Journal of Phytopathology and Pest Management 2(3): 32-42, 2015
pISSN:2356-8577 eISSN: 2356-6507
Journal homepage: http://ppmj.net/

Efficacy of emamectin benzoate, pyridalyl and methoxyfenozide on pomegranate butterfly, *Virachola livia* (Klug) (Lepidoptera: Lycanidae) in cultivated and reclaimed lands

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Abstract

The pomegranate butterfly, *Virachola livia* (Klug) (Lepidoptera: Lycanidae), is one of the most important pests in Egypt. Under pomegranate field conditions, the study was conducted from 2012 to 2013 to compare the efficacy of methoxyfenozide, pyridalyl and emamectin benzoate against pomegranate butterfly in cultivated and reclaimed lands at Assiut Governorate, Egypt. These insecticides demonstrated a significant low infestation of the pomegranate butterfly during 2012 and 2013 seasons compared to control field. Emamectin benzoate and pyridalyl were found to be highly effective for controlling *V. livia* with an average infestation of 0.33- 4.33% and 1.00- 6.67% compared to methoxyfenozide (0.33- 17.67%) in both cultivated and reclaimed lands, respectively. Data indicated that, the infestation was higher in the cultivated land than reclaimed land during 2012 and 2013 seasons. The change in temperature and relative humidity may affect the susceptibility of pomegranate fruits to the infestation by *V. livia*. In addition, results showed that all pomegranate trees under treated field had a significant increase in the average weight fruits by about 70-90% compared to control field. Methoxyfenozide, pyridalyl and emamectin benzoate can be considered as promising candidates to control the pomegranate butterfly, *V. livia* infestation in both cultivated and reclaimed lands.

Key words: *Virachola livia, Punica granatum*, pyridalyl, methoxyfenozide, emamectin benzoate, pest management

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Introduction

Pomegranate tree (*Punica granatum* L., Punicaceae) is one of the most important cash fruits at Assiut Governorate, Egypt. In 2010, the total area of pomegranate in Egypt was about 4238 thousand ha “76% of this area located in Assiut Governorate” and the production was around 52 thousand tons of which 91% was produced by Assiut Governorate. The pomegranate trees are attacked by several insect species, which decrease the quality and quantity of its product. The pomegranate butterfly, *Virachola livia* (Klug) (Lepidoptera: Lycanidae) is the most important insect pest attacking pomegranate fruits and date palm in Egypt (Sayed & Temarek, 2007). Current management programs depend on chemical and biological insecticides as well as beneficial insects. Recently several insecticides, (e. g. spinosad and methoxyfenozid), were ineffective at managing pomegranate butterfly population in date palm fruits in the New Valley area in Egypt (Sayed & Temarek, 2007). Methoxyfenozide [N-tert-butyl-N’-(3-methoxy-o-toluoyl)-3,5-xylohydrazide] is the newest and most efficacious member of the diacylhydrazine class (Le et al.,1996; Carlson et al., 2001) which acts as an ecdysone agonist. It is highly effective against lepidopteran pests, such as beet armyworm, *Spodoptera exigua* and codling moth *Laspeyresia pomonella*, and selective toward beneficial organisms (Schneider et al., 2003, 2004; Pineda et al., 2009). Methoxyfenozide is used and recommended for insect control on pome fruits, date palm and cotton (Sayed & Temarek, 2007; Talebi-Jahromi, 2007). Pyridalyl [2,6-dichloro-4-(3,3-dichloroallyloxy) phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy] propyl ether] is a novel synthetic unclassified insecticide not related to existing compounds (Sakamoto et al., 2003; Saito et al., 2004). Pyridalyl has contact and ingestion toxic effects and is effective for controlling lepidopteran and thysanopteran pests (Shigeru et al., 2004; Isayama et al., 2005; Abdel-Rahim, 2011; Gad et al., 2013). Emamectin benzoate [(4’’R)4’’-deoxy-4’’-(methylamino) avermectin B1 benzoate] is also a novel macrocyclic lactone insecticide derived from the avermectin family and similar to abamectin. It affects the nervous system of arthropods by increasing chlorideion flux at the neuromuscular junction, resulting in cessation of feeding and irreversible paralysis (Ishaaya et al., 2002). It is highly effective against lepidopteran pests such as *Plutella xylostella* (L), *Trichoplusia ni* (Hübner), *Heliothis virescens* (F), *Spodoptera exigua* (Hübner) and *Spodoptera littoralis* (Boisduval). The objective of the present study is to determine the efficacy of methoxyfenozide, pyridalyl and emamectin benzoate against native infestations of pomegranate fruit butterfly, *V. livia*, in cultivated and reclaimed lands.

