Detection of toxicity and effects of some insecticides to local honey bee race (*Apis mellifera jemenatica*)

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Abstract: Toxicity of some insecticides (Azadirachtin, Imidacloprid and Methoxyfenozide) were tested on the foragers honeybee *Apis mellifera jemenatica*, under laboratory conditions. All these three insecticides were used at the same concentrations: 0.5, 2.5, 7.5 and 10 ppm and control group (0 ppm) and comparing the mix of the three insecticides. Assessments were made after 1,2,3,4,5,6,8,12,24,48,72 and 96 hours after treatment. There were differences in foragers honeybee workers mortality between the control and all insecticides treatments, also mortality differences were found among the various treatments were the most dangerous insecticides after 48 h. is the Imidacloprid whose mortality rate was the highest reaching to 100% at 7.5 and 10 ppm. At 0.5 ppm, Azadirachtin and Methoxyfenozide had the lowest mortality rate. Foragers honeybee workers mortality increased with time after treatment. Anyway, at 7.5 ppm, the LT_{50}= 2.714, 5.061, 7.052 and 8.687 hours in Imidacloprid, Mix, Methoxyfenozide and Azadirachtin, respectively. The control group (Control) showed the longest age of the honey bee workers having an LT_{50} = 1749.421 hours. These findings indicate that Imidacloprid then Methoxyfenozide greatly affect forager honeybees workers, whereas Azadirachtin was less effective because it was associated with lower death rate and a longer life span seen with the honeybees workers. In conclusion, the present study clearly showed that these were the most effective insecticides at the rate each was tested.
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1. Introduction

Honey bees (*Apis mellifera*) provide vital pollination services to crops and wild plants and are thus important components for food security and the maintenance of biodiversity. The importance of honeybees, *Apis mellifera*, to the global world economy far surpasses their contribution to honey production, because bees are used for the pollination of many major crops. Loss of beneficial pollinators, including honeybees, will have serious adverse impact on the agricultural production. Honeybees are exposed to many risks, including insecticides while they leave to look for food in the agricultural fields **(Colin, *et al*., 2004)** and any chemical exposure that compromises workers' abilities to carry out these tasks could impact colony performance**. (Rabea, *et al*., 2010).** In a study conducted by **(Brittain, *et al*., 2010, Mullin, *et al*., 2010 and Whitehorn, *et al*., 2012),** which focuses primarily on the sub-lethal effects that are induced by pesticides on pollinating insects especially honeybees, it was found that insecticides cause a decrease in the numbers of honeybees. Insecticides impose a series of sub-lethal effects on these beneficial insects which are often overlooked. Though these harsh chemicals are targeted towards pests, non-target beneficial insects are often exposed to these and are relentlessly incapacitated. Residues of the pesticides may be brought back to the hive and fed to the brood – thereby imposing an additional threat to the population. Hazards arise mainly from the damage resulting from their misuse of total insecticides/pesticides out of a total consumption of 98,221.89 tonnes among 23 Asian nations **(FAO, 2015).** In recent years, in many countries of the world, there is phenomenon led to the deaths of individuals honey bees, which caused many of losses in honey bee colonies **(Mullin, *et al*., 2010).** Scientists have pointed to many factors may be responsible for Phenomenon known as "Colony Collapse Disorder" (CCD) including pesticides, diseases, parasites and other factors that affect the health and life of honeybees. And I conducted many of studies to know the causes of this phenomenon. As well as, to evaluate the insecticide residue in the honeybee, this which could make them responsible for the deterioration of the health of bees. **(Van Engelsdorp, *et al*., 2009).** When you focus on the side effects of the older insecticides such as synthetic organophosphate, carbamate and pyrethroid, we find they are more harmful to the environment and beneficial to the insects, especially honeybees. Asimilar trend can also be seen for the newer generation of insecticides (IGR and neonicotinoids), the biological insecticides, which are thought to be less harmful to humans and the environment, which observable that honey bees are more sensitive insecticides. Based on that, they can be used as an

indication of honeybee toxicity. **(Mommaerts, and Smagghe, 2011).** Biopesticidesare alternative to chemical pesticides, and are typically derived from living organisms, microorganisms and other natural sources. As can control in Many types of pests especially for their harmful pests that want to eliminate them. Some of the additional benefits of biopesticides are their action against target pests and efficient resistance management to extend the product life of conventional pesticides. Biopesticides pose less risk to people and the environment as compared to synthetic pesticides, and hence gain global attention as a new tool to kill insects and plant pathogens **(Mehrotra, *et al.*, 2017).** The new generation of Insecticides, including the insect growth regulators (IGR) come from a blend of synthetic chemicals or from other natural sources. In addition to the challenge industries face to develop compounds that provide a more environmentally or ecologically sound insect pest control, growth regulating properties may adversely affect insects by regulating or inhibiting specific biochemical pathways or processes essential for insect growth and development. Some insects exposed to such compounds may die due to abnormal regulation of hormone-mediated cell or organ development **(Mehrotra, *et al.*, 2004).** Therefore, scientists and growers are seeking alternative materials that are effective against pests, safe to humans, environmentally friendly, and compatible with targeted pest management (IPM) practices. Resistant management programs is the use of biorational control agents such as synthetic insect growth regulators (IGR) and those based on naturally derived products. IGR are claimed to be safer for beneficial organisms than conventional products, and they have been successfully used in IPM programs (**EL-Khayat, *et al.*, 2012).** Following exposure to lethal and sublethal concentrations of insecticides can be directly affected was observed on adult longevity **(Gradish, *et al.*, 2010),** observed a shortened life-span when adult workers were fed on Imidacloprid-treated pollen by scoring the number of dead workers. Several neonicotinoids, however, show very strong toxicity to pollinating insects and in particular to the honey bee (*Api smellifera* L.), causing other effects which are seldom easily identifiable, such as behavioural disturbances, orientation difficulties and impairment of social activities **(Desneux, *et al.*, 2007; El-Hassani, *et al.*, 2008 and Maini, *et al.*, 2010).** Pesticides are extremely toxic to pollinators when administered in high doses. Chronic exposure to pesticides may reduce adult longevity in the insects as a consequence of sublethal toxicity. In honey bees, as mentioned before, contaminated food may be stored in hive for longer term **(Chauzat, *et al.*, 2006 and Claudianos, *et al.*, 2006).** The lack of any scientific literature on the biological consequences of combinations of pesticides, suggest strongly for urgent changes in regulatory policies regarding pesticide registration and monitoring procedures as they relate to pollinator safety.

