# Use of Neonicotinoids in Bees: A Review

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**Review Article** 

#### ABSTRACT

Received: 10-Jan-2023, Manuscript No. JAAS-22-86488; Editor assigned: 12-Jan-2023, Pre QC No. JAAS-22-86488 (PQ); Reviewed: 26-Jan-2023, QC No. JAAS-22-86488; Revised: 18-Apr-2023, Manuscript No. JAAS-22-86488 (R); Published: 25-Apr-2023, DOI: 10.4172/2347-226X.12.2.011

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Neonicotinoid insecticides are widely used in controlling insect pests in a variety of agricultural crops; however, they not only adversely affect insect pest population but also non-target organisms such as pollinators. This review encapsulate more than 15 years of research on the risk of neonicotinoids to bees including bumble bees, honey bees and solitary bees. The main focus of the paper is on different aspects determining the risks of neonicotinoid concentrations for bees and other pollinator populations; the neonicotinoid residue levels in bees and bee products; the reported side effects with special attention to sub-lethal effects and effect on different biological parameters such as biology, foraging behaviour, queen reproductive success, honey production, effect on morphology etc.

**Keywords:** Neonicotinoid; Encapsulate smore; Pollinator populations; Morphology; Agricultural crops

#### INTRODUCTION

Abundance and diversity of wild bees as well as domesticated bees are declining worldwide. Bees, including honey bees, bumble bees and solitary bees, are economically most important as they are largest pollinator groups worldwide. More than 35% of the world food crop production is dependent on pollinators, accounting for an annual value of 153 billion Euros. Bees also provide key insect pollination services to wild plants, of which in Europe 80% pollination need entotomophily, which confirms their ecological significance. The decline of pollinators, which has grown over the last decades, may lead to a parallel decrease of plant biodiversity, or vice versa. Moreover, decline of the honey bee, *Apis mellifera* all over the world is a great concern because of its high economic importance <sup>[1]</sup>.

Abundance of insect pollinators in the environment is also influenced by multiple factors for instance biotic like pathogens, parasites, availability of resources due to habitat fragmentation and loss; and abiotic factors such acclimate change and pollutants. Although the putative causes are still currently analyzed, the extensive use of chemical pesticides against insect pests for crop protection may be considered as one of the important factor causing the loss of pollinators <sup>[2]</sup>.

With increase in human population, the need to have extensive food security is important and chemical insecticides play most significant role by increasing the crop productivity in intensive farming systems where they safeguard about one-fifth of the crop yield. Within the vast variety of insecticide classes used, the neonicotinoid insecticides, which include imidacloprid; acetamiprid; clothianidin; thiamethoxam; thiacloprid; dinotefuran and nitenpyram; are most important group of neurotoxins specifically acting as antagonists of the insect nicotinic Acetylcholine Receptors (nAChR). Neonicotinoid insecticides were developed in the 1990's as an alternative to broader spectrum systemic pesticides. They are the synthetic analogue of nicotine, which is a naturally occurring insecticide, and target the nicotinic Acetylcholine Receptors (nAChRs) of a cell and trigger a response by that cell. In insects, these receptors are limited to the central nervous system. Nicotinic acetylcholine receptors are activated by the neurotransmitter acetylcholine receptors in the insect nervous system. They are registered for use on a variety of crops and are effective against a wide range of sucking insects as well as chewing insects such as beetles and some Lepidoptera, particularly cutworms. Neonicotinoid insecticides can be applied as either a foliar spray or a seed coating <sup>[3]</sup>.

#### LITERATURE REVIEW

This review provides important information regarding brief knowledge of the data published over the last 15 years by different scientists on concentrations of neonicotinoid insecticides recovered in plants, bees and their products. This analysis of the literature took into consideration the different crops, the methods of application and the importance of metabolism, and covered data from different parts of the world. Second, the publicly available data on side effects of the different neonicotinoid insecticides towards honey bees, bumble bees and other bee species are summarized. and critically analyzed with a special emphasis on sub-lethal effects on reproduction, foraging behavior, memory/learning abilities and overwintering success. A third part focuses on the potential applicability of the new stepwise risk assessment scheme as proposed for systemic pesticides, for more adequately assessing risks for side effects by neonicotinoid insecticides. The latter assessment took into account the characteristics of doses of neonicotinoid insecticides in their field realistic range and followed the classical approach from the laboratory to field related conditions and from exposure of individual bees to the colony level. The importance of the use of adults and larvae (brood) together with the scoring of lethal and sub lethal biological endpoints is also discussed. Points of comparison and experimental advantages and difficulties between honey bees, bumble bees and other bees are discussed. Attention is paid to the use of mixtures containing neonicotinoid insecticides that can synergize their hazards for bees. Our paper concludes with some targets for research and recommendations for future risk assessment studies, specifically with the aim to assess the global bee colony health status [4-13].

