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The Research on the Distribution, Abundance and Some Ecological Characteristics of *Neuroterus* Species (Hymenoptera: Cynipidae) in Oak Forest of West Azerbaijan (Iran)

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Abstract: Oak gall wasps (Hymenoptera: Cynipidae), with more than 1300 species, represent one of the largest radiations of gall inducing insects. Many studies have documented galling species lists and richness, especially gall wasps, but few studies have been directed toward understanding how the gall-inducing species are locally distributed. In this survey, the oak gall wasps were collected from April to November (2009 and 2010) in West Azerbaijan of Iran. Among these collected galls, five species of oak gall wasps which belonged to the genus *Neuroterus* sp. were identified as: *Neuroterus lanuginosus* Giraud, *N. numismalis* Geoffroy, *N. saliens* Kollar, *N. laeviusculus* Schenck and *N. quercus-baccarum* Linnaeus. All of the galls in this survey are leaf galls. These galls were produced by the asexual reproduction and were collected in summer and fall seasons. The highest species richness was recorded in Ghabre-hossein and Vavan stations. Also, the highest density of gall formation was recorded at the southern direction. The multiple-sites similarity measures indicated that similarity in gall composition and community between collecting sites was generally between 0.57 and 0.90. The highest multiple site similarity was recorded between Vavan vs Rabat vs Dare-ghabr station. The authors showed that the very cold and humidity climate is not appropriate to *Neuroterus* species activity and the south direction is the best side for their survival.

Key words: Oak, gall wasps, distribution, population, *Neuroterus* sp., forest.

1. Introduction

Quercus brantii Lindl. and *Q. infectoria* Olive. are among the most important and predominant trees in the Zagros vegetations [1]. These wasps' species are of considerably high distribution throughout West Azerbaijan province where various animal populations are encountered in association with these trees. A group of insects found on these oak trees are gall-inducing wasps which belonged to Cynipidae family, a family of about 1300 species identified [2]. The important trait of gall-inducing wasps is the formation of an abnormal structure called galls on host plant [3]. In fact, gall is the abnormal growth of plant tissue that is induced because of the plant reaction to the oviposition by the female wasp as well as to the secretions by gall-inducing wasps larvae [4]. The oak gall-inducing wasps of the tribe Cynipini are active on the plant species which belonged to the family Fagaceae, and with a thousand of species identified

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comprise around 80% of the known gall-inducing wasps [5]. There are about 1000 oak gall wasps species that belong to 41 genera (with a mean of 24 species per genus). Some genera are very large, with 300 species in *Andricus*, 150 species *in Callirhytis*, and 100 species in *Neuroterus* [6]. Studies on Cynipid faunas associated with American oaks show that hosts with greater geographic ranges support richer cynipid communities [3]. *Neuroterus, Cynips, Andricus*, and *Plagiotrochus* are among the genera which belonged to the tribe Cynipini that are found biologically active on different species of oak trees such as *Q. infectoria* and *Q. libani*, where they induce various galls on various parts of oak trees [2, 3]. The structure of a single gall includes larval cavity and outer part. Larval cavities are similar in most galls and the observed morphological differences of the galls induced by oak gall-inducing wasps are resulted from the different growth states of the outer part [7, 8]. Each gall-inducing wasp initiates galls on a specific organ and therefore, different species are recognized based on the shape as wells as the location of their galls on host plants [9]. Gall protects the gall-inducer from unfavorable environmental conditions and natural enemies [10]. Mature cynipid galls may be as small as 2 or 3 mm or as large as 10 cm in diameter. Galls of some species house only a single larva, whereas several hundred inhabit others. Cynipids induce galls on virtually all plant organs, although most species are very specific about the location of their galls. Many of them have impressive surface structures, such as spines of variable shapes, or glands that secrete sugary or otherwise sticky compounds [8]. Many studies have documented galling species lists and richness, especially gall wasps, but few studies have been directed toward understanding how the gall-inducing species are locally distributed [11]. Beta diversity is generally thought of as the change in diversity among various Alpha diversities (variation in species composition among sites in a geographic region) [12, 13]. There exists a wide variety of methods for measuring Beta diversity, among which similarity measures are the simplest and the most commonly used for calculating Beta diversity from abundance or presence/absence data [12, 14]. The classical Jaccard and Sørensen indices were based on both the number of species present in samples and the numbers only seen in each of them [12]. Sorenson's measure is regarded as one of the most effective presence/absence similarity measures [14]. Most European gall-inducing wasps are belonged to one of the following 4 genera: *Andricus* (116 species), *Plagiotrochus* (14 species), *Neuroterus* (13 species), and *Cynips* (8 species) [15]. The oak cynipid gall wasp fauna of Iran remains little-studied [1]. Thirty six species of oak cynipid gall wasps were recorded in Iran by Chodjai (1980) [16]. The recent surveys were conducted about the Cynipids fauna in Iran [17-19] but the oak cynipid gall wasps ecological characteristics remain little-studied. Gall wasps of *Neuroterus* genus are of several species and induce galls of different structures and shapes on different species of oak trees. In the present research, the galls induced by *Neuroterus* sp. species in West Azerbaijan have been collected and identified, and some characteristics of these galls have been studied.