Honeybees are constantly exposed to a wide range of vital and non-vital pressures that may interact with each other and affect the health or survival of the insects. Pesticides are the main danger for the insects, were selected to examine the effect of pesticides on workers' longevity three insecticides, i.e., Deltamethrin, Malathion, and Abamectin, in different concentrations. The study found that the type and concentration of the insecticides that are found in the honeybees' food had a significant impact on the time of survival of the insects. as the variation in the intensity of the effect of the insecticide on the bees appeared with the severity of the effect diminishing in the order of Abamectin followed by Malathion followed by Deltamethrin. The longevity of a worker honeybee depends on the health and safety of all of the members of the beehive. **(Aljedani, and Almehmadi, 2016)**. **(Aljedani, 2017)** evaluating the toxicity of some insecticides (Abamectin and Deltamethrin) on the lethal time (LT_{50}) and midgut of foragers honeybee workers of *Apis mellifera jemenatica* were studied under laboratory conditions. That results found the abamectin most toxicity on health and vitality to honeybees colony. **(Aljedani, and Almehmadi, 2014)**. Insecticides Considered the main danger especially when used in places frequented by bees, causing the death of bees to evaluate the toxic effects of insecticides which were (Deltamethrin, Malathion and Abamectin) by using four different concentrations of each insecticide, were follow up after every 24,48 and 72 hours. The results of study showed that the different tested insecticides showed a strong effect on the bee's life. The results indicated that Abamectin was the most affecting and dangerous among tested insecticides against the foragers worker honeybees, followed by Deltamethrin and finally Malathion. That study showed that the seriousness of using insecticides on the vitality and ability of the foragers worker honeybees to carry out their duties.

Some reports graphically state how mortality rate varies during the time of pesticide exposure **(Suchail, *et al.*, 2001).** Studies by **(Chakrabarti, *et al.*, 2014)** have reported lethal time 50 (LT_{50}) instead of LC_{50} of a particular pesticide treatment group in *Apis cerana* and *Apis dorsata* laboratory populations exposed to pesticides. This study also showed the cumulative survival functions of these two species exposed to the various pesticide treatment groups. There are numerous reports **(Sandrock, *et al.*, 2014)** from many bee keepers all over the world as to how their colony faces extinction due to severe adult honey bee death

exposed to pesticides in the crop fields. The purpose of this study is to determine the effects of some botanical insecticides on the activities of honey bees, especially foraging honey bees adult workers because they are more affected by external environmental pollutants. Thus, bees may act as an indicator of environmental contamination. The aim of the present study is to determine which insecticides from the new generation of insecticides are used in the current study, the concentrations used them pose impact on the health and lives of honeybees and survival the longest period. Few studies have been to study the lethal effects Insecticides on the local bee from Saudi Arabia. This study was conducted to find out and determine the effect of some insecticides on honey bees of activities, especially foraging honey bee workers adult individuals because they are more affected by external environmental pollutants. Bees may act as an indicator of environmental contamination. The aim of the present study is to investigate to determine which insecticides which of the new generation are used in the current study, the concentrations used them pose impact on the health and lives of honeybees and survival the longest period.

2. Materials and Methods

The present investigations were carried out in the apiary and in labs. *Apis mellifera jemenatica* (Hymenoptera: Apidae) sample collection of forager bees consisted of workers (Local species) that were collected from an apiary at a research station of Hada Al-Sham, Faculty of Meteorology, Environment and Agriculture of the Dry Zones. In addition, the study was conducted at the Laboratory of Entomologyin King Abdulaziz University, Jeddah (Saudi Arabia).

2.1. Individuals used in the study

The samples were collected under normal colony conditions, the forager bees are workers with an age 21 days, at which time they shift to perform out-colony tasks including water, nectar, pollen or resin collection. The samples were directly transferred to the Entomology lab, Where the experiment was conducted under laboratory conditions, at a temperature 28± 3^{o}C and at relative humidity 50±5 RH. Wooden cages were used for breeding, taking into account that one face of the wooden box is covered with metal wire mesh, while the opposite face would be of glass, based on what was mentioned by **(Kakmand, *et al*., 2008),** and the measurements of the cage were (30 × 30 × 30 cm).

2.2. Material and food administration

The cage was provided, on the top side, with two plastic medical syringes (50 ml), one of which with water, and the other syringe with sugar solution of 50% w/v based on what was conducted by **(Bortolotti, *et al*., 2003 and Pohorecka, 2004**), laced with insecticide solution under test. The control group was provided with sugar solution without any additives. The cage was also provided with a natural protein nutrition (pollen).

2.3. Time period of experiment conductance

It has follow-up samples after 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72 and 96 hour (h.). The samples were followed up, as a bee would be considered dead when it remains motionless for 10 seconds of the observation period, after moving it gently by a fine brush (**Laurino, *et al*., 2013).** The longevity of the foraging honeybee worker, staying alive after its exposure, was calculated by follow-up and comparing to the control group. The experiment was using 60 insects.

2.4. Insecticides used:

Insecticides used in this study (Azadirachtin, Imidacloprid and Methoxyfenozide) are among the main insecticides used to control pests in Saudi Arabia. These insecticides are one of the newest generation of IGR and botanical insecticides used to control pests.

1. **Azadirachtin.**

Azadirachtin 1 % (EC), from the botanical insecticide group, Trade name: Amen. Amen is considered to be a part of the botanical insecticide group and is environmentally friendly. Contact and stomach pesticide, it is effective on all larval stages and pupae, they reduce crop damage by repelling and deterring feeding of all stages of insects. Azadirachtin**:** is effective as a soil drench for controlling soil-borne insect larvae. Azadirachtin**:** Organic insecticides has fungicidal and miticidal properties.

2. **Imidacloprid**.

Imidacloprid (20% SL), it is an insect neonicotinoid, Trade name: Imidaclorin. Is a systemic insecticide which acts as an insect neurotoxin and belongs to a class of chemicals called the neonicotinoids and it is widely used for pest control in agriculture.

3. **Methoxyfenozide**.

Methoxyfenozide: (24% SC) is an insect growth regulator (IGR), Trade name: Runner: Suspension Concentrate (SC). Common name: Methoxyfenozide. Chemical name: 3-methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl) hydrazide.

2.5. Concentrations and Doses used

All these thre einsecticides were used at the same concentrations: 0.5, 2.5, 7.5 and 10 ppm.

The dose that have been submitted with the sugar solution. When insecticide were used alone, the dose was ($LD_{25:50}$); (25 ml. insecticide and 25 ml. sugar solution), when it was mixed with other insecticides, the dose was ($LD_{12.5:\ 50}$); (Mix of three insecticides;

12.5ml. Azadirachtin, 12.5 ml. Imidacloprid**,** 12.5ml. Methoxyfenozide) and 12.5 ml. sugar solution).

