# An Artificial Intelligence Based System to Neutralize Pesticides and Sustain Honey Bee Populations


## Abstract

Honey bees are one of the significant contributors of pollination in our ecosystem. Among the various factors for their decline, neonicotinoids based pesticides are one of the greatest threats. Neonicotinoids bind to nicotinic acetylcholine receptors (nAChRs), which are expressed in the central nervous system causing neurological damage and eventual death. To overcome these detrimental effects on honey bees, we present a novel combination of (a) synthetic nAChRs peptides that bind to neonicotinoids thereby keeping it from binding to the bee's receptors and (b) bacteria comprising of specific enzymes that rapidly degrade neonicotinoids. In order to make this process work, an automated machine learning and artificial intelligence based drone system is utilized to dispense the formulations of synthetic peptides and bacteria to desired locations on the crops. The outcome of this project will be a thriving, healthy, and sustainable honey bee population for maintaining our agricultural needs and biodiversity.

## A. Present Technology:

More than 75% of the leading crop species worldwide are dependent on pollinators [1]. Although humans have made significant progress on increasing the productivity of crops by our scientific understanding of plant growth, use of fertilizers, improved water schemes, and prevention from pests and diseases, most plants and crops still rely on pollinators. Among these pollinators comprising of birds, bats, butterflies etc., bees are responsible for the pollination of approximately 70% of all crop species worldwide and contribute to over $20 billion in crop production annually in the US. Out of more than 25,000 globally known species of bees, honey bees (Apis mellifera) rank as the highest single species of pollinator in the US [2].

In the past decade, global declines in honey bees have been linked to pathogens, climate change, habitat fragmentation, and pesticide use [3]. A 2019 study [4] found that 40% of their honey bee colonies loss was attributed to the use of pesticides. Honey bees are exposed to pesticides via numerous pathways including direct exposure, exposure through the pollen and nectar of plants treated with contact or systemic pesticides [5]. Figure 1 shows the different ways the pesticides affect honey bee leading to decay of their colonies.

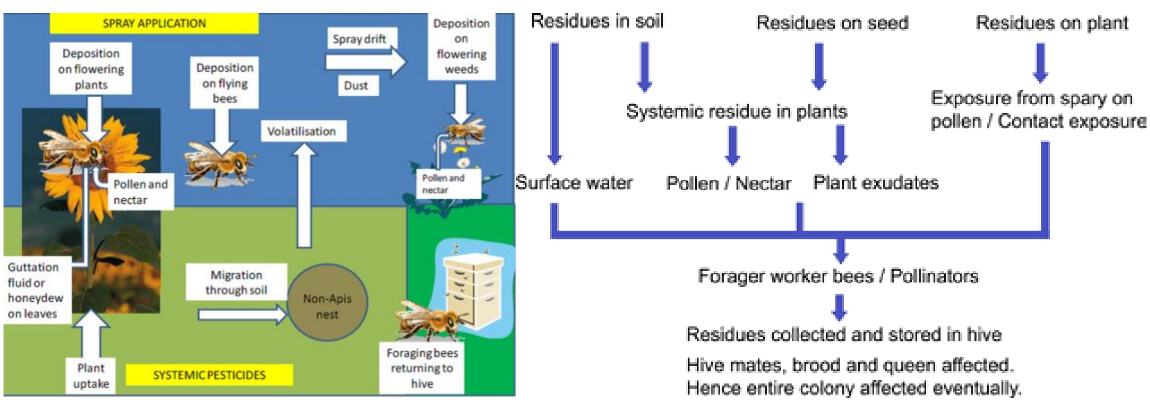


*Figure 1. Pesticide exposure to bees and the steps affecting their health and colonies [5].*

