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ALTERNATIVE METHODS FOR CONTROLLING POMEGRANATE BUTTERFLY *Virachola livia* KLUG (LEPIDOPTERA LYCAENIDAE)

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ABSTRACT

Pomegranate is the most important fruit crop in Kalubeia gevernorate. The fruits are always attacked by the pomegranate butterfly, *Virachola livia* Klug, causing great loss to the famers. The pomegranate butterflies lay their eggs mostly on the lower half of the fruit body with more preference on north and east directions. In the present work three different methods were used for controlling this pest: First; (by bagging Fruits) as a mechanical method which resulted in 100% protection. The second; by testing neem extract and suspension of *Bacillus thuringiensis* which gave mor than 95% protection to the treated fruits. The third; by the release of the eggs parasite *Trichogramma brassicae* at Kalube, (kalubeia govenorate) for two seasons which resulted in reduction of the infestation from 31.3% in 2010 to 3% in 2011. The cost/benefit ratio estimated for the different control methods in this work indicated that spraying with the bacteria B.t was of the least cost in relation with yield production followed by releasing the egg parasite of *Tricogramme bressica*

INTRODUCTION

The pomegranate butterfly *Virachola livia* klug is the most important insect pest on the pomegranate *(Puinca gramatum)* in Kalubeia governorate. It lays its eggs singly on the sking of the fruits, almost at any stage of the fruit growth. The average number of eggs per one female is about 99 eggs (Awadallah, 1966). The newly hatched larvae burrows through the skin of pomegranate fruit and feed inside, discharging its excretion outside on which the fungus develops and eventually finds its way through the larval entrance hole, causing fermentation and decaying of the fruit seeds. The growing larva may migrate from one fruit to another that causes a great increase in the number of damaged fruits. Larvae may eat the fruit of *Eriobotrya japonica,* or the small date fruits (Larsen, 1980) In Egypt Awadallah, (1966) stated that, pomegranate butterfly attacks pomegranate fruits *Punica gramatum*, dates *phoenix dactylifera*, green pods of utna, *Acacia farnesiana,* and green pods of sunt, *Accua nilotica. Virachola livia* has been reported on pomegranate, dates, acacia and sidr *(Ziziphus* sp.) in Saudi Arabia (Abdel Salam, 1993). This insect pest has been recorded previously on *Acacia edgworthi* in Aden by Gough, (1913); *Lycopersicum esculentum* (tomato) in Plastine by Buxton, (1913); and it was recorded in Egypt on *Inga dulcis* by Andres, (1916) and on *Acacia mellifera, A. lasiopetala, A. modesta, A.Nubica, A. horrida, A. catechu, A. spadicigera, Cassia bicapularis, Dichrostachys mutans; Eriobotrya japonica, Prosopis piralis,* and *Punica vulgaris* by Hanna (1939). The present work had begun in May 2000 and continued till September 2002. The main objective was to find out the possibility of developing an IPM programme against. *V. livia* to control it and to

keep its damage below the economic level with the maximum safety for the environment, by using non- hazardous control methods.

The nature of eggs deposition and the infestation rate were studied under the conditions of Kalubeia governorate, protecting the fruits by bagging them as a cultural practices and spraying with non-toxic substances; such as; azadirachtin (neem extract); the use of the biological larvicide, *Bacillus thurngiensis,* was the third method of this work. The release of this bio agent was conducted during 2001 and 2002 seasons at Kalubeia.

The cost/ benefit ratio was estimated in this work to make clear comparisons helping for pest management decision.

MATERIALS AND METHODS

A. Bagging :

Natural extract: Azadiraction : Biological arvicide : *Becillens thuringiensis*, Egg parasitoid *Trichogramma brasiae*

Materials :

Determination of egg deposition on pomegranate fruits: Pomegranate trees in the farmer's orchards were inspected to study the deposition behavior by *V. livia* throughout the season to find out where exactly the females lay their eggs on the fruit body. This study was carried out from the time of the fruit setting till the harvest season. Inspection of pomegranate fruits in the field has been done for two seasons: from 30th May to 30th August 2001 and from 1st of June to 11th September 2011. A number of 100 fruits were randomly inspected at every two weeks through this study, using hand lens 5x to count the number of the eggs and describe the egg deposition site on the fruit then removed by finger. Seven sites on the fruit were considered, as shown in Fig, 1, as; 1 = apex 2 = upper half, 3 = lower half, 4 = bottom, 5 = calyx, 6 = calyx edge and 7 = intra-calyx. The number of eggs and the entrance holes made by the larvae were recorded on each sits. The direction of the infested fruit location on the tree; north (N0), east (E), south (S) and west (W) was recorded as well.

