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INSECTICIDAL ASPECT TO *DIPRION PINI* L. (HYMENOPTERA; DIPRIONIDAE) AND *APIS MELLIFERA* L. (HYMENOPTERA; APIDAE)

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Abstract

The substance 1,3-Diazaspiro[4.4]nonane-2,4-dithione was tested in laboratory conditions for insecticidal activity against *Diprion pini* L. (Hymenoptera; Diprionidae) and *Apis mellifera* L. (Hymenoptera; Apidae). High mortality of 93.1 % was found against the larvae of *D. pini* with a concentration 0.01 %. The lifespan of the bees consumed food containing 0.01 % of the tested substance was up to 8 hour, with an average of 3.6 hour. The highest mortality was reported up to the 3rd hour, while the highest value was established at the 2nd hour. The bees fed on clean food lived up to 168 hour, an average of 120 hour. The highest mortality was reported at the 120th hour.

Key words: honey bee, insecticidal effect, sawfly, spirohydantoin.

Introduction

Pesticides are chemicals used against weeds, insects and pathogens. Over 75 % of them are applied in agriculture for plant protection. After more than six decades of use, their side effects onto ecosystems are visible (Sánchez-Bayo 2011). The use of pesticides is associated with a risk of environmental pollution and adverse effects on non-target organisms (Tilman et al. 2011). It is estimated that only 0.1 % of the pesticides used affect target organisms, while the remaining 99 % affect the environment (Pimentel 1995). In Persia, it was found that the extract from chrysanthemum flowers had insecticidal activities against crop pests (Gabriel and Mark 1996). During recent years, spirohydantoin compounds have found application as pesticides in agriculture (Marinov et al. 2012). Hydantoins are organic compounds with wide application, most significantly in the area of medicine (Sarges et al. 1988).

The purpose of this study is to establish the potential insecticidal effect of 1,3-Diazaspiro[4.4]nonane-2,4-dithione, a substance of the spirohydantoin group, on sawfly *Diprion pini* L. and *Apis mellifera* L. as a non-target organism.

Materials and Methods

An *in vitro* study was done in laboratory conditions in 2015 of the insecticidal activity of a substance from spirohydantoin group with respect to *D. pini*. The substance was tested in five concentrations

ranging from 0.001 % to 0.01 %, in order to determine the highest efficacy. Each concentration was tested on 10 larvae placed on filter paper in 100×20 mm Petri dishes. Each test variant was repeated three times. The larvae of each test variant were sprayed with the respective solution of the substance. Petri dishes were covered and left at room temperature of 20–21 °C. Mortality was taken at the 24th, 48th and 72nd hour after treatment. The efficacy of the substance was calculated using Abbott's formula (Abbott 1925):

$$E(\%) = \frac{(T - t) \cdot 100}{T},$$

where: T is mean number of alive larvae on control, t is mean number of alive larvae on each treatment.

The concentration with highest efficacy was tested against *A. mellifera*. In two groups of five wooden cages (10×10×10 cm) 20 newly hatched bees were placed and fed on 50 % sugar syrup. To both groups, in quantity of 20 mL sugar syrup 2 g of *Helianthus annuus* L. pollen with protein content of 14.83 % (Radev 2021) was added. The food was delivered to the cages through a 2 mL pipette. Tested substance was added to the food of the first group at a concentration of 0.01 %, while the bees from second group consumed just pure food. The syrup was renewed daily. The cages were covered and left at room temperature of 20–21 °C. During the first day, the bees were monitored an hour, later every 24 hour subsequently.

The results were statistically processed by Excel and Anova.

Results and Discussion

The laboratory results show an insecticidal effect on the larvae of *D. pini*. According to Abbott's formula (Abbott 1925) and the obtained data, the dose-response curve displays the insecticidal activity at the highest concentration. No insecticidal effect is found at concentration of 0.001 %, and at 0.003 % and 0.005 % the efficacy is low. Good toxicity is measured at concentration 0.008 %, while the highest efficacy of 93.1 % is measured at concentration 0.01 % of the tested agent ($F > F_{crit}$, Anova: Single factor) (Fig. 1).

