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Some aspects of integrated management to control the pomegranate fruit worm *Ectomyelois ceratoniae* (Zell.) (Lepidoptera: Pyralidae).

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Abstract

The biological efficiency of the parasitoid *Apanteles plutellae* and the Insect Growth Inhibitor IGR Trigard 75 WP to control the pomegranate fruit worm *Ectomyelois ceratoniae*(Zell.) was studied. The duration of exposure of the host larvae positively affected the efficiency of the parasite and its biological performance with different exposure durations of 72, 48, and 24 hours. The rate of eggs laid by females also increased directly with increasing time period, as the percentage of larvae infected by the parasite reached 90% at the time period exposure for 72 hours. The number of parasite larvae and pupae produced reached 52.50 and 35.75, respectively, for the same period, while the percentage of infected host larvae was 35.75%. The number of parasite larvae and pupae was 12.00 and 8.0, respectively, at 24-hour exposure period. The IGR Trigard showed a clear effect in the second larval instar, as the rates of cumulative mortality in larvae increased, the duration of the larval instar increased, and the rates of pupation and adult emergence rates decreased with increasing concentrations of the IGR compared to low concentrations. The results showed a direct relationship between the concentration of the IGR Trigard rates with the larvae's mortality, as the highest cumulative mortality rate reached 85.00% after 12 days of treatment at a concentration of 5000ppm. The duration of the larval instar was 4.33 days, the pupation rate was 6.22% at the same concentration, and the emergence rate of adults was 0.00%. As for the effect of the IGR on the fourth larval instar, the highest cumulative mortality rate was 80.00% at the concentration of 5000ppm after 12 days of treatment, which differed significantly from the other treatments, as well as the control treatment, which recorded a mortality rate of 20%. As for the effect on the duration of the fourth larval instar, the results of the statistical analysis indicated that there were no significant differences between the concentrations, and the highest duration was 12.33days, the results of adult emergence were the lowest, 0.00% at the two concentrations of 4000 and 5000 ppm, compared to 90.00% in the control treatment.

Keywords: pomegranate fruit worm, Trigard, parasitoid, IPM.

Introduction

Agricultural development is one of the main pillars of comprehensive economic development in Iraq and the Arab countries, given the importance of the agricultural sector and its role in meeting the growing food needs of society, and providing inputs for many food industries. Pomegranate cultivation is one of these products because of its ability to achieve a surplus for export, and its economic importance is increasing pomegranate's nutritional value[1].Pomegranate cultivation faces many challenges, including the spread of the pomegranate fruit worm *Ectomyelois ceratoniae*, as it causes significant economic losses to the crop in quantity and quality, as the butterfly, at the beginning of fruit set, lays eggs on the fruit and upon hatching, the larvae work to penetrate the fruit and feed on it from the inside. The fruits rot due to of the entry of bacteria and fungi[2]. The pomegranate fruit worm is one of the most important agricultural pests that infect pomegranates. It is one of the economic insects that harm production and cause losses to farmers. The moth is spread in all parts of the world due to its transmission inside fruits and other food materials during shipment. It also infects pomegranates carob fruits, walnuts, almonds, chestnuts, dates, figs, apples, pears, quince, citrus fruits, figs and dried apricots. Parasitoids are an effective means of control because they attack the eggs or larvae of the host, thus preventing the emergence of adult insects and avoiding the expected loss of the crop, therefore controlling the pest without economic damage[3]. Due to the large economic losses resulting from this pest, the Integrated Pest Control (IPM) program was implemented to control the pomegranate fruit moth, focusing on biological control using the parasitoid *A. plutellae*, which is one of the most important parasitoids used in combating many insect pests. IGRs are a group of chemicals that inhibit basic physiological processes such as growth, development, moulting and reproduction in arthropods. Those working in the field of insect pests have begun using them as an alternative to chemical pesticides that cause great harm to the ecosystem. One of the IGR is Trigard 75 WP, which has been used in the control of the order Lepidoptera and Diptera showed a significant decrease in the hatching rates of tomato fruit worm eggs *H. armigera*[4].

Materials and Methods

Preparing the insect farm for the pomegranate fruit worm

The pomegranate moth was raised on an artificial diet based on wheat bran, yeast, sucrose, salt mixture, vitamin C, aureomycin, methylparaben, lysine, and glycerin, with distilled water. The rearing conditions were: 25±1C^{o}, photoperiod (15L:9D)h, and relative humidity 65±5%[5]. The artificial food was placed in three volumetric flasks with a capacity of 100 ml, then inside glass boxes with a capacity of 120x120x120 cm containing a side opening covered with a velvet cloth to prevent the exit of insects. Five pairs (5males + 5females) of newly emerged insects were released into each box to establish the colony, also placed pieces of medical cotton, pieces of pomegranate peels, and Petri dishes containing a quantity of moist soil for the larvae pupation.

