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Toxicity and Persistency of Thiodicarb Insecticide, on Cotton Plants to Conserve Parasitoid Wasp, *Bracon hebetor* Say

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ABSTRACT

***Bracon hebetor* Say is one of the most important parasitoids of cotton bollworm, *Helicoverpa armigera* (Hübner) in cotton fields. Using reduced-risk chemicals with low toxicity and persistency, is a principal tool to conserve and increase population of this parasitoid in cotton fields. This study was carried out to investigate residual lethal and sublethal toxicity and persistency of thiodicarb insecticide, on cotton plants against this parasitoid wasp. All experiments were carried out at the Entomological Research and Biological Control Labs. Department of Plant Protection and Greenhouse of the Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran. Cotton seeds (variety Golestan) were sown under greenhouse condition and cotton plants were sprayed with insecticides at recommended concentration rates (2000 and 500 ppm, respectively). Sprayed cotton leaves were removed at one-day intervals and female parasitoids were exposed to them within a container under laboratory condition (25±1°C, 60±5% RH, and 16:8 h L: D). The lethal (mortality percent) and sub lethal (reduction in female fecundity and paralyzing ability) effects of each insecticide were recorded every day until the "total effect" (mortality + fecundity reduction) reach to harmless category (<25%), according to IOBC standards. Data were analyzed and means were compared using a completely randomized experimental design and then insecticides toxicity and persistency were categorized according to the IOBC standard classes. Residual lethal toxicity of thiodicarb on *B. hebetor* (up to 77.9% mortality). Moreover, thiodicarb significantly reduced parasitoid fecundity and paralyzing ability (with 100 and 98.1 percent reductions compared to control, respectively) and was categorized as a harmful insecticide (group 4) based on total effect. Thiodicarb was also highly toxic to immature stages of parasitoid and reduced egg hatching and survival of parasitoid larvae by 95.9% and 82.8%, respectively compared to control. Residual persistency of insecticide was significantly different on cotton plants. Thiodicarb had longer persistency on cotton plants and its "total effect" on parasitoid was more than 25% for 22 days after spraying. Therefore, it was classified as a "moderately persistent" (group 3) insecticide according to the IOBC standard classes.**

Because of high toxicity and long-term persistency, thiodicarb is not recommended to use in cotton field against cotton bollworm in integrate program with mass release of *B. hebetor*. Of course, because of adverse effects of thiodicarb on parasitoid fecundity and paralyzing ability, a 25-day waiting period was recommended between spraying with this insecticide and mass releasing of *B. hebetor* in cotton fields.

Keywords- Side-effects, cotton, thiodicarb, *B. hebetor*, persistency.

I. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important and strategic crops that is cultivated in 69 countries of the world. China (25 percent), America (20 percent), India (16 percent), Pakistan (9 percent), Brazil (5 percent), Turkey (4 percent) and Uzbekistan (4 percent) are the largest producers of this product, respectively. are considered The area under cultivation and the amount of cotton production in the world are reported as 2.31 million hectares and 6.23 million tons, respectively, and it is predicted that by 2027, the area under cultivation will reach 1.33 million hectares. And its production amount will reach 28 million tons. The global average cotton yield is reported to be about 600 kg/ha, but the range of yield in different countries is very different and varies from about 1670 kg/ha in Australia to 350 kg/ha in India and African countries (Deguine et al., 2008; FAO, 2021).

The employment potential of cotton and its side industries is reported to be very high and about six times that of wheat. About 150 million people in more than 75 countries of the world work in sectors related to this product. In addition to providing the raw material for textile industries, cotton has many products that are used in the food, pharmaceutical and health industries. In 2019, the global value of cotton production was reported to be about 46 billion dollars and the value of its global trade was about 15 billion dollars. Most of the world's cotton is produced by small-scale farmers in developing countries and in small farms (with an area of less than one hectare). Despite all management measures, about 30% of the final cotton crop in the world is lost due to various factors, especially insects and weeds (OECD-FAO, 2016; FAO, 2021).

Cotton bollworm, *Helicoverpa armigera* (Hübner) is a ubiquitous pest whose activity has been reported from all continents (Deguine et al., 2008; Czepak and Albernaz, 2013). This pest is very polyphagous and about 200 plant species have been reported as its hosts, the most important of which are cotton, tomato, soybean, corn, tobacco and peas. Kurdish (EPPO, 2003). This pest is known as a key and dangerous pest in many parts of the world due to its omnivorousness, high mobility, multigenerational, nutritional preference on fruit (harvestable product) and resistance to most insecticides. (Cherry et al., 2003; Kumar et al., 2009), so that up to 60% yield reduction by this pest has been reported in Indian cotton fields (Mohapatra, 2006). The damage of this pest on other crops is also significant, so that up to 40% damage in chickpea fields and up to 55% damage in tomato fields have been reported (Riaz et al., 2021).

The complete insects of this pest have a very high flight and migration power, and their flight radius is reported to be up to two thousand kilometers. In addition, the reproductive power and reproduction rate of this insect is very high, and a female insect can lay up to a thousand eggs during her lifetime and if the environmental conditions are suitable. The incubation period of eggs (length of embryonic development period) is reported to be 3-4 days. This insect has a larval age of 5-6 and the larvae have a very high nutritional capacity and usually prefer reproductive organs (buds, flowers and fruits) for feeding. The pupae of this pest, which are formed in the soil, can enter voluntary diapause in the face of adverse environmental conditions. In most parts of the world, this pest completes between 4 and 6 generations per year on different crops, although in some tropical climates, up to 11 generations per year have been reported (Riaz et al., 2021).