Materials and methods

Experimental field sites: The current study was conducted in two pomegranate fields during the years 2012 and 2013. The cultivated and reclaimed lands were localized at Assiut Governorate, Egypt. The cultivated land is the experimental farm of the Faculty of Agriculture, Assiut University. The reclaimed land is the farm of Arab El-Awamer, which is located 25 Km northeast of Assiut city, in the eastern desert. Both orchards were cultivated in 2005 and have 7-year old 'Manfalouty' cultivar pomegranate trees spaced by 5 × 5 m. Irrigation is performed by a drip irrigation. The trees of the orchards were of uniform vigour and size.

Infestation and susceptibility of pomegranate cultivar 'Manfalouty' to *V. livia*: The pomegranate infestation was initiated after fruit-set on May 23 and terminated in October (at harvest) in cultivated and reclaimed lands. Ten sampling dates at two week's intervals were taken during the study time. Twelve trees were used per each sampling date for each insecticide and from each tree 20 fruits were randomly selected and examined for *V. livia* infestation. The susceptibility of pomegranate cultivar 'Manfalouty' to *V. livia* was estimated by harvesting all fruits of each tree at the end of the 2012 and 2013 seasons. The infestation rate of fruits was calculated by dividing the number of infested fruits by the total fruit numbers for each tree separately.

Impact of physical factors on *V. livia* infestation: The data of different physical factors (e. g. temperature and relative humidity) obtained from the meteorological observatory of Faculty of Agriculture, Assiut University, Assiut, Egypt were subjected to a standard statistical analysis with the infestation rate to determine the effect of these factors on *V. livia* infestation under pomegranate field conditions in both cultivated and reclaimed lands.

Insecticides: Three pesticides were tested (trade name, formulation type and percentage of active ingredient and application ratio): pyridalyl (Peillio, 50% EC, 0.2 mlL^{-1}), methoxyfenozide (Runer 24% SC, 0.5 mlL^{-1}) and emamectin benzoate (Emperior, 0.5 % EC, 0.8 mlL^{-1}) (Fig. 1). The experimental area in the different locations was divided into plots of 5 × 5 m, each includes four trees. Tested insecticides were distributed in a randomized complete block design (RCBD) in three treated replications (12 trees for each insecticide) and control (12 trees). The knapsack sprayer with one nozzle covering 20 liter per replicate was used in the application. Four sprays were carried out in the 2012 and 2013 seasons at 3-week intervals until harvest in both cultivated and reclaimed lands. The first spray was applied on May 23 after fruit onset and the other three sprays on June 13, July 4 and July 25. Average *V. livia* infestation was calculated from May 23 to September 25 and at harvest day. The fruit weight (total fruits examined 100) was recorded during harvest in treated and control pomegranate trees.

Statistical analysis: Data were analysed using one-way ANOVA and presented as mean ± S.E.M (Standard Error of Mean). Means were separated by Tukey's multiple comparison test ($P < 0.05$) whenever differences were indicated. Figures and statistical analysis were done using Graph Pad Prism 5™ software (San Diego, CA). Regression analysis of *V. livia* infestation were undertaken with SPSS for Windows Version 16.0 (SPSS Inc.®, 2012).

