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Eco-Phytosociology Method and it New Application in Determination and Discrimination of Intraspecific Diversity, Case Study: *Astragalus Verus and Astragalus Glaucops*

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Eco-Phytosociology Method and it New Application in Determination and Discrimination of Intraspecific Diversity, Case Study*: Astragalus Verus* and *Astragalus Glaucops*

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Abstract - This study carried out for determination and discrimination of intraspecific diversity of *Astragalus verus and Astragalus glaucops* by Eco-phytosociological method from west of Iran. In this order, application of Endogenous milieu (special station) for data collecting and then their analyzing permit us only determine existence of inter and intraspecific diversity. Then for determinating kind and level of intraspecific diversity (Ecophene, Chemotype, Cytotype, Ecotype …), can use other studies such as: morphological, anatomical, phytochemical, cytological and etc. In this survey, 31 special stations were studied. Then floristic-ecologic data collected from each 31 special stations and analyzed by Anaphyto software (F.C.A, A.H.C, Marquag methods). Comparison of obtained results on multiple coordinate axes from F.C.A method with results from Marquag and A.H.C methods led to determination of 7 main groups of Endogenous milieus (special station). Flavonoid analyses were used for determination kind and level of intraspecific diversity in 7 discriminated groups. Leaves flavonoid components of all collected individuals of *Astragalus verus and Astragalus glaucops* were investigated by TLC method. Obtained data from flavonoid survey analyzed by MVSP package with WARD and UPGMA methods. Finally, the results of flavonoid studies confirmed the same groups that identified by floristical composition study and showed intraspecific diversity in chemotype level.

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I. Introduction

During the past forty years or more much of the attention of continental plant ecologists has been devoted to devising and systematizing methods for the description and classification of plant communities. So impressive is the body of material collected that schemes have been proposed to establish rules for the correct description and nomenclature of vegetation units comparable for those in effect for taxonomic species, genera, families etc. (Barkman, 1950; Du Rietz, 1930, 1936). The view expressed by Tuxen (1942) that the plant can measure habitat factors better than any instrument is symptoma - tic of the skepticism with which the sociologist regards intensive ecological investigation, in spite of the fact that the only exact knowledge which he possesses of the tolerance of species has been obtained by extrapolation (often unjustified) from original instrumental measure - ments.

The knowledge of the floristic composition of an area is a perquisite for any ecological and phytogeographical studies and conservation management activities. In studying any particular piece of vegetation, from an ecological point of view, our first step must be to determine the facts as they exist on the ground: facts regarding the vegetation, on the one hand; facts regarding the habitat, on the other (Nicholes, 1930). If there is any one set of facts which is more susceptible to direct study and exact characterization than any other, it is the floristic composition of the vegetation.

In mentioned studies did not use a special method in plant specimens collecting process, while for collecting correct and precise floristic-ecologic data, we must apply an appropriate method that be according to factors governing nature and can be used for determination and discrimination existence of inter and intraspecific diversity. In this order, we used the unit of study (Endogenous milieu) in Eco-phytosociological method (Atri, 1996, 1999).

The aim of this project, was study on exist of intraspecific diversity (biodiversity point of view) in species, *Astragalus glaucops* and *Astragalus glaucops* in wes Iran. It was carried out from two different aspects, the studies of floristic- ecologic diversity in this species belong to their stations and investigation on the diversity of flavonoid patterns in their populations. The next aim was if floristic- ecologic data and obtained groups for each species can be distinguished intraspecific diversity of the species separately.

II. Eco-Phytosociology Methodology

By 1996, after extensive plant sociological studies in the Iran, Atri had formed definite ideas about the fundamental concepts of his system. Which he published under the title, a presentation of some aspects of the application of neosigmatiste method in pedology, systematics and chorology. In new method

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namely Eco-phytosociology for determine plant association after diagnosis individuals association, in each individual's association; Endogenous milieus determine. Endogenous milieu in Eco-phytosociological method is an area of vegetation that is homogenous view point of floristic- ecologic. Establishment of releves (stands) carry out randomly in each Endogenous milieu for floristic-ecologic data collecting.

