ECOLOGIA BALKANICA

International Scientific Research Journal of Ecology

Volume 4, Issue 1
June 2012

UNION OF SCIENTISTS IN BULGARIA – PLOVDIV

UNIVERSITY OF PLOVDIV PUBLISHING HOUSE

International Standard Serial Number
Print ISSN 1314-0213; Online ISSN 1313-9940

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The current issue of Ecologia Balkanica is published with the financial support of Bulgarian Science Fund, Ministry of Education, Youth and Science, project №НП03 014.

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ECOLOGIA BALKANICA – Volume 4, Issue 1 / 2012

CONTENTS

Research Articles

Short Notes

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 1-7

Determining the Heavy Metal Pollution in Mascara (Algeria) by Using Casuarina equisetifolia

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Abstract. In this study, *Casuarina equisetifolia* needles were evaluated as the possible biomonitors of heavy metal air pollution in Mascara (Algeria). The needles were sampled from seven locations with different degrees of metal pollution (near roads) and from a control site. The concentrations of lead, zinc, copper and nickel were measured by using a flame atomic absorption spectrophotometer. The maximal values of these four metals were found in the samples collected near the roads and the minimal values were found in the control site. Furthermore, sites with high traffic density and frequency of cars stoppage showed high heavy metal concentrations. However, the comparison of concentrations of all metals showed that the zinc one had the highest concentration of all. The cluster analysis divided the selected sampling sites in three distinct clusters. With regard to the results of this study, *Casuarina equisetifolia* can be successfully applied in biomonitoring of air pollution.

Key words: *Casuarina equisetifolia*, Mascara, biomonitors, pollution, heavy metals.

Introduction

Heavy metal pollution represents an important environmental problem due to toxic effect of metals, and their accumulation throughout the food chain leading to serious ecological and health problems. In developing countries an estimated 0.5-1.0 million peoples die prematurely each year as a result of exposure to urban air pollution (KOJIMA, 2001). The emission of toxic substances into the environment has spread mainly from industrialized countries. However, many industrial plants and especially road traffic may emit heavy metals into the atmosphere. LEYGONIE (1993) noted that, fossil fuels contain many kinds of heavy metals which are emitted during the combustion of those fuels. Furthermore, the wear of auto tires, degradation of parts and especially paint, and metals in catalysts are all suspected as potential sources of heavy metal pollution (SADIQ *et al.*, 1989; WEI & MORRISON, 1994; MONACI *et al.*, 2000; OZAKI *et al.*, 2004; SUZUKI *et al.*, 2009). Generally, traffic related pollutants include toxic metals like lead, cadmium, copper and zinc (VIARD *et al.*, 2004). On the other hand, some trace metals are essential in plant nutrition, but plants growing in a polluted environment can accumulate them at high concentrations (HOVMAND *et al.*, 1983; HUCKBEE *et al.*, 1983; KABATA-PENDIAS & PENDIAS, 1984; ALLOWAY, 1990; VOUSTA *et al.*, 1996; SHARMA *et al.*, 2004).

The first attempts for (in the early 1960s) biomonitoring and assessment of environmental pollution coming from exhaust gases of automobiles in road traffic were based on the analyses of different trees, grasses and vegetables that grow near highways and in the cities. Since then,

phytomonitoring is increasingly used as an alternative to the traditional methods, for studying the regional deposition of natural and anthropogenic pollutants from the atmosphere to the terrestrial environment (PACHECO *et al.,* 2001; DOGAN *et al.,* 2007). An advantage of plants as biomonitors is that they are effective collectors which reflect the summarized effect of environmental pollution and accumulation of toxicants from the atmosphere (deposition, binding and solubility of metals on the leaf surface).

Recently, different bio-indicators are used in monitoring of the air pollution especially in urban areas. Botanical materials such as fungi, lichens, tree rings and leaves of higher plants have been used to detect pollution level (HUSEYINOVA *et al.,* 2009). The use of higher plants, especially different parts of trees, for air monitoring purpose is becoming more and more widespread. Tree leaves have been widely used as indicator of atmospheric pollutions (KOVÁCS, 1992; PETROVA, 2011), and they are effective alternatives to the more usual monitoring methods, including mosses and lichens. Trees are long-lived organisms, which can take up trace elements from the soil, water, or air, and retain them for a long time (MADEJON *et al.,* 2006). However, the foliage of tree species from contaminated regions can be considered as an accumulation monitor where significant amount of chemical elements is cumulated on the leaf surface (MANKOVSKÁ *et al.,* 2004). According to SRINIVAS *et al.* (2009) atmospheric metals are deposited on plant surfaces by rain and dust. On the other hand, airborne pollutants can retain on leaf surfaces and some elements could enter via the stomata and accumulate in leaf tissues.
The aim of this study was to assess *Casuarina equisetifolia* needles as possible biomonitors of heavy metal pollution in Mascara city (Algeria).