Breeding of the parasitoid *A. plutellae*

Adults of the date moth *E. cautella* were raised inside glass cages with a capacity of 50 x 50x50 cm, inside of which were containers containing the artificial nutritional medium consisting of wheat grits 80% (2.250kg), dry yeast 1% (60g), molasses 6% (200g), and glycerol 14% (400g).

When the larvae were observed emerging for pupation, they were collected to be used to maintain the parasitoid population according to the method. [6] One pair (male x female) of newly emerged *A. plutellae*, 1-24 hours old, was placed in a 4x 25cm glass tube with 3 larvae of date moths (the last larval instar) and a small piece of cotton saturated with a 5% sugar solution was placed to feed the adults of the parasite. The tubes containing moth larvae infected by the female parasitoid were placed in an incubator at a temperature of 27± 1C^0 and a relative humidity of 50-65% until the parasitoid adults emerged.

Testing the efficiency of the parasitoid *A. plutellae* on larvae

The last instar larvae of the moth were used to test the efficiency of the parasitoid *A. plutellae* when it was released on the larvae over different periods of time (24, 48 and 72 hours). The larvae were placed inside glass tubes (3 x 22cm) and released in one pair (male x female) of the parasitoid at 1-24 hours of age, and the tube was closed with a piece of cotton saturated with a 5% sugar solution to feed the parasitoid adults. 3 groups were created, and each group had 5 replicates for each time period, in addition to the control treatment. Each group remained for the required period of time, and the parasite pair was withdrawn after the end of the period. All tubes were placed in an incubator with a temperature of 25±1C^0 and a relative humidity of 50-65%. The infected moth larvae were counted, and the parasitoid larvae feeding on the moth larvae were counted. After 6 days, they were isolated to calculate the number of parasitoid pupae and the number of parasitoid adults.

Prepare concentrations of IGR

Trigard 75% wettable powder is a systemic IGR in the form of a soluble concentrate. The active ingredient is Cyromazine at a concentration of 0.4 g/L. The growth inhibitor was obtained from the agent of Shanghai Bosman Industrial Co., Ltd. Baghdad branch. Four different concentrations were prepared (2000, 3000, 4000, and 5000ppm) by the dilution method according to the information, which depends on dissolving each weight of the pesticide in distilled water and completing the volume to a liter. For example, the concentration of 1500 is equivalent to 2g of the commercial preparation Cyromazine/L of distilled water[7].

Testing the effectiveness of IGR Trigard in the second and fourth larval instars:

To study the effect of growth inhibitor concentrations on the second and fourth larval instar of the insect, (30) larvae were taken from each colony stage and divided into five replicates. Each replicate contained 5 larvae in addition to the control treatment. They were placed inside Petri dishes containing filter paper and equipped

with 50g of food prepared for the instar incomplete. The dishes were sprayed with the required concentrations of IGR, amounting to 5ml. For the control treatment, they were sprayed with distilled water. The dishes were completely covered to prevent the larvae from emerging. The same experiment was repeated with the fourth-instar larvae. The growth of second and fourth-instar larvae treated with different pesticide concentrations was monitored until the emergence of adult insects. The cumulative percentage of destruction was recorded after (3,6,9,12) days of treatment, the mortality rates were corrected based on equation[8]. The results were analyzed using a completely randomized design (CRD)[9], and rates were compared according to Duncan's multinomial test at the probability level (0.05), in addition to using Chi-square to evaluate sex ratios.

% of pesticide effectiveness = 100(1-(no. of larvae in the control before treatment x no. of larvae in the treatment))/(no. of larvae in the comparison after treatment x no. of larvae before treatment). The percentages of inhibition of adult emergence were also calculated by calculating the corrected percentage of emergence inhibition[10].

Corrected percent inhibition of emergence(IE%) = 100-T/C ×100 (T = % of emergence in the treatment: C = % of emergence in the control.

