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Evaluation of Certain Synthetic and Natural Pesticides Against Whitefly *Bemisia tabaci* (Genn.) on Green Bean and Effect on Honeybee *Apis mellifera* L.

Sharihan MM Ahmad[1], Walaa El-Sayed[2], Ahmed MT Ahmed[1], Sawsan M Abdelmegeed[2*]

1- Department of Vegetable Pests - Plant Protection Research Institute - Research Center Agricultural, Cairo, Egypt

2- Plant Protection Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt

*Corresponding author: sawsan_ali@agr.asu.edu.eg

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Abstract: The green bean crop (*Phaseolus vulgaris*) is infested with many insects such as whiteflies (*Bemisia tabaci*). An investigation was carried out to study the insecticidal activity of whitefly (*Bemisia tabaci*), five pesticides were used, namely Thiamothoxam, Sulfoxaflor, Spirotetramat, Pymetrozine, and Flonicamid, and three commercial natural pesticides, namely Superrego, Topnine, and Bernastar. Pesticides and natural pesticides were sprayed during the two seasons of 2020 and 2021. The obtained results in terms of the reduction rate of the insects' number were as follows: The highest rate of reduction appeared in Thiamothoxam and Sulfoxaflor treatments, where it reached above 80%, while the other pesticides were in the range of 40% to 60%, and the lowest reduction rate was 34.33% and 36.00 % for Bernastar during season 2020 and 2021 respectively. When evaluating the toxicity of these pesticides and natural pesticides against honeybees (*Apis mellifera*), the pesticides did not reveal any toxicity to honeybees, except for Thiamothoxam, Sulfoxaflor, and Superrego The LC_{50} and LD_{50} values against honeybees were 0.2 ppm and 0.000005µg/one honeybee for Thiamothoxam, 4.5 ppm and 0.0001125 µg/one bee for Sulfoxaflor, and 0.95 ppm and 0.00002375 µg /one bee for Superego respectively.

1 Introduction

Green bean (*Phaseolus vulgaris* L.) production has increased in recent years in many countries all over the world; agricultural operations including pest control have crucial effects on crop productivity (Altieri et al 1977, Binnie and Clifford 1981, Begum et al 2023). Green beans are infested with many insects, the most important of which are sucking and piercing insects such as white flies, which causes great losses in the green bean crop (Altieri et al 1977).

The most important pesticides used against white fly, which works to reduce the infestation rate significantly, are Sulfoxaflor insecticide **(**Zewain et al 2013), Flonicamid insecticide (El-Zahi et al 2017), lambda – Cyhalothrin insecticide (Bughdady et al (2020) and neonicotinoid insecticides (Barman et al 2021). Recently, the trend has been to use natural pesticides with different origins e.g. plant extracts due to their low toxicity to humans and living organisms in the surrounding environment (Abd-Allah et al 2005, Sayed et al 2020, Al-Ghamdi et al 2021, Noureldeen et al 2022).

Consideration shall be taken when selecting pesticides to reduce the whitefly population, in terms of the toxicity against honeybee insects (Abd-Allah et al 2005, Xavier et al 2015, Tosi and Nieh 2017, Saleem et al 2020).

The research aims to determine the relative efficacy of selected insecticides (Thiamothoxam, Sulfoxaflor, Spirotetramat, Pymetrozine, and Flonicamid,) and natural pesticides (Superrego, Topnine, and Bernastar) against *Bemisia tabaci* under field conditions and to assess acute toxicity of the selected synthetic insecticides and natural pesticides to honeybee workers under laboratory conditions.

2 Materials and Methods

2.1 Pesticides used to control whitefly

2.1.1 Chemical Pesticides

Thiamethoxam

Molecular formula: $C_8H_{10}ClN_5O_3S$ (**Fig 1)**.
Trade name: Lex 25% WG (Thiamothoxam) is produced by (Starchem Industrial Chemicals– Egypt). It was used at the recommended rate of 60g/100L.