Between 1960 and 1990, the use of non-selective pesticides was the main method of controlling cotton bollworm, and more than 20 sprayings were carried out against this pest in one crop season. Becoming resistant to pesticides, the destruction of the population of natural enemies and the outbreak of secondary pests and environmental problems caused by the excessive use of pesticides made important cotton-producing countries to control bollworms. turn to integrated management systems. In these systems, in order to reduce bollworm population below the economic threshold, a series of measures are performed outside the planting season and during the planting season (Wilson et al., 2018; Vyavhare et al. al., 2018).

With the implementation of integrated pest management programs, although the use of insecticides in cotton fields has decreased significantly, chemical control is still one of the main pillars of integrated management of cotton bollworm (Dhawan et al. ., 2009b; Wilson et al., 2018; Deguine et al., 2008;). Indoxacarb and thiodicarb insecticides are used in the chemical control of the larvae of butterflies, including the cotton bollworm, in different parts of the world (Brickle et al., 2001; Cordero et al., 2007; Aheer et al., 2009; Gadhiya et al., 2014; Sattar et al., 2017). In Iran, the effect of these two insecticides on cotton bollworm and other pests have been investigated and various results have been reported. Javan Moghaddam et al. (2002), Mohaghegh Neishaburi et al. (2009) and Heravi et al. (2012) have reported indoxacarb and thiodicarb as two effective insecticides against cotton bollworm larvae.

For the biological control of bollworm in the cotton fields of the world, various strategies have been used, including the protection of natural enemies, classical biological control, and mass breeding and release of natural enemies. Due to the fact that the cotton plant is one-year-old and the lack of permanent establishment of natural enemies in it, classical biological control has not been very effective, but the implementation of protection strategies in addition to mass breeding and release of natural enemies, especially the parasitoid bees Braconidae and Trichogrammatidae, have been successful in many cases. (Deguine et al., 2008).

The wasps of the Braconidae family have 18,000 described species, most of which play a valuable role in the biological control of pest lepidopterans (Yu et al., 2006; Ghimire and Phillips 2010). In most cases, the bees of this family have been used as a supplement along with the egg parasitoid bees, especially individuals of the Trichogramma genus, in order to control the cotton bollworm (Vyavhare et al., 2018). In the list of agricultural pests of Iran and their natural enemies, 64 species have been reported from different parts of Iran, and in the list of extinct species of Iran (Fallahzadeh and Saghaei, 2010), 200 species of bees have been reported from different parts of Iran. Among them, *B. hebetor* species has a wider geographical distribution and host range.

The wasp *B. hebetor* is better known compared to other species of Braconidae and has been reported as a parasitoid of *H. armigera* larvae on various crops, especially cotton and tomato, from different parts of the world (Uwais et al., 2006; Gimire and Phillips, 2010; Saxena et al., 2012). In Europe, since 1980, this bee has been commercially and mass-reared to control the insect pest (van Lenteren, 2008). According to valid taxonomic studies, placing this bee in the genus Habrobracon is not correct and this bee still belongs to the genus Bracon taxonomically (Gimire and Phillips, 2010, 2014). Currently, this bee is mass and commercially bred in Iran's insectariums and released in order to control the larvae of *H. armigera* in various crops such as cotton, soybeans and tomatoes (Rafiei Dastjardi et al. 2009 Najafi Navaei et al., 2002;).

II. MATERIALS AND METHODS

This research was carried out during the years 2020 and 2021 in the biological control and entomological research laboratories of the Department of Plant protection and the greenhouse of the Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources. The purpose of this research is to investigate the toxicity and persistency of thiodicarb insecticide, on cotton plants in order to determine the appropriate time to release the parasitoid wasp *B. hebetor* was in the cotton fields. Experiments began with rearing an alternative host, the Mediterranean flour moth, *Ephestia kuehniella Zeller*. Cultivation of alternative host in room conditions (temperature of 2±26 degrees Celsius and relative humidity of 10±50 percent) and main tests (measurement of toxicity and durability of insecticides) in a germinator with temperature conditions of 1±25 degrees Celsius, relative humidity of 5±60% and a photoperiod of 16 hours of light to 8 hours of darkness were carried out (Figure 1).

Figure1: Placement of cups containing sprayed cotton leaves, *B. hebetor* bee and Mediterranean flour moth larvae (alternative host) related to different treatments inside the germinator (orginal).

III. ALTERNATIVE HOST BREEDING

In this research, Mediterranean flour moth larvae were used as alternative hosts. Alternative host eggs were obtained from a private insectarium in Gorgan city to create the primary infection. In order to breed this month, first, according to Yazdanian et al.'s method (1379), 0.5 kg of wheat bran and 1.5 kg of wheat flour were placed in a plastic pan with an opening diameter of 35 cm and a height of 13 cm. meters were poured (the ratio of bran to wheat flour was 1 to 3 in each pan). After mixing flour and bran, Mediterranean flour moth eggs were sprinkled uniformly on its surface at the rate of approximately 0.2 grams of eggs per kilogram of food. The opening of the pans was blocked with a black cloth, and to maintain the humidity inside the pans, the black cloth at their opening was moistened with a hand spray containing water every day. The decontamination process was repeated every 10 to 14 days and 3 to 5 pans were prepared each time. The pans were placed in room conditions with a temperature of 26±2 degrees Celsius and a relative humidity of 10±50% (Figure 2). 45 to 60 days after the beginning of contamination of the pans and the larvae reached the fourth and fifth ages, the larvae were collected from the pans with the help of tweezers and given to the parasitoid bee as a host. In order to prepare moth eggs to continue the breeding process of the alternative host, the larvae were allowed to complete their development and become full insects in some pans. The removed moths were collected from contaminated pans daily with the help of a vacuum cleaner and placed inside a disposable plastic container with a lid with an opening diameter of 18 cm and a height of 11 cm. The opening of the containers was blocked with a net and then the containers were placed on a piece of A4 paper (Figure 3). The host moth eggs were collected daily from the surface of the paper and after being stored in the refrigerator (temperature of 5 degrees Celsius), They were gradually used to contaminate new pans.