Neonicotinoids [6] are the current generation of synthetic pesticides widely used all over the world. There are five neonicotinoids (imidacloprid, acetamiprid, clothianidin, thiamethoxam and dinotefuran) that are used in the United States for crop protection [7]. Neonicotinoids act by binding to nicotinic acetylcholine receptors (nAChRs), which are expressed in the central nervous system and are involved in synaptic transmission [8]. Under normal conditions, the receptors first get activated upon binding of a neurotransmitter known as acetylcholine (ACh) followed by inactivation when ACh is broken down by an enzyme called acetylcholinesterase (AChE), forming acetate and choline. Similar to ACh, neonicotinoids can bind and activate nAChRs. But, unlike ACh, they cannot be broken down by AChE. As a result, they cause over-stimulation of the nervous system leading to impaired feeding, impaired locomotion, altered learning and memory, impaired foraging, reduced immunity, and eventually, death. Figure 2 shows the structure of the 5 neonicotinoids and the toxicity following interaction with the receptor highlighting the affects neonicotinoids have at different stages of life cycle of insects.

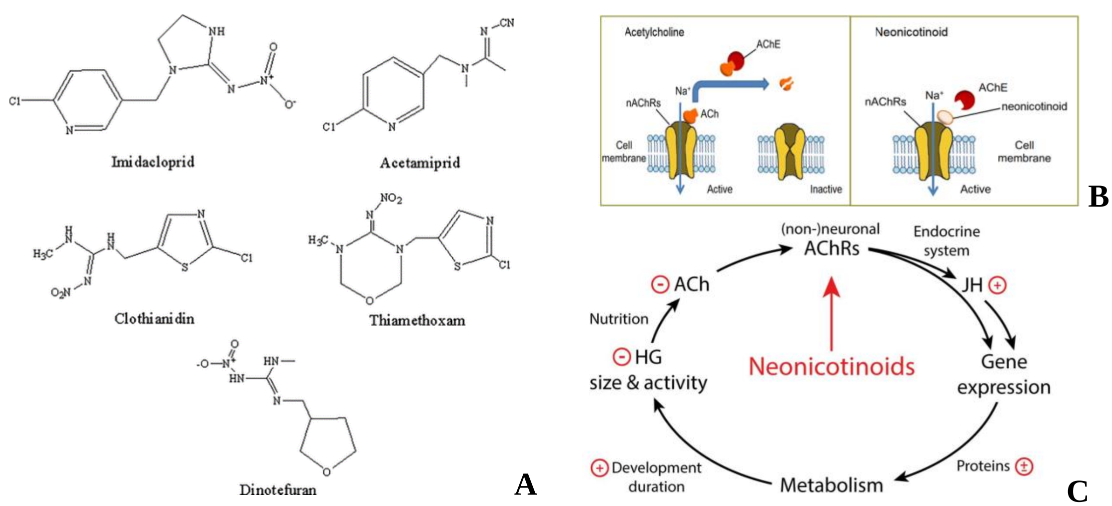


*Figure 2. A. Chemical structures of neonicotinoids [7]. B. Mechanism of interaction with acetylcholine receptor [8] leading to toxic effects. C. Metabolic and endocrine functions of acetylcholine (ACh) in honey bee adults and larvae and its disturbances by neonicotinoid highlighting reduction of hypopharyngeal gland (HG) size and its ACh secretion [9].*

Current Mitigating Process and Limitation

Current methods [10] to limit exposure of honey bees to neonicotinoids include (a) application in the late evening, night, or very early morning when fewer bees are foraging (b) limiting spraying during windy conditions (c) using ground based spraying instead of aerial application (d) using a formulation that dries quickly and does not leave residue that can adhere to the body of the honey bees, (e) establishing apiaries far away from crop fields and (f) use of organic pesticides. However, all of these methods are not suitable for widespread farm use and also do not address the pesticide toxic effects on the honey bees. Hence, there is a critical need to develop a solution that will allow use of pesticide but save the pollinators and especially the honey bees. Loss of honey bees will not only be detrimental to the agricultural industry that is facing challenges to the ever growing human population but will also cause major havoc on the diversity of life observed on our planet.

