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Research Article

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Study and Comparison of the Lethal Effect of the Insecticide Chlorpyrifos on *Aphis fabae* in Laboratory and Greenhouse Conditions

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ABSTRACT

Aphis fabae is one of the most damaging pests that attacks more than 200 plant species and carry more than 50 types of plant diseases. Considering the indiscriminate use of chemical toxins against this pest, particularly Chlorpyrifos, determination of a proper dose of this toxin for farmers' uses seems necessary, so that we may be able to minimize the remaining amount of this toxin in the environment. Chlorpyrifos is a part of the group of OP insecticides exposure, gastrointestinal effect and a little fumigant property. It plays its role by affecting the normal function of the nervous system. In this research a completely randomized scheme with seven treatments and five replications has been used in order to find the proper concentration of the toxin Chlorpyrifos to control the aphis fabae in laboratory and greenhouse. In both conditions (laboratory and greenhouse) after 96 hours, a significant difference was observed on the n probability level of one percent between treatments. The results showed that the 0.07ppm concentration is the most effective concentration of the Chlorpyrifos toxin in laboratory and greenhouse conditions, and also with the help of Polo-PC software, Chlorpyrifos' LC_{50} in laboratory and green house conditions for aphis fabae was respectively obtained 0.008 and 0.05ppm.

Keywords: *Aphis fabae*, Chlorpyrifos, Laboratory and greenhouse conditions.

INTRODUCTION

Pesticides are an essential component of the agricultural world. Although the request for production and distribution of pesticide for enhancing the improvement of agricultural quality and efficiency is evident, the possibility of inappropriate and unreasonable use of them is quite high. Population growth and following that increase of food consumption, especially agricultural products, has made the farmers to enhance their yields. Increase of products' cultivation has subsequently caused an increase in pesticide toxins [1].

In fact, they use the fastest way to destroy the pest which is due to their lack of awareness. One of these lacks of awareness is using doses on the package of the toxins against pests other than the pests that are suggested on the package [2]. On the other hand, one of the harmful insects of the agricultural world that makes the farmers to face some challenges for fighting it is aphis. Among the group of the aphids, the aphis fabae (scientific name) which exists in all areas and in addition to beets, it exists on peas, beans, broad beans, potatoes, boxwood and etc., attacks more than 50 plant species in Iran and more than 200 plant species in the world [3]. Also by studying the used toxins in the beets farms against various pests including the aphis fabae, it was clear that Chlorpyrifos plays an important role among these toxins and this is totally obvious the in remains of this toxin in the roots of the beets farms in the sugar factory of Isfahan. On the other hand, the remains of Chlorpyrifos in the underground waters of the same region [4] is a good evidence of the widespread use of this toxin in Iran. "Dursban" with the generic name "Chlorpyrifos" is the OP insecticide with an exposure, gastrointestinal effect and a little fumigant property. This

insecticide is in the chemical group of the organophosphorus that plays its role by affecting the normal function of the nervous system by discontinuation of acetylcholine, which is a neurotransmitter. In this research, we tried to determine the proper dose of Chlorpyrifos with accurate experiments in order to find a way to prevent the indiscriminate use of it.