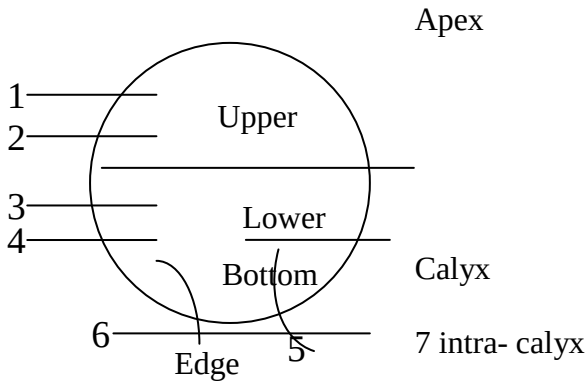

Fig. 1: Inspection of laid eggs on the pomegranate fruits to describe the sitecs egg deposition by *V. livia* females.

B. Fruit protection trials

These trails were conducted for the two successive seasons of 2000 and 2001. three different treatment were applied in this trial as follow:

1. Cultureal practice (mechanical control)

Bagging of the fruit was applied using insect-proff material (white perforated polythen sheet) to make bags (25 x 25 cm). this material (Insectennet) described by the manufacturer as insects repel net, high light transparency, maximum air transmission and long durability due to maximum UV., addititives, Each fruit was enclosed in a bage, then the top was firmly closed to keep the fruit inside the bag and to prevent the butterfly from touching the fruit. At the time of bagging, the fruit was inspected carefully to be sure that there were no eggs of the pest on the fruit, and removed them by finger. Bagging was done just after fruit setting and kept till the harvest.

2. Spray with naturally-originated substance :

Spraying with azadirachtin (Neem extract), which is of a botanical origin, is extracted from the seeds of neem tree, *A zadiracta indica*, is safer for the environment than the conventional insecticides. Azadirachtin is used as anti-feedant against a wide range of insect pests in different parts of the world. The used trade formulation in this trial was Neemosan (80% azadiractin) Produced by (Petrolium R.P.Co.) at the rate of 5 ml/liter of water.

3. Spry with biological larvicide

Bacillus thusringiensis suspension (BT) was used in the field trial to control the larvae of *V.livia.* This material consists of both the spores and toxicant of the bacteria *B, thuringiensis* iun *korstaki* (32000 international units of potency per milligram). It is a specific bacteria against the larval stage of Lepidopteran insecte. The formulation Diple 2X is produced by a company in Bon City, Germany.

The spray with NE and BT were applied 4 times at two weeks interval. In season 2010, the applications were carried out on 15, 27 July, 11, and 31 August. The trial was repeated for confirmation in 2011 on 7, 19 July, 8 and 23 August. These trials were designed with four replications, and the experimental until was one mature tree. An untreated tree was kept in each block for comparison. In each treatment the fruits were labeled and three fruits were selected from each direction (N, E, S, W) in each tree (12 fruit total in each tree). The sprays were directed to the labeled fruits by using hand sprayer 1 (1.25) capacity per-pressurized type, wetting agent: Citowett was added at the rate of 0.25 ml/L, to improve the contract and adherence of the compounds on the skin of the fruits.

Rate of infestation, fruit characters (weight total soluble solids [TSS] and acidity) were evaluated at the harvest time. Four fruits (representing the treated fruits) from each tree were selected and their juice was extracted. TSS was determined using refactometer (0- 50%). Acidity was determind by Titration 5 ml of the fruit juce with NaOH (0.1 Normal) and phenolphthalein (0.25 % in 50 percent ethanol) as indicator to determine the acidity of the fruits. Total acidity was calculated according to the following formal: % Acidity as citric acid (mg/L) = (vol. of NaOH x 100) I5)

C. Release of *Trichogramma brassicae ;*

The general egg of *V.livia* in Amar area (containing about 4485 pomegranate trees). Where number of 500.000 parasitoid individuals were released from 3rd to 10th of July 2001, and one million were released in the same orchards from 12 June to 10 July 2002. The parasitism on the butterfly's eggs and infestation rate were estimated.

