Evaluation of the effect of some biopesticides on alfalfa weevil larvae, *Hypera postica* (Gyllenhal.) in laboratory and field conditions

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Abstract: Alfalfa weevil (*Hypera postica* Gyllenhal.) control is traditionally based on the use of chemical insecticides. Due to the side effects of these compounds, there are many motivations for the use of integrated control methods, which may include the use of biopesticides (microbial and botanical). The result of the effect of seven doses of the tested biopesticides (500, 1000, 1500, 2000, 2500, 3000 and 3500 ppm) under laboratory conditions indicated that the insecticides Azadiractin® (1859.971 ppm) and Biobit® (2000.57 ppm) were of the highest, while Basiokara® (3178.697 ppm) and Naturalis® (2973.246 ppm) were of the lowest lethality. Field experiments were carried out based on the randomized complete block design with10 treatments and four replications. The treatments included Palizin® (2.5 mL L^{-1}), Azadiractin® (2 mLL^{-1}), Tondaksir® (2.5 mL L^{-1}), Basiokara® (3 mL L^{-1}), Biobit® (2.5 mL L^{-1}), Naturalis® (3 mL L^{-1}), two-component treatments, Azadiractin® (1 mL L^{-1}) + Basiokara® (1.5 mL L^{-1}), Biobit® (1.25 mL L^{-1}) + Tondaksir® (1.25 mL L^{-1}), and the chemical insecticide Cypermethrin® (2 mL L^{-1}) in two year studies (2015-2016). Water was used as a control treatment. Sampling was done before pesticide application and on the third, seventh and fourteenth days after application and live larvae were counted in each plot. The percentage of the efficacy was calculated by Henderson-Tilton equation. The analysis of variance showed experimental treatments were of significantly different impacts at a probability level of 1% over two years. Comparison of the average mortality rate of alfalfa larvae showed that the highest ratios of the insecticides Cypermethrin®(69.375%), Azadiractin® (57.875%) and Biobit® (46.25%) were of the highest lethalities, while that of Naturalis® (27.52%) and Basiokara® (22%) led to the lowest mortalities of alfalfa weevil larvae. Based on LC_{50} values and the results of field experiments, Azadiractin® and Biobit® are recommended as a high toxic insecticide useful in the integrated management of the pest in organic alfalfa production system.

Keywords: biopesticide, control, forage, lucerne, management

Citation: Alizadeh, E., O. Valizadegan, S. Aramideh, M. H. Safaralizadeh, and S. E. Sadeghi. 2019. Evaluation of the effect of some biopesticides on alfalfa weevil larvae, *Hypera postica* (Gyllenhal.) in laboratory and field conditions. Agricultural Engineering International: CIGR Journal, 21(4): 161–168.

1 Introduction

Alfalfa, also called Lucerne (*Medicago sativa* L.) is an important agricultural forage crop which plays a vital role in human life by providing livestock food, stabilizing nitrogen, reducing soil erosion, and etc. This valuable cropis always threatened by various pests, the most important of which is Alfalfa weevil, *Hyperapostica* Gyllenhal. (Summers and Godfrey, 2004). The use of insecticides due to the chemical contamination of forage and the required costs is not a reliable way (Pandey and Singh, 1984). Also, several side effects such as pest resistance, insect outbreaks, groundwater pollution and human health threats have been observed due to the excessive use of chemical insecticides (Gouamene-Lamine