#### Ways of exposure

Neonicotinoids are applied as seed coatings, foliar sprays, soil drenches or granules, as well as by direct injection into tree trunks or by chemigation. Due to this wide variety of application methods, their systemic action and their low toxicity to vertebrate species, neonicotinoids are increasingly used for crop protection against insect pests in Europe and all over the world. Thus, honeybees are frequently exposed to these systemic substances. While successfully controlling a variety of agricultural crop pests, these applications do not only affect insect pests population but also non-target organisms like insect pollinator species observed that residues of neonicotinoids tend to be incorporated into weeds growing within or next to treated fields, which indicates either a deposition of neonicotinoids on the flowers, or an uptake by the root system, or both. These systemic insecticides have longest duration of action in comparison of other pesticides. Hopwood, et al., also proved that these insecticides can persist in plant tissues for months or even for more than a year. In addition to that, neonicotinoids are able to remain in soils over longer periods of time (Table 1). Untreated plants are at risk of residue uptake from previous uses of pesticides still remaining in the soil. Due to water soluble nature, they can migrate to surface water bodies. Besides filtrate, water sources may also

be contaminated by over sprays, drifts or field run offs. In summer days, bees generally collect water to cool their hives contaminated water bodies are thus additional routes of exposure [14-17].

Girolami, et al., investigated leaf guttation drops of corn plants germinated from neonicotinoid coated seeds. They found that the concentration of neonicotinoids in guttation drops can be near those of active ingredients commonly applied in field sprays for pest control, or even higher <sup>[18]</sup>.

#### Effects on morphology

Study conducted by Annelise de Souza Rosa, et.al., showed that thiamethoxam significantly affect head width and in tertegular span of the worker of sting less bee. Higher doses of thiamethoxam exhibited significantly lower values for both the head width and intertegular span of bees. Wing pattern and the centroid size were also significantly affected in treated and non-treated treatments. Furthermore, increased wing symmetry was also observed by Higginson and Barnard that resulted in reduced flight efficiency <sup>[19-23]</sup>.

#### Effects on reproductive success

Ian Laycock, et. al., observed effects of dietary imidacloprid on ovary development and fecundity to arrange between 0 to 125  $\mu$ g L<sup>-1</sup> and found that microcolonies showed a dose dependent decline in fecundity, with environmentally realistic dosages in the range of 1  $\mu$ g L<sup>-1</sup> capable of reducing brood production by one third. They also emphasized that ovary development was unimpaired by dietary imidacloprid except at the highest dosage <sup>[24]</sup>.

Wu, et al., observed that worker bees reared in brood comb containing high levels of many pesticides showed multiple health effects including reduced adult longevity, increased brood mortality, delayed larval development, and higher fecundity of *Varroa* mites. Delayed development was observed in the early stages (day 4 and 8) of worker bee that leads to reduced adult longevity by 4 days in bees exposed to pesticides during development. Higher brood mortality and delayed adult emergence in bees also observed in insecticides treated in compared to control comb <sup>[25]</sup>.

#### Effect on foraging activity

Decreased foraging activity has been observed in worker honey bee fed with sugar solution with 24 µg/kg of imidacloprid and negative effects on olfactory learnt discrimination task was also observed by Decourtye, et al.

The waggle dancing has often been considered an important cognitive behaviour of honeybee foraging activity. The waggle dance is an important signal in communication that provides information to honey bees about the presence of good food sources, activates private navigational information in followers, facilitates the acquisition of information about food odours, and indicates the location of the food source. Unusual and diminished waggle dancing adversely affect colony food source and reduce stored honey weight gain in situations where recruitment is necessary, and also reduce colony fitness overlong term. Eiri, et al., have found bees ingested sucrose solution contained imidacloprid (0.21 mg/bee) had higher SR thresholds. Bees ingested imidacloprid (0.21 ng/bee) have also been found reduce waggle dancing circuits (10.5 and 4.5 fold fewer for 50% and 30% sucrose solutions, respectively in compared to control after 24 hours treatment [<sup>26</sup>].

