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Review paper

INFLUENCE OF INSECTICIDES IN PRODUCTION OF HONEY BEES

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ABSTRACT: In recent decades insecticides have entered into wide use in agricultural production. In addition to the positive effects such as increased yields, there was a far-reaching negative implications for biodiversity, agro-biodiversity, the environment and human health. Toxic chemical compounds, which are components of insecticides contaminating pollen and nectar, and thus have a negative impact on the bee population and their survival. Through bees insecticides are becoming an integral part of the bee products consumed by man. Insecticides weaken resistance of bees, encourage the emergence of diseases for example Nosema ceranae, and can lead to the collapse of the colony. Further contamination of ecosystems insecticides can jeopardize the whole economic and ecological system.

Key words: *Insecticides, bee production, biodiversity, environment*

INTRODUCTION

Number of bees that are important for pollination has declined in the last 15 years, and that phenomenon is explained by different factors, including pesticide use, parasites, lack or improper use of veterinary drugs, reduction of habitats, nutrition and climatic changes. As bees are insects, many insecticides that are used for pests control in agricultural crops are toxic also to them. The attempt to present necessity of honey bees' existence, as insects that are invaluable and significant for humanity is reality and it should be observed from more aspects. In agriculture, especially in pomiculture honey bees have an invaluable role in pollination of fruit trees. Bees have a key role in agricultural production. From economic aspect, bees are the most important pollinators. In USA, pollination 20-30 billions of dollars in plant production at the annual level (Walker, 2013).

Maintenance of healthy population of honey bees and other pollinators is of crucial importance for the future of agricultural production and for securing of sufficient food quantities (Walker, 2013). All our fruits (apricot, peach, plum, cherry, sour cherry, apple, etc.) attract bees to a great extent by their pollen and nectar. Bee keeping and pomiculture are branches which ideally complement each other. The central strategy for income increase in agriculture is restriction of losses caused by negative effects of weeds, diseases and pests. Since the mid-20th century, the main principle in plant protection has been an increase in the use of pesticides.

Table 1. Pollination of some agricultural crops in year 2006

	Value in billions US $	% of bees participation in pollination
Soybean	19.7	5
Cotton	5.2	16
Grape	3.2	1
Almond	2.2	100
Apples	2.1	90
Oranges	1.8	27
Peanuts	0.6	2
Apricots	0.6	2
Strawberries	0.5	90

These positive effects of Green revolution have also their dark side. It is evident that contemporary agriculture is faced with a serious crisis in the environmental field. An increasing number of people in the world and in our country are concerned for the long-term sustainability of such a system of food production.

PESTICIDES

Pesticides represent selectively toxic chemical compounds that are used for control of harmful insects, agents of plant and animal diseases and in control of weed population, i.e. in agriculture, forestry, veterinary medicine and community hygiene (Sanford, 2012). Scientific researchers have confirmed that pesticide use, even at low ''allowed'' rates in the long term cause the extinction of bees. Lately, bees population reduce fast, and this phenomenon is called ''Colony Collapse Disorder'' and refers to the whole planet (Zivadinovic – www.spos.info). In the Northwest California, highly toxic insecticides with the residual toxicity longer than 8 hours are responsible for the greatest part of the poisoning of bees. Pesticide poisoning of wild bees can remain undetected (Hooven et al., 2013). In the study on the impact of neonicotinoid pesticides to bees populations, that were headed by Dave Goulson it was found that the treated group was lighter, suggesting that less food entered the hive. At the end of the experiment, the treated groups in average were 8-12% smaller than control ones and had almost 85% less of bee quines (Goulson, 2015). The problem is that recommended pesticide doses are often not respected by farmers, i.e. doses that are used are on the limit of the mortality of bees and the consequences of doses that do not kill them instantly, but can cause disorders in behaviour due to which they are gradually killed are ignored. The main reason for poisoning of bees is often lack of information or consciousness, more than intention to harm. Majority of pest control programs in crops can be modified so that bees poisoning, without unnecessary costs or inconvenience for the farmer is very unlikely (Hooven et al., 2013). Depending on the target group of pests that control, pesticides are divided in to phytoncides, i.e. fungicides and herbicides and zoocides, that are according to the same criteria divided (Trkulja et al., 2015): Insecticides (pesticides used for to kill insects), acaricides (pesticides used to kill mites), nematicide (pesticides used to kill plant-parasitic nematodes), limacides (pesticides used to kill snails), rodenticides (an agent that kills, repels, or controls rodents), avicides (any substance which can be used to kill birds).

