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Insecticidal effect of some plant extracts on the Alfalfa Weevil *Hypera postica* (Coleoptera: Curculionidae)

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Abstract
This study was conducted to examine the three types of plant extracts against the Alfalfa weevil *Hypera postica* under laboratory condition, namely: Neem *Azadirachta indica* (Meliaceae), Oleander *Nerium Oleander* (Apacynaceae) and Eucalyptus *Eucalyptus camaldulensis* (Myrtaceae). Concentrations of 0, 10, 20, and 40 were tested for all plant extracts. Mortality rates for larvae were determined at 2–4 days and for adults at 2-8 days after treatment. This study showed that Neem leaves extract was the most effective, causing 73.33% after 4 days larvae mortality within 4 days and 96.66% adult mortality within 8 days after treatment among all the tested biorationals. Our results suggested that Neem leaves extract insecticide could be applied for H. postica control as an alternative insecticide for chemicals. In general, the toxicity results of all tested plant extracts against all instars and adult phase of *H. postica* in this research showed a good relationship between mortality percentage and number of days after treatment. The mortality rate increased with number of days for all examined biorationals.

Keywords: Alfalfa weevil, *Hypera postica*, biorationals, plant extracts

Introduction
Alfalfa (*Medicago sativa* L.), which is referred to as the "Queen of Forages" and known as Lucerne, is one of the most important forage crops worldwide. Though Alfalfa as a perennial crop normally lives for 4 to 8 years, it can live longer (more than 20 years) depending on variety and climate [1]. Alfalfa is most valued and grown forage worldwide due to its being wide adaptation, biological nitrogen fixation, soil improvement and benefits to subsequent crops for being often grown in rotation system that provides benefits to farmers and agro-ecosystems. Additionally, the plant provides high energy and safe feed and is in a high demand by meat and dairy industry of a wide range of ruminant livestock (such as cows, sheep and goats) and non-ruminant (such as horses) as hay, silage, green chop, pelletized cubed or grazed [2]. Recently, the increase of meat and dairy production industry led to increase of alfalfa cultivation in Iraq and Kurdistan region. The cultivated forage area in Iraq was about hundred thousand hectares in 2011 [3]. Previous studies show that only a few numbers of insects infect alfalfa while these insects can cause a significant yield and quality losses when presented in a high number. The most important pests include the alfalfa weevil in spring, alfalfa caterpillar larvae in the warmer months and a complex of aphids throughout the year. Meanwhile, alfalfa plants support insect diversity that some of them are beneficial insects in providing biological pest control [4].
Alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera; Curculionidae) is a destructive pest on alfalfa (*Medicago sativa* L.) and closely related legume plants [5]. Both larvae and adults of alfalfa weevil, *H. postica* are voracious feeders damaging foliage, and a new crown shoot of alfalfa plant [4]. In fields with severe infection may appear silver or white, because most alfalfa leaves consumed or skeletonized; retarding new growth and resulting in low plant vigor and yield in subsequent harvests [6].
The objective of this study is to investigate the efficacy of some plant extracts treatment methods to substitute synthetic chemical pesticides with plant extracts as a part of integrated pest management and avoid using insecticides and reduce its risks to people, water resource and wild life.

Materials and Methods

This study conducted at the Insect Laboratory of Horticulture Department/College of Agriculture/ Sulaimani Polytechnic University SPU and Central Laboratory of Faulty of Agricultural Sciences/ University of Sulaimani.

Plant materials and extraction methods

Preparation of powder and extracts

The powder preparation: Eucalyptus, Neem, and Oleander plant Leaves were collected during summer season in 2015. The harvested leaves were washed with distilled water and then shade air dried at room temperature 25 ± 3 ^{0}C for 15 days. Thereafter the leaves ground to a fine powder using home grinder followed by sieving. Powders were kept in a properly sealed polythene bag at room temperature to prevent quality losses [7].