2.6. Study groups division

The research experiences were divided into five groups:

1. Namely: a non-exposed (Control group).
2. An exposed group to Azadirachtin.
3. An exposed group to Imidacloprid.
4. An exposed group to Methoxyfenozide.
5. An exposed group to Mix of three insecticides.

2.7. Study procedures

In this study, the foraging honeybees workers *Apis mellifera jemenatica* were exposed to some insecticides and various concentrations, to check the long-term survival of honeybees when exposed to different insecticides. The survival data of caged bees under chronic exposure to three insecticides (Azadirachtin, Imidacloprid and Methoxyfenozide), and comparing the mix of the three insecticides, had three replicates and four concentrations (10, 7.5, 2.5, 0.5ppm) and control group (0 ppm).

***1*-Account the percentage mortality:** The adult mortality was assessed after 1, 2, 3, 4, 5, 6, 8, 12 hours (h.), assessments were made 24, 48, 72 and 96 hours after treatment, respectively**.**

Exposure to the treatments was conducted through prodding the insects with a fine hairbrush. The adults were considered dead if they were unable to move after the prodding stimulation. The exposure period of 72 h. was chosen because bees in the control treatments showed the minimum survival during this period (80%, which is the minimum recommended survival rate for a preliminary lethal effect bioassay**. (Galdino, *et al.*, 2011).**

***2*- Lethal concentration (LC_{50}):** It was determine its value LC_{50} at 4, 6, 8, 12, 48 and 72 h.

***3*-Lethal time (LT_{50}):** Adult longevity determined its value LT_{50} at 0.5 and 7.5 ppm.

2.8. Statistical analysis

The results were analyzed using the toxicity value after the correction rate of death was determined using Abbott's formula (**Abbott, 1925)**. The values of LC_{50} and LT_{50} were determined using the mortality regression lines drawn according to **(Finney, 1971)** method, and by program of **(Bakr, 2007)** Ldp line.

3. Results

Insecticides are frequently used in the external environment, especially in agricultural fields to combat insect pests, believing they are safe for the environment and eco-friendly, but did not look at the negative aspects caused by the threat to the most important economic insects that play an important role in the pollination of flowers, the most important honey bees and especially workers honeybees. In this study, we were to study the effects of three insecticides on forager honeybees workers (*Apis mellifera jemenatica*) at the same concentrations under laboratory conditions, Where he found for these insecticides varying effects different with the passage of time.

1.1. Mortality:

After one hour (1h.), we found that the most dangerous insecticides is Imidacloprid which caused the highest percentage of death (mortality**)**, 35% in a concentration of 7.5 ppm. In contrast, the mortality of Azadirachtin in all concentrations, was the lowest of all insecticides.

Imidacloprid showed a high mortality rate of 48.33 % and 50% after 2 and 3 hours treatment under 7.5ppm concentration, respectively.

After 4 h., 5 h., 6 h. 8 h. and 12 h. we found that Imidacloprid had mortality rate between 50% to 88.33% under 7.5 and 10 ppm concentration, respectively. At 10 ppm, Methoxyfenozide had moratlity rate between 70% to 98.33%. In contrast, at 0.5 ppm, morality rate was the lowest in both Azadirachtin and Methoxyfenozide.

After 24 h. the mortality rate of Azadirachtin, Methoxyfenozide and Imidacloprid was 100%, 100% and 95% respectively at 10 ppm. Whereas at 0.5 ppm, the lowest mortality rate was seen in Azadirachtin.

Also, we found that the most dangerous insecticides after 48 h. is the Imidacloprid whose mortality rate was the highest reaching to 100% at 7.5 and 10 ppm. At 0.5 ppm, Azadirachtin and Methoxyfenozide had the lowest mortality rate.

After 72 h. and 96 h. in most concentrations we found that the mortality rate reach 100% in Azadirachtin, Imidacloprid and Methoxyfenozide. Whereas the lowest mortality rate was seen in Azadirachtin at 0.5 ppm. At the mortality in the control group in early hours of treatment to 0%. While, at 12h., 24h., 48h., 72h. 96h. arrived to 1.66, 3.33, 3.33, 6.66 and 11.66% respectively. Table (1).

Mix of three insecticides

When comparing the mix of the three insecticides: Azadirachtin, Imidacloprid and Methoxyfenozide, it was found that the mortality rate was lower than the one in Imidacloprid when it was used alone., But you must take into account the dose that have been submitted with the sugar solution. The lethal dose (LD) of the Imidacloprid when used alone was $LD_{12.5:50}$; however, when mixed with other insecticides, it had $LD_{25:50}$. Therefore, there was no death of individuals in the early hours of the test, but when after 12-hour to 96-hour the mortality rate emerged, although in normal limits and rates ranging from 1.66% to 11.66%. Table (1).

1.2. Compared with insecticide at similar time lengths:

Table (1): Mortality of foragers honeybee workers orally exposure to different concentrations of some insecticides.

Insecticides	Con. (ppm)	% Mortality											
		after 1 h.	after 2 h.	after 3 h.	After 4 h.	After 5 h.	after 6 h.	after 8 h.	after 12 h.	after 24 h.	after 48 h.	After 72 h.*	After 96 h.*
Azadirachtin	0.5	0	0	1.66	1.66	1.66	1.66	8.33	10	28.33	33.33	48.21	100
	2.5	0	15	18.33	21.66	25	46.66	75	78.33	91.66	96.66	100	___
	7.5	0	5	10	10	30	50	55	63.33	83.33	91.66	100	___
	10	1.66	18.33	21.66	25	36.66	36.66	45	66.66	100	___	___	___
Imidacloprid	0.5	0	0	0	16.66	30	45	55	66.66	78.33	91.66	94.64	100
	2.5	1.66	1.66	5	15	20	51.66	61.66	75	86.66	95	100	___
	7.5	35	48.33	50	55	58.33	61.66	75	80	88.33	100	___	___
	10	5	13.33	25	50	50	73.33	81.66	88.33	95	100	___	___
Methoxyfenozide	0.5	0	0	0	1.66	3.33	5	8.33	15	30	43.33	64.28	100
	2.5	1.66	6.66	15	18.33	23.33	26.66	33.33	45	58.33	75	94.64	100
	7.5	8.33	11.66	16.66	20	21.66	40	55	76.66	93.33	100	___	___
	10	16.66	25	45	70	85	95	96.66	98.33	100	___	___	___
Mix of three insecticides	0.5	0	0	5	6.66	26.66	51.66	51.66	60	65	78.33	85.71	100
	2.5	0	3.33	15	25	33.33	46.66	53.33	63.33	86.66	98.33	100	___
	7.5	11.66	33.33	38.33	41.66	45	50	60	65	91.66	98.33	___	___
	10	5	13.33	20	50	53.33	55	58.33	66.66	73.33	100	___	___
Control	0.0	0	0	0	0	0	0	0	1.66	3.33	3.33	6.66	11.66

***(*)** Correction mortality rate using equation (Abbott, 1925). **(Con.)** concentrations**.(h.)** hours.

Table (2): Comparing of efficacy of some insecticides against foragers honeybee worker sat same time (h.).