In this method, by employing physiognomic criterion, the exiting formations (principle and secondary formations) are specific. By employing the floristic criterion in each formation, the homogenic areas are determines in terms floristic composition and their delimitations are specific on the map as association individuals respect. Then, by using ecological criterion in each association individuals, based on observation of any changes in one or more ecological factors, the exiting endogenic milieu(s) can specific in each association individuals. Then, any endogenic milieus, which show homogeneity in floristic-ecologic term, the releve are place at random. To determine the minimal area of each releve, by using the area-species method on basis of area-species curve and Cain method are apply (Cain, 1959). The necessary floristic-ecologic information and data (including plant species, texture class, OM %, OC%, pH, EC, moisture Altitude, exposition and slope degree) are collect for each releves and are duly enter in the relative forms. In the next stage, the species and samples of soil identify and duly study so that they can prepare to analysis by computer software after labeling and coding of the releves. Must, Pay attention that, each own Endogenous milieu can be one releve or in each Endogenous milieu establish several releve. Finally, data analyses lead to know plant associations of vegetation study.

III. Eco-Phytosociology Applications

Employing ecologic and phytosociologic criteria as eco-phytosociology (Atri, 1996) are not only suitable and exact in the data collection stage to determine the placement of releves, but also it is able to provide results which conform and agree to the rules that govern the nature in the analysis and result interpretation stage. Some Investigations by use this method (Atri, 1996, 1999., Atri et al., 2006, 2007, Fakhre-Tababaei et al, 2000; Safidkon et al, 2003 and 2005; Kalvandi et al, 2004) show that this method can suitable for ecological studies such as chorology, auteocology, pedology, biosystematics, plant diversity (intra and interspecific variations such as, ecotype, cytotype, ecophene, chemotype,…).

IV. Eco-Phytosocilogy and Plant Diversity

The environmental factors and its influence in plant variation (plant diversity) have been extensively studied. Some of these studies include: (Turesson, 1922; Mooney and Billings, 1959; Koch and Bernhardt, 2004; Semmar *et al.*, 2005; Telascrea *et al.*, 2006).

In mentioned studies did not use a special method in plant specimens collecting process, while for collecting correct and precise floristic-ecologic data, we must apply an appropriate method that be according to factors governing nature and can be used for determination and discrimination existence of inter and intraspecific diversity. In this order, we can use the unit of study (Endogenous milieu) in Eco-phytosociological method (Atri, 1996, 1999).

In vegetations study, endogenous milieu determine by physiognomic-floristic-ecologic criteria. Establishment of relieves (stands) carry out randomly in each Endogenous milieus for floristic-ecologic data collecting (figure 1). Finally, data analyses lead to know plant associations of vegetation study. While for studying inter and intraspecific diversity, an Endogenous milieu (special station) determine base on the presence of individual of studied species in its stations (Atri and Asgari Nematian, 2006). Data collecting in inter and intraspecific diversity study is based on floristical composition in each special station (figure 2). floristical composition as floristical marker is good marker, because any kind of changing floristical composition in different special stations show existence of different ecological factors in them, that lead to inter and intraspecific diversity. We have done some research by this method (Atri, 1996, 1999; Atri et al, 2006, Atri et al, 2007).

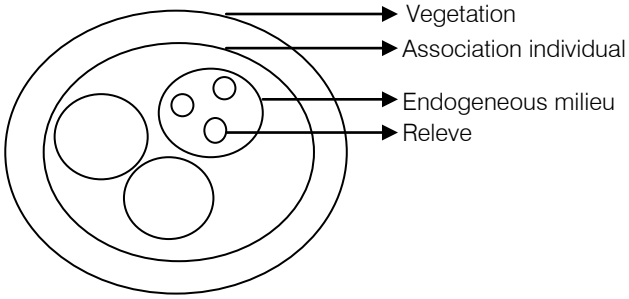

Figure 1 : The use Eco-phytosociology method for determine association

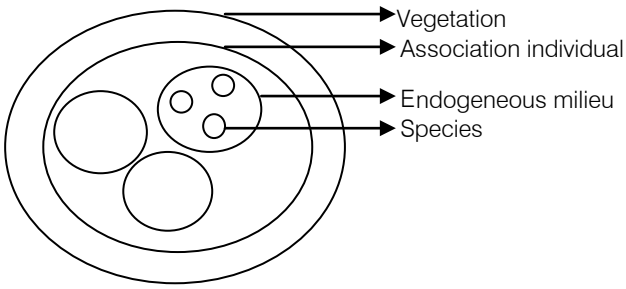

Figure 2 : The use Eco-phytosociology method for determination and discrimination intra and interspecific variations