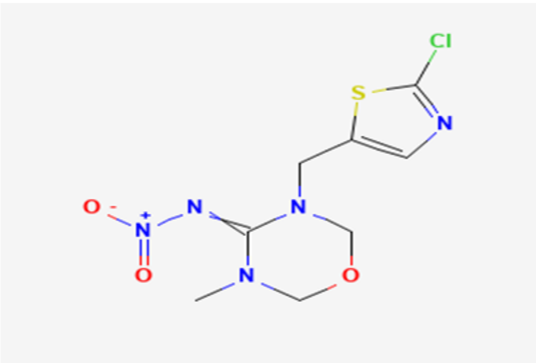

Fig 1. Chemical structure of (Thiamothoxam)

Sulfoxaflor

Molecular formula: $C_{10}H_{10}F_3N_3OS$ (**Fig 2**).
Trade name: Closer™ 24% SC (Sulfoxaflor) is produced by (Dow AgroSciences USA). It was used at the recommended rate of 50cm^3/fed.

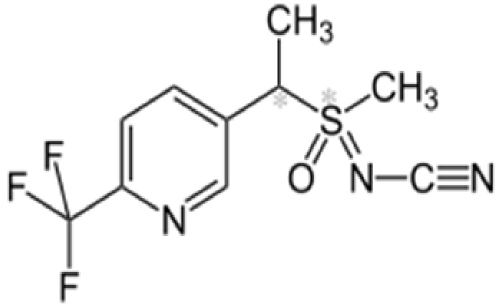

Fig 2. Chemical structure of Sulfoxaflor

Spirotetramat

Molecular formula: $C_{21}H_{27}NO_5$ (**Fig 3**)
Trade name: Movento® 10% SC (Spirotetramat) is produced by (Bayer CropScience). It was used at the recommended rate of 75cm^3/100L.

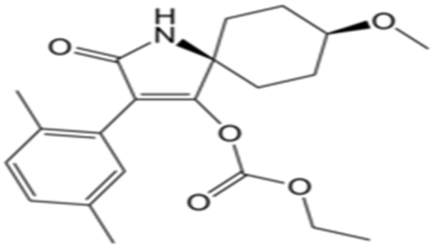

Fig 3. Chemical structure of Spertetromate

Flonicamid

Molecular formula: $C_9H_6F_3N_3O$ (**Fig 4**).
Trade name: Teppeki 50% WG (Flonicamid) is produced by (Starchem Industrial Chemicals– Egypt). It was used at the recommended rate of 20gm/100L.

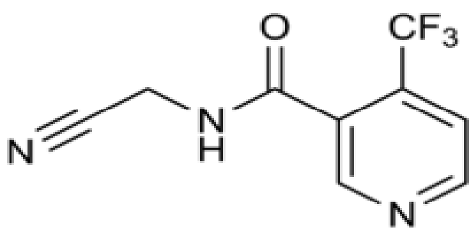

Fig 4. Chemical structure of Flonicamid

Pymetrozine

Molecular formula: $C_{10}H_{11}N_5O$ **(Fig 5).**
Trade name: Tedo 50%WDG (**Pymetrozine**) is produced by (Starchem Industrial Chemicals – Egypt). It was used at the recommended rate of 50gm/100L.

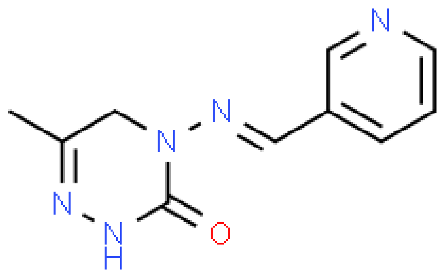

Fig 5. Chemical structure of Pymetrozine

2.1.2 Natural pesticides

BernaStar

Trade name: BernaStar 32% (Coconut pulp extract 40%, Avocado seed extract 38%, Sulfur compounds of plant origin 8% and Auxiliary materials and water 14%) is produced by (Starchem Industrial Chemicals – Egypt). It was used at the recommended rate of 1L/fed.

