LABORATORY TOXICITY AND FIELD EFFICACY OF LUFENURON, DINOTEFURAN AND THIAMETHOXAM AGAINST *HYPERA POSTICA* (GYLLENHAL, 1813) (COLEOPTERA:CURCULIONIDAE)

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ABSTRACT: Toxicity of the insect growth regulator lufenuron and two neonicotinoids, dinotefuran and thiamethoxam, for the alfalfa weevil, *Hypera postica* (Gyllenhal, 1813), was determined through exposure of the second instar larvae and adults to dipped alfalfa leaves under laboratory conditions at 25±1 °C, 60±5% RH ,16:8 L:D. Based on mode of action, the mortality of the treated larvae and adults was recorded after 72 hours for lufenuron, and after 24 hours for dinotefuran and thiamethoxam. LC_{50} values for second instar larvae were 34.32, 24.91.32.9, 15.82 AI L-1 and for adults were 175.67, 289.76, 164.02 AI L-1 for dinotefuran, thiamethoxam and lufenuran, respectively. Results showed that lufenuron was the most toxic to both larvae and adults of *H. postica* among insecticides tested. Once the height of alfalfa field reached to about 20 cm, a single treatment was made on May 11th. All insecticides reduced the mean number of alfalfa weevil. According to results of laboratory and field experiments, lufenuron might be a more valuable chemical to adequately control *H. postica* with little adverse effects on environment. lufenuron may be considered as alternative chemicals to other compounds with a high potential for controlling certain pests and with less adverse effects on natural enemies.

KEY WORDS: Alfalfa weevil, Dinotefuran, LC_{50}, Lufenuron, Thiamethoxam.

Alfalfa, *Medicago sativa* L., is one of the important forage crops cultivated in most regions of the world. Lucerne is another name sometimes used for alfalfa, which was first cultivated in Iran. Because of its importance among forage crops, alfalfa is referred to as the 'Queen of Forages' (Caddel et al., 2003). Alfalfa is an extremely adaptable plant and can be grown under a wide range of soil and climatic conditions. It is annually attacked by a diversity of arthropod pests. Among arthropods attacking alfalfa, *Hypera postica* (Gyllenhal) is the most damaging phytophagous pest and the major limiting factor in alfalfa production in the most regions of the world (Blodgett et al., 2000; Danielson et al., 2006).

The alfalfa weevil is a snout beetle that is usually univoltine (Radcliffe & Flanders, 1998; Caddel et al., 2003). Both larvae and adults of alfalfa weevil are voracious feeders damaging terminals, foliage and new crown shoots. The larvae cause indirect damage by feeding on and removing the highly digestible solute portion of the cell, which is intended for livestock (Summers, 1998). Direct alfalfa weevil damage is caused by adults and larvae feeding on the growing tips, leaves and buds of alfalfa, which removes crop biomass and reduces harvested yield (Fick & Liu, 1976).

Multiple measures have been examined to manage alfalfa weevil populations. Tolerant cultivars are currently available, often do not provide sufficient protection from alfalfa weevil larval damage (Blodegett et al., 2000). Although biological agents have reduced weevil populations below economic injury thresholds in most regions of world (Richardson et al., 1971), limited commercial utility of biological, cultural and biotechnological control options Iran means that growers remain heavily reliant on insecticides. Application of insecticides has been an essential component of the control programs, and it has prevented economic damage to the alfalfa crop in Iran (Karimpour, 1994). In order to prevent resistance, effective chemical control of the pest requires new insecticides with novel modes of action. Organophosphates, carbamates, pyrthroids (Armbrust & Grysco, 1965; Pass, 1966; Stenhauer & Blickenstaff, 1967; Windbiel et al., 2005) and different compounds such as dieldrin, diazinon, aldrin, trichlorfon and lindane have been used against the pest recently (Vodjdani & Daftari, 1963). In the late 60s, the weevil developed resistance to heptachlor and dieldrin, and these agents no longer provided satisfactory control (Alder & Blickenstaff, 1964; Dorsey, 1966). The effects of some other insecticides on the pest have been studied by several researchers in Iran (Esmaili, 1970; Habibi, 1976; Karimpour & Pourmirza, 2000).