IV. *B. HEBETOR* BEE BREEDING.

In order to breed and prepare the parasitoid bees needed in this research, in cooperation with a private insectarium in Gorgan city (Muhammadabad village), a number of breeding cabins were provided to this research. Each rearing cabin consisted of a wooden cage 90 cm long, 50 cm wide, and 100 cm high, which was covered with net fabric (Figure 4). Inside each cabin, there were two movable plates made of plastic mesh with a wooden frame, on which pieces of flour and bran infected with host larvae were placed. After the larvae of the Mediterranean flour moth reached the fourth to fifth larval stage (about 45 to 60 days after contamination of the pans), flour and bran inside them which were woven together with wire, were divided into smaller pieces and placed on the net plates inside the cabin (Figure 4). After the pieces of flour and bran were placed inside the rearing cabins, a suitable number of bees (around 500 per pan) were released into each cabin and its door was closed. After releasing the bees in the cabin, the bees started laying eggs on the bodies of the larvae, and 9-11 days after the release, a new generation of bees appeared, some of which were used in the main experiments. and another number of them were returned to the cycle of bee breeding in order to continue breeding and maintaining the colony.

Figure 2. Breeding of Mediterranean flour moth larvae on a mixture of flour and wheat bran in plastic pans (main).

Figure 3. The laying of eggs from the Mediterranean flour moth in order to re-contaminate pans containing flour and bran (main).

Figure 4. Placing flour and bran slices infected with alternative host larvae inside a wooden cabin for the purpose of breeding the parasitoid *B. hebetor* bee (main).

V. PLANTING COTTON PLANTS

Cotton seeds (Golestan variety, one of the common cultivars in Golestan province) were obtained from the Cotton Research Institute of the country and planted in March 2019 in plastic pots with a height of 25 cm and a diameter of 18 cm. The planting of the pots was done in several stages so that when spraying with each of the two insecticides, a sufficient number of plants of the same size would be available. In order for the plants to grow better, the pots were kept in greenhouse conditions for the first 45 days of planting and when the weather warmed, they were moved to the open space and exposed to natural sunlight (Figure 5). In order to protect the plants from rain and prevent the insecticide from washing off the plant, the pots were placed under a canopy on the days when there was a possibility of rain. After the height of the bushes reached about 30 cm, the bushes were sprayed with the desired insecticide using a manual sprayer. The sprayed leaves were separated from the plants at regular intervals and used in the relevant experiments.

Figure 5. Planting cotton plants in pots and spraying them with the desired insecticides (main).

VI. PREPARING INSECTICIDE SOLUTION AND SPRAYING COTTON PLANTS

In this study, the toxicity and persistence of thiodicarb insecticide, thiodicarb (DF 80%, with the brand name Larvin and manufactured by Henan Agrochemical Company, China) on *B.hebetor* bee and cotton plants were measured (Figure 6). According to the recommended dose/concentration of this insecticide (thiodicarb, one kilogram per hectare), according to the IOBC guidelines (Sterk *et al*., 1999; Hassan *et al*., 1991) and based on spraying 500 liters of solution per hectare, the solutions of this insecticide were prepared in concentrations of 500 ppm respectively and sprayed on cotton plants. According to the type of formulation of this insecticide, to prepare the thiodicarb solution in the desired concentration, first, the necessary amount of this insecticide is measured using a digital scale (ADAM®) with an accuracy of one ten-thousandth of a weight. And it was dissolved in one liter of distilled water and mixed completely using a magnetic stirrer to create a uniform solution of insecticide (Figure 5). The prepared solutions were sprayed on the cotton plants using a sprayer so that both the lower and upper surfaces of the leaves were completely covered with the insecticide and the insecticide solution on the leaves of the plant became liquid.

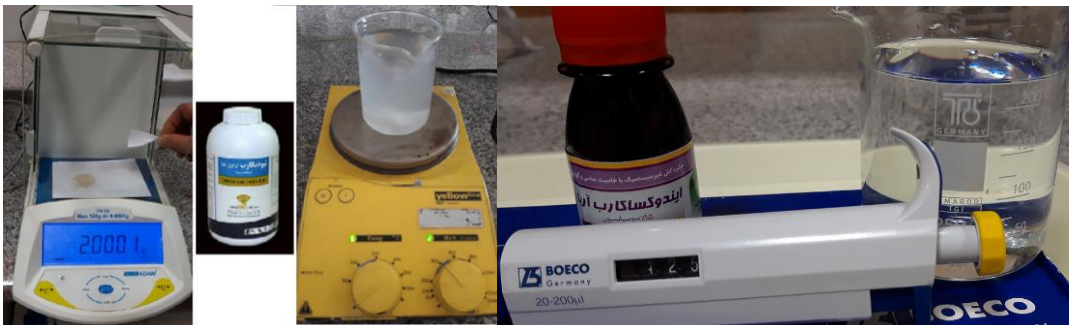

Figure 5. Insecticide used in the research (thiodicarb) and its solution preparation for spraying cotton plants (main).

