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Certificate

This is to certify the work recorded in this thesis entitled **"Effect of type of sugars on the toxicity of some neonicotinoids to honey bees (*Apis mellifera* Linn.*)*".** Submitted by Satinder Kaur (Reg. number- 11718218) in partial fulfilment of the requirements for the award of Degree of Master of Science (Agriculture) in Agriculture Entomology of Lovely Professional University, Phagwara, Punjab is the faithful and bonafide research work carried out under my personal supervision and guidance.

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SYNOPOSIS

Effect of type of sugars on the toxicity of some neonicotinoids to honey bees (*Apis mellifera Linn.)*

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Certify that topic entitled "Effect of type of sugars on the toxicity of some neonicotinoids to honey bees (*Apis mellifera Linn.)''* has been decided and formulated by the student himself and is appropriate for his programme.

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EFFECT OF TYPE OF SUGARS ON THE TOXICITY OF SOME NEONICOTINOIDS TO HONEYBEES *Apis mellifera Linn.*

INTRODUCTION:

The domestic honeybee is a eusocial insect, belonging to the order Hymenoptera and superfamily Apoidea. At present, honeybees are considered to constitute one of the most complex society among the invertebrates, with a strict caste division and a highly developed communication capacity. Caste and sexual dimorphism are well pronounced, so that within the colony we can easily distinguish the queen bee, the drones and the worker bees.

Honeybee have a key role in agriculture and in environmental preservation. Honeybees pollinate 73 of the 100 most common crops that account for 90% of the world's food supply, making honeybees the most economically important pollinator. Worldwide crop production is also affected by 35% where as 87% output increase in the leading food crops by pollinators (FAO, 2009). Gallai *et.al.* (2009) revealed that the value of crop pollination was estimated worldwide at €153 billion ($200 billion). The area covered by pollinator dependent crops has increased by more than 300% during the past 50 years (Aizen and Harder 2009). But the decline in bee population occurred due to unexpected colony losses from 2006. The losses may be due to the genetically modified crops, pesticides, habitat fragmentations etc. Out of all these factors, the neonicotinoids are at the top in causing severe bee losses because they directly harm effect of these chemicals on the nervous system of the honeybees. Bee

mortality occurs due to the direct spraying of neonicotinoids on the plants or by seed-dressing.

The evaluation of side effects on honeybees is in fact essential to estimate the environmental danger of pesticides. In the last decade, in particular, new chemical classes of pesticides that have been released on the market, have raised awareness about the complexity of the lethal and sub-lethal effects that may threat honeybees. To the aim of risk assessment, two fundamental issues are to be considered: the risk of exposure of honeybees to pesticides and the assessment of the toxic effects of these products on honeybee health. Neonicotinoids a new generation pesticides, have been around since Shell developed them in the 1980s and Bayer began production in 1990s. They are now the most commonly used insecticides. The neonicotinoids are 10 thousand times more toxic than DDT, they cause rapid paralysis and death. A mere 4 Nano-grams of clothianidin, the most commonly used neonicotinoid, kill half of all the honeybees that feed on it. Loss of pollinators especially honeybees, may have adverse bearing on agricultural economy and may also affects wild crop diversity, crop production, food security and overall ecosystem stability.

OBJECTIVES:

1. To judge the effects of different concentrations of ordinary commercial sugar, sucrose, glucose and fructose on the toxicity of neonicotinoids to *Apis mellifera* Linn.
2. To see, the effect of the mixtures of main sugars in varying proportion on the toxicity of neonicotinoids to *Apis mellifera* Linn.
3. To correlate the results obtained with the nectar present in various plants.