Lufenuron, a chitin synthesis inhibitor, can control immature stages of many pests with relatively low harm to beneficial arthropods and environment (Catangui et al., 1996; Consoli et al., 2001; Wakgari & Gilimore, 2003). An assessment of the potential impact of lufenuron on *Shistocerca gregaria* showed satisfactory impact on it (Hamadah et al., 2012). Dal pogoetto et al. (2012) and Abd El-Mageed et al. (2011) studied susceptibility of some insecticides on *Spodoptera littoralis* and reported lufenuron as the most effective toxicant. Neonicotinoids are a unique chemical class for several insect pest controls owing to their broad spectrum of activity (Elbert et al., 2008). Dinotefuran and thiamethoxam belong to the neonicotinoids and are neurotoxins (Arthur et al., 2004; Tomizawa & Casida, 2005). These chemical compounds, with their specific mode of action, are relatively new in Iran. In the current study, new groups of chemical compounds were tested against *H. postica*.

MATERIALS & METHODS

Insecticides

Three relatively new chemical insecticides in Iran, i.e. lufenuron (Match® 5EC, 200ml/ha, Syngenta Crop Protection, Greensboro, NC), dinotefuran (Dinotefuran MTI-446® 20SG, 120g/ha, Syngenta Crop Protection, Greensboro, NC) and thiamethoxam (Actara® 25WG, 60g/ha, Syngenta Crop Protection, Greensboro, NC) were obtained from Iranian Research Institute of Plant Protection, Tehran and were used in the present studies.

Laboratory Bioassays

Laboratory experiments were conducted at 25±1 °C, 60±5 RH and photoperiod of 16:8 L:D conditions at the Entomology Laboratory of Mohaghegh Ardabili University. A laboratory colony of *H. postica* was established in May 2011 from 1st instars collected from experimental field of Mohaghegh Ardabili University in the suburb of Ardabil, Iran. To assess the insecticidal activity of the chemicals, different concentrations were prepared based on preliminary experiments. The freshly moulted 2nd instars larvae were used in the experiments. The leaf dipping technique was used (Morse et al., 1986; Munger, 1942; Immaraju et al., 1989). Alfalfa leaves were dipped in different concentrations of tested insecticides for 30s, were left to dry at room temperature before being subject to *H. postica* larvae and adults. The control was only dipped in water. Each experiment consisted of five concentrations and control by four replications (15 larvae per dose). Each replication was done on a different day and the solutions used for the treatments were freshly prepared each time. Lufenuron, dinotefuran and thiamethoxam concentrations ranged from 30-3000, 25- 2500, 5-2100 AI L-1 for larvae and 800-500, 150-3000, 200-3500 AI L-1 for adults respectively. Based on insecticides mode of action, mortality was recorded after 72h for lufenuron and after 24h for dinotefuran and thiamethoxam. The mortality percentage of larvae and adults was corrected by using Abbott formula (Abbott, 1925). Probit analysis was performed using a software to calculate LC_{50} and LC_{90} and slope values of the tested chemicals (Finney 1971).Statistical analysis was conducted by SPSS (2004). Confidence intervals for toxicity ratios of LC_{50} values were determined by the method of Robertson and Preisler (1992). If the 95% confidence interval was 1, then the difference between LC_{50}s was considered insignificant (Robertson & Preisler, 1992).

Field Experiments

Field trials were conducted during the spring and summer of 2011 at the experimental field of Mohaghegh Ardabili University, located in a suburb of Ardabil, Iran (38 and 19´ N, 048 and 50´ E and 1340 m altitude). The 20 plots were 5×5 m^2, arranged in a randomized complete block design with five replications, three treatments and one control for each replication. The treatments randomly allocated to the plots were as follows: 1. Recommended dose of lufenuron (200 ml/ha), 2. Recommended dose of thiamehoxam (120g/ha), 3. Recommended dose of dinotefuran (60 g/ha), 4. Control that was treated only with equal amount of distilled water that was used in insecticide applications.

