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EFFECT OF FOLIAR FERTILIZERS APPLICATIONS ON THE NECTAR PRODUCTION OF SUNFLOWER (*Heliathus Annuus L.*) AND ON THE FORAGING BEHAVIOUR OF HONEYBEES (*Apis Mellifera L.*)

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Abstract: The effect of fertilizer applications on nectar production and foraging behaviour of honeybees were investigated in four sunflower hybrids at two locations (Mocsa, Komárom-Esztergom County; and Töltéstava, Győr-Moson-Sopron County) in Hungary in the years 2014 and 2015. Four foliar fertilizers $[Cu(NH_3)_4]^{2+}$+OH^{2-}+$CaCO_3$, $ZnCO_3$+$MgCO_3$, $[Cu(NH_3)_4]^{2+}$+OH^{2-}, and $Zn(OH)_2$+$ZnCO_3$ produced from industrial by-products at high purity were applied. The production of sunflower nectar was performed with glass capillaries prepared for sampling. We measured 24-hours nectar production from insect-isolated heads. The nectar samples were taken from the flowering circles of the same developmental stage and in the flowering phase. The daily distribution of bee visits was carried out every two hours between 8:00 and 16:00 in both years. Foliar fertilizer applications containing copper and zinc significantly raised the nectar production of sunflower hybrids ($p < 0.05$) and the higher nectar content of flowers significantly increased the honeybee visitation at the treated plots ($p < 0.05$). Their physiological effect to sunflower plants foliar fertilizer applications increased the nectar production of the experimental sunflower hybrids. Significantly more nectar gatherers were observed at plots treated with foliar fertilizers. Increased bee visitations caused significant increase in the average time that honeybee foragers spent in the flowering heads. More abundant honeybee populations with higher number of nectar foragers would be resulted in higher honey yields, too.
Keywords: bee visitation, foliar fertilizer, foraging behaviour of honeybees, nectar production, pollination, sunflower

1. INTRODUCTION

Sunflower – behind soybean - is the second most important entomophilous oil plant in the world (Pham-Delegue *et al.*, 1990; Corbet *et al.*, 1991; Yadav *et al.*, 2002; Baydar and Erbaş, 2005). Hybrid sunflower seed production is a special problem in Europe since economic pollination can only be achieved by honeybees (*Apis mellifera*) (Benedek and Manninger, 1972; Du Toit, 1990; Free, 1993; Benedek, 2002; Chambó *et al.*, 2011; Ali *et al.*, 2015). Kumar *et al.* (2002) established that among *Apis* species honeybees are the most frequent flower visitors at sunflower fields. Honeybees, being eutrophic, polylectic flower visitors, can easily utilize the nectar and pollen produced by the stereomorphic flowers of sunflower. While visiting flowers in sunflower heads honeybees carry pollen grains from the anthers to the surface of receptive stigmas and so they are effective pollinating agents at this crop (Free 1993, Benedek and Manninger, 1972; Goras *et al.*, 2016).

Nectar is very important in attracting honeybees to sunflower fields in bloom. Thus, factors affecting the nectar production can influence honeybee visitation and foraging behaviour of honeybees at the crop. Nutrient supply is one of the factors influencing the nectar production of plants and the sugar content in the nectar, but the method of nutrient application has no effect to the nectar production (Farkas and Zajácz, 2007). Shuel (1955; 1961) has shown that nectar production of plants is mainly affected by macro-element supply. According to the literature, two microelements, zinc and copper have also decisive influence on the nectar production of insect-pollinated plants. For example, the nectaries of Chinese hibiscus secrete intensely copious quantities of nectar the precursors of which are provided mainly from the zinc content phloem in the subglandular tissue. Zinc greatly affects nectar secretion, indicating that it may play an important role in nectar production (Sawidis *et al.*, 2014). Chorbiński and Liszewski (2014) have found that nectar the nectar production of buckwheat was higher on plots supplied with nitrogen and copper foliar fertilizer applications than on untreated plots. Among entomophilous oil plants the effect of macro- and micro-elements on the nectar production has been studied with foliar fertilizer application at oilseed rape crops. In the experiments of Viik *et al.* (2002) the nectar production of oilseed rape flowers was significantly higher on plots treated with copper and magnesium foliar fertilizers than on unfertilized plots. For sunflowers, however, no similar results have been available so far. Accordingly, we have made experiments to study the effect of two micro-elements as foliar fertilizers to the nectar production of sunflowers and on the foraging behaviour of honeybees at the treated crop fields.

2. MATERIAL AND METHODS

— Foliar fertilizer application

Field experiments were conducted in 2014 and 2015 at commercial sunflower crops. In 2014 the experimental field (47°40’37.9’’N 18°10’52.0’’E) was near the village Mocsa (county Komárom-Esztergom, Hungary). In 2015 one other commercial sunflower field was used (47°37’06.3’’N 17°43’38.1’’E) near the village Töltéstava (county Győr-Moson-Sopron, Hungary). The experimental crop fields were cultivated with the Clearfield technology by Dow AgroSciences. 150 kg ha^{-1}

NPK fertilizer was applied as top-dressing in doses of 10:20:20 on the sunflower stand. Total germ count per hybrid amounted 50 000 germs ha^{-1}. In both years of the study, experiments were carried out with the following four sunflower hybrids: 8N 358 CLDM, 8H 288 CLDM, MG 305 CP, 8M 449 CLDM. On both sites 60 plots (2x5m) were involved into the trials in randomised block design, leaving an isolation distance of 1.4 m between them.