EXPERIMENTAL SECTION

First some samples of the aphis fabae (against which a toxin has not been used) were collected from the beets farms of the sugar factory of Isfahan and they were recognized by all species of the aphis [5]. These aphids were released on a number of grown plants of the sugar beet which were on the 4 to 6 leaves level. After the first reproduction, the bioassay tests were performed for determining the required concentration of deaths between 10 to 90 percents. Six concentrations were obtained, 0.001, 0.004, 0.007, 0.01, 0.04 and 0.07ppm. For doing this research in the laboratory, a completely randomized scheme with seven treatments and five replications was used. Treatments included six concentrations and one treatment was control (distilled water). In order to calculate the lethality effects in the laboratory, petri dishes that each were used. Each of those petri dishes included a disc of a leaf of the beets [6]. Before putting the leaf in the petri, each leaf was dipped in a toxic solution of the obtained concentrations for 30 seconds [7] and then they were exposed to air for 30 minutes so that they would be completely dry [8]. In each petri, for keeping the humidity and healthiness of the leaf discs, a space was created by using the petri dish's lid (under the lid). The space was filled with water; petiole was put in the water through the hole that has been created at the bottom of the petri dish [9]. The aphis fabae has four instars [10] for this purpose, 10 instars of an aphis fabae were placed in each leaf in the petri and its lid was encapsulated with a Para film. Then the petries were put in a germinator with the temperature of 25±2ºC, relative humidity 65±5, and the lighting conditions of 16 hours light and 8 hours darkness. The petries were took out of the germinator every 24 hours and the number of the dead insect was counted. In this research the aphids were irritated with a brush and in case there was a movement of their body or themselves, they were considered alive and otherwise they were considered dead. After 96 hours, the obtained results were analyzed by the SPSS and Polo-PC software [7]. For the greenhouse part, a small hand sprayer with a volume of 2.5 liters was used for spraying the pots, which was calibrated before the application. The proper distance for spraying was evaluated 50cm so that the toxic solution would cover the entire aimed plant with the perfectly fine particles, without the solution shedding from the plant. After spraying doses and control with the toxin, the plants' drying, polluting them was done such as the laboratory conditions. Each leaf was put into a disposable cup in order to prevent the insects from escaping the leaves. The mouth of the cup was closed by a lace. We tried to place the insects on various leaves to keep the random process. After 96 hours passed, the obtained results were analyzed [11].

RESULTS AND DISCUSSION

The average of the percentage of the aphis fabae's death was corrected with the help of the "Abot" formula [12] and it was used for various concentrations of Chlorpyrifos in laboratory conditions after 24, 48, 72 and 96 hours and the group of 96 hours was considered as the basis of the calculations.

Table (1) – Average of the percentage of the corrected death of the aphis fabae for various concentrations of the Chlorpyrifos in laboratory conditions after 24, 48, 72 and 96 hours

Composition	Chlorpyrifos			
Time	24	48	72	96
Concentrations				
0.001ppm	0	2	4.2	10.6
0.004ppm	0	8.2	10.6	19.1
0.007ppm	10	26.5	38.3	53.2
0.01ppm	16	34.7	44.7	65.9
0.04ppm	20	24.8	55.3	76.6
0.07ppm	22	44.9	74.5	93.6
Control	0	2	6	6

Table (2) – The results of the analysis of the variance of the determination test of the proper concentration of Chlorpyrifos on the death of the aphis fabae after 96 hours in the laboratory

Sources of variation	degrees of freedom	Sum of squares of treatment	Average of the squares of treatment	F
Treatment (concentration of the toxin)	6	35938.22	5989.70	58.88**
Error	28	2848.32	101.73	

***it means significant in the probability level of one percent.*

Analysis of the variance and comparing the average of the percentage of the losses in various concentrations of Chlorpyrifos showed that this composition has a significant effect among the treatments in the probability level of one percent (Table 2) and (Table 3).

Table (3) – The result of comparing the average of the percentage of the aphis fabae's losses for various concentrations of Chlorpyrifos after 96 hours in the laboratory

Concentrations (ppm)	Average (percent)
Control	6^{e}
0.001	10.7^{de}
0.004	19.1^{d}
0.007	52.6^{c}
0.01	65.5^{bc}
0.04	76.5^{b}
0.07	93.6^{a}

✓ *Variation of the column's numbers which have mutual letters is no statistically significant in the level of one percent.*

In table 3, the significant variation among treatments shows that the toxicity of Chlorpyrifos are different in the concentrations that were being tested with the confidence level of 99 percent. Therefore, it was the most lethal rate related to the treatment 0.07ppm that created 93.56 percent of the losses. Minimum rate of death (10.66 percent) is also about the treatment with a concentration of 0.001ppm.

And also the results of the average of the percentage of the corrected death of the aphis fabae for various concentrations of Chlorpyrifos in the greenhouse conditions after 24, 48, 72 and 96 hours was specified in Table 4 and the group of 96 hours was the basis of the calculations.

Table (4) – The average of the percentage of the corrected death of the aphis fabae for various concentrations of Chlorpyrifos in the greenhouse conditions after 24, 48, 72 and 96 hours

Composition	Chlorpyrifos			
Time	**24**	**48**	72	96
Concentrations				
0.001ppm	0	0	6.1	8.3
0.004ppm	0	0	8.2	12.5
0.007ppm	2	2	16.3	25
0.01ppm	6	10.2	24.5	35.4
0.04ppm	10	18.4	36.7	45.8
0.07ppm	12	28.6	46.9	60.4
Control	0	2	2	4

Analysis of the variance and comparing the average of the percentage of the losses in various concentrations of Chlorpyrifos in the greenhouse conditions showed that this composition has a significant effect among the treatments in the probability level of one percent (Table 5) and (Table 6).