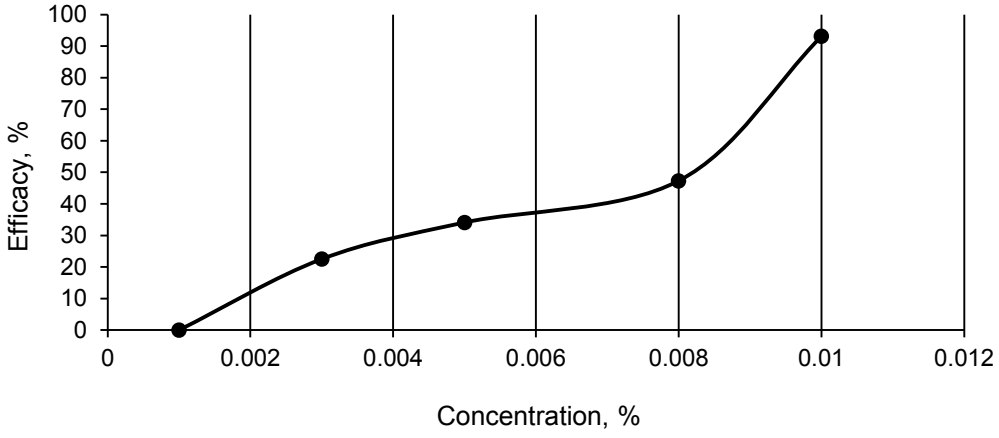

Fig. 1. Insecticidal efficacy of 1,3-Diazaspiro[4.4]nonane–2,4–dithione against larvae of *D. pini*.

Due to the limitation of conventional means for plant protection, it is necessary to investigate and create alternative means for control of plant pests. These means must be selective to the beneficial entomofauna and toxic to the highest degree to the harmful. In this connection, the tested substance is applied for testing at concentration 0.01 % with representatives of *A. mellifera* in order to determine the insecticidal effect.

The results show a lifespan of worker bees of up to 8 h, with an average of 3.6 h. The highest mortality was reported up to the 3rd hour, while the highest value was at the 2nd hour (means ±SD 7.8 ±1.9) Fig. 2). The bees fed on clean food lived up to 168 h, an average of 120 h. The highest mortality was measured at the 120th hour (means ±SD 6.2 ±1.6) (Fig. 3).

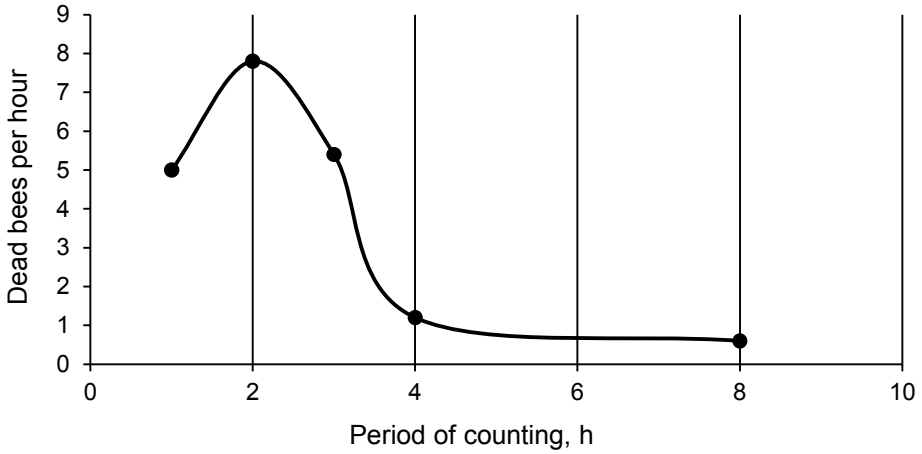

Fig. 2. Insecticidal impact of 0.01 % 1,3–Diazaspiro[4.4]nonane–2,4–dithione to *A. mellifera*.

