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Study of the stimulating effect of *Tribulus terrestris* L. on honey productivity of bee colonies

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Abstract: Honey bee (*Apis mellifera*) colony development and honey productivity depend on both nutritional resources and the availability of bioactive compounds that support brood rearing and physiological regulation. While supplemental feeding with sugar syrup provides carbohydrates, it does not supply essential phytoactive substances. *Tribulus terrestris* L., a medicinal plant rich in furostanol saponins such as protodioscin, flavonoids, and glycosides, has demonstrated hormone-like and adaptogenic properties in various organisms, yet its potential as a natural stimulant in apiculture remains largely unexplored. This study evaluated the effect of *T. terrestris* extract on honey productivity of bee colonies over two consecutive seasons (2024–2025) in northeastern Bulgaria. Experimental groups received inverted sugar syrup supplemented with either *T. terrestris* extract or the Startovit mineral-vitamin stimulant, while a control group received syrup only. Results indicated that colonies receiving *T. terrestris* extract achieved significantly higher honey yields - up to 35% more than controls in 2024 and 43% in 2025. The findings demonstrate that *T. terrestris* extract can safely and effectively enhance colony performance and honey production, offering a cost-efficient, natural alternative to synthetic stimulants in apiculture.

Keywords: honey productivity, plant extract, *Tribulus terrestris*, stimulation, *Apis mellifera*

Introduction

The use of medicinal plants and their bioactive properties have been documented since ancient times. Archaeological and ethnobotanical evidence suggests that plants were used for therapeutic purposes as early as the Neanderthal period [1]. In recent decades, growing scientific interest has been directed toward plant-based compounds as natural alternatives to synthetic substances in medicine, animal production, and agriculture, driven by concerns related to safety, sustainability, and regulatory restrictions [2, 3].

In apiculture, one of the key challenges for maintaining colony health and productivity is the availability of nutritionally balanced forage. Normal development of honey bee (*Apis mellifera*) colonies requires not only carbohydrates and proteins but also a continuous supply of physiologically active substances, including vitamins, minerals, and phytohormones, which are naturally present in pollen and nectar during periods of abundant flowering [4, 5]. These compounds play an essential role in brood rearing, wax secretion, and overall colony growth.

Periods of limited or absent flowering result in significant disturbances in colony dynamics. Reduced pollen intake leads to decreased queen egg-laying, suppression of brood development, interruption of comb construction, and delayed colony expansion, effects that are particularly detrimental during early spring when colony buildup is critical for subsequent productivity. Although supplemental feeding with sugar syrup is a common management practice, it provides primarily carbohydrates and fails to compensate for the deficiency of biologically active compounds required for normal physiological regulation [6, 7].

To address these limitations, several studies have explored the use of phytohormones and biologically active plant-derived substances as dietary supplements for honey bees. Early work by Mitev [8] demonstrated that supplementation with gibberellic acid during spring development enhanced queen egg-laying, brood production, colony strength, and honey and wax yield. However, the application of synthetic hormonal substances in animal production has become increasingly restricted due to potential side effects and food safety concerns, emphasizing the need for safe, natural alternatives of plant origin [9].

Tribulus terrestris L. is an annual herb widely distributed in arid and semi-arid regions of Europe, Asia, and the Americas. It has a long history of use in traditional medicine for its tonic, adaptogenic, and hormone-modulating effects. Phytochemical analyses have identified a complex profile of bioactive compounds, including steroidal saponins, flavonoids, glycosides, and alkaloids [10-12]. The pharmacological activity of the plant is mainly attributed to furostanol and spirostanol saponins such as protodioscin, diosgenin, and gitogenin, whose concentrations vary depending on geographical origin and plant part used [13-15]. Notably, Bulgarian populations of *T. terrestris* have been reported to contain particularly high levels of protodioscin [16]. Recent studies further confirm the antioxidant, hormone-like, and regulatory potential of *T. terrestris* extracts [17-18]. Promising research studies on the influence of *T. terrestris* on queen bee egg production were conducted by Hristakov [19-21].

Despite extensive research on the effects of *T. terrestris* extracts on reproductive, metabolic, and endocrine functions in humans and terrestrial animals, their potential impact on honey bee colony development and productivity remains largely unexplored. To date, there is a lack of experimental data evaluating the application of *T. terrestris* derived bioactive compounds in honey bee nutrition, particularly with respect to honey production.

