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Study on the bioecology of alfalfa leaf weevil *Hypera postica* Gyll. (Coleoptera: Curculionidae) and Its natural enemies populations

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Abstract
Alfalfa leaf weevil (ALW) *Hypera postica* Gyll. (Coleoptera: Curculionidae) is regarded as one of the most important pests of alfalfa in West Azerbaijan Province, Iran. In order to investigate the population dynamics of the pest, the present study was performed in two regions Saadel (Chaldoran) and Shoat (Maku) during 2014-2015. ALW as well as other insects were collected through regular weekly samplings from alfalfa fields in the regions using nets for catching insects. Based on the behavioral characters of the weevil, 100 net-hits were performed along field diameters in early mornings. The density of the weevil larvae was determined per net-hit. Aspirators were applied to gather mature ALWs from field soil surface. The pest begins its activity in Maku on 4th April and in Chaldoran on 5th May. Its peaks of activity were determined in 19th May and 11th June in Maku and Chaldaran, respectively. The study on the dynamics of the populations of the important parasitoids, *Bathyplectes curculionis* Thomson, *B. anurus* Thomson, and *Oomyzus* (=*Tetrasticus*) *incertus* Ratzeburg showed that th most and the least population densities belonged to *B. anurus* and *O. incertus*. The population peak of *B. curculionis* and *B. anurus* were recorded in Maku at the 15th of May and the 7th July, respectively. In Chaldoran, the population peak of *B. curculionis* and *B. anurus* were observed on the 8th June and 6th July. The population densities of the predators in 2014 and 2015 were similar, however, the population density of *Coccinella septempunctata* was higher than that of *Chrysoperla carnea* in both regions and years.

Keywords: alfalfa, *Hypera postica*, bioecology and natural enemies

1. Introduction
Alfalfa (*Medicago sativa*) has found particular importance among other fodder plants because of its high quality, scrumptiousness, and nutritive content such as various minerals, protein matters, and different types of vitamins [1]. ALW, *Hypera postica* (Coleoptera: Curculionindae) is regarded as one of the most dangerous pests of alfalfa plants. The insect feeds on shoots in all its larval and mature stages, and if the population density of the pest exceeds 50 larvae per square meter, all leaves of the attacked plant are eradicated and dried, and finally a net of leaf veins remains hanging from the plant. In some cases, more than 90% of the yield in the first harvest is lost as the result of the pest invasion [2, 3]. The damage season of the pest starts since February in warm regions of Khuzistan, and Sistan & Baluchistan, and lasts till May, while it is postponed to mid-late March dependant on prevalent temperatures of spring in cold mountain areas of northern and central Iran [3].
More than twelve parasitic species have globally been identified on ALW of which a few have been applied in the biological control of the pest [4-6]. Van den Bosch and coworkers [7] listed five wasp species parasitic on ALW in Iran: *Patasson* sp. (an egg parasite, Hym.: Mymaridae), *Bathyplectes curculionis* Thom. (parasitic on juvenile larvae, Ichneumonidae, Hym.), *B. anurus* Thom. (parasitoid of larvae, Ichneumonidae), *Dibrachoides druso* Walk. (parasitoid of the larva inside cocoon, Pteromalidae), and finally *Habrocytus* sp. (parasitic on prepupae and pupae, Pteromalidae).
Because of the importance of alfalfa in West Azerbaijan as well as the significance of ALW damage in the Province, the present study was performed in order to determine the local dynamism of ALW population and to identify the diverse parasitoid and predator insect species in the region, where the dynamism of most important species populations were also studied.

In the United States of America, a wasp species, *Anaphes luna* (Hym.: Mymaridae) has been collected as a parasite on the pest eggs and its efficacy in the biological control of ALW has been studied [8]. The wasp parasites 6% to 8% of ALW eggs [9]. The parasitoid wasp, *Bathyplectes curculionis* has been gathered from ALW larvae in northern and southern Europe [10], as well as from northern and southern Africa [11]. Also, the efficacy of the parasitoids belonged to the genus *Bathyplectes* has been evaluated [5]. *B. curculionis* Thompson, *B. anurus* Thompson, and *Oomyzus* (=*Tetrastichus*) *incertus* (Ratzeburg) from the family Eulophidae are considered as the most important natural enemies of the pest [12].