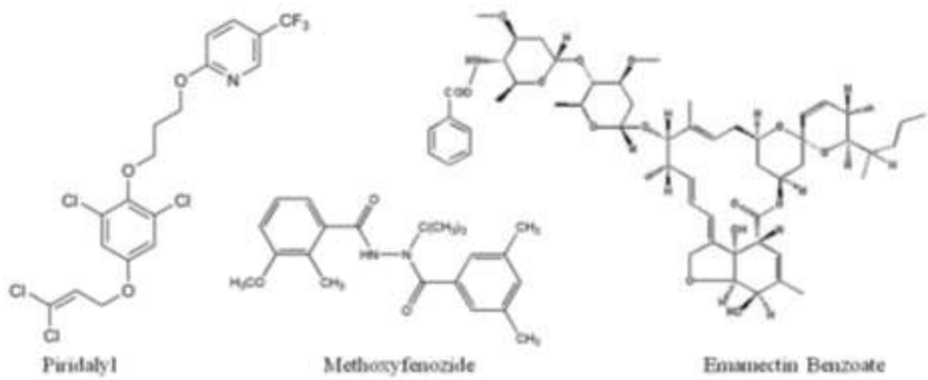

Figure 1: Chemical structures of pyridalyl, methoxyfenozide and emamectin benzoate.

Results

Susceptibility and development of pomegranate cultivar 'Manfalouty' fruits to the infestation by *V. livia*: The damage of pomegranate fruits caused by *V. livia* larvae are described as a red circular shape of 1-2 cm diameter which turns into dark colour at the late developing stage (Fig. 2). In addition a black colour in the skin of the fruit appears because larvae produce wastes where many fungi grow on fruits. The susceptibility and the development of pomegranate cultivar 'Manfalouty' fruits infested by *V. livia* from May 23 to September 25 in cultivated and reclaimed lands during 2012 and 2013 seasons are shown in Figure 3A and B. Fruit infestation caused by *V. livia* appeared more or less May 23 and June 5 in cultivated and reclaimed lands. The infestation increased at the end of May and continued until September 25 in both cultivated and reclaimed lands. In the 2012 season, the average percent infestation at harvest were 52.33 ± 1.45% and 40.00 ± 0.57% in cultivated and reclaimed lands, respectively (Fig. 3A). However, in 2013 season, the average percent infestation at harvest were 49.00 ± 2.85 and 35.67 ± 1.41% in cultivated and reclaimed lands, respectively (Fig. 3D).

Figure 2: Damage of *V. livia* in treated (A) and untreated (B) pomegranate fruits (cultivar Manfalouty).

Impact of physical factor on *V. livia* infestation: In the present study, the infestation of *V. livia* according to temperature and relative humidity in both cultivated and reclaimed lands was estimated (Fig. 3). The minimum infestations were found at the temperatures of 32.4 and 34.20°C and relative humidity of 42% and 38% in cultivated and reclaimed lands during 2012 season, respectively. The maximum infestations 52.33 and 40.00% were found at temperature of 41.40°C and 45.20°C and relative humidity 46% and 42% (Fig. 3B, C). In 2013 season, the minimum infestations of 1.33 and 0.67% were found at the temperature of 33.20°C and 37.40°C and relative humidity of 44% and 40% in cultivated and reclaimed lands, respectively. The maximum infestations 49% and 35.67% were found at 39.4°C and 43.50°C and a relative humidity of 48% and 44%, respectively (Fig. 3E, F).