2. Materials and Experiment

At first, some stations were determined for the collection of oak gall-wasp galls in west-southern region of West Azerbaijan province, Sardasht and Piranshahr. The areas of study in this research were Ghabre-hossein, Mirabad, Vavan, Rabat and Dare-ghabr (Table 1).

The Cynipids galls in these areas were gathered during these inspections made since the early growth season till the end of the season (2009 and 2010). The best number of samples have been determined to be 20 trees base on Southwood and Henderson formula (2000) [20] so that $N = (t \times s/D \times m)^2$, where t is student's T-test of standard statistical tables, D is predetermined of the confidence limit for the estimation of the mean express as a decimal, m is sampling mean and s is the

Table 1 Locations in West Azerbaijan where the survey was carried out.

Sites	Situation	Climate	*Quercus* (host)
Ghabre-hossein	36°28′N 45°18′W	Humidity and cold	*Q. infectoria, Q. brantii*, *Q. libani*
Mirabad	36°15′N 45°22′W	Very cold and humidity	*Q. infectoria, Q. brantii*
Rabat	36°14′N 45°33′W	Mediterranean humidity	*Q. infectoria, Q. brantii*
Vavan	36°16′N 45°28′W	Mediterranean humidity	*Q. infectoria, Q. brantii*
Dare-ghabr	36°11′N 45°24′W	Humidity and cold	*Q. infectoria, Q. brantii*, *Q. libani*

standard deviation. In each tree, as unit of sampling, all of the occurred galls by *Neuroterus* species, on four branches (each branch length was 50 cm) in four geographical directions were counted in the stations. After identifying species, abundance and distribution of each gall were recorded. Diameter of the galls was measured with the ocular micrometer to the nearest 0.07 mm. With these data, comparisons of the mean values for: (1) density of the galls (in all stations for the *Neuroterus* species abundance), (2) geographical direction of gall occurrence and, (3) difference between galls diameter of these five species were conducted using a one way ANOVA (among samplings' direction and among station abundance of the galls). When necessary, Turkey's HSD test was used for the multiple comparisons of the mean values. If homogeneity of variances between groups was not certain, Kruskal-Wallis test was used. The information related to oak gall collected samples (host plant, date of collection and etc.) was recorded and the samples were transferred to the laboratory. The galls were separated based on their morphology and put inside separate plastic cylinders with aeration to get access to gall-inducing agents and their parasitoids. The galling wasps appeared after about 10 days were isolated and transferred into tubes containing Pumple's solution. The traits such as gall diameter, color, shape, collection date and location were recorded and the total number of larval chambers per gall was determined through cross-sections made with each gall. Also, pair-wise comparisons of species similarity were performed for the different dates using Sorensen's similarity quotient that SQ = 2J/(2J + A+ B), where J is the number of species shared by two samples. A is only the number of species in the location A, and B is only the number of species in the location B. This similarity quotient is for qualitative data and ranges from 0 (there are no species in common) to 1 (where all species are shared) [13, 21]. If similarity are high (with considering that the vegetation are the same in all of sites), then indicated climate variation and oak sub-species are important and a strong effect on gall's distribution as the first reasons. Pair wise comparison of neighboring sites will suffice if the goal is to look at how species composition changes along a physical or environmental gradient. The level of similarity among the sites sampled (multiple site similarity), based on presence of galls was calculated using a multiple site similarity measure (range = 0-1), according to Diserud and Ødegaard (2006) [22]:

$$C_s^T = \frac{T}{T-1}\left(\frac{\sum_{i<j} a_{ij} - \sum_{i<j<k} a_{ijk} + \sum_{i<j<k<l} a_{ijkl} - \cdots}{\sum_i a_i}\right),$$

where a_i is the number of species in site A_i, i=1, …, T; a_{ij} is the number of species shared by sites A_i and A_j; and a_{ikj} is the number of species shared by sites A_i, A_j and A_k, etc. in T sites.

For a given number of sites T, C_s^T decreases with increasing number of 'rare' species, i.e. species observed in only one or a few sites. Conversely, $\overline{C_s^T}$ increases with increasing number of species observed in several sites [22].

Cluster analysis is used when research is being conducted on more than one site and starts with a table or matrix giving the similarity between each pair of sites (by using any similarity coefficient). The two most similar sites are combined to form a single cluster. The analysis then proceeds by successfully combining

similar sites until all are combined into a single cluster. Cluster analysis was measured with hierarchical cluster and cluster method was base on Ward's method (SPSS 17). The multiple-sites similarity value and mean gall diameter were used in a cluster analysis to illustrate similarity patterns at the five sites and to grouping the gall size. The similarity was calculated with Ecological Methodology software. Stability of species was measured base on this formula: S= (N/P)*100 where S is percent of stability, N is the number of samples that the species is present, and P is the total of samples. If S is less than 25, species is temporary and if value of S is more than 50, in this case would be sustainable. Stability shows that what would be the species present in the area.

3. Results

Since early March synchronous to the development of catkins of oak trees, galls formed on catkins were gathered, and with the raise of the temperature and opening of leaf buds, galls developed on leaves and twigs of oak trees were collected. Five different kinds of galls resulted from the activity of *Neuroterus* sp. species were collected and identified based on their morphology indicator of gall-inducing wasp species:

(1) *Neuroterus lanuginosus* Giraud

Date of collection: 21/August/2009-2010; Location of collection: Dare-ghabr, Vavan, Rabat and Ghabre-hossein; No. Larval chambers per gall: 1; Gall diameter: 4-6 mm; Overall shape of gall: spherical; Place on the host: leaf undersurface.

(2) *Neuroterus saliens* Kollar

Date of collection: 5/August/2010; Location of collection: Rabat and Vavan; No. Larval chambers per gall: 1; Gall diameter: 3-4 mm; Overall shape of gall: spherical and ellipsoidal; Place on the host: leaf upper surface.

(3) *Neuroterus laeviusculus* Schenck

Date of collection: 8/October/2009; Location of collection: Ghabre-hossein; No. Larval chambers per gall: 1; Gall diameter: 3-4 mm; Overall shape of gall: spherical vs flat; Place on the host: laterally on an upper sides of the leaf.

(4) *Neuroterus numismalis* Geoffroy

Date of collection: 21/September/2009-2010; Location of collection: Ghabre-hossein, Vavan and Dare-ghabr; No. Larval chambers per gall: 1; Gall diameter: 2-4 mm; Overall shape of gall: spherical and of central dint; Place on the host: leaf upper side.

(5) *Neuroterus quercus-baccarum* Linnaeus

Date of collection: 8/October/2009-2010; Location of collection: Dare-ghabr, Vavan, Rabat and Ghabre-hossein; No. Larval chambers per gall: 1; Gall diameter: 4-6 mm; Overall shape of gall: spherical and prominent; Place on the host: leaf upper side.