Plant leaves extracts were prepared according to Harborne (1984) [8]. 100 g of each powdered leaves was separately weighed and transferred to 1 liter of distilled water and stirred for 15 minutes then left for 24 hours. The solution was filtered using muslin cloth. This solution was then centrifuged at 3000 rpm for 10 min. The supernatant was decanted. The pooled extracts were made up with distilled water to a final volume. The pellet was discarded. The pooled aqueous extract was evaporated to dryness at a temperature 45 °C in the oven. The dried extract was used in stock solution preparation 100% concentration. Further dilutions were made for bioassays.

Sampling of alfalfa weevil adults population

Alfalfa adults were collected during October, November and December 2015 in Reshien village, located in Halabja city, Kurdistan region, Northern Iraq. Alfalfa weevil actively appears in late autumn in Kurdistan region/Northern Iraq [9, 10]. Normally, Alfalfa weevil have variability in oviposition and may deposit a greater number of eggs from late October to mid-March, in other words, oviposition occurs during the fall, winter and spring seasons [9-12].

Rearing insects

After the insects have been collected, they were taken to the laboratory for rearing inside a wooden cage (dimensions: 40 * 40 * 80 cm) with a cloth cover and a circular whole left to deal with the insect under laboratory conditions (temperature 27 – 30 °C, and humidity rate 65 -70%). The insects were reared and fed on alfalfa planted in the small pods and placed inside the cage until 2nd and 4th instar larvae and adult stages. Then each of 2nd and 4th instar larvae and adult insects were transferred into a clear disposable plastic box (dimensions: 4.5 * 8 * 10 cm) for spraying according to research plan.

Toxicity of Extracts on *H. Postica*

Toxicity tests were done in the laboratory. Three replicate plastic box (dimensions: 4.5 * 8 * 10 cm), containing a total of 30 of each 2nd and 4th instar larvae and adults separately, were sprayed using perfume sprayer with 2ml of each Eucalyptus, Neem, and Oleander plant extracts in four different concentrations 0, 10, 20, and 40%. Plastic boxes were lined with moistened filter-paper. Each plastic box was contained similar adequate amount of alfalfa stem and leaves as larval food and changed every couple days. The plastic boxes were kept under the same laboratory conditions as used for insect rearing. The corrected mortality percentages of larvae were recorded after two days of treatment. Abott's formula was used to adjust for control mortality [13].

(Abott's formula)

$$\text{Corrected\%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

Where: n=Insect population, T=Treated, Co=Control

Statistical Analysis

Data were analyzed based on completely randomized design (CRD) using (SAS) software. Mean comparison was done using Duncan's multiple range tests at $p \leq 0.05$.

Results and Discussion

All the plant extracts used in this experiment had an effect on the correct mortality of alfalfa weevil; however there was a difference in their efficacy against the alfalfa weevil larvae (See Table 1 & 2) and (Fig. 1 & 2). The effectiveness of the tested extracts rising correct mortality was; Neem > Olender > Eucalyptus, compared to the controls.

The results presented in Table 1 shows different affectivity of plant extracts causing different correct mortality percentages within 2 and 4 days after treatment among all the tested plant extracts at 0, 10, 20 and 40% concentration.

As presented in table 1, the mortality percentage of 2nd instar larvae after 2 and 4 days of treatment with Neem, Olender and Eucalyptus leaves extracts at 0, 10, 20 and 40% concentration were differences in the correct mortality percentages for each treatment.

As illustrated in table 1, the highest rate of correct mortality of 2nd instar larvae was recorded for Neem extract 36.67 ± 3.33 at 40% concentration after 2 days, which is not significantly different from the mortality percentage recorded for Oleander extract 30.00 ± 5.00 at the same concentration.

The mortality percentages of larvae treated with Neem, Oleander, and Eucalyptus extracts after 2 days at 20% concentration were 26.66 ± 3.33, 16.66 ± 5.00 and 0.00 ± 0.00 chronologically after 2 days of treatment. Meanwhile, the lowest mortality percentage was recorded for larvae treated with Eucalyptus leaves extract 0.00% at 10 and 20% concentration followed by Neem extract 6.66± 5.00 at 10% concentration after 4 days of treatment