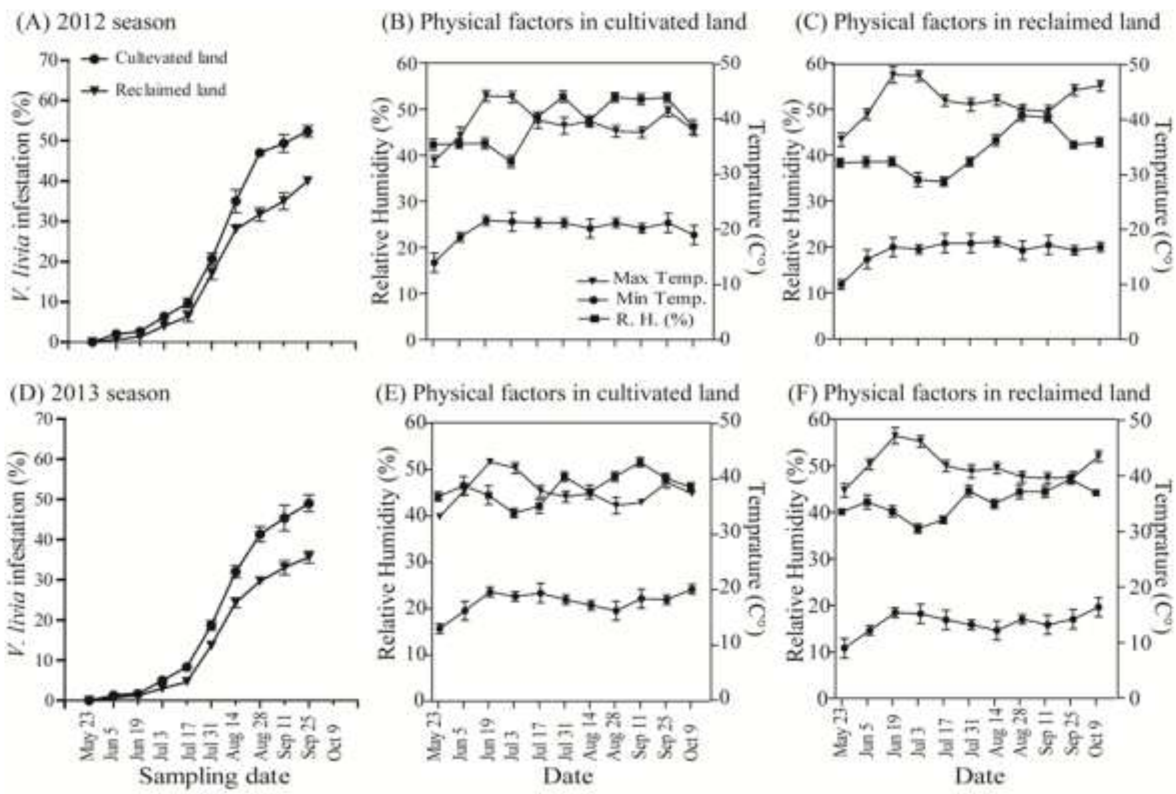

Figure 3: Susceptibility and development of pomegranate cultivar ‘Manfalouty’ fruits infested by *V. livia*. Data are collected from May 23 to September 25 in cultivated and reclaimed land during 2012 (A), Physical factors (B, C) and 2013 (D), Physical factors (E,F) seasons.. Data are presented as mean ± SEM.

Efficacy of methoxyfenozide, pyridalyl and emamectin benzoate on *V. livia*: Data presented in Figure 4 show that methoxyfenozide, pyridalyl and emamectin benzoate caused a significant reduction in pomegranate fruit infestation caused by *V. livia* compared to control from May 23 to September 25 which ranging from 0.33 to 4.33 and from 0.67 to 3.00% in cultivated and reclaimed lands during 2012 season (Fig. 4A, B). Pyridalyl was the second insecticide, which caused a significant reduction of the infestation which ranging from 0.33 to 6.67% and from 0.67 to 6%. Methoxyfenozide had less effectiveness with a percent infestation ranging from 0.33 to 17.67% and from 0.33 to 14.33% in both cultivated and reclaimed lands. In 2013 season, emamectin benzoate was the most effective in reducing the infestation range of *V. livia*, which was from 0.33 to 3.33% and from 0.33 to 2.33% in cultivated and reclaimed lands respectively. Followed by pyridalyl reduced the infestation value which ranged from 0.67 to 6.33% and from 0.67 to 5.00%. The third insecticide was methoxyfenozide, which caused a reduction of the infestation rate that ranging from 0.33 to 16.33% and from 0.67 to 13.33% and it was significantly different from pyridalyl and emamectin benzoate treatments, respectively (Fig. 4C, D). In all cases, the highest infestation rates were in the control field.

At harvest day (September 25) the percent infestation of *V. livia* in control and in three insecticide treatments was calculated as well. Data in Figure 5 show that, emamectin benzoate caused a significant reduction in pomegranate fruits infested by *V. livia* with 4.33 and 3.00 % in both cultivated and reclaimed lands during 2012 and 3.33 and 2.33% during 2013 seasons respectively (F= 415.3, df 3,11; F= 240.6, df 3,11) (Fig. 5A, B). Pyridalyl caused a reduction infestation with 6.67 and 6% in 2012 season and 6.33 and 5% in 2013 season in both cultivated and reclaimed lands, respectively. Methoxyfenozide caused the lowest infestation rate with 17.67 and 14.33% during 2012 season and with 16.33 and 13.33% during 2013 season in both cultivated and reclaimed lands, respectively (F= 669.5, df 3,11; F= 249.1, df 3,11) (Fig. 5A, B).

