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**Study of the effect of the plant extract of *Tribulus terrestris* L. on the foraging activity of bees
(summer flight activity)**

Ivailo Hristakov

Department of Agricultural Technology, Angel Kanchev University of Ruse,

8 Studentska Str., 7017 Ruse, Bulgaria

Abstract: Honey bee (*Apis mellifera* L.) colony productivity and foraging activity are influenced by both external factors, such as climatic conditions and nectar availability, and internal factors, including colony strength and queen fertility. While plant-based supplements have been studied for their effects on colony health and productivity, the impact of *Tribulus terrestris* L. on summer flight activity remains unknown. This study evaluated the effect of *T. terrestris* extract on honey bee foraging behavior over two consecutive seasons (2024–2025) in three Bulgarian apiaries. Three groups were established: a control fed with inverted sugar syrup, an experimental group supplemented with *T. terrestris* extract, and a second experimental group receiving the Startovit mineral-vitamin stimulant. Flight activity was measured by counting bees departing each hive during three-minute intervals. Results showed that *T. terrestris* supplementation significantly increased summer flight activity compared to the control and “Startovit” groups, with the highest increases observed in 2024. These findings provide the first experimental evidence that *T. terrestris* extract can enhance honey bee foraging behavior, suggesting its potential as a natural, non-hormonal stimulant to improve colony performance and resource utilization.

Keywords: stimulation, flight activity, honey collection activity, plant extract, *Tribulus terrestris*, honey bees

Introduction

The development and productivity of bee colonies depend on a complex set of external and internal factors. The main external factors are the natural-climatic conditions and the honey-gathering potential of the region [1]. Most of these factors are either impossible for humans to influence or their modification is

economically unjustified [2]. The availability and nutritional quality of pollen resources are also critical determinants of colony development and foraging behavior [3, 4].

Another group of factors is created within the nest as a result of the vital activity of the bee colony. The main internal factors are: 1) the number of bees (colony strength); 2) the fertility of the queen bee; 3) the quantity and quality of food reserves; 4) the honey-gathering activity of worker bees.

Unlike external factors, these are largely controllable by humans.

A thorough understanding of the complex interaction between internal and external factors is necessary for the year-round management of strong and productive bee colonies [2, 5-8]. Modern automated monitoring systems further allow precise assessment of foraging flight activity as an indicator of colony health and productivity [9].

Increasing honey productivity is one of the main goals of every beekeeper. This type of productivity depends on multiple factors, among which colony strength and the optimal age structure of worker bees are particularly important. Environmental factors- especially honey resources and climatic conditions - have a strong and sometimes decisive influence on honey yield; however, they are beyond the beekeeper's control [10].

Various internal and external morphological and physiological characteristics of bees are also related to honey productivity and flight activity [11-22]. A correlation has been demonstrated between honey productivity and summer flight activity, honey stomach volume, proboscis length, live body mass, and the amount of brood reared both before and during the main honey flow [2, 23-27]. Recent reviews also emphasize that environmental contaminants and trace elements may directly affect foraging intensity and flight performance [28].

Honey productivity also depends on the applied beekeeping technology and the breed of bees. Under identical honey flow and climatic conditions, different breeds exhibit differences in productivity and flight activity [22]. Effective beekeeping practices must therefore be adapted to the specific characteristics of the honey-gathering behavior of different bee breeds [29].

Of great importance are the honey resources of the region and the botanical composition of the nectar-producing vegetation, as they determine the maximum expression of honey productivity and flight activity. It has been demonstrated that, depending on the breed, bees accumulate honey reserves differently and shift between major nectar flows in distinct ways, affecting the efficiency of resource utilization [30]. Modern melissopalynological and DNA-based studies further confirm that ecological conditions significantly shape foraging patterns [3, 4].

