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The toxicity of three current insecticides against third larval stage of alfalfa weevil, *Hypera postica* Gyllenhal (Col.: Curculionidae)

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Abstract
Because alfalfa forage is a high quality crop in terms of nutrients for livestock, it is important to manage the crop efficiently in the absence, as well as the presence, of serious pests. The alfalfa weevil *Hypera postica* Gyllenhal is a worldwide variable pest that is established in almost all Iranian alfalfa fields and Alfalfa weevil larvae affects quality by attacking the upper canopy. In this research, three insecticides include Deltamethrin, Cypermethrin and Chlorpyrifos were evaluated for their efficacy in the control of 3rd larval stage of *H. postica*, under field conditions. The LC_{25} and LC_{50} values of Deltamethrin, Cypermethrin and Chlorpyrifos were estimated on 3rd larval insect. Furthermore, LC_{25} and LC_{50} values for Deltamethrin, Cypermethrin and Chlorpyrifos on 3rd larval insects were 587.89, 6.14, 186.84 and 1142.64, 110.17, 636.56 ppm for 24 hours and 333.30, 12.03, 135.49 and 772.37, 50.24, 370.47 ppm for 48 hours, respectively. To evaluate effects of three insecticides one experiment with completely randomize design in three replicate by recommendation doses carried out in field conditions. The results show that there were significant differences between treatments (Deltamethrin, Cypermethrin, Chlorpyrifos and control) after 24, 48 and 72 hours. After 24 and 48 hours Cypermethrin was more lethal in comparison to other treatments, and after 72 hours the Chlorpyrifos was lethal. The result showed that for control of larval stage of *H. postica* in fields conditions Chlorpyrifos recommended.

Keywords: *Hypera postica*, deltamethrin, cypermethrin, chlorpyrifos, LC_{25} and LC_{50}

1. Introduction
Alfalfa (*Medicago sativa* Leyss) is a major forage crop fed primarily to dairy cows and other livestock that require high quality feed [7], which was first cultivated in Iran. Alfalfa is also one of the more prolific nitrogen fixing legumes, making it useful in long term rotations as a soil builder that can supply nitrogen to ensuing crops. Because of its importance among forage crops alfalfa is referred to as the 'Queen of Forages' [3]. Alfalfa is an extremely adaptable plant and can be grown under a wide range of soil and climatic conditions. In Iran, more than 600,000 hectares of alfalfa are planted yearly across large parts of Iran [14]. Every year several Iranian alfalfa farms, as well as farms around the world, face hug damages from *Hypera postica* Gyllenhal commonly known as alfalfa weevil [2, 8]. In the alfalfa weevil is a snout beetle that is usually univoltine [3, 13]. *H. postica* not only decreases yield and quality of the first cutting, but can also harm subsequent cuttings [4]. Both larvae and adults damage terminals, foliage and new crown shoots, thereby lowering crop yield and quality [11]. However, the larvae caused the most damage [12]. The weevil causes substantial costs to producers in many regions in terms of both forage yield and feeding value. Alfalfa weevil larvae affects quality by attacking the upper canopy with its emerging leaf buds, which removes emerging leaflet mesophyll that contains the leaf fractions with the highest concentrations of crude protein and soluble nutrients, leaving behind less digestible fibrous components and reducing overall digestibility. Defoliation of early spring growth also damages the alfalfa plant because it further depletes reserve energy that initiated spring growth and reduces prime photosynthetic area that is needed to replenish reserves for regrowth after the first cutting, which can result in reduced stand life [7]. Much alfalfa weevil damage occurs in the first cutting, which is often the most productive. First-generation larvae from eggs laid in stem bases during fall and open periods of winter and early spring cause most of this damage. The eggs hatch when they receive adequate heat units [6], sometime after alfalfa growth has begun.

In this research, the effects of Deltamethrin, Cypermethrin and Chlorpyrifos on 3rd larval stage of *H. postica* were evaluated.

2. Material and Methods

The present study was carried out in Noshan village, Urmia city, West Azerbaijan province, Iran.

2.1 Determine of LC_{50} and LC_{25} values

To estimate the LC_{50} and LC_{25} five concentrations from each insecticides after primary experiments with distill water as control in three replications used in filter paper dipping method on 10 larvae in Petri dish and mortality recorded after 24 and 48 hours.

2.2 Fields bioassay with pesticides on 3rd larval stage

Field trials were conducted during the spring of 2016 at the experimental field located in a suburb of Urmia, Iran. The 12 plots were 4×6 m^2, arranged in a randomized complete block design with three replications, three treatments and one control for each replication. The treatments randomly allocated to the plots were as follows: 1. Recommended dose of Deltamethrin (0.5 Li/ha), 2. Recommended dose of Cypermethrin (0.2 Li/ha), 3. Recommended dose of Chlorpyrifos (2.0 Li/ha) and 4. Control that was treated only with equal amount of distilled water that was used in insecticide applications. Before experiment by quadrat (25×25 cm^2) population of 3rd larval stage was estimated. After 24, 48 and 72 hours spraying pesticide percentage of 3rd larval insect mortality recorded by quadrat.