Therefore, the aim of the present study is to evaluate the effect of *T. terrestris* extract on the honey productivity of honey bee colonies.

Material and Methods

The subject of the research is the stimulating effect of *T. terrestris* extract on bee colonies in combination with inverted sugar solution.

The experimental part of the research was carried out in northeastern Bulgaria in an area with rich honey-bearing vegetation. Near the apiary with coordinates 43.534449° N and 25.753916° E there are linden and acacia forests, and rapeseed and sunflower are sown from agricultural honey-bearing crops. This determines four main bee pastures for the region. The climatic conditions in the region are favorable for the development of beekeeping.

When conducting the experiment with the stimulating feedings of one experimental group, in addition to inverted sugar solution, the preparation *Vemo Herb-Bees* dry plant extract of *T. terrestris* produced by the VEMO-99 company, Sofia, was used. This is an original non-hormonal preparation.

Its active components are furostanol saponins, defined as protodioscin in the product with a content of not less than 50%.

The second experimental group was fed with inverted sugar syrup and the *Startovit* veterinary medicinal preparation contains: Natrii chloridum - 0.450 g, Cobalti chloridum - 0.0366 g; Dinatrii phosphas - 0.010 g and Excipients ad 5.0 g. It is produced by the Primavet-Sofia company.

For the experiment, one control and two experimental groups with 7 bee colonies were formed, equalized by the method of analogues. The method is based on our experience from previous experiments in which we equalize the colonies based on food supply, bee spacing and brood quantity and origin of the queens. The bee colonies were settled in hives according to the Dadan-Blatt system.

The bee colonies participating in the experiments were fed according to the following scheme:

- Group No. 1. Control - every three days 200 ml of inverted sugar solution;
- Group No. 2. Experimental - every three days 200 ml of invert sugar solution with the addition of *T. terrestris* extract in an amount of 10 mg per 1 kg of bees, with dose adjustment every 12 days depending on the mass of the bees in each colony;
- Group No. 3. Experimental - fed with 200 ml of inverted sugar solution with the addition of the *Startovit* stimulant at a dose of 1g per 2 l of syrup.

The dose of 10 mg per 1 kg of bee mass is based on our previous studies related to the use of *T. terrestris* to stimulate the egg-laying activity of queen bees. All groups of hives participating in the experiment are located in one apiary and are exposed to an identical nectar flow.

The first part of the experimental work was carried out during the period from February 28 to May 23, 2024. In the previous year, 21 bee colonies were prepared with queen bees of equal age and origin. At the beginning of the beekeeping season of 2024, the three groups of colonies were located in one apiary under the same conditions. The feeding of the three groups of bee colonies was stopped after April 17, 2024, when the stores for the first main rapeseed pasture were placed.

Honey productivity (kg) – was determined separately for each bee colony included in the tests. It represents the difference between the mass of the combs with honey removed from the nest and the mass of the combs after centrifugation of the honey. At the end of the honey collection period, the amounts of honey obtained from each extraction were summed up to determine the total amount.

The test was repeated with the same bee colonies (respectively the same queen bees) during the period from May 1 to May 24, 2025. The methodology for conducting the experiment and reporting the results are the same as in the previous year. Stimulating feeding was discontinued after April 18, 2025, when the first stores were placed on the hives.

After May 24, 2025, due to a large number of bee colonies included in the test falling into a swarming state, all queen bees were separated into new hives with a part of the bees, with an equal amount of honey and newly built combs without brood. The study involved old queen bees, each of which remained in the same group in which it was the previous year.

Stimulating feedings of the three groups continued from June 9, 2025 to September 7, 2025.

In 2025, no commercial honey was extracted from the newly established bee colonies on June 9, 2025. The total amount of honey in the group was determined as the difference between the amount of honey in the bee nests when the colonies were created and the amount of honey during their wintering.

Results and Discussion

The results for the honey productivity of the bee colonies included in the test from the first main pasture (rapeseed) for 2024 are shown in Table 1. A 52.88% higher honey yield was found for the colonies stimulated with *T. terrestris* extract, and 38.74% for the group receiving the *Startovit* stimulant, compared to the control. The trend for higher honey productivity in the experimental groups (fed with the addition of plant extract of *T. terrestris* and *Startovit* stimulant) is maintained for the other main bee pastures, as well as for the entire honey collection period (Fig. 1).