Table (5) – The results of the analysis of the variance of the determination test of proper concentration of Chlorpyrifos on the death of the aphis fabae after 96 hours in the greenhouse

Sources of variation	degrees of freedom	Sum of squares of treatment	Average of the squares of treatment	F
Treatment (concentration of the toxin)	6	13192.27	2198.71	31.74**
Error	28	1939.57	69.27	

******** *it means significant in the probability level of one percent.*

Table (6) - The result of comparing the average of the percentage of the aphis fabae's losses for various concentrations of Chlorpyrifos after 96 hours in the greenhouse

Concentrations (ppm)	Average (percent)
Control	4^{d}
0.001	8^{d}
0.004	12.22^{d}
0.007	24.66^{d}
0.01	35.32^{bc}
0.04	45.76^{b}
0.07	60.22^{a}

✓ *Variation of the column's numbers which have mutual letters is no statistically significant in the level of one percent.*

In table 6, the significant variations among treatments show that the toxicity of Chlorpyrifos are different in the concentrations that were being tested with the confidence level of 99 percent. Therefore, it was the most lethal rate related to the treatment 0.07ppm that created 60.2 percent of the losses. Minimum rate of death (8 percent) is also about the treatment with a concentration of 0.001ppm.

On the other hand the calculations showed that in the laboratory conditions, the insecticide Chlorpyrifos has LC_{50} equal to 0.008ppm (with a confidence level of 0.006 and 0.009) and in the greenhouse conditions, it has LC_{50} equal to 0.005ppm (with a confidence level of 0.004 and 0.06). Of course, calculation of the LC_{50} value is not enough for determining toxicity and the slope of the regression line is another parameter that is used for comparing [13].

As it is seen the clarification coefficient (R^2) has also been close to one in both conditions. Therefore a high coordination exists between the obtained data relative to the regression line or the data that the model has predicted. The probit regression line shows that with increasing the concentration of the toxin, the rate of death in aphis' instars will also increase.

The results show that the most lethal rate is related to the treatment 0.07ppm which created 93.56 percent of the losses, in this regard, "Leena" and her colleagues evaluated the effect of the 0.25mm dose of Chlorpyrifos on the kitchen garden's aphis in the year 2001. The spray of one hundred percent death was created on the same day. And yet our highest dose in the laboratory conditions was 0.07ppm, in the first 24 hours to be 22 percent, in 48 hours 44.9 percent and in 72 hours 74.5 percent death was observed. And in greenhouse conditions on the same day, in the first 24 hours 12 percent, in 48 hours 28.6 percent and in 72 hours 46.9 percent death was observed. Considering this issue, in Peshawar, Pakistan, the 5 milliliter per liter Chlorpyrifos on the aphis of canola was observed to be 96.1 percent of death in the first 24 hours, in 48 hours 95 percent death and in 72 hours 92.3 percent death was seen [14]. In another research, the effect of two insecticides Thiacloprid and Fenvalerate on the aphis fabae was evaluated. The results of this evaluation shows that after 24 hours, respectively 89.21 and 81.14 death has been observed [15]. The conditions of exposure, dose and duration of exposure, age and species of the living creature and so many other factors can be considered as the reasons for variation in results [16].

CONCLUSION

It can be said that the bioassay of the toxin Chlorpyrifos in the laboratory and greenhouse conditions on the aphis fabae in this research after 96 hours showed that the concentration of 0.07ppm is the most effective concentration of it in the laboratory and greenhouse conditions. And as the variation of the death rate in laboratory and greenhouse conditions was stated in this section earlier, it can be believed that this difference is caused by the effect of the toxin (concentration of the toxin and size of the particles), drift and etc. On the other hand, LC_{50} (the concentration that causes the creation of 50 percent of the death in pest population) of the toxin Chlorpyrifos for aphis fabae in laboratory and greenhouse conditions was respectively equal to 0.008 and 0.05ppm. The usage of each of these concentrations caused fifty percent of the death in the aphis fabae population after 96 hours.

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