RESULTS AND DISCUSSION

A. Egg deposition and infestation;

In the season 2001, the largest number of eggs were recorded on the last week of June (42 eggs/100 fruits). The egg-laying was continued till the second week of August but no egg was detected in the there of ter (Fig. 2). The majority of the eggs were laid during June where 70% of the eggs were recorded from the end of May till the end of June, (21, 24 and 42 eggs at 30th May, 15th June and 29th June, respectively). Tow and three eggs on one fruit were recorded on 18.5% and 2.5% fruits, respectively. The enterance holes made by young larvae were visible on the fruits from 15th June in the season 2001 and their numbers were gradually increased. On the first week of July, the first peak of hole numbers was recorded. Infested fruits that having 2, 3 and 4 holes formed 25, 5.6 and 0.9% , respectively.

شكل

Fig. 2: Number of eggs and enterance holes of *V.livia* on the pomegranate fruits during the activity seson of 2010 at Kalube

In the following season, the highest number of eggs was recorded during June 2011 where 19 eggs were recorded at 4th and 18th June, respectively, 52.8% of the total number of recorded eggs throughout the season (Fig. 3). No eggs were noticed after August 2011. On 4th June. The highest number of holes was recorded, but only one hole was recorded at 18 June. This could owe to spread of the egg parasitoid *T. brassicae* that was released in Kalube on 12th June 2011 in the farmer's orchards. It was found that in 9.7%, 1.8% and 0.9% of the infested fruits a number of 2.3 and 4 eggs were recoded, respectively. Also about 5.9% of the infested fruits were having 2 to 3 holes.

These results show that June is the critical period for the infestation with *V.livia* on pomegranate fruits and it is the proper time to apply the control measures against this pest.

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Fig. 3: Number of eggs and enternace holes of *V. livia* on the pomegranate fruits during the activity season of 2011 at Kalube

Deposition sites:

The statistical analysis proved significant differences between the numbers of eggs laid on the different sites of the fruit in the season of 2010. the maximum egg-laying (33.7%) was recorded on the fruit bottom followd by the lower half (23.7%). The least egg-laying was recorded on the calyx edge (Table. 1). The numbers of holes on the different sites were significantly different. The maximum searching of larval entrance holes were mainly recorded on lower helfs and bottoms. No holes were recorded on the calyx edges because they are borned from skin only with no flesh underneath. In the season 2011, the highest percentage of eggs were recorded on the bottom fruit, followed by on sites top half and lower half, respectively, and no egg was found on the calyx edge (Table 2).

Table 1: Relative percentage of eggs laid by *V.livia* and the numbers of larval holes on the difference sites on the pomegranate fruit throughout the activity season 2010 at Kalube

	Site of ovipostion	Percentage	
No	**Site on the fruit**	**egg-laying**	**Holes numbers**
1-	**Apex**	3.4 ± 0.6 e	0.5 ± 0.4 b
2-	**Top half**	14.5 ± 2.7 bc	13.9 ± 5.1 b
3-	**Lower half**	23.7 ± 7.6 ab	45.1 ± 11.5 a
4-	**Bottom**	33.7 ± 6.3 a	39.5 ± 7.6 a
5-	**Calyx**	3.9 ± 1.4 e	0.5 ± 0.4 b
6-	**Edge of calyx**	2.2 ± 0.8 c	0.0 ± 0.0 b
7-	**Inside calyx**	19.1 ± 3.5 ab	0.5 ± 0.4 b

Means in column not followed by the same letter are significantly different (Duncan's multiple test, p ≤ 0.05)

Table 2: Relative percentage of eggs laid by *V.livia* and the numbers of larval holes on the difference sites on the pomegranate fruit throughout the activity season 2011 at Kalube

No	Site of oviposition on the fruit	Percentage	
		egg-laying	Holes numbers
1-	Apex	5.4 ± 2.0 e	1.6 ± 0.4 b
2-	Top half	20.9 ± 4.2 bc	8.1 ± 1.6 b
3-	Lower half	19.6 ± 3.2 ab	38.71 ± 3.0 a
4-	Bottom	30.5 ± 7.7 a	45.4 ± 4.9 a
5-	Calyx	13.5 ± 4.3 e	6.2 ± 1.6 b
6-	Edge of calyx	0.0 ± 0.0 c	0.0 ± 0.0 b
7-	Inside calyx	10.1 ± 0.8 ab	0.0 ± 0.0 b