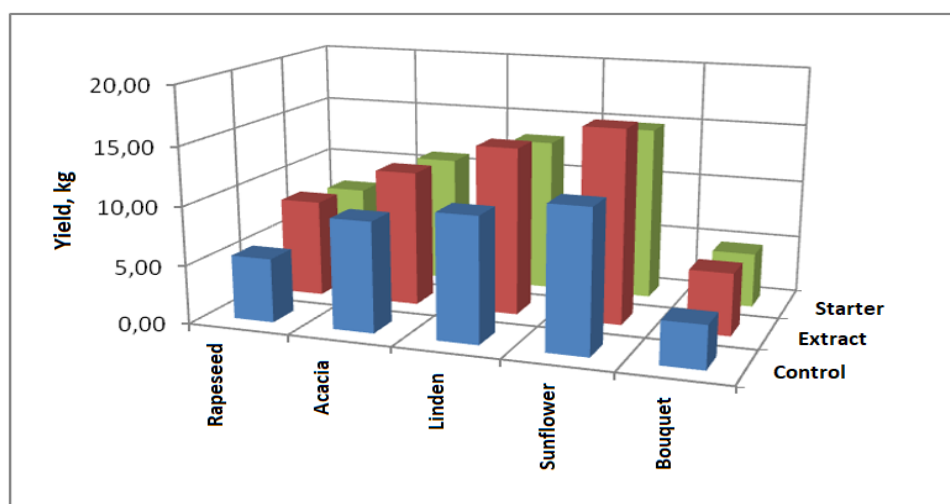


Figure 1. Average honey productivity values from the different main pastures (2024)

Figure 2 shows the increase in honey yield (in %) of the families from the two experimental groups compared to the control, from which it can be seen that throughout the entire testing period the group stimulated with *T. terrestris* extract had the highest honey productivity. At the end of the honey collection period, a total of 290.6 kg of honey was obtained from the control group, from the group fed with *T. terrestris* extract - 392.3 kg, which is 34.99% higher yield compared to the control, from the families stimulated with *Startovit* stimulant - 358.8 kg of honey in total or 23.47% more than the control. The reported differences are statistically significant (Table 1).

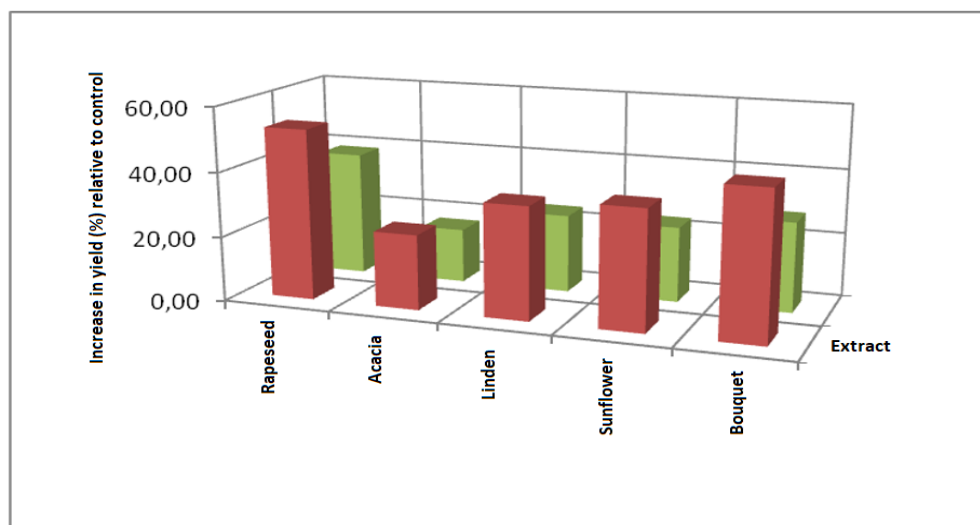


Figure 2. Honey productivity (%), compared to the control (2024)

Table 1. Honey productivity of bee colonies (2024)

