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Visitation of *Apis mellifera* L. in Runner Bean (*Phaseolus coccineus* L.) and Its Exposure to Seasonal Agrochemicals in Agroecosystems

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Abstract: Plant species and abiotic factors including season appear to be the most important variables influencing the frequency of visits by honeybees (*Apis mellifera* L.). In the present study, we evaluated the activity of honeybee workers visiting runner bean (*Phaseolus coccineus* L.) local cultivar 'Piękny Jaś'. The runner beans are widely cultivated in south-eastern Poland, and are an important forage plant for honeybees in agroecosystems. We aimed at a comprehensive monitoring of the health of colonies and symptoms in *A. mellifera* in response to acute exposure to pesticides. The most numerous visits of *A. mellifera* were observed at the highest flower opening of the runner bean. A very weak positive correlation was observed between the number of honeybees on *P. coccineus*, the number of visited flowers, the time spent per flower and air temperature. The visitation rates of honeybees were more frequent at mid-day and decreased after 15:00. Signs of poisoning were detected in two out of seven apiaries monitored for acute pesticide exposure symptoms on runner bean plantations. The analysis of dead honeybee samples revealed the acute exposure of honeybees to the imidacloprid (neonicotinoid) and chlorpyrifos (organophosphorus) insecticides, which are highly toxic and banned in the European Union. Hazard quotient (HQ) screening showed an elevated burden of imidacloprid and chlorpyrifos corresponding to 7.1% and 10% of the LDD_{50}, respectively, most likely indicating bee poisoning due to chronic exposure to these substances with contaminated food. Noteworthy was the presence of three fungicides that could pose a risk of poisoning in honeybees.

Keywords: honeybee; runner bean; frequency of visits; pesticide exposure; imidacloprid; chlorpyrifos

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1. Introduction

Referring to the concept of ecosystem services, the western honeybee (*Apis mellifera* L.) performs regulatory (plants pollination), supplying (api-products delivery) and cultural (api-tourism) functions. The most important role is plant pollination, which affects the production and diversity of agriculture, and in the case of wild plants, sustains biodiversity [1,2]. Pollination is a mutualistic relationship between a pollinator and the plant being pollinated, in which the insect benefits from obtaining food and the plant benefits from the flow of pollen between flowers. Thus, floral function refers to the attractiveness of a flower to visitors (pollination quantity), and it concerns the efficiency of pollen transfer during a single visit (pollination quality) [3]. The honeybee is a predominant insect species that enhances agricultural production and yields [4]. The number of pollinator visits necessary for plant pollination is a fundamental metric of crop performance, and it can be determined by developing an effective practice for monitoring flower visitors [5]. However, the number of flower visits depends on pollinator and plant species, as well as abiotic factors [6]. For

this reason, expanding knowledge of pollinator activities and efficacy in individual agroecosystems is crucial. This allows an understanding of how agricultural practices translate into investments in the long-term sustainability of agroecosystems. It can also contribute to standardising pollinator visitation rates per crop [7]. The foraging activity of honeybees relates to worker bees and is associated with their age-related division of labour, as well as the colony's needs. During the first 2–3 weeks after emergence, workers first clean the cells and then begin to perform other activities in the brood nest, such as brood feeding, comb building and food processing [8]. The older workers (>3 weeks old) are involved in foraging, food storage and colony defence until they die at approx. 5 to 7 weeks of age [9]. Foragers rely on a combination of sensory cues, including visual and olfactory cues, such as colours, floral shapes and odours, along with innate or learned behaviours to locate and select floral nectar sources [10].

One aspect of beekeeping management is the transport of honey bee colonies from one forage site to another for pollination services and/or to increase honey yield and produce monofloral honey preferred by consumers [11]. The distances depend on the area of the country and the crops visited [12]. The most extreme cases occur in the USA, where colonies are transported across several states by trucks to various monocultures (e.g., blueberries, cranberries, almonds or citrus) over months [13]. In Europe, it depends on the country. For example, in Spain, about 80% of honeybee colonies are moved over distances of at least 400 km in the summer, from the warmer regions in the south to the northern regions with a milder climate and plants flowering later in the season [14]. In Poland, 25–28% of beekeepers move honeybee colonies during the season each year. About 90–95% of them rely only on one to two additional sources (mainly *Brassica napus* L. var. *napus* and *Robinia pseudoacacia* L.) for their bees. However, there are at least 24 crops whose use is possible and depends on the inventiveness of the beekeepers themselves [15]. Among those crops, runner bean (*Phaseolus coccineus* L.) (Fabaceae) is one of five domesticated *Phaseolus* plant species, native to Central and South America. In Poland, it is grown in small-scale agriculture and cultivated for its edible dry seeds. Additionally, it is valued as an ornamental plant due to its striking inflorescences and the unique characteristics of its leaves, fruits and seeds, including their shape, appearance, size and colour [16]. The runner bean is characterised by its exceptionally long vegetation season with a flowering period spanning nearly 2 months. Flower colours can be white, red, purple or lilac [17]. The inflorescences rise above the leaves, ensuring that the flowers are readily available to visiting pollinators. Moreover, these magnificent flowers serve as a significant source of both nectar and pollen, making them highly attractive to bees [16]. The flowers in a one-hectare area are sufficient to produce approximately 200 kg of runner bean honey [18].

Honeybees, in both migratory and stationary hives, are exposed to a various biological and environmental factors that, acting alone or in combination, affect colony health and mortality [19]. In addition, the risks associated with the movement of honeybees concern the dissemination of honeybee pathogens and pests, spread of resistance genes in pest/pathogen populations, exposure to agrochemicals and their synergistic toxicity, season and geographical region [20]. In addition, honeybees collecting contaminated food resources can serve as bioindicators of pesticide exposure in agroecosystems [21]. The side effects of sub-lethal pesticide exposure include reduced brood comb longevity, the delayed development of adults and their motor skills, reduced memory and behavioural impairments [22]. The extent of pesticide exposure in honeybees varies due to their foraging behaviours and the timing and type of pesticide applications. Therefore, it is crucial to examine the impact of plant protection products used in agroecosystems under the conditions of natural bee foraging. This study was conducted taking into account the aforementioned aspects and involved the analysis of visitation rates of honeybees and their exposure to pesticides on the runner bean. Direct observations were carried out to achieve the following objectives: (i) to contribute to the knowledge of the relationship between *A. mellifera* and runner bean flowers, (ii) determine the activity of honeybee workers visiting runner bean flowers and (iii) assess the risk of *A. mellifera* exposure to pesticides in agroecosystems.