With the onset of the main honey flow, the intensity of flight activity in strong colonies increases sharply due to the mobilization of previously inactive worker bees. In weak colonies, such an increase is not observed because a larger proportion of young bees remain engaged in brood rearing [2]. Consequently, strong colonies

collect more honey not only per season but also per unit of live weight. For example, with a bee mass of 1 kg at the beginning of the honey flow, 7 kg of honey were obtained, whereas with 4 kg of bees, 49 kg of honey were collected [31]. It has been shown that increasing colony live weight up to 5 kg leads to increases in both total honey yield and honey yield per 1 kg of live weight. However, when colony mass reaches 8–10 kg, productivity per 1 kg of live weight decreases [31, 32].

A characteristic feature of strong colonies is that, with the onset of the main honey flow, young bees begin actively participating in nectar collection and processing as early as five days of age [31, 33]. Under strong nectar flow conditions, bees can collect up to three times more nectar with lower relative energy expenditure. For example, with an average daily honey yield of 1 kg, bees return with approximately 7.1 mg of nectar in the honey stomach; with 1–2 kg, they return with 15.5 mg; and with 4 kg, with up to 28 mg. This demonstrates that increasing nectar availability significantly enhances collection efficiency [34].

The use of stimulants in bee colonies may influence queen egg-laying activity and, consequently, overall colony development, flight activity, and productivity. Mineral-vitamin stimulants (e.g., *Startovit* stimulant) have shown positive effects; however, in strong colonies during spring, they may induce undesirable swarming. Research indicates that administering stimulants with inverted sugar syrup reduces bee exhaustion, prevents protein deficiency, prolongs lifespan, and improves stimulation efficiency. Dietary phytochemicals have also been shown to influence honey bee longevity and pathogen tolerance, indicating that plant-derived bioactive compounds may affect colony vitality and behavioral performance [35]. Recent experimental studies demonstrate that supplementation with plant extracts can positively affect certain colony parameters [36].

A review of the available literature indicates that many hormonal preparations for stimulating animal productivity are prohibited due to adverse side effects. This necessitates the search for new, harmless stimulants of plant origin. *Tribulus terrestris* L. is known to contain biologically active compounds with antioxidant, antimicrobial, and enzyme-modulating properties [37–39]. However, despite the demonstrated biological activity of *T. terrestris* extracts in various experimental models, there is currently no clear evidence regarding their influence on the flight (foraging) activity of honey bees.

Despite the growing interest in plant-based supplements in apiculture and the documented biological activity of *T. terrestris*, there is currently no experimental evidence regarding its effect on the foraging (summer flight) activity of honey bees. While previous studies have focused mainly on colony health, antimicrobial properties, and general productivity parameters, the direct influence of this plant extract on behavioral activity and nectar-gathering intensity remains unexplored. This lack of data defines a clear research gap concerning the potential role of *T. terrestris* as a natural stimulant of flight activity in honey bee colonies.

The aim of the present study is to investigate the effect of the herbal preparation *T. terrestris* on the foraging (summer flight) activity of honey bees.

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 three apiaries located in the village of Brestovitsa, Borovo municipality, Ruse region. The apiary is located at an altitude of about 290 m above sea level.

When conducting the experiments with the stimulating feedings of one experimental group, in addition to inverted sugar solution, a dry plant extract of *T. terrestris*, produced by the VEMO-99 company, Sofia, was also 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 other experimental group, together with inverted sugar syrup, was administered the veterinary medicine *Startovit* stimulant containing: 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 each experiment, one control and two experimental groups were formed with 7 bee colonies, equalized by the method of analogues. The bee colonies were settled in hives of the Dadan-Blat 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 inverted sugar solution with the addition of *T. terrestris* extract at 10 mg per 1 kg of bees, with a 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 *Startovit* stimulant at a dose of 1 g per 2 l of syrup.

The bee colonies participating in the experiment are located in an apiary in a checkerboard pattern, with the distance between the individual groups participating in the experiment being about three meters, and the lateral distance of the hives from one group from each other being about two meters. Invert sugar syrup is obtained by adding the enzyme invertase to 65% sugar syrup. The inverting process is carried out at a temperature of 55°C in a vessel providing constant stirring, which guarantees good homogenization and uniformity.

The first part of the experimental work was carried out during the period 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 discontinued after April 17, 2024, when the stores for the first main rapeseed pasture were placed.