Means in column not followed by the same letter are significantly different (Duncan's multiple test, $p \leq 0.05$)

Statistical analysis confirmed the significance of the difference between them. The percentages of the hole numbers on sites 3 and 4 were close and both are significantly different from the other sites. Such results indicate that fruit bottom is the most preferable for egg deposition by the female butterfly as mall as for the larvae to bore the entrance hole. Awadalla, (1966) stated that the deposition site differs according to size of the fruit. At early stages of fruit growth the eggs are often laid inside and around the calyx. In more developed fruits, the eggs are preferably deposited on the fruit surface rather than the calyx. In the present work, the number of eggs founded on both the calyx and the lower half or the bottom on 4th June 2011. Scarcely, the butterfly lays eggs on the leaves that in touch with the fruits.

Direction of Oviposition site :

The direction of the infested pomegranate fruit was taken in consideration; since there were significant difference between the numbers of eggs laid on the different directions. In season 2001, the highest number of eggs were recorded on the fruits in the north and the east dirctions of the trees (Table 3) where 34.7% and 31.2 of the total number of eggs were recorded. The least number of eggs on the fruits of the western direction (18.6%). The same trend was observed in the case of holes where 33.5%, 31.5%, 17.% and 18% of the holes made by the larvae, were recorded on the north, east, south and west directions, respectively. The same behaviour was founded in the season 2011 where the percentages of 37.3%, 35.2%, 11% and 16.5% of the eggs were recorded in north, east, south and west directions respectively. In case of entrance holes the percentages of 35.6%, 32.2%, 15.5% and 16.7% were recorded in north, east, south and west directions, respectively. These results pointed to the superiorty of the north and south directions in harboring most of deposited eggs and larval entrance holes. This may be refer to the fact that the north and east directions are less exposed to sunlight. The estimation of the percentage of the infested fruits that having eggs and holes in the four directions are illustrated where most of these fruits located in north and east directions.

Table 3: The relative percentage of numbers of eggs and holes found on different direction on pomegranate tree at Kalube in the seasons 2010 and 2011.

	Percentage			
	Egges		Holes	
Direction	2001	2002	2001	2002
North	34.7 ± 7.2 a	37.3 ± 9.2 a	33.5 ± 4.6 a	35.6 ± 3.6 a
East	31.2 ±11.9 a	35.2 ± 7.9 a	31.5 ± 8.1 a	32.2 ± 6.5 A
South	15.5 ± 6.1 b	11.0 ± 5.3 b	17.0 ± 5.6 b	15.5 ± 1.5 b
Weast	18.6 ± 4.3 b	16.5 ± 7.1 b	18.0 ± 4.2 b	16.7 ± 3.2 b

Means in column not followed by the same letter are significantly different (Duncan''s multiple range test, P ≤ 0.05).

B. Fruits protection trials :

Field trial of 2010 :

The main objective of these trails is to reduce the economic damage on the yield with the maximum safety to environment. The bagging of pomegranate fruits gave complete protection against the pomegranate butterfly where no infestation was recoded (Table 4), this was followed by lower infestations in NE treatment (2.1%) and BT treatment (4.2%) when compared with the untrated plots (31.3%). The statistical analysis showed significant differences between the treated and untreated polts. No effects on the fruit characters or weight were observed. Tss and total acidity of the juice of the fruits were analysed in all plots. Traces of sooty mould were observed on 97.1% of the fruits in case of bagging treatment indicating previous aphid infestation. The percentage was 2.1% in BT treatment and 100% in the NE treatment.

Table 4: The effect of different treatments against *V.livia,* on the fruit weight, Tss and acidity in fruit juce in the seasons 2010.

Treatment	Infestation %	Fruit weight (g)	TSS	Acidity me/1
Bagging	0.0 b	335.0 a	14 a	61.0 a
BT	4.2 b	327.5 a	13 a	61.0 a
NE	2.1 b	343.9 a	14 a	61.0 a
Control	31.3 a	332.6 a	14 a	61.5 a

BT = *bacillus thruingiesis* *NE-Neem extract*

Means in column not followed by the same latter are significantly different (Duncan's multiple range test, P ≤ 0.05).

Field trail of 2011 :

The repeated trial gave the same indication, where the infestations were 0%, 2.1 % and 2.1% in bagging, Bt and NE plots, respectively. They were significantly different from the control where 18.8% were infested with *V. livia* (Table 5). Also, there was no effect on the fruit charactes. Sooty mould was found on 35.4% of the bagging fruits only.