Group	Parameters	Rapeseed kg	Acacia kg	Linden kg	Sunflower kg	Bouquet kg	Total per kg	Honey per kg
Control	Average values	5,46	9,40	10,61	12,04	3,64	41,51	290,6
	Minimum values	4,00	8,00	9,00	11,00	3,00	35,00	
	Maximum values	6,50	10,20	12,00	13,00	4,50	45,20	
	Estimation of disp. σ	0,96	0,80	1,22	0,55	0,48	15,39	
	Standard deviation Sx	0,98	0,89	1,10	0,74	0,69	3,92	
	Confidence interval	4,73	8,74	9,80	11,49	3,13	38,61	
	Confidence interval	6,18	10,06	11,43	12,59	4,15	44,42	
<i>T. terrestris</i> extract	Average values	8,34	11,59	14,31	16,50	5,30	56,04	392,3
	Minimum values	7,20	10,00	13,50	15,00	4,50	51,20	
	Maximum values	10,00	13,00	16,00	18,50	6,00	62,90	
	Estimation of disp. σ	0,81	1,12	0,76	1,33	0,22	16,04	
	Standard deviation Sx	0,90	1,06	0,87	1,15	0,47	4,00	
	Confidence interval	7,68	10,80	13,67	15,64	4,95	53,08	
	Confidence interval	9,01	12,37	14,96	17,36	5,65	59,01	
	P(F)	0,84	0,69	0,58	0,30	0,37	0,96	
	P(t)	0,0001	0,0013	0,0000	0,0000	0,0002	0,0000	
	% relative to control	52,88	23,25	34,86	37,01	45,49	35,00	
<i>Startovit</i> stimulant	Average values	7,57	10,99	13,19	14,86	4,66	51,26	358,8
	Minimum values	7,00	10,50	12,40	14,00	4,00	50,00	
	Maximum values	8,50	11,60	14,00	16,00	5,60	52,50	
	Estimation of disp. σ	0,27	0,14	0,28	0,56	0,49	0,86	
	Standard deviation Sx	0,52	0,37	0,53	0,75	0,70	0,93	
	Confidence interval	7,19	10,71	12,80	14,30	4,14	50,57	
	Confidence interval	7,95	11,26	13,58	15,41	5,17	51,95	
	P(F)	0,14	0,05	0,10	0,98	0,98	0,00	
	P(t)	0,0003	0,0010	0,0001	0,0000	0,0181	0,0005	
	% relative to control	38,74	16,87	24,23	23,37	27,84	23,47	

The results of repeating the test in 2025, using bee swarms with queen bees from 2024, are reflected in Table 2.

From the data in the table, it is clear that the families in the control group for the period from their creation to their wintering have collected an average of 8.71 kg of honey per family, those from the groups stimulated with plant extract and with the *Startovit* stimulant, respectively by 43.44% and 27.04% more than the control (Fig. 3).

The increase in honey yield in the two experimental groups compared to the control group is shown in (Fig. 4), from which it is evident that the highest percentage is the increase in honey productivity in the group of bee families stimulated with *T. terrestris*. The total honey yield in the families in the control group is 61.0 kg, in the group with plant extract 87.5 kg and those stimulated with the *Startovit* stimulant - 77.5 kg (Table 2).

Table 2. Honey productivity of bees warms (2025)

Group	Parameters	Reporting date		Total for the period kg	Yield per kg
		9.6.2025	17.10.2025		
Control	Average values	5,36	14,07	8,71	61
	Minimum values	4,50	13,00	7,50	
	Maximum values	6,00	15,00	10,00	
	Estimation of disp. σ	0,31	0,45	0,65	
	Standard deviation Sx	0,56	0,67	0,81	
	Confidence interval	4,95	13,57	8,11	
	Confidence interval	5,77	14,57	9,31	
<i>T. terrestris</i> extract	Average values	5,36	17,86	12,50	87,5
	Minimum values	4,50	17,00	12,00	
	Maximum values	6,00	19,00	13,00	
	Estimation of disp. σ	0,31	0,48	0,17	
	Standard deviation Sx	0,56	0,69	0,41	
	Confidence interval	4,95	17,35	12,20	
	Confidence interval	5,77	18,37	12,80	
	P(F)	1,00	0,95	0,12	
	P(t)	1,0000	0,0000	0,0000	
	% relative to control	0,00	26,90	43,44	
<i>Startovit</i> stimulant	Average values	5,50	16,57	11,07	77,5
	Minimum values	5,00	15,50	10,00	
	Maximum values	6,00	17,50	12,00	
	Estimation of disp. σ	0,17	0,45	0,37	
	Standard deviation Sx	0,41	0,67	0,61	
	Confidence interval	5,20	16,07	10,62	
	Confidence interval	5,80	17,07	11,52	
	P(F)	0,47	1,00	0,50	
	P(t)	0,5939	0,0000	0,0000	
	% relative to control	2,67	17,77	27,05	

The higher values obtained for the honey productivity trait of the bee colonies from the two experimental groups compared to the control group are of high significance ($P \leq 0.001$), both during wintering (17.10.2025) and overall, for the study period.