Received date: 2018-08-03 **Accepted date:** 2018-09-11

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et al., 2003; Edwards et al., 2008). Hence, the use of healthy and low-risk compounds for pests such as alfalfa weevil is essential and the use of bio-insecticides as a part of biological control has found a special place in pest control, and this group of substances has been distinguished from other insecticide groups with their impacts (Ware and Whitacre, 2004). The most important of these insecticides is microbial pesticides based on various strains of *Bacillus thuringiensis* and *Beauveria bassiana* (Balsamo). In a three-year study, *B. bassiana* caused an average loss of 38.1% of all overwintering adult alfalfa weevils, and resulted in the average mortality of 86%, 56% and 38.5% of respectively adult weevils, larvae and pupae under conditions of artificial infestations (Saeidi, 2007). The fungus *Erynia phytonomi* (Thomon) first reported in Ontario, Canada in 1974 (Harcourt et al., 1974) has rapidly extended to the northern parts of the United States (Muka, 1976; Puttleret al., 1978; Barneyet al., 1980; Los and Allen, 1983; Nordinet al., 1983). Now, this fungi is known as the most important pathogen of alfalfa weevil (Carruthers and Soper, 1987). *Zoophthora phytonomi* (Thomson) is another fungus pathogen which infects all larval stages of the alfalfa weevil, but the outbreak of the disease is significant when large larval numbers are high and disease under certain environmental conditions causes a significant loss in pest population (Harcourt, 1990; Los, 1982). In a survey, Cothran and Gyrisco (1966) introduced *Beauveria bassiana* (Balsamo) fungi as a good controlling element of *H. postica*. Another study showed that extraction protein titled cry6b (BGSC-4D8) from *Bacillus thuringiensis* with LC_{50}=280 ng mL^{-1} significantly controlled the alfalfa weevil larvae (Sharma et al., 2010). One of the main advantages of biochemical insecticides is their safety for non-target species, so that in a test of sub-lethal concentration of 3×10^5, the concentration of *B. bassiana* had no negative effect on the biochemical parameters of predator *Andrallus spinidens* Fabricius (Gholamzadeh-Chitgar et al., 2016). Additionally, in another study, the effect of Naturalis-L, a microbial insecticide based on *B. bassiana* on European honeybee was investigated and the results showed that the recommended concentration of Naturalis-L insecticide in the field did not have a contact and oral hazard for honey bee (Ariyana et al., 2003). Neima and Ahmed (2016) studied the effect of *Eucalyptus camaldulensis* (Myrtaceae), *Oleander nerium* (Apacynaceae) and *Azadirachtaindica* (Meliaceae) on larvae and adults of alfalfa weevil. Results showed that *A. indica* extract had a greater effect in comparison with other treatments, so that it resulted in the larval mortality of 73.33% within 4 days, and in the 96.66% mortality of adult insects within8days. In another survey, the toxicity of *Lavandulastoechas* L. and *Heracleum persicum* Desf.ex Fischer extracts on adult of *H. postica* were studied and the results showed that the extract of both plants had significant toxicity against adult weevils (Ebadollahi and Tajmiri, 2016). Therefore, the necessity of maintaining bio-security, food safety and environmental protection have limited the use of chemical compounds, and incited researches to find and use organic, selective and environmentally safe insecticides. Accordingly, the use of biological insecticides against alfalfa weevil, if successful, by reducing the adverse effects of chemical insecticides can be considered as a promising strategy for controlling the alfalfa weevil.

2 Materials and methods

2.1 Geografical location

The present study was carried out at third cropping season alfalfa (cv. Hamedani) field infested with alfalfa weevil in an area of 1.46 ha in the village of Gurt-Tappe in the northeast of Urmia city. There was about 1295 to 1296 meters above free sea level.

This experiment was planned as a randomized complete block design with ten treatments and four replications. The tested formulations were as follows:

-Palizin®, SL 70%, 2.5 mL L^{-1}: a concentrated soap of coconut oil with peppermint and eucalyptus extract (Kimia-Sabzavar Co., Iran).

-Tondaksir®, EC. 85%, 2.5 mL L^{-1}: an insecticide and miticide containing garlic and pepper extract (Kimia-Sabzavar Co., Iran).

-Azadiractin (Neemarin®), EC 15%, 2 mL L^{-1}: based on neem seed extract (Pre-tree folio Co., Germany).

-Cypermethrin (Ripcord®), EC 40%, 2 mL L^{-1}: a contact-digestive insecticide from the family of Pyrotroids (Tragusa Co., Spain).

-Biobit®, WP, 2.5 mL L^{-1}: containing the active ingredient of spores and crystals of *B. thuringiensis subsp. tenebrionis* (45 × 10^6 CFU sporesg^{-1}) (Fanavariye Zistiye Tabiat Gera Co., Iran).

-Basiokara®, WP, 3 mL L^{-1}: containing *B. bassiana*spores (1×10^8 CFU sporesg^{-1}) (Sanaye-zistfanavariye-karaCo., Iran).

-Naturalis®, WP, 3 mL L^{-1}: Based on *B. bassiana* ATCC 74040 spores (2.3 × 10^8 CFU sporesg^{-1}) (Troy Bioscience Co., USA).

Effective concentrations of each insecticide in this study were investigated with a preliminary test to determine the most effective concentration on alfalfa weevil before the application of them under field conditions.