**B. History:**

Pesticides have been used since before 2500 B.C. [11], with the first ones consisting mainly of sulfur. Later on, heavy metal compounds such as those that contained arsenic, lead, or mercury were also used as pesticides. These interrupted biological processes including enzymatic activity focused on energy generation such as ATP production. However, these pesticides [12] often persisted a long time, did not degrade easily, and often made their ways into local ecosystems, which negatively affected the lives of many animals.

At the beginning of the 19$^{th}$ century, newer pesticides manufacturing methods were developed resulting in the manufacturing of Dichlorodiphenyltrichloroethane or DDT [13]. This pesticide became very prominent in the agriculture world by 1945. After a decade of widespread use, in 1962 Rachel Carson, a marine biologist and conservationist published a book focused on the harmful effects of pesticides on the environment and the animals and pollinators. However, without an oversight and monitoring agency, pesticide use continued widespread. In 1970, President Nixon formed the Environmental Protection Agency (EPA) to monitor and enforce the use of harmful chemicals in the environment. In 1972, the EPA banned DDT [14] which led to development of the class of pesticides called neonicotinoids in the 1980s which were touted to be safer than DDT to mammals. Among the different class of pesticides comprising of organophosphates, carbamates, sulfonylureas and pyrethroid, neonicotinoids account for more than 25% of use worldwide. Neonicotinoids [6] are the current generation of synthetic derivatives of nicotine and were launched in 1991 to overcome the rapid degradation and resistance encountered with nicotine by being persistent following application. They are also effective at very low concentrations and less toxic to mammals. However, these neonicotinoids were identified to be toxic to the honey bees (Table 1) resulting in the need for development of mitigation strategies. Therefore, many alternatives to these pesticides and methods to control them are currently being developed.

Table 1. Known Toxicity of Neonicotinoids to Honey Bees [6]

| **Neonicotinoid** | **Toxicity Levels** | **Contact $LD_{50}$** | **Oral $LD_{50}$** |
|---|---|---|---|
| Acetamiprid | Medium | 7.1-8.09 µg | 8.85-14.52 µg |
| Clothianidin | High | 0.022-0.044 µg | 0.00379 µg |
| Dinotefuran | High | 0.024-0.061 µg | 0.0076-0.023 µg |
| Imidacloprid | High | 0.0179-0.243 µg | 0.0037-0.081 µg |
| Thiamethoxam | High | 0.024-0.029 µg | 0.005 µg |

**C. Future Technology:**

To reduce/eliminate the effects of neonicotinoids on honey bees, our technology relies on integration of biochemical interactions observed in nature coupled with an Artificial Intelligence (AI) based system. The first aim is to develop synthetic peptides that replicate the receptors of nAChRs. These synthetic peptides will bind to neonicotinoids strongly thereby eliminating their interaction with the nAChRs of the honey bee and hence any toxicity. The amino acid sequence information for the binding site (Figure 3) will be used to make the peptide using solid phase synthesis. The peptide will be tested by incubating with the neonicotinoids and measuring the binding efficiency using a competitive fluorescence labeled assay. From the different peptides synthetized, the one with the highest affinity will be selected for use on honey bees.