THE IMPACT OF INSECTICIDES TO HONEY BEES (*Apis mellifera* L.)

Insecticides are intended for control of harmful insects and vectors of human, animal and plant disease agents. Depending on the way of its introduction, we distinguish: Contact insecticides – insects can be poisoned by touch or contact with the treated area. Digestive insecticides – the poisoning occurs after food intake into digestive tract. Inhalation insecticides - cause poisoning after being inhaled, e.g. when spraying mosquitoes. Toxicity of insecticides depends also on the mode of penetration into bee's organism. They can penetrate digestive tract by nectar and pollen or by introduction to the trachea (breathing system of bees). In some modes of penetration, there exist differences in toxicity, but the fact that new pesticides penetrate in all three modes is important. The basic criterion for insecticide toxicity is medium oral lethal dose (LD-50) sufficient for killing of 50% of experimental insects. Based on the level of toxicity, all insecticides are divided into 4 classes. (Laurino et al., 2011; Suchail et al., 2001; Tennekes and Sanchez-Bayo, 2011): Insecticides of Toxicity Class I (most toxic) - (LD-50 lower than 2 mg per bee). Bees suffer very much if the treatment is performed during their flight, but they can be killed also in the following 5 days due to high toxicity of the products. Insecticides of Toxicity Class II (moderately toxic) - (LD50 is between 2 and 11 mg per bee). In cases of correct use, doses, time and method of application they do not represent direct danger, but should not be directly applied to bees or bees' communities. Insecticides of Toxicity Class III (slightly toxic) - (LD-50 is higher than 11 mg per bee). When used near the bees can cause minimum damage. Lower insecticide doses do not necessarily cause rapid death, colonies are more likely to fall ill, and their immunity weakens which can cause their extinction that occurs after a few weeks or months. Weakened immune system is favourable situation for development of different diseases such as *Nosema cereanea*, when synergistic effect leads to the collapse of colonies (Sluijs et al., 2013). In the study of Pettis et al. (2013), it is stated that 8 pesticides (22.9%) were linked with the increased distribution of *Nosema cereanea,* while the remaining 14 were linked with its decrease. Formamidine ormamidine (DMPF) and two of three piretrioides (bifenthrin and fluvalinate, but not esfenvalerate) are linked with the increased risk of nosema infection. Carbamate (carbaryl), all neonicotinoids (acetamiprid, imidacloprid and thiacloprid), organophosphates (coumaphos, diazinon and phosmet) and oxadiazine (indoxacarb) were associated with a reduced risk of nosema infection. In other research (van Engelsdorp at al., 2012), the increased risk of nosema infection was associated with the consumption of DMF (formamide), fluvalinate and miticide that were applied by beekeepers to help control high-destructive Varroa mite. These results suggest that insecticides have different effects to the function of the bees' immune system. Insecticides are the most dangerous to bees, because bees belong to insects. Phytoncides are less dangerous, and fungicides practically do not cause poisoning. There are more evidences of negative effects of real doses of the most frequently used insecticides to properties such as reproductive levels, search for feed and navigation in bees. When bees feed by plants that are treated by pesticides, low concentration in nectar and pollen can also be found (Goulson, 2013; Henry et al., 2012; Mommaerts et al., 2010; Whitehorn et al., 2012). Penncap-M applications should not be performed in fields and: when bees are in search for feed, fruits in the phase of flowering, plant branches contain more than five flowers per quadrat meter or when on sweet corn are tassels and silk (Adams and Bartholomew, 2004).