The most number of gall wasps species (local diversity) which belong to the genus *Neuroterus* sp. were gathered from Ghabre-hossein and Vavan stations (4 species), and the least number of them were encountered in Mirabad station (without species). There was no significant difference in the gall's number in each station ($df = 3$; $P > 0.05$) so that the number of oak gall wasps species was the same in Ghabre-hossein, Vavan, Rabat and Dare-ghabr stations. Among the species, *N. quercus-baccarum* in Ghabre-hossein and Dare-ghabr sationas and also, *N. lanuginosus* in Rabat and Vavan stations had the highest abundance in 2009 (Fig. 1). Fig. 2 shows the abundance of *Neuroterus* species in 2010. The highest density was related to *N. quercus-baccarum* (9.2 galls per trees) (Table 2) meanwhile, the stability of this species was about 47 percent (Table 3) in Ghabre-hossein station in 2009. This result shows that this species forms the galls with high density on the number of a few trees. Among the species, *N. saliens* in Vavan station and *N. lanuginosus* in Rabat station had the highest stability in 2009 (Table 3). Also, there was significant difference in the direction of the gall's occurrence ($df = 3$; $P < 0.01$).

The highest density of gall formation was recorded with the southern directions (mean ± SD; 4.01 ± 0.97)

and the northern (mean ± SD; 3.16 ± 0.72) as well as eastern (mean ± SD; 3.27 ± 0.89) direction are in the

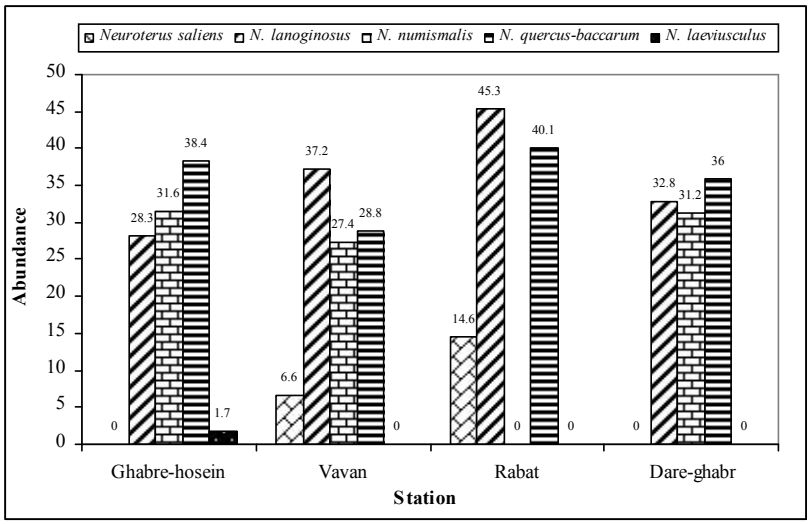

Fig. 1 Percent of abundance of *Neuroterus* species in different stations, 2009.

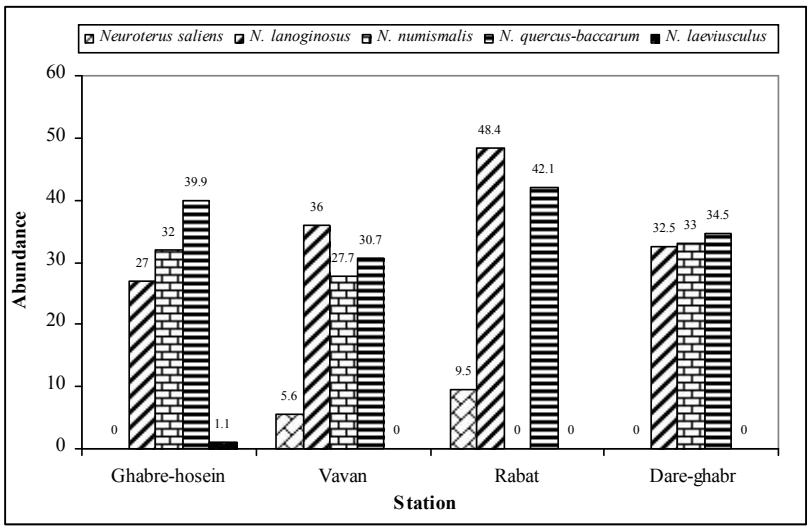

Fig. 2 Percent of abundance of *Neuroterus* species in different stations, 2010.