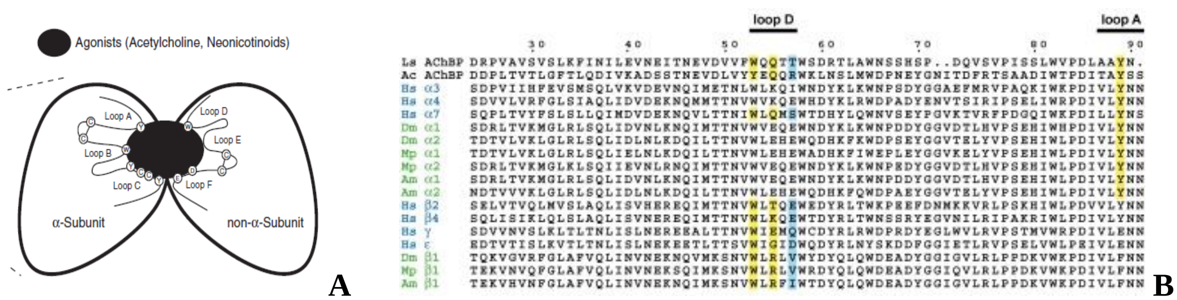


*Figure 3. Representations of a nAChRs binding sites. A. Schematic showing the interaction of the different peptides of the subunits [15]. B. Sequence alignments of acetylcholine receptor α and non-α subunits for different species[16]. Direct intercations are highlighted in yellow, while indirect interactions are shown in light blue background. Am is honey bee (Apis mellifera).*

The next aim is to integrate bacterial species with efficient enzymes for degradation of neonicotinoids. In a study [17] conducted by researchers at Bayer AG (one of the company that manufactures neonicotinoid insecticides), honey bees were found to be more than 1,000 times less sensitive to the neonicotinoid thiacloprid than imidacloprid. Using genomic and biochemical methods, they showed that this was due to select enzymes that were able to rapidly degrade thiacloprid. These were found to be a subfamily of classical enzymes known as cytochrome

P450s which are found to have different selectivity for each of the pesticides. Based on our literature review [18]; there are several enzymes that break down pesticides with the end product being carbon dioxide and water. In addition, there are several bacterial species [19] that rapidly break down the pesticides. Figure 4 shows examples of common enzymes and the bacterial species. For each of the neonicotinoids, the bacteria with the highest specificity will be selected by monitoring the degradation rate of the pesticide. The bacteria will be then converted into the spore form for easy storage, transport and use as a spray on the flowers.

| Enzymes | Source | Neonicotenoid compound |
|---|---|---|
| CYP6ER1 | *Nilaparvata lugens* | Imidacloprid, Thiamethoxa, dinotefuran |
| CYP6G1 | *Drosophila melanogaster* | Imidacloprid |
| CYP6CM1 | *Bemisia tabaci* | Imidacloprid |
| CYP6Y3 | *Myzus persicae* | Neonicotinoids |
| CYP353D1v2 | *Laodelphax striatellus* | Imidacloprid |
| CYP6CY14 | *Aphis gossypii* | Thiamethoxam |

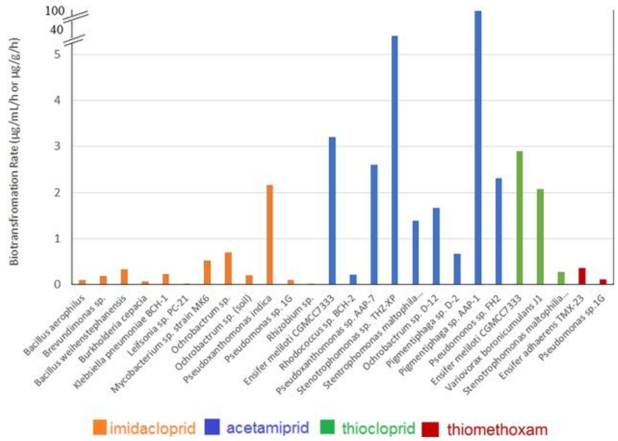


*Figure 4. Biological degradation of neonictonoids. Left panel shows common enyzmes [18]. Right panel shows example of bacteria and their degradation rate for the pesticides [19].*