Bagging provides a very effective protection for the pomegranate fruits. This result conforms the finding of Sukla and Prasad (1983). The disadvantage of this methods is the high percentage of the bagged fruits

having traces of sooty mould on the skin and aphid's eluvia. If aphids succeeded to penetrate inside the bags they colonize the fruits and contaminate them with their wastes, honeydew and their exuvia since they will be protected from their natural enemies. The way by which the aphids enter the bags is not clear, but it is possible that the first nymphal instar of the aphids could be perpetrated to the fruit or inside the calys and it is not easy to be detected during bagging.

Table 5: Infestation with *V. livia,* fruit weight, Tss and acidity in fruit juice in different treatments, and the impact on fruit characters during 2010 trial

Treatment	Butterfly Infestation %	Fruit weight (g)	TSS	Acidity Mel/1
Bagging	0.0 b	34.1 a	14 a	64.8 a
BT	2.1 b	328.6 a	15 a	68.0 a
NE	2.1 b	332.8 a	14	67.3 a
Untreated cheek	18.8 a	329.5 a	15 a	63.5 a

BT = Bacillus thuringiesis **NE= Neem extract**

Means in column not followed by the same latter are significantly different (Duncan's multiple range test, P $\leq$ 0.05).

C. Releasing the egg parasitoid *Trichogramma brassicae:*

T. brassicae was released at Kalube during two successive seasons of 2010 and 2011. They were distributed in the famer orchards. In 2010 the first release was on 3rd July (250.000 parasitoid individuals) and these the second (250.000) was released on 10th July. In the following season, the release (500.000) was carried out earlier on 12th June 2011 and the second release (500.000) on July. At the harvest season in 2001 the rate of infestation was 6.6% compared with 13.3% in 2010 (Table 6). In Amar area, the infestation estimated 25.5% in 2010. In the second release of *T. brassicae,* where (500.000) where on 12th June and another 500.000 were released on 10th July 2011. the parasitism rate on the eggs of *V.livia* estimated 84.4% and 82.1% on the 5th and 24th July 2011, respectively, in the farmers' orchards.

Table 6: Infestation with *V. livia,* fruit weight, Tss and acidity in fruit juice in different treatments, and the impact on fruit characters during 2010 trial

Season	Date of release	No of released	Date of Inspection	Rate of Parasitized eggs	Rate of Infestation at harves(%)	Infestation at the preveious season (%)
2010	0.3th July	250.000	-	-	6.60%	31.10%
	10th - July	250.000	-	-		
2011	12th - Jun	500.000	05th – July	84.40 %	3.00 %	6.60 %
	10th - Jun	500.000	24th - July	82.10 %		

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الطرق البديلة لمكافحة فراشة ثمار الرمان

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تعتبر فراشة ثمار الرمان من أهم الآفات التى تهاجم ثمار الرمان وتسبب خسائر كبيرة للمزارعين.

ومن دراسة سلوك الحشرة فى وضع البيض وجد أنها تضع أكثر بيضها على النصف السفلى من الثمرة كذلك فإنها تفضل الثمار التى فى الجهة الشمالية والشرقية وقد تم استخدام ثلاثة طرق مختلفة لمكافحة الآفة: الأولى باستخدام التكييس كمكافحة ميكانيكية والذى أعطى حماية بنسبة 100%. الثانية باستخدام مواد آمنة مثل مستخلص النيم (من أصلى نباتى) ومعلق بكتريا الباسيلس حيث أعطت هذه أكثر من 95% حماية للثمار. وفى الطريقة الثالثة من هذا العمل تم إطلاق طفيل البيض *Brassicae Trichogramma* لمدة موسمين بقليوب بالقليوبية حيث أدى إلى انخفاض الإصابة بهذه المنطقة من 31.3% فى موسم 2000 إلى 3% فقط فى موسم 2002.

وقد تم حساب نسبة التكلفة إلى الفائدة لكل وسيلة من الوسائل المستخدمة فى هذا العمل التى أوضحت أن المعاملة ببكتريا الباسيلس كانت أقلها تكلفة بالنسبة لما يترتب عليها من زيادة فى الإنتاج يليها إطلاق الطفيل ثم التكييس.

قام بتحكيم البحث

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