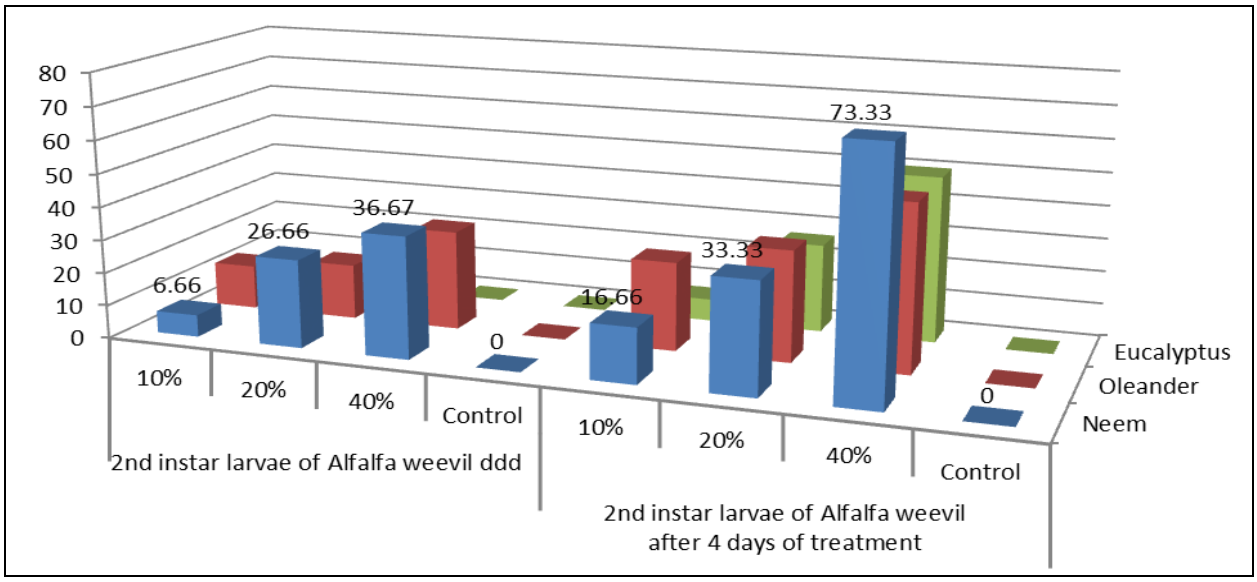

Fig 1: The 2nd instar larvae mortality percentage and time changes with different concentrations for all treatments

Table 1: The differences among the extracts type and concentration on the *H. postica* individual's mortality percentage

Concentration / Extract plant	Correct mortality							
	2nd instar larvae of Alfalfa weevil After 2 days of treatment				2nd instar larvae of Alfalfa weevil after 4 days of treatment			
	10%	20%	40%	Control	10%	20%	40%	Control
Neem	6.66± 5.00ef	26.66 ± 3.33 abc	36.67 ± 3.33 a	0.00 ± 0.00 f	16.66± 10.00de	33.33 ± 3.33 c	73.33 ± 3.33 a	0.00 ± 0.00 f
Oleander	13.33 ± 0.00 de	16.66 ± 5.00 cde	30.00 ± 5.00 ab	0.00 ± 0.00 f	26.66 ± 5.00 cd	33.33 ± 10.00 c	50.00 ± 5.77 b	0.00 ± 0.00 f
Eucalyptus	0.00 ± 0.00 f	0.00 ± 0.00 f	0.00 ± 0.00 f	0.00 ± 0.00 f	6.67 ± 3.33 ef	26.66 ± 3.33 cd	50.00 ± 5.77 b	0.00 ± 0.00 f

Table 2: The differences among the extracts type and concentration on the *H. postica* Adult phase mortality percentage