Once the alfalfa reached about 20 cm in height in each plot, a single treatment was made on 11 May, 2011, with a compressed air-powered hand-sprayer with 2 nozzles on a 2.4m boom at a pressure of 10psi. Assessments were done one day before and 3, 7, 14, 21 and 28 days after treatment. Plots were sampled for larval density by counting all living and dead larvae collected from 20 stems randomly selected from each plot. The stems were shaken against a white surface to dislodge the larvae from the plants, and the number of larvae per stems was recorded exactly. Data were subjected to analysis of variance for significance, and SNK test was applied to separate pair comparison. All analyses were performed using the SPSS Version 16.0 (2004) software package.

RESULTS

Laboratory Bioassays

LC_{50} values for 2^{nd} instar larvae and adults of *H. postica* are shown in Tables 1 and 2 respectively. According to the LC_{50} values, the 2^{nd} larvae instar were more susceptible to the insecticides than the adults. The LC_{50} values of the insecticides for adults were about 5-10 times higher than those for 2^{nd} instars. Figs. 1-2 present the relationship between the probit of percentage mortalities and the logarithm of the concentrations of insecticides tested. Lufenuron and thiamethoxam were more toxic than dinotefuran for 2^{nd} larval instars, especially lufenuron was the most toxic compared with two other insecticides with a different mode of action. Lufenuron was also the most toxic compound for alfalfa weevil adults followed by dinotefuran and thiamethoxam, respectively (Table 2).

The slopes of the dose-response lines of the compounds tested were quite steep (Fig. 2).

Results showed that among the insecticides tested, lufenuron was the most effective at low concentrations against larvae and adults of *H. postica*. Neonicotinoids compounds, dinotefuran (with LC_{50} values of 34.320 AI L-1) for larvae of *H. postica* and thiamethoxam (with LC_{50} values of 289.768) for adults, had the least lethal effect on alfalfa weevil.

Field Experiments

Population density of alfalfa weevil during the sampling period was significantly different among treatments (P<0.01) (Fig. 3). Mean number of live and dead alfalfa weevils in different treatments and control in different sampling dates is shown in Table 3. These results indicated that all insecticide treatments significantly reduced the densities compared with control (P<0.05). During the 2011 season, significant differences in *H. postica* catches were observed among treatments and dates, but there were no significant differences between mean number of alfalfa weevils in different plots before insecticide spraying (F= 1.61; df=19; P=0.225). The number of *H. postica* in lufenuron treatment was significantly different just three days after treatment (Table 3), and not on any other day (P≤ 0.05). The date had no observed effect on the impact of lufenuron and two other insecticides on alfalfa weevil after May 10. Although mortality of *H.postica* in insecticide-treated plots resulted in significantly fewer number of weevils compared with the control in all days of sampling (P≤ 0.05), there was no difference among three tested insecticides.

Larvae densities of pest in control increased from May 10 to May 25 (Fig. 3). In lufenuron, thiamethoxam and dinotefuran treatments, density of weevil decreased respectively from 16, 81, 11.96 weevils per plant before treatment to 0.03, 0.05, 0.02, respectively (Fig. 3). Therefore, these chemicals can be used as an alternative to the traditional chemical insecticides to control this pest. So, the application of recommended dose of lufenuron (F=28.64; df= 29; P=0.000), thiamethoxam (F=8.00; df=29; P=0.000) or dinotefuran (F=11.69; df= 29; P= 0.000) reduced significantly the alfalfa weevil population. Also in all insecticides treated plots number of injured stems reduced significantly. Lufenuron had the least injury in stem at 3 days after treatment (F=115.07; df= 29; P=0.000). But in thiamethoxam (F=61.53; df= 29; P=0.000) an dinotefuran (F=22.66; df= 29; P=0.000) there was significant difference among pre-treatment, 3 days after treatment and other date of sampling. Plots were not sampled after 28 days from treatment because many of weevils were beginning to move to diapause phase, and further sampling would not have been meaningful.