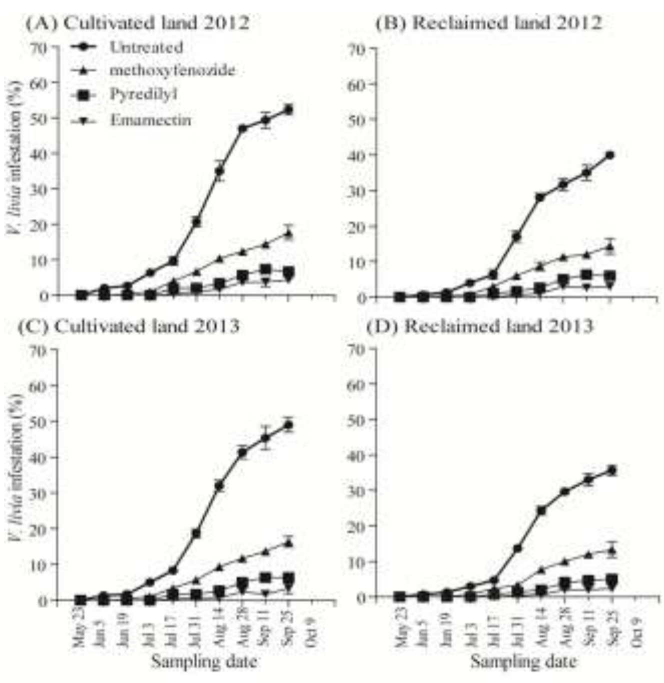

Figure 4: Efficacy of methoxyfenozide, pyridalyl and emamectin benzoate on *V. livia* infestation from May 23 to September 25 in cultivated and reclaimed lands during 2012 (A, B) and 2013 (C, D) seasons. Data are presented as mean % infestation ± SEM.

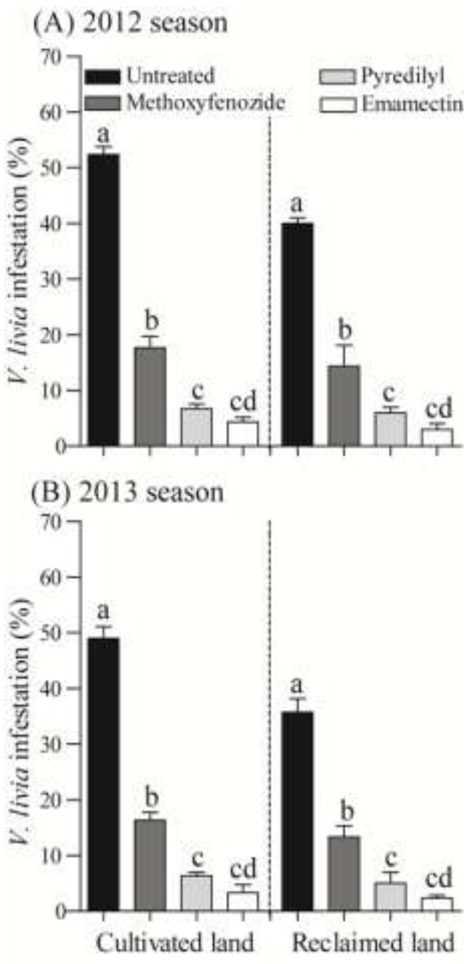

Figure 5: Impact of methoxyfenozide, pyridalyl and emamectin benzoate on *V. livia* infestation at the harvest time in cultivated and reclaimed lands during 2012 (A) and 2013 (B) seasons. Data are presented as mean % infestation ± SEM. Columns headed by the same letter within the same seasonal graph are not significantly different (p<0.05, Tukey's test).