TopNine

Trade name: TopNine 100% (An innovative formula of natural materials containing chitosan) is produced by (Starchem Industrial Chemicals – Egypt). It was used at the recommended rate of 750 cm/fed.

SuperRego

Trade name: SuperRego 100% (Matrine and Chitosan). Matrine is a kind of plant-sourced pesticide; it is a kind of alkaloid extracted from leguminous plant (*Sophora flavescens*) root, stem and fruit by ethanol and other organic solvent. SuperRego is produced by Starchem Industrial Chemicals – Egypt. It was used at the recommended rate of 125ml/100L.

2.2 Evaluation of the efficacy of pesticides on Green beans (*Phaseolus vulgaris*) crop in the field

A field experiment was carried out to evaluate the efficacy of Lex 25% WG, Closer™ 24% SC, Movento® 10% SC, Teppeki 50% WG, Tedo 50% WDG, BernaStar 32%, TopNine 100% and SuperRego 100% against the nymphs of whitefly (*Bemisia tabaci*) during 2020 and 2021 growing season of green beans at the agricultural experiment station, Faculty of Agriculture, Cairo University, Giza, Egypt. Seeds of green beans (*Phaseolus vulgaris* L.) Bronco varieties were cultivated in the field on 21st August 2020 and 21st August 2021. The experimental area was divided as a complete randomized block design including nine treatments and three replicates for each treatment. A plot size of 350 m^2 of a green bean plant and the standard package of practices as described by AL-Kherb (2011) and Bughdady et al (2020). A hand sprayer was used in applying the tested pesticides and natural pesticides at their recommended application rates separately. The insecticides were applied just after the mean infestation counts of 10 Whitefly insects shoot/ plant or above. Fifteen leaves of each replicate for each treatment from the green beans plant were selected randomly for recording the Whitefly insects. The pre-count was performed 24 hr. before spray and post-counts at the intervals of 1, 3, 7 and 10 days after treatment. Treated leaves were put in paper bags to preserve the samples until they reached the laboratory for examination with a binuclear. The reduction of the population (mortality percentages) was calculated according to the equation of Henderson and Tilton (1955).

2.3 Evaluation of the toxicity of chemical and natural pesticides on honeybees in the laboratory

Laboratory experiments were carried out against honeybee workers of *Apis mellifera* L. The workers were obtained from bee hives maintained at the apiary of the Faculty of Agriculture, Ain-Shams University. After collection, an appropriate number of bees were placed into a large plastic box and transferred to the laboratory. The bees were distributed in experimental plastic jars in groups and worker bees were placed into an experimental jar, three replicates were maintained for each concentration. The bees are starved for 2 hours before the initiation of the experiment so that all bees are equal in terms of their gut contents at the start of the experiment. Oral exposure for each concentration for 4 hours. After the period of exposure to the insecticide at different concentrations, the treated honeybees were fed a 50% (w/v) sucrose solution without pesticides for 72 hours. Mortality percentage was recorded after 4, 24, 48 and 72 hours of treatment. Bees were fed with 50 % (w/v) sucrose solution only were used as a control. The tests were carried out under laboratory conditions at 28 ± °C and 70 % RH according to guidelines No. 213 of the Organization for Economic Co-operation and Development OECD (1998).

Preliminary tests were carried out to determine the range of concentrations that produce 20 to 80 % mortality in honeybees (Laurino et al 2013, Delkash-Roudsari et al 2022). The concentrations used were from Thiamethoxam 0.1, 0.2, 0.3, 0.4 and 0.6 µl/L. While the concentrations of Sulfoxaflor were 1, 2, 4, 6, 8 and 10 µl/L. The used concentrations of Superrego were 0.125, 0.25, 0.5, 1, 5 and10 µl/L. Each concentration of each tested agent was replicated three times in addition to the control treatment. The rest of the pesticides did not appear toxic to bees and were safe.