B. curculionis is an endoparasite of ALW larvae that is of a single whole generation in the spring sometimes followed with the second [13]. It prefers the larvae in the first to the third instars for parasitization and the female wasp uses its long ovipositor to find larvae in early instars concealed inside alfalfa buds. The wasp species has been named as one of the parasitoids of the pest in Karaj [14]. The wasp is believed to be of 95% share of the activity of all weevil parasitoids in Virginia, the USA [15]. Implying to the endoparasitic feature of *B. anurus*. Harcourt and Ellis [16] have reported more collection of *B. anurus* compared with *B. curculionis* from south Ontario and recorded its rate of parasitism in a range of 0.1% to 15% with an average rate of 4.6%. Investigating in south Iowa, Giles and colleagues [17] reported the activity of three wasp species *B. anurus* and *B. curculionis* (parasitoids on larvae), as well as *Microctonus aethiopoides* (parasitoid on mature insects) on ALW. They calculated the rates of parasitism as 21% for each of the *Bathyplectes* species, and up to 26% for *M. aethiopoides*.

B. anurus is an individual selective endoparasite on the second and third instar larvae of ALW larvae. It has two diapause stages, one in summer and the other in winter, and is of good competitiveness with *B. curculionis* [6]. The abundance of eggs in female oviduct, the shortness of the period required to access and parasite its host, as well as the shortness of the time to search for and to find the host are among the reasons underlying its high competitiveness to *B. curculionis* [18].

O. incertus is another wasp endoparasitic on ALW larvae that can colonize inside its host body and grow and develop there in aggregation. The species develops three to four generations per year, while a fraction of larvae enter diapauses per generation. Female wasps choose larvae in the second, third and fourth instars to parasitize and lay several eggs inside each individual larva [19].

Materials and Methods

During the years 2014 and 2015, the dynamics of ALW population was studied through weekly samplings performed in two regions in West Azerbaijan, Iran: Saadel 1860 meters higher than free sea surface, and seven kilometers far from and located in the southwestern direction of Chaldoran; and an area 900 meters higher than free sea surface located in southeastern direction of, and 25 kilometers far from Shoat. Samplings were made within determined hours of the day. To collect mature and larval stages of ALW and other insects from the districts under study, an entomological insect net was used. Netting was performed within early morning hours considering behavioral characteristics of the insect and because of the heterogeneity the rate of infestation in different parts of the field was pre-studied in several points before sampling from the field, performing 100 net-hits with an angle of 90° along the diameters of the field area. To collect mature ALWs from the soil surface, an aspirator was used in field studies.

The trapped insects were transferred into a small net sac and subsequently into a container containing a killer material (ethyl acetate, chloroform, and ether). After transfer to the laboratory, all the gathered insects were separated on a ridged metal sheet. The number of the insects was recorded in tables, and the larval density per net-hit was determined. The trapped and killed parasitoid and predator species were kept inside glasses of 75% ethyl alcohol. The parasitoids kept in such a manner were used in morphological studies made taking advantage of binoculars of 250× magnification power and in the preparation of drawings with the drawing apparatus of the binoculars, the insect samples were identified to the level of their families and finally specified based on the accordance between the samples and the available. The identification of insect species was finally re-confirmed by Dr. Monajemi at Institute of Plant Protection, Tehran, Iran.

The statistical data obtained through samplings made per region were separately recorded with ALW, parasitoids, lady beetles, and other predators (and bugs). The curves of the population dynamics of the above-mentioned groups were drawn taking advantage of the Software Excel 2003. The analyses of data and the correlations among the studied populations were performed using the software SPSS 18.

Results and Discussion

As illustrated in Fig. 1, ALW begins its activity in Maku since 4th April, and in Chaldoran since 5th May. The higher altitude and consequently colder weather of Chaldoran compared with Maku are the most relevant reasons for the delay in the emergence of the pest observed in Chaldoran. Since the beginning of the growth season, and with the increase of temperature, the population of ALW gradually rises up and reaches its peak in Maku area in 19th May, and in Chaldoran as affected by the cold weather in 11th June. The activity of the pests finished in Maku and Chaldoran respectively 30th June and 8th August, so that the population rate of the pest came down to zero.