VII. MEASURING THE TOXICITY AND PERSISTENCE OF THIODICARB INSECTICIDE ON COTTON PLANTS

In general, in this research, the lethal and sub lethal effects of the desired insecticide residues on the *B. hebetor* bee were measured on different days after spraying the cotton plants. For this purpose, at regular intervals one day after spraying the cotton plants, one to several leaves were separated from the sprayed plants (proportionate to the size of the leaves) and placed in a plastic cup. It was placed with an opening diameter of 10 cm and a height of 15 cm, so that about two-thirds of the bottom and the wall of the glass were covered with sprayed leaves. Unsprayed leaves were used in the control treatment. Then, inside each glass, 30 bees of one-day release females and the mouth of the glass are blocked with net fabric and elastic and inside the germinator (temperature 1±25 degrees Celsius, relative humidity 5 ±60 percent and photoperiod 16 The light hours were set to 8 hours of darkness (Figure 6). After 24 hours, the number of dead bees in each glass (repetition) was counted and their mortality percentage was calculated and recorded. This experiment was done in 5 repetitions (5 glasses each containing 30 one-day-old female bees). The work of calculating and recording the percentage of parasitoid bee mortality started one day after spraying the cotton bushes and continued until the insecticide residues were neutralized (bee deaths were below 25%). The trend of daily changes in the percentage of mortality of female bees after modification with Abbott's formula (Abbott, 1925) was drawn.

Figure 6. Placing sprayed cotton leaves in plastic cups and releasing parasitoid bees in them in order to measure the lethal and sublethal effects and the durability of the insecticide (main).

The method of measuring the sub lethal effects of insecticide residues on the bee *B. hebetor* was almost the same as the lethal effect. For this purpose, a large leaf or several smaller leaves were separated from the plants sprayed with the desired insecticide and placed in plastic cups with an opening diameter of 5.8 cm and a height of 12 cm, so that about two-thirds of the bottom and walls of the glass were covered with sprayed leaves.

Then a pair of one-day-old male and female bees were released inside each glass, and the mouth of the glass was blocked with lace fabric and elastic. The glasses were arranged on the bottom of a plastic tray and the trays containing the glasses were placed in the germinator. If the leaves in the glass rot (usually every 4-5 days), the leaves in the glass were replaced with a new leaf that had been sprayed for the same number of days. In order to feed the whole bee insects, a hole was made in the wall of the glasses and a piece of cotton dipped in 20% honey water was placed on the opening of this hole. In order to record the laying rate of female bees, 10 large larvae of the host were given to the bees in each glass on a piece of paper. After 24 hours, the old larvae of the host were removed from under the glass and replaced by new larvae. The number of paralyzed larvae and the number of eggs laid on them were counted daily and compared with the control. This work continued until the death of the male and female bees in the glasses.

VIII. STATISTICAL ANALYSIS

Comparing the toxicity of insecticides

Whole insects: The effect of the type of insecticide used on mortality, fertility (number of eggs laid during the life of a female bee) and paralyzing power of female bees (number of host larvae paralyzed by a female bee) in three different time periods after spraying (24, 48 and 72 hours) factorial (the first factor is the type of insecticide and the second factor is the time after spraying) and in the form of a completely randomized design with six treatment combinations, analysis Variance and mean comparison.

Comparison of the durability of insecticide on cotton plants

In this section, the durability of the desired insecticide residues on cotton plants was measured and compared. For this purpose, the lethal (percentage of mortality) and sub-lethal effects of the desired insecticide (reduction in the longevity of male and female bees and reduction in reproduction and paralyzing power of female bees) in different periods of time after spraying. As an experimental treatment) were measured and in the form of a completely random design, analysis of variance and mean comparison.

Rating of toxicity and persistency of thiodicarb insecticide

To rank the level of toxicity of thiodicarb insecticide from the combination of the International Organization for Biological Control (IOBC) standard in greenhouse conditions (Sterk et al., 1999) and the "total effect" index (Overmeer and van Zon, 1982; Moscardini) et al., 2013) was used. For this purpose, first, the percentage of mortality and reduction in reproduction of female bees after spraying the cotton plants with the desired insecticides were recorded. Then, based on the value of these two parameters and using the relation E=100-(M-100). R, the total effect of insecticide was calculated and the trend of changes in its value on different days after spraying was drawn. In this regard: M: mortality percentage of female bees after modification with Abbot's formula (1925) and R: reproduction ratio of female bees in the spraying treatment to reproduction in the control treatment. Based on the amount of total effect, insecticide were classified in terms of toxicity as follows: below 25%, harmless; between 25 and 50 percent, low damage; 51 to 75% is semi-harmful and more than 75% is harmful. Also, according to the standard of the International Organization for Biological Control (Sterk et al.,

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1999), in order to measure the persistence of the desired insecticide on cotton plants, the time interval between spraying the cotton plants and The damage of the insecticide to the parasitoid bee was measured (the total effect value reaching below 25%) and the insecticide were ranked in terms of persistence as follows: unstable for less than 5 days; 5 to 15 days short-lasting; 16 to 29 days semi-stable and 30 days or more, stable.

The software used

All statistical analyzes were performed using SAS software (SAS Institute, 2003) and LSD test was used to compare the averages and Excel 2013 software was used to plot the trend of daily changes in the values of all traits.

IX. RESULTS AND DISCUSSION

Comparison of the toxicity of tested insecticide on Adult bee of B. hebetor insect.

The results of variance analysis of the data showed that in the first three days after spraying, the type of effect of the insecticide and the duration after spraying, as well as their interaction (type of insecticide × duration after spraying) It was significant on the percentage of death of female bees (Table 1-4). Also, during this period of time, the reproduction of female bees (the total number of eggs laid during their longevity) and their paralyzing power (the number of paralyzed host larvae) are also significantly affected by the type of insecticide. But the effect of time after spraying and the interaction effect of type of insecticide × time after spraying were not significant (Tables 2-4 and 4-3).