In control treatment, rates of density increased gradually until May 25th but after that time, they gradually decreased due to aging till June 8th. After treatment, all plots continued to have significantly fewer weevils than the untreated control (Fig. 3).

All insecticides reduced the mean number of alfalfa weevils and had a significant effect on its mortality.

DISCUSSION

The LC_{50} values were invariably lower for lufenuron than for thiamethoxam and dinotefuron, indicating that the former is more toxic to weevil larvae and adults.

Results showed that with a fairly small increase in insecticides concentration, the mortality would increase considerably. This requires more careful use of these chemicals in the field to prevent exerting a high selection pressure that could eliminate the susceptible insects and lead to selection of resistant ones (Alyokhin et al., 2007).

Recently developed neonicotinyl insecticides such as dinotefuran, thiamethoxam and Clothianidin have been shown to be effective against different pests (Delgrade & Rouland-Lefevre 2002; Corbel et al., 2004; Nault et al., 2004; Wilde et al., 2004). However, there are no reported data on susceptibility of *H. postica* to these chemicals; several insecticides representing various classes of chemistry have been evaluated to estimate LC_{50} of these insecticides against other coleopteran pests. For example, the efficiency of dinotefuran and thiamethoxam were evaluated on adults of a cerambycid beetle, *Anoplophora glabripennis,* and the LC_{50} values were 2.2 ppm for dinotefurran and 1.0 ppm for thiamethoxam. Wang et al. (2005) and Mcleod et al. (2002) also found toxicity of thiamethoxam against the eggplant flea beetle, *Epirtrix fuscula* crotch on eggplant foliage. There are also many other studies conducted evaluating the effect of these insecticides on coleopteran pests (Alyokhin et al., 2007; Acda, 2008; Hoffmann et al., 2008). Since these compounds do not belong to the groups of chemical compounds conventionally applied for *H. postica* control in Iran, so they can be used in rotation with other insecticides. This would confirm that for an effective *H. postica* management program, the same class of insecticides should not be applied more than once within a growing season.

Insect growth regulators (IGRs) are known to be highly effective against many agricultural pests with a relatively low toxicity to mammals and natural enemies (Ishaaya, 1990). Lufenuron is an IGR assessed against several insect pests, such as summer fruit tick, *Adoxophyes orana* (Charmmillot et al., 1991; Ioriatti et al., 1993), cat flea and *Ctenocephalides felis* (Hink et al., 1991). However, there are different results about the effects of these acylurea compounds on the pests. Present experiments indicated that lufenuron, a chitin synthesis inhibitor with demonstrated selectivity in favor of beneficial insects, can provide very good protection of alfalfa from the pest and gives the best control. Control of larvae is especially important since this stage is usually responsible for over 90% of the defoliation caused by *H. postica* (Fick, 1976; Blodgett & Lenssen, 2004). Several studies have been done about field efficacy of several chemicals against alfalfa weevil (Koehler et al,. 1959; Armbrust et al., 1965; Abu Yaman & Naser, 1970; Depew, 1987), but there were not any reports about our tested chemicals on this pest. However, these chemicals have been tested on other coleopteran pest such as efficacy of lufenuron on snout beetles (Echeverri-Molina & Santolamazza-Carbone, 2010).

Thiamethoxam has been applied successfully to other coleopteran pests such as cerambyciidae and chrysomelidae (Alyokhin et al., 2007; Acda, 2008; Hoffmann et al., 2008). Over the last 3 years, sales of the total group have nearly doubled, and future expansion will be driven by growth of the established neonicotinoids, which also will open new opportunities in low-price markets (Elbert et al., 2008). Combined with active life-cycle management such as optimized formulations and new combinations, neonicotinoids could be important chemical for insect control.