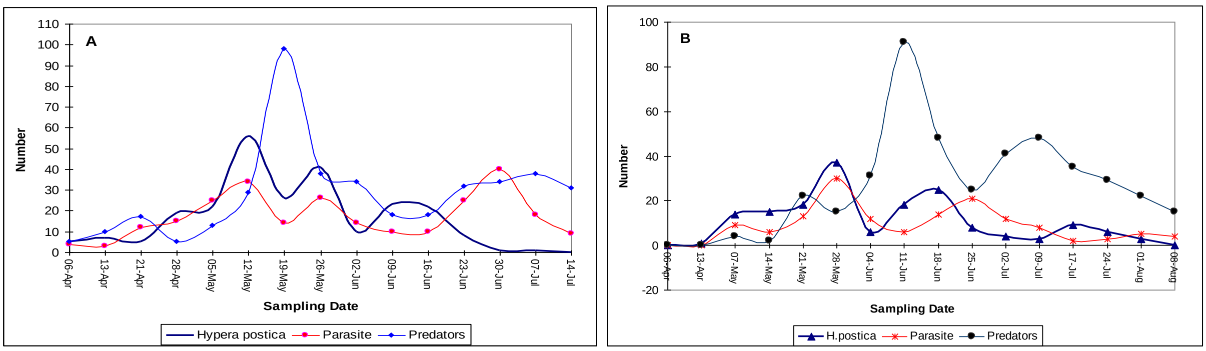

Fig 1: Population dynamics curve of *Hypera postica* and its natural enemies in West Azerbaijan (2014), A: Maku and B: Chaldoran

In Maku, in the first inspection made on 25th Februrary 2013, old remnants of alfalfa shoots and superficial field soil were sampled and several cases of ALW eggs were observed. The eggs encountered with inside old remnants of alfalfa shoots were overwintered eggs that their grayish green color inferred that those were laid some while ago and were near to hatch. With other remnants of alfalfa old shoots, the collected eggs were lemon yellow indicating those were newly laid. Despite there was still no alfalfa plant emerged at the time of the first inspection, however because of the favorite weather conditions in the region, adult overwintered weevils had started their activity earlier beginning to lay their eggs. In surveys made later, several cases of laying eggs in the remaining of older shoots of alfalfa plants were observed as before. On 8th April the tiny larvae of the pest were firstly seen inside terminal buds of alfalfa shoots that had grown up to an average length of 2cm. On 18th April, the signs of pest larval feeding and damage were completely apparent on young shoots (averagely 5cm in length) of alfalfa plants. Keeping on the survey studies, the first pupa cocoons of the pest were found on alfalfa shoots of average length of 25cm. The quality of the cocoons was so as those have just recently been formed. The leaves on alfalfa shoots had become net-like and after feeding on different parts of alfalfa shoots, the larvae in the fourth instar had fallen from on to the soil surface. Therefore, pupa cocoons were mainly observed on the leaves of various parts of alfalfa shoots fallen on the surface of field soil. The pupa cocoons were mostly formed on the leaves of lower parts of the shoots as well as on the leaves of alfalfa and weed plants fallen on the soil surface in the field, and a less number of pupa cocoons were found in the upper parts of shoots. The first inspection in Chaldoran was made on 23'd April, when the field was rather green and the length of alfalfa shoots was recorded about 3 cm. The adult overwintered weevils were rarely seen under plant debris scattered on the field soil surface. However, no sign was found that the pest has laid eggs in alfalfa shoot remnants or and green young shoots. On 16th May, the first eggs resulted from the spring activity of the adult overwintered weevils were enfaced inside delicate and thin remnants of alfalfa plants remained since last year. At this time, some adult overwintered weevils were found among other netted insects implying to their activity and feeding on alfalfa shoots of the recorded average length of 10 cm. The tiny larvae of the pest were gathered on 23'd May, when mature weevils were active on alfalfa shoots averagely 20 cm in length. On 2'd June, ALW larvae of different instars beside of abundant population of the overwintering mature weevils were observable on alfalfa shoots with leaves indicating perfectly obvious marks of their grazing. Fig. 2 represents the curves of population dynamics of ALW and its natural enemies in 2015. As observed, the curve of population dynamics of ALW in 2009 well corresponded to that obtained in the previous year of study with a difference that the occurrence of population peak of the pest had happened a week earlier (in 4th June), compared with that recorded in the last year. The activity of the pest ended in Maku and Chaldoran regions on 14th July and 8th August, respectively. This termination of activity was so severe that the population rate of the pest fell down to zero during these dates. It was notable that the termination date of activity in 2015 occurred ten days later than that in previous year. This might be explained as the impact of the warmer conditions in 2015. There was a significant difference between ALW populations in two years of study ($P<0.05$) indicating the favorability of the environmental conditions for the growth and development of the pest in 2015 that led to higher mean population than that in 2014. The fauna of natural enemies of ALW investigated in this study included three wasp species, a lacewing species, a lady beetle species, and finally a predator bug species.