2.3 Statically Analyses

The LC_{50} and LC_{25} values (with 95% confidence limits) were calculated by using Probit Analysis Statistical Method and mortality data of different treatments subjected to analysis of variance (One Way ANOVA) and mean separation tests were conducted with Tukey's HSD with SPSS statistical analysis software (Version 22.0).

3. Results

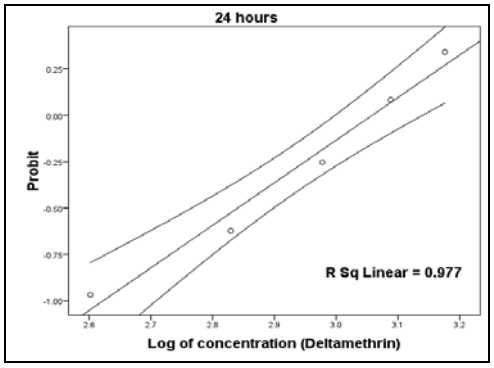
3.1 LC_{50} and LC_{25} pesticides on 3rd larval stage

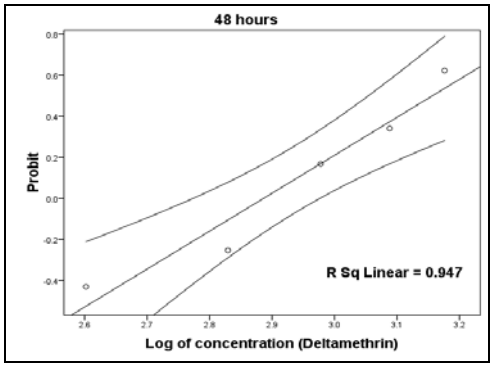
LC_{50} and LC_{25} of Deltamethrin, Cypermethrin and Chlorpyrifos on 3rd larval stages in 24 and 48 hours are shown in Table 1.

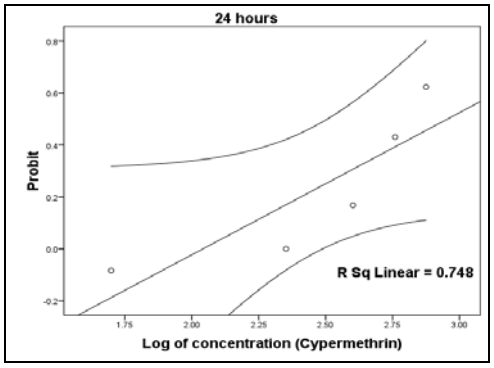
Table 1: LC_{50} and LC_{25} values calculated Deltamethrin, Cypermethrin and Chlorpyrifos effect on 3rd larval stage of *H. postica* within 24 and 48 hours.

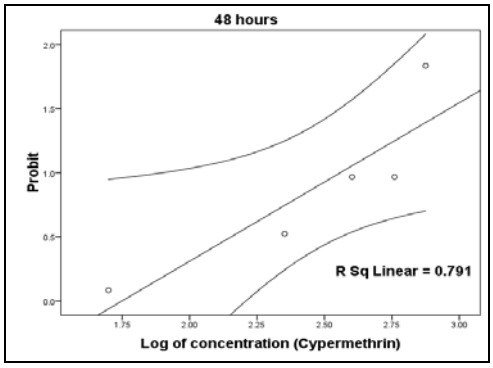
Insecticide	Time Hrs	Total insect	χ^2 (df=3)	Slope±SE	Intercept (a)	LC_{50} (PPM)	LC_{25} (PPM)
Deltamethrin	24	30	0.42	0.56±2.33	7.14	1142.64	587.89
	48	*30*	*0.71*	*0.57±1.84*	*5.33*	*772.37*	*333.30*
Cypermethrin	*24*	30	1.60	0.24±0.53	1.09	110.17	6.14
	48	30	2.73	0.27±1.08	1.84	50.24	12.03
Chlorpyrifos	*24*	30	1.13	0.42±1.26	3.55	636.56	186.84
	48	30	2.17	0.43±1.54	3.96	370.47	135.49

The LC_{50} show that the Cypermethrin was more toxic as compare to Deltamethrin and Chlorpyrifos after 24 and 48 hours.