Time	No	Line name	LC_{50}	Index	RR	Slope	Slope +/-
After 4 h.	1	Mix-4 h.	9.669	100	1	1.171	0.161
	2	Imidacloprid - 4 h.	9.763	99.037	1.01	0.923	0.145
	3	Methoxyfenozide - 4 h.	9.962	97.059	1.03	1.551	0.202
	4	Azadirachtin -4 h.	136.04	7.107	14.07	0.681	0.179
After 6 h.	1	Mix-6 h.	0.85	100	1	0.037	0.123
	2	Imidacloprid - 6 h.	1.201	70.774	1.413	0.496	0.125
	3	Azadirachtin-6 h.	9.428	9.016	11.092	1.029	0.15
	4	Methoxyfenozide - 4 h.	9.962	8.532	11.72	1.551	0.202
After 8 h.	1	Mix-8 h.	0.353	100	1	0.156	0.123
	2	Imidacloprid-8 h.	0.392	90.051	1.11	0.549	0.128
	3	Methoxyfenozide- 8h.	3.634	9.714	10.295	1.866	0.172
	4	Azadirachtin -8 h.	4.5	7.844	12.748	0.794	0.132
After 12 h.	1	mix-12 h.	0.0051	100	1	0.127	0.125
	2	Imidacloprid - 12 h.	0.074	6.892	14.51	0.478	0.135
	3	Methoxyfenozide - 12 h.	2.249	0.227	440.98	1.865	0.159
	4	Azadirachtin - 12 h.	2.521	0.202	494.314	1.122	0.135
After 48 h.	1	Imidacloprid - 48 h.	0.0082	100	1	0.739	0.234
	2	mix-48 h.	0.138	5.942	16.829	1.452	0.267
	3	Azadirachtin -48 h.	0.712	1.152	86.829	1.968	0.19
	4	Methoxyfenozide - 48 h.	0.713	1.15	86.951	1.874	0.181
After 72 h.	1	Imidacloprid-72 h.	0.111	100	1	1.972	0.623
	2	Mix-72 h.	0.154	72.078	1.387	2.107	0.637
	3	Methoxyfenozide-72 h.	0.308	36.039	2.775	1.724	0.191
	4	Azadirachtin-72 h.	0.513	21.637	4.622	4.173	1.232

After 4 h., 8 h., 12 h. and 72 h. we found that the most profound insecticides is the Mix and Imidacloprid with the highest mortality rates. For example, after 4 h., the value of LC_{50} reached to 9.669 and 9.763 ppm, respectively. Similarly, after 4 h., the value of LC_{50} in Methoxyfenozide reached to

LC_{50}=9.962 ppm. The lowest value of LC_{50} was seen in Azadirachtin with LC_{50}=136.04 ppm.

After 6 h. and 48 h., we found that the most profound insecticides is Imidacloprid with the highest mortality rate. For example, after 48 h., the value of LC_{50} reached to 0.0082 ppm. Similarly, the Azadirachtin had LC_{50}= 0.138 and 0.712 ppm, respectively. The lowest value of LC_{50} was seen in Methoxyfenozide with LC_{50}= 0.713 ppm. Table (2), Figure (1).

1.3. Compared with insecticide at different times:

After 48 h., 24 h., 12 h., 8 h., 6 h. and 4 h., the LC_{50} value in Azadirachtin reached to 0.712, 0.889, 2.521, 4.5, 9.428 and 136.04 ppm, respectively. However, the mix of the insecticides found that the effect of insecticides was uneven after 12 h. and 24 h. highest mortality but When 8 h. and 48 h. the percentage of medium-death. But, at 4 h. and 6 h. arrived to lowest death was at this time. This depends on the value of $LC_{50.}$ Table (3), Figure (1).

1.4. Lethal time (LT_{50}) after exposure to some insecticides after 96 h. at 0.5 and 7.5 ppm of for agers honeybee workers.

Been compared to of insecticides effect when concentrates 0.5 and 7.5 ppm. It were found most effect it were; At 0.5 ppm, the lethal time (LT_{50}) was LT_{50}= 10.348, 12.19, 37.984 and 47.535 hours in Imidacloprid, Mix, Methoxyfenozide and Azadirachtin, respectively. Table (4) Figure (3). Whereas at 7.5 ppm, the LT_{50}= 2.714, 5.061, 7.052 and 8.687 hours in Imidacloprid, Mix, Methoxyfenozide and Azadirachtin, respectively. The control group (Control) showed the longest age of the honey bee workers having an LT_{50} = 1749.421 hours. Table (5), Figure (4).