V. Materials and Methods

Plant materials: At the first phase, different stations of *Astragalus verus and Astragalus glaucops* were determined in the west of Iran by using the accessible references, Herbaria and existence information. Then we referred to the different stations in study area, along 2004-2006 years, in growth season for collecting floristic-ecologic data. Totally between studied stations, 31 stations selected for investigation in Hamadan, Kermanshah, Kordestan and Markazi provinces from west of Iran (Table 1 and 2). Data collecting from 31 selected station carried out by using the unit of study in Eco-phytosociological method (Atri, 1996, 1999; Atri and Asgari Nematian, 2006) that is named Endogenous milieu (special station). In each station, location of establishment for each relive (stand) determined on base of presence of individual study species.

Then for determination of special station of individual study species, minimal area determined by using the area-species method with area-species curve and Cain method (Cain *et al.*, 1959). All ecologic-floristic data (the studied species and its companion species as floristical markers) were collected of each special station. Plant specimens deposited in the Herbarium, of Bu-Ali Sina University in Hamadan, Iran. Studied ecological factors included (elevation, pH, EC, texture of soil, slop direction and slop percent) in each special station.

Flavonoid aglycone study: The plant leaflet of different individuals of *Astragalus verus* and *Astragalus glaucops*, that collected from different special stations in west of Iran, were separated and then ground in a grinder. Flavonoid aglycone analysis was taken on all individuals of *Astragalus glaucops* and *Astragalus glaucops* listed in (Table 1 and 2). Briefly, 2 g dried powder of leaves boiled in 50 mL 2 M HCL for 45 min. Hydrolyzed leaf extracts were allowed to cool to room temperature. The extracts were then washed 3 times with equal volumes of ethyl acetate. The pooled ethyl acetate fractions were evaporated to dryness in a fume hood. The residue of each plant sample was taken up in an equal volume of 95% ethanol (Joseph *et al.*, 2003). The analysis was performed on Silica gel plates 25 Fuelled aluminum CCM (20x20), Gel de silica 60 F_{254} (Merck). Replicate plates were developed in BAW (n-butanol: acetic acid: water, 4:1:5, top layer used). Quercetin, flavone and rutin used as standards. The plates were developed at room temperature in a vertical separating chamber to the height of approximately 14 cm from the start. After drying, visualization was performed by:

- Spraying with 1% methanolic diphenylboryloxyethyl-amine
- and 5% ethanolic polyethyleneglycole 4000

Chromatograms were interpreted in long wave UV light (366 nm), then were measured R_f of each bands (Medica-Saric *et al.*, 2004).

Data analysis: For determination and discrimination of intraspecific diversity of *Astragalus verus* and *Astragalus glaucops*, were applied correspondence, cluster, classification and discriminate analysis. Floristical composition data (as floristical marker) analyzed by using Anaphyto software version 95 (Briane, 1995) by means FCA (Factorielle Correspondence Analysis), AHC (Ascendant Hierarchical Classification) and Marquage methods. In studying phytochemical data, once presence and absence of different bands were determined on chromatogram for different individuals of *Astragalus glaucops* and *Astragalus glaucops*. Then phytochemical data analysis was taken by MVSP softwares by means UPGMA method. Ecological data analyzed by MVSP software with CCA method and Anaphyto software with FCA method.

VI. Results

Floristical results: Obtained results base on floristical composition analyses of 31 special stations showed seven main groups by using FCA method (Fig. 1). Group (A) include special station number 0031, 0022, 0020, 0015, 0014 group (B) number 0002, group (C) numbers 0042, 0043, 0044, 0045, 0046, 0047, 0048, 0049, 0050, 0018, 0007, group (D) number 00392, 0030, 0024, 0023, 0011 group (E) numbers 0032, group (F) number 0041, 0037, 0038, group (G) numbers 0006, 0005, 0004, 0003, 0001. It must indicate that the mentioned 7 groups obtained base on similarity and dissimilarity of their floristic composition (as floristical marker). The obtained results from FCA method completed by AHC and Marquage methods (Fig. 2, 3). These 7 main groups evidence existence of intraspecific diversity for *Astragalus verus* and *Astragalus glaucops* in study area