Fatality due to contact with thiodicarb residues were significantly higher, in the first to third days after spraying with thiodicarb, 77.89%, 51.53% and 30.87% of bees died, respectively.

The negative effect of thiodicarb residues on reproduction and paralyzing power of female bees was also significantly higher, so that in the first to third days after spraying with thiodicarb, the reproduction of female bees, respectively 100, 91.2 and 87.4 percent and their paralyzing power decreased by 98.1, 82.5 and 79.2 percent respectively.

The total effect (total mortality and reproductive reduction) of thiodicarb residues in the first to third days after spraying is 100, 95.7 and 91.3% respectively and its total effect based on the power of paralysis (total casualties and reduction of paralyzing power) were calculated as 99.6%, 91.5% and 85.6%, respectively, and in both cases, according to the IOBC standard, they were classified in the group of harmful insecticides (level 4) (Table 5-4).

Table 1-4 Variance analysis of the data related to the effect of the type of insecticide and the duration of time after spraying (24, 48 and 72 hours) on the percentage of mortality of *B. hebetor* female bees.

Source of change	**Degree of freedom**	**sum of squares**	**mean of squares**	**F**
Insecticide type	1	9746/1	9746/1	**72/2
Duration after spraying	2	4852/7	2426/3	** 17/9
Duration after spraying× Insecticide type	2	1477/1	738/5	5/46**
Error	24	3246/6	135/3	
Total	29	19340/5		

*and ** are respectively significant at five and one percent probability levels,

Table 2-4 Analysis of variance of the data related to the effect of the type of insecticide and the duration of time after spraying (24, 48 and 72 hours) on the reproduction of *B. hebetor* female bees.

Source of change	**Degree of freedom**	**sum of squares**	**mean of squares**	**F**
Insecticide type	1	26334/1	26334/1	****42/2**
Duration after spraying	2	304/2	152/1	**0.26^{ns}**
Duration after spraying× Insecticide type	2	151/9	75/9	**0.13^{ns}**
Error	54	32197/9	596/3	
Total	59	58988/2		

** and ^{ns} are respectively significant and non-significant at the one percent probability level.

Table 3-4 Variance analysis of the data related to the effect of the type of insecticide and the duration of time after spraying (24, 48 and 72 hours) on the paralyzing power of *B. hebetor* female bees.

Source of change	Degree of freedom	sum of squares	mean of squares	F
Insecticide type	1	53103/8	53103/8	**44/6
Duration after spraying	2	411/2	205/6	0.17^{ns}
Duration after spraying× Insecticide type	2	1674/3	837/2	0.71^{ns}
Error	54	64332/9	1191/3	
Total	59	119522/2		

** and ^{ns} are respectively significant and non-significant at the one percent probability level.

Table 5-4: Toxicity ranking of the residues of thiodicarb insecticide, on the bee *B. hebetor* based on the IOBC standard and the total effect criterion.

Insecticide/ Duration time after spraying	Total effect (Mortality + fertility reduction)	Toxicity Ranking	Total effect (Mortality + reduction of paralyzing power)	Toxicity Ranking
Thiodicarb (24 hours)	100	4 (harmful)	99/6	4 (harmful)
Thiodicarb (48 hours)	95/7	4 (harmful)	91/5	4 (harmful)
Thiodicarb (72 hours)	91/3	4 (harmful)	85/6	4 (harmful)

X. THE INSECTICIDE PERSISTENCY ON COTTON LEAVES

Thiodicarb

Based on the results of analysis of variance, the mortality percentage of *B. hebetor* female bees on different days after spraying cotton plants with thiodicarb had a significant difference with each other at the probability level of 1% (Table 6- 4). The percentage of Mortality of female bees decreased in different days after spraying and reached from 77.9±5.8% on the first day to less than 1% on the 24th day. In the first seven days after spraying, the percentage of mortality was higher than 25% (the maximum acceptable mortality to consider the residues of an insecticide harmless according to the IOBC standard), but on the eighth day, the mortality of female bees was It reached below 25% (17.98±7.2%) and remained below 25% until the 24th day after spraying (Figure 1-4).

Based on the results of data variance analysis, reproduction (number of eggs laid during longevity) and power of paralysis (number of paralyzed host larvae during longevity) of female *B. hebetor* bees on different days after The spraying of cotton plants with thiodicarb had a significant difference with each other at the probability level of one percent (Tables 7-4 and 8-4, respectively). With the increase of time after spraying, the reproduction of female bees increased and reached from zero on the first day after spraying to 61.7±7.1 eggs/female bee on the 24th day after spraying. The pattern of changes in the power of paralysis of female bees was similar to reproduction and its value was from 1.8±0.9 host larvae/female bee on the first day after spraying to 87/9±1/03 host larvae/female bee arrived twenty-fourth days after spraying. (Figure 2-4). According to the value of these two parameters in the control treatment (respectively, 68.9 eggs/female bee and 94.3 host larvae/female bee), the reproduction of female bees compared to the control on the first day after spraying was 100% and after 24th days of spraying it decreased10.45%. In the same period of time, the reduction rate in the paralyzing power of female bees reached from 98.1% on the first day after spraying to 7.63% on the 24th day after spraying. In general, up to 22 days after spraying with thiodicarb, egg-laying power and paralysis of female bees decreased by more than 25% compared to the control. The trend of changes in the total effect of thiodicarb (percentage of losses + percentage reduction in reproduction) was similar to its effect on reproduction and paralysis, so that the numerical value of the total effect reached from 100% on the first day after spraying to 11.34% on the 24th days. In other words, until the first 22 days after spraying, the total effect of thiodicarb was higher than 25% and on the 23rd day it reached a harmless level (below 25%) (Figure 1-4). In short, the duration time of thiodicarb residues on cotton plants was measured based on the percentage of mortality, reduction in reproduction and the total effect on the parasitoid bee, 22, 7 and 22 days, respectively. And based on IOBC ranking and total effect index, this insecticide was ranked in 3rd level (Semi-permanent) In terms of longevity (Table 9-4). The longevity of *B. hebetor* female and male bees on different days after spraying cotton plants with