Concentration	Adult phase of Alfalfa weevil after 2 days of treatment				Adult phase of Alfalfa weevil after 4 days of treatment				Adult phase of Alfalfa weevil after 6 days of treatment				Adult phase of Alfalfa weevil after 8 days of treatment			
Extract plant	10%	20%	40%	Control	10%	20%	40%	Control	10%	20%	40%	Control	10%	20%	40%	Control
Neem	10.00 ± 10.00 cd	23.33 ± 3.33 b	46.67 ± 3.33 a	0.00 ± 0.00 e	26.66 ± 5.00 c	56.67 ± 6.67 b	76.67 ± 3.33 a	0.00 ± 0.00 f	43.33 ± 5.00 b	83.33 ± 3.33 a	93.33 ± 6.67 a	0.00 ± 0.00 e	46.66 ± 5.00 bc	93.33 ± 3.33 a	96.66 ± 6.67 a	0.00 ± 0.00 f
Oleander	3.33 ± 3.33 de	6.66 ± 5.00 cde	13.33 ± 3.33 c	0.00 ± 0.00 e	10.00 ± 5.00 ef	16.66 ± 5.00 cde	23.33 ± 0.00 cd	0.00 ± 0.00 f	20.00 ± 5.00 cd	26.00 ± 5.00 c	30.00 ± 0.00 c	0.00 ± 0.00 e	26.66 ± 5.00 e	40.00 ± 5.00 cd	53.33 ± 5.00 b	0.00 ± 0.00 f
Eucalyptus	0.00 ± 0.00 e	0.00 ± 0.00 e	3.33 ± 3.33 de	0.00 ± 0.00 e	6.67 ± 3.33 ef	6.67 ± 3.33 ef	13.33 ± 3.33 de	0.00 ± 0.00 f	13.33 ± 3.33 d	13.33 ± 3.33 d	23.33 ± 3.33 cd	0.00 ± 0.00 e	26.66 ± 3.33 e	33.33 ± 3.33 de	46.66 ± 3.33 bc	0.00 ± 0.00 f

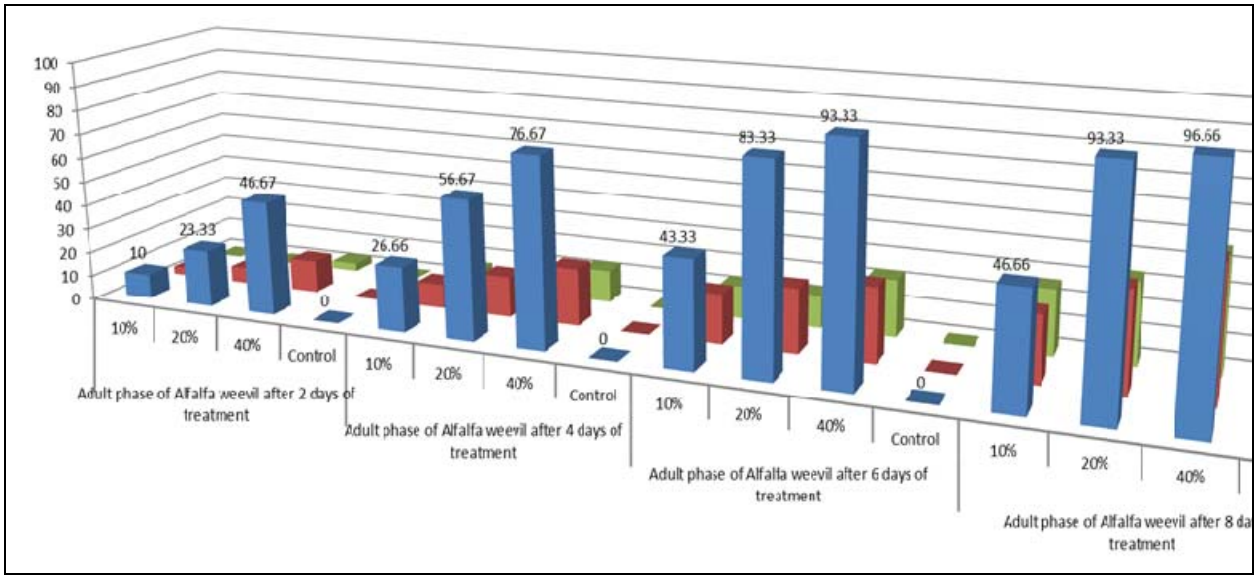

Fig 2: The adult phase mortality percentage and time changes with different concentrations for all treatments

Among all tested biorational insecticides, Neem caused the highest mortality to *H. postica* adults, reaching 96.66% mortality at 40% concentration after day 8 and also caused the highest mortality to *H. postica* 2nd instar larvae reaching 73.33% mortality at 40% concentration after day 4 of treatment (Table. 1 and 2).