2.2 Determination of LC_{50} and LC_{25} of six bio-insecticides and cypermethrin on second larval stage of alfalfa weevil

2.2.1 Determination of concentration range

To find the appropriate concentration range, first preliminary tests were performed with seven doses of 500, 1000, 1500, 2000, 2500, 3000 and 3500 ppm. Then, the concentrations causing 25% and 75% mortality were chosen and analyzed by SPSS software Ver. 22.

2.2.2 Bioassay

The impact of Palizin®, Tondaksir®, Azadiractin®, Cypermethrin®, Biobit®, Basiokara® and Naturalis® treatments on the second larval stage of *H. postica* was investigated following the standard bioassay method (Robertson and Preisler, 1992). The effect of the mentioned formulations, each in seven concentrations, was tested on second larval stage of alfalfa weevil (3 mm in body length, with a brown head capsule 0.2±0.02 mm in diameter). The experiment was carried out in three replicates, where each replicate included ten larvae applied without gender determination in plastic containers of 8×10×6 cm, wherein fresh alfalfa impregnated with insecticide solution were placed. The experiment was performed at a temperature of 22°C±3°C and a relative humidity of 60±5 percentage under light conditions of 8-16 h. The mortality rate was determined in 24 h intervals (24, 48, 72, 96, 120, 144, 168 and 192 h) touching larvae with a warm needle and immobile larvae were considered as dead so the percentage of mortality was calculated for each test plot.

2.3 Field experiment

For this purpose, 10 treatments containing Palizin® 2.5 mL L^{-1}, Tondaksir® 2.5 mL L^{-1}, Azadiractin®2 mL L^{-1}, Biobit® 2.5 mL L^{-1}, Basiokara®3 mL L^{-1}, Naturalis® 3 mL L^{-1}, Cypermethrin® 2 mL L^{-1} and two combined treatments of Azadiractin® 1 mL L^{-1}+ Basiokara® 1.5 mL L^{-1}, Tondaksir® 1.5 mL L^{-1} + Biobit® 1.5 mL L^{-1} with water as control were tested. Based on Hilburn (1985) statistical method, repeats were in form of 4 × 3 m plots which before treatment implementation. Considering a 25 cm- broad band of marginal effect, the number of live larvae of each plot was counted using 1×1 m wooden frame and simultaneous with the activity of live larvae of different ages, the plots were sprayed and the live larvae were sampled in the third, seventh, and fourteenth days after treatments. In order to correct the mortality rate of treatments based on the mortality rate in control plots, the efficiency percentage of each compound was calculated by the following Henderson-Tilton formula (Henderson and Tilton, 1955):

$$\text{Efficiency percentage} = (1-\frac{Ta}{Ca}\times\frac{Cb}{Tb})\times 100 \qquad (1)$$

where, *Ca* (%) and *Cb* (%) were infection rates in control plots before and after spraying, and *Ta* (%) and *Tb* (%) were the infection rates in treatment plots before and after spraying.

2.4 Data analysis

Data of the second larval stage mortality were corrected by the Abbott formula (Abbott, 1925) and analyzed by probit analysis using the software SPSS 22 and LC_{50} and LC_{25} values were calculated. Also, if the mortality rate data resulted from different treatments applied under laboratory conditions were not normally distributed and were meaningless in Levene test, the data were first transformed to $Arc\sin\sqrt{x}$ form. The analysis of variance (One way ANOVA) and comparison of means by Tukey method (HSD-TUKEY) at a confidence level of 95% were performed taking advantage of the software. Finally correcting the mortality rates in the treatments calculated with Henderson-Tilton formula.

3 Results

3.1 Lethality test (bioassay) results

According to results of probit analysis of different concentrations of the insecticide Cypermethrin® and six biological insecticides on the second larval stage of alfalfa weevil after 8 days, the highest lethal concentration with the highest LC_{50} and with values of 1859.971 and 1478.279 ppm respectively related to Cypermethrin® and Azadiractin®,whilethe lowest lethal concentration with lowest LC_{50} and with values of 3187.697 and 2973.246 ppm belonged to Naturalis® and Basiokara®, respectively. Also, Cypermethrin®, Azadiractin®, Palizin® and Tondaksir® showed their higher impacts up to 72 h and then became ineffective. Also, Naturalis® and Basiokara® did not show any effect during the first 48 h but after then, they had the highest lethality up to 168 h. Additionally, Biobit® had no impact in the first 24 h but after then, it became the most effective till up to 96 h (Table 1).