Table 2 Density (number of the galls per tree) of *Neuroterus* species in different stations in 2009 and 2010.

Species		Station				
		Ghabre-hossein	Mirabad	Vavan	Rabat	Dare-ghabr
1	*Neuroterus saliens*	0 (0)	0 (0)	1.6 (1.1)	2.7 (1.5)	0 (0)
2	*N. lanoginosus*	6.8 (5.6)	0 (0)	8.7 (7.1)	8.4 (7.4)	7.7 (6.8)
3	*N. numismalis*	7.6 (6.7)	0 (0)	6.4 (5.5)	0 (0)	7.4 (6.6)
4	*N. quercus-baccarum*	9.2 (8.3)	0 (0)	6.8 (6.1)	7.4 (6.5)	8.5 (6.9)
5	*N. laeviusculus*	0.4 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)

Density in 2010 was located in parenthesis.

same statistical group (Fig. 3). Mean gall diameter of the galls of *Neoroterus* species was significant different ($df = 3$; $P < 0.01$). Mean diameter of gall were highest for southern direction (mean ± SD; 2.10 ± 0.82)

and the least diameter was found in 3 other directions (north, west and east directions that were in one

Table 3 Stability of *Neuroterus* species in different stations in 2009 and 2010.

Species		Stations				
		Ghabre-hossein	Mirabad	Vavan	Rabat	Dare-ghabr
1	*Neuroterus saliens*	0 (0)	0 (0)	90 (70)	85 (77)	0 (0)
2	*N. lanoginosus*	70 (60)	0 (0)	80 (73)	90 (80)	60 (50)
3	*N. numismalis*	37 (33)	0 (0)	37 (50)	0 (0)	37 (33)
4	*N. quercus-baccarum*	47 (50)	0 (0)	40 (50)	47 (57)	37 (30)
5	*N. laeviusculus*	23 (17)	0 (0)	0 (0)	0 (0)	0 (0)

Stability in 2010 was located in parenthesis.

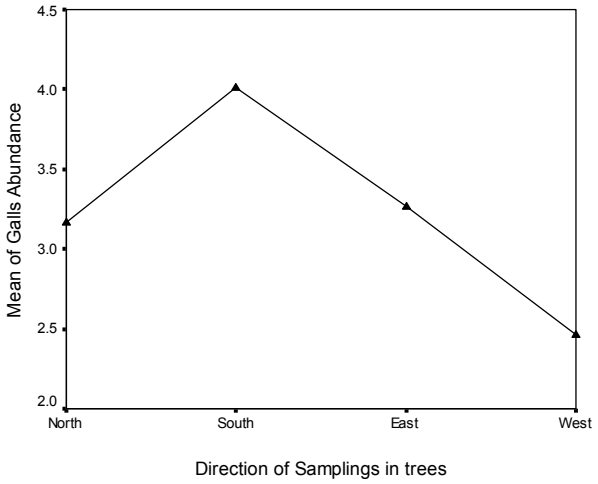

Fig. 3 Means plot of geographical direction of gall occurrence.

statistical group "b"). In common case, it seems that the female of oak gall wasps prefers to lay eggs in the southern side where the sun rises. The cluster analysis of gall diameter showed that the collected galls were tow groups. The highest mean diameter was related to the galls of *N. lanuginosus* and *N. quercus-baccarum* galls, so were in a single cluster (Fig. 4). Community similarity estimated by Sorensen ranged from 0 to 1 similarity. The highest similarity index was recorded between three pare wise similarity comparison as: Ghabre-hossein vs Dare-ghabr, Rabat vc Dare-ghabr, Vavan vs Dare-ghabr and no similarity was seen between Mirabad and others sites (Table 4). Also, multiple site similarity showed that the highest value of this index was found among Vavan vs Rabat vs Dare-ghabr stations (Fig. 5).

