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Preeti Kaundil
Department of Entomology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Raj Kumar Thakur
Department of Entomology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Corresponding Author:
Preeti Kaundil
Department of Entomology, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India

Techniques for assessing insecticidal poisoning in honey bees and their products

Preeti Kaundil and Raj Kumar Thakur

Abstract
Honeybees are quite valued resource-insect that assist in pollination, growing fruit yield and also reduce fruit drop in many fruit crops. But in recent years, there has been a worldwide decline within the honey bee population. Honey bees are uncovered to different forms of herbal and artificial chemical materials along with pesticides in the course of foraging. Over 98% of sprayed pesticides reach a destination other than their target species, including non-target species, air, water and soil. Compared to other insects, honey bees are extremely sensitive to pesticides, due to a deficiency in the number of genes encoding detoxifying enzymes. Most pesticides are applied as sprays, droplets and dust, which can fall directly on the bees that fly across the treated fields. Pesticides affect the cognitive, behavioural and physiological functions of honey bees. Laboratory bioassays have end up increasingly essential due to their predictive value, producing comparative toxicity facts on many chemicals in incredibly short times at distinctly low expense. The laboratory investigations offer a better information of insect-insecticide or insect-plant-insecticide interactions. It is a simple, versatile, easy and touchy approach for determining toxicity of wide variety of chemical compounds, which greatly allows the determination of the LD50, LC50 or some other deadly concentration/dose. The principle of bioassay research is to evaluate the toxicity of pesticides with diverse mode of action to the equal species beneath the equal test conditions. Due to growing makes use of highly poisonous insecticides to control negative insect pests, it is crucial to invest igate not only the technical and formulated materials however additionally minute quantities in their residues on/in plant and animal tissues.

Keywords: Honeybees, pollination, pesticides, bioassay methods

Introduction
The pollinators including honey bees are very important in crops yield whereby honey bee, *Apis mellifera* L., used as insect in pollination of crops [14]. According [22] these insects have been contributed high production in agriculture services. In recent years, the bee population has decreased throughout the world due to several factors which considered as the potential causes of this decline [21]. The use of organosynthetic pesticides is one of the factor which decrease the population on pollinators including honey bees. In [20] study they revealed that when bee larva and adults are contaminated with pesticides, the pesticide will cause death or sublethal effects in pollen or nectar. The impact of insecticides on the growth and development of honey bees causes the great hazards on growth of honey bees. The application of organosynthetic insecticides in controlling insects does not kill only targeted insects but also affects the environment and non-target organisms such as bees [11]. The application and use of natural products as an alternative pest control strategy, mainly in developing countries will help to ameliorate the pollinators including honey bees [12]. The exposure of honey bees in pesticides can be detected using toxicological sampling [34] which affects bees through different ways such as ingestion, contact exposure or through respiratory openings. Different studies revealed that ingestion and contact exposure are the main routes of contamination on honey bees [24].

At present the global pesticide market is around 0.6 billion $, which is highest among other agricultural inputs. Though the per hectare pesticide consumption in India is very low yet the scenario is quite unhealthy, because we are still using and manufacturing a number of hazardous pesticides which are banned elsewhere. Hill farmer use only a small quantity of pesticide in comparison to other states however the chances of pollution are very high because of fragility of hill ecosystem. Besides this farmer use newly introduced chemicals without knowing their hazards to consumer and safety to non-target organisms and environmental pollution they cause.

Such imprudent utilize of plant assurance chemicals cause harming and other side impacts to useful insects including honey bees [30].

The exposure of honey bees to pesticides may occur through contaminated pollen/ nectar or by their direct contact on the unevenly sprayed field crops [18]. The presence of pesticides residues even on the low amount inside the honey bee frame systems may also prove unfavourable to the their critical metabolic functions. It is also known that the contaminated pollen fed to the brood may poison and kill larvae in the early stages of development. Sometimes the effects may be delayed so that the death occurs in pre- pupae, pupae and newly emerged bees. Contamination of colony's bee wax and stored food with pesticides may also cause long term effects due to persistent physiological changes or delayed exposure and are responsible for weakened and lost colonies from killed brood and adult bees [4].

To prevent hazard in pollinators including honey bees, natural pesticides including insecticides are recommended due to the fact that they have low impact to the environment as well as pollinators [12]. Different research studies agree that there are little effects on natural pesticides including botanical insect [17]. The natural pesticides contribute positive changes including lying of eggs, feeding habits of several arthropods pests [17]. Therefore, [17, 12] revealed that biological pesticides will greatly bring positive impacts on pests control while improving the growth and production of pollinators including honey bees in order to improve crop production.