MATERIAL AND METHODS:

Material: The following chemicals are used in the present investigation:

1. Sugars: a. Ordinary sugars
   b. Sucrose
   c. Glucose
   d. Fructose
2. Insecticides: Neonicotinoids :Imidacloprid, Acetamiprid and Thiamethoxam.
3. Others: a. Emulsifier (triton-X-100)
   b. Benzene

Methods:

To evaluate the effects of the type and extent of various sugars on the toxicity of neonicotinoids, stomach toxicity method will be used. Emulsion concentrations of the insecticides will be prepared in 65, 35 and 10% (W/V) syrups of ordinary commercial sugar, sucrose, glucose and fructose with the help of benzene and emulsifier 'triton-X-100'. A complete test finally comprised three replications (15 bees per replication) of each 5 concentrations and the control. 15 treated bees will be held in cage in an incubator at 27+-1 degree centigrade. These bees will be provided with 50% syrup as food after an hour of treatment and mortality counts to be taken after 24hrs of treatment.

a.) Sucrose dominant: 4:1:1 (sucrose: glucose: fructose)
b.) Balanced with 3 sugars: 1:1:1 (sucrose: glucose: fructose)
c.) Glucose and fructose dominant: 0.4:1:1 (sucrose: glucose: fructose)

The average mortality % for each treatment will be corrected by Abbott's formula (1925) and the data subjected to probit analysis (Finney, 1952).

REVIEW OF LITERATURE:

Medrzycki *et.al.* (2003) found significant reductions in mobility of honeybees that lasted for one to several hours and bees remained stationary for a longer period or moved very slowly due to exposure to imidacloprid.

Contact LD50 value of imidacloprid 0.005 and 0.0024ug/bee (Suchail *et.al.*2000) and 10-40ug/bee (Jeschke *et.al.*2001) have been reported after 48hrs of exposure to imidacloprid

Axel Decourtye *et al.*, (2003) conducted the acute and chronic oral tests on caged honeybee workers (*Apis mellifera* L.) using imidacloprid and a metabolite, 5-OH-imidacloprid, under laboratory conditions. Five times higher LD50 (153mg/bee) was observed by them with the metabolites as compared to imidacloprid (30mg/bee) in 48hrs. The lowest observed effect concentrations (LOEC) of the chemicals on mortality of winter bees were 24 and 120 $\mu g\, kg^{-1}$ respectively. They also study the behavioral effects of both the chemicals were studied using the olfactory conditioning of proboscis extension response at two periods of the year. Greater sensitivity of honey-bees behavior in summer bees LOEC of imidacloprid (12µg/kg) compared to winter bees (48µg/kg) was observed by them. The sub-lethal effects of imidacloprid and delta methrin were compared in both semi-field and laboratory conditions by Decourty *et.al.* (2004). They offered a sugar solution containing 24µg/kg of imidacloprid or 500µg/kg of deltamethrin to a colony which was set in outdoor flight case. They found that imidacloprid and deltamethrin had lethal effects on worker bees. They also reported that negative effects of imidacloprid were observed in an olfactory learnt discrimination task.

Faucon *et.al.* (2005) supplied two different concentrations of imidacloprid in saccharose syrup during summers to two groups of 8 honey bee colonies (each colony was given 1lt. of saccharose syrup containing 0.5 micro g/lt or 5 micro g/lt of imidacloprid on 13 occasions). Adult bee activity, capped brood area, mortality etc. parameters were followed. Comparing with non-supplemented syrup, non-significant higher activity index of adult bee and larger number of capped brood cells were observed. When imidacloprid was no longer applied, activity and pollen carrying were re-established at a similar level.

Daniela laurino *et.al.* (2010) concluded that the LD50 value of clothianidin and thiametoxam were lower than that of imidacloprid and there was a slight difference among different honey-bee strains. Exposure of non-lethal doses cause high mortality in honey-bees due to non-returning of bees to home to such an extent that the colonies had a risk of collapsing (Cresswell *et.al.*2010). Significant detrimental short and long-term impacts on colony performance and queen's fate suggest that neonicotinoids may contribute to colony weakening in a complex manner due to their sub-lethal exposure (Sandrok *et.al.*2014).