Results and Discussion

The results of the study (Table1) indicated that the parasitoid *A. plutellae* increases its parasitic activity as the period of time available for exposure of the host larvae to the parasite increases, as the duration of exposure of the host larvae positively affected the efficiency of the parasite and its biological performance with different exposure durations (24, 48, 72 hours). As the rate of eggs laid by females also increased proportionally with increasing duration of time, as the percentage of larvae infected by the parasite reached 90% at an exposure period of 72 hours. The number of resulting larvae and pupae of the parasite reached 32.50 and 25.00, respectively, for the same period. In contrast, the percentage of larvae in the infected host was 35.75%, and the number of parasite larvae and pupae was 12.00 and 8.00, respectively, at an exposure period of 24 hours (Table1). The sex ratios avored males at exposure periods of 48 and 72 hours, but they were not significant when conducting the chi-square test. The results agreed with what was stated by[11] when he studied the parasitoid *B. hebetor*, that the rate of eggs, larvae, and pupae of the parasitoid increased with the increase in the time period available to it. At the same time, the sex ratio showed an increase in favor of the male parasitoid individuals over the females.

Table (1): Efficiency of the parasitoid *A. plutellae* and its biological performance on the larvae of the *E. ceratoniae* at different time periods.

Exposure duration /hour	Sex ratios ♀	Sex ratios ♂	Average No. parasite virgins ± standard error	No. parasitoid larvae * Average ± standard error	No. parasitoid eggs Average ± standard error*	% of infected host larvae Average ± standard *error
Contr.	*1:1		b 0.0±0.00	b 0.0±0.00	c 0.0±0.00	c 0.0±0.0
24	1: 1		b 0.0 ± 8.00	b 0.1±12.00	c 0.1 ± 20.0	c0.75±35.75
48	1: 1.5		a 1.03 ±18.75	b 1.32 ± 36.25	b 3.2 ± 45.5	b1.3±66.50
72	1: 1.25		a 0.48 ±25.00	a 0.65 ± 32.50	a 0.75 ± 80.4	a1.20±90.00

The rates or ratios marked with the same letters and in the same column do not differ significantly according to the Duncan multinomial test at the probability level (0.05). * The Chi-square test did not indicate any statistically significant differences.

Parasitoids can use a number of mechanisms to regulate the laying of eggs on the host, including the method of visual sensory sensing, such as compound and simple eyes, or feeling the host, or using jaws to insert them into the host's body. The reason for the difference in the acceptance of parasitoid females for different larval ages is also due to reasons that are that small larval ages may not show any defensive movements compared to older larval stages, which can show sudden reactions such as turning around and biting the intruder, or secreting various fluids that affect the behavior of the intruder[12].

The results of Tables(2,3) showed that the IGR Trigard had a clear effect in the insect's second and fourth larval instar, as the cumulative mortality rates in larvae increased and the duration of the larval stage decreased. The pupation rates and adult emergence rates also decreased with increasing concentrations of the IGR compared to low concentrations.

Table (2): Effect of the interaction between different concentrations of IGR Trigard on the second larval instar and the rates mortality of *E. ceratoniae*.

% corrected for emergence inhibition ± standard error*	% pupation ± standard error*	Average larval Stage duration/ day± standard error*	*% mortality after/day ± standard error				Concen. ppm
			12	9	6	3	
±89.00 a 0.75	1.3±82.30 a	1.20±12.10 b	±10.00 a 0.1	0.1±10.00 a	±0.00 a 0.0	±0.00 a 0.0	Contr.
1.44±67.20 b	±32.33 b 0.80	±8.33 a 1.03	±45.00 c 0.75	±40.00 b 0.65	±20.33 b 0.1	1.75±8.10 a	2000

±42.30 c 5.89	±20.00 c 1.70	b 0.48±6.66	±50.10 b 3.2	0.6±40.10 b	±30.10 b 1.03	3.3±12.00 b	3000
d1.44±4.00	±10.30 d 1.46	b1.3±5.66	±72.00 c 0.1	3.2±43.07 b	±35.00 c 0.75	±30.10 c 1.03	4000
d0.00±0.00	0.91±6.22 d	±4.33 c 0.75	±85.00 d 0.65	±59.21 d 1.32	±46.92 c 3.2	3.3±38.20 c	5000

The rates or ratios marked with the same letters and in the same column do not differ significantly according to Duncan's multinomial test at the probability level (0.05).

