The Effect of Plant Supplements on the Development of Artificially Weaken Bee Families

Liviu Al. Mărghitaş, Otilia Bobiş, Melinda Tofalvi

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Animal Husbandry and Biotechnology, 400372 Cluj-Napoca, Mănăştur str. 3-5, Romania

Abstract
In this study, infusions from nettle, thyme and *Echinacea*, fresh juice of onion and garlic, and Protofil (alcoholic extract of different plants enriched with vitamins and mineral elements), were used in supplementary feeding of artificially weaken bee families. Correlation between total phenolic content, total flavonoid content and antioxidant activity of the supplements used in honeybee feeding and uncapped, capped and total brood surface of experimental groups were established. The highest content of biologically active compounds exhibit nettle infusion, which present the most effective growth in field experiments.

Keywords: bee families, biological evolution, plant supplements, feeding.

1. Introduction

The honey bee has a definite place in our modern world. Its products of honey and wax are useful to man, although perhaps not essential to all men. However, the pollination activities of bees affect the lives of most of the people of the world.
The natural food of the honey bee consists of pollen, nectar or honey, and water. In early spring, before pollen and nectar are available or at other times of the year when these materials are not available for bees in the field or in the hive, supplementary feeding may help the colony survive, or make it more populous so it will produce more honey or be better able to pollinate crops.
The essential requirements for successful wintering of honeybee colonies are an ample supply of sufficient food, a good cluster of young, vigorous honeybees with a young queen coming from a strain selected for wintering abilities, free disease and parasites [1-2].
Supplemental foods are fed to honey bees to supply the nutritive requirements of colonies in areas and at times when natural food sources (pollen, nectar, or honey) are inadequate or not available. The brood rearing activity and nutritional state of the colony, the quantity and quality of incoming pollen and nectar, and the food reserves in the hive will determine whether the bees need supplemental foods.
Colonies are usually fed supplemental foods for one or more of the following reasons: to ensure continued colony development in places and times of shortage of natural pollen and nectar; to develop colonies with optimum populations in time for nectar flows; to develop colonies with optimum populations for pollination of crops; to build up colony populations for autumn and spring division; to sustain brood rearing and colony development during inclement weather; to build colonies to high populations for queen and package-bee production; to maintain colonies and extend the season for high drone populations for queen makings; to maintain colonies in feedlot

* Corresponding author: Otilia Bobiş, tel. 0264-596384/224; fax 0264-593792; e-mail: obobis@usamvcluj.ro

situations; to build up colonies after pesticide losses [3].
The literature data reveals the effect of additivefeeding on honey bee colonies, wintering abilities and survival rates [4].
Diferentiate maintenance of bee families, according to the conditions that our country offers for bioapiaries, has a great importance in the beekeeping economy, as insures, for every type of collecting, a specific methodology for bee breeding, which differ greatly, as time and technique, for apiaries situated in plain zone, to those situated in hilly or mountain zone [5-6].
There are many studies regarding supplementary feeding of honey bee colonies with different formulas consisting of glucidic, proteic and vitaminic mixtures, but there are fewer studies made on the use of different plant infusions and extracts used in supplementary feeding of bee colonies, especially in different stages of their biological development.
In our study we tested the effect of Protofil, onion and garlic fresh juice, nettle, thyme and *Echinaceea* infusion and organic selenium, on honeybee colonies development (artificially weaken families and artificial swarms).
Protofil is a natural product obtained by hydro alcoholic extraction. Through the substances obtained from different plants (*Taraxacum officinalis* – dandelion; *Thymus vulgaris* – savory; *Achillea millefolium* – chamomile; *Ocimum basilicum* – basil and others), by the vitamins and microelements from its composition, this product prevents the development of *Nosema apis*, inhibits the intestinal pathogen flora and stimulate the digest enzymatic secretion of bees and larvae [7].

2. Materials and methods

The biological material was represented by 40 artificially weaken bee families divided in 8 experimental batches and 40 artificial swarms.
The artificially weaken bee families were made by eliminating the frames covered with bees from the nest, until they reach ¼ from the initial population (the queen and 4 frames with sapling, honey and afferent working bees).
For this biological stage, in the experiment, 2 control groups were made and 6 experimental groups which were fed differently with plant supplements added in the conventional feed.
Sometimes, in honeybee supplementary feeding are introduced medicinal plants with the purpose of preventing or treating different diseases, or to stimulate the development of the colonies. Regarding the effect that these plants have, it can be distinguished:
- Plants with trophy action and general stimulation: nettle, dandelion, wild rose, box thorn, blackberries, raspberry, wild strawberry
- Plants with antibacterial action: garlic, onion, chamomile, mug worth, linden, horse tail
- Plants with astringent, disinfectant and stimulative action on digestive tract: mug worth, balm mint, tansy, horsemint, wild rose, nettle, oak, birch

Biologically active substances may be extracted from these plants by using different solvents: water, alcohol, oil, using several processes (extraction, infusion, maceration).
Natural biostimulators used for experimental schemes were:
- Nettle infusion: prepared from fresh nettle plant (100 g fresh nettle in 1000 ml hot water). The infusion was used for preparing the syrup
- Onion extract: was obtained by grounding the fresh onion, intermingling it for obtaining the juice, using 5 ml of juice in one kg of syrup
- Garlic extract was obtained in the same manner, using the same quantity per kg of syrup
- Thyme infusion was prepared from dry plant, fine grounded and infused in hot water, obtaining a 10% solution used for syrup preparing.
- *Echinacea* infusion was obtained infusing 10 g of plant in 1000 ml of hot water, used afterwards for syrup preparation
- Protofil was purchased from the specialized stores for beekeepers, using 17 ml of extract in one kg of sugar syrup.