The reported data for the two studied seasons (2024 and 2025) show that the bee colonies from the group that received invert sugar solution and the addition of *T. terrestris* extract had the highest honey productivity. This also correlates with a larger number of bees and, accordingly, stronger bee colonies in the respective group, but this is the subject of our other studies.

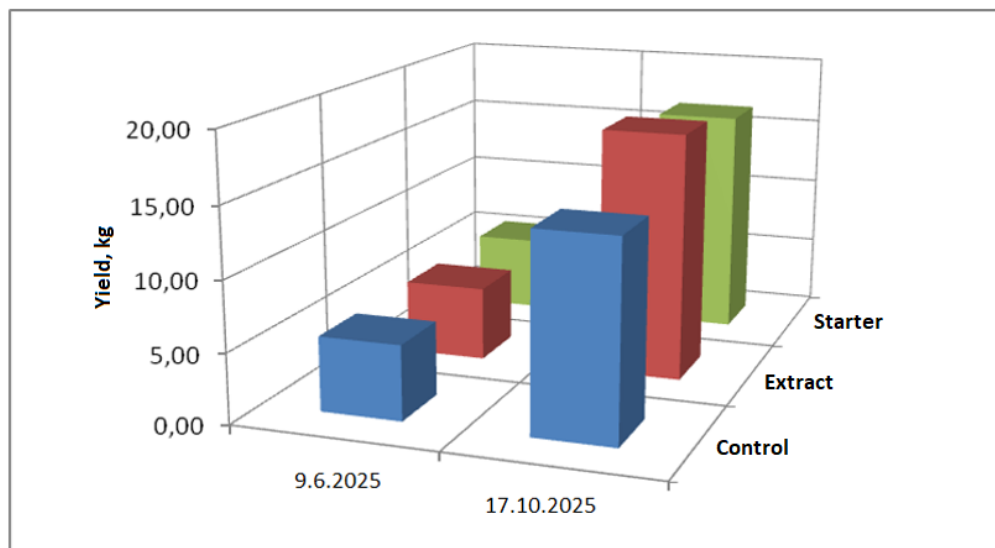


Figure 3. Average honey productivity values for bees warms (2025)

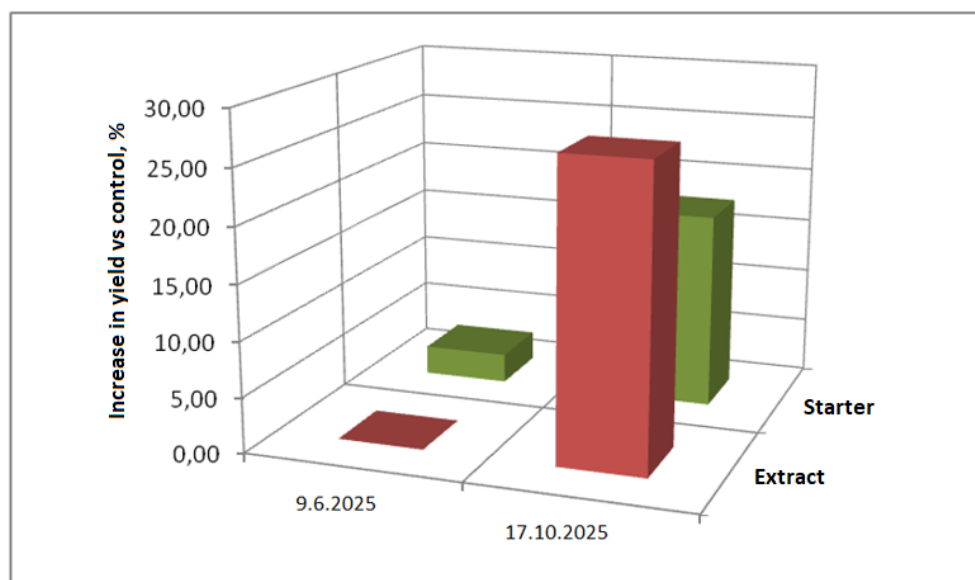


Figure 4. Honey productivity in bees warms (%) compared to the control (2025)

Economic efficiency of applying *T. terrestris* extract in stimulating feeding of bee colonies.