The test was repeated with the same bee colonies (respectively the same queen bees) during the period from Mart 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 the 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.

The study was continued with the newly created colonies using the same methodology. Stimulating feedings of the three groups continued from June 9, 2025 to September 7, 2025.

The flight activity of the worker bees was determined in 2024 (from May 29 to June 5 after the acacia grazing) and in 2025 (from 27 to August 3 after the sunflower grazing). The measurement was carried out daily between 10 and 12 o'clock, and the number of bees leaving each hive in 3 min was recorded [20].

Results and Discussion

Beekeeping season 2024

For the 2024 beekeeping season, the data in Table 1 show that at the first reading, the average value of the summer activity trait for the bees from the experimental group stimulated with the plant extract was 144.71 bees, and for the group with *Startovit* stimulant was 167.57 bees. Throughout the entire study period, the bees from both experimental groups had greater flight activity compared to those from the control group - respectively by 89.41% for the group with *T. terrestris* and by 65.17% for the group with *Startovit* stimulant (Fig. 1 and Fig. 2). The results regarding the statistical significance of the obtained differences in the values of the summer activity trait of bee colonies from the control and both experimental groups show the following:

- medium to high degree of reliability between the control group and the group receiving *T. terrestris* extract ($P \leq 0.01$ - $P \leq 0.001$);
- low to high degree of reliability between the control group and the group receiving *Startovit* stimulant ($P \leq 0.05$ - $P \leq 0.01$ - $P \leq 0.001$);
- high degree of reliability between the control group and both experimental groups for the total testing period ($P \leq 0.001$);
- low degree of statistical significance between the two experimental groups ($P \leq 0.05$).

Based on the results obtained, it can be assumed that the stimulating feeding of bee colonies with inverted sugar solution and plant extract of *T. terrestris* leads to the development of strong colonies that use the available bee pasture very well. The data reported in the study are confirmed by Farrar [5] and Krivtsov & Lebedev [20], according to which the flight activity of bees depends on the strength of the colony, the amount of brood, grazing in nature and can be considered as an indirect indicator of the honey productivity of bee colonies.

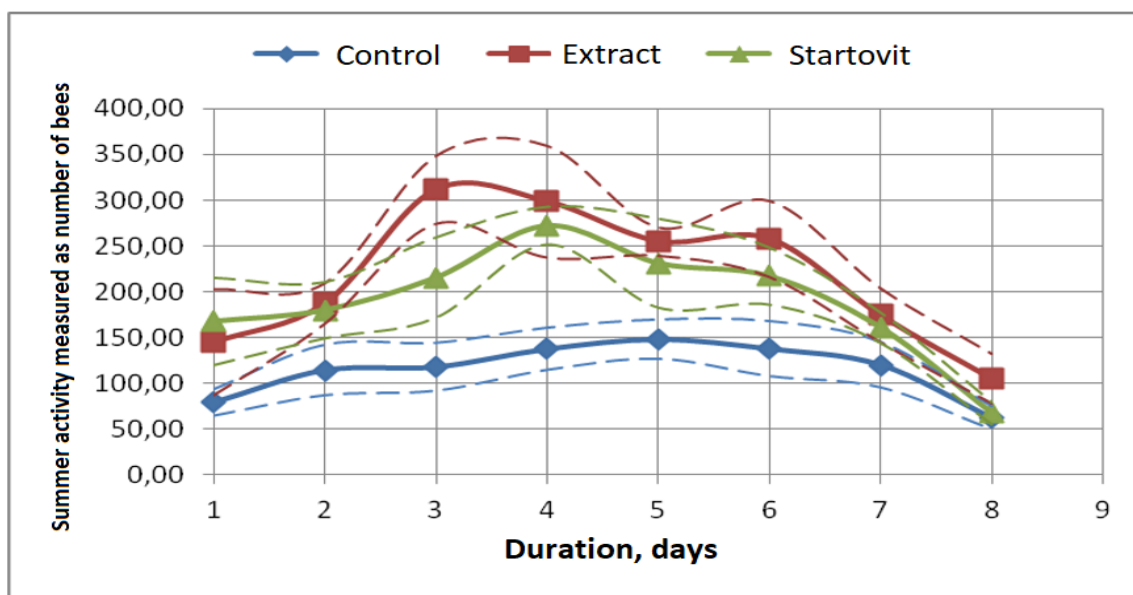


Figure 1. Average values and confidence intervals for summer activity (2024)

