FORESTRY IDEAS, 2022, vol. 28, No 1 (63): 84–87

STUDY ON TOXIC TOLERANCE OF BENEFICIAL ENTOMOFAUNA (*APIS MELLIFERA* L.) THROUGH USAGE OF POLLEN FROM A FORESTRY AREA

Zheko Radev[1*] and Tsvetanka Raycheva[2]

[1]Tobacco and tobacco products institute, 4108 Markovo, Bulgaria. [*]E-mail: zhekoradev@abv.bg
[2]Agricultural University-Plovdiv, 4000 Plovdiv, Bulgaria. E-mail: raicheva@abv.bg

Received: 22 January 2022 Accepted: 22 February 2022

Abstract

Toxic tolerance study of 1,3-Diazaspiro[4.4]nonane-2,4-dithione of *Apis mellifera* L. was performed in a laboratory using pollen with different protein content. There is higher toxic tolerance when bees consumed pollen with higher protein content. The lifespan of bees after application of the substance varies from 6 h for those which consumed pollen from *Chondrilla juncea* L. with 12.3 % protein content to 36 h for those which consumed pollen from *Cornus sanguinea* L. with 27.7 % protein content. When bees consumed pollen with a protein content of more than 21 %, lived longer. It seems that bees which consumed pollen with a protein content of 14.2 % had a lower toxic tolerance.

Keywords: honey bee, bee-pollen, lifespan.

Introduction

Modern agricultural practices have an impact on biodiversity, and must be assessed in order to protect wildlife and increase agriculture production (Norris 2008). Agroecosystems must play a main role in conserving biodiversity (Fischer et al. 2006). In the last 60 years, the use of pesticides increased in agriculture (Tilman et al. 2001). Use of pesticides leads to direct and indirect effects on the environment (Woodcock et al. 2016). The amount and balance of macro- and micronutrients and secondary metabolites in the diet of insects affects their condition (Simpson and Raubenheimer 2012). There is a connection between insect's sensitivity to toxins and the protein:carbohydrate ratio (Deans et al. 2017). Secondary metabolites may increase insect resistance to various pesticides (Johnson et al. 2012). This is extremely important for *Apis mellifera* L., the main pollinator on the Earth (Hung et al. 2018).

The aim of this study was to investigate toxic tolerance of beneficial entomofauna (*Apis mellifera* L.) depending on protein content of consumed pollen.

Materials and Methods

A laboratory experiment was performed in 2015. Pollen with established botanical origin and protein content were used: *Chondrilla juncea* L. pollen with protein content of 12.3 %, *Cichorium intybus* L. – 14.2 %, *Cornus mas* L. – 21.7 %, *Pyrus malus* Borkh. – 26.1 % and *Cornus sanguinea*

L. – 27.7 % collected from forestry area Borushtitsa (42°73′38" N and 25°57′29" E) (Radev 2021). As a toxic agent for bees 1,3-Diazaspiro[4.4]nonane-2,4-dithione was used in a concentration of 0.01 %.

Five experimental groups were used, every group consisted of three wooden cages 10×10×10 cm. Twenty newly hatched bees were placed in every cage and fed on 50 % sugar syrup for three days. On the fourth day tested substance was added to the food of all five groups in a concentration of 0.01 %. Every group also received 20 mL sugar syrup containing 2 g of pollen: 1st group received from *C. juncea*, 2nd group from *C. intybus,* 3rd group from *C. mas,* 4th group from *P. malus* and 5th group from *C. sanguinea*. The food was delivered to the cages through 2 mL pipette. The syrup was renewed daily. The cages were covered and left at room temperature of 20–21 °C. During the first 3 days, the bees were monitored once a day when changing the syrup, on the 4th day every hour and on the 5th day every 3 hours.

The results were statistically processed by Excel and Anova and method of low significant difference (LSD) at $p \leq 0.05$.

Results and Discussion

The results show higher toxic tolerance of *A. mellifera* to the applied substance when consumed pollen with higher protein content. The lifespan of bees fed on the substance varies from 6 h for those which consumed *C. juncea* pollen with 12.3 % protein content to 36 h for those which consumed *C. sanguinea* pollen with 27.7 % protein content (Table 1).

Table 1. Lifespan of bees fed on 1,3-Diazaspiro[4.4]nonane–2,4–dithione 0.01 % and pollen with different protein content, %.

Period of counting live bees	Group I, survived bees	Group II, survived bees	Group III, survived bees	Group IV, survived bees	Group V, survived bees
Till 1st h	73.0	76.5	100.0	100.0	100.0
Till 2nd h	41.5	56.5	100.0	100.0	100.0
Till 3rd h	21.5	36.5	100.0	100.0	100.0
Till 4th h	2.3	16.5	100.0	100.0	100.0
Till 5th h	11.5	11.5	100.0	100.0	100.0
Till 6th h	0.0	8.5	100.0	100.0	100.0
Till 8th h	NF	0.0	96.5	100.0	100.0
Till 10th h	NF	NF	76.5	81.5	96.5
Till 14th h	NF	NF	55.0	12.6	88.0
Till 18th h	NF	NF	21.5	63.0	71.5
Till 24th h	NF	NF	5.0	30.0	45.0
Till 27th h	NF	NF	0.0	23.0	23.0
Till 30th h	NF	NF	NF	8.0	15.0
Till 33th h	NF	NF	NF	0.0	11.5
Till 36th h	NF	NF	NF	NF	0.0
$LSD_{p \leq 0.05}$	0.65	0.65	0.69	0.69	0.69

Note: NF – not found.