2.4 Statistical analysis

The reduction of the population (mortality percentage) was calculated according to the equation of Henderson and Tilton (1955) and The Data were analyzed using the SAS (2006) statistical analysis system, version (9), separating the means using Tukey.

Alive and dead nymph (*Bemisia tabaci*) were counted after 24 mortality percentages and Alive and dead honeybees were counted daily for three days and mortality percentages were corrected by Abbott's formula (1925). Mean percentages of mortalities after correction were plotted on a probit scale against the log of concentration. According to Finny (1971), the regression lines, Ld-p lines, were drawn. The slope and median lethal concentrations were calculated. Each concentration of each tested agent was replicated three times, in addition to the control. For estimation of the lethal concentration (LC_{50}) was used where elapsed time for 50% kill of honeybee workers was determined for accumulated mortality because of Thiamothoxam, Sulfoxaflor, Spirotetramat, Fonicamid, Pymetrozine, Superego, Bernastar and Topnine treatments. The LC_{50} values were calculated by probit analysis Finny (1971).

The lethal dose (LD_{50}) to kill 50% of the treated honeybees for cumulative mortality was determined for Thiamethoxam, Sulfoxaflor, Spirotetramat, Fonicamid, Pymetrozine, Superego, Bernastar and Topnine, the LD_{50} values can be calculated through the amount needed to feed one bee per day, which is 25 µg. By substituting in the following equation, the dose taken by the bee can be deduced from the pesticide.

$$LD_{50} = 25 \text{ µg for one bee} * LC_{50} \backslash 1000000$$

3 Results and Discussions

3.1 Efficacy evaluation of the tested pesticides and natural pesticide agents to nymphs of *Bemisia tabaci* (Genn) on green beans during the seasons 2020 and 2021

Table 1 shows the rate of reduction of whitefly infestation during the season of 2020, as the number of insects decreased on green bean leaves when using chemical pesticides and plant extracts. The results showed that the rate of reduction in whitefly infestation was increased by increasing the number of sprays, as it was the highest possible in the third spray compared to the first and second spray.

The highest reduction rate was found, up to more than 80%, in Thiamethoxam and Sulfoxaflor pesticides, and the reduction rate reached about 40% to 60% in the rest of the pesticides used (Spirotetramat, Pymetrozine, Flonicamid, Superego, and Topnine), while the lowest percentage of reduction in infestation in the case of the Bernastar pesticide was 34%. Almost the same results appeared in the percentage of rate reduction in infestation during the season of 2021 (**Table 2)**.

Neonicotinoids compounds are among the newly spread and widely used compounds, which have significant effects against sucking and piercing insects, especially the whitefly (Barman et al 2021), and the most important of them is Thiamethoxam, as El-Zahi et al (2017) found that it had the highest effect in reducing the infestation of the whitefly on the cotton crop, and LC_{50} against nymphs (The second age) reached 30.37 ppm, and then the flonicamid pesticide, reached 226.24 ppm. Bughdady et al (2020) also used thiamethoxam pesticide against the whitefly on the tomato crop, which resulted in a decrease in infestation after 14 days of treatment (Zewain et al 2013). Sulfoxaflor pesticide 24% SC was used at the recommended rate of application against whitefly on cucumber plants and showed its effectiveness on the rate of infestation reduction as well. Salazar-López et al (2016) and Iftikhar et al (2022) found that Spirotetramat pesticide was very effective against sucking-piercing insects such as aphid

Table 1. Efficacy evaluation of the tested pesticides and natural pesticides agents to nymphs of *Bemisia tabaci* (Genn) on green bean during August 2020