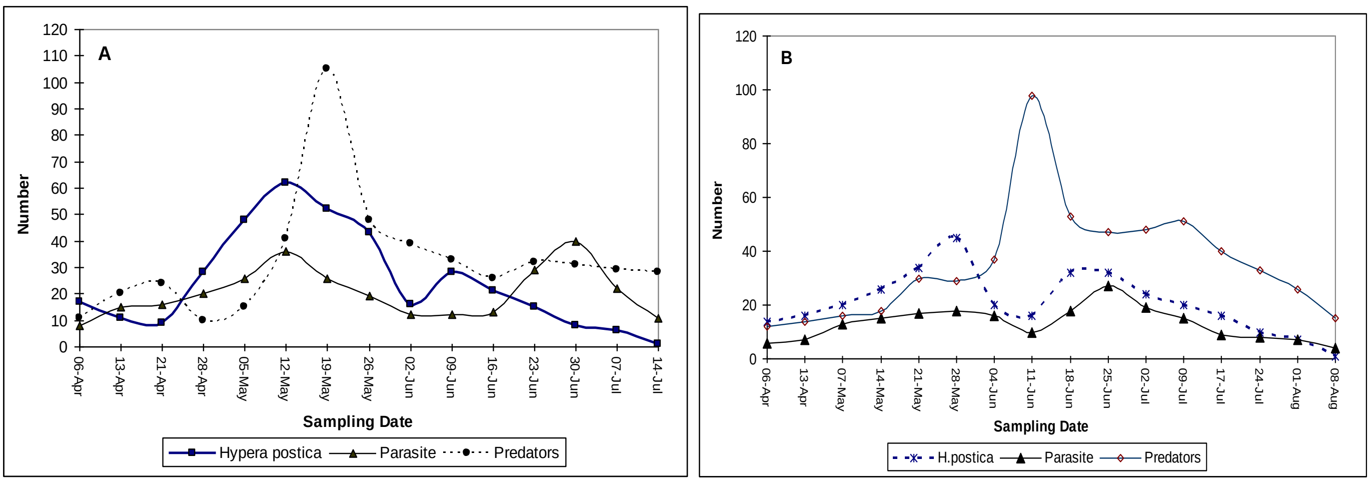

Fig 2: Population dynamics curve of *Hypera postica* and its natural enemies in West Azerbaijan (2015), A: Maku and B: Chaldoran

Fig. 3 and 4 indicate the population dynamics of three parasitoid wasp species of relevant activities on ALW, *Bathyplectes curculionis*, *B. anurus*, and *Oomyzus* (= *Tetrasticus) incertus* studied in the years 2014 and 2015. While no significant difference was found between parasitoid wasp populations in the years of study ($P<0.05$), the densities of three wasp populations were of statistically considerable differences ($P<0.05$). Comparison of data means indicated that *B. anurus* and *O. incertus* respectively had the highest and the lowest population densities in both years. In Maku region, the peaks of population density and activity of *B. curculionis* and *B. anurus* were recorded in 15th May and 6th July in both years of investigation. In Chaldoran area, the peak of population densities of both *Bathyplectes* species was determined in 8th June and 6 July two year study. The emergence of these two wasp species in this region occurred with a delay of a month that might be the consequence of lower environmental temperatures and higher elevation of the area. The first netting of *O. incertus* species in Maku was recorded on 23th May and its peak of activity occurred on 26th

June, 2014. In chaldoran, the emergence and the peak of activity of this insect were respectively recorded on 18th May and 8th June, 2014. It seems that the emergence of this species is antedated in the regions of higher elevations.