Honey bees (*A. mellifera*) perform the vital task of pollinating agricultural crops and native species and are important for the commercial production of honey and beeswax. Every day, 10,000 to 25,000 honeybee workers make a mean of 10 journeys to explore roughly 7 km^2 in the place surrounding their hive, amassing nectar, water, and pollen from owers. During this process, diverse microorganisms, chemical products, and particles suspended in the air are intercepted by these workers and retained in the hair of their body surface or inhaled and attached in their trachea. Due to this, bees may be used as one bio indicator to display environmental stress because of biological, chemical and physical factors, together with parasites, industrial contaminants, or pesticides [19].

Bee keeping

The beekeeping is an art and skill of retaining the bees in modern moveable frame hives for hobby or fascination, production of hive products (honey, beeswax etc.) and for pollination services. Beekeeping is a high profit enterprise; it does not interfere with other agricultural activities and provides income. It is a prime tool to achieve sustainable development [19, 16]. It plays a high-quality role in agricultural diversification. Moreover, beekeeping contributes to the provision of pollination services, assuring crop yields and helping maintain plant biodiversity in natural ecosystems [15].

Benefits of beekeeping

Honey is a nutritious complete food with medicinal, antioxidant and antibacterial properties. It is used in cooking, baking and as an addition to diverse beverages inclusive of tea and as a sweetners in some commercial drinks. Honey can be used as instant energizer because it consists of sugar which can be quickly absorbed via our digestive system and transformed into energy. The bee wax is more valuable, utilized in candle, pharmaceutical and beauty industry. Bee venom is used for remedy of arthritis, rheumatic and pains (treatment called Apitherapy), royal jelly is nutritious, increase energy and vitality/fertility. Bee pollen is reffered as exceptional meals. Propolis is a resinous substance used as gum. Honey and hive merchandise are source of earning to the farm family, facilitates employment generation.

Pesticide

Pesticide as any substance of chemical or organic origin supposed for preventing, destroying, repelling, attracting or controlling any pest such as unwanted species of flora or animals inflicting harm. They are chemical compounds with precise mode of action, meaning they may be designed to particularly manage a target institution of organism by using interfering with specific metabolic pathways.

Toxicology of pesticides

Pesticides are toxic. The toxicity of each sort of pesticide, however, isn’t always one-of-a-kind to target institution of organism: different species that percentage compareable metabolism is affected as well, although typically to a lesser degree. The potency of a pesticide to any species is defined by the dose of toxic chemical that is lethal to 50 per cent of individuals of that species (LD50), and such dose varies from species to species. Doses lower than the LD50 are considered ‘sublethal’, but they can also cause mortality on a certain proportion of the species population, i.e., 20 or 30 per cent of individuals may die. In general, sublethal doses cause poisonous consequences that do not kill the organism however still have an effect on their ordinary functioning and health. For example, exposure of bees to sublethal doses of neurotoxic insecticides may motive stress [8] paralysis or abnormal behaviours without killing the bees [35].

Pesticide toxicology in honeybees

The dose at which 50 per cent of the population dies (LD50) is a useful metric for quantifying pesticide-specific lethality and evaluating sublethal exposure in adult honey bees. Estimates of LD50 can vary by length of exposure and mode of delivery, so knowing the oral and dermal specific LD50 of individual pesticides can make a useful predictor of pesticide associated risk. Furthermore, by means of evaluating LD50 obtained for pest and beneficial insect species, we can better investigate the trade-off between supposed goal species and any collateral damage to pollinators. When combined with pesticide software rates, these values are useful for calculating the risk of pesticide use to pollinators. The Hazard Quotient (HQ = utility rate/LD50) is a feasible metric to calculate area use threat of pesticide software but can be erroneous along variable LD50 values.

Most insecticides are implemented as sprays over the crop canopy; however sprays of herbicides and fungicides are commonly applied without delay at the soil earlier than the planting of crops. In these types of cases, droplets and dust from the programs can fall without delay at the bees that fly throughout the dealt with fields or nearby due to the fact wind can convey the tiny droplets and dirt particles hundreds of meters away from the crop [10]. A single droplet of insecticide could also be sufficient to kill a bee because the spray solutions contain concentrated doses of those chemicals which cause behind the bee incidents reported within the literature [23]. Granular pesticides which can be incorporated into soil (e.g, herbicides) have no direct publicity to bees.