2. Materials and Methods

2.1. Plant Selection

The scarlet runner or runner bean (*Phaseolus coccineus* L. syn. *Phaseolus multiflorus* Willd.) local cultivar 'Piękny Jaś' is an annual climbing vegetable in a moderate climate zone cultivated for edible dry seeds with a high content of protein (approx. 22%), vitamins B_1, B_2, C and E, as well as magnesium (Mg) and phosphorus (P). It is commonly cultivated in south-eastern Poland due to its optimal soil and climatic conditions. The most suitable regions for cultivation are those where the isotherm is 16.5 °C in June, 18 °C in July and 17 °C in August; the frost-free period lasted at least 140 days, and rainfalls in August did not exceed 90 mm [16,23]. Moreover, the honey made from the nectar of the 'Piękny Jaś' variety of runner bean flowers is on the traditional product list of the Polish Ministry of Agriculture and Rural Development, which includes products whose quality or unique characteristics and properties result from the use of traditional production methods [24].

2.2. Visitation Rate

The study was conducted in Lubelskie Voivodeship, i.e., south-eastern Poland, in 2020. Honeybee foraging data were collected in the field of an average size of 1.4 ha located in the village of Dyniska (50°24′45.1″ N; 23°42′59.0″ E). The experimental field was bordered by several trees to the north, a wheat field to the east and farm buildings to the west and south. There were no large areas with other melliferous plants within the honeybees' flight zone; only a home garden with melliferous ornamental plants was present. Despite the close proximity of the garden to the runner bean plantation, it did not pose a risk to the intensity of bean flower pollination by honeybees. There were two apiaries belonging to the beekeeping farm "Bees gifts", one consisting of 12 hives located 300 m to the east, and the other with 8 hives located 500 m to the west of the runner bean plantation. The runner bean was sown at the end of April on land that had previously been used for wheat cultivation. The spacing between rows was 45 cm. Weeds were controlled manually and no pest control practices were applied.

The observations were conducted from 30 June to 1 August, during the flowering of the runner bean, which was divided into three periods: (1) the beginning of blooming (flowers buds outnumbering developed flowers), (2) peak of the flowering period and (3) end of blooming (appearing of pods). Five plots (replicates; transects) of the runner bean, each 5 m long and 1 m wide, were selected in the study area. The survey involved the daily counting of bees and flowers they visited during a single walk along each plot at 9:00, 12:00, 15:00 and 18:00 UTC + 2 h. A flower was considered visited when a honeybee landed on it and extended its proboscis towards the flower. The number of flowers visited by one honeybee was counted until it disappeared from sight. To measure foraging activity, a stopwatch was used. The time spent by a single honeybee on nectar collecting was tracked, from inserting its proboscis to withdrawal. Honeybees that perched on flowers to clean themselves or perform other activities were not taken into account. In addition, prevailing weather conditions (temperature, humidity, wind strength and direction, cloud cover and rainfall) were recorded using an outdoor thermometer placed at the farm building close to the runner bean plantation as well as the "weather & radar" phone application during each sampling period.

2.3. Monitoring of Honeybee Health and Behaviour including Signs of Pesticide Poisoning

The field assessment regarding the risk of *A. mellifera* being exposed to pesticides in agroecosystems was based on a comparison of the condition of these insects in seven test apiaries (owned by the family apiary "Bees gifts" from Dyniska) in 2021. Our goal was to determine what substances honeybees were exposed to in agrocenoses (in different sites), assuming that farmers apply the principles of good agricultural practice. This is valuable for beekeepers to estimate the hazard to which honeybees are exposed. Healthy and properly developing honeybee colonies housed in Wielkopolska-type hives, equipped with multifunctional bottoms with hygienic inserts for monitoring honeybee] mortality,

were selected for the experiment. There were 20 honeybee colonies in each apiary, resulting in 140 honeybee colonies being examined in total. These selected colonies were introduced into seven runner bean plantations in the districts of Tomaszów and Hrubieszów (Lubelskie Voivodship). The honeybee colonies were introduced to these plantations at the early onset of the flowering period of this plant (28 June). Locations of these planation are presented in Table 1.

Table 1. Precise location of *Phaseolus coccineus* L. plantations where honeybee colonies were introduced during 2021.

Site	Site Name	Coordinates
1	Korczmin Osada	50°25′41.3″ N, 23°52′15.5″ E
2	Wasylów	50°32′32.1″ N, 23°52′20.4″ E
3	Żerniki	50°28′14.2″ N, 23°43′40.7″ E
4	Dutrów	50°32′30.5″ N, 23°50′33.2″ E
5	Machnówek 1	50°24′59.2″ N, 23°54′39.8″ E
6	Machnówek 2	50°25′59.8″ N, 23°54′20.0″ E
7	Łasków	50°37′45.4″ N, 23°56′23.8″ E

The mean distance between particular sites was approximately 22 km, while the minimum distance between Site 5 and Site 6 was 2 km. Individual plantations were surrounded by wheat, maize, sugar beets or carrot crops. The health of the implemented honeybees colonies was assessed on a weekly basis. This assessment included the evaluation of flight activity at the hive entrance, behaviour of honeybees, overall fitness of the colonies, signs of honeybees poisoning and mortality. The measurement of flight activity at the hive entrance was conducted via a visual assessment of individuals entering and exiting the hive. Honeybees were counted in the afternoon between 14:00 and 15:00 in 5 min intervals until no bees entered or left the hive during three consecutive observations. Flight activity was scored as follows: low (1–15 incoming foragers), medium (16–30 incoming foragers) and high (>30 incoming foragers). The behaviour of bees in the apiary was defined as follows: very calm (normal movements), calm (higher overall activity; wing buzzing), aggressive (rapid movements; threatening behaviour towards the target) and very aggressive (aggressive rapid movements; stinging and defection). Colony fitness was assessed on the basis of average nest size (the number of honeybees in the hive and the number of frames occupied by honeybees), and it was defined as follows: poor (1–2 occupied frames), moderate (3–4 occupied frames), good (5–6 occupied frames) and very good (7–8 occupied frames).