Four foliar fertilizers were applied (Table 1) in the following dosages: 3 l ha^{-1} of $[Cu(NH_3)_4]^{2+}+OH^{2-}$, 20 l ha^{-1} of $Zn(OH)_2+ZnCO_3$, 10 l ha^{-1} of $ZnCO_3+MgCO_3$ and 10 l ha^{-1} of $[Cu(NH_3)_4]^{2+}+OH^{2-}+CaCO_3$. We applied three repetitions of each foliar fertilizer treatment at each hybrid. For each hybrid three control plots were inserted. Each foliar fertilizer was applied at four plots at both sites and four untreated plots were used as untreated check. Foliar fertilizers were applied in the stage of bud-forming in flowers of star-like appearance (R1) on 13th June in 2014 (64th day after sowing) as well as on 18th June in 2015 (on 66th day after sowing).

Table 1. Foliar fertilisers used

Foliar fertilizer compound	Content of active substances
$[Cu(NH_3)_4]^{2+}+OH^{2-}+CaCO_3$	5.57% Cu; 2.41% Ca
$ZnCO_3+ MgCO_3$	1.2% Mg; 1,18% Zn
$[Cu(NH_3)_4]^{2+}+OH^{2-}$	5.65% Cu
$Zn(OH)_2+ ZnCO_3$	10.71% Zn

The experimental foliar fertilizers were manufactured from high purity by-products of industrial processes (Szakál *et al.*, 1988). ICP-AES method was used to analyse the active substances of micro- and macro-elements in the foliar fertilizers in compliance with the standards of environmental soil analysis (MSZ 21470-50:1998). Samples of 1; 2.5; 5 ml were taken for digestion in water of crystallization in a micro-wave device. 3.0 cm^3 of concentrated hydrochloric acid and 1.0 cm^3 concentrated nitric acid were given to the weighed samples.

The samples were left for 2 hours in the vessels for digestion under a hood and the digestion was carried out with the apparent programme. After finishing digestion vessels were chilled and their contents were filtered into measuring flasks of 25 cm^3. Vessels and filter were washed out with some cm^3 of nitric acid solution in a concentration of 0.5 mol (dm^3)$^{-1}$. Finally, the measuring flasks were filled with distilled water till the mark. The prepared digested samples were subjected to ICP-AES system in three different dilutions (1x, 10x, 100x). Table 1 shows the results of the analysis.

— Nectar sampling

Nectar samples were taken from florets in sunflower heads furnished with spacer and covered with tulle net 24 hours before sampling (as recommended by Free and Simpson 1964). We covered 1 sunflower heads per plot being in the same flowering stage. Samples were taken twice a day, late morning and early afternoon. To avoid double sampling, we took samples in the morning from the upper half of the head, and in the afternoon on the lower half of the head. Samples were separately taken every day from floret of male and female phase. Each capillary tube were used to take nectar sample from 5 indiviudul florets together. These samplings were treated together as sampling units. Samples were repeatedly taken on 3 days in both years, on 3-5-7th July in 2014 and on 6-8-10th July in 2015.

We applied three replications of each foliar fertilizer treatment at each hybrid. For each hybrid three control plots were also inserted. Number of nectar samples was 720 in total in both experimental years. Samples were taken with glass capillary tubes (made of glass tubes of 2.2 mm inner diameter with tampered tips). Glass capillaries were equipped with small beeswax balls at both ends and were weighed prior to field samplings on a four-decimal precision digital analytic scale, type Ohaus Adventure Pro AV264 and then they were given an ordinal number. After collecting nectar samples, the glass capillaries were closed with their beeswax balls and then they were put into a cooler box and were carried into the laboratory. Capillaries with nectar samples and their beeswax balls were weighted on the same precision analytic scale in the lab. One fifth of the difference of capillary masses prior to and after nectar sampling indicated the nectar content of one floret. Altogether 1440 glass capillary tubes were used for the experiments (1 flowering head/plot x 2 times a day x 2 samples, one male and one female x 3 days x 60 plots x 2 years).

— Bee visitation and the foraging behaviour of honeybees

In both years, 10 medium strong bee colonies were moved to the experimental fields as close to the sampling plots as possible. However, there were also other apiaries in the nearby in both years of the study. Counting all bee colonies in the close vicinity, the honeybee population was some 10-12 bee colonies per hectare at both sunflower crops.

Bee visitation of sunflower heads was recorded three times at both fields, at the beginning, in the middle and at the end of the flowering period, respectively, on the everyday of the nectar sampling (i.e., on 3, 5, 7th July in 2014, and 6, 8, 10th July in 2015).

Observations on bee visitation were carried out on 3 sunflower heads being in the same flowering stage for 10 minutes periods every two hours a day from 8 am till 4 pm, 5 times a day, as recommended in the literature (Benedek et al., 1972; Free, 1993; Ali et al., 2015). At each census the number of incoming honeybees and their foraging behaviour was recorded. The foraging behaviour was categorized as follows:

1. pollen gatherers,
2. nectar gatherers (without pollen loads), and
3. mixed behaviour (nectar foragers with pollen loads).

Also the duration of time that honeybee foragers spent at individual sunflower heads (in seconds) was recorded.