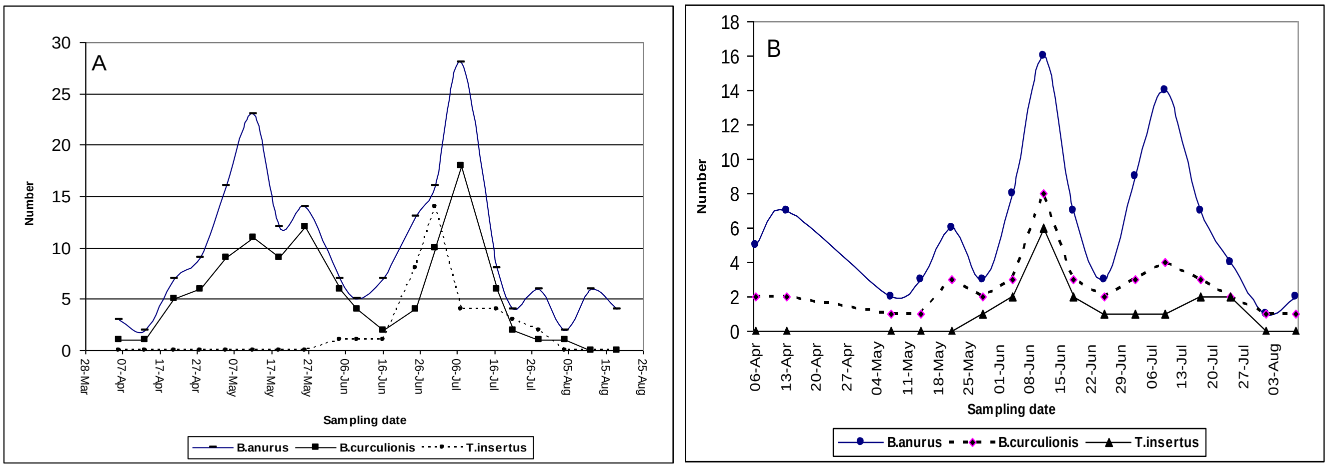

Fig 3: Population dynamics curve of *Bathyplectes curculionis*, *B.anurus* and *Oomyzus incertus* in West Azerbaijan (2014), A: Maku and B: Chaldoran

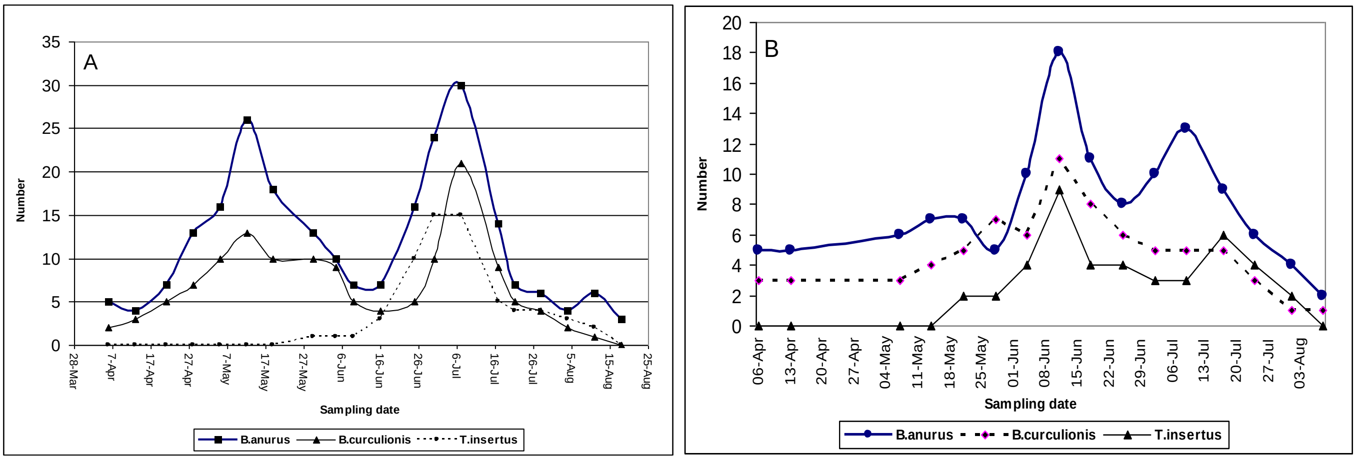

Fig 4: Population dynamics curve of *Bathyplectes curculionis*, *B. anurus* and *Oomyzus incertus* in West Azerbaijan (2015), A: Maku and B: Chaldoran