### D. Breakthroughs:

Although much of the technology required for this system is already available to make the desired peptide and the combinatorial enzyme/bacterial species for neonicotinoids degradation, some components still require breakthroughs. These include (a) development of a formulation of the peptide and the enzyme/bacterial species for stability in environments ranging from hot to cold weather and (b) automated identification of plants, stem, branches, leave, flowers and petals via machine learning. Although there is a lot known in the literature about protein formulation, there is very little information or knowledge for formulation of peptides. Even though they are smaller, they do present significant challenges for the formulation including chemical instability resulting in degradation and self-association often resulting in gel formation. In order to develop a stable delivery system for the peptides and the bacteria, a hydrogel based encapsulation strategy will be used. The peptide and the bacteria species will be encapsulated in the hydrogel using either an emulsification process or lyophilization to create nano sized particles. They will be characterized for stability and bioactivity against the neonicotinoids. The data obtained will be compared with the free peptide and bacteria experiments for validation.

Artificial Intelligence and machine learning is a composite of different processing methods, comprising neural networks, probabilistic models, and a variety of unsupervised and supervised feature learning algorithms for desired pattern recognition [20]. These methods have been used for image analysis and also for automated classification of flowers [21] as shown in Figure 5.

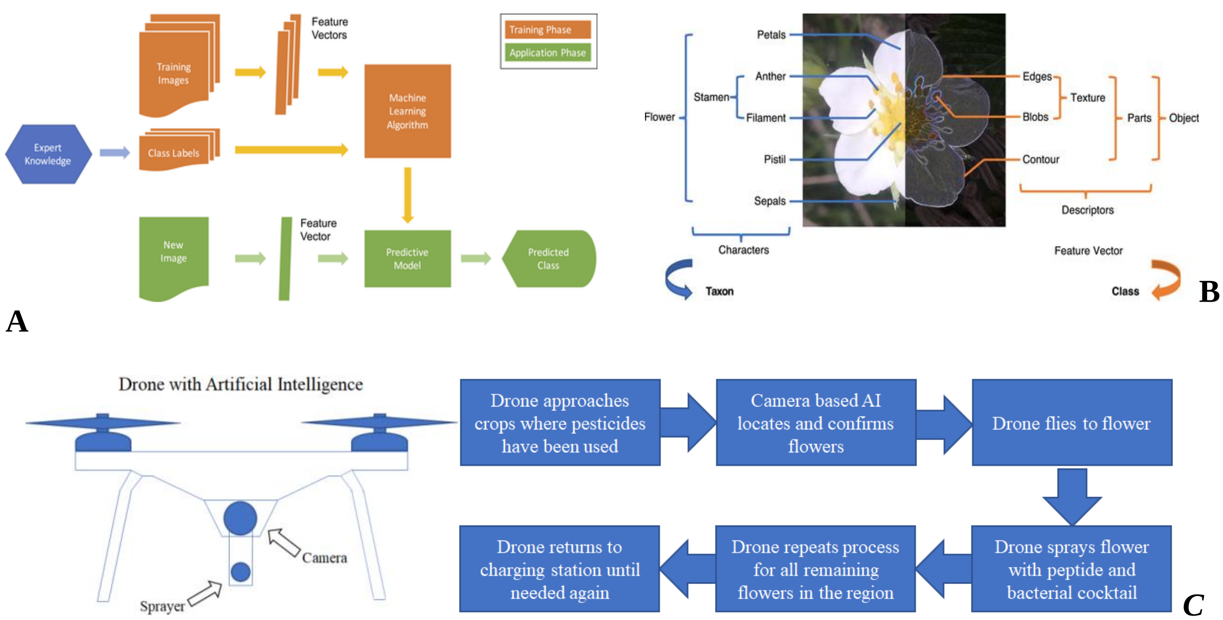


*Figure 5. Fundamental steps for AI based machine learning for image-based flower identification [20]. B. Examples showing the flower image recognition based on a botanist (left) versus machine learning (right) description of flowers [21]. C. Process highlighting the steps for the drone based AI system for biodegradation of pesticides.*

The algorithm developed here will be interfaced with the drone having a camera for automated identification. The drone will be modified to have a chamber for spraying the emulsified or lyophilized mix of the synthetic peptides and the cocktail of bacterial species on the flowers. Once the on-board system on the drone identifies a plant, it will scan for the presence of flowers and use artificial intelligence to spray the flowers with the optimized concentration of the synthetic peptide and the bacterial cells based on the surface area.