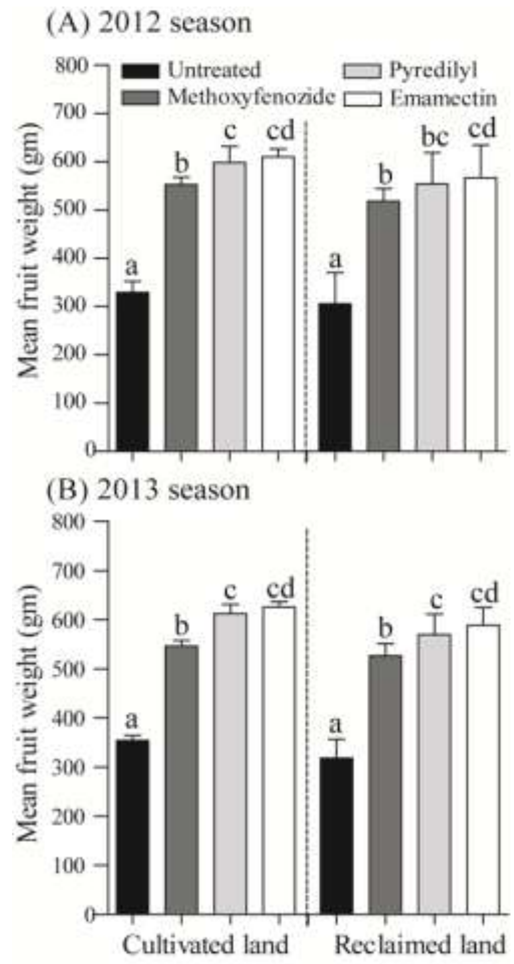

Figure 6: Mean fruit pomegranate weight (g) in control and after methoxyfenozide, pyridalyl and emamectin benzoate treatments in cultivated and reclaimed lands during 2012 (A) and 2013 (B) seasons. Data are presented as mean fruit weight ± SEM. Columns headed by the same letter within the same seasonal graph are not significantly different ($p < 0.05$, Tukey's test).

Effect of foliar applications of methoxyfenozide, pyridalyl and emamectin on pomegranate fruit weight: Results presented in Figure 6 indicated that, the application of methoxyfenozide (0.5 mlL^{-1}), pyridalyl (0.2 mlL^{-1}) and emamectin (0.8 mlL^{-1}) against the pomegranate butterfly *V. livia*, under field conditions increased the average weight fruits (e.g., 552.5, 598.25, 610 and 517.75, 553.50, 566.5 gm/fruit in cultivated and reclaimed lands, respectively) during 2012 season ($F= 90.27$, df 3,11; $F=257.8$, df 3,11) compared the control (Fig. 6A). In 2013 season, methoxyfenozide, pyridalyl and emamectin also increased the average weight fruits (e.g., 546.25, 612.25, 625.38 and 526.56, 569.26, 588.89 gm/fruit in cultivated and reclaimed lands, respectively) ($F= 312.1$, df 3,11; $F= 578.2$, df 3,11) (Fig. 6B). The application of these insecticides under field conditions to suppress the pomegranate butterfly, *V. livia*, infestation increased the yield production of pomegranate by about 70–90% compared to the control.