Material and methods

Study area and sampling sites

This study was carried out in the city of Mascara which is located in the North-West (NW) part of Algeria at N 35°26', E 02°11' (Fig.1). It is one of the most populated cities of Algeria (over 365 000 inhabitants), with Mediterranean climate and a mean annual precipitations of about 450mm. The average annual temperature is 13.1°C.

Nowadays, *Casuarina equisetifolia* covers the majority of urban trees in Mascara and it has a very wide range of horticultural use. The needles of *Casuarina equisetifolia* were collected in June 2011 from seven locations (sites S1 to S7), near the roads and from one control site (St) located far from road traffic and other anthropogenic sources of metal contamination (Fig.1). Cars were the dominant vehicles in all the seven sites. No other sources of pollutants were noted. At each site needles samples were taken from the lower part of the tree crown at the 2-3m height in all directions. They were cut directly from the branch, at about 1cm from needle base. All the samples were stored separately in clean cellulose bags to avoid further contamination and were transported to laboratory in the same day.

Chemical analysis

In laboratory, about 1g of dried and milled plant material was put into Erlenmeyer's flasks with 6 ml mixture of 65 % Nitric acid (HNO_3) and 70% Perchloric acid ($HClO_4$). Later, they were incubated in hot water (52°C) for at least 2 hours. Then these digests were filtered by a Whatman filter paper. The obtained filtrates were completed by deionized distilled water and the new solutions were stored in glass bottles and used to determine heavy metals concentrations (MAATOUG *et al.,* 2007). The concentrations of Pb, Zn, Cu and Ni were measured by a flame atomic absorption spectrophotometer (Perkin-Elmer, 280 model).

Statistical analysis

Pearson correlation coefficient was used to analyze and establish inter-metal relationship. Cluster analysis was performed to classify and identify relatively homogenous groups with similar properties. For all statistical analysis the STATISTICA 6.0 statistical package was used (STATSOFT, 2001).

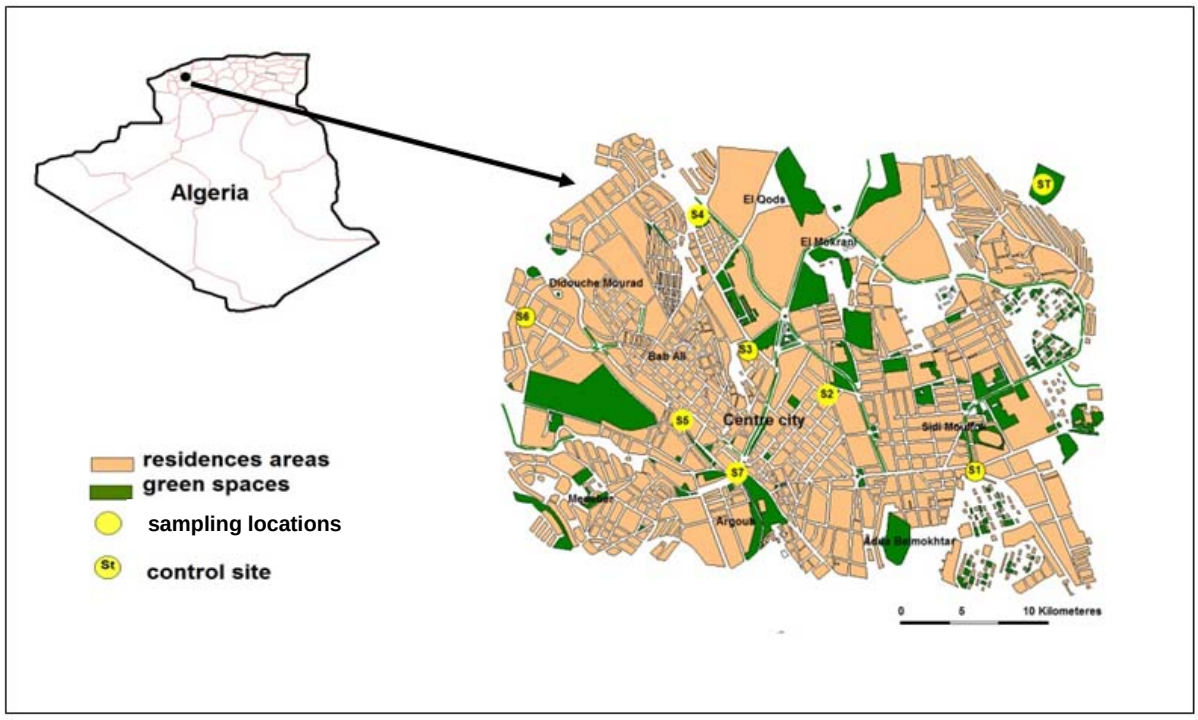

Fig.1. Geographical location of the study area and sampling sites.