Overall, all three insecticides tested in present study significantly reduced density of alfalfa weevil. According to LC_{50} values, Lufenuron with the least value can be suggested as the most potent insecticide. Lufenuron is recommended with satisfactory control due to low values of toxin needed for more mortality percentage, relatively wider margin of safety, reducing cost and risk of insecticide for *H. postica* in Iran. Although additional research with these insecticides is needed, the results presented in this study should aid producers in making alfalfa weevil management decisions.

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Table 1. LC_{50} and LC_{90} values of insecticides on 2nd instars of *Hypera postica* after using the leaf dip method.

Insecticide	LC_{50}[1]	95% C.L.[1,2]		Slope±SE	LC_{90}[1]	X[3]	*df*[4]
		upper	lower				
Lufenuron	15.82[a5]	554.50	187.53	0.69±0.11	1108.37	1.56	3
Dinotefuran	34.32[a]	262.52	104.80	0.82±0.11	1256.41	0.88	3
Thiamethoxam	24.91[a]	174.86	56.60	0.64±0.08	2354.78	2.54	3

[1] mg AI L-1, [2] Confidence Limits, [3] Chi square values, [4] Degrees of freedom, [5] Data in the same letter are not statistically different ($P \geq 0.05$)

Table 2. LC_{50} and LC_{90} values of insecticides on adults of *Hypera postica* after using the leaf dip method.

Insecticide	LC_{50}[1]	95% C.L.[1,2] upper	95% C.L.[1,2] lower	Slope±SE	LC_{90}[1]	X^3	*df* [4]
Lufenuron	164.02[a5]	4190.88	2740.92	2.09±0.29	670.31	3.28	3
Dinotefuran	175.67[a]	44471.54	250.20	1.00±0.15	3338.23	4.84	3
Thiamethoxam	289.76[a]	4886.75	502.78	1.39±0.20	2387.79	2.54	3

[1] mg AI L-1, [2] Confidence Limits., [3] Chi square values, [4] Degrees of freedom, [5] Data in the same letter are not statistically different ($P \geq 0.05$)

Table 3. Efficacy of selected insecticides for control of alfalfa weevil, *H. postica* on alfalfa in 2011.

Treatments	Rate/ha	Mean number (±SE) alfalfa weevil plant: 10 May (1 DBT[1])	14 May (3 DAT[2])	18 May (7 DAT)	25 May (14 DAT)	1 June (21 DAT)	8 June (28 DAT)
Control	-	20.83 ±0.98[aA]	20.49 ±0.09[aA]	28.48 ±1.23[aA]	24.29 ±1.42[aA]	7.45 ±0.08[aA]	2.03 ±0.02[aA]
Lufenuron	200ml	11.96 ±1.65[bA]	11.4 ±0.19[bB]	0.13 ±0.04[bC]	0.22 ±0.03[bC]	0.06 ±0.01[bC]	0.03 ±0.00[bC]
Thiamethoxam	120gr	12.81 ±1.98[bA]	12.71 ±0.17[bB]	0.05 ±0.01[bB]	0.38 ±0.06[bB]	0.09 ±0.01[bB]	0.05 ±0.1[bB]
Dinotefuran	60gr	16.85 ±1.21[bA]	16.23 ±0.09[bB]	0.92 ±0.13[bB]	0.56 ±0.06[bB]	0.44 ±0.05[bB]	0.02 ±0.01[bB]

[1] Day before treatment , [2] Day after treatment, Means with lower letters within the column are significantly different at P<0.05 in comparison with control, Means with capital letters within the row are significantly different at P<0.05 in different date, probability level were estimated using SNK tests