The conducted research and the obtained results show that when adding the *Startovit* stimulant and *T. terrestris* extract to the inverted sugar solution during the stimulating feeding of the experimental groups of bee colonies increased the strength and honey productivity, with a significantly greater effect when stimulated with the plant extract.

When reporting honey productivity in 2024, the group stimulated with extract produced 98.3 kg more honey than the control group, and received 372.38 leva more when selling it. In the group of bee colonies receiving *Startovit* stimulant, the honey yield was 68.2 kg higher, and received 287.88 leva more when selling it.

In 2025, no commercial honey was obtained from the newly established bee colonies. Nevertheless, when considering the honey productivity (Table 2), it can be seen that the group of colonies stimulated with *T. terrestris* extract collected 26.5 kg more honey than the control group for the period. With an average selling price of honey of 4 leva, the value of the larger amount of honey obtained by them is 106 leva. The colonies fed with the *Startovit* stimulant collected 16.5 kg more honey worth 66 leva.

Despite the fact that commercial honey was not obtained from the newly established bee colonies, their stimulation with the *T. terrestris* extract leads to better development, successful wintering and the production of stronger colonies the following year.

Group No. 1 - Control

Rapeseed honey total - $38.2 \text{ kg} \times 3.8 \text{ leva} = 145.16 \text{ leva}$

Acacia honey total - $65.8 \text{ kg} \times 4.4 \text{ leva} = 289.52 \text{ leva}$

Linden honey total - $74.3 \text{ kg} \times 4.2 \text{ leva} = 312.06 \text{ leva}$

Sunflower honey - $84.3 \text{ kg} \times 4.0 \text{ leva} = 337.20 \text{ leva}$

Bouquet total - $25.5 \text{ kg} \times 4.0 \text{ leva} = 102.0 \text{ leva}$

Total leva from honey sold = 1185.94 leva

Group No. 2 - Stimulated with *T. terrestris* extract

rapeseed honey total - $58.4 \text{ kg} \times 3.8 \text{ leva} = 221.92 \text{ leva}$

acacia honey total - $81.1 \text{ kg} \times 4.4 \text{ leva} = 356.84 \text{ leva}$

linden honey total - $100.2 \text{ kg} \times 4.2 \text{ leva} = 400.8 \text{ leva}$

sunflower honey - $115.5 \text{ kg} \times 4.0 \text{ leva} = 462.00 \text{ leva}$

bouquet total - $37.1 \text{ kg} \times 4.0 \text{ leva} = 148.4 \text{ leva}$

Total leva from honey sold = 1558.32 leva

Group No. 3 - Stimulated with the *Startovit* stimulant

Rapeseed honey total - 53.0 kg x 3.8 leva = 201.4 leva

Acacia honey total - 76.9 kg x 4.4 leva = 338.36 leva

Linden honey total - 92.3 kg x 4.2 leva = 387.66 leva

Sunflower honey - 104 kg x 4.0 leva = 416.00 leva

Bouquet total - 32.6 kg x 4.0 leva = 130.4 leva

Total leva from honey sold = 1473.82 leva

In the 2024 experiments, about 20 g of the *Startovit* stimulant were used to stimulate 7 hives, with the market price of 25 g being 3 leva. In the 2024 stimulation of the families with *T. terrestris* extract, 3000 mg of extract worth 6.5 leva were used.

In our study, the cost of the inverted sugar syrup used was not taken into account, nor was the cost of the labor involved, but they were the same for all three groups participating in the experiment. Our goal was to determine the effect of the stimulants used and their value relative to the value of the larger production obtained.

Taking into account, on the one hand, the results obtained for better development of bee colonies (larger amount of brood reared and available bees) and higher honey productivity, and on the other hand, the low cost of the stimulants used, the potential of their use for increasing the honey productivity of bee colonies is shown.

Conclusion

1. The highest honey productivity was achieved by bee colonies from the group receiving inverted sugar solution and the addition of *T. terrestris* extract. The reported values for the honey productivity trait compared to the control group are of high significance ($P \leq 0.001$).

2. The results indicate potential economic feasibility of applying *T. terrestris* extract and the *Startovit* stimulant in stimulating feeding of bee colonies.

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