Results and Discussion

The chemical analysis revealed a significant difference in Pb, Zn, Cu and Ni concentrations in needles samples collected from different sites. This can be attributed to the different traffic density between the seven sites (Table 1).

Table 1. Heavy metal concentrations (ppm) in needles of *Casuarina equisetifolia.*

Sites	Zn	Pb	Cu	Ni
Site1	141.07	23.73	22.5	17.7
Site2	144	51.01	20.4	11.7
Site3	175	51.07	21.5	17.22
Site4	178	31.22	21.01	20
Site5	453	67.5	23.5	21
Site6	146	43.9	37.02	16.3
Site7	440	60.7	16.3	21.07
Control	2.33	0.54	0.21	0.60

However, comparison of concentrations of all metals showed that the zinc one had the highest concentration of all. The average highest value of Zn (453 ppm) were detected in samples collected from site 5, whilst the lowest (2.33 ppm) was measured in samples collected from the control site. It was observed that the second highest values were found in samples collected from site 7 (440 ppm). The environmental pollution of Zn greatly influences the concentrations of this metal in plants (SRINIVAS *et al*., 2009). Zn arises mainly from atmospheric deposition and could also be derived from vehicular traffic (CONTI *et al*., 2008). Zinc levels can be enhanced in automobile exhaust, may be elevated near roadways due to tire wear. On the other hand, zinc is an essential element for plants and is considered as an important factor in the biosynthesis of enzymes, auxins and some proteins. But when their concentrations reach a certain level, they become toxic to plants and reports produce various physiological and biochemical changes in plants. A critical toxic level of Zn in the leaves is about 100ppm (ALLEN *et al*., 1974; YILMAZ & ZENNGIN, 2004). According to these values, the Zn concentrations found in our study are higher than the normal limits. Therefore, it can be supposed that all the seven sites studied were heavily polluted with Zn.

The Pb concentrations were the highest at site 5 (67.5 ppm), and the lowest at the control site (0.54 ppm), whereas the second highest value was found in site 7 (60.7 ppm). Lead pollution on a local scale is caused by

emissions from motor vehicles using leaded gasoline (KOEPPE, 1981; VIARD *et al.*, 2004; YILMAZ & ZENGIN, 2004). In Algeria, the addition of lead in gasoline is 0.45g/L (SEMADI & DERUELLE, 1993). Lead is known as a deadly and cumulative poison even when consumed in small quantities and is capable of deadening nerve receptors in man (NWAEDOZIE, 1998). The relationship between lead concentrations and traffic intensity has been demonstrated in detail by many authors (GROMOV & EMELINA, 1994; LI *et al.*, 2001; VIARD *et al.*, 2004). ALLEN (1989) considered that the normal content of Pb in plants is less than 3ppm. In general, Pb concentrations in vegetation grown in industrial and urban areas have increased in recent decades owing to human activities and road traffic. According to our results, there is lead pollution in Mascara city.

The average highest value of Cu (37.02 ppm) was found in samples collected from site 6, whilst the lowest (0.21 ppm) was measured in the needles collected from the control site. Copper is an important component for many enzymes, which catalyze oxidation and reduction reactions. The main sources of Cu are home tools production, metal manipulating, road traffic and ashes (AKSOY *et al.*, 2005). KABATA-PENDIAS & PIOTROWASKA (1984) reported that the normal content of Cu in plants ranges to be 2-20 ppm, but in most cases it is in a narrower range of 4-12 ppm. According to these values, the Cu concentrations found in this study are higher than the normal limits. So we can conclude that there is copper pollution in Mascara city.

The average highest value of nickel (21.07ppm) was found in samples collected from site 7, whereas the lowest mean value was determined in the control site (0.60ppm). It was observed that the second highest values were found in samples collected from site 5 (21ppm). These results indicate that the origin of nickel in the investigated locations is related to vehicular traffic. Ni is essential element for plants in low concentrations and is absorbed easily and rapidly by them (GUNES *et al.*, 2004). According to AL-SHAYEB & SEAWARD (2001), the highest concentrations of nickel are attributed to emissions from motor-vehicle that use nickel gasoline and by abrasion and corrosion of nickel from vehicle parts. The results further revealed that the sources of nickel in Mascara city are emissions from motor vehicle running on petroleum and diesel fuel.