In general, the toxicity results of all tested plant extracts against all instars and adult phase of *H. postica* in this research showed a good relationship between mortality percentage and number of days after treatment. The mortality rate increased with number of days for all examined biorationals [14-18]. Generally, lethal time decreased with increasing concentrations for all treatments (See Fig. 1, 2 and 3). The previous studies showed that the Eucalyptus and Neem plant extracts contain compounds that are toxic and time dependence on insect larvae [14, 15, 17- 22].

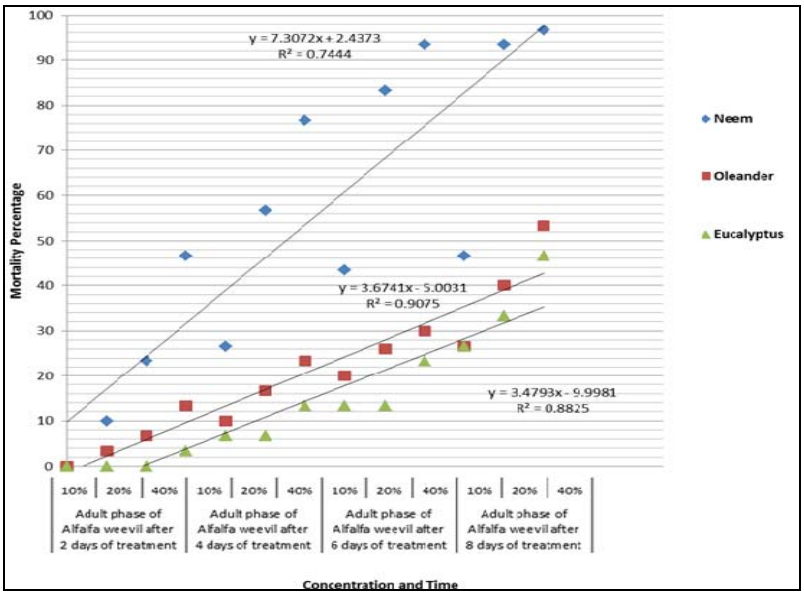

Fig 3: Scatter plot of the adult phase mortality percentage and time changes with different concentrations for all treatments

Neem plant extract was found the most effective biorational insecticide compared to other tested plant extracts (Table 1 and 2). Among other extracts, based on lethal concentrations estimated at day 4 for 2nd instar larvae and day 8 for adults, Oleander plant extract was second most effective biorational insecticide followed by Eucalyptus (Table 2) (See Fig, 2 and 3). The result showed that none of the treatments had effectiveness at par compared with Neem plant extract. In this study, Neem caused 93.33% mortality of *H. postica* adults within 8 days after treatment. Based on the relative effectiveness, Neem was the most effective among the treatments. This extract is known to be effective on other pests or natural enemies like; moths [17, 19], aphids [19, 20] and thrips. The active ingredient in Neem, has been observed to be remarkably benign to pollinators especially bees, though it has low toxicity to many beneficial insects [19]. These results agree with Oroumchi and Lorra [21] who reported that aqueous extracts of Neem seed kernels and leaves, and chinaberry *Melia azedarach* L. (Meliaceae) leaves applied to alfalfa leaves in the laboratory caused high mortality in the larval stages of *H. postica*.

Conclusion

In conclusion, these used natural extracts in this study can be considered as potential pesticide for Alfalfa weevils control and other insects as a part of IPM control especially Neem extract, as has been previously shown by different authors for other insect pests.

Recommendation

Further studies need to be carried out on the nature of plant extracts and their phytotoxicity potential to fruit and foliage for better understanding of their mode of action and limitation of the chemical pesticides use.