As protein content in pollen increased lifespan of bees also increased. When bees consuming pollen with protein content more than 21 %, lived longer. It seems that bees which consumed pollen with protein content up to 14.2 % have low toxic tolerance. In groups I and II the highest mortality was reported by the 3rd h and in groups III, IV and V a gradual loss of bees was observed, starting after 6th h. There is no difference between groups I and II ($F \leq F_{crit,}$ Anova: Single factor), as well as between groups III, IV and V ($F \leq F_{crit,}$ Anova: Single factor). A difference was found when comparing groups I and II with groups III, IV and V ($F > F_{crit,}$ Anova: Single factor). According to the obtained results, it is necessary to conduct field experiments work with bee hives, as the results may be different from the laboratory.

In the present study, the effect of protein content in the pollen on pesticide toxicity was considered, taking into account its positive effect. Wahl and Ulm (1983) first mentioned that pollen increases the tolerance of honey bees to pesticides. Consumption of pollen reduces the susceptibility of bees to chlorpyrifos compared to bees fed without pollen (Schmehl et al. 2014). Carrying out research work in this direction is imperative, having in mind bee colonies losses worldwide. Barascou et al. (2021) found a reduction in pesticide toxicity in honey bees depending on different pollen. Honey bees have been exposed to a number of stressors in recent years, including pesticides (Goulson et al. 2015).

Conclusions

The protein-rich pollen feeding could somewhat increase the toxic tolerance in bees. As protein content in pollen increased lifespan of bees also increased.

References

Barascou L., Sene D., Barraud A., Michez D., Lefebvre V., Medrzycki P., Di Prisco G., Strobl V., Yañez O., Neumann P., Le Conte Y., Alaux C. 2021. Pollen nutrition fosters honeybee tolerance to pesticides. Royal Society Open Science 8(9), 210818. DOI: 10.1098/rsos.210818

Deans C., Behmer S., Tessnow A., Tamez-Guerra P., Pusztai-Carey M., Sword G. 2017. Nutrition affects insect susceptibility to Bt-toxins. Scientific Reports 7(1), 39705. DOI: 10.1038/srep39705

Fischer J., Lindenmayer D., Manning A. 2006. Biodiversity, ecosystem function, and resilience: Ten guiding principles for commodity production landscapes. Frontiers in Ecology and the Environment 4(2): 80–86. DOI: 10.1890/1540-9295(2006)004[0080:BEFART]2.0.CO;2

Goulson D., Nicholls E., Botías C., Rotheray E. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science 347(6229), 1255957. DOI: 10.1126/science.1255957

Hung K-L., Kingston J., Albrecht M., Holway D., Kohn J. 2018. The worldwide importance of honey bees as pollinators in natural habitats. Proceedings of the Royal Society B: Biological Sciences 285(1870), 20172140. DOI: 10.1098/rspb.2017.2140

Johnson R., Mao W., Pollock H., Niu G., Schuler M., Berenbaum M. 2012. Ecologically appropriate xenobiotics induce cytochrome P450s in *Apis mellifera*. PLoS ONE 7(2), e31051. DOI: 10.1371/journal.pone.0031051

Norris K. 2008. Agriculture and biodiversity conservation: opportunity knocks. Conservation Letters 1(1): 2–11. DOI: 10.1111/j.1755-263X.2008.00007.x

Radev Zh. 2021. Comparing protein content in bee pollen from different forestry areas. Forestry Ideas 27(2): 293–297.

Schmehl D., Teal P., Frazier J., Grozinger C.

2014. Genomic analysis of the interaction between pesticide exposure and nutrition in honey bees (*Apis mellifera*). Journal of Insect Physiology 71: 177–190. DOI: 10.1016/j.jinsphys.2014.10.002

Simpson S., Raubenheimer D. 2012. The Nature of Nutrition: a Unifying Framework from Animal Adaptation to Human Obesity. Princeton, NJ, Princeton Univeristy Press. 256 p.

Tilman D., Fargione J., Wolff B., D'Antonio C., Dobson A., Howarth R., Schindler D., Schlesinger W., Simberloff D., Swackhamer D. 2001. Forecasting agriculturally driven global environmental change. Science 292(5515): 281–284. DOI: 10.1126/science.1057544

Wahl O., Ulm K. 1983. Influence of pollen feeding and physiological condition on pesticide sensitivity of the honey bee *Apis mellifera carnica*. Oecologia 59(1): 106–128. DOI: 10.1007/BF00388082

Woodcock B., Isaac N., Bullock J., Roy D., Garthwaite D., Crowe A., Pywell R. 2016. Impact of neonicotinoid use on long-term population changes in wild bees in England. Nature Communications 7(1), 12459. DOI: 10.1038/ncomms12459