Pesticides	First spray				Second spray				Third spray			
	1day	3day	7day	10day	1day	3day	7day	10day	1day	3day	7day	10day
Thiamothoxam	50.00^{a} ±1.6329	52.00^{a} ±1.4142	55.33^{a} ±0.9428	58.00^{a} ±0.8164	61.33^{a} ±0.9285	63.33^{a} ±1.2472	68.53^{a} ±1.7987	71.06^{a} ±0.8219	72.16^{a} ±1.5542	75.00^{a} ±1.4142	77.00^{a} ±2.1602	84.86^{a} ±0.9843
Sulfoxaflor	51.30^{a} ±0.4966	52.80^{a} ±0.5656	54.03^{a} ±0.8576	57.0^{a} ±0.8993	53.56^{ab} ±4.0301	62.30^{a} ±0.4966	65.00^{a} ±1.2247	67.66^{a} ±0.9428	69.36^{a} ±1.2391	72.83^{a} ±0.4642	74.11^{a} ±0.6861	80.40^{b} ±1.3366
Spertetromate	29.66^{c} ±0.4714	32.33^{c} ±0.4714	35.03^{c} ±0.8178	38.33^{c} ±1.2472	44.20^{cd} ±5.2484	44.16^{c} ±0.6236	49.20^{c} ±0.8640	51.86^{c} ±0.6599	54.83^{b} ±0.9030	57.36^{b} ±1.0498	58.96^{b} ±0.8576	60.00^{c} ±0.8164
Pymetrozene	31.0^{c} ±0.8379	32.00^{c} ±0.9899	35.20^{c} ±1.2472	37.16^{c} ±0.8576	37.33^{de} ±0.8164	37.23^{d} ±0.8164	36.50^{e} ±0.9201	37.73^{d} ±1.7454	38.50^{c} ±1.4290	39.33^{d} ±1.2657	35.53^{de} ±1.2391	40.63^{e} ±0.6531
Flonicamid	34.13^{b} ±0.8379	40.30^{b} ±0.9899	40.66^{b} ±1.2472	48.96^{b} ±0.8576	51.00^{bc} ±0.8164	54.00^{b} ±0.8164	54.70^{b} ±0.9201	56.30^{b} ±1.7454	57.96^{b} ±1.4290	57.63^{b} ±1.2657	57.63^{b} ±1.2391	59.00^{c} ±0.6531
Superrego	9.00^{d} ±0.8164	40.00^{b} ±0.8164	41.76^{b} ±1.3572	40.20^{c} ±0.5887	40.33^{d} ±1.2229	42.93^{c} ±1.3695	45.00^{d} ±0.8164	42.0^{d} ±1.6329	40.80^{c} ±0.5887	47.26^{c} ±1.7632	50.06^{c} ±0.8993	52.20^{d} ±0.8640
Topnine	9.00^{d} ±0.8164	9.83^{d} ±1.1785	12.10^{d} ±1.2027	28.96^{d} ±0.8576	31.66^{e} ±1.2762	32.60^{e} ±1.8402	32.36^{f} ±0.9877	33.13^{e} ±0.8379	32.00^{d} ±1.6329	36.13^{de} ±1.7987	38.80^{d} ±1.0708	40.13^{e} 0.8379
Bernastar	8.00^{d} ±0.8164	11.00^{d} ±1.6329	15.16^{d} ±0.6236	17.96^{e} ±0.0471	19.83^{f} ±0.4642	21.3^{f} ±0.4714	23.23^{g} ±0.5557	26.36^{f} ±1.6739	29.16^{d} ±0.6236	32.00^{e} ±0.8164	33.30^{e} ±0.9201	34.33^{f} ±0.4714
F.value	772.34**	480.31**	478.32**	454.88**	56.80**	338.18**	381.76**	327.41**	375.67**	347.70**	386.75**	968.19**
L.S.D	3.1218	3.654	3.5505	3.1383	8.5579	3.891	3.9563	4.3782	4.1889	4.2778	4.1893	2.9281

Table 2. Efficacy evaluation of the tested pesticides and natural pesticides agents to nymphs of *Bemisia tabaci* (Genn) on green bean during August 2021.