The signs of poisoning taken into account were the following: extended proboscis, expanded wings, tucked up abdomen, extended/curled legs, the gathering of bees on grass and hive walls, and uncoordinated and uncontrolled movements [25]. The mortality of honeybees was determined by counting dead bees in front of each hive and then dividing this count by the number of hives in the apiary.

Honeybee samples from two locations, Dutrów (Site 4) and Łasków (Site 7), with suspected poisoning from plant protection products were handled in the presence of a veterinarian according to the relevant guidelines developed and provided by the National Veterinary Research Institute—State Research Institute in Puławy, Poland [26], where chemical residue analysis was performed. Hazard quotients (HQs) were calculated by directly dividing the concentration of pesticides in honey bees by the acute oral median lethal dose (LD_{50}), in the worst case from 24, 48 and 72 h values, or 1the 0-day chronic median lethal dietary dose (LDD_{50}), and screening benchmarks such as relevant pesticide load (a HQ greater than 50) or elevated pesticide load (a HQ greater than 1000) were adopted next for the interpretation of the results [27,28].

After observing the poisonings, honeybees (adult and larvae) were fed with warm syrup in the form of a mixture of sugar and water (3:2) without any residues of harmful substances.

2.4. Pesticide Residue Analysis

Dead honeybees were analysed for the residues of 200 pesticides and their metabolites via the dedicated acetate buffered QuEChERS method developed and validated for poisoned honeybee samples [29]. The method is used within the framework of Poland's functioning system for diagnosing cases of suspected bee poisoning by pesticides, implemented on behalf of the Ministry of Agriculture and Rural Development. Samples of poisoned honeybees were collected by official commission designated to authenticate each poisoning case. A veterinary inspector was responsible for the overall inspection of the colony's health and the collection of honeybee samples. A plant health and seed inspector examined whether or not plant protection products were used properly in the surrounding area. A beekeeper was represented by a member of the beekeepers association. Samples of dead honeybees were pooled per apiary and stored on dry ice, then brought to the laboratory where these were frozen in liquid nitrogen until being processed. Briefly, a 5 g sample of thoroughly homogenized dead honeybees was spiked with a mixture of internal standards, 10 mL of water and glass beads were added and the sample was shaken in a vertical mechanical disrupter for 3 min. Samples were extracted by adding 10 mL of a 1% acetic acid mixture in acetonitrile and shaking them in the mechanical disrupter for 3 min. Afterwards, 1 g of sodium acetate and 4 g of magnesium sulphate were added and the sample was shaken and centrifuged at 3500 rpm and −12 °C for 20 min. Later, 7 mL of the supernatant was cleaned up using a dispersive solid-phase extraction sorbent mixture containing 350 mg of PSA, 350 mg of Z-Sep+ and 1050 mg of $MgSO_4$. After shaking and centrifugation, part of the supernatant was analysed via liquid chromatography tandem mass spectrometry (LC-MS/MS). Another part of the supernatant was concentrated, solvent-exchanged to n-hexane and analysed via gas chromatography tandem mass spectrometry (GC-MS/MS). LC-MS/MS analyses were carried out using an Agilent 1260 HPLC system (Waldronn, Germany) equipped with a Phenomenex Luna Phenyl-Hexyl (3 micron, 150 mm × 2.0 mm) column and AB Sciex QTRAP® 6500 mass spectrometer (Framingham, MA, USA). GC-MS/MS analyses were carried out using Agilent Technologies 7890A+ gas chromatograph (Palo Alto, CA, USA), equipped with a 7693B series autosampler, split/splitless injector and 7000B tandem mass spectrometry detector. Chromatographic separation was achieved on a HP-5MS UI capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness, Agilent, Santa Clara, CA, USA). The LC-MS/MS and GC-MS/MS parameters were adopted as used in a previous study [29].

2.5. Statistical Analysis

The non-parametric Kruskal–Wallis test was applied after rejecting the normality assumption of the data for the mean number of honeybees (NB), mean number of visited flower by one honeybee (NVF) and mean foraging time of one honeybee (FT). Afterwards, post hoc multiple comparisons were applied. The strength of the relationship between weather conditions (temperature and humidity) and NB, and NVF and FT was estimated using Spearman's rank correlation coefficient (r_S). All statistical analyses were performed using Statistica for Windows v. 13.1 (StatSoft, Kraków, Poland) [30] with a significance threshold at $\alpha = 0.05$.

3. Results

3.1. Visitation Rate

The honeybees visited the flowers of runner bean during all blooming phases from 30 June to 1 August; however, the intensity of visits varied across the flowering stages. The number of visits during the observation period exhibited significant variation ($H_{(30,n=620)} = 206.12$, $p < 0.001$). Likewise, the mean number of flowers visited by one honey bee ($H_{(30,n=620)} = 184.29$, $p < 0.001$) and mean time spent on flowers during foraging ($H_{(30,n=620)} = 158.53$, $p < 0.001$) also showed significant differences. Between 30 June and 14 July (beginning of the flowering) and after 26 July (end of flowering), the presence of honeybees on the plantation was low. During the initial flowering period of *P. coccineus*,

individual bees were observed to visit an average of five to six flowers, spending approximately 8–12 s on foraging activity (Figure 1a–c). *A. mellifera* visits were the most numerous between July 18 and 23, when the number of opened flowers was the highest. During this period, the highest mean number of honeybees observed within a 5 min interval was 17.5 (Figure 1a). It should be emphasized that between 18 and 22 July, one honeybee visited only 1.9–3.5 flowers (Figure 1b), spending as long as 25–48 s foraging (Figure 1c). The highest humidity (83%) during this period was recorded on 18 July, with subsequent days showing a gradual decrease to 41% by 22 July. The average air temperature during the hours of bee activity ranged from 20 to 25 °C (Figure 1a–c).