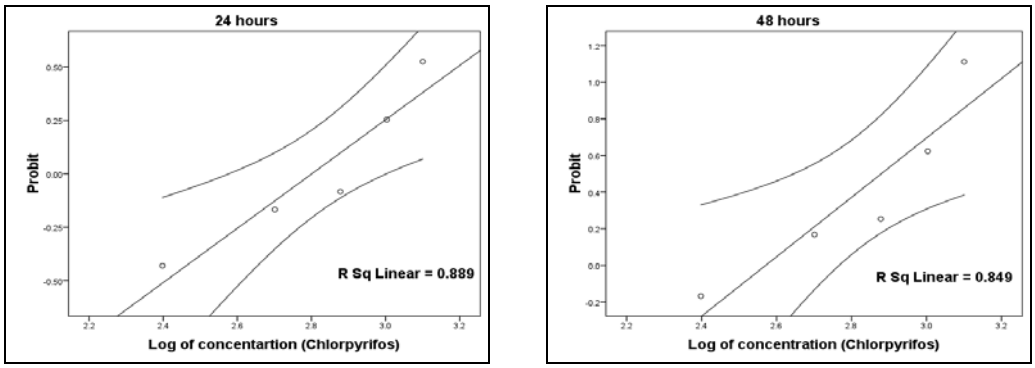


Fig 1: Relationship between log of concentration and Probit in three pesticides with R square

The dose-response and R square show that the relation between concentration and mortality of Deltamethrin had best fitted.

3.2 Fields bioassay with pesticides on 3rd larval stage

Effects of treatments, Deltamethrin LC_{50}, Cypermethrin LC_{50} and Chlorpyrifos LC_{50} and control on 3rd larval insect stage of *H. postica* was evaluated and counting the percentage mortality after 24, 48 and 72 hours. The results showed that there was a significant difference between treatment with 95% confidence in 24, 48 and 72 hours with [F (3, 8) = 1.462, p=0.001)], [F (3, 8) = 3.719, p=0.001)] and [F (3, 8) = 1.894, p=0.001)], respectively (Fig 2).

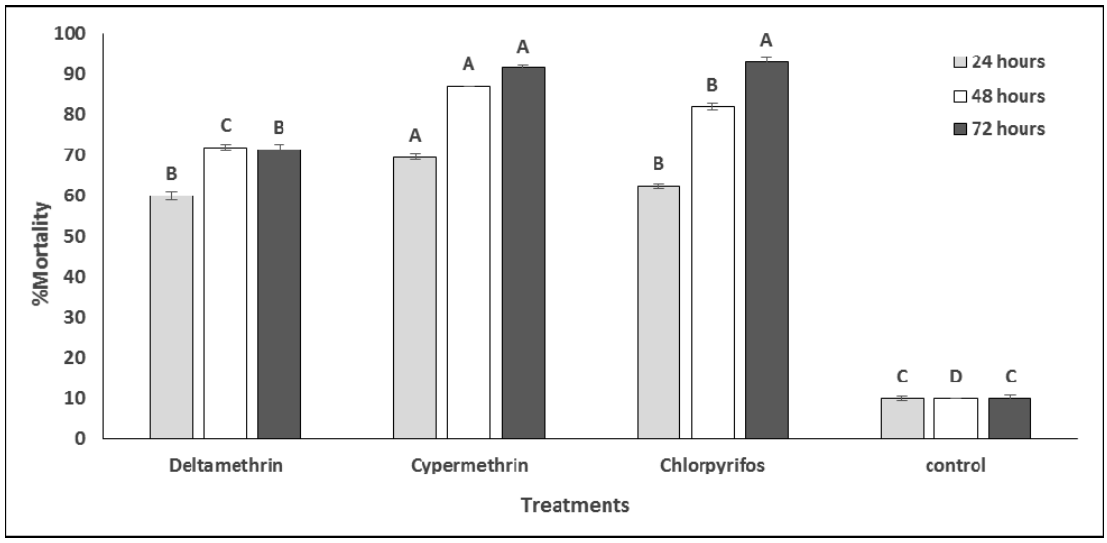


Fig 2: Mortality percentage ± SE of different treatments include Deltamethrin, Cypermethrin, Chlorpyrifos and control on 3rd larval stage after 24, 48 and 72 hours.

The results show that there were significant differences between treatments (Deltamethrin, Cypermethrin, Chlorpyrifos and control) after 24, 48 and 72 hours. After 24 and 48 hours Cypermethrin was more lethal in compare other treatments, and after 72 hours the Chlorpyrifos was lethal (Fig 2).

4. Discussion

According to results of laboratory and field experiments, Cypermethrin and Chlorpyrifos were more valuable chemical to adequately control 3rd larval stage *H. postica*. Cypermethrin and Chlorpyrifos may be considered as alternative chemicals to other compounds with a high potential for controlling certain pests. For controlling *H. postica* in Iranian alfalfa felids, several pest management strategies have been suggested [8, 9, 10]. But the best management method is use of effective pesticides in integrated pest management in outbreak of pest [10]. For this aim we assessed three insecticide against this pest. The LC_{50} values were invariably lower for Cypermethrin than for Deltamethrin and Chlorpyrifos, indicating that the former is more toxic to 3rd larval stage. 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 [1].

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.

Overall, all three insecticides tested in present study significantly reduced density of alfalfa weevil. According to LC_{50} values after 24 and 48 hours Chlorpyrifos and Cypermethrin with the least value can be suggested as the most potent insecticide. Cypermethrin 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 [10].

In general, our study showed that the tested pesticide can be used to manage larval stage of *H. postica*. Further research is needed to determine the impact of these insecticides on non-target insects and natural enemies.

5. Acknowledgements

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