Pesticides	First spray				Second spray				Third spray			
	1day	3day	7day	10day	1day	3day	7day	10day	1day	3day	7day	10day
Thiamothoxam	54.96^{a} ±1.6739	57.63^{a} ±0.4496	60.00^{a} ±0.8164	59.600^{a} ±0.4320	63.00^{a} ±0.9285	66.33^{a} ±0.9428	70.20^{a} ±1.2961	72.70^{a} ±1.2027	73.40^{a} ±1.1775	76.00^{a} ±0.8164	78.33^{a} ±1.6996	89.16^{a} ±1.1085
Sulfoxaflor	54.00^{a} ±0.8164	55.46^{a} ±0.8993	55.30^{a} ±0.9201	58.400^{a} ±0.4320	59.16^{a} ±0.8498	65.30^{a} ±0.4966	66.83^{a} ±1.4337	68.00^{a} ±1.4142	69.00^{b} ±1.6329	72.00^{a} ±1.4142	75.00^{a} ±1.6329	81.20^{b} ±1.0708
Spertetromate	33.00^{c} ±1.63291	36.66^{c} ±1.8856	35.70^{d} ±0.4242	39.00^{d} ±0.8164	40.86^{d} ±0.6599	44.00$^{\pm d}$ ±0.8164	49.33^{c} ±0.9428	53.33^{c} ±1.8856	55.00^{cd} ±0.8164	57.10^{b} ±0.9416	59.63^{bc} ±1.2918	61.66^{c} ±1.6996
Pymetrozene	37.33^{b} ±1.2472	36.63^{c} ±0.9672	37.36^{d} ±1.2918	38.166^{d} ±1.17851	41.66^{d} ±1.6996	43.96$^{\pm d}$ ±1.4613	45.33^{d} ±1.2472	46.30^{d} ±1.7454	47.83^{e} ±0.6236	49.00^{c} ±0.8164	50.66^{d} ±0.4714	51.30^{d} ±0.4966
Flonicamid	40.00^{b} ±0.8164	42.66^{b} ±1.2472	43.66^{c} ±1.2472	49.00^{b} ±0.8164	51.00^{b} ±0.8164	54.63$^{\pm b}$ ±1.2119	55.66^{b} ±0.9428	58.66^{b} ±1.2472	58.46^{c} ±1.2036	59.13^{b} ±0.8379	60.00^{b} ±0.8164	63.10^{c} ±0.6976
Superrego	12.33^{d} ±1.2472	42.00^{b} ±1.6329	42.30^{c} ±1.6872	45.33^{c} ±1.2472	46.33^{c} ±1.6996	49.33^{c} ±1.2472	49.96^{c} ±0.8178	52.00^{c} ±1.6329	53.10^{d} ±1.6062	54.33^{b} ±3.2998	55.33^{c} ±1.6996	59.00^{c} ±0.8164
Topnine	13.00^{d} ±0.8164	14.66^{d} ±1.2472	18.00^{e} ±0.8164	31.33^{e} ±1.2472	34.00^{e} ±0.8164	34.60^{e} ±0.9933	34.76^{e} ±1.1145	36.13^{e} ±1.9754	39.66^{f} ±0.4714	40.00^{d} ±0.8164	40.66^{e} ±1.2472	41.30^{e} ±0.4966
Bernastar	10.00^{d} ±0.8164	14.00^{d} ±1.6329	16.16$^{\pm e}$ ±0.6236	18.63^{f} ±0.9672	20.40^{f} ±1.1775	22.66^{f} ±1.6996	25.33^{f} ±1.2472	28.70^{f} ±0.5715	28.90^{g} ±0.8286	32.66^{e} ±1.2472	36.66^{e} ±0.4714	36.00^{f} ±2.1602
F.value	474.85**	303.22**	444.12**	422.69**	272.99**	323.43**	343.96**	192.47**	339.24**	191.61**	271.52**	456.83**
L.S.D	4.105	4.5713	3.6353	3.2713	3.9697	4.0297	3.9731	5.2649	3.8798	5.198	4.3759	4.165

and whitefly. Plant extracts have been chosen instead of pesticides, and they give positive results in controlling pests such as fermented plant extracts of neem leaf and wild garlic on tomato plant (Nzanza and Mashela 2012).