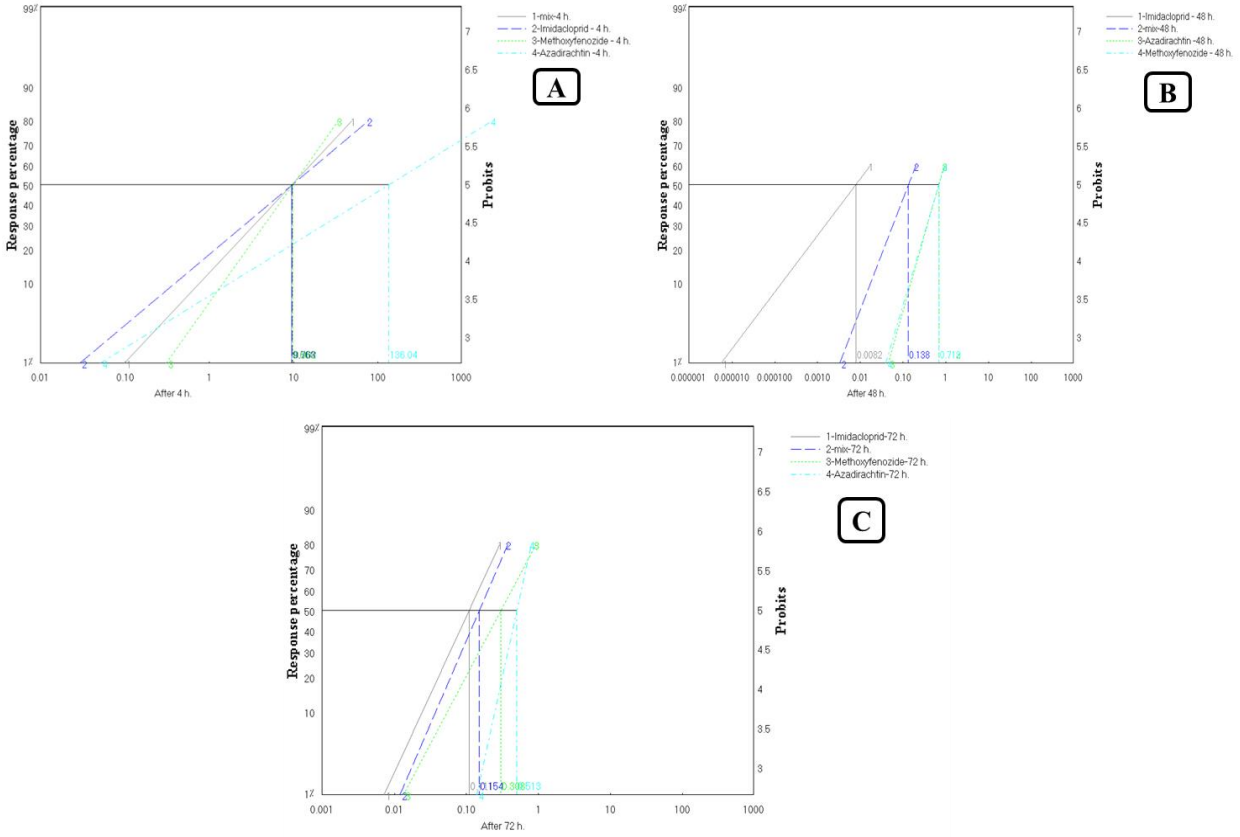

Figure 1. Comparison of some insecticides against foragers honeybee workers using the mortality of LC_{50} at same time (h.); (A) after 4h. (B) after 48h. (C) after 72h.

Table (3): Comparing of efficacy of some insecticides at different times against for agers honeybee workers.

Insecticides	No	Line name	LC_{50}	LC_{90}	Index	RR	Slope	Slope +/-
Azadirachtin	1	Azadirachtin -48 h.	0.712	3.187	72.051	1.388	1.968	0.19
	2	Azadirachtin- 24 h.	0.889	6.284	57.705	1.733	1.509	0.153
	3	Azadirachtin - 12 h.	2.521	34.951	20.349	4.914	1.122	0.135
	4	Azadirachtin -8 h.	4.5	184.647	11.4	8.772	0.794	0.132
	5	Azadirachtin-6 h.	9.428	165.787	5.441	18.378	1.029	0.15
	6	Azadirachtin -4 h.	136.04	10398.11	0.377	265.185	0.681	0.179
Imidacloprid	1	Imidacloprid - 48 h.	0.0082	0.442	100	1	0.739	0.234
	2	Imidacloprid - 24 h.	0.015	5.272	54.667	1.829	0.502	0.153
	3	Imidacloprid - 12 h.	0.074	35.493	11.081	9.024	0.478	0.135
	4	Imidacloprid-8 h.	0.392	84.264	2.092	47.805	0.549	0.128
	5	Imidacloprid - 6 h.	1.201	461.509	0.683	146.463	0.496	0.125
	6	Imidacloprid - 4 h.	9.763	238.469	0.084	1190.61	0.923	0.145
Methoxyfenozide	1	Methoxyfenozide - 48 h.	0.713	3.441	43.198	2.315	1.874	0.181
	2	Methoxyfenozide - 24 h.	1.308	10.109	23.547	4.247	1.443	0.156
	3	Methoxyfenozide - 12 h.	2.249	10.941	13.695	7.302	1.865	0.159
	4	Methoxyfenozide- 8h.	3.634	17.667	8.476	11.799	1.866	0.172
	5	Methoxyfenozide - 6 h.	4.875	22.357	6.318	15.828	1.938	0.192
	6	Methoxyfenozide - 4 h.	9.962	66.762	3.092	32.344	1.551	0.202
Mix of three insecticides	1	Mix-12 h.	0.0051	6.81E+07	100	1	0.127	0.125
	2	Mix-24 h.	0.023	46.096	22.174	4.51	0.389	0.136
	3	Mix-48 h.	0.138	1.051	3.696	27.059	1.452	0.267
	4	Mix-8 h.	0.353	6.15E+07	1.445	69.216	0.156	0.123
	5	Mix-6 h.	0.85	4.97E+34	0.6	166.667	0.037	0.123
	6	Mix-4 h.	9.669	120.14	0.053	1895.882	1.171	0.161