Flavonoid results: Determination of level and kind of intraspecific diversity were used by flavonoid studies. Prepared chromatograms by TLC method showed different flavonoid bands and also different quantity of bands in different individuals of *Astragalus verus* and *Astragalus glaucops* in study area. Different bands and their R_f measured. Analyzing of flavonoid data separate 5 groups (Fig. 4). The obtained groups of flavonoid results had a good correlation with floristical composition groups that confirm them and showed intraspecific diversity in chemotype level. These 7 groups are different regarding quality, quantity and R_f of flavonoid bands.

Ecological results: Ecological factors data that were collected by applied method analyzed by MVSP software with CCA method. The obtained results showed between studied ecological factors (elevation, pH, EC, texture of soil, slop direction and slop percent) elevation factor has the most important role in separating different determined groups (Fig. 5, 6).

VII. Discussion

Creation of inter and intraspecific diversity are the main origin and storage of speciation. In this order, creation, inter and intraspecific diversity in different levels cause to richness of taxa in an area. For determination and discrimination of inter and intraspecific diversity, should applicate and suitable method that the obtained results of applied method be correct, precision and spends lower expenses (Atri et al, 2006, 2007).

Many studies carried out for determination intraspecific diversity (Turesson, 1922; Mooney and Billings, 1959; Perez-Alonso *et al*., 2003; Koch and Bernhardt, 2004; Semmar *et al*., 2005; Telascrea *et al*., 2006), but most of them do not apply a special method in plant data collecting process and carry out by time consumer and expensive experiments. While, application the floristical marker and the unite of study in Eco-phytosociological method (special station) in this kind of studies led to correct and precision results because that is according to factors governing nature. In the other hand, it prevents of more expenses and time consumer experiments (Fakhre-Tabatabaei *et al*., 2000; Sefidkon *et al*., 2003; Kalvandi *et al*., 2004; Sefidkon *et al*., 2005; Ebrahimzadeh *et al*., 2006; Atri et al, 2006, 2007).

In regard to applied principles in this method for data collecting, we can certainly declare that floristic markers without application other markers can determine intraspecific diversity existence. Then for determinating kind and level of intraspecific diversity (Ecophene, Chemotype, Cytotype, Ecotype …), between obtained floristical groups, we can use other studies such as: morphology, anatomy, phytochemistry, cytology and etc.

Present results show that *Astragalus verus* and *Astragalus glaucops*, has high diversity in the west of Iran. According to our results of floristical analyses, there are 7 distinctive different groups of *Astragalus verus* and *Astragalus glaucops* individuals in study region. At second phase, phytochemical studies create seven kinds of chemotypes which conform and affirm the obtained results of floristical studies.

In picture 1_ 3, Special stations of *Astragalus verus* and *Astragalus glaucops* separated from each others. Group (C) include numbers 0042, 0043, 0044, 0045, 0046, 0047, 0048, 0049, 0050, 0018, 0007 separated by floristic composition was belonged to *Astragalus glaucops* that conformed with flavonoid analysis (Fig 4). On the other hand, flavonoid profile of *Astragalus verus* and *Astragalus glaucops* separated from each others that conformed to floristical composition.

The phenomena such as interactions, substitution, stenoece and euryece nature of species and existence of intra-specific and inter-specific relations, consideration of the ecological factors as the base and pillar by focusing on one or a number of predetermined ecological factors to study vegetation, could not express the existing reality in all times. On the other hand, with respect to homogeneity of the environment for dominant species and its non – homogeneity for other species, the possibility of careful determination of association individuals or the homogenic surface in ecologic- floristic term is low. With respect to the aforesaid instances in studying effective ecological factors on the vegetation, eco-phytosociology method was employed. In this approach, it became possible to determine principal ecological factors. Between studied ecological factors, elevation is the most important ecological factor in creation intraspecific diversity. For *Astragalus glaucops*, elevation starts of 2450m to 2750m. The present study shows that in studying the vegetation and determining ecological factors, employing ecological and phytosociologic criteria as eco-phytosociology (Atri, 1996) are not only suitable and exact in the data collection stage to determine the placement of releves, but also it is able to provide results, which conform and agree to the rules that govern the nature in the analysis and result interpretation stage.