— Weather records

Air temperature, air humidity and rainfall were measured with standard meteorological instruments in every two hours during the flowering period of the experimental sunflower fields. The weather was typical to the moderate climate during the experimental period in both years. In the sampling period mean air temperature was 30.5 ± 4.8 C° in 2014, and 30.5 ± 2.2 C° in 2015. The air humidity was around 82.4 ± 5.5 % (min. 75.0 %) and 80.4 ± 8.6 % (min. 72.5 %) in 2014 and 2015, respectively. No rainfall was registered in either of the experimental years during the flowering period of our sunflower fields. As seen, the weather data resembled in both years.

— Weather

The weather conditions were favourable during the periods of experimental periods for honeybee activity and for collecting nectar samples as well as for observing bee visitations (c.f. Hedtke and Pritsch, 1993; Zajácz et al., 2006).

— Statistical analysis

Experimental data were registered with Microsoft Office Excel 2007 programme. All statistical analyses were carried out using IBM SPSS Statistics 20 software. Data the nectar samples and the of bee visitations normality records were analysed with the Kolmogorov-Smirnov test. The means were tested using the One-Way ANOVA test, and multiple comparisons with LSD (Least Significant Difference) test. Comparisons of parameters for non-parametric groups were conducted using the Mann-Whitney U-test or the Kruskal-Wallis test on a significance level of α=0.05. For all analyses, p-value <0.05 was considered significant.

3. RESULTS

In the experimental year of 2014 the staminate and pistillate flowers showed to following results. In the 8H 288 CLDM hybrid the mean nectar yields significantly (One-Way ANOVA test, staminate: F=2.852 df=4 SS=0.664 p<0.05; pistillate: F=5.909 df=4 SS=2.454 p<0.05) increased by the effect of $[Cu(NH_3)_4]^{2+}$+OH^{2-}, $Zn(OH)_2$+$ZnCO_3$, and $ZnCO_3$+$MgCO_3$ compound in both flower circles. In the 8M 449 CLDM hybrid the mean nectar yields significantly increased (One-Way ANOVA test, staminate: F=4.594 df=4 SS=1.402 p<0.05; Kruskal-Wallis test pistillate: p<0.05) by the effect of $[Cu(NH_3)_4]^{2+}$+OH^{2-}, and $Zn(OH)_2$+$ZnCO_3$ compound in both flower circles. In the MG 305 CP hybrid the mean nectar production yields significantly increased (One-Way ANOVA test, staminate: F=3.856 df=4 SS=0.737 p<0.05; pistillate: F=2.254 df=4 SS=0.815 p<0.05) by the effect of $[Cu(NH_3)_4]^{2+}$+OH^{2-} compound in both flower cicles. In the 8H 288 CLDM hybrid the $Zn(OH)_2$+$ZnCO_3$, in the MG 305 CP, and 8M 449 CLDM hybrids the $[Cu(NH_3)_4]^{2+}$+OH^{2-} foliar treatment resulted a higher nectar production at pistillate flowers than at stamniate flowers (Figure 1).

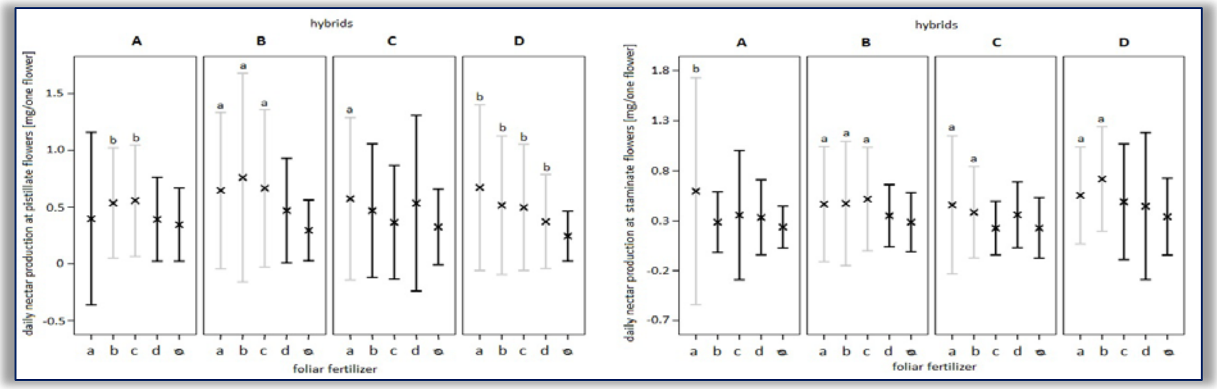

Figure 1: Effect of foliar fertilizer applications on the nectar production of sunflower pistillate (left) and staminate (right) flowers at Mocsa, 2014 (mean ±SD)

Hybrids: A=8N 358 CLDM, B=8H 288 CLDM, C=MG 305 CP, D=8M 449 CLDM

Foliar fertilizers (a= $[Cu(NH_3)_4]^{2+}$+OH^{2-}, b= $Zn(OH)_2$+$ZnCO_3$, c= $ZnCO_3$+$MgCO_3$, d= $[Cu(NH_3)_4]^{2+}$+OH^{2-}+$CaCO_3$), ⊘=untreated check,

Gray marking[a] LSD test, gray marking[b] Mann-Whitney test is significant (p<0.05)

In the experimental year of 2015 the staminate and pistillate flowers showed the following results. In the MG 305 CP hydrid the mean nectar production yields significantly (One Way ANOVA test, staminate: F=4.283 df=4 SS=0.738 p<0.05; pistillate: F=2.437 df=4 SS=0.611 p<0.05) increased by the effect of $[Cu(NH_3)_4]^{2+}$+OH^2 compound in both flower circles. In the 8H 288 CLDM MG, 305 CP and 8M 449 CLDM hybrids by the effect of $[Cu(NH_3)_4]^{2+}$+OH^2 foliar treatment the nectar production was higher in the pistillate flowers than in the staminate flowers (Figure 2).