Poisoning of bee colonies by insecticides depends on several factors (Trkulja et al., 2015): Toxicity of insecticides, mode of their penetration into bees' organism, the form of the insecticide, application method, plant species being treated and the plants phenophases, time of day and season of the year when insecticidal treatment is performed.

NEONICOTINOIDS

Neonicotinoids are relatively new herbicide class. They belong to the group of synthetic and systemic contact insecticides; in the central nerve system of insects they cause blockade of receptors and weakening of the memory. They are similar to natural nicotine and belong to the group of most toxic. They have negative impact to motor activity; disrupt navigation and orientation of bees (Decourtye et al., 2004; van der Sluijs et al., 2013; Goulson, 2013). Michael Henry came to the result for the bees with a small dose of neonicotinoid often failed to find their way to the hive, due to which they died (Yang et al., 2008). Exposure to thiamethoxam showed that a morphological brain damage occurs in bees (Oliveira et al., 2013). Neonicotinoids are insecticides are the most widely used insecticides in the world, and their market value amounts to several billion dollars a year. Dozens of independent, professional studies evaluated that neonicotinoids are highly toxic to bees and other pollinators, and their use can have fatal effects (Walker, 2013). The effects of neonicotinoid pesticides on pollinators are cause for alarm. Neonicotinoids are a class of insecticide that are used to control aphids and other insects. For less than 20 year, neonicotinoids became the mostly distributed pesticides in the world with more than 25% of market share in 2010, with the growing trend of production and sell (Casida and Durkin, 2013). Neonicotinoids are persistent in the soil, sediments and water and as such they are highly toxic to environment and living organisms (Tomizawa and Casida, 2011; Walker, 2013). Imidacloprid was registered in 1992 as the best known insecticide in this class, nowadays there is a whole range of new neonicotinoids registered since then (clothianidin, acetamiprid, thiamethoxam, thiacloprid, dinotefuran, etc.). Their use has increased dramatically in recent years and now they are the most widely used group of insecticides in the United States (Frazier, 2007; Sluijs et al., 2013.) Imidacloprid which is the oldest and most widely used neonicotinoid insecticide is directly linked to poor health of bees and their reproduction (Walker, 2013). It can certainly damage the nervous system of bees already at extremely low concentrations, reducing their activities, communication, memory and the ability to smell (Decourtye et al., 2004). Its acute oral toxicity LD-50 was established within the limits of 3.7 and 102 ng/bee (Suchail et al., 2001). California is the number one in use of imidacloprid for pests' control. Imidacloprid and clothianidin are highly toxic to bees. For example, clothianidin is highly toxic to bees on acute bases (LD50 = 4 ng/bee). It reaches nectar and pollen and has a high potential of toxicity for honey bee, as well as for other pollinators. The outcome can be lethal, and negative effects can be to larvae, as well as for reproductive effects for queen bees. In 2002 a study on pesticide residues in pollen was carried all over France. Imidacloprid is the most frequently found insecticide in even 49% of samples (Chauzat et al., 2006). The queen bee production is lower in colonies exposed to high concentrations of neonicotinoids. A decline of 85% in queen bees' production was registered in conditions of their exposure to real levels of imidacloprid during 2 weeks in a field (Goulson, 2015). The study of the University of North Carolina showed that some neonicotinoids in combination with certain fungicides (Terraguard and Procure) in laboratory conditions synergistically increase toxicity to bees over 1.000 times (Iwasa et al., 2004).

The occurrence of non-toxicity or partial and "negligible" toxicity to bees is called insecticide selectivity. Selective insecticides that are used for treatments of destructors in honey bee production are (Sanford, 2012):

- AMITRAZ (C19H23N3) (Bivarol, Mitac, Varamit, Hemovar, Varolik) is non-systemic acaricide and insecticide widely used in honey bee production. LD-50 for one bee is 12 000 ng in 48 hours of action.
- COUMAPHOS (0.0-diethyl-0-/3-chlor-4-methyl-7 cumarinyl/thiophosphat) (Perizin, Apiprotekt) proved to be one of the more dangerous systemic to bees se. LD-50 is 3 000 ng per bee.
- FLUVALINAT (C26H22CIF3N2O3) (Klartan, Mavrik, Varotom, Apistan, Apihelt) is the representative of pyrethroids, the insecticides initially isolated from certain plants and later also artificially synthetized. Medium oral lethal dose for a bee is 2 000 ng.