Table 1 Probiotic analysis of different concentrations of six bio-insecticides and chemical Cypermethrin® on the second larval stageof *H. postica* in the laboratory after 8 days

Treatment	Larvae	Time (h)	std.error± slope	Intercepts+5	X^2 (df)	LC_{25}(ppm)	LC_{50} (ppm)
Cypermethrin®	210	24	0.001±0.310	4.458	0.049(3)	–433.061	1776.11
		48	0.001±0.310	4.54	0.047(3)	–742.245	1586.489
		72	0.001±0.314	4.348	0.168(3)	–50.788	1478.279
		96	0.001±0.314	4.348	0.168(3)	–50.788	1478.279
Azadiractin®	210	24	0.001±0.315	4.273	0.298(3)	215.818	2529.864
		48	0.001±0.319	4.003	0.137(3)	731.872	2234.711
		72	0.001±0.318	4.064	0.378(3)	519.536	1859.971
		96	0.001±0.318	4.064	0.378(3)	519.536	1859.971
Palizin®	210	24	0.001±0.339	4.17	0.063(3)	1667.184	3935.363
		48	0.001±0.323	4.147	0.053(3)	765.235	3620.537
		72	0.001±0.320	3.992	0.137(3)	768.792	2324.865
		96	0.001±0.320	3.992	0.137(3)	768.792	2324.865
Tondaksir®	210	24	0.001±0.330	4.019	0.036(3)	1228.806	3936.395
		48	0.001±0.318	4.131	0.040(3)	619.783	2721.613
		72	0.001±0.316	4.126	0.138(3)	507.772	2220.017
		96	0.001±0.316	4.126	0.138(3)	507.772	2220.017
Naturalis®	210	24	-	-	-	-	-
		48	-	-	-	-	-
		72	0.001±0.450	4.136	0.093(3)	4015.423	6291.886
		96	0.001±0.362	4.427	0.130(3)	2003.167	3507.091
		120	0.001±0.348	3.593	0.110(3)	1745.508	3353.535
		144	0.001±0.327	3.647	0.099(3)	1479.012	3379.951
		168	0.001±0.327	3.925	0.009(3)	1108.033	2973.246
Basiokara®	210	24	-	-	-	-	-
		48	-	-	-	-	-
		72	0.001±0.478	2.859	0.109(3)	3632.902	5302.478
		96	0.001±0.386	3.267	0.260(3)	2417.5	2839.58
		120	0.001±0.364	3.47	0.240(3)	2150.28	3846.35
		144	0.001±0.349	3.638	0.260(3)	1861.35	3688.41
		168	0.001±0.334	3.803	0.016(3)	1331.779	3187.697
Biobit®	210	24	-	-	-	-	-
		48	0.001±0.425	4.221	0.036(3)	3451.82	5559.17
		72	0.001±0.340	3.69	0.184(3)	1512.03	3116.82
		96	0.001±0.319	3.997	0.102(3)	678.45	2073.25

3.2 Field evaluation results

Results of the combined variance analysis of the data showed no significant difference between the years, but showed significant difference in simple effects of treatments and the interaction between the year × experimental treatments in the third, seventh and fourteenth days after spraying at the probability level of 1% (Table 2). All insecticides used in this experiment showed mortality on alfalfa weevil. Comparison of treatment means showed that after Cypermethrin®, the highest mortality after three days of spraying were related toAzadiractin® and Tondaksir®, after seven days to Azadiractin® and Azadiractin® + Basiokara®, and after fourteen days related to Naturalis® and Biobit® (Table 3). Three days after spraying Cypermethrin®, Azadiractin® and Tondaksir® wererespectively placed in the groups A, B and C. Also, seven days after spraying Azadiractin® + Basiokara® in the group A, both Biobit® and Biobit® + Tondaksir® were located in group B and Tondaksir® in the group C. Fourteen days after spraying Biobit®, Naturalis® and all Biobit® + Tondaksir®, Basiokara® and Cypermethrin® were placed in the groups A, B and C respectively (Table 3). Evaluation of insecticides showed that Cypermethrin® was three day safer spraying sooner than other treatments exhibited the highest efficiency which had reduction rate up to 14th day so lost its lethality soon, also such function occurred about Azadiractin®, Tondaksir® and Palizin®, while Naturalis®, Biobit® and Basiokara® had less efficiency during first three days but later (14thday), reached to its highest impact and thus maintained its lethality more (Table 3).