— Bee visitation

Observing the bee visitations in both years of the study, similar tendencies were recorded at plots treated with foliar fertilizers compared to those untreated.

Due to the foliar treatment the bee visiting increased compared with the control parcels. The intensity of the visit was concentrated at 10:00; 12:00 and 14:00 observation times (Figures 3-4). Staminate flowers were more intensely visited by incoming honeybee foragers than pistillate flowers in both years of the experiment. Namely, mean intensity of bee visitation was double in 2014 and more than double in 2015 at the staminate compared to the pistillate flowers (Tables 3-4).

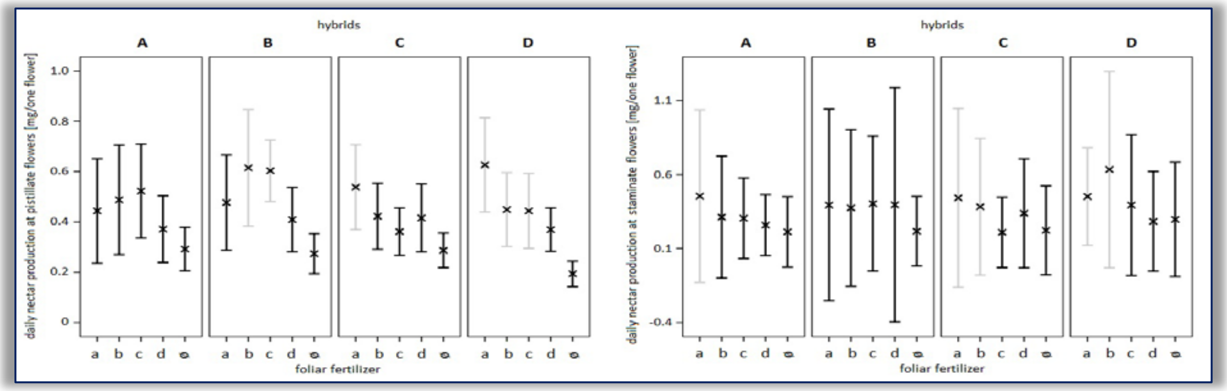

Figure 2: Effect of foliar fertilizer applications on the nectar production of sunflower pistillate (left) and staminate (right) flowers at Töltéstava, 2015 (mean ±SD)
Hybrids: A=8N 358 CLDM, B=8H 288 CLDM, C=MG 305 CP, D=8M 449 CLDM)
Foliar fertilizers (a= $[Cu(NH_3)_4]^{2+}+OH^{2-}$, b= $Zn(OH)_2+ZnCO_3$, c= $ZnCO_3+MgCO_3$, d= $[Cu(NH_3)_4]^{2+}+OH^{2-}+CaCO_3$), ⊗=untreated check, Gray marking LSD test is significant ($p<0.05$)

In the case of the hybrid 8N 358 CLDM due to the foliar fertilizer applications the intensity of honeybee visitation was found significanly more intense at staminate flowers circles as compared to untreated plots in both experiment year, in 2014 and 2015 (One-Way ANOVA test, 2014: F=7.843 df=4 SS=62.747 $p<0.0001$; 2015: F=3.896 df=4 SS=21.52 $p<0.0001$). Also significant difference was found in the intensity of honeybee mean visitation at the staminate flower circles due to foliar fertilizer applications at the hybrid 8M 449 CLDM in 2014 (One-Way ANOVA test, F=14.967 df=4 SS=67.28 $p<0.0001$).
Results of Kruskal-Wallis test on the effect of foliar fertilizer applications to the honeybee visitation number is shown in Table 2.

Table 2. Results of Kruskal-Wallis test on the effect of foliar fertilizer applications to the honeybee visitation number of selected sunflower hybrids

<table>
<tr><th rowspan="3">Hybrids</th><th rowspan="3">Foliar fertilizers</th><th colspan="2">Field experiment Mocsa, 2014</th><th colspan="2">Field experiment Töltéstava, 2015</th></tr>
<tr><th>Bee visiting at staminate flowers</th><th>Bee visiting at pistillate flowers</th><th>Bee visiting at staminate flowers</th><th>Bee visiting at pistillate flowers</th></tr>
<tr><th>p value</th><th>p value</th><th>p value</th><th>p value</th></tr>
<tr><td rowspan="5">A</td><td>a</td><td rowspan="5"></td><td rowspan="5">0.024</td><td rowspan="5"></td><td rowspan="5">0.024</td></tr>
<tr><td>b</td></tr>
<tr><td>c</td></tr>
<tr><td>d</td></tr>
<tr><td>e</td></tr>
<tr><td rowspan="5">B</td><td>a</td><td rowspan="5">0.0002</td><td rowspan="5">0.080</td><td rowspan="5">0.719</td><td rowspan="5">0.003</td></tr>
<tr><td>b</td></tr>
<tr><td>c</td></tr>
<tr><td>d</td></tr>
<tr><td>e</td></tr>
<tr><td rowspan="5">C</td><td>a</td><td rowspan="5">0.004</td><td rowspan="5">0.013</td><td rowspan="5">0.005</td><td rowspan="5">0.0004</td></tr>
<tr><td>b</td></tr>
<tr><td>c</td></tr>
<tr><td>d</td></tr>
<tr><td>e</td></tr>
<tr><td rowspan="5">D</td><td>a</td><td rowspan="5"></td><td rowspan="5">0.070</td><td rowspan="5">0.015</td><td rowspan="5">0.220</td></tr>
<tr><td>b</td></tr>
<tr><td>c</td></tr>
<tr><td>d</td></tr>
<tr><td>e</td></tr>
</table>