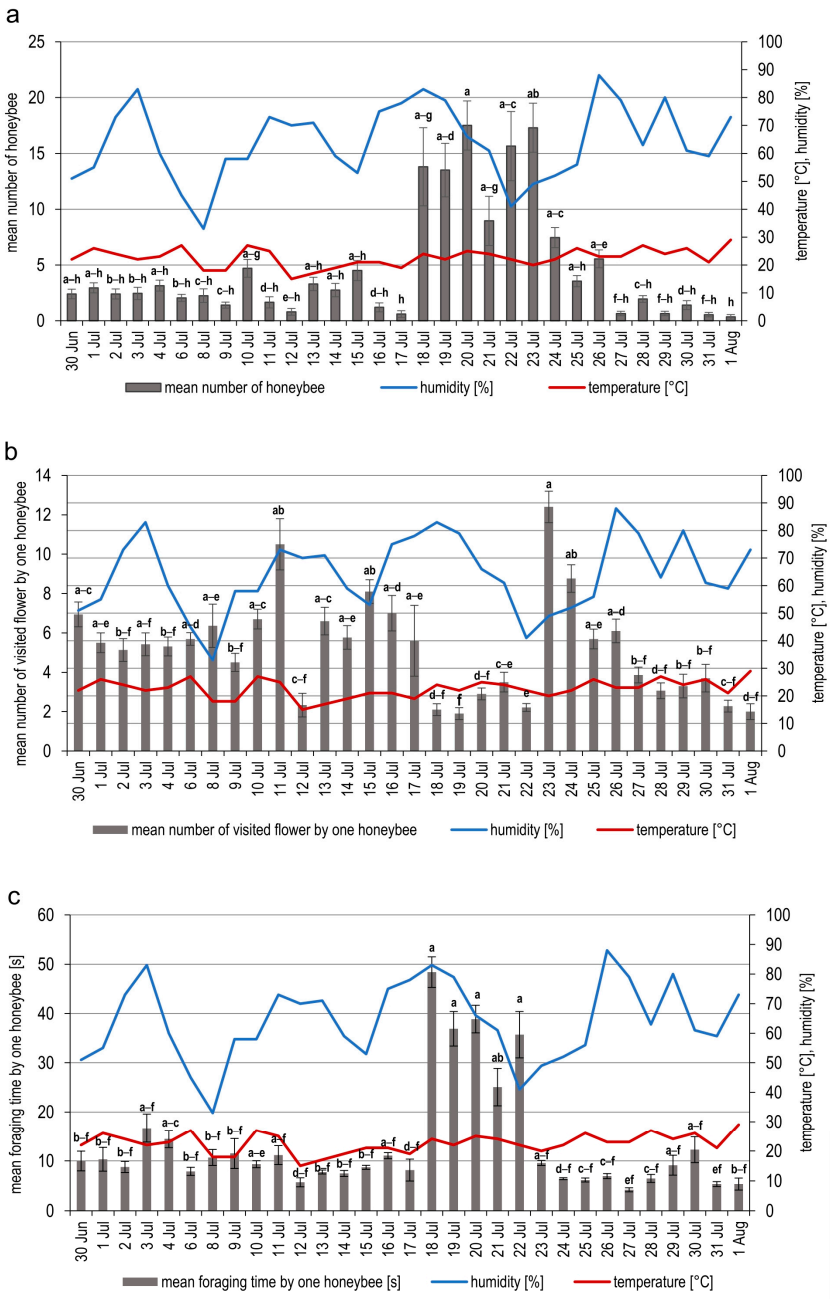

Figure 1. Seasonal variation of *Apis mellifera* L. visiting flowers of *Phaseolus coccineus* L.—mean number (±SE) of honeybees (**a**), mean number (±SE) of flowers visited by one honeybee (**b**) and mean foraging time (±SE) by one honeybee (**c**). Values with different letters are significantly different at $p < 0.05$, applying multiple comparisons after a non-parametric Kruskal–Wallis test.

Significant differences were found in the mean number of bees visiting runner bean flowers, the mean number of visited flowers and the duration of foraging at a particular time of the day (Table 2). During the observations, honeybees visited flowers from 9:00 to 18:00. One honeybee visited 1.2 to 4.47 flowers on average, depending on the flowering period and time of the day. Honeybees visited runner bean flowers in greater numbers at mid-day, and their abundance significantly decreased after 15:00 (Figure 2a). No differences were observed in the mean number of flowers visited by one honeybee between 9:00 and 15:00, but at 18:00, they visited significantly less flowers (Figure 2b). The duration of honeybee foraging activity varied throughout the day and lasted from 3.67 to 13.03 s. It was markedly higher at 12:00, while at 9:00 and 15:00, honeybees spent similar amounts of time per flower for nectar collection. The significantly shortest foraging time was recorded in the late afternoon (Figure 2c).

Table 2. Test statistics for mean number of *Apis mellifera* L. (NB), mean number of flowers visited by one honeybee (NVF) and time spent on flowers during foraging (FT) for observation hours during the day.