Experiments were developed in two different series, during 2 years. In the first experiment as supplementary feeding were used nettle infusion, fresh onion and garlic juice, and Protofil.
The second experiment, as supplementary feeding was used thyme and Echinacea infusions.
For all control and experimental lots, uncapped brood surface, capped brood surface and total brood surface was measured, using Netz frame.
Laboratory testing of fresh juice, water infusions and alcoholic extract were done for biologically active constituents, responsible for antioxidant and antibacterial activities, as it is known from human consumption.

Total phenolic content from samples (water infusions and alcoholic extracts) was quantified using Folin-Ciocâlteu colorimetric method with some modifications [8]. Results were expressed as average of three determinations, in Gallic acid equivalents/100 g plant.

Total flavonoid content was determined after Kim and col. (2003)[9] method, using $NaNO_2$, $AlCl_3$ and NaOH as reagents. Results were expressed as average of three determinations, in mg Quercetin equivalents/100 g plant.

Antioxidant activity of water infusions and alcoholic extracts was correlated with chemical composition and compared with the known action of natural and synthetic antioxidants. Radical scavenging activity was measured by DPPH assay. The scavenging activity (H/e^- transferring ability) against DPPH radical was evaluated according to the method of Brand-Williams (1995)[10], with some modifications. This method is one of the shortest available to investigate the overall hydrogen/electron-donating activity of antioxidants [11].

In the presence of antioxidants, the blue color of 2,2-diphenyl-1-picrylhydrazyl decays, and the change of absorbency can be monitored spectrophotometrically at 517 nm.

The effect of supplements upon artificially weaken bee families were made utilizing methods from beekeeping practice: measurement of uncapped brood surface, capped and total brood surface, at the beginning of the experiment and after supplemental feeding

The results were statistically analyzed using one-way analysis of variance (ANOVA), a *p* value of 0.05 was considered as significant.

3. Results and discussion

3.1. Results obtained after laboratory determinations on plants. Fresh juice, water extracts and alcoholic extract of the plants used in the experiment were subjected to chemical analysis for total phenolics, total flavonoid content and antioxidant activity. Table 1 show the results obtained in laboratory testing.

Table 1. Total polyphenolic content (mgGAE/100 g), total flavonoid content (mg QE/100 g) and antioxidant activity expressed as % Inhibition of DPPH radical of analyzed plant supplements

Plant	Total polyphenols (mgGAE/100 g)			Total flavonoids (mg QE/100 g)			Antioxidant activity (% Inhibition)		
	Extract	Infusion	Juice	Extract	Infusion	Juice	Extract	Infusion	Juice
Echinacea	549.0	570.0	-	183.0	325.9	-	53.38	75.47	-
Thyme	555.0	530.0	-	131.0	123.2	-	74.13	50.70	-
Nettle	192.0	310.0	-	94.3	268.0	-	74.59	63.08	-
Onion	33.0	-	150.9	4.4	-	1.6	29.84	-	84.85
Garlic	88.0	-	69.0	11.3	-	4.4	20.98	-	95.10
Protofil	576.0	-	-	87.3	-	-	55.48	-	-

As it can be observed from Table 1, *Echinacea* infusion (10 g of ground plant, infused for 30 min in 100 ml hot water) exhibit a high amount of polyphenols (570 mgGAE/100 g plant), followed by thyme extract (555 mgGAE/100 g plant) and *Echinacea* extract (549 mgGAE/100 g). Onion and garlic fresh juice are not a rich source of polyphenols (150.9 and 69 mgGAE/100g), or flavonoids (1.6 and 4.4 mgQE/100 g), despite their high antioxidant activity (84,85 – 95.1 %). The highest amount of total polyphenols was found in 95.1%), followed by Echinacea infusion (74,47%, Thyme (74.13%) and nettle alcoholic extracts (74.59%). The product Protofil exhibits a moderate antioxidant activity, inhibiting the DPPH radical in a percentage of 55.5%.