Impact of Pesticides on bees

The uses of pesticides affect the bees in several ways as explained hereunder:

Herbicides cast off the weed flora which serves as superb food source for bees especially at some stage in dearth period. The direct exposures to insecticidal sprays bring about the loss of life of bees and occasionally lead to the full destruction of bee colonies. Contamination of water sources may additionally also affect water carriers. Contamination of nectar and pollen also causes brood mortality, considerable use of chemical compounds also contaminates the hive products and indiscriminate use of insecticides threatens the integrity of bee flower mutualistic system [32].

Factors affecting pesticide poisoning

Pesticide Formulation, application time, and application method

Dusts are notably risky to bees due to their tendency to rift to vast distances and particles continue to be adhered to plant surface for long. So is the case with wet desk powders which also remain unabsorbed at the plant surface for longer periods than emulsifiable concentrates. The emulsifiable concentrate is comparatively more stable. The granular insecticides are safest. However, granular pesticides with systemic action can also contaminate nectar and may additionally bring about losses to bees, foraging upon them. The fumigant-effect insecticides can also be harmful to bees. Microencapsulated granules implemented on flowers are sometime gathered by bees and stored inside the hives, where they'll beaten via adult bees or fed to brood causing high mortality besides causing long term infection of hive elements and hive products.

Insecticides when carried out to crops in flower can be risky to bees or when pesticides are implemented to a non-flowering crop however having big variety of appealing flowering weeds or hedges inside the fields or inside the adjacent fields. The bees also are affected if they pass via a field handled or sprayed with insecticides. Little foraging happens early in the morning or late in the evening. Application of insecticides throughout late evening or early morning provides relative safety. This avoids direct deposition of pesticides at the bee body and even residues on the dealt with surfaces are rendered less harmful, mainly in case of brief residual pesticides. Aerial application of pesticides has been appeared as greater hazardous than ground application. Bees get much less time to get away the drift of insecticides. Safety of pollinators implemented at the blooming crop may motive hazards to the bees. Fine sprays are more stable than coarse sprays.

Weather and bee colony factors

The warm and sunny weather is conducive to bee foraging. When there's a protracted dull weather the foraging activity is reduced considerably and insecticides is applied on the crop. Temperature is maybe the foremost significant factor causing differences within the toxicity of pesticides. An immediate effect is also much greater at higher temperatures whereas, residual effects are likely to be less because the toxic materials break down more quickly. Populous colonies often experience greater losses than small colonies, as they are exposed to insecticides by more foragers. Newly emerged bees are more prone to insecticides than older bees. Honey bee mortality is inversely proportional to the space of colonies from treated fields. Farther is that the crop from colony, less likely is to draw in sizable amount of foragers.

Minimizing the threats to bees / management activities of pesticides

Proper understanding of above-mentioned principles can go a protracted way in reducing pesticide hazards to honey bees. The idea is that, if possible, honey bees should not be exposed to the harmful effects of insecticides. Reducing the injury to honey bees from pesticides requires contact and cooperation between beekeepers and farmers. Since both mutually get pleasure from honey bees, the beekeeper in terms of its products and also the farmer in terms of increased production of crops. While it is unlikely that poisoning is often avoided, a balance must be struck between the effective use of insecticides, the preservation of pollinators, and the rights of all beekeepers, farmers, and the community as well.

Methods for the bioassay of laboratory insecticide toxicity assessment [26]

- Topical application
- Potter’s tower method
- Injection method
- Contact or Residual method
- Film method

Topical application

A commonly used method is topical application where the insecticide is dissolved during a relatively non toxic and volatile solvent such as acetone and tiny, measured droplets are applied at a selected location on the body surface of individual third-stage larvae with micro-applicator operation. A motor driven topical applicator is on the market with micrometer-driven precision syringe. The advantage of these methods is the high degree of accuracy and reproducibility that can be achieved, the large number of tests performed over a relatively short period of time, the small number of insects required per replication (10- 20), the simple and cheap equipment required, the toxicant touch and the solvents used [13].