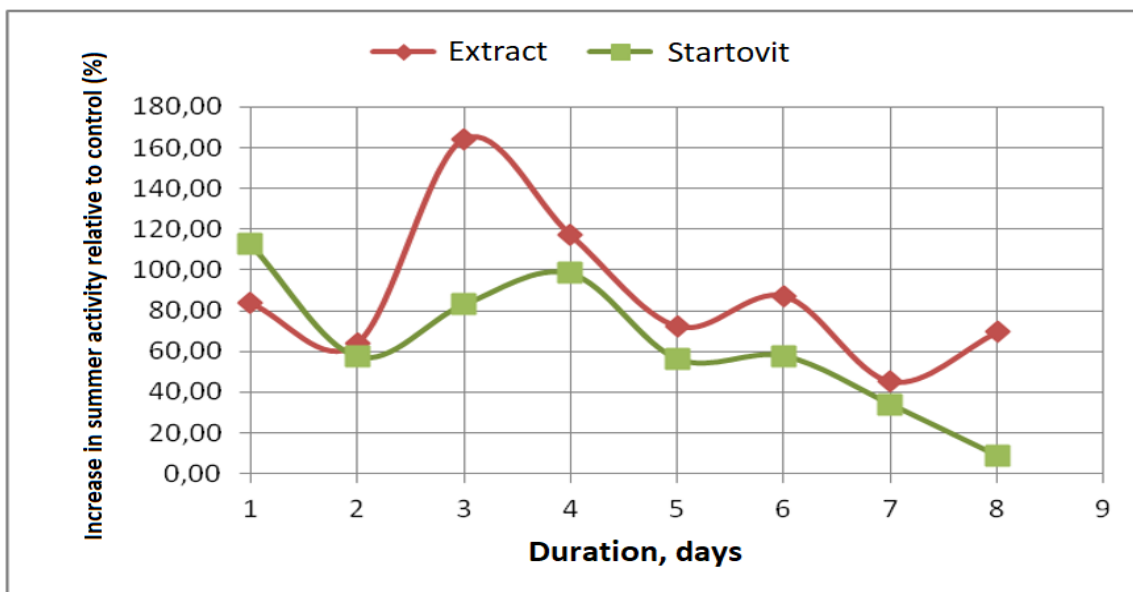


Figure 2. Increase in flight activity compared to the control (2024)

Table 1. Summer activity of bee colonies (2024)