Discussion

The development of resistance to all classes of agricultural chemicals is a significant and increasing problem prohibiting the effective management of pest populations (Rouch & Tabashnik, 1990). Although insecticides play a critical role in the management of pomegranate butterfly, *V. livia*, their frequent use has prompted the development of resistance. The present study showed that the development of pomegranate cultivar ‘Manfalouty’ fruit infestation caused by *V. livia* significantly started at the end of May and it continued until the end of September in cultivated and reclaimed lands. the present data indicated also that, the infestation was higher in cultivated land than reclaimed land in both seasons due to the presence of the date palm (*Phoenix dactylifera*) plantation as well as acacia green pods (*Acacia nilotica*) which they are the primary hosts of *V. livia* in cultivated land, but these hosts are not found frequently in reclaimed land (Hanna, 1939; Temerak & Sayed, 2001). In addition, the impact of weather factors (e.g., daily maximum and minimum temperatures, daily relative humidity) on the infestation of *V. livia* was highly significant in both cultivated and reclaimed lands. The change in temperature and relative humidity may increase the susceptibility of pomegranate fruits to infestation by *V. livia*. Interestingly, the results of our study indicated that the foliar application of methoxyfenozide, pyridalyl and emamectin against pomegranate butterfly, *V. livia* caused a significant reduction in the pomegranate fruit infestation in both cultivated and reclaimed lands during 2012 and 2013 seasons. Emamectin was found to be highly effective for controlling the pomegranate butterfly, *V. livia*. There was no significant difference between emamectin and pyridalyl. The highest infestation rate was in the control treatment which was significantly different from all other insecticides. Field assays have shown that emamectin (equivalent to 25gAI $ha^{-}1$) has potential for controlling *S. littoralis*, with special efficiency against *H. armigera* (Ishaaya et al., 2002). Jansson et al. (1997) reported that emamectin benzoate was efficient in controlling the diamond back moth *P. xylostella* at a rate of 8.4 gAI $ha^{-}1$ in cabbage fields, and at a similar concentration for controlling *S. exigua*, *Heliothis virescens* F. and *Trichoplusia ni* (Hübner) (Leibee et al., 1995). In addition, emamectin was found to have good field potency and it has less cross-resistance with the commercially used insecticides (White et al., 1997). These results correlate with other investigations, e. g. Ishaaya et al., 2002; Argentine et al., 2002. Emamectin is also having the lowest coefficients indicating that this insecticide had much more persistent effect over time followed by pyridalyl and methoxyfenozide. Under pomegranate field condition, pyridalyl had contact and ingestion toxic effects and was effective for controlling *V. livia*. It was also used against lepidopteran and thysanopteran pests (Isayama et al., 2005; Gad et al., 2013). Methoxyfenozide is an IGR that shows a high selectivity, but it takes longer to reduce insect populations in comparison with conventional insecticides (Dent, 2000). In the present study, methoxyfenozide caused a moderate infestation and there was a significant deference between pyridalyl and emamectin. The habit of *V. livia* larvae to enter the fruits reduces the possibility to come in contact with insecticides. These results do not agree with those of Hussein et al. (1983) who found that the use of altoside (IGR) gave more pronounced direct toxic action against pupae rather than larvae. At harvest of pomegranate fruits, methoxyfenozide, pyridalyl and emamectin caused a significant reduction in the pomegranate fruit infestation compared to control. Emamectin induced a maximum lowest infestation compared to methoxyfenozide and pyridalyl in cultivated and reclaimed lands. There was no significant difference between emamectin and pyridalyl but there was significant difference with methoxyfenozide. The pomegranate butterfly *V. livia* causes a serious damage to the yield if no control measures are applied to limit the pest population development. This insect lays its eggs on the fruit of pomegranate and when the egg hatch, the larvae penetrate the skin of the fruit and damage the fruit. Secondary fungal infection of the insect entry holes may lead to fruit decay (Samy, 2004). However, the foliar application of these insecticides against *V. livia* was found to save the fruits against the larvae and to increase significantly the average weight of the fruits by about 70-90%. Our results indicate that, emamectin and pyridaly caused a highly increase average weight of the fruits and there was a significant difference with methoxyfenozide. The highest reduction rate of weight fruits was in the control treatment which was significantly different from all other insecticides. In conclusion, results obtained in this study indicate that, the foliar application of methoxyfenozide, pyridalyl and emamectin gave the highest efficiency against pomegranate butterfly *V. livia* under cultivated and reclaimed land conditions during 2012 and 2013 seasons. Our results suggested that these insecticides caused a significant reduction of the pomegranate fruit infestation. In addition, emamectin benzoate and pyridalyl were highly effective for controlling *V. livia* compared to methoxyfenozide. However, results showed that all treatments caused a significant increase in the average weight of the fruits compared to the control. Therefore, these insecticides will be currently used to control pomegranate butterfly.

Acknowledgements

The author wishes to thank the Plant Protection Department Faculty of Agriculture, Assiut University, Egypt, for providing a partial funding support for this study. Many thanks are extended also to Dr. Essam Abd-Elzaher, Department of Horticulture (Pomology), Faculty of Agriculture, Assiut University, for his help and for providing the equipment to treat the pomegranate trees through this study. Author would like also to thank all people in the same Horticulture Department for their kind help.

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