Mortality percentage of alfalfa weevil during two years, and three days after spraying has shown in Figure 1. Also results showed that bio-insecticides on third day and chemical insecticides on fourteenth day had the most mortality on *H. postica* (Figure 1).

Table 2 Analysis of variance effects of different treatments on second larval stage of *H. postica*

S.O.V	df	Day 3	Day7	Day14
Year	1	6.050^{ns}	2.450^{ns}	151.250^{ns}
First error	6	10.817	7.958	6.15
Treatment	9	1464.717^{**}	230.500^{**}	294.478^{**}
Treatment*Year	9	69.661^{**}	18.172^{**}	21.332^{**}
Second error	54	6.104	4.829	2.502
Sum	79			
C.V		14.34	17.94	14.96

Table 3 Comparison of mean mortality percentages of different biological insecticide treatments on *H. postica* 3, 7 and 14 days after spraying during two years (2016-2017) in field using Tukey test at probability level of 1%

Treatment	Mortality percentage±SE		
	Day 3	Day 7	Day 14
Naturalis®	3.125^{F}±0.186	8.125^{DE}±0.156	16.250^{B}±0.286
Tondaksir®	22.75^{C}±0.276	12.25^{C}±0.136	6.625^{E}±0.096
Azadiractin®	31^{B}±0.145	17.25^{AB}±0.115	9.625^{D}±0.135
Palizin®	19.375^{D}±0.012	10.125^{CD}±0.122	3.125^{F}±0.167
Basiokara®	2^{FG}±0.184	7.750^{E}±0.131	12.25^{C}±0.166
Biobit®	8.750^{E}±0.012	15.375^{B}±0.078	22.125^{A}±0.186
Tondaksir®+Biobit®	18.875^{D}±0.284	14.875^{B}±0.154	12.25^{C}±0.186
Azadiractin®+Basiokara®	24.750^{C}±0.284	18.750^{A}±0.143	10.625^{CD}±0.186
Cypermethrin®	41.250^{A}±0.184	16.50^{AB}±0.191	11.625^{C}±0.176
Testifier	0.3750^{G}±0.084	1.5^{F}±0.063	1.25^{G}±0.028

Note: In each column, the treatments marked with the same letter are not significantly different.

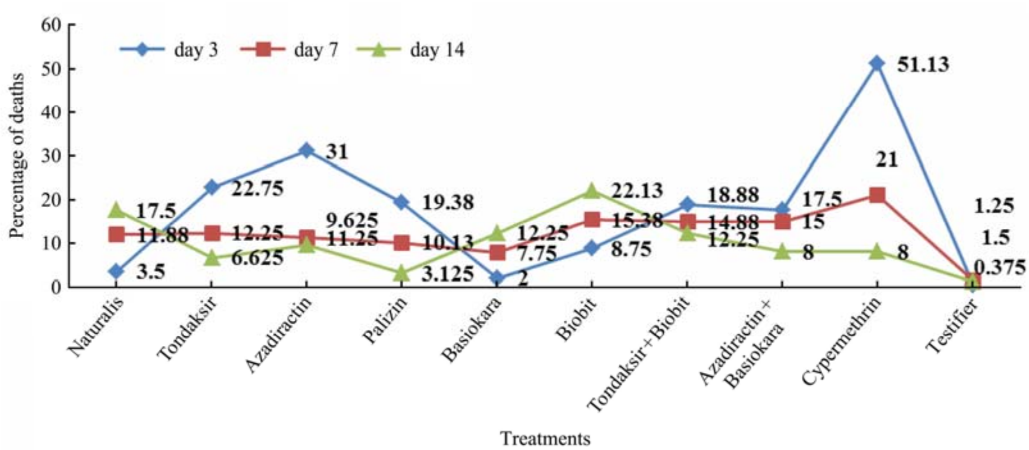

Figure 1 Mortality percentage of second larval stage of *H. postica* 3, 7 and 14 days after spraying during 2015-2016.