4. Discussion

In all sites, *Q. infectoria* and *Q. brantii* had formed about 90 and 10 percent of the local vegetation, respectively. These oak species are of several sub-species which are under identification in the term of a comprehensive research plan. As hypothesis to explain local gall-forming insect species richness is suggested: galling insects may preferentially select those plant species with characteristics such as chemical toxicity, mechanical strength, or longevity that can be manipulated to benefit the galler [11, 23]. Locality climate affects gall wasp diversity and impacts on the distribution of some gall wasp species. *Neuroterus laeviusculus* was collected only from Ghabre-hossein station. In order to considering that the vegetation cover in these stations is the same, distribution of *N. laeviusculus* is under sub-species of the *Quercus* sp. and climate effects. The very cold and humidity climate is not appropriate to the *Neuroterus* species. It is expectable that there are no species in Mirabad station. *Neuroterus saliens* was collected from Rabat and Vavan station that have similar climate. This species is active in the Mediterranean humidity climate of these two stations. Also, it seems that regional climate was not so effective on some gall wasp species such as *N. numismalis* whose galls encountered in two types of climate. It is reported that species richness in hot, dry environments and cooler are the same [6]. But according to another study [26], combination moisture

and the high temperature, with the potential for hygrothermal stress, may also be important.

Climate change can influence plant productivity both directly and indirectly. Experiments have shown

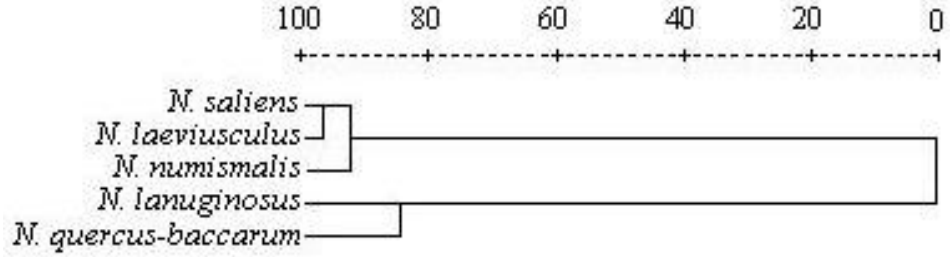

Fig. 4 Cluster analysis of mean gall diameter of the *Neuroterus* species (grouping the galls).

Table 4 Sorensen pair wise similarity between stations.

Station	Ghabre-hossein	Mirabad	Vavan	Rabat	Dare-ghabr	Number of *Nueuroterus* species per site
Ghabre-hossein	1.00	0.00	0.75	0.57	0.86	4
Mirabad	0.00	1.00	0.00	0.00	0.00	0
Vavan	0.75	0.00	1.00	0.86	0.86	4
Rabat	0.57	0.00	0.86	1.00	0.67	3
Dare-ghabr	0.86	0.00	0.86	0.67	1.00	3

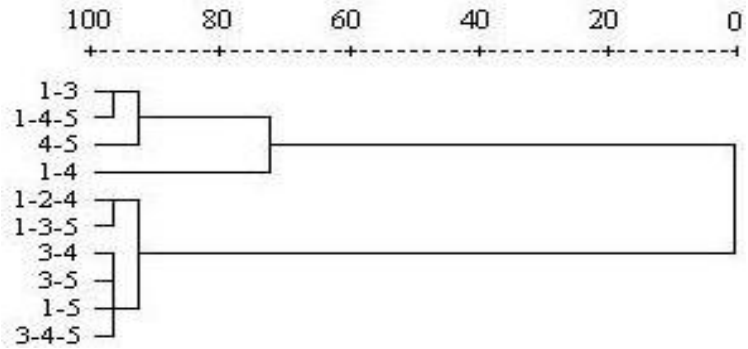

Fig. 5 Cluster analysis of the multiple size similarity coefficients. [1-Ghabre-hossein, 2-Mirabad, 3-Vavan, 4-Rabat, and 5-Dare-ghabr].