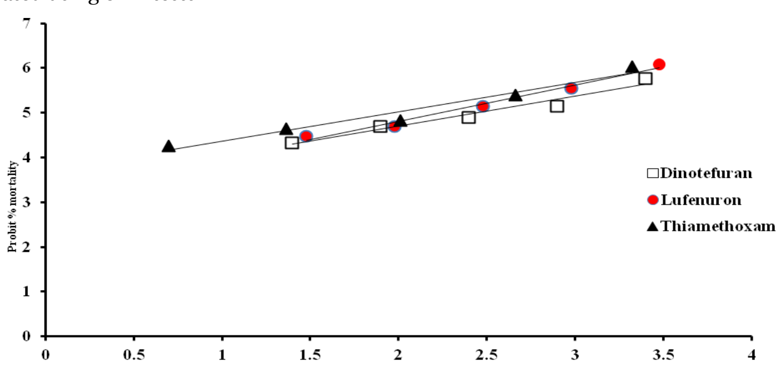

Figure 1. Concentration-response relationship between three insecticides and the 2nd instars of *Hypera postica,* after treatment.

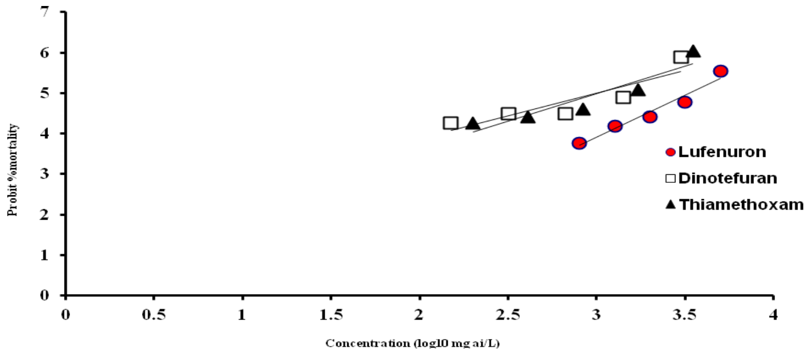

Figure 2. Concentration-response relationship between three insecticides and the adult of *Hypera postica,* after treatment.

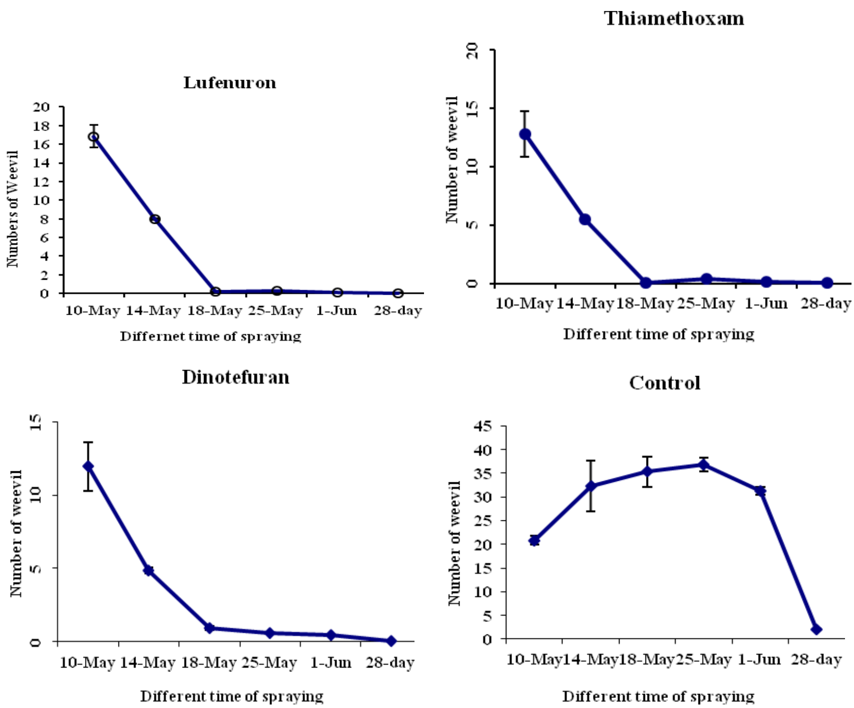

Figure 3. Comparison of fluctuation of densities of the alfalfa weevil at different treatments during 2011.