Response Variable	Kruskal—Wallis Test			
	H	***n***	**df**	***p***
NB	98.2419	620	3	$p < 0.001$
NVF	94.0695	620	3	$p < 0.001$
FT	81.5352	620	3	$p < 0.001$

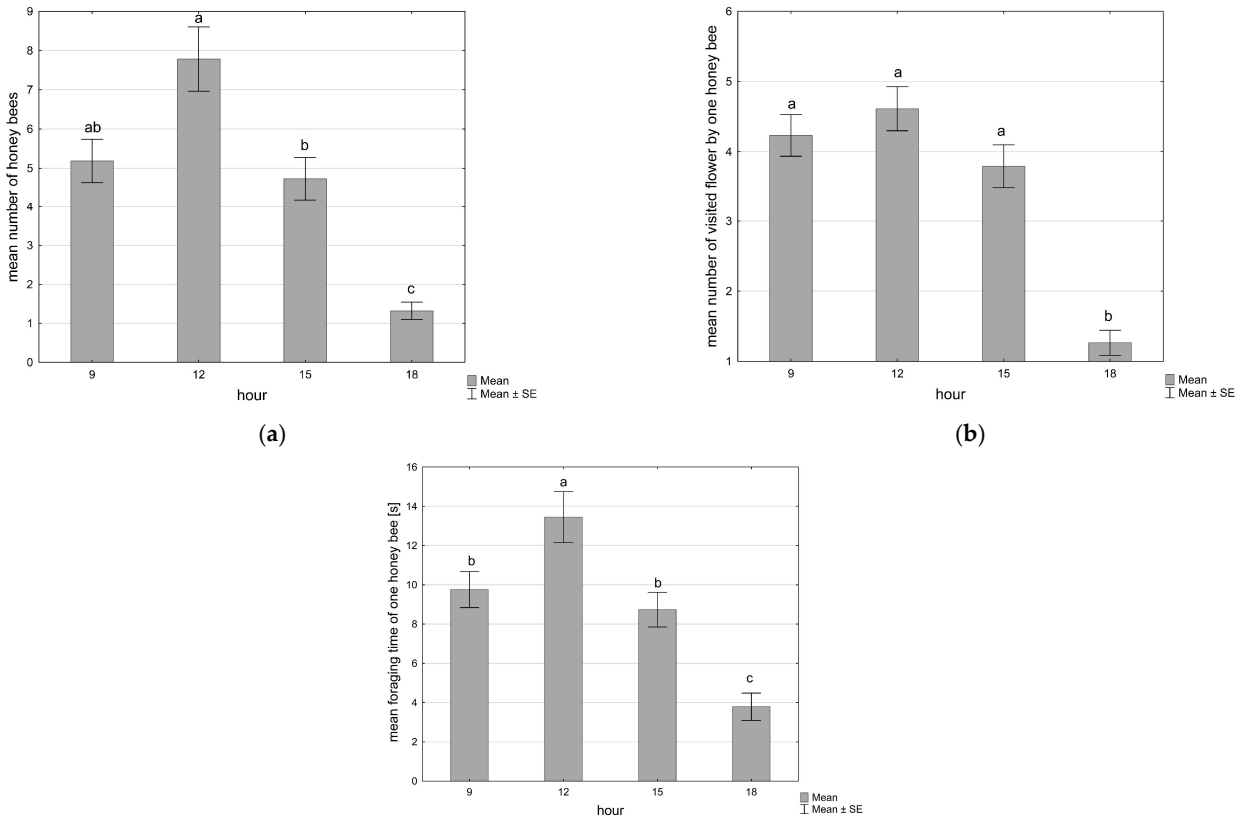

Figure 2. The daily pattern of *Apis mellifera* L. visits to *Phaseolus coccineus* L. flowers—mean number (±SE) of honeybees at tested plots (**a**); mean number (±SE) of flowers visited by one honeybee (**b**); mean foraging time (±SE) of one honeybee (**c**). Values with different letters are significantly different at $p < 0.05$, applying multiple comparisons after a non-parametric Kruskal–Wallis test.

The total number of visits was strongly positively correlated with feeding time. A weak positive correlation was found between the number of honeybees (NB), number of flowers visited by one honeybee (NVF), feeding time of one honeybee (FT) and temperature. A weak negative correlation was found between relative humidity and NVF (Table 3). There were 13 days with rainfall and strong gusts of wind, which made it difficult for honeybees to fly. On days when rainfall persisted from morning to noon, honeybee activity in the subsequent hours was very low (max. four individuals) or no insects were observed. During this time, one honeybee visited a maximum of five flowers, with the longest foraging time being 8.48 s.

Table 3. Correlation coefficients obtained between temperature (T), humidity (H), number of honeybees (NB), number of flowers visited by one honeybee (NVF) and feeding time of one honeybee (FT) observed on runner bean (*Phaseolus coccineus* L.).

Trait	T	NB	NVF	FT
H	−0.184 *	−0.054	−0.295 *	−0.011
T		0.114 *	0.089 *	0.149 *
NB			0.339 *	0.781 *
NVF				0.205 *

* significant at $p < 0.05$.

3.2. Comprehensive Monitoring of Honeybee Health and Behaviour including Signs of Pesticide Poisoning

In the first week of observation (28 June), honeybees were moderately active and their behaviour in all apiaries was very calm (Table 4). The colonies developed properly, with brood present at all stages of development, showing no signs of underdevelopment. The flying activity of bee workers increased as the number of developed runner bean flowers increased. During the second week of observation on 6 July, honeybees on *P. coccineus* plantations at Sites 1, 2, 3, 5 and 6 continued to work normally and developed well. No signs of poisoning were observed in the bees. However, during the inspection of the apiary located at Sites 4 and 7, typical symptoms of honeybee poisoning were noted. There was a significant increase in the number of deceased honeybees in front of the hives and on the frames under the roof of hives. Over 50 dead individuals per hive were noted in Site 7 and 44 deceased bees in Site 4 during one inspection (Table 4). These honeybees exhibited extended proboscis, raised abdomens and curled legs. The aggressiveness of surviving honeybees towards siblings and depopulation was also recorded from the bees of Site 4 and Site 7. We also observed that honeybees returning from the field were removed from their hives by patrolling guards or housekeeping bees. They were grouped on the grass or on the outer walls of the hive. These affected honeybees displayed paralyzed, folded wings, positioned unnaturally at rest, rendering them unable to fly. During the subsequent apiary inspection on 13 July, we continued to observe abnormalities in the hives. Honeybees in Sites 4 and 7 were still aggressive, but their mortality had decreased (Table 4). The flight activity of honeybees remained at a low level, only with sporadic flights out of the hive. Symptoms of damaged brood were also observed, potentially resulting from poisoning through contaminated food sources. In the following weeks, the mortality decreased to 7–10 individuals/hive in the poisoned apiary, while the flight activity of bees increased. Honeybees were fed warm water–sugar syrup and their condition began to improve. In turn, honeybee colonies at Sites 1, 2, 3, 5 and 6 developed dynamically, showing no signs of intoxication during their time on runner bean plantations.