Although DDT and given pesticides for control of Varroe destructor toxic also to bees, in comparison to neonicotinoids their toxicity is negligible. For example, imidacloprid is more than 7.000 times more toxic than DDT, and more than 800 times more toxic than Coumaphos. The demand of honey beekeepers is that farmers use insecticides carefully, as the contamination of honey bees' colonies will cause losses due to the lack of pollination. Transformed agro-ecosystem will reduce biodiversity and possibility of quality food production (Sluijs at al., 2013). Many countries have intensified and taken preventive measures to protect bees and other pollinators from the negative impact of neonicotinoids. On 29 April 2013 The European Union (EU) has approved a minimum two-year moratorium on the use of certain neonicotinoid chemicals all over the continent. The use of the three neonicotinoids such as imidacloprid, thiamethoxam, clothianidin has been suspended (Walker, 2013; Henry et al., 2012). The moratorium should result in better performances of bees' colonies over the course of time, if neonicotinoids residues reduce (Goulson, 2015). In countries in which neonicotinoids have so far not been banned, and a policy of protection of honey bees' colonies is implemented, the possible solution is increase in price of insecticides (or introduction of additional taxes) or change of the type of insecticides that are used on farms (Kleczkowski at al., 2013). Non-governmental organizations for environmental protection consider that temporary moratorium is insufficient and that ban should be permanent, because only that "can help in restoration of bees' populations.

SYMPTOMS OF INSECTICIDE BEE POISONING AND GUIDELINES FOR BEEKEEPERS AND ANYONE WHO WANTS THE SURVIVAL OF MANKIND AND BEES

Majority of insecticides toxic to bees act on nerve system. After exposition, sooner or later occurs a paralysis of bees' vital organs. Initially suffer tentacles, legs and wings, and then oral apparatus and the other parts of the body. Some products cause spasms and wrapping of bees' body. At the beginning, poisoned bee becomes more active, then the body convulses, legs are uncoordinated, it falls over onto its back and dies quickly (Mommaerts et al., 2010). As a rule, the common sign of bees poisoning is the appearance of excessive number of dead honey bees in front of the hives. Poisoned bees die in all hives at the same time, almost simultaneously (Sanford, 2012; Goulson, 2015).

If the bees are poisoned by neonicotinoids, they die on the way to the hive. Dead bees are not visible in front of the apiary, but declining numbers of them alerts us that there was a poisoning. Mass bees poisoning occur during treatment of plants in flowering phase. Also, large poisoning of bees is recorded during plant treatments in the late flowering phase, i.e. when at least 80% of flower petals fall off. The biggest poisoning of bees cause amateur fruit growers, who do not follow label instructions on the use until the end, but are interested only in the part about the dosage which they often increase. If the poison with slower action or less toxic is used for the treatment, bees can intake it with nectar into the hive, and then also die bees that nurture and grow a litter.

Farmers depend on bees (for pollination of their crops), and they must permanently maintain delicate equilibrium between protection of their crops from pests and protection of insects that are necessary for pollination of these crops (Frazier, 2007). Farmers that treat plants must inform about it beekeepers in surroundings, or beekeepers society, at least two days before the treatment. All hives that are less than 5 km from the treated area are endangered. Informed beekeepers must move or close their bee societies. Beekeepers are obliged to attach a table beside an apiary with his name, address and telephone number. The treatment is also not allowed at a distance less than 60 meters from the apiary. The attention is also to be paid those bees that are leaving for their feed, do not pass through pesticide cloud (Goulson, 2015). After being informed on pesticide treatment, for protection of bee communities, a beekeeper has two possibilities (Sanford, 2012): Moving of bee communities 7-10 km from the edge of the treated zone, or closing of hives (jute cloth) in order to prevent bees' flight to treated areas for 1-2 days.