that plant productivity can be increased with higher levels of CO_2; however, these high levels can reduce plant productivity through increased temperature levels and reduction of water accessibility. Indirectly, climate change can cause changes in the phenology the relationship between climatic settings and periodic biological events of certain regions [24]. Then the direct and indirect effects of climate must be considered and studied. Host specialization of oak gall wasps was confirmed and these insects have also selected your host for gall formation on a specific organ skillfully [3]. The kind of oak subspecies will be the first reason of the different distribution of *N. numismalis*. Rabat and Vavan stations are of similar climate, however, the specific diversity of gall wasps was found very different and *N. numismalis* there is not in Rabat station. It shall be believed that gall wasps are skilled plant taxonomists and recognize sub-species of plant genus. The effect of different oak sub-species and locality climate are effective on the absence/presence of *N. laeviusculus* and *N. saliens* in Ghabre-hossein and Vavan station (Table 2). It is the reason of reduction of Sorensen similarity index between these two sites from 1 to 0.75. The stations such as Vavan and Rabat are similar in oak species and climate but similarity between them is (0.86), not high, and *N. numismalis* is

absent in Rabat. The reason of absence this species may be the effect of the oak sub-species *N. numismalis* in distribution. The factors like altitude and regional climate are similar in some areas under comparison whereas the differences in the geographical distribution of oak sub-species may be attributed to the difference in the presence of the regions. This implies to the important role of the host plant sub-species distribution in the determination of gall wasp distribution. Gall-inducing insects have long been known to exhibit a high degree of host-plant specificity [3]. *N. lanuginosus* induces the formation of small spherical galls underside of *Q. brantii* leaves. The gall is of a single larval chamber in its central part and superficially covered with tiny lint. The wasp only reproduces asexually [9]. The galls induced on *Q. brantii* trees as the result of *N. saliens* activity are morphologically rather spherical and dimensionally small. These galls formed on upper side of host leaves are light in color. The species is of both asexual and sexual reproduction [3]. The galls collected in this research were the result of activity of asexual generation of this species. Bioactivity of *N. numismalis* leads to the formation of small spherical galls with on the under side of leaves. The gall is superficially covered with very tiny lint's and of a pin-head shaped central the dint. The occurred galls induced by *N. quercus-baccarum* are light brown to creamy in color. These galls are found in the lower side of leaves and the larval chamber is triangular [3, 8]. The galls of *N. laeviusculus* are brown to red in color, flat and spherical in shape. These galls develop on the upper side of leaves and are the result of the activity of asexual generation. All galls of this species are of only one larval chamber [8]. The highest numbers of hole in the occurred galls in the south show these gall wasps successfully exit and gall formation was been complete. The general ecological theory must be tested about the selection of the southern by the genus *Neuroterus* sp. to activity and gall formation. We tested and showed this matter that the species which belong to the *Neuroterus* genus prefer south geographical direction to other direction for activity and gall forming. This subject is important to us that know why this species selects south direction and other Cynipids also select it for activity? At last, the diversity in the local population of oak trees at the level of species and inferior shall be considered as one main factor involved in the distribution of gall wasp species beside other factors like regional climate, annual temperatures, and vegetation. Some studies indicate that the species richness gall-inducing insect's increases as environments become hotter and drier while others suggest that these factors have no effect. Study of galling insect distribution in tropical rain forests became more important in an attempt to test some hypothesis on galling insect diversity. The most important point is that the role of the oak subspecies, in distribution of oak gall wasps should not be ignored. Therefore, the diversity in the local population of oak trees at the level of species and inferior shall be considered as one main the factor involved in the distribution of gall wasp species beside other factors like regional climate, annual temperatures, and vegetation. At the smaller scales, vegetation has a moderating influence on local climates and may create quite specific micro-climates. Some organisms are dependent on such micro-climates for their existence. Diversity of gall inducing insects to be intimately bound to plant diversity, since the presence of a larger number of plants in a given environment would denote more ecological niches to be explored [25]; other studies, however, do not find a link between these variables, host finally the structural complexity and altitudinal/latitudinal variation [26]. Temporal changes in the quality and/or quantity of host plants also regulate the resource availability to herbivores, and phonological synchrony of herbivores with the host plants is thus likely to be a determinant of the patterns of herbivore abundance [27, 28].

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