References

1. USAID Inma. Alfalfa production guide for Iraq. United State Agency for International Development. Inma agribusiness program, 2011, 138.
2. Putnam D, Russelle M. The Importance and Benefits of Alfalfa in the 21st Century. California Alfalfa and Forage Association, 2001. Available at http://agric.ucdavis.edu/files/242006.pdf (Viewed on 30th September 2016)
3. Omer TMA. FAO: Country Pasture/Forage Resource Profile, 2011. Available at http://www.fao.org/ag/AGP/AGPC/doc/Counprof/Iraq/Iraq.html. (Viewed on 30th September 2016)
4. Summers CG, Godfrey LD, Natwick TE. Managing Insects in Alfalfa University of California, Division of Agriculture and Natural Resources, 2007, 8295,
5. Byrne HD, Blickenstaff CC. Host- plant preference of the alfalfa weevil in the field. J Econ. Entomol. 1968; 61:334-335.
6. Ignoffo CM. Entomopathogens as insecticides, Environ. Lett, 1975; 8:23-40.
7. Chayengia B, Patgiri P, Zeenat Rahman, Saurabh Sarma. Efficacy of different plant products against *Sitophilus oryzae* (Linn.) (Coleoptra: Curculionidae) infestation on stored rice, Journal of biopesticidies, 2010; 3(3):604-609
8. Harborn JB. Phytochemical methods aguids to modern techniques of plants analysis. 2nd ed. Chapman and Hall, London, New York, 1984, 288.
9. Khaphy AA. Ecological, Biological and Chemical Control Studies on Alfalfa Weevil Hypera fascocinera (Marsh.) (Coleoptera: Curculionidae) in Ninevah. M.Sc thesis. College of Agriculture and Forestry, Mosul University, Iraq, 1981, 178.
10. Mustafa RA. Eco- Biological Study of Alfalfa Weevil

Hypera postica Gyllenhal (Coleoptera: Curculionidae) and It's Control by some Entomopathogenic Fungi in Duhok Governorate. M.Sc. Thesis. University of Duhok, Iraq, 2013; 50:147-149.
11. Woodside AM, Bishop JL, Pienkowski RL. Winter oviposition by the alfalfa weevil in Virginia. J Econ. Entomol, 1968; 61:1230-1232
12. Stark JA, Berberet RC, Cuperus GW. Mortality of overwintering eggs and larvae of the alfalfa weevil in Oklahoma. Environ. Entomol, 1994; 23:35-40
13. Abbott WS. A method of computing the effectiveness of an insecticide. J. Econ. Ent, 1925; 18:265-267.
14. Tang YQ, Weathersbee AA. Effect of Neem Seed Extract on the Brown Citrus Aphid (Homoptera: Aphididae) and its *Parasitoid Lysiphlebus testaceipes* (Hymenoptera: Aphidiidae). Environmental Entomology, 2002; 31(1):172-176.
15. Elbanna SM. Larvaecidal effects of Eucalyptus extract on the larvae of Culex pipiens mosquito. International Journal of Agriculture and Biology. 2006; 8:896-897.
16. Younis GH. The Effect of Some Extracts on the Stone Fruit Aphid Hyalopterus Pruni in Duhok Region. International Journal of Pure and Applied Sciences and Technology. 2013; 18(2):39.
17. Mustafa Rebwar A, Neima Hemin A. Sustainable control of pomegranate carob moth with some biorationals, Journal of Entomology and Zoology Studies. 2016; 4(5):748-751
18. Reddy GVP. Evaluation of toxicity of biorational insecticides against larvae of the alfalfa weevil. Toxicology Reports, 2016; 3:473-480.
19. National Research Council. Neem: A Tree for Solving Global Problems. National Academy Press, Washington, D.C., 1992.
20. Lowery Thomas D, Isman Murray B. Insect growth regulating effects of Neem extract and azadirachtin on aphids. Entomol. exp. Appl, 1994; 72:77-84.
21. Oroumchi S, Lorra C. Investigation on the effects of aqueous extracts of Neem and chinaberry on development and mortality of the alfalfa weevil *Hypera postica* Gyllenh (Col., Curculionidae), J Appl. Entomol. 1993; 116:345-351.
22. Wickramananda IR, Mordue AJ, Allan EJ. Insecticidal Activity of Leaf Extracts of Neem (*Azadirachta indica* A. Juss.), Tropical Agricultural Research. 1998; 10:282-290