The emergence of *O. incertus* in Maku and Chaldoran was respectively recorded on 28th May and 22th May, 2015, 7-10 days earlier than that in 2014. The correlations between the population of ALW and populations of *B. curculionis* and *B. anurus* species for both Maku and Chaldoran regions have been presented in the Fig. 5, and 6. As observed in these figures, a high correlation was found between populations of ALW and *B. anurus* in Maku (R^2= 0.841). The correlation between populations of the pest and *B. curculionis* was lower (R^2= 0.661) in Maku. In Chaldoran, the correlation between the populations of ALW and *B. anurus* was R^2= 0.782 lower than that obtained in Maku, but similarly higher than the correlation between the populations of ALW and *B. curculionis* (R^2= 0.612) in Chaldoran again lower that that obtained in Maku.

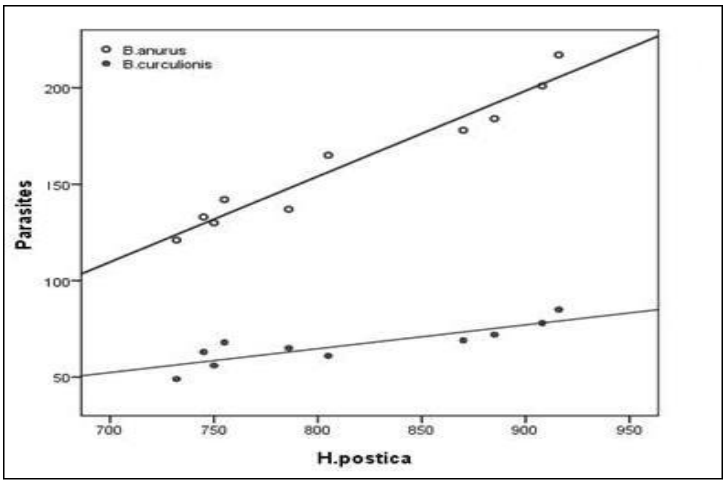

Fig 5: Correlation between *Hypera postica* population and those of *Bathyplectes curculionis* and *B. anurus* in Maku

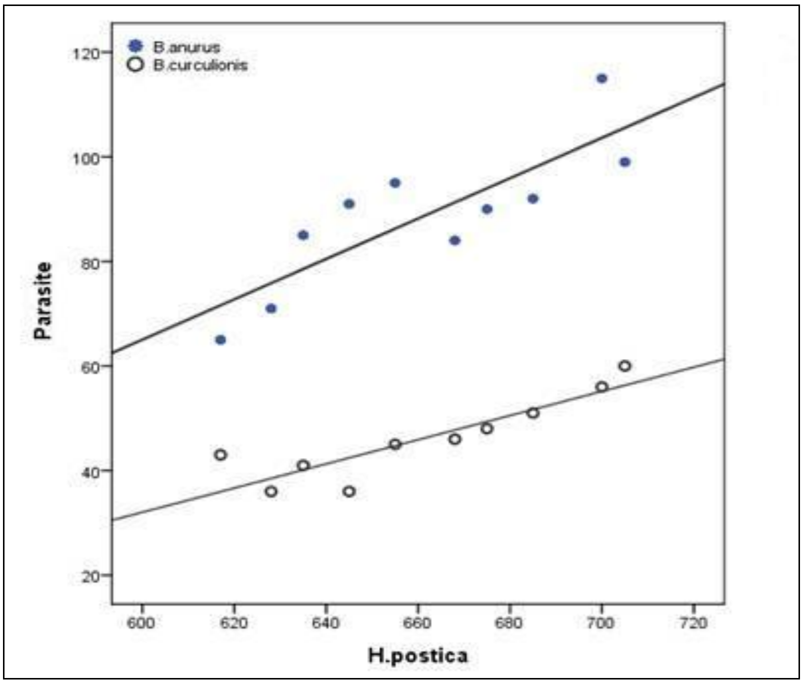

Fig 6: Correlation between *Hypera postica* population and those of *Bathyplectes curculionis* and *B. anurus* in Chaldoran