The results showed that there was a direct relationship between the concentration of IGR Trigard on the one hand and the rates of larval mortality (Table2), as the highest cumulative mortality rate reached 85.00% after 12 days of treatment at the concentration of 5000ppm, while the duration of the larval stage was 4.33 days. The pupation rate was 6.22% at with the same concentration, the adult emergence rate was 0.00%. As for low concentrations (4000ppm), the cumulative larval mortality rates were 45.00%, and the duration of the larval stage and pupation rates were 8.33 days and 32.33%, respectively, while the adult emergence rate was 67.20% at the same concentration. The reason for the death of larvae is that they reach a lethal concentration of growth inhibitor by feeding them on processed food. It also hinder the transition of larvae from one age to another.

Table (3): Effect of the interaction between different concentrations of IGR Trigard on the fourth larval instar and the rates mortality of *E. ceratoniae*.

% corrected for emergence inhibition ± standard error*	% pupation ± standard error*	Average larval Stage duration/ day± standard error*	% mortality after/day ± standard error*				Concen. ppm
			12	9	6	3	
4.40±90.00 a	±80.00 b 3.68	±15.75 c 0.91	±20.00 b 0.54	±20.00 b 0.54	±0.00 a 0.00	±0.00 a 0.00	Control
±26.66 b 2.39	±42.33 c 1.80	±12.33 a 3.18	±45.00 c 2.39	±40.00 a 12.24	±20.00 c 0.65	±8.85 b 3.68	2000
2.5±13.33 c	±20.00 c 0.54	±10.66 a 1.77	±49.00 c 0.80	±40.00 a 12.25	±30.00 c 4.40	±8.85 b 6.23	3000
0.00±0.00 d	±10.00 d 0.27	±9.00 a 12.24	±60.00 d 1.59	±43.07 a 6.32	±35.00 c 1.59	±17.70 c 4.0	4000
0.00±0.00 d	±0.00 d 0.00	±8.30 a 2.5	±80.00 d 3.18	±59.21 b 3.68	±46.92 b 1.74	±8.85 b 1.03	5000

The rates or ratios marked with the same letters and in the same column do not differ significantly according to the Duncan multinomial test at the probability level (0.05).

[13]mentioned that the IGR is characterized by its specificity to the larvae of the Lepidoptera order, as it stops the larvae's moulting and transition from one instar to another due to its effect on the process of chitin formation. As for the results of the fourth larval instar (Table3), the highest cumulative mortality rate was 80.00% at the concentration of 5000ppm after 12days of treatment, which differed significantly from the other treatments, as well as the control treatment, which recorded a mortality rate of 20%. As for effect on the duration of the fourth larval instar, the results of the analysis indicated statistically; there were no significant differences between the concentrations, and the highest duration was 12.33 days at the concentration of 2000ppm, while the control treatment differed significantly from the rest of the treatments and amounted to 15.75 days. As for the results of the percentages of adult emergence, the lowest was 0.00% at the two concentrations of 4000 and 5000ppm, compared to 90.00% in the control treatment. The speed of killing depends on the time between treatment and subsequent moulting, as the IGR also interferes with the production of chitin during the formation of the cuticle, causing a failure in its formation, which leads to the death of the larvae during the moult. It reaches its highest level when used early on larvae of the first ages. The results of the current study showed that the second larval instar is more affected by the IGR Trigard than the fourth larval instar due to the nature of the thin chitinous wall, which makes it easier for the inhibitor to affect the wall of those larvae.

IGRs combat many insect pests, individually or in combination with other methods. Trigard also affects the fertility of adult insects and reduces the hatching rates of resulting eggs. The growth regulator Trigard was also used to reduce the infection rate of bean plants with the black bean aphid *A. fabae*, as it inhibited the formation of chitin[14]. [15]also mentioned that the chitin formation inhibitor Applaud showed an increase in the rate of inhibition of emergence in house flies with increasing concentrations. The substance also has an effect after mating, as this effect is transmitted to the second generation. [16]indicated that the IGR Trigard hinders the process of formation of chitin in the ice of the whitefly's body, which prevents the moulting process as a result of the insect's inability to get rid of the old cuticle, and also affects the nutrition of the larvae as they refrain from feeding. [17]mentioned that when treating the first, third, and fifth larval instar of the beetle with the growth inhibitor Trigard at a concentration of 0.30 g/L, the mortality rates reached 100% for the first and third instar and 90% for the fifth larval instar. [18]Trigard has a good effect in controlling the black bean aphid, as the highest mortality rate reached 84.2% at a concentration of 0.5%. It was also mentioned [19]that the first larval instar of the hairy grain beetle (Khapra) is more sensitive than the fourth and fifth larval instars, when treated with Trigard, and that low concentrations caused a prolongation of the duration of the larval instar and a decrease in the pupation rates of the first instar larvae.

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