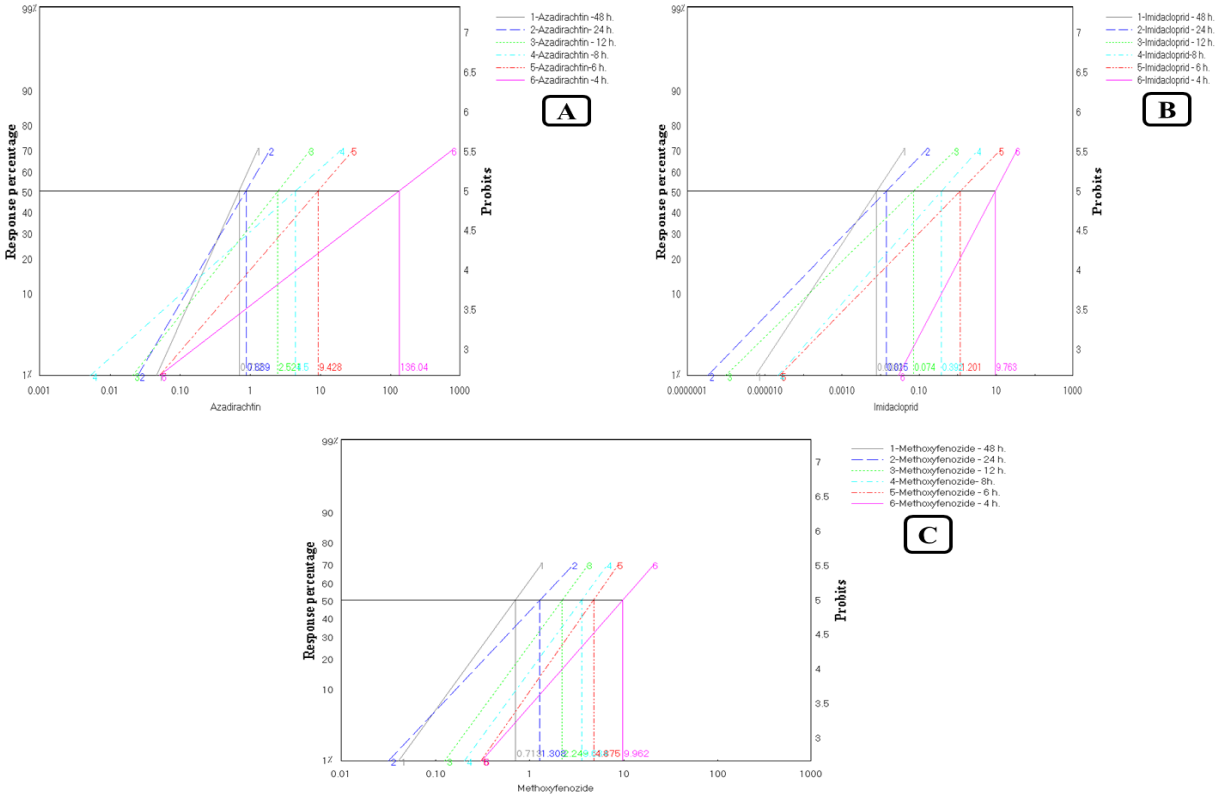

Figure 2. Comparison of some insecticides against foragers honeybee workers using the mortality of LC_{50} at different times (h.); (A) Azadirachtin (B) Imidacloprid (C) Methoxyfenozide.

Table (4): **Comparing of efficacy of some insecticides against foragers workers of honey bee.**

No	Line name	LT_{50}	LT_{75}	LT_{90}	Lower limit	Upper limit	1	2	3	4	5	Index	RR	Slope
1	Imidacloprid–0.5 ppm	10.348	19.731	35.271	7.751	14.544	*	*				100	1	2.407
2	Mix -0.5 ppm	12.19	28.012	59.235	8.728	17.599	*	*				84.889	1.178	1.867
3	Methoxyfenozide-0.5 ppm	37.984	77.411	146.93	29.796	50.784			*	*		27.243	3.671	2.181
4	Azadirachtin–0.5 ppm	47.535	99.126	192.075	34.989	72.378			*	*		21.769	4.594	2.113
5	Control	1749.42	7292.648	26358.65	456.19	59628.8					*	0.592	169.05	1.088

Index compared with Imidacloprid -0.5 ppm. Resistance Ratio (RR) compared with Imidacloprid -0.5 ppm.

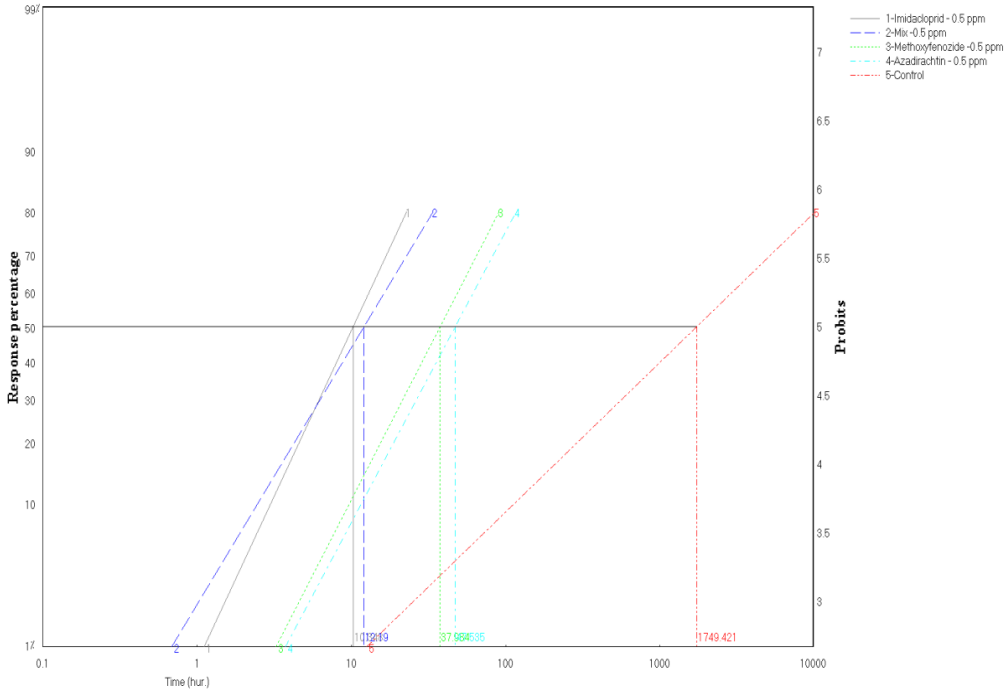

Figure 3. Lethal time (LT_{50}) after exposure to some insecticides after 96 h. at 0.5 ppm of for agers honeybee workers.

Table (5) Comparing of efficacy of some insecticides at different times against foragers workers of honey bee.

No	Line name	LT_{50}	LT_{75}	LT_{90}	Lower limit	Upper limit	1	2	3	4	5	Index	RR	Slope
1	Imidacloprid-7.5 ppm	2.714	8.418	23.316	2.25	3.178	*					100	1	1.372
2	Mix-7.5 ppm	5.061	12.509	28.244	4.50	5.661		*				53.626	1.865	1.716
3	Methoxyfenozide-7.5 ppm	7.052	13.181	23.145	5.68	8.916			*	*		38.486	2.598	2.483
4	Azadirachtin-7.5ppm	8.687	16.442	29.198	7.21	10.676			*	*		31.242	3.201	2.434
5	Control	1749.421	7292.648	26358.65	456.19	59628.84					*	0.155	644.5	1.088

Index compared with Imidacloprid - 7.5 ppm. Resistance Ratio (RR) compared with Imidacloprid - 7.5 ppm.