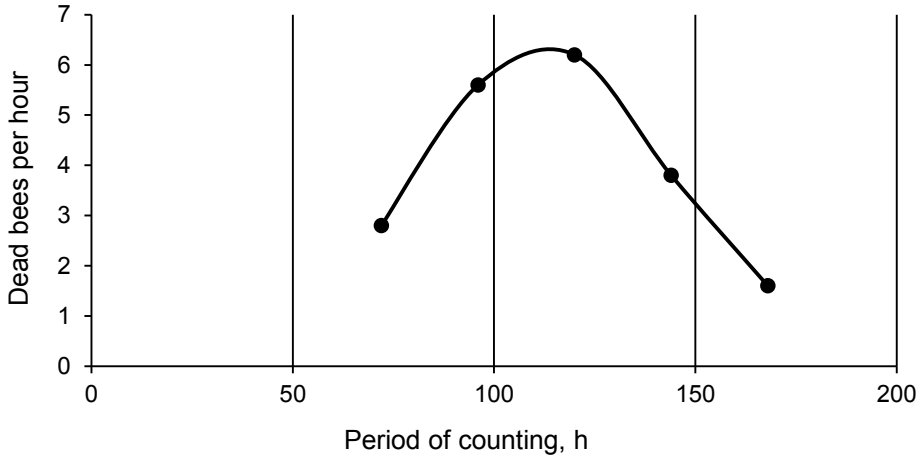

Fig. 3. Lifespan of *A. mellifera* fed on pure food.

These results show difference in the effect of the substance tested on the lifespan of worker bees ($F > F_{crit}$, Anova: Single factor). The data obtained confirm the findings established by Radev (2020). Numerous studies have found toxicity of many groups of pesticides to honey bees (Smarta and Stevensona 1982, Blacquière et al. 2012). Experimental outdoor work with bee colonies is mandatory and the results to be obtained may vary from the laboratory ones.

Conclusions

High mortality of 93.1 % was found against the larvae of *D. pini* with concentration 0.01 % of the tested agent as well as much lower lifespan of the bees which consumed it.

References

Abbott S. 1925. A method for computing the effectiveness of an insecticide. Journal of Economic Enthomology 18: 367–271.

Blacquière T., Smagghe G., Gestel C., Mommaerts V. 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. Ecotoxicology 21(4): 973–992.

Gabriel K. L., Mark R. 1996. Environmental toxicology of pyrethrum extract. In Pyrethrum flowers: production, chemistry, toxicology, and uses. New York NY: Oxford University Press: 277–283.

Marinov M., Ganchev D., Marinova P., Krustev S., Atanasova N., Zlateva M., Stoyanov N. 2012. Synthesis of cyclopentanespiro-5-(2,4-dithiohydantoin) and *in vitro* insecticidal activity against *Cladius pectinicornis*. Bulgarian Journal of Agricultural Science 18(6): 929–933.

Pimentel D. 1995. Amounts of pesticides reaching target pests: environmental impacts and ethics. Journal of Agriculture and Environmental Ethics 8: 17–29. DOI: 10.1007/BF02286399

Radev Zh. 2020. Helpful entomological fauna reaction to spirohidantoins. New knowledge journal of science 9(2): 203–207.

Radev Zh. 2021. Protein content in bee-collected pollen of some plants in forestry region. Forestry ideas 27(1): 68–73.

Sánchez-Bayo F. 2011. Impacts of Agricultural Pesticides on Terrestrial Ecosystems. In: Ecological Impacts of Toxic Chemicals: 63–87. DOI: 10.2174/978160805121210063

Sarges R., Schnur R., Belletire J., Peterson M. 1988. Spiro hydantoin aldose reductase inhibitors. Journal of Medicinal Chemistry 31(1): 230–243. DOI: 10.1021/jm00396a037

Smarta L., Stevensona J. 1982. Laboratory Estimation of Toxicity of Pyrethroid Insecticides to Honeybees: Relevance to Hazard in the Field. Bee World 63(4): 150–152.

Tilman D., Balzer C., Hill J., Befort B.L. 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences of the United States 108(50): 20260–20264.