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thiodicarb had a significant difference with each other at the 1% probability level (Tables 10-4 and 11-4, respectively). The Changing Procedure in the longevity of female and male bees were similar to a large extent, from one day on the first day after spraying to 5.5±0.4 days (female parasitoid) and 1.2±0.13 days (male parasitoid). It arrived on the 24th day after spraying (Figure 3-4).

According to the longevity of female and male bees in the control treatment (2.3±0.3 and 7.6±0.94 days, respectively), the rate of reduction in the longevity of female bees is between 86.84% on the first day to the 27.6 % on the 24th day and the rate of decrease in the longevity of male bees fluctuated from 56.52% on the first day to 0% on the 21st day after spraying.

Table 6-4, analysis of variance of the data related to the mortality percentage of *B. hebetor* female bees after contact with thiodicarb residue on different days after spraying cotton plants.

SOURCE OF CHANGE	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN OF SQUARES	F
Treatment	23	52210/3	2270/01	****32/33**
Error	96	6741/1	70/2	
Total	119	58951/3		

** are respectively significant in probability, at one percent levels.

Table 7-4, analysis of variance of the data related to the Fertility percentage of *B. hebetor* female bees after contact with thiodicarb residue on different days after spraying cotton plants.

Source of change	**Degree of freedom**	**sum of squares**	**mean of squares**	**F**
Treatment	23	53309/9	2317/8	**3/39
Error	216	147753/1	684/04	
Total	239	201062/9		

** are respectively significant in probability, at one percent levels.

Table 8-4, analysis of variance of the data related to the paralyzing power of *B. hebetor* female bees after contact with thiodicarb residue on different days after spraying cotton plants.

Source of change	**Degree of freedom**	**sum of squares**	**mean of squares**	**F**
Treatment	23	53309/9	2317/8	**3/39
Error	216	147753/1	684/04	
Total	239	201062/9		

** are respectively significant in probability, at one percent levels.

Table 9-4: The persistency time (days) of the residues of thiodicarb insecticide used on cotton plants based on the type of their negative impact on the *B. hebetor* bee.

Type of negative effect				
Insecticides type	**Mortality percentage**	**Reduction Fertility**	**Total Effect**	**Ranking according to IOBC total effect**
Thiodiocarb	7	22	22	Semi-stable (group 3)

Table 10-4, analysis of variance of data related to the longevity of *B. hebetor* female bees after contact with thiodicarb residue on different days after spraying of cotton plants.

Source of change	**Degree of freedom**	**sum of squares**	**mean of squares**	**F**
Treatment	23	296/65	12/89	**4/66
Error	216	597/2	2/76	
Total	239	893/8		

** are respectively significant in probability, at one percent levels.

Table 11-4, analysis of variance of data related to the longevity of *B. hebetor* Male bees after contact with thiodicarb residue on different days after spraying of cotton plants.

Source of change	Degree of freedom	sum of squares	mean of squares	F
Treatment	23	46/5	2/02	**2/91
Error	216	150/3	0/695	
Total	239	196/8		

** are respectively significant in probability, at one percent levels.

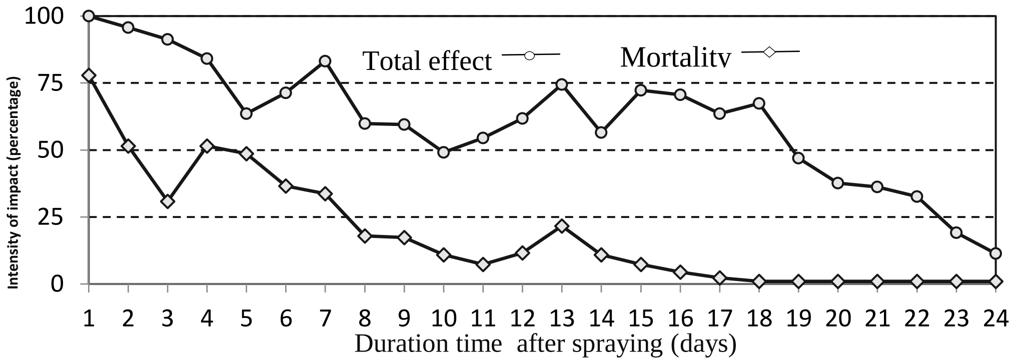

Figure 1-4: The process of changes in the mortality rate and the total Effect (mortality + reduction in fertility) of thiodicarb on *B. hebetor* female bees in different days after spraying cotton plants.