Some concrete recommendations for beekeepers are provided (Frazier, 2007; Johansen, 2013; Hooven et al., 2013; Adams and Bartholomew, 2004; Sanford, 2012):

- Hives should not be left near fields or orchards that are likely to be treated by pesticides toxic to bees.
- Local beekeepers must be informed on the plan of treatments during vegetation period.
- It should be informed on pesticides and their toxicity to bees.
- The label should be red and the instruction is to be followed.
- Neonicotinoid pesticides are not to be used in the phase of flowering of crops or weeds, if bees are present. Pesticides are to be applied only after petals fell off, when horticultural plants are less attractive to bees. This will reduce a risk of bees contact with pesticides.
- If the treatments in flowering phase are needed, as it is the case with silking of sweet corn, a pesticide with short action should be chosen.
- Use of pesticides belonging to the group of neonicotinoides is not recommended before flowering, immediately before bees are not actively foraging.
- If unusually low temperatures or dew are expected, insecticides are not to be applied.

- If one of these pesticides must be used, choose the one with lower toxicity to bees (acetamiprid or thiacloprid) and use it only when bees are not in search for food, preferably in late evening hours.
- Pesticides are not to be used until bees are not removed from the crop.
- Water springs are to be protected from pesticide contamination.
- Use of alternative pesticides (bio-pesticides) and integral crop protection are to be considered, with the aim of elimination of reduction of damaging treatments, or those that are not needed.
- If it is established that bee communities are poisoned, they are to be fed by rare sugar syrup (1:1), for due to the loss of foragers, such bee communities do not have enough water, and sometimes are also without feed. Feeding is to be continued for 5 days. If high mortality of bees in a hive is noticed, the frames with fresh nectar and pollen that still contain the poison must be immediately removed (Decourtye, 2012).

As bee losses due to poisoning by pesticides increase every year, the right choice for its prevention is that agricultural organizations and farmers understand significance of bees for pollination of plants and begin, not only to keep wit not only to comply with legal norms, but also to use less toxic and non-toxic pesticides for bees. Economy of food still requires the use of pesticides. It is often stated that humanity has two roads ahead: one is to poison itself slowly by low pesticide doses whose residues are in fruits and to die slowly, and the other is to accept the concept of organic or ecological agriculture, i.e. production of healthy food in which use of toxic substances (pesticides) is strictly forbidden (Losey and vaughan, 2006). Human consciousness is necessary to be influenced by education on significance of biodiversity preservation, intensification and providing support to organic food production, as the only sustainable way of humanity survival.

CONCLUSIONS

Concerning the significance of pollination for production of organic matter and agriculture production the measures for protection of pollinating insects, among which bees are the most important, should be undertaken. Mass extinction of tens of thousands of bee colonies devastated many beekeepers and they remained without the quantity and quality of honey. Numerous studies suggest that chemical contamination is one of the reasons for destroy of bee populations. This includes chemicals that are used in hives for control of mites and diseases, as well as chemical pesticides that are used on crops, and that often accidentally reach the hive. It is needed to take care on insecticide application in agricultural production, especially neonicotinoides that are the most toxic. During their application should be taken appropriate measures, and instructions that will protect stability of agro-ecosystem should be followed. As our survival depends on healthy pollinators, we must all in our power to solve this problem. Situation requires multiple strategies to protect the health of bees and to give support to original pollinators. If we wish to preserve these amazing creatures, we must act fast. Not only to reduce economic losses due to collapse of bee communities, but to preserve biodiversity and resources on which life and health depend. Beekeeping begins to be highly demanding agricultural activity whose results depend on numerous factors, among which one of the most important is good cooperation between producers (farmers, fruit growers) and beekeepers. Both need to be aware that a world without bees, is also a world without fruit, vegetables, cereals, flowers - a world without life.

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