Group	Parameter	Summer activity of bee colonies, number in 3 min. as of date								Average for the period
		29.5.2024	30.5.2024	31.5.2024	1.6.2024	2.6.2024	3.6.2024	4.6.2024	5.6.2024	
	Duration, days	1	2	3	4	5	6	7	8	-
Control	Average values	78,71 a	114,14	117,71	137,29	147,86	137,57	120,00	62,00 a	114,41
	Minimum values	52,00	75,00	47,00	89,00	101,00	95,00	93,00	42,00	74,25
	Maximum values	105,00	186,00	162,00	170,00	192,00	187,00	187,00	88,00	159,63
	Estimation of disp. σ	400,57	1417,48	1278,90	988,57	857,14	1671,29	1143,33	320,67	-
	Standard deviation Sx	20,01	37,65	35,76	31,44	29,28	40,88	33,81	17,91	-
	Confidence interval	63,89	86,25	91,22	113,99	126,17	107,29	94,95	48,73	-
		93,54	142,03	144,21	160,58	169,55	167,86	145,05	75,27	-
<i>T. terrestris</i> extract	Average values	144,71 a	187,14 a	311,29	298,43 a	255,00 a	257,71 a	174,29 a	105,14	216,71
	Minimum values	82,00	146,00	242,00	157,00	223,00	187,00	106,00	69,00	151,50
	Maximum values	301,00	237,00	378,00	386,00	290,00	334,00	223,00	167,00	289,50
	Estimation of disp. σ	6172,57	892,48	2423,57	6692,29	422,00	3095,24	1594,90	1373,48	-
	Standard deviation Sx	78,57	29,87	49,23	81,81	20,54	55,63	39,94	37,06	-
	Confidence interval	86,51	165,01	274,82	237,83	239,78	216,50	144,70	77,69	-
		202,92	209,27	347,75	359,03	270,22	298,93	203,87	132,60	-
	P(F)	0,00	0,59	0,46	0,03	0,41	0,47	0,70	0,10	-
	P(t)	0,0695	0,0017	0,0000	0,0014	0,0000	0,0006	0,0178	0,0169	-
<i>Startovit</i> stimulant	% relative to control	83,85	63,95	164,44	117,38	72,46	87,33	45,24	69,59	89,42
	Average values	167,57	179,86 a	215,43	272,29 a	231,29 a	217,14 a	160,86 a	67,43 a	188,98
	Minimum values	102,00	123,00	138,00	235,00	149,00	173,00	122,00	54,00	137,00
	Maximum values	298,00	231,00	302,00	314,00	320,00	301,00	181,00	102,00	256,13
	Estimation of disp. σ	4121,62	1688,48	3450,29	731,24	4224,24	1729,14	491,48	274,29	-
	Standard deviation Sx	64,20	41,09	58,74	27,04	64,99	41,58	22,17	16,56	-
	Confidence interval	120,01	149,42	171,91	252,25	183,14	186,34	144,43	55,16	-
		215,13	210,30	258,94	292,32	279,43	247,95	177,28	79,70	-
	P(F)	0,01	0,84	0,25	0,72	0,07	0,97	0,33	0,85	-
	P(t)	0,0097	0,0089	0,0027	0,0000	0,0092	0,0036	0,0203	0,5669	-
	% relative to control	112,89	57,57	83,01	98,34	56,43	57,84	34,05	8,76	65,18

* The difference is statistically significant if the variants do not share the same letters.

Beekeeping season 2025

The data from the measurement of the number of departing bees from the bee colonies participating in the experiment during the beekeeping season of 2025 are shown in Table 2, with the starting date of the experiment being July 27, 2025 after the sunflower grazing, the last main grazing for the study area.

The average number of departing bees from the colonies of the control group is 68.7 pcs., for the group stimulated with the plant extract - 90.1 pcs. (31.18% more) and for the colonies stimulated with *Startovit* stimulant - 74.4 pcs. (8.31% more) (Table 2).

The graphical presentation of the results (Fig. 3) shows that bees from the groups stimulated with plant extract and *Startovit* stimulant maintained higher values for the summer flight activity trait throughout the study period. The percentage difference varied during the different days of reporting (Fig. 4). On average for the experimental period, bee colonies from the group fed with the addition of *T. terrestris* extract had 22.82% higher flight activity, and those from the experimental group with *Startovit* stimulant had 9.37% more compared to the activity of bees in the control.