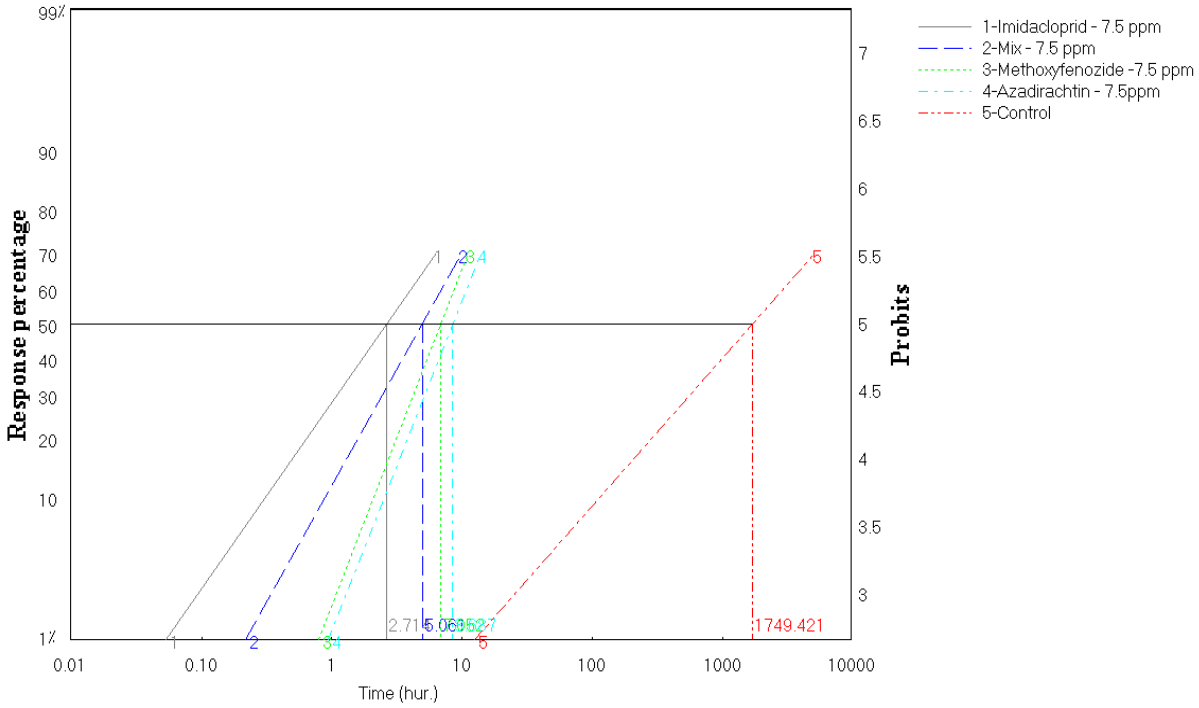

Figure 4. Lethal time (LT_{50}) after exposure to some insecticides after 96 h. at 7.5 ppm of foragers honeybee workers.

4. Discussion

Insect pollination is an ecosystem service with a high economic value. Many hymenopteran pollinators are threatened worldwide, and suffer from a decline; therefore, lead to higher production costs of crops. In addition, plant biodiversity and ecosystem stability may be at risk. Honeybees of the genus *Apis* are among the most important pollinating hymenopterans. The insecticides pose dangers and a threat to the life of the honey bee. Our results demonstrate the effects of three insecticides on forager honeybees workers of *Apis mellifera* at the same concentrations under laboratory conditions. By knowing the effects of the insecticides on bees, it will serve to benefit the bees' health and investigate the interactions of pesticides with other stressors, especially nutrition. In addition, diet quantity, quality, and diversity, greatly affect the health of honey bees **(Alaux, *et al.*, 2010; Brodschneider, and Crailsheim, 2010 and Di Pasquale, *et al.*, 2013)**. Many highly eusocial bees such as honeybees (Apinae) practice age polyethism, in which different groups of individuals perform a different ensemble of tasks as they age. Young workers, for example, are responsible for brood and queen care and nest maintenance, while older workers are involved in foraging activities. Since JH is involved in the regulation of age polyethism in the honeybee, *Apis mellifera* L.**(Robinson, and Ratnieks, 1987).**

After one hour (1h.) we found that the lowest mortality was associated with Azadirachtin in all concentrations. These results conform with the work of **(Rembold, and Czoppelt, 1981**) who studied the effects of Azadirachtin on honey bee larvae. They purified the compound from neem seeds and treated third instar larvae by topical application. Larvae were fed with a royal jelly and yeast mixture and reared in the incubator. The lowest dose causing observable effects was 0.25 mg. larva–1. **(Naumann, and Isman, 1996)** did not use seed extracts with unknown amounts of Azadirachtin, but an emusifiable concentrate with an undiluted Azadirachtin content of 46000 mg. kg–1. Oral application of increasing doses of Azadirachtin on first and fourth instar larvae resulted in larval ejection by nurse bees in a dose dependent manner. The LD_{50} for both instars was 37 mg. g–1 body weight and 61 mg. g–1 body weight. Similarly, in the study by **(Bacci, *et al.*, 2016)** which proved the Insecticides derivative of biological acting (Spinosyn) are characterized by a broad spectrum of action, but they are also characterized by a low toxicity for natural enemies. Trials on bees demonstrated a low toxicity also for these insects.

In the present study, it concluded that the Imidacloprid was the most damaging insecticide among the others, and had the exhibited a high mortality rate of honey bees. For example, after 4 h., 5 h., 6 h. 8 h. and 12 h, Imidacloprid produced high

mortality rates that range between 50 % to 88.33 % in concentrate 7.5 and 10 ppm, respectively**.** As well as exposure to pesticides may also affect colony survival **(Sandrock, *et al*., 2014 and Goulson, *et al*., 2015).** In particular the application of neonicotinoid insecticides, which has increased substantially on a global scale over the last decade **(Mullin, *et al*., 2010; Jeschke et al., 2011; van der Sluijs, *et al*., 2013andGoulson, *et al*., 2015),** has been suspected to represent a major threat to honey bee survival **(Desneux*et al*., 2007; Goulson, 2013 and Pisa, *et al*., 2015).** For the neonicotinoids, *Apis mellifera* was most sensitive to Imidacloprid and this agrees with **(Hardstone, and Scott, 2010)** who concluded that *Apis mellifera* was among the most sensitive for Imidacloprid. Imidacloprid was recorded as highly toxic insecticide against *Apis floria*, *Apis mellifera* and *Apis dorsata* which causes 100% mortality after 6h. at high concentrations. No comparable results has been noted. The results by **(Husain, *et al*., 2014)** differ from those of (**Pistorius, *et al*., 2009**) and this difference may be due to the use of different insecticides on different species. Study by (**Blacquière, *et al*., 2012**) focused on Imidacloprid which is widely used as a systemic and is highly toxic to honey bees. Moreover, the maximum Imidacloprid residues ingested by the different types of bumble bee would reach the oral LD_{50} within their respective life spans, while average residues in honey result in LT_{50} of 11 days for nectar foragers, indicating than half of them would probably die before reaching the end of their lives. **(Sanchez-Bayo, and Goka, 2014).** In addition, the neonicotinoid(Imidacloprid) was even less toxic to the extent that it could not be used for inhibitor assays or to determine an LC_{50} – on diet with 100 µg/g imidacloprid, and mortality was only 10% after 48 hours **(Dana, 2016).** Experimental evidence has shown that mixtures of imidacloprid and lambda-cyhalothrin increase mortality of bees and reduce brood production in their colonies more than when fed on pollen contaminated with only one insecticide**. (Gill, *et al*., 2012).** However, the effects of insecticide mixtures are additive, not synergistic. Some experimental evidence indicates that the reproductive output of bee queens is seriously curtailed when fed on pollen contaminated with imidacloprid. Presumably, queens would be affected in a similar way as larvae, because both consume royal jelly and pollen, with the queens consuming larger quantities**. (Gill, *et al*., 2012).**