So this study and other studies that done base on this method until to now, show the high efficiency of it in determination and discrimination of inter and intraspecific diversity existence. By using this method after determinating floristic groups, we should characterize kind and level of intraspecific diversity only between obtained floristic-ecologic groups and this in require us of testing all of the individuals of studied species and expending long time and much money in this way.

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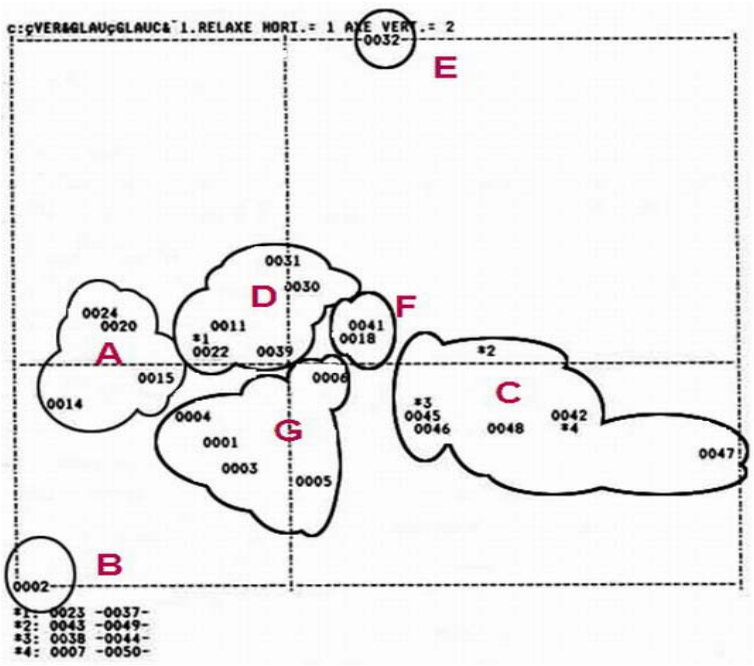

Figure 1: Results of floristical composition data analysis by FCA method on1, axes

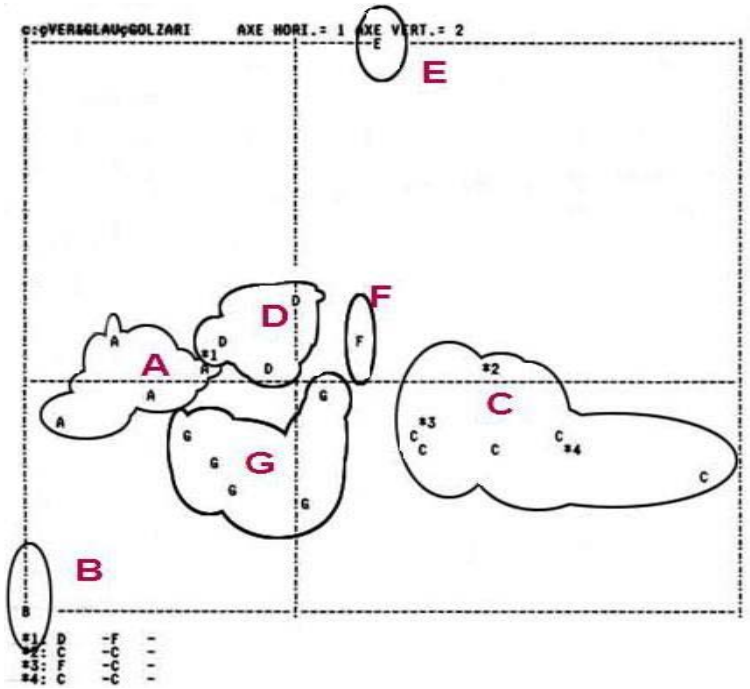

Figure 2 : Results of floristical composition data analysis by Marquage method on1, axes