4 Discussion

Applying biological and microbial insecticides against alfalfa weevil is one of the best controlling strategies, hence Moradi-Vajargahet al. (2013), Kamangar and Habibi (2006) and Reddy et al. (2016) bioassayed the second, third and fourth larval stage of alfalfa weevil, respectively. Bioassays carried out with the second larval stage (Reddy et al., 2016) indicated that the best time for the chemical control of the pest was since the end of the first and the initiation of the second larval stage till just before transformation to the third stage. Entrust® and Xpectro® bio-insecticides showed the highest and Met®52 and Mycotrol® showed the lowest lethality on *H. postica* (Reddy et al., 2016) and in this survey, Azadiractin® (2 mL L^{-1}) and Biobit® (2.5 mL L^{-1}) with 57.88% and 46.25% had the highest lethality while Naturalis® and Basiokara® with 27.5% and 22% led to the lowest mortalities of the pest, respectively.According to Danay-Tous et al. (2013), Palizin® and Tondaksir® led to more acceptable results in controlling pistachio common psylla (*Agonoscena pistaciae*. Burckhardt and Lauterer) compared to the insecticide Acetamiprid® 20% sp, all tested under field conditions. Palizin® applied at the ratios of 1500 and 2500 ppm decreased greenhouse population of *Aphis gossypii* Glover by 75.9% and 90.6%, respectively (Baniameri, 2008). Palizin® and Tondaksir® reduced the population of *Aphis punicae* Passerini by 73% and 55% in pomegranate gardens in Iran (Farazmandet al., 2012). Also in this study, Tondaksir® (2.5 mL L^{-1}) and Palizin® (2.5 mL L^{-1}) decreased alfalfa weevil population by 41.62% and 27.5%, respectively. According to Homayonfar and Zohdi (2012), neem extract applied at the ratio of 75 ppm and 100 ppm well controlled the nymph and adult stage of pistachio psylla in laboratory, respectively. Also, Ebrahimi et al. (2012), showed that essential oil of neem, eucalyptus and laurel could control *A. gossypii* well, but the impact of laurel extract was less than the others. Commercially formulated neem extract controlled alfalfa weevil in a manner comparable to the insecticide, Ecamet®, while it additionally had anti-nutritional effects and increased product (Oroumchi and Lorra, 1993). In this research, neem-based Azadiractin® controlled *H. postica* well. Three commercial formulations of *B. thuringiensis* (Dipel®, Turex® and Costar®) led to acceptable rates of mortality of three larval stage of *Tuta absoluta* and decreased its damage (Gonzalez-Cabreraet al.,2011). Biobit® as a *B. thuringiensis*-based insecticide resulted in a mortality rate in Alfalfa weevil comparable to other insecticides. According to Javvi and Pourmirza (2005), *B. thuringiensis* and neem extract caused 22.5% and 25% mortality of *Leptinotarsa decemlineata*, respectively, which in this study Biobit® also caused 46.25% mortality in alfalfa weevil. *B. bassiana* strain GHA alone and combined with *B. thuringiensis* decreased 68% of larval population of *T. absoluta* (Torres-Gregorioet al., 2009) and in this investigation, Basiokara® and Naturalis®led to 22% and 27.5% mortalities in the second larval stage of *H. postica* under field conditions, respectively.

In general, all treatments applied in this study were efficient in reducing the density of the alfalfa weevil and according to LC_{50} obtained under field and laboratory conditions, Azadiractin® and Biobit® found highly poisonousness, and are suggested as acceptable choices in Integrated Pest Management (IPM) and organic agriculture. The results of this study can help alfalfa producers in making decision and managing *H. postica*.

References

Abbott, W. S. 1925. A method for computing the effectiveness of an insecticide. *Journal of EconomicEntomology*, 18(2): 265–267.

Ariyana, A., G. H. Tahmasebi, H. Asgari, M. Khalili-Mahani, and M. Ghazavi. 2003. Laboratory evaluation of contact and oral effects of Naturalis-lbased on *Beauveriabassiana*strain ATCC-74040combinationon European honey bee. In *3rd National Conference of Biological Materials Application and the Effective Use of Fertilizer and Toxin in Agriculture*. pp: 430–431. Karaj, Iran: Agricultural Education Publication.

Baniameri, V. 2008. Study of the efficacy of different concentrations of insecticidal soap,in comparison Oxydemeton-methyl to control *Aphis gossypii* in greenhouse cucumber. In *Intergrated Control in Protected Crops, Temperate Climate*, 32, 13–16. Sint Michielsgestel, the Netherlands, 21-25 April.

Barney, R. J., P. L. Watson, K. Black, J. V. Maddox, and E. J. Armbrust. 1980. Illinois distributionof theFungus *Entomophthoraphytonomi* (Zygomycetes: Entomophthoraceae) in larvae of the alfalfa weevil (Coleoptera: Curculionidae). *Great Lakes Entomology*, 13(3): 149–150.