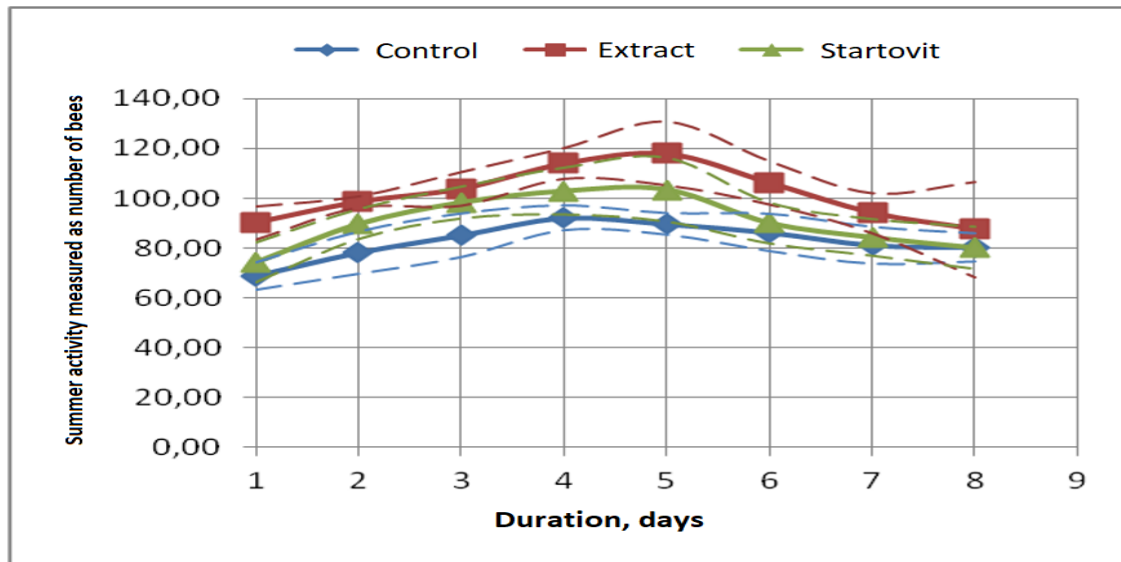


Figure 3. Mean values and confidence intervals for summer activity (2025)

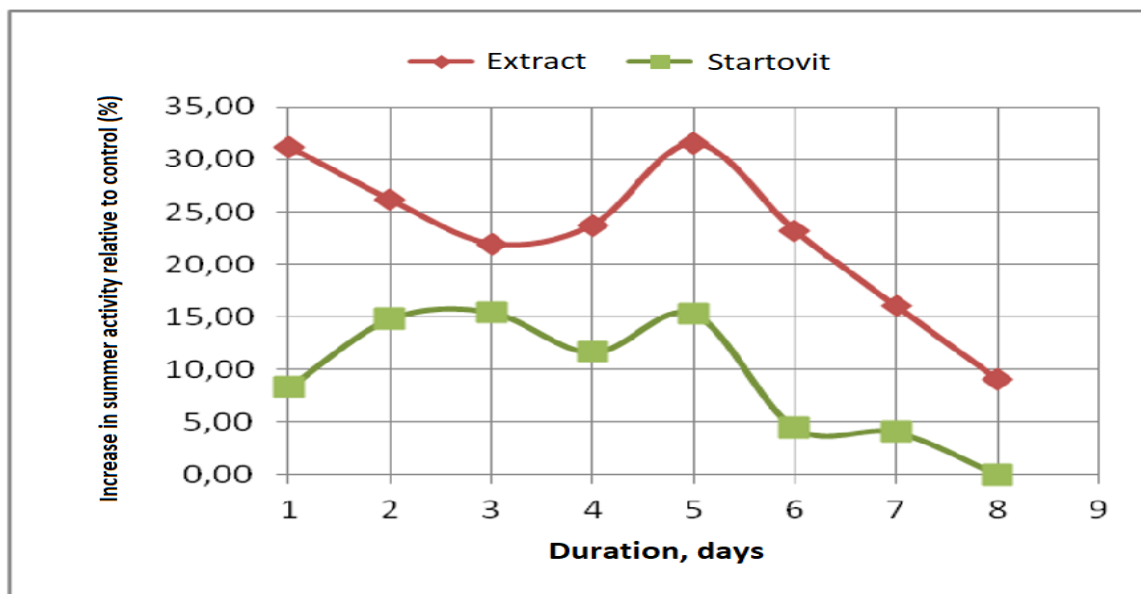


Figure 4. Flight activity of bees from the experimental groups (%), compared to the control (2025)

Table 2. Summer activity of bee colonies (2025)