Potter’s tower method

Uniform spraying or dusting on the body of insect can be done by mean of potter’s tower. Potter designed a spray tower with a twin-fluid nozzle mounted centrally at the top of an open ended metal tube where the sprays falls vertically and deposits on horizontal plane. Using the potter's tower, the topical application on the entire insect body can be done by keeping the Petri dish containing known number of insects below the bottom part of the tower and spraying inside through a nozzle fitted in the bottom side by maintaining a specific pressure. This method simulates area publicity conditions and subsequently is informative for pest management. The method has emerged as one of the most convenient methods of meting out known quantity of toxins appropriately on insects. The Potter spray tower has been named after C. Potter, who has developed this spray equipment at Rothamsted Experimental Station, Harpenden, Herts, England. This sort of equipment is required for studying the biological effects of touch poisons on organisms. Potters spray tower is evolved to save you the operator exposure and infection to the toxins/ pesticides [27, 28].

Injection method

In this method the insecticide is immediately injected into the insect body (thorax in insects) with the aid of hypodermic needle. The technique employs a very first-rate chrome steel needle (27 or 30 gauge thickness and 0.41 or 0.30 mm in diameter) is used and the amount of toxicant required is measured by using micrometer. In small insects small glass needles with a diameter of 0.1- 0.16 mm are used for injection. The insecticide is generally dissolved in propylene glycol or peanut oil and injected intraperitoneally (into the frame cavity). Care has to be taken to keep away from bleeding via the insect. This approach is mainly employed to recognize the exact amount of toxicant needed in the body of the insect.

Contact or Residual method

In this method, the formulated insecticide is diluted in a risky solvent (acetone) and the insecticide solution is coated inner a glass vial. The solvent is allowed to evaporate by way of rotating the field so that the insecticide is unfold frivolously over the entire surface leaving a residual film. The dose is varied by means of the attention of insecticide solution delivered to the vials. Insects are launched directly to the handled surface and for that reason get uncovered to residual film. Alternatively, the insecticide is applied frivolously directly to the leaf, glass, filter paper, wood panel or other types of building materials and permitted to dry until the residual deposits are exposed to the insects [31].

Film method

The technique entails insecticide answer is typically deposited on glass surface including Petri dish, flask, vial, wide mouth jar etc. Petri dishes are most normally used for comparing insecticide efficacy. In this technique Petri dishes (5 cm diameter) are lined with one milliliter answer on their internal facets and the answer is authorized for uniform spreading inside the Petri dish through swirling it gently and then allowing it dry up at room temperature. The target take a look at insects are then launched onto the film of the toxicant inside the container. Then; the known number of insects are subjected to the recalculation for 18- 24 hours [5].

Methods for testing hazards of pesticides to bees

Feeding test

1. Minimum cage size to be 2*2*3m is taken. Pure compounds or commercial formulations are tested.
2. Bees feed with sterilized 50 % sucrose solution, although candy and water might be used after dosage.
3. Starve bees for up to two hours before tests.
4. Bees dosed individually or in groups of 10 to 50 depending on the size of cage.
5. Bees should not be kept individually for more than one hour.
6. At least three groups of 10 or more bees to be used at each concentration to provide a regression line and LD50.
7. Dose at 10 to 20 cm per bee.
8. Supply fresh 50 per cent sucrose solution after dose has been taken and replaced daily.

Contact tests

1. Test with measured drop, measured spray or measured dust is used, where exact amount of compound that is applied to the bee can be measured, are preferred.
2. Contact with sprayed paper or leaves are also useful and may assist in estimation of hazard as well as toxicity.
3. Solution in acetone was acceptable.

Cage test

1. Use small colony of at least three full frames.
2. Minimum 3 mm mesh size for cage to prevent escape of bees, but allow adequate ventilation.
3. Ideally no field bees should be introduced into the cage to reduce" trapping" on ceiling. Plastic coated netting on the roof can also be used to discourage "trapping".
4. Plants growing in soil are preferred, but potted plants are sometimes used.
5. Apply pesticide spray during day with bees flying.
6. Use dead bee traps and count bees dying in test of cage.

Field test

1. For discipline test, use as a minimum 4 colonies for treatment. They need to be healthy, nicely fed, queen-right, and contain as a minimum 10,000 to 15,000 bees in keeping with season.
2. Each colony consists of 10 to twelve frames, including 5 to six brood frames.
3. The area of the plot for the check ought to be 1500 meter square and minimal distances need to be at least one km.A toxic standard and an untreated control are desirable.
4. Spray when bees are foraging actively.
5. Counting bees on frames and estimating impact on brood. (Felton *et al*., 2016)

Analytical Methods for Pesticide Residue Determination in Bee Products [13]