The correlation between ALW and *O. incertus* populations in Maku and Chaldoran were low (R^2= 0.493 and R^2= 0.684, respectively), lower than those studied with *Bathyplectes* species. The calculated determination coefficient for the correlation between populations of *O. incertus* and ALW was higher in Chaldoran probably because of higher population of *O. incertus* in Chaldoran district compared with that in Maku region (Fig 7).

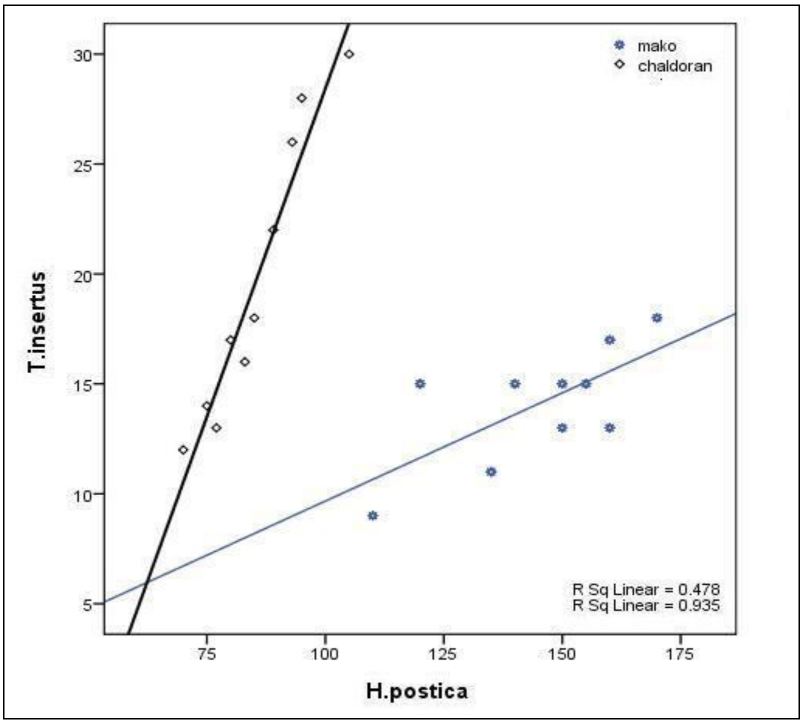

Fig 7: Correlation between *Hypera postica* and *Oomyzus incertus* populations is in Maku and Chaldoran

Figures 8 and 9 represent the population dynamics of seven spotted lady beetle *Coccinella septempunctata* and that of green lacewing *Chrysoperla carnea* in 2014, and 2015. No significant difference was found between trapped predator populations in each of the years of study (P<0.05), however, the rate of population density of *C. septempunctata* was statistically significantly different from that of *C. carnea* (P<0.05). Comparison of means indicated that seven spotted lady beetles were of higher population mean in both regions in both years. In Maku, the peak of activity of *C. septempunctata*, and *C. carnea* occurred on 11th May and 1th June, 2014 inferring a delay in the activity started of green lacewing. In Chaldoran in the same year, the activity peaks of *C. septempunctata* and *C. carnea* were recorded on 10th June and 15th June, respectively. The later peak of activities recorded were explained as the result of higher elevation of

Chaldoran area and colder climate of the region. In 2015, the peak of seven-spotted lady beetle and green lacewing was recorded in Maku on 16th May, and in Chaldoran respectively on 15th June and 5th June. Collectively, it was concluded that the differences in the altitude compared with free sea surface, and even a few degrees in environmental temperature as density independent factors could obviously affect the emergence of pests, of their natural enemies as well as the population density of both.