Table 4. The assessment of the health of honeybees (*Apis mellifera* L.), their behaviour and signs of poisoning depending on the location of the apiary on runner bean (*Phaseolus coccineus* L.) plantations.

Site	Date	Average Mortality (Individuals/Hive)	Behaviour	Flight Activity *	The Colony Fitness	Signs of Poisoning/ Visible Abnormalities
1	28.06	≤5	very calm	++	very good	not visible
	06.07	≤5	very calm	+++	very good	not visible
	13.07	≤5	very calm	+++	very good	not visible
	19.07	9	very calm	++	very good	not visible
	26.07	7	very calm	++	very good	not visible
2	28.06	8	very calm	++	very good	not visible
	06.07	≤5	very calm	+++	very good	not visible
	13.07	10	very calm	+++	very good	not visible
	19.07	≤5	very calm	++	very good	not visible
	26.07	≤5	very calm	++	very good	not visible
3	28.06	15	very calm	++	very good	not visible
	06.07	≤5	very calm	+++	very good	not visible
	13.07	≤5	very calm	+++	very good	not visible
	19.07	≤5	very calm	++	very good	not visible
	26.07	≤5	very calm	++	very good	not visible
4	28.06	10	very calm	++	very good	not visible
	06.07	44	aggressive	+	poor	present/extended proboscis, expanded wings
	13.07	15	very aggressive	+	poor	present/young bees remove the larvae from the hive
	19.07	9	very calm	++	moderate	not visible
	26.07	7	very calm	++	moderate	not visible
5	28.06	≤5	very calm	++	good	not visible
	06.07	≤5	very calm	+++	very good	not visible
	13.07	≤5	very calm	+++	very good	not visible
	19.07	≤5	very calm	++	very good	not visible
	26.07	≤5	very calm	++	very good	not visible
6	28.06	7	very calm	++	good	not visible
	06.07	≤5	very calm	+++	very good	not visible
	13.07	8	very calm	+++	very good	not visible
	19.07	10	very calm	++	very good	not visible
	26.07	≤5	very calm	++	very good	not visible
7	28.06	≤5	very calm	+++	very good	not visible
	06.07	≥50	aggressive	+	poor	present/no flying bees, dead bees with outstretched tongues, large losses of bees in the hives, uncoordinated and uncontrolled movements
	13.07	44	calm	+	poor	present/large losses of bees in the hives
	19.07	10	very calm	+	poor	not visible
	26.07	≤5	very calm	++	moderate	not visible

* + low (1–15 incoming foragers), ++ medium (16–30 incoming foragers) and +++ high (>30 incoming foragers).

In apiaries where honeybee poisoning was not observed, honey was collected on 21 July. The average amount of honey was 28–30 kg per hive. On 26 July, a final inspection of honeybee colonies was carried out before they were transported to their wintering site. Poisoned colonies from Site 4 and 7 were excluded from the production of honey and other honeybee products.

3.3. Pesticide Residues in Dead Honeybee Samples

Chemical analyses of the poisoned honeybees revealed the residues of seven pesticides including three insecticides (chlorpyrifos, imidacloprid and p,p'-DDT), three fungicides (azoxystrobin, carbendazim and difenoconazole) and a herbicide, pendimethalin (Table 5). Pesticides analysed in honeybee samples but not quantified in terms of concentrations above respective LOQ values are listed in Table S1 (Supplementary Materials). The sample of dead honeybees from Site 4 contained residues of three pesticides, while the sample from Site 7 contained residues of as many as six pesticides at one time. Pesticides were determined at low concentrations, slightly above the limits of quantification of the analytical method. Observed poisoning symptoms indicated honeybees' exposure to pesticides with food, so reference values of the acute oral median lethal dose (LD_{50}) or chronic median lethal dietary dose (LDD_{50}) from the literature data were used to further evaluate the results obtained. Only two pesticides showed any HQ value and these were the insecticides chlorpyrifos and imidacloprid. The concentration of chlorpyrifos in dead honeybees when considering their acute oral exposure corresponded to a HQ value of 13, while when interpreted under a chronic oral exposure scenario, it corresponded to a HQ value of 1000. In the case of imidacloprid, its concentration value in dead honeybees corresponds to an HQ value of 541 and 709 for acute and chronic oral toxicity, respectively. The concentrations of the other pesticides whose residues were determined in the dead honeybee samples did not indicate any risk from them in the interpretation based on HQ.

Table 5. Results of the pesticide residue analysis in samples of dead honeybees together with the classification and toxicity of particular pesticides.