The developed system will be tested in collaboration with farmers who use neonicotinoids, raise honey bees farms, and have faced decline in their population. The tests will be conducted over a one year period to ensure a complete cycle of the crops. A control group of farmers will not receive these synthetic peptides or the bacterial cocktail. At the end of the year, results will be compared to test the success of the developed system.

## E. Design Process:

Throughout the completion and optimization of our project, we had to make many decisions along the way. Although these decisions were not always easy to make, we chose the ones that we felt would benefit our technology the most.

One of the first ideas that our team considered for our project was to put the neutralizing agent in the water around the plant rather than on the flower of the plants. This way, the agent would be absorbed into the plant itself when the plant absorbs water from the soil through its roots. However, we decided against this because this method would mean that the entire plant no longer contained the pesticide and therefore would not be able to kill the pests. This led us to choose our current method of spraying the spray on only the flowers of plants. This way, the pesticide will still kill insects on other parts of the plant, but will not harm the bees on the flowers, which is where most bees tend to go on plants.

Another idea that was considered by our team for our project was having a manual or mass spraying system in order to spread the neutralizing agent. A manual spraying system would allow for people to control exactly where they wanted to spray the neutralizing agent and how much is used in different locations. However, this was decided against because a manual spraying method would not allow for the task to be accomplished in a timely manner and would be very inefficient. On the other hand, a mass spraying system would allow for a large area of crops or plants to be covered in a short amount of time which would be very efficient. However, this has a similar problem to putting the spray in the water because it would not be very accurate and would make the entire plant not have any of the pesticide rather than just the flowers. Due to this, we chose to use an automated drone interfaced with an artificial intelligence camera instead. This choice allows for the spray to be utilized and spread in a timely manner while maintaining the accuracy that is needed to ensure that it is only sprayed on the flowers of plants.

Furthermore, another idea that was considered by our team for our project was having a manually controlled drone rather than a completely autonomous drone. We thought about including this because it would give the farmers or people complete control over exactly where the drone goes and allow the farmer to see what the drone is seeing. This would allow them to make judgements regarding whether or not the neutralizing agent is needed which is something that the autonomous version cannot do. However, we chose to have the autonomous drone rather than a manually controlled drone because it requires no user input and as stated previously, would be much more efficient than having a human control it.

**F. Consequences:**

Our technology provides a solution for the current EPA approved pesticide use while providing a sustainable environment for the pollinators including honey bees. The developed peptide and bacteria based dual targeting system will enable automated degradation of neonicotinoids by successful completion of the following milestones.

**Milestone 1:** Development of peptides that bind with neonicotinoids with higher affinity than the native nAChRs present in the honey bees for eliminating toxicity.

**Milestone 2**: Development of bacteria samples in powder form for rapid spraying capabilities with maximal efficiency for biodegradation of neonicotinoids.

**Milestone 3:** Successful demonstration of an automated drone system that can specifically spray the developed neonicotinoid degrading component only on the flowers of the plants.

Farmers can continue to use the pesticides as desired and the pollinators so crucial for survival of our ecosystem can perform their routine pollination with no concerns of pesticide toxicity.

As with all technologies, there are some negative effects. First, the synthetic peptide may be carried over by the honey bees back to their hive and may pass onto the honey being collected. Second, the bacteria being used overgrow and cause health issues on humans who consume the produce from the crop. Finally, every farmer may not prefer to use drones due to privacy concerns. However, overall, we believe that the positivity impact for the benefit of the honey bees far outweighs the negative effects which can also be managed effectively. The developed system will make the entire community work together to help and create a better and healthy life for honey bees and directly benefit the future of our ecosystem.

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