Henry *et.al.* (2014) stated that the risk assessment of plant protection products on pollinators is currently based on the evaluation of lethal doses through repeatable lethal toxicity laboratory trials. They showed that the sub-lethal effects of neonicotinoid pesticide are modified in mangnitude by environmental interactions specific to the landscape and time of exposure events. Chensheng *et.al.* (2014) found that honey bees in both control and neonicotinoid-treated groups progressed almost identically through the summer and fall season and observed no acute morbidity or mortality in either group until the end of winter. Bees from half of the neonicotinoid-treated colonies had abandoned their hives and eventually died with symptoms that resembled with that of CCD.

Cresswell *et.al.* (2014) observed low amount of the imidacloprid in the body of honey bees as compared to the bumble bees (0.2ng

versus 2.4ng/bee respectively). They conducted that the feeding and movement of bumble bees was reduced, however the bees had a stable behavior.

Mc Indoo (1916) stated that honeybee workers were not killed by spraying pure nicotine but when fed with 10ml mixture of nicotine and honey (1:100), all the bees died on an average in 33hrs. Ginsberg *et al.* (1935) applied 0.1% anabasine sulphate as contact to bees and found that 100% of them were dead in 24hours and the mortality was only 10% at 0.2% anabasine sulphate.

M.R. Bajiya and D.P. Azrol studied the effect of direct spray of insecticides on mortality of honeybee, *Apis mellifera* on mustard crop cultivar- DGS-1 under field like conditions. The application of methyl-demeton resulted in 100% bee mortality within one hour of spraying followed by imidacloprid (76.5%, 100%), acetamiprid (55%, 62.5%), diamethoate (47.5%, 59%) and thianethoxam (42.5%, 51.5%). After 3hrs the mortality was 100% of methyl-demeton and imidacloprid followed by acetamiprid (77.5%, 80%), dimethoate (70%, 79%) and thiamethoxam (50%, 56.5) whereas no mortality was observed in control treatment. It is thus evident that methyl-demeton followed by imidacloprid, acetamiprid and diamethoate were more toxic to honeybee foragers as compared to thiamethoxam which was found to be less toxic to honeybee foragers.

Mr.Gregor *et al.* (1947) found that the bees were not killed by exposure to the applied dust or to Sulphur fumes when screened cages of bees placed in cotton field to which a dust of 7.5% parisgreen with Sulphur as a diluent was applied.
Acetamiprid and thiacloprid caused higher mortality than the untreated controls only in oral toxicity to honeybees starved for 2hrs (Daniela Laurino *et.al.*2011). Johnson Stanley *et.al.* (2014) concluded that spraying of pesticides at their field recommended doses on potted mustard plants showed monocrotophos to be highly toxic insecticide with 100% mortality even with 1hour exposure followed by thiamethoxam, dichlorvos, profenofos and chloropyriphos which are not recommended for use in pollinator attractive flowering plants.

Arzone and Patetta (1984) tested the toxicity of mancozeb along with cartap and fenitrothion by ingestion and by indirect contact on foraging honeybees under laboratory conditions. Mencozeb was found to be non-toxic to honeybees either by ingestion or by indirect contact. Cartap was highly toxic by ingestion and moderately toxic by indirect contact.

In 2001 Tasei *et.al* showed that the recommended dose of imidacloprid as seed-coating of the sunflower crop, didn't affect the foraging behavior and colony development in the *Bombus terrestis* (Hymenoptera).

Suchail *et.al.* (2004) reported that imidacloprid can be determined in various body parts of *Apis mellifera* viz. head, thorax, abdomen, haemolymph and rectum within 24hrs of treatment(@ 100ug/kg bee). It was observed that haemolymph had the lowest and rectum had the highest level of total imidacloprid. Decourtye *et.al.* (2004) concluded that 30mins after oral treatment of honeybees with imidacloprid, the olfactory learning performances in a proboscis extension reflex (PER) procedure were impaired and further concluded that imidacloprid administered 15 min or 1 hr after a one-trial conditioning of PER impaired the medium-term olfactory memory. Yang *et.al.* (2008) concluded that abnormal foraging behavior of bees could occur in the field through multiple visits to imidacloprid contaminated flowers. Imidacloprid (0.15-6ng/bee) and clothianidin (0.05-2ng/bee) led to a significant reduction of foraging activity and to longer flights at doses of >0.5ng/bee (clothianidin) and >1.5ng/bee (imidacloprid) during 3hrs after treatment (Christof *et.al.*2012).