Pesticide			Toxicity Endpoints		Concentration in Dead Bees ± Expanded Uncertainty (mg/kg)		HQ	
Name	**Type, Systemicity**	**Group**	**Oral Acute LD_{50} (µg/Bee)**	**Oral Chronic 10-day LDD_{50} (µg/Bee/Day)**	**Site 4**	**Site 7**	**Acute Oral**	**Chronic Oral**
Azoxystrobin	fungicide, systemic	strobilurin	>25 [31] *	–	–	0.0030 ± 0.0015	0	-
Carbendazim	fungicide, systemic	benzimidazole	>100 [31] *	–	0.0020 ± 0.0010	0.0010 ± 0.0005	0	-
Chlorpyryfos	insecticide, non-systemic	organophosphates	0.15 [31] *	0.002 [31] *	–	0.0020 ± 0.0010	13	1000
Difenoconazole	fungicide, systemic	conazole	>177 [31] *	–	–	0.0010 ± 0.0005	0	-
Imidacloprid	insecticide, systemic	neonicotinoids	0.0037 [31,32] *	>0.00282 [32] *	0.0020 ± 0.0010	–	541	709
p,p'-DDT	insecticide, non-systemic	organochlorine	5.1 [31] *	–	–	0.0020 ± 0.0010	0	-
Pendimethalin	herbicide, systemic	dinitroanilines	>101.2 [31] *	>96.5 [31] *	0.0020 ± 0.0010	0.0020 ± 0.0010	0	0

* references.

4. Discussion

P. coccineus flowers are known for their high secretion of nectar (ca. 41 mg of sugars per 10 flowers) [33], making them part of an excellent melliferous plant that can support

beekeeping during summer [16]. However, this plant has been the subject of few scientific investigations focused specifically on its role as a forage plant for pollinators. The available studies have primarily examined the diversity and number of floral visitors [33,34], the pollination efficacy of bumble bees and honeybees [35] and their behaviour [36]. There have also been investigations into the foraging and pollination behaviour of species belonging to the genus *Xylocopa* as the most common solitary bee visiting runner bean flowers [37,38]. According to the available literature, various pollination insects are attracted to the runner bean as a forage plant, but the dominant species are *A. mellifera* and species belonging to the genus *Bombus* [33]. In our study, the number of honeybees visiting the runner bean was the highest when the plants were in the peak of the flowering period. This dependency was confirmed in studies conducted, among others, on *Crocus vernus* (L.) Hill [39], *Fagopyrum esculentum* Moench [40] or *Syzygium guineese* var. *guineese* [41]. Larger-display plants are more easily recognized by honeybees and are perceived as offering greater rewards in terms of pollen and nectar [42], which likely explains the observed preference for runner bean flowers by honeybees. On the other hand, learning and memory are also vital components of honeybee foraging behaviour. Honeybees must learn the locations and times of the day when sustainable food sources are available to make repeated visits and increase their numbers [8,10]. The foraging activity of honeybees throughout the day depends on a combination of factors that include temperature, light intensity, insect behaviour and physiological state, amount of floral resources [43], floral structure, corolla depth [44] and flower volatiles [45]. Our analysis of the daily pattern of honeybee visits to runner bean flowers revealed that both the number of honeybees and the number of visited flowers were influenced by the time of day and peaked at 12:00. Before and after that period, foraging activity was less intense. Our results are generally consistent with those of previous research by Alves et al. [46], Ribeiro et al. [47] and Khan et al. [48], who observed the foraging activity of *A. mellifera* on different plants under field conditions. Interestingly, this foraging activity does not necessarily correlate with the period of the highest availability of nectar in the flowers of *P. coccineus*. According to Kołtowski [33], freshly opened flowers in the morning contain nearly 70% of the total sugar content, with sugar resorption starting in the evening. The nectar/sugar content in flowers can be used as a measure of reward for honeybees, and determines their flower fidelity [49].

Another aspect that we studied was the length of time a honeybee spent on the flower. The average duration of a visit per flower varied throughout the blooming period, ranging from just a few seconds to as long as 1.5 min. The time spent per flower also varied throughout the day and was significantly longer in the middle of the day. Hennessy et al. [50] observed that an increasing wind speed led to greater flower movement, which significantly increased the time spent by honeybees on one flower. This resulted in a reduction in the number of flowers visited. In addition, the availability of nectar prolongs the time spent by pollinators on a particular flower [51].

The importance of temperature and relative humidity for the internal and external activities of honeybees is well documented [52,53]. These factors can also affect the quantity and concentration of sugar in the flower nectar, subsequently affecting the foraging behaviour of honeybees [54]. In our study, we found a very weak positive correlation between the number of honeybees on the runner bean, the number of flowers visited, the time spent per one flower and air temperature. A weak negative correlation was observed between relative humidity and the flight activity of *A. mellifera*. Several authors observed a positive correlation between temperature and the flight activity of honeybees [46,55,56]. For instance, Begna et al. [56] described that honeybee visitation rates were relatively high and stable when temperatures ranged from 20 to 25 °C, with honeybees peaking at 29–33 °C. In our study, the temperature during individual observations was generally within the optimal range for honeybee activity, typically between 20 and 29 °C.

The abundance of honeybees in agroecosystems can be negatively affected by field composition and configuration, as well as by local farming practices, particularly the use of pesticides. Consequently, beekeepers must make informed decisions regarding the

placement of their apiaries within intensive agroecosystems [57]. In Poland, the rate of honeybee losses caused by pesticide poisoning was reported to be 9825 colonies in 2020 [58]. Most poisoning incidents were associated with the spraying of oilseed rape (especially spraying against the rape pollen beetle, *Brassicogethes aeneus* F.). There were also a few reported cases of bee poisoning linked to fruit trees, beans and berry crops (e.g., raspberry). During 2020–2021, the application of fungicides containing active substances like captan, fludioxonil, azoxystrobin, tebuconazole, difenoconazole, fludioxonil, trifloxystrobin and fluopyram, as well as insecticides with active substances such as acetamiprid, deltamethrin and cypermethrin, was permitted in bean crops in Poland [59].