Carruthers, R. I., and R. S. Soper. 1987. Fungal disease. *InEpizootiology of Insect Disease*, eds. J. R. Fuxa, and Y. Tanada, ch. 12, 357–416. New York: John Wiley and Sons.

Cothran, W. R., and G. G. Gyrisco. 1966. An entomogenous fungus observed attackin galfalfa weevil adults in New York. *Journal of Economic Entomology*, 59(1): 243–247.

Danay-Tous, A. H., H. Farazmand, A. Oliaei-Torshiz, and M. Sirjani. 2014. Effect of red pepper and garlic extract impact on pistachio psylla nymph *Agonoscena pistacian* in field conditions. *Biocontrol in Plant Protection*, 1(2): 91–99.

Ebadollahi, A., and P. Tajmiri. 2016. Managementof Alfalfa weevil *Hypera postica* (Gyllenhall) by somenative plants to increase the production efficiency. In *First National, Agriculture and Entrepreneurship Conference Agriculture Faculty of Tarbiat ModaresUniversity*, pp: 10. 14-16 November, Tehran

Ebrahimi, M., M. H. Safaralizadeh, and O.Valizadehgan. 2012. Neem, eucalyptus and laurel essential oils impacts on biological parameters of *Aphis gossypii* Glover. In *First National Conference of Environmental Protection and Planning of Hamedan*, 17-19 August. Islamic Azad University of Hamadan.

Edwards, O. R., Franzmann, B., Thackray, Debbie, Micic, S. 2008. Insecticide resistance and implications for future aphid management in Australian grains and pastures: a review. In *Australian Journal of Experimental Agriculture*. Vol. 48, No. 12. pp. 1523–1530.

Farazmand, H., G. R. Golmohammadi, and A. Moshiri. 2012. The efficacy of organic pesticides for control of St Pomegranate aphid. *Aphis punicae*Passerini (Hem.: Aphididae). In *Proc. of the1stArdebil Organic National Congress*, 408–411. Ardebil, Iran, 17–18 October.

Gholamzadeh-Chitgar, M., J. Hajizadeh, M. Ghadamyari, A. Karimi-Malati, and H. Hoda. 2016. Effect of sublethalconcentration of *Beauveriabassiana* on cellular energyallocation in the predatory bug, Andrallus spinidens Fabricius (Hem.: Pentatomidae). *Plant Pest Research*, 6(1): 23–35.

González-Cabrera, J., O. Mollá, H. Montón, and A. Urbaneja. 2011. Efficacy of *Bacillus thuringiensis* (Berliner) for controlling thetomatoborer, *Tutaabsoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Biocontrol*, 56(1): 71–80.

Gouamene-Lamine, C. N., K. S. Yoon, and J. M. Clark. 2003. Differential susceptibility toabamectin andtwo bioactive avermectin analogs in abamectin-resistant andsusceptible strains of Colorado potatobeetle, *Leptinotarsadecemlineata* (Say) (Coleoptera: Chrysomelidae). *Pesticide Biochemistry andPhysiology*, 76(1): 15–23.

Harcourt, D. G., J. C. Guppy, D. M. MacLeod, and D. Tyrrell. 1974. The fungus *Entomophthoraphytonomi*pathogenicto the alfalfa weevil, *Hyperapostica*. *The Canadian Entomologist*, 106(12): 1295–1300.

Harcourt, D. G. 1990. Displacement of *Bathyplectescurculionis* (Thoms.) (Hymenoptera:Ichneumonidae) by *B. anurus* (Thoms.) in Eastern Ontaiopopulations of the alfalfa weevil, *Hyperapostica* (Gyll.) (Coleoptera: Curculionidae). *The Canadian Entomologist*, 122(04): 641–645.

Henderson, C. F., and E. W. Tilton. 1955. Tests with acaricides against the brown wheat mite. *Journal of Economic Entomology*, 48(2): 157–161.

Hilburn, D. J.1985. Population dynamics of overwintering life stages of the alfafa weevil, *Hyperapostica* (Gyllenhal). Ph.D. diss. Blackburg, Virginia, USA:Virginia Polytechnic Institute and State University.

Homayonfar, F., and H. Zohdi. 2012. The used of extract of *Azadirachtaindica* in control *Aganoscena pistaciae* at laboratory conditions. In *Proc. of the 20th Iranian Plant Protection Congress*, 366. Shiraz, 25-28 August.