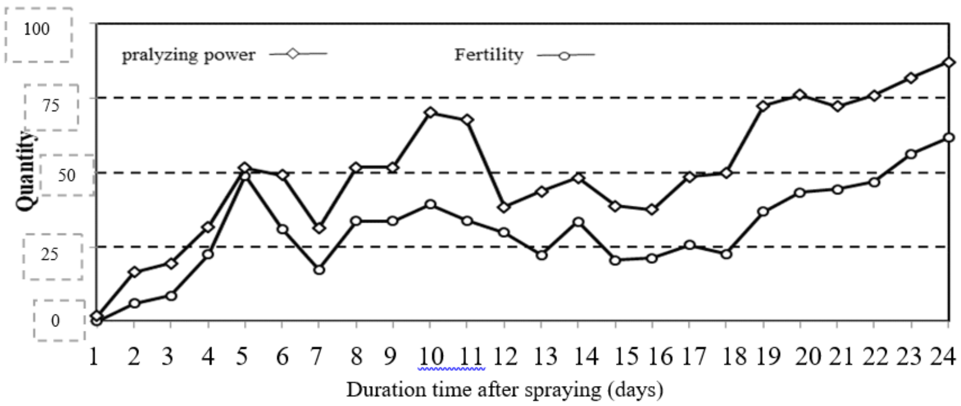

Figure 2-4: Changes in reproduction (number of eggs laid during longevity) and paralytic power (number of paralyzed host larvae during longevity) of female *B. hebetor* bees on different days after spraying the Cotton plant with thiodicarb.

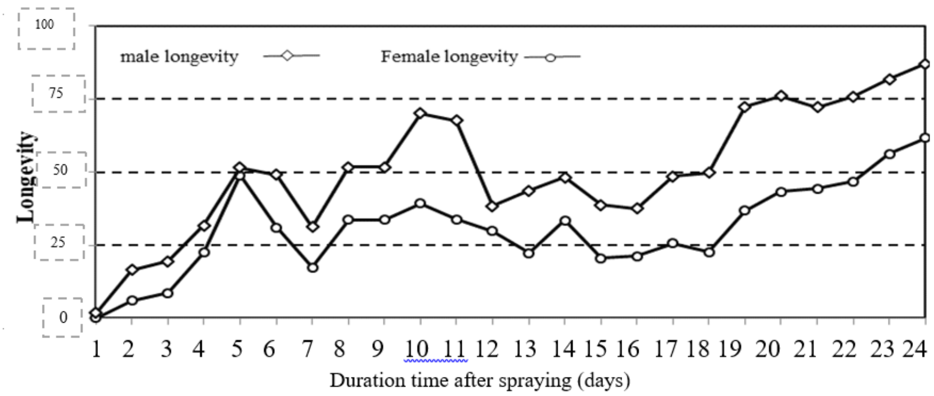

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Figure 3-4: Changes in longevity of male and female *B. hebetor* bees on different days after spraying cotton plants with thiodicarb insecticide.

XI. DISCUSSION

With Considering the importance of the parasitoid bee *B. hebetor* in the biological control of the cotton boll worm (*H. armigera*), awareness of the toxicity of the insecticides recommended against this pest in cotton fields and the removal of high-risk insecticides from the Spraying program, can help protect this parasitoid better against insecticides and increase their population. For this reason, the lethal and sub lethal effects of a number of common insecticides in cotton fields on this parasitoid bee have been investigated (Rafiee Dastjerdi et al., 2009 a,b; Khan et al., 2009; Mahdavi, 2013; Gharib, 2015). Of course, most of these researches have been conducted in laboratory conditions, and there are very few greenhouse studies (like the present research) and field studies in this field. In addition to the toxicity of insecticides, measuring the duration of their residues on cotton plants is also very important in terms of the timing of the release of the parasitoid bee *B. hebetor* in the fields. Knowing how long the insecticides last on the cotton plants allows a proper time interval to be observed between spraying the cotton fields and releasing this parasitoid bee, and this, in turn, Reducing the harmful effects of insecticides and making integrated management of this pest will help more efficient. In general, the persistence of an insecticide on a crop plant has been reported to be different depending on the type of plant, the environmental conditions after spraying (such as temperature changes and the occurrence or non-occurrence of rainfall) and the species of the target natural enemy. To discuss it, each of these three components should be considered (Narendra et al., 2015). Different factors such as the size of the crown of the plant, the angle of the leaves, the number and shape of the small structures on the leaf (such as trichomes, hairs, etc.). And the thickness of the epidermis layer in the leaves of plants may have an effect on the duration of persistence of insecticide residues on plants. Thiodicarb is a carbamate insecticide that, due to its high ovulation and larvicide capability, farmers tend to use it against boll worm in cotton fields (Joanmoghadam et al., 2008; Mohaghegh Neishaburi et al., 2009). Based on the results of the present study, the lethal (mortality percentage) and sub lethal (longevity reduction, parasitism and paralyzing power) effects of thiodicarb residues on cotton plants was strong against complete *B. hebetor* insects, so that its total effect (mortality + reduction in fertility) fluctuated between 87 and 100% in the first three days after spraying. And based on the IOBC classification, it was ranked among the harmful insecticides (group 4). Before conducting this research, there were several reports about the severe toxicity of thiodicarb on Complete insects and immature stages of parasitoid of *B. hebetor* (Khan et al., 2009; Rafiee Dastjerdi 2009 a,b; Mahdavi, 2013; Gharib, 2015) as well as several species of Trichogramma bees (Hassan et al., 1991; Consoli et al., 1998; Suh et al. ., 2000; Bastos et al., 2006; Afshari et al., 2017) was published in laboratory conditions. In the present study, according to the instructions of the International Organization for Biological Control (IOBC) about the need to measure the toxicity of pesticides on natural enemies step by step (Hassan et al., 1994; Sterk et al., 1999), The lethal and sublethal effects of this insecticide were measured in greenhouse conditions and on cotton plants, and its toxicity to the *B. hebetor* was also confirmed in these conditions. In line with the results of the present research, the extreme toxicity of the residues of this insecticide on the parasitoid *B. hebetor* bee on tomato plants (Khosrovabadi, 2019) and the *T. brassicae* bee on cotton plants (Nakunam et al., 2019) Reported under greenhouse conditions. Injection of venom and permanent killing or paralyzing of host larvae is an important behavioral characteristic of idiobiont parasitoids such as *B. hebetor* (Gimire, 2010). The results of this research showed for the first time that the contact of the female bees of this parasitoid with the residues of thiodicarb on cotton plants significantly reduced their paralyzing power. The only previous report published in this regard indicates the negative effect of thiodicarb residues on tomato plants on the paralyzing power of this parasitoid (Khosrovabadi, 2019). The summary of the results of this research is in line with the results of other published reports about the toxicity of thiodicarb (Khosroabadi, 2019; Nekonam et al., 2020) indicating the severe toxicity of this insecticide on two important parasitoids of the cotton boll worm in cotton fields. And it is necessary to review the chemical control programs with this insecticide. The results of the present research showed that the persistence of thiodicarb residues on cotton plants was long-term, so that until the first seven days after spraying, between 33.7 and 77.9 percent of female bees died daily. And until 22 days after spraying, their fertility and paralyzing power decreased significantly.