3.2 Evaluation of the toxicity of chemical and natural pesticides against honeybees under laboratory conditions

The toxicity of the investigated chemical pesticides and plant extracts against honeybee workers was evaluated and expressed as LC_{50} and LD_{50}. The following pesticides (Spirotetramat, Pymetrozine, Flonicamid, Bernastar, and Topnine) did not show toxicity to honeybees, therefore their use is safe in pest control without causing harm to bees. However, Thiamethoxam, Sulfoxaflor, and Superrego were expressed severe toxicity effects on bees as high death rates of worker honey bees were detected.

Thiamethoxam pesticide showed high mortality, reaching 83.9% at a concentration of 0.6 ppm, and the lethal concentration and lethal dose for half of the bees were 0.2 ppm and 0.000005µg/one bee respectively (**Fig 6)**. Sulfoxaflor pesticide showed high mortality, reaching 72.4% at a concentration of 10 ppm, and the lethal concentration and lethal dose for half of the bees were 4.5 ppm and 0.0001125 µg/one bee respectively, which is the most toxic to worker bees compared to other pesticides (**Fig 7)**.

Superrego pesticide showed high mortality, reaching 100% at a concentration of 30 ppm, and the lethal concentration and lethal dose for half of the bees were 0.95 ppm and 0.00002375 µg/one bee respectively (**Fig 8)**.

Chemical and natural pesticides should be studied and their effectiveness against bees or beneficial insects evaluated, as Saleem et al (2020) found that neonicotinoid pesticides such as Thiamethoxam and Imidacloprid affect the activity of honeybees and feeding at different temperatures. He also found that safe concentrations of these pesticides on bees in the summer are deadly to honey bees in winter. Tosi and Nieh (2017) studied the effect of Thiamothoxam for two days on the behavior and activity of bees and found that it led to a weakness in the movement of bees inside and outside the hive.

Plant extracts are considered safer for humans, beneficial insects and bees, such as neem compounds, which did not show any negative effect on the activity of honeybees (Abd-Allah et al 2005). But not all plant extracts are safe and should be studied on honeybees, in this concern, Xavier et al (2015) found that andiroba oil, garlic extract, and neem oil have high toxicity on honeybee larvae, except for eucalyptus oil, and these oils affected the activity and movement of honeybees adult.

Conc /ppm	mortality%
0.1	33.7%
0.2	50.5%
0.3	73.9%
0.4	79.6%
0.6	83.9%

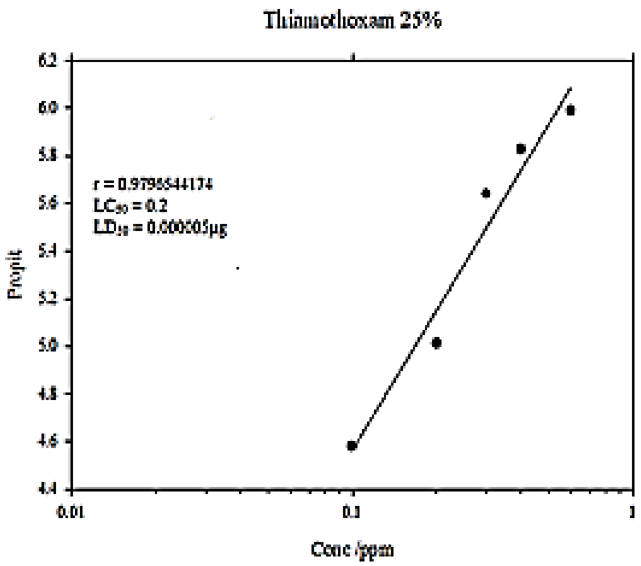

Fig 6. Mortality and the lethal concentration and lethal dose for fifty percent (LC_{50} &LD_{50}) of worker honeybees when they were fed a sugar solution with different concentrations of Thiamethoxam pesticide