Research on the impact of pesticides on pollination insects has focused on their mortality effect; however, a risk assessment of sublethal effects is also important because they can affect the stability of bee populations [60]. Lethal doses of pesticides are indicators of acute exposure and the direct effects of this can be observed within as little as 45 min after exposure [61]. When pesticide levels are sublethal, honeybees survive, but their activity and behaviour are disturbed even at low pesticide concentrations that may not be readily detected through chemical analysis [62]. For this reason, honeybees and their associated matrices subjected to low pesticide exposure can be used for the short- and long-term monitoring of their health status and behaviour [63]. In the present study, we monitored honeybee colonies in runner bean crops to collect information on the overall health status of honeybees and to determine whether or not plant protection products used in the agroecosystems of south-eastern Poland might be contributing to colony losses. These studies are in line with the current concept of monitoring apiaries to examine regional differences in the effects of pesticide exposure on honeybees' health. Our results showed that colony mortality was associated with the presence of highly toxic imidacloprid and chlorpyrifos. The interpretation of pesticide concentrations in bee samples using HQ revealed that imidacloprid and chlorpyrifos showed relevant a pesticide load (a HQ greater than 50). HQ 50+ was successfully applied in the interpretation of pesticide load in honeybees, which enabled an interpretation of whether concentration of pesticides found in dead honeybees indicate poisoning or not [28]. The concentration of chlorpyrifos in dead bees interpreted under a chronic oral exposure scenario corresponded to a HQ value of 1000. A HQ greater than 1000 showed an elevated pesticide load [27]. A previous study showed that the maximum HQ calculated for live honeybee samples was 181 and HQ values greater than 1000 were indicated only in the case of poisoned honeybee samples [28]. Therefore, the concentration of imidacloprid interpreted through HQ indicates that it is the cause of honeybee poisoning at Site 4 in both chronic and acute oral exposure scenarios. Chlorpyrifos concentrations interpreted through HQ can only be considered a cause of bee poisoning at Site 7 in the case of a scenario of chronic oral exposure to its residues.

Numerous studies support the notion that neonicotinoids, including imidacloprid, are the primary cause of abnormal foraging behaviour and the main source of poisoning in agroecosystems [64–66]. Imidacloprid has an exceptionally low lethal dose value, with an oral LD_{50} of 0.007 µg/bee and a contact LD_{50} of 0.018 µg/bee [67]. Thus, the use of the neonicotinoids has been progressively restricted in the European Union since 2013, culminating in a complete ban [60,68,69]. Poisoning symptoms observed in honeybees, in which imidacloprid was detected, included initial aggressive behaviour, followed by slower movements, an extended proboscis and expanded wings. Similar findings have been reported in other works [70]. In our study, the risk of poisoning could have been increased due to contamination with imidacloprid and the fungicide carbendazim. According to Tong et al. [71], fungicides, including carbendazim, posed a moderate risks to honeybees when sprayed in oilseed rape fields; however, it is well documented that they can increase the toxicity of neonicotinoids through a synergistic toxic effect [72,73]. Of particular concern is the presence of carbendazim in our samples, given that it has been banned in the European Union since 2016 [74] due to its toxicity and persistence. The detection of this substance in our samples in 2021 can be explained in several ways. Although carbendazim is a non-approved active substance, it is a metabolite of thiophanate methyl; thus, the

presence of its residues could be attributed to the use of plant protection products containing thiophanate-methyl, which was non-renewed according to Regulation (EC) No 2020/1498, with a maximum grace period until 3 February 2021 [75]. Moreover, carbendazim persists in bare soils for a long time due to its slow degradation, with half-lives ranging from 6 to 12 months on bare soil and 3 to 6 months on turf [76]. Lastly, there is also a possibility of illegal and unregistered pesticide use by farmers, as attempts to use banned or counterfeit pesticides have occurred and been documented in Poland [77].

In one of our observation areas, we detected chlorpyrifos and three fungicide residues (carbendazim, azoxystrobin and difenoconazole) in dead honeybee samples. Chlorpyrifos is a highly persistent organophosphate whose toxicity through oral exposure (LD_{50}) has been reported to be 0.33 µg/honeybee over 48 h [78]. The observed acute toxic effect on honeybee colonies expressed via the high number of dying/dead individuals in front of the hive was indicative of the intensive use of pesticides at this site. These results are consistent with findings reported by Porrini et al. [79], Kiljanek et al. [28] and Kadalikova et al. [69]. The fungicides azoxystrobin and difenconazole are commonly applied in agriculture and are considered to have low acute toxicity for honeybees [80,81]. However, morphological damage in the midgut of honeybees exposed to azoxystrobin was reported [82], as were significant effects of difenconazole on the survival, morphology and immunity of honeybee larvae [83] and delayed metabolic disruptions, especially when honeybees were exposed to residual concentrations of other pesticides (imidacloprid and glyphosate) [84]. There is increasing evidence that fungicides are not completely safe for honeybees, and the toxicity criteria for fungicides should be re-evaluated.

Residues of pendimethalin were also found in samples of dead honeybees at both sites. Residues of this herbicide were also detected in wax, pollen and bee samples from North America and Germany [85–87]. According to He et al. [88], exposure to pendimethalin had no significant effect on the development of honeybee larvae; however, its detection in honeybees and hive products seems to pose a risk to honeybees. We also detected DDT derivatives (residues of p,p' DDT) in samples of dead honeybees. Its presence in honey and beeswax samples has been reported previously [89–91]. The use of DDT has been banned in Europe for decades; however, its metabolites are still present in the environment and can be picked up from soil and plant surfaces by foraging honeybees [92].

In conclusion, this study underscores the significance of environmental monitoring. Both aspects examined and discussed in this research are important because agroecosystems can vary from season to season, affecting the quality, ease of access and time of availability of their resources. A systematic sampling approach to studying honeybee activities in a specific agroecosystem allows the gathering of more data to support risk assessments and improve honeybee health under local conditions and agricultural practices. Pesticide hazards to honeybees during crop pollination can be significant, making it vital to understand where honeybees come into contact with high-risk pesticides in agroecosystems [93]. This issue appears to be more acute in landscapes dominated by annual crops that undergo intensive pesticide applications [94].

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture13112138/s1. Table S1: List of analysed pesticides with their category of use (AC—acaricide; AL—algicide; VMP—veterinary medicinal product; FU—fungicide; HB—herbicide; IN—insecticide; MO—molluscicide; NE—nematicide; PG—plant growth regulator; RE—repellent; ST—soil treatment), their metabolites (MET) and LOQ values.

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