Javvi, E., and A. A.Pourmirza. 2005. Studies on the effect of *Bacillus thuringiensis*var. *kurstaki*on different larval instars of Colorado potatobeetle, *Leptinotarsadecemlineata* (Say),and the role of synergists in enhancement of its efficiency under laboratoryconditions. *Journal of Water and Soil Science-Isfahan University of Technology*, 8(4): 187–199.

Kamangar, S., and J. Habibi. 2006. Investigation some methods in controlling alfalfa weevil, *Hyperapostica* (Col.: Curculionidae). *Journal of Entomological Society of Iran.* 26(1): 1–12.

Los, L. M. 1982. Evaluation of several natural control agents of the alfalfa weevil *Hyperapostica* in Virginia alfalfa fields. M.S. thesis. Blacksburg, VA: Virginia Polytechnic Institute and State University.

Los, L. M., and W. A. Allen. 1983. Incidence of *Zoophthoraphytonomi* (Zygomycetes: Entomophthorales) in *Hyperapostica* (Coleoptera: Curculionidae) larvae in Virginia. *Environmental Entomology*, 12(5): 1318–1321.

Moradi-Vajargah, M., H. Rafiee-Dastjerdi, A. Golizadeh, M. Hassanpour, and B.Naseri. 2013.LaboratoryToxicity and Field Efficacy of Lufenuron,Dinotefuran and Thiamethoxam against *Hypera postica* (Gyllenhal, 1813) (Coleoptera: Curculionidae). *Munis Enthomology and Zoology Journal*, 8(1): 448–457.

Muka, A. A. 1976. *A disease of the alfalfa weevil in New York*. Forage Insect Research Conference, 18: 28–29.

Neima, H. A., and R. A.Mustafa. 2016. Insecticidal effect of some plant extracts on the Alfalfa Weevil *Hyperapostica* (Coleoptera: Curculionidae). *Journal of Entomology and Zoology Studies*, 4(6): 545–549.

Nordin, G. L., G. C.Brown, and J. A.Millstein. 1983. Epizotic phenology of Ervynia disease of the alfalfa weevil Hyperapostica (Gyllenhal) (Coleoptera: Curculionidae), in central Kentucky. *Environmental Entomology*, 12(5): 1350–1355.

Oroumchi, S., and C. Lorra. 1993. Investigation on the effect of aqueous extracts of neem and chinaberryon development and mortality of the alfalfa weevil *Hyperapostica* Gyllbnh (Col., Curculionidae). *Journal of Applied Entomology*, 116(1-5): 345–357.

Pandey, K. C., and A. Singh. 1984. Laboratoryevaluation of medics for resistance to lucerne weevil. *The Indian Journal of Genetics and Plant Breeding*, 44(2): 253–258.

Puttler, B., D. L. Hostetter, S. H. Long, and R. E. Pinnel. 1978. *Entomophthoraphytonomi*, a fungal pathogen of the alfalfa weevil in the mid-great plains. *Environmental Entomology*, 7(5): 670–671.

Reddy, G. V. P., F. B. Antwi, G. Shrestha, and T. Kuriwada. 2016.

Evaluation of toxicity of biorational insecticides against larvae ofthe alfalfa weevil. *Toxicology Reports*, 3: 473–480.

Robertson, J. L., and H. K. Preisler.1992. *Pesticide Bioassays with Arthropods*. 4th ed. CRC Press.

Saeidi, K. 2007. Preliminary studies on natural enemies of alfalfa weevil *Hypera postica* (Gyllenhall), in Yasouj. *Journal of FarmAnimal Nutrition and Physiology*, 3(1): 1–13.

Sharma, A., S. Kumar, and R. K. Bhatnaga. 2010. *Bacillus thuringiensis*protein Cry6B (BGSC ID 4D8) is toxic to larvae of *Hyperapostica*. *Current Microbiology*, 62(2): 597–605.

Summers, C. G., and L. D. Godfrey. 2004. University of California IPM Online; Statewide Integrated Pest Management Program. Ebruary.

Torres-Gregorio, J., J.Argente, M. A. Diaz, and A. Yuste. 2009. Application de *Beauveria bassiana*en lalucha biologica contra *Tutaabsoluta*. *Agrícola Vergel:Fruticultura, Horticultura, Floricultura*, 326(129): 129–132.

Ware, G. W., and D. M. Whitacre. 2004. An introduction to insecticides. In *The Pesticide Book*, ed. Tomlin C, 6th ed, ch. 12, 496–505. Willoughby, Ohio:Meister Media Worldwide.