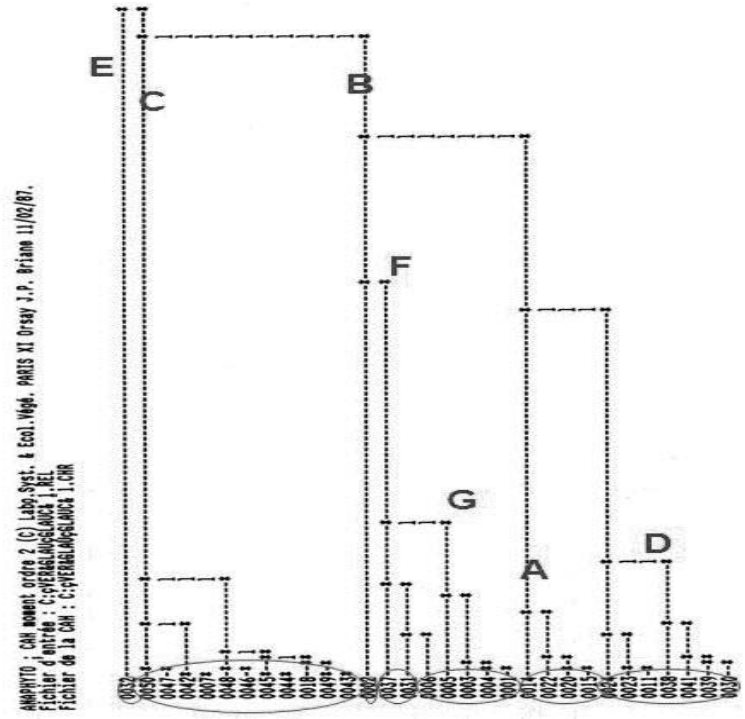

Figure 3: Results of floristical composition data analysis by AHC method on1, axes

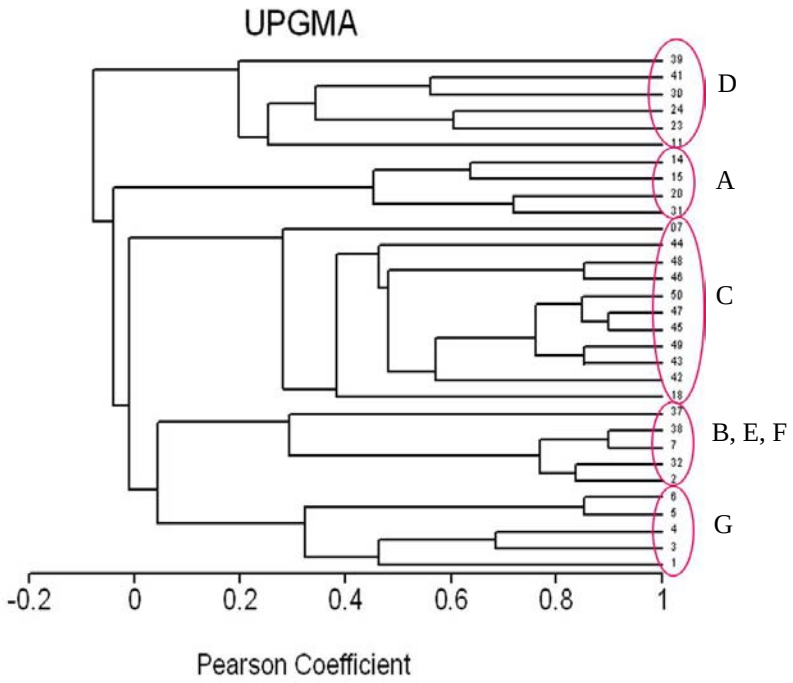

Figure 4. 2 : Resulted cluster of aglycoside flavonoids studies of *Astragalus glaucop and Astragalus verus* individuals by UPGMA method