Hybrids: A=8N 358 CLDM, B=8H 288 CLDM, C=MG 305 CP, D=8M 449 CLDM; Foliar fertilizers: a= $[Cu(NH_3)_4]^{2+}+OH^{2-}$, b= $Zn(OH)_2+ZnCO_3$, c= $ZnCO_3+MgCO_3$, d= $[Cu(NH_3)_4]^{2+}+OH^{2-}+CaCO_3$), ⊗=untreated check, Boldfaced marking is significant; *-Kruskal-Wallis test

As an effect of foliar fertilizer fertilization applications significant increase was found in the mean honeybee visitation at the staminate and/or pistillate flower circles in the two experimental years. In the case of the foliar fertilizer applications with $[Cu(NH_3)_4]^{2+}+OH^{2-}$ and $ZnCO_3+MgCO_3$ significant increase was found in the mean honeybee visitation both at the staminate and the pistillate flower circles in the hybrid 8N 358 CLDM in both years. Similarly, in the case of the MG 305 CP hybrid significant increase of mean honeybee visitation was observed at the staminate and the pistillate flower circles as affected by the foliar application of $[Cu(NH_3)_4]^{2+}+OH^{2-}$ in both years. Contrarily, in the case of the hybrid 8N 358 CLDM the foliar application of $Zn(OH)_2+ZnCO_3$ and $[Cu(NH_3)_4]^{2+}+OH^{2-}+CaCO_3$ has resulted in a significant increase of mean bee visitation only at the staminate flowers and in the case of the hybrid 8H 288 CLDM the foliar treatments with $[Cu(NH_3)_4]^{2+}+OH^{2-}$ and $[Cu(NH_3)_4]^{2+}+OH^{2-}+CaCO_3$ increased the mean honeybee visitation at the pistillate flowers only (Tables 3-4).

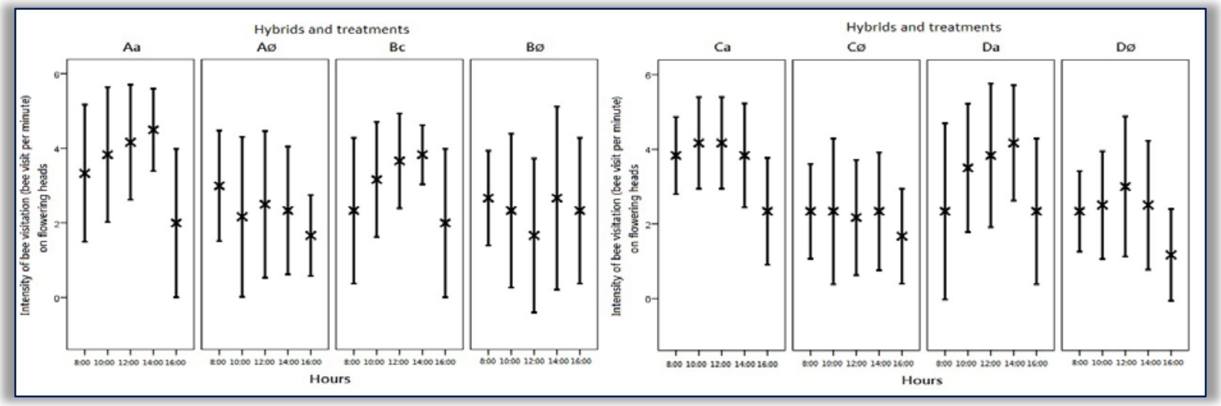

Figure 4: The effect of foliar fertilizer applications on the intensity of honeybee visitations at sunflower (mean±SD) at Mocsa (left) and Töltéstava (right) in 2015.
Hybrids: A=8N 358 CLDM, B=8H 288 CLDM, B=8H 288 CLDM, C=MG 305 CP, D=8M 449 CLDM
Foliar treatments: a= $[Cu(NH_3)_4]^{2+}+OH^{2-}$, b= $Zn(OH)_2+ZnCO_3$, Ø= untreated check

— Duration of bee visits

The mean duration of honeybee visits at sunflower heads is shown in Tables 3 and 4. The duration of mean honeybee visits was much longer in most cases (28 out of 32) at treated than at the untreated plots ($p<0.05$). There were only five exceptions. In three cases the duration was similar (100-103 %): MG 305 CP hybrid with $ZnCO_3+MgCO_3$ fertilizer in Table 3, 8N 358 CLDM hybrid with $[Cu(NH_3)_4]^{2+}+OH^{2-}$ fertilizer, MG 305 CP hybrid with $ZnCO_3+MgCO_3$ fertilizer in Table 4).