Group	Parameter	Summer activity of bee colonies, number in 3 min. as of date								Average for the period
		27.7.2025	28.7.2025	29.7.2025	30.7.2025	31.7.2025	1.8.2025	2.8.2025	3.8.2025	
	Duration, days	1	2	3	4	5	6	7	8	-
Control	Average values	68,71 a	78,14	85,14	92,14 a	89,71 a	86,29 a	81,14 a	80,29 a	82,70
	Minimum values	61,00	61,00	62,00	82,00	84,00	71,00	67,00	70,00	69,75
	Maximum values	82,00	93,00	95,00	101,00	102,00	97,00	95,00	91,00	94,50
	Estimation of disp. σ	53,90	131,14	141,81	47,48	35,90	103,90	101,14	58,90	-
	Standard deviation Sx	7,34	11,45	11,91	6,89	5,99	10,19	10,06	7,67	-
	Confidence interval	63,28	69,66	76,32	87,04	85,28	78,73	73,69	74,60	-
<i>T. terrestris</i> extract		74,15	86,63	93,96	97,25	94,15	93,84	88,59	85,97	-
	Average values	90,14	98,57 a	103,86 a	114,00	118,00	106,29	94,14	87,57 a	101,57
	Minimum values	75,00	94,00	90,00	100,00	97,00	95,00	78,00	65,00	86,75
	Maximum values	102,00	102,00	120,00	125,00	140,00	130,00	112,00	141,00	121,50
	Estimation of disp. σ	83,14	10,29	85,81	72,67	303,33	141,24	119,81	667,62	-
	Standard deviation Sx	9,12	3,21	9,26	8,52	17,42	11,88	10,95	25,84	-
	Confidence interval	83,39	96,20	96,99	107,69	105,10	97,48	86,03	68,43	-
		96,90	100,95	110,72	120,31	130,90	115,09	102,25	106,71	-
	P(F)	0,61	0,01	0,56	0,62	0,02	0,72	0,84	0,01	-
	P(t)	0,0004	0,0027	0,0066	0,0002	0,0043	0,0055	0,0392	0,4975	-
<i>Startovit</i> stimulant	% relative to control	31,19	26,14	21,98	23,72	31,53	23,18	16,02	9,07	22,82
	Average values	74,43 a	89,71 a	98,29 a	102,86 a	103,43 a	90,14 a	84,43 a	80,29 a	90,45
	Minimum values	58,00	81,00	85,00	87,00	84,00	75,00	65,00	61,00	74,50
	Maximum values	89,00	104,00	107,00	121,00	130,00	106,00	95,00	92,00	105,50
	Estimation of disp. σ	114,95	64,57	72,90	157,81	293,95	119,81	97,29	128,90	-
	Standard deviation Sx	10,72	8,04	8,54	12,56	17,15	10,95	9,86	11,35	-
	Confidence interval	66,49	83,76	91,96	93,55	90,73	82,03	77,12	71,87	-
		82,37	95,67	104,61	112,16	116,13	98,25	91,74	88,70	-
	P(F)	0,38	0,41	0,44	0,17	0,02	0,87	0,96	0,36	-
	P(t)	0,2673	0,0491	0,0352	0,0713	0,0835	0,5080	0,5487	1,0000	-
<i>Startovit</i> stimulant	% relative to control	8,32	14,81	15,44	11,63	15,29	4,47	4,05	0,00	9,37

* The difference is statistically significant if the variants do not share the same letters.

Conclusion

This study evaluated the effect of *T. terrestris* extract supplementation on the foraging flight activity of honey bee colonies over two consecutive beekeeping seasons. The results demonstrate that colonies receiving the plant extract exhibited significantly higher flight activity compared to both the control group and the group treated with the *Startovit* veterinary preparation. The observed differences were statistically significant in both experimental years, indicating a consistent stimulatory effect of the extract.

Although the magnitude of the differences was lower during the second season, probably due to structural changes in the experimental colonies, the overall trend remained stable. The findings therefore support the hypothesis that supplementation with *T. terrestris* extract enhances foraging intensity in honey bee colonies under summer conditions.

From a practical perspective, the use of *T. terrestris* extract as a feed supplement may represent a promising natural strategy for stimulating colony activity during key nectar flow periods. Further studies are required to investigate long-term effects, colony productivity, and potential physiological mechanisms underlying the observed increase in flight activity.

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