validity under preservation conditions, in dark and dry places at a temperature of maximum 25°C.

**Ways of administration:** In all monitored colonies **Protofil** was administered as syrup 20 ml./l and as candy 40ml/kg, repeated for 2-4 times, 250-500 ml(g) syrup (candy) with drugs/colony by season.



In autumn it was administered in the syrup for stimulation and to complete the beehive food and in spring, in sugar pasta and in the syrup for feeding and stimulation.

The total quantity of **Protofil** that was administered to one bee colony has been of 50-80 ml/season, in spring and autumn, depending on its amount and condition.

The initial level of infection was determined by performing the clinical exam in the apiary and estimated after carrying out the laboratory tests on representative samples.

In the laboratory the examination of the samples pathologic material has been made in conformity with the specific working procedure including the homologated I.D.A.H. method (Mardare A., Chioveanu G, 1992) and the OIE method (Manual of Standards for Diagnostic test and Vaccines, 3<sup>rd</sup> edition, 1996).

The samples were taken randomized from the combs from inside of the hive. Homogenates from the bee guts were carried out, considering 1 ml. distilled water/ bee.

The examination was carried out at the standard light microscope with magnified x 400 (**Photo 4**).

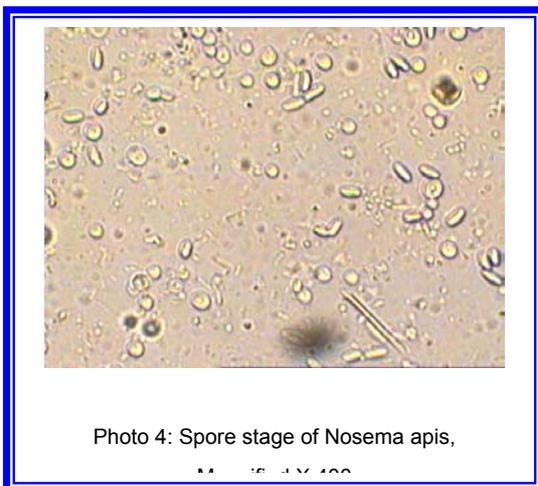


Photo 4: Spore stage of *Nosema apis*,

Intensivity of the parasitism has not been calculated by the OIE 2000 method because the monitoring of the colonies was begun before the 4-th edition of the OIE Manual of Standards appeared and the equivalence between the two quantitative diagnosis methods couldn't be established.

In none of the apiary monitored before the mention period no treatment with prophylactic and therapeutic effect for nosemosis was administered.

**In apiary I** the initial level of infection was of 35-100 spores/unit., with a media of 65 spores /unit. In this apiary have been administered 80 ml

Protofil/ season, both in spring and in autumn.

At the initial clinical exam, the 1998 spring all the 120 colonies presented: development on 5-7 frames, depopulation underweight bees and with digested symptoms, great abdomen flight incapacity, paralyzing signs of the legs, depilation and diarrhoea with light faeces, brown colour and fetid smell, irregular disseminated on the hive components.

Queens laid eggs but it wasn't uniformity on the combs and had low vitality; death bees had black chitin thoracic and abdominal depilation, the legs tightened under the thorax and the wings extended in flight position.

**In apiary II** the initial level of infection was of 8-25 spores/ spores unit, with a media of 18 spores /unit.

At the clinical exam the 150 colonies presented: development on 7-9 frames, with normal development, without colour modifications of the chitin, faeces with normal aspect but the queen laid eggs on the frames, with irregular dispose and the percentage of mortality was high than the normal and physiological one. In this apiary were administered 50 ml. Protofil, each in spring and autumn too.

## RESULTS:

In tables 3 and 4 are presented synthetically express by percent: the level of infection/apiary/season, initial and after administered Protofil and the efficiency of the treatment after each administered.

**Table 3:** : Mean levels of *Nosema apis* infection in apiaries I and the efficacy of Protofil treatments

Year	PERIOD	Mean levels of <i>Nosema apis</i> infection in colonies treated with Protofil SPORES / UNIT.	EFICACY % ± 0,50	Mean levels of <i>Nosema apis</i> infection in control colonies SPORES / UNIT.	OBSERVATIONS
1998	Spring	28	57	68	-
	Autumn	20	69	75	-
1999	Spring	36	45	84	-
	Autumn	23	65	86	-
2000	Spring	28	57	98	30% queens replace
	Autumn	21	68	69	-
2001	Spring	26	60	89	-
	Autumn	19	71	78	-
2002	Spring	24	63	94	-
	Autumn	17	74	93	-

Initial level of *Nosema apis* spores infection: **68 spores/unit**

Apiary I, total colonies: **120**

Colonies treated with Protofil: **108**Control colonies: **12****Table 4:** Mean levels of *Nosema apis* infection in apiaries II and the efficacy of Protofil treatments

Year.	PERIOD	Mean levels of <i>Nosema apis</i> infection in colonies treated with Protofil SPORES / UNIT.	EFICACY % ± 0,50	Mean levels of <i>Nosema apis</i> infection in control colonies SPORES / UNIT.	OBSER- VATIONS
1998	Spring	3	83	18	-
	Autumn	1	94	20	-
1999	Spring	8	56	41	-
	Autumn	5	72	32	-
2000	Spring	10	44	43	30% queens replace
	Autumn	3	83	28	-
2001	Spring	8	56	32	-
	Autumn	1	94	21	-
2002	Spring	5	72	58	-
	Autumn	2	89	53	-

Initial level of *Nosema apis* spores infection: **18 spores/unit**Apiary I, total colonies: **150**Colonies treated with Protofil: **135**Control colonies: **15**

## CONCLUSIONS:

1. The Protofil product was well tolerated by bees; being a natural product there is no possibility of appearing residues in honey or in the other product of the hive.
2. Due to the characteristics of the medicinal plants used for obtained Protofil this product interrupts the development of the *Nosema apis* endoparasite and stops the evolution of the disease. Also it has a stimulant effect on the development of the bee-colonies.
3. A long with the monitoring of the apiary have be noticed the clear attenuation of the clinical signs of the disease especially during the first year of administration (apiary I) and the complete disappearing of these, in apiary II.
4. The continues decrease of the infection with *Nosema apis* spores, as after 10 series of administration in the dosage indicated in the paper, the therapeutic effect of the medicine assessed on the basis of the laboratory exam was: apiary I – 74%; apiary II – 89%.

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