- LLE (Liquid -liquid extraction)
- SPE (Solid phase extraction)
- SPME (Solid phase micro-extraction)
- SFE (Supercritical fluid extraction)
- Gas chromatography

Liquid-liquid extraction

This is the classic approach to extract pesticides from honey, wax and bee samples. Water immiscible solvents are used for liquid partioning eg. Hexane, dichloromethane, ethyl acetate, petroleum ether or a mixture which include benzene + isopropanol or methanol + ethyl acetate. Hexane offers the cleanest extract, however polar pesticides are not completely extracted. Polarity of hexane is increased by way of addition of acetone. There also are other factors that influence the extraction, like addition of salts along with sodium sulphate or sodium chloride- they cut out pesticide from aqueous solutions Ph of water impacts the solubility of ionizable insecticides.

Solid-phase extraction

Before using SPE on bees or bees' products, it is necessary to obtain aqueous solution by extraction with water or miscible solvents such as methanol, acetone and acetonitrile. Reversed phase c 18 – cartridge used to pre-concentrate a single compound. Solvent used to extract the pestcides depends on partioning co-efficient of the pesticide. Most common solvent used is hexane, ehthyacetate etc.

Solid-phase microextraction

It is a rapid, solvent-free technique. It concentrates the organic compounds from aqueous samples. Fuse silica fiber coated with polymeric film is exposed to the sample. Pesticide adsorbed in the stationary phase is thermally de absorbed in the injector of a gas chromatograph.

Supercritical fluid extraction
In this technique, a supercritical fluid is used, commonly carbon dioxide with or without an natural modifier. The supercritical fluid has higher fluid diffusivites and lower viscosity, thus there is speedy mass switch and quicker solute extraction. It is an alternative to the classic technique to measure organophosphate and carbamate in bees.

Gas chromatography
GC is the most broadly used technique for multi residue screening because of the variety of pesticides that are amenable to this approach, the high separation power, and the provision of selective detectors. These detectors respond to the compounds containing a particular or defined variety of businesses or elements, which includes nitrogen-phosphorous detector (NPD), flame ionization detector, atomic emission detector (AED), or MS detector. Although an ECD has been applied extensively for halogenated compounds. Because it's very sensitive to molecules that incorporate electronegative atoms, its lack of specificity requires next conformation of high-quality findings. The NPD enhances the detection of organophosphorous, carbamate, urea, and triazine pesticides [1, 3], however conformation is essential due to feasible interferences of excessive levels of N-containing compounds.

Management in order to avoid pesticide impacts
The various threats calculated above offer us some hints about the most dangerous kind of exposure to the different castes of bees inside the hives. Spray drift is that the main reason behind incidents involving mortality of forager worker bees [25], whereas ingestion of contaminated pollen, nectar and water also affects many apiaries of the planet affecting mainly the nurse workers and therefore the queen specifically [29]. Additionally, the acaricides utilized in Varroa treatment pose a major risk mainly to the bee larvae, and consequently to the long-term sustainability of the colonies. Awareness of these threats can assist beekeepers and farmers draw precise management plans to keep away from pesticide impacts. Beekeepers should undergo in thoughts of the landscape surroundings on which their managed bees forage, bearing in mind that an outsized proportion of the land in advanced and growing international locations is employed for agricultural production where pesticides of every type are used on an regular basis. Since usage of those plant protection products can not be stopped, as they're necessary for agricultural production, a rational approach must have a look at minimizing the risks of such agrochemicals to bees. The risk of pesticides to bees is minimized by spraying the crops at night while bees are not foraging.

Conclusion
Beekeeping and pesticides are both important inputs of current agriculture management technology. Ignoring both of the two, global meals production could be critically impaired however insecticides affect the cognitive, behavioural and physiological capabilities of honey bees. Laboratory bioassays have become more and more important due to their predictive value, producing comparative toxicity information on many chemical substances in tremendously quick instances at distinctly low expanse. The laboratory investigation presents a better understanding of insect-insecticide or insect-plant-insecticide interactions. Systemic pesticides applied this way are taken up through the crop plant life as they develop and their residues are present in all components of the treated plant, which include the flowers, pollen and nectar [6]. Not best the crop flowers but additionally the weeds and trees that grow in the place are affected [7] because additionally they absorb small amounts of residues that spread via the soil through lateral water flow or are contaminated through dust/ spray drift. In addition, some flowers can produce guttation drops within the early hours of the morning (instance maize, strawberries), and systemic insecticides seem in such drops in multiplied concentrations [33] which are able to killing the bees.

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