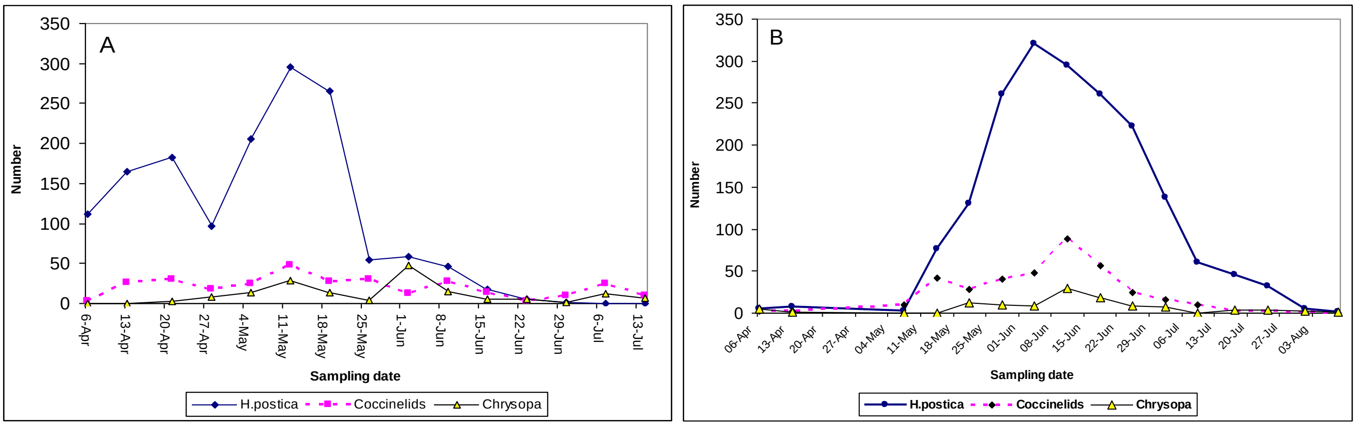

Fig 8: Population dynamics curve of *Hypera postica*, *Coccinalla septempunctata* and *Chrysoperla carneae* in West Azerbaijan (2014), A: Maku and B: Chaldoran

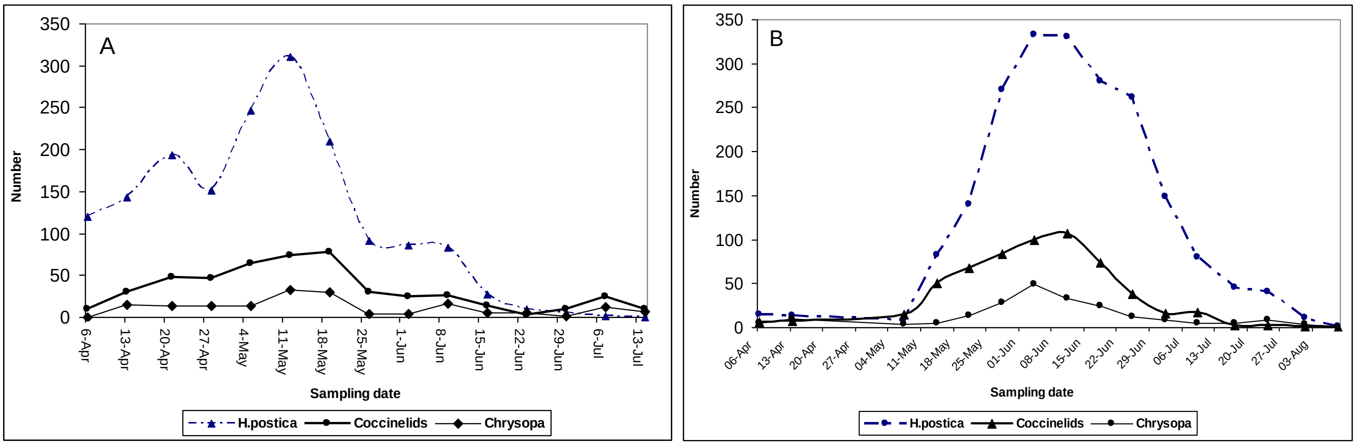

Fig 9: Population dynamics curve of *Hypera postica*, *Coccinella septempunctata* and *Chrysoperla carnea* in West Azerbaijan (2015), A: Maku and B: Chaldoran

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