Conc /ppm	% mortality
1	13.7 %
2	20.9 %
4	44 %
6	61.7 %
8	70.7 %
10	72.4 %

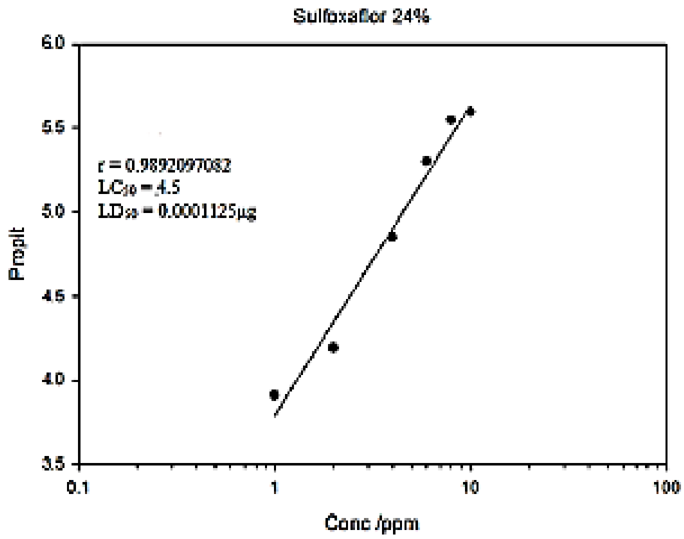

Fig 7. Mortality and the lethal concentration and lethal dose for fifty percent (LC_{50} &LD_{50}) of worker honeybees when they were fed a sugar solution with different concentrations of Sulfoxaflor pesticide

Conc / ppm	% mortality
0.125	18.7 %
0.25	39.1 %
0.5	31.9 %
1	54.4 %
5	70.5 %
10	78.6 %
15	89.3 %
20	94.1 %
30	100 %

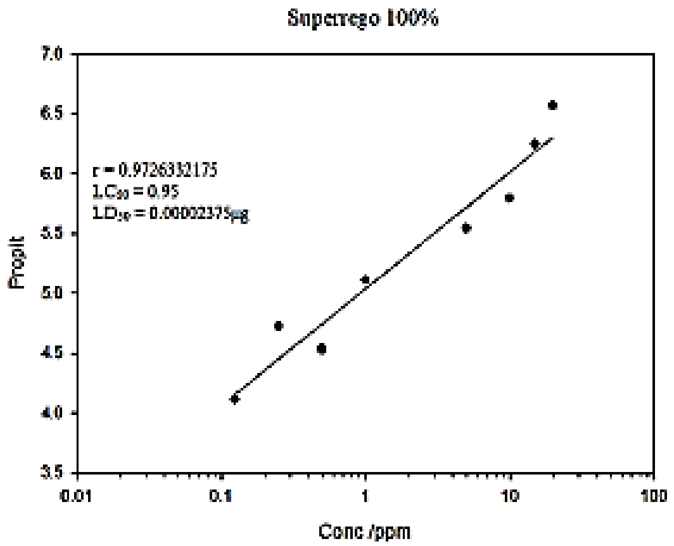

Fig 8. Mortality and the lethal concentration and lethal dose for fifty percent (LC_{50} &LD_{50}) of worker honeybees when they were fed a sugar solution with different concentrations of Superrego pesticide

4 Conclusion

Thiamethoxam and Sulfoxaflor pesticides showed their effectiveness against whiteflies, but unfortunately, it is advised not to use them due to their toxicity to honeybees. Also, plant extracts are not necessarily safe, and this appeared in the pesticide Superrego, where the results showed that it is less effective against whiteflies, but it is highly toxic to honeybees when compared to other pesticides used. Therefore, it is recommended to use pesticides such as Spirotetramat, Pymetrozine, Flonicamid and Topnine because they are effective in reducing whitefly infestation on green bean crops, in addition to being non-toxic to honeybees.

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