Table 3. Foraging behaviour of honeybees at Mocsa, 2014

Hybrids	Foliar fertilizers	Mean No. of bee visits in 10 minutes periods mean±SD		Flower visiting behaviour of bees mean±SD			Mean±SD duration of honeybee visits at blooming sunflower heads (sec)
		At staminate flowers	At pistillate flowers	Nectar gatherers	Pollen gatherers	Mixed behaviour (nectar and pollen gethering)	
A	a	5.8±1.9	1.7±1.2	3.00±2.6	0.53±0.1	0.20±0.6	64.79±27.62
	b	4.7±1.4	2.0±1.2	2.77±1.8	0.60±1	0.00±0	72.73±41.91
	c	4.9±1.0	1.5±1.2	2.70±1.8	0.37±0.7	0.07±0.3	105.95±91.51
	d	4.0±1.6	1.5±1.2	2.27±1.6	0.47±0.7	0.03±0.2	68.92±41.87
	Ø	3.1±0.9	0.7±0.7	1.50±1.2	0.33±0.5	0.03±0.2	52.88±49.13
B	a	4.9±1.0	1.9±1.0	2.93±1.7	0.37±0.8	0.07±0.4	112.34±79.88
	b	5.7±0.9	1.7±1.5	3.03±1.9	0.60±1	0.03±0.2	113.83±68.78
	c	5.3±1.3	1.7±1.5	2.87±2	0.57±0.9	0.07±0.3	103.88±63.46
	d	4.1±1.5	1.9±1.5	2.37±1.8	0.60±1	0.03±0.2	100.66±58.59
	Ø	3.6±1.5	0.8±0.7	1.60±1.6	0.57±1	0.00±0	58.37±41.77
C	a	4.3±0.8	2.1±1.0	2.60±1.3	0.57±0.9	0.03±0.2	112.02±58.98
	b	4.0±1.6	1.5±1.2	1.93±1.7	0.83±1.1	0.00±0	53.57±48.18
	c	2.7±1.0	0.9±0.7	1.33±1	0.47±0.7	0.03±0.2	59.23±39.92
	d	3.5±1.2	1.4±1.1	1.77±1.2	0.63±0.9	0.07±0.3	76.75±38.75
	Ø	3.1±1.2	1.1±0.8	1.47±1.3	0.47±0.8	0.13±0.3	59.13±31.12
D	a	5.1±1.0	1.6±1.2	2.27±1.7	1.00±1.3	0.10±0.3	116.19±64.22
	b	4.7±0.9	1.9±1.2	2.30±1.3	0.90±1.1	0.10±0.3	126.32±169.08
	c	4.7±0.9	2.1±1.4	2.90±1.7	0.47±0.8	0.00±0	139.22±47.43
	d	4.5±1.2	2.2±1.6	2.57±1.9	0.67±1.1	0.13±0.3	140.67±39.46
	Ø	2.7±1.0	1.1±0.7	1.37±1	0.43±0.7	0.13±0.3	66.55±25.09

Hybrids: A=8N 358 CLDM, B=8H 288 CLDM, C=MG 305 CP, D=8M 449 CLDM; Treatments: a= $[Cu(NH_3)_4]^{2+}+OH^{2-}$, b= $Zn(OH)_2+ZnCO_3$, c= $ZnCO_3+MgCO_3$, d= $[Cu(NH_3)_4]^{2+}+OH^{2-}+CaCO_3$), ø=untreated check, Boldfaced marking Mann-Whitney test and italic marking LSD test is significant.

There were no more than two cases when the bee visitation was a bit less intense at the treated than at the untreated plots (91 per cent with 8N 358 CLDM hybrid treated with $Zn(OH)_2+ZnCO_3$ fertilizer in Table 4; 89 per cent with MG 305 CP hybrid treated with $Zn(OH)_2+ZnCO_3$ fertilizer in Table 3).

Table 4. Foraging behaviour of honeybees at Töltéstava, 2015

Hybrids	Foliar fertilizers	Mean No. of bee visits in 10 minutes periods mean±SD		Flower visiting behaviour of bees mean±SD			Mean±SD duration of honeybee visits at blooming sunflower heads (sec)
		At staminate flowers	At pistillate flowers	Nectar gatherers	Pollen gatherers	Mixed behaviour (nectar and pollen getharing)	
A	a	4.7±1.2	2.4±1.4	3.30±1.7	0.23±0.4	0.03±0.2	78.34±34.76
	b	4.6±0.8	2.1±1.1	3.00±1.6	0.33±0.7	0.03±0.2	96.94±64.35
	c	4.7±1.4	1.9±1.3	2.97±1.7	0.30±0.6	0.07±0.3	89.85±43.87
	d	3.6±1.3	1.9±1.1	2.37±1.4	0.30±0.7	0.10±0.3	85.33±28.22
	Ø	3.6±1.1	1.1±0.8	1.93±1.5	0.27±0.6	0.13±0.5	76.69±45.89
B	a	4.0±0.8	2.4±1.2	2.70±1.4	0.43±0.9	0.07±0.3	102.26±36
	b	3.9±0.6	2.6±1.1	2.83±1.2	0.37±0.7	0.03±0.2	115.46±58.95
	c	4.2±0.6	1.8±1.3	2.40±1.6	0.30±0.7	0.3±0.7	94.47±43.85
	d	4.0±0.8	2.1±1.6	2.60±1.5	0.37±0.8	0.10±0.4	91.45±41.93
	Ø	3.8±1.2	0.9±0.8	1.90±1.7	0.37±0.8	0.07±0.3	58.48±34.33
C	a	4.5±1.0	2.9±1.1	3.20±1.5	0.40±0.8	0.07±0.3	103.76±40.28
	b	3.5±1.2	2.5±1.3	2.33±1.2	0.50±0.8	0.17±0.6	85.11±54.54
	c	3.0±1.2	1.1±1.2	1.67±1.4	0.20±0.5	0.20±0.5	68.75±37.41
	d	3.6±0.7	1.6±1.3	2.07±1.4	0.37±0.6	0.17±0.4	81.51±34.34
	Ø	3.2±0.9	1.1±1.0	1.83±1.4	0.27±0.5	0.07±0.3	66.38±30.25
D	a	4.7±0.9	1.7±1.3	2.70±1.7	0.33±0.6	0.13±0.3	105.45±52.47
	b	4.5±0.9	2.3±1.4	2.87±1.5	0.40±0.8	0.13±0.3	114.56±49.64
	c	4.5±0.8	2.1±1.5	2.70±1.5	0.43±0.8	0.13±0.3	137.49±41.13
	d	4.3±1.0	2.3±1.5	2.87±1.5	0.33±0.6	0.13±0.4	131.19±43.94
	Ø	3.3±1.2	1.3±0.8	1.73±1.3	0.43±0.7	0.13±0.3	66.73±25.87