Before conducting this research, no report had been published regarding the duration of thiodicarb residues on cotton plants in terms of the effect on the *B. hebetor* bee. But the results of the research conducted on other parasitoids and host plants showed that the durability of the residues of this insecticide is different depending on the host plant species and the parasitoid species. Persistence of the residues of this insecticide on tomato plants in terms of causing mortality and reducing fertility in female *B. hebetor* bees 6 and more than 16 days were reported respectively (Khosroabadi, 2019). which is close to the results of the present study. On the other hand, the duration of the residue of this insecticide on cotton plants in terms of the lethal effect on the parasitoid *T. brassicae* bee is two weeks (Nakunam et al., 2020) and on the *T. chilonis* wasp on tomato plants. It has been reported ten days for *B. hebetor* (Narendra et al., 2015), which is longer than the seven days reported in the present study for *B. hebetor*. This difference may be due to the larger size of the *B. hebetor* compared to the bees of the genus Trichogramma, and as a result, it is more resistant to thiodicarb residues or the host plants are different .More toxicity of thiodicarb insecticide on *T. brassicae* (parasitoid of cotton bollworm eggs) than on *B.*

hebetor (parasitoid of cotton bollworm larvae) in the research of Afshari et al. (2018) and Gharib (2016). Also has been reported. Despite the fact that in the present study, the lethal effect of thiodicarb residues on *B. hebetor* reached a "harmless" level (below 25%) on the eighth day after spraying the cotton plants, but the sub lethal effects It continued for a longer time, so that until 22 days after spraying the cotton plants with thiodicarb, the reproduction and paralyzing power of the female bees was significantly reduced compared to the control, and the value of the "total effect" index was still higher than 25%. Based on the results of the present research, based on the "total effect" (mortality + reduction in reproduction) in the IOBC classification, with 22 days of persistence on cotton leaves, it classified as a "semi-persistent" insecticide. The Long-term durability persistence of thiodicarb residues and its main side product (methomyl) in the soil (24 and 30 days, respectively) has been confirmed using gas chromatography method (Chopra et al., 2013; Bisht et al., 2015). Also, it has been reported that the persistence of this insecticide on cotton plants is long-term in terms of its effect on pest insects, so that its residues on cotton can cause more than 50% losses in cotton bollworm population up to 27 days after spraying (Brevault et al., 2009) and up to 24.5 days after spraying. Causing losses in cotton mealybug population were *Phenacoccus solenopsis* (Dhawan et al., 2009a). In general, based on the results of the present study, the residues of thiodicarb, in addition to being highly toxic (lethal and sub lethal) to the parasitoid *B. hebetor* bee, had a long-term durability persistence on cotton plants, and for this reason, its use in Integrated bollworm management programs are not recommended. The severe toxicity of this insecticide on the most important egg parasitoid active in cotton fields, namely *T. brassicae* (Afshari et al., 2018; Nekonam et al., 2020) and the gradual resistance of cotton bollworm larvae to this insecticide (Ramasubramanian, and Regupathy, 2003; Moslinejad et al., 2016) are among other reasons that justify not recommending this insecticide in cotton fields.

XII. CONCLUSION

The results of this research showed that the residues of thiodicarb on cotton plants had more paralyzing power against *B. hebetor* bee. In addition, the residues of this insecticide had a strong negative effect on the longevity and reproduction of this parasitoid bee. And as a result, according to the standard of the International Organization for Biological Control (IOBC) And based on the sum of lethal and sub-lethal effects (total effect), it was classified as a harmful insecticide (group 4).

The tested insecticide in this study had differences in terms of longevity. Thiodicarb residues caused losses above 25% (acceptable level according to IOBC) in the parasitoid bee population up to seven days after spraying cotton plants and until 22 days after spraying, it reduced the reproduction of female bees by more than 25%, and according to the "total effect" index, it was classified in the group of "relatively stable" insecticides (group 3).

SUGGESTION

- Due to the extreme toxicity of thiodicarb on the parasitoid bee *B. hebetor* and the long-term persistence of its residues on cotton plants, it is recommended to remove this insecticide from the insecticides lists in order to protect this parasitoid bee in cotton fields.
- Measuring the toxicity and longevity of other insecticides used in cotton fields and other agricultural ecosystems should be considered as a joint and permanent program between the plant protection organization and research centers.
- In addition to the *B. hebetor* bee, the toxicity and persistence of insecticides on other natural enemies (especially predators) should also be measured.
- It is recommended that in measuring the toxicity and longevity of insecticides on *B. hebetor* and other natural enemies, in addition to the mortality factor, the sub-lethal effects such as the reduction of reproduction and paralyzing power of the parasitoid should also be considered.

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