Henry, et al., tested sub-lethal effect of thiamethoxam on the foraging behaviour of honey bees. They found that sublethal dose of thiamethoxam increases hive mortality rate indirectly because of homing failure in foraging honey bees. They observed daily intoxication events that bees would have received by a field realistic, sub-lethal dose of 0.07 ppb of thiamethoxam. Radio Frequency Identification (RFID) method was used to monitoring foraging behaviour of honey bees. Mortality due to post exposure homing failure was derived from the proportion of non-returning foragers and corrected by data from non-treated bees for other causes of homing failure in treated foragers such as natural mortality, predation, or handling stress a significant mortality was observed in comparison with controlled foragers.

Bortolotti, et al., found that honey bee workers got confused and disoriented when exposed to imidacloprid. A sublethal dose of imidacloprid in sucrose solution did not affect both the foraging and homing ability of honey bees but only 500 to 1000 ppb of insecticide in syrup was sufficiently cause the workers to fail to return to their hive or to the feeding site. In addition, an imidacloprid solution as low as 100 ppb could delay honey bee workers for up to 24 hrs from returning to their hive or arriving at their feeding site. Tan, et al., demonstrated that sub-lethal concentrations of imidacloprid can also significantly affect honeybee (*Apis cerana*) in decision making by increasing the probability of a bee visiting a dangerous food source <sup>[27]</sup>.

#### Effect on colony growth

William G Meikle, et al., evaluated the effects of different imidacloprid concentrations in sugar syrup using cage and field studies, and across different environments. Honey bee colonies were fed with sub-lethal concentrations of imidicloprid (0, 5, 20 and 100 ppb) over 6 weeks in field trials were monitored with respect to colony metrics, for instance adult bee and brood population sizes, as well as pesticide residues. Colonies fed 100 ppb imidacloprid had significantly lower adult bee populations, brood surface areas and average frame weights, and reduced temperature control, compared to colonies in one or more of the other treatment groups, and consumption rates of those colonies were lower compared to other colonies although no differences in capped brood or average frame weight were observed among treatments. Colonies fed 5 ppb imidacloprid had less capped brood than control colonies, but

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## e-ISSN: 2347-226X p-ISSN: 2319-9857

contamination of control colonies was detected. In contrast, significantly higher daily hive weight variability among colonies fed 5 ppb imidacloprid. Imidacloprid concentrations in stored honey corresponded well with the respective syrup concentrations fed to the colonies and remained stable within the hive for at least 7 months after the end of treatment.

#### DISCUSSION

#### Neurological impairment

Behaviour and learning ability of honey bees was adversely affected by sub-lethal concentration of neonicotinoids. Decreased acquisition and the retention performances were observed in the conditioned Proboscis Extension Reflex (PER) paradigm treated with sub-lethal dose of imidacloprid. El Hassani, et al., observed reduced sucrose sensitivity in honey bee workers at the dose of 1 ng/bee of fipronil treated 1 hour after a thoracic application. They also observed that a sub-lethal dose (0.5 ng/bee) could impair the acquisition and retention performances of PER paradigm most probably due to deterioration of olfactory learning. They also stated that sub-lethal concentration of acetamprid could affect gustatory, motor, and mnemonic functions in the honeybee.

A sub lethal dose of imaidacloprid and coumaphos independently did not affect learning ability but when bees exposed to these two insecticides simultaneously, the additive response was observed. Williamson and Wright have found that simultaneous exposure of these two insecticides negatively affect the neurological behavior of worker honey bees, suggesting that pollinators decline could be result of failure of neural function.

#### CONCLUSION

Many scientists have conducted various experiments regarding lethal and sub-lethal effects of neonicotinoids on the foraging behaviour, learning and memory abilities of bees, while a little knowledge about effects in field studies at field realistic dosages were conducted. Study on molecular level has not been conducted till now. It is essential to conduct future research studies with field realistic concentrations, relevant exposure and evaluation durations. Molecular markers can be used to improve risk assessment by a good understanding of neonicotinoids in bees leading to the identification of environmentally safer compounds.

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