Hybrids: A=8N 358 CLDM, B=8H 288 CLDM, C=MG 305 CP, D=8M 449 CLDM); Foliar fertilizers: a= $[Cu(NH_3)_4]^{2+}+OH^{2-}$, b= $Zn(OH)_2+ZnCO_3$, c= $ZnCO_3+MgCO_3$, d= $[Cu(NH_3)_4]^{2+}+OH^{2-}+CaCO_3$), Ø=untreated check, Boldfaced marking Mann Whitney test and italic marking LSD test is significant. Boldfaced marking Mann-Whitney test and italic marking LSD test is significant.

In general foliar fertilizer application increased bee visitation time at flowering heads with 150-170 per cent. The mean rate of increase was 163 per cent in 2014 and 147 per cent in 2015, resp. All the four foliar fertilizers had very similar effect on the bee visitation of flowering sunflower plots, namely, the mean rate of increase was between 155-171 per cent in 2014 and between 146-151 per cent in 2015.

— Foraging behaviour of honeybees

Most honeybees visiting sunflower heads were nectar gatherers and the rate of pollen gathering bees amounted only one fifths or one sixth of their number while the rate of the mixed behaviour bees (nectar gatherers with pollen loads) amounted always only one tenth of the foraging honeybee population (Tables 3 and 4).

Due to the application of foliar fertilizers significant increase was recorded in the number of nectar gatherer honeybees in all cases, but no relationship was found between the effect of the treatments and the numbers of pollen gatherers or mixed behaviour honeybees (Tables 3 and 4).

4. DISCUSSION

Foliar fertilizer applications with copper and zinc increased the nectar production of sunflowers in our experiments as it has been indicated in the case of some other cultivated crops plant species earlier. Zinc as foliar fertilizer has been found to increase the nectar production of Chinese hibiscus (*Hibiscus rosa-sinensis* L.) by Sawidis et al. (2014), because zinc as specific and non-specific enzyme activator had an effect on the carbohydrate metabolism and in the transformation of sugars (Brown et al. 1993). Also the positive effect of copper on the nectar production has been found in buckwheat (*Fagopyron esculentum* L.) by Chorbiński and Liszewski (2014), because copper similarly to zinc has been an important enzyme activator, consequently in the absence of copper the synthesis of carbohydrates has decreased during the vegetative period (Brown and Clark, 1977). Among oil plants also a positive effect of both mentioned micro elements was described on the nectar production of spring rapeseed (*Brassica napus* L. var. *oleifera*) by Viik *et al.* (2012).

Similarly to Benedek and Manninger (1972) we found that the nectar gathering honeybee foragers prefer to visit the circles of staminate flowers, for this reason a huge amount of pollen is sticking on their bodies when visiting sunflower heads. As foliar fertilizer applications containing copper and zinc can cause more intense nectar production of pistillate flowers foliar fertilizer applications can make sunflower heads more attractive to honeybee foragers. Consequently, nectar gatherers are effective pollinators on the flowers in the pistillate phase.

It was found that the increased mean honeybee visitation significantly increased the mean duration of honeybee visits at sunflower heads, so it increased the time that honeybee foragers spent in individual flowering sunflower heads. Increased mean honeybee visitation, and longer time of honeybee visits is favourable for more effective pollination (Free, 1993), so increased honeybee visitation contributes to more effective pollination of flowers, thus favourable effect of higher honeybee densities can raise the yields of sunflower fields as well as the oil content of sunflower seeds (McGregor, 1976; Parker, 1981; Benedek, 2002; DeGrandi-Hoffman and Chambers, 2006).

Finally it can be concluded that due to their physiological effect to sunflower plants foliar fertilizer applications increased the nectar production of the experimental sunflower hybrids. This is definitely favourable to the honeybee visitation of the sunflower fields because more intense honeybee visitation can be expected at plots with higher nectar production caused by treatments with foliar fertilizers. Higher nectar production of sunflowers due to foliar fertilizer applications also affects the foraging behaviour of honeybees because significantly more nectar gatherers have been observed at plots treated with foliar fertilizers. So, more abundant honeybee population with higher number of nectar foragers attracted by higher nectar content in sunflower florets would be resulted in higher honey yields too. Consequently due to foliar fertilizer applications higher honey harvest can be resulted in honeybee colonies moved to sunflower fields.

References

[1] Ali H, Owayss AA, Khan KA, Alqarni AS (2015). Insect Visitors and Abundance of Four Species of Apis on Sunflower *Helianthus annuus* L. in Pakistan. Acta Zoologica Bulgarica 67(2):235-240.

[2] Baydar H, Erbaş S (2005). Influence of seed development and seed position on oil, fatty acids and total tocopherol contents in sunflower (*Helianthus annuus*). Turkish Journal of Agriculture and Forestry 29(3):179-186.