Yang *et.al.* (2012) reported that the residue of imidacloprid in the nectar and pollens of the plants is toxic not only to the adult honeybees but also the larvae. The brood-capped rates of the larvae decreased significantly when the dosages increased from 24 to 8000 ng/larva. But no significant effects of 0.4ng of imidacloprid/larva on the brood capped, pupation and enclosion rates were observed.

Kessler *et.al.* (2015) reported that the honey bee *Apis mellifera* and the buff-tailed bumble bee *Bombus terrestris*, do not avoid nectar-relevant concentrations of three most commonly used neonicotinoids i.e. imidacloprid (IMD), thiamethoxam (TMX) and clothianidin (CLO) in food. Moreover, bees of both the species prefer to eat more of sucrose solutions laced with IMD or TMX than sucrose alone. Thiacloprid (24hrs oral exposure, 200ug/l) and imidacloprid (1ug/l) reduced haemocyte density, encapsulation response and antimicrobial activity even at the field realistic concentrations .Clothianidin had an effect on their immune parameters only at higher than field realistic concentrations (50-100ug/l) (Annely Brandt *et.al.* 2016). Simone tosi *et.al.* (2017) reported that acute or chronic exposure to a neonicotinoid alone can significantly alter bee flight. Such exposure may impair foraging and homing, which are vital to normal colony and ecosystem services.

Schmuck *et.al.*2001 reported that seed-treated sunflower had no residual level of imidacloprid in their nectar and pollen and concentration of 0.020mg/kg while comparing with field residue of <0.0015mg/kg. Maus *et.al.* (2003) observed that imidacloprid seed-dressing posed only negligible risks by 10days exposure of honeybee colonies to the treated sunflower, the residue of imidacloprid and its secondary metabolites i.e. olefin and hydroxyl imidacloprid were detected in the traces (<1.5ug/kg bees).

The residues dynamics of endosulfan (525g a.i./ha), imidacloprid seed treatment (21g a.i./kg), lambda-cyhalothrin (75g a.i./ha) and spiromesifen (225g a.i./ha) in nectar and pollen of mustard, *Brassica juncea* L. grown in HP showed that imidacloprid seed treatment was practically harmless to honey bees, whereas a waiting period of 5 days must be observed on crops sprayed with these chemicals during

blooms to avoid any accidental hazards to honey bees (Choudhary and Sharma, 2008).

Girolami *et.al.* (2009) found that leaf guttation drops of all the corn plants germinated from neonicotinoid-coated seeds contained amounts of insecticide between 10mg/l and100mg/l for thiamethoxam and clothianidin, and up to 200mg/l for imidacloprid. When bees consume guttation drops, collected from plants grown from neonicotinoid-coated seeds, they encounter death within few minutes. Reetz *et.al*. (2011) demonstrated that guttated water of plants germinated from seeds dressed with neonicotinoids contain their residues. Maize seeds treated with clothianidin (Poncho 0.5mg/seed and Poncho Pro 1.25 mg/seed) resulted in neonicotinoid concentrations upto 8000ng m/l in the guttated fluid. Triticale seeds treated with imidacloprid contained small quantities of this active agent upto 13 ng m/lin the gutatted fluid. During the sampling of guttation fluid, no bees were observed collecting these droplets from triticale or maize. Alburaki *et.al.* (2015) stated that honey bee colonies located in neonicotinoid treated cornfields expressed higher pathogen infection than those located in untreated cornfields. AChE levels showed elevated levels among honeybees that collected corn pollens from treated fields. They also found that neonicotinoids weaken honey bee health by inducing physiological stress and increasing pathogen loads.

Keywords: honeybees, neonicotinoids, imidacloprid, toxicity, pollinator, seed dressing, mortality, oral exposure etc.

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