The insecticide treatments of Methoxyfenozide, at different doses, were ranked slightly harmful to harmful after 48 hours of their application. Same kind of experiment was done by **(Ahmed and Ahmad, 2006),** amongst new chemistry insecticides, complete mortality (100%), was observed in the adults treated with the high dose rate after 48 hours of application. The higher dose-rates (10% above the recommended) of Methoxyfenozide (110 ml/acre), proved to be slightly harmful, as the percentage mortality, in the adults, treated with these dose rates ranged between 50-79%, after 12 hours of interval. The insecticide, like, Methoxyfenozide (100 ml/acre) were also slightly harmful, at their recommended dose rates (**Khan, *et al*., 2009).** The stomach poisonous impact of some insect growth regulators (IGR) and biocides was tested under laboratory and semi-field conditions against cotton leaf worm and the obtained results revealed according to the LC_{50} value. The rest compounds gave moderate effects in this respect. Data concerning the initial and residual activity of the tested insecticides including, Methoxyfenozide against 4th in star larvae of field strain cotton leafworm. The initial effect calculated as the cumulative mortalities at zero time recorded 100, 100, 92, 88 and 26 % for Methoxyfenozide, the untreated check recorded 2% Methoxyfenozide and Chlorpyrifos gave the highest significant mortalities effects comparing to the untreated, Chlorpyrifos and Methoxyfenozide were detected the highest significant mortalities effect as general residual as compared to other insecticides **(EL-Khayat, *et al*., 2012).** IGR are classified as more selective due to their interference with insect-specific targets however only 47% of the compounds tested has been found non-toxic. Within the IGR, three different groups can be distinguished: chitin synthesis inhibitors (CSIs), juvenile hormone analogs (JHAs), and ecdysteroid agonists or also called molting-accelerating compounds (MACs) **(Mommaerts, and Smagghe, 2011).** IGR are highly selective, but their potential adverse effects on beneficial organisms cannot be discounted. However, this type of IGR is generally safe for non-target and beneficial organisms (e.g., honeybees, ants, and predaceous mites) **(Mordue-Luntz, and Blackwell, 1993).** Study by **(Yousif-Khalil, *et al*., 2010)** detected the toxic effect of compounds of newer generation of insecticides, i.e. Methoxyfenozide (Runner), a newer class of IGRs were used commonly in controlling the pest. The results showed that Methoxyfenozide is safe to honeybee workers in general, as the percentage of mortality in exposed workers did not exceed 3%.

The causes of the current global decline in honey bee health are unknown, one of major group of hypotheses invokes the pesticides to which this important pollinator species is often exposed on throughout the adult life of honey bees **(Forkpah, *et al*., 2014).** The present results of this study has been compared to the effects of insecticides when concentrates 0.5 and 7.5 ppm. It was found that the most effect were; Imidacloprid, Mix, Methoxyfenozide and Azadirachtin, with values of

LT_{50}= 10.348, 12.19, 37.984 and 47.535 hours, respectively at 0.5 ppm. When the concentrate was 7.5 ppm, the values of were LT_{50}= 2.714, 5.061, 7.052 and 8.687 hours. The control group (Control) was longest of age, reaching the age of the honey bee workers to LT_{50} = 1749.421 hours. The present results agree with studies carried out by **(Husain, *et al*., 2014)** which indicate that the chronic toxicity induced by insecticides laboratory bioassay showed that Imidacloprid was the most toxic at their high dose (1000 ppm) with LT_{50} of 4 hours in each case for *Apis mellifera*. Also, Imidacloprid was the most toxic at high dose (1000 ppm) with LT_{50} value of 5 hours.

In the current study, when it was compared between the mix of three insecticides (Azadirachtin, Imidacloprid and Methoxyfenozide), we found that the mix had lower effects when compared to Imidacloprid when it was used alone. Imidacloprid when used alone, the lethal dose was ($LD_{12.5:50}$), however, when it was mixed with other insecticides, the lethal dose value was ($LD_{25:50}$). Furthermore, the results showed that there has been no death for individuals in the early hours of the test, but when the 12-hour to 96-hour passed, the mortality rate began to increase, but within the normal limits and rates ranging from 1.66% to 11.66%. Various studies have revealed new insights into the sub-lethal impacts of pesticides including the effects of a high dose of pesticide that would result in the death of the foragers or would cause an aversion to foraging from the affected patches. However, it is the sub-lethal dose that poses a major threat to the survival of the entire colony **(Desneux, *et al*., 2007).**

This further calls for emergency funding to address the myriad holes in our scientific understanding of pesticide consequences for pollinators. The relegation of bee toxicity for registered compounds to impact only label warnings, and the underestimation of pesticide hazards to bees in the registration process may well have contributed to widespread pesticide contamination **(Mullin, *et al*., 2010).** Pesticides are extremely toxic to pollinators when administered in high doses. Chronic exposure to pesticides may reduce adult longevity in the insects as a consequence of sublethal toxicity. In honey bees, as mentioned before, contaminated food may be stored in hive for longer term **(Chauzat, *et al*., 2006 and Claudianos, *et al*., 2006).** Hence, death in honey bees due to acute toxicity is only a partial measure as chronic sub-lethal effects may be further critical. Discerning long term pesticide effects on honey bee survival is however not easy and there are statistical issues of analyzing survival data **(Desneux, *et al*., 2007)** and there has been several approaches to tackle this. Often the end result is stated in chronic toxicity tests **(Schmuck, 2004).**

5. Conclusion:

The mortality rate results indicate that Imidacloprid then Methoxyfenozide greatly affect foragers honeybee workers (*Apis mellifera jemenatica)*, whereas Azadirachtin was less effective because it was associated with lower death rate and a longer life span seen with the honeybees workers.

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