[3] Benedek P, Manninger S (1972). Sunflower pollinating insects and honeybee activity on sunflower. Növénytermelés 21(2):145-157.

[4] Benedek P (2002). A review of the pollination research on temperate zone crop plants in the past decade: results and the need of further studies. International Journal of Horticultural Science 8(2):7-23.

[5] Benedek P, Manninger S, Nagy B (1972). The number of colonies and the density of honeybees in sunflower fields in relation to the pollination of the crop. Journal of Applied Entomology 71(1-4):385-389.

[6] Brown JC, Clark RB (1977). Copper as essential to wheat reproduction. Plant Soil 48:509–523.

[7] Brown PH, Cakmak I, Zhang Q (1993). Form and function of zinc plants. Developments in Plant and Soil Sciences 55:93-93.

[8] Chambó ED, Garcia RC, Oliveira NTED Duarte-Júnior JB (2011). Honey bee visitation to sunflower: effects on pollination and plant genotype. Scientia Agricola, 68(6):647-651 http://dx.doi.org/10.1590/S0103-90162011000600007

[9] Chorbiński P, Liszewski M (2014). Effect of copper and manganese foliar supplementation on selected properties of buckwheat nectar and yield. Medycyna Weterynaryjna, 70(12):786-790.

[10] Corbet SA, Williams IH, Osborne JL (1991). "Bees and the pollination of crops and wild flowers in the European Community." Bee World 72(2):47-59. dx.doi.org/10.1080/0005772X.1991.11099079

[11] DeGrandi-Hoffman G, Chambers M. (2006). Effects of honey bee (*Hymenoptera: Apidae*) foraging on seed set in self-fertile sunflowers (*Helianthus annuus*). Environmental Entomology, 35(4):1103-1108. http://doi.org/10.1603/0046-225X-35.4.1103

[12] Du Toit AP (1990). The importance of certain insects as pollinators of sunflower (*Helianthus annuus).* South African Journal of Plant and Soil, 7(3):159-162. http://dx.doi.org/10.1080/02571862.1990.10634559

[13] Environmental protection. Testing of soils. Sampling (MSZ 21470-50:1998)

[14] Farkas A, Zajácz E (2007). Nectar production for the Hungarian honey industry. The European Journal of Plant Science and Biotechnology, 1(2):125-151.

[15] Free JB (1993). Insect Pollination of Crops. 2nd Academic Press, London, UK.

[16] Free JB, Simpson J (1964). The pollination requirements of sunflower (*Helianthus annuus*). Empire Journal Experimental Agriculture, 32:340-342.

[17] Goras G, Tananaki C, Dimou M, Tscheulin T, Petanidou T, Thrasyvoulou A (2016). Impact of honeybee (*Apis mellifera*) density on wild bee foraging behaviour. Journal of Apicultural Science, 60(1):49-62. http://doi.org/10.1515/jas-2016-0007

[18] Hedtke C, Pritsch C (1993). Quantitative and qualitative investigation of insects foraging buckwheat (Fagopyron esculentum Moench). Apidology, 24:476-477.

[19] Kumar M, Chand H, Singh R, Ali MS (2002). Effect of different modes of honeybee pollination on oil content in seeds of sunflower (*Helianthus annuus*). Journal of Entomological Research, 26(3):219-221.

[20] McGregor SE (1976). Insect pollination of cultivated crop plants *(Vol. 496)*. Agricultural Research Service, US Department of Agriculture.

[21] Parker FD (1981). Sunflower pollination: abundance, diversity and seasonality of bees and their effect on seed yields. Journal of Apicultural Research, 20(1):49-61. http://dx.doi.org/10.1080/00218839.1981.11100473

[22] Pham-Delegue MH, Etievant P, Guichard E, Marilleau R, Douault PH, Chauffaille J, Masson C (1990). Chemicals involved in honeybee-sunflower relationship. Journal of Chemical Ecology, 16(11):3053-3065.

[23] Sawidis T, Papadopoulou A, Voulgaropoulou M (2014). Effect of zinc on nectar secretion of Hibiscus rosa-sinensis L. Protoplasma 251(3):575-589.

[24] Shuel RW (1955). Nectar Secretion in Relation to Nitrogen Supply, Nutritional Status, and Growth of the Plant 1. Canadian Journal of Agricultural Science 35(2):124-138. http://doi.org/10.4141/agsci-1955-0018
[25] Shuel RW (1961). The influence of calcium and magnesium supply on nectar production in red clover and snapdragon. Canadian Journal of Plant Science 41(1):50-58. http://doi.org/10.4141/cjps61-008
[26] Szakál P, Schmidt R, Barkóczi M (1988). The agricultural utilization of Zn-containing industrial waste. (pp. 1355-1359). Amsterdam: Elsevier Science Publishers B. V.
[27] Viik E, Maend M, Karise R, Laeaeniste P, Williams IH, Luik A (2012). The impact of foliar fertilization on the number of bees (*Apoidea*) on spring oilseed rape. Zemdirbyste Agriculture 99:41-46.
[28] Yadav RN, Sinha SN, Singhal NC (2002). Honeybee (*Apis spp.*) pollination in sunflower hybrid seed production: Effect of planting design on honeybee movement and its operational area. Apimondia Standing Commission of Pollination and Bee Flora.
[29] Zajácz E, Zaják Á, Szalai ME Szalai T (2006). Nectar production of some sunflower hybrids. Journal of Apicultural Science 50(2):7-11.

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