Protofil (as expected)(576 mgGAE/100g), because of the complex medicinal plant composition. The highest amount of total flavonoids was found again in *Echinacea* infusion (325.9 mgQE/100 g plant), followed by nettle infusion (268 mgQE/100g) and *Echinacea* alcoholic extract (183 mgQE/100g). This time we could observe that Protofil is not a good source of flavonoids (87.3 mgQE/100 g), as well as onion and garlic fresh juice. Highest antioxidant activity exhibited fresh juice of onion and garlic (84.85 –

3.2. Results regarding the effect of nettle, Protofil, garlic and onion upon bee artificially weaken bee colonies. As it was stated above, artificially weaken bee families were obtain by eliminating

the frames covered with bees, until remained ¼ from the initial family. The remaining nest consisted of 4 frames covered with brood, honey and worker bees, together with the existing queen. At the beginning of the experiment the approximate surface of brood was 102 dm^2/lot.
25 weaken families were created, divided in 5 lots, each lot consisting of 5 families. Control lot was fed with sugar syrup 1:1, 1000 g/family, during 10 days, after the last administration the measurements were made for uncapped brood surface, capped brood surface and total brood surface.
Figure 1 present the results obtained in the experiments, where supplementary feeding of artificially weaken bee families were nettle infusion, Protofil, fresh juice of onion and garlic. Control group was fed only with sugar syrup.

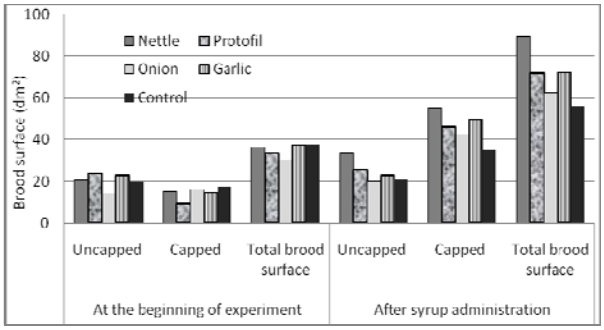

Figure 1. Uncapped, capped and total brood surface in artificially weaken bee families from the first experiment

3.3. Results regarding the effect of Echinacea and thyme upon artificially weaken bee colonies. In the second experiment, infusion of *Echinacea* and thyme was experimented in the supplementary feeding, towards a control, fed only with sugar syrup. Results obtained are presented in Figure 2. Control and experimental groups as well as measurement of the results were made similar to first experiment, changing only the plant supplements.

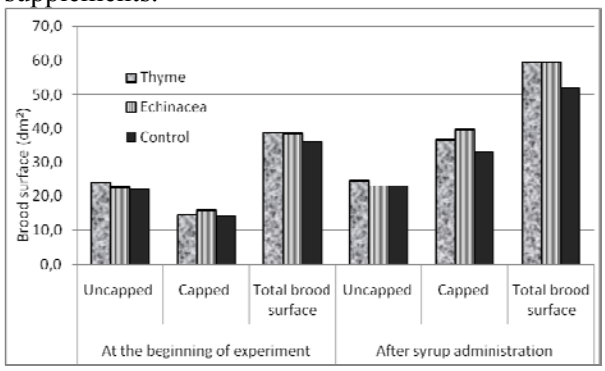

Figure 2. Evolution of uncapped, capped and total brood surface in the second experiment, when using *Echinacea* and thyme infusion in supplementary feeding toward a control group fed with sugar syrup

Tabel 2 centralizes the data obtained in the first experiment when testing upon artificially weaken bee families the plant supplements: nettle, onion, garlic, Protofil towards the control group fed only with sugar syrup.
Tabel 3 present the results obtained in the second experiment, regarding average growts/group at bee colonies from experimental groups in comparison with control.
Figure 3 present comparatively a frame with brood from control group (A) and the experimental lot fed with nettle extract. It can be seen the difference in brood surface between the experimental lot and control.

Table 2. Centralised data regarding average growth/group at bee colonies from the first experiment in comparison with control

Analyzed characteristic	Nettle	Protofil	Onion	Garlic
Uncapped brood surface (dm^2)	+11.6	+0.4	+4.6	-1.2
Capped brood surface (dm^2)	+21.8	+18.6	+8.4	+17.0
Total brood surface (dm^2)	+33.4	+19.0	+13.0	+15.8
SUPPLEMENTS CLASIFICATION	**I**	**II**	**IV**	**III**

Table 3. The centralised data regarding mean growth/group at weaken bee families from the second experiment in comparison with control group

Analyzed characteristic	Thyme	*Echinacea*
Uncapped brood surfaced (dm^2)	-0.6	-0.5
Capped brood surfaced (dm^2)	+3.2	+4.7
Total brood surface (dm^2)	+4.8	+5.2
SUPPLEMENTS CLASIFICATION	**II**	**I**

Figure 3. Comparison between control frame (A) and experimental frame of the lot fed with nettle extract

4. Conclusions

The purpose of the present research was to determinate in the laboratory and in the field the effect of different plant infusions and extracts on honeybee colonies productivity. Studied supplements were nettle (*Urtica dioica*), Protofil, onion (*Allium cepa*), garlic (*Allium sativum*), thyme (*Satureja hortensis*), Echinacea (*Echinacea sp.*). Experimental groups were build out of artificially weaken bee colonies.

Laboratory determinations show different polyphenolic content in the studied plants, which were correlated with the field experiments regarding the production of brood surface.

If we make a classification of the used supplements, regarding the growth of brood surface comparing to control groups, we can say that nettle infusion induced the highest growth, followed by Protofil, garlic, onion, *Echinacea* and thyme.

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