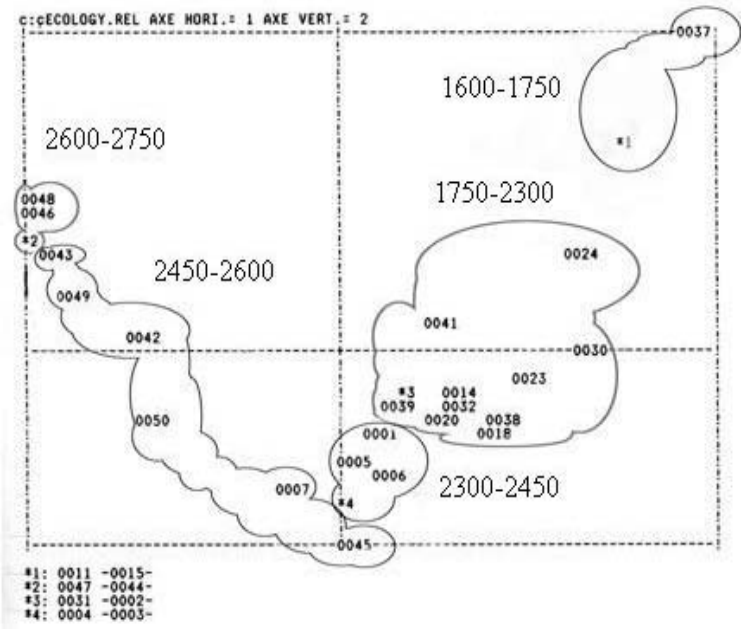

Figure 5 : Results of ecological factors studies by FCA method

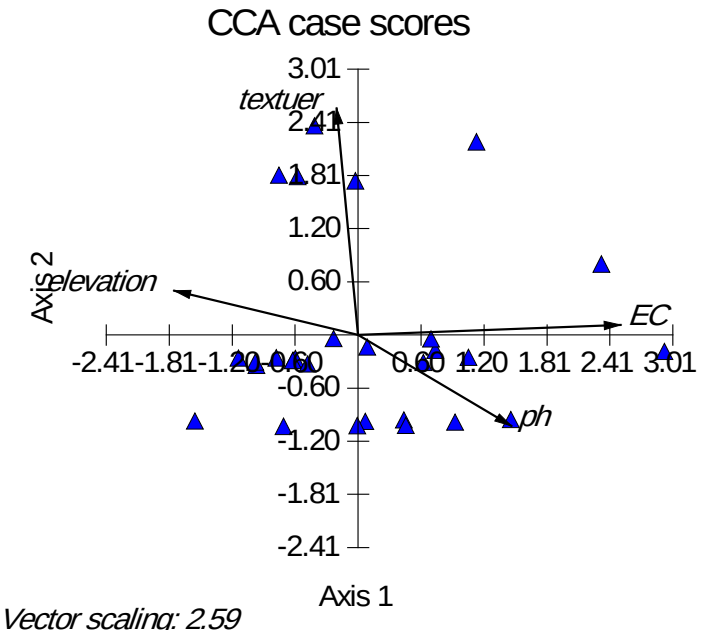

Figure 6 : Results of ecological factors studies by CCA method

Table 1: The different studies special stations for *Astragalus verus*

Releve NO.	Voucher No.	Altitude	Place
1	7286	2425	Hamedan, Asad- Abad
2	7287	2417	Hamedan, Asad- Abad
3	7288	2408	Hamedan, Asad- Abad
4	7289	2424	Hamedan, Asad- Abad
5	7290	2416	Hamedan, Asad- Abad
6	7291	2344	Hamedan, Asad- Abad
7	7292	2563	Hamedan, Alvand
11	7293	1723	Hamedan, Nahavand
14	7294	1898	Hamedan, Razan
15	7295	1723	Hamedan, Razan
20	7296	1898	Hamedan, Touyserkan
23	7298	2213	Hamedan, Divijin
24	7299	2220	Hamedan, Divijin
30	7300	1840	Kermanshah
31	7301	1850	Kermanshah
32	7302	1800	Kermanshah
37	7303	1610	Kordistan, Sanandaj
38	7304	1995	Arak
39	7305	1940	Arak
41	7306	2008	Arak

Table 2 : The different studies special stations for *Astragalus*

Releve NO.	Voucher No.	Altitude	Place
7	7307	2563	Hamedan, Alvand
18	7308	2300	Hamedan, Touyserkan
42	7312	2540	Hamedan, Alvand
43	7310	2536	Hamedan, Alvand
44	7313	2547	Hamedan, Alvand
45	7309	2600	Hamedan, Alvand
46	7311	2650	Hamedan, Alvand
47	7314	2660	Hamedan, Alvand
48	7315	2723	Hamedan, Alvand
49	7316	2570	Hamedan, Alvand
50	7317	2598	Hamedan, Alvand