According to the cluster analysis (Fig 2), three distinct groups can be identified namely "cluster 1", "cluster 2" and "cluster 3" (Table 2). Cluster 2 includes the control site. This site has a good air quality (low concentrations of metals) which is explained by the absence of any sources of such contamination. Cluster 3 includes sites S1, S2, S3, S4, and S6. The mean values of Zn, Pb, Cu and Ni in this cluster are higher in comparison with data from cluster 2, but this cluster has a good air quality when is compared to cluster 1. However, all the sites of cluster 3 are characterized by low slope and low traffic density, for this reason, low concentrations of heavy metals were found. Cluster 1 includes sites S5, S7, where the highest levels of lead, zinc, copper and nickel are found. All the sites of cluster 1 are located in the central area of Mascara which is with high traffic density, frequency of cars stoppage, more abrasion of asphalt tire and brake.

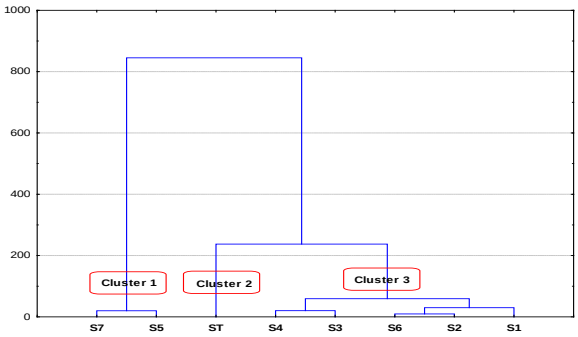

Fig.2. Diagram of cluster analysis.

In addition, these sites are characterized by important slopes. The slope requires the vehicle to develop more power, therefore emit more heavy metals (MADANY *et al.*, 1990). The braking in the slope increased the emissions of copper, from fine particles deposited on the leaf surface (MAATOUG *et al.*, 2007). AIJUAN *et al.* (2011) noted that low-speed or high-acceleration operations lead to higher emission.

Table 2. Mean of heavy metals (ppm) in the three clusters.

Clusters	Pb	Zn	Cu	Ni
Cluster 1	64.10	446.50	30.26	21.03
Cluster 2	0.54	10.33	0.21	0.6
Cluster 3	40.18	156.81	20.34	16.58

Correlation analysis showed that elemental pairs Pb/Zn (r=0.75); Pb/Cu (r=0.56); Pb/Ni (r=0.81); Zn/Cu (r=0.73); Zn/Ni (r=0.70); Ni/Cu (r=0.54) were significantly correlated with each to another. This is clear indication that the origin of metal contamination in the investigated area is related to vehicular traffic. SESHAN *et al.* (2010) noted that positively correlated metals became from the same anthropogenic source.

Conclusions

The results of this study show that the highest and the lowest concentrations of Pb, Cu, Zn and Ni were found near the road and the control site respectively. This indicates that the vehicular traffic has been major source of heavy metal contamination in urban areas. Correlation coefficients were calculated for each element-element combination. They showed positive correlation which indicated that the origin of studied metals in the investigated area is related to road traffic.

The cluster analysis divided the eight sites in three major clusters according to their characteristics, where sites with high traffic density, frequency of cars stoppage and important slope, showed the highest metal concentrations. The results of our study confirmed that *Casuarina equisetifolia* needles can be used as biomonitors of heavy metal pollution in urban areas.

Acknowledgements

Special thanks to undergraduate laboratory assistant Miss. Drir Fatima Zohra, and research assistant Mr Bouakkaz Mohamed for rechecking language of the document.

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Received: 05.10.2011
Accepted: 03.01.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 9-20

An Investigation into Eco-tourism Potential of the Alamut Region of Iran using SWOT Analysis Model

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Abstract. The present research examines the potentials and limitations of tourism in the Alamut region, offering suggestions and strategies for the promotion of tourism in this region. Research methodology adopted in this study is based on analytical-descriptive approach. To formulate and establish a sound research theoretical framework as well as to review the research literature, a questionnaire tooling method was used and the results were analyzed using the SWOT technique. While introducing the tourist attraction in the province, the influential factors in the region categorized into the four headings of: strengths; weaknesses; opportunities and threats were included in our considerations and strategies or guidelines for a sustained tourism development in the region provided. The research findings, taking into consideration the present status of the Alamut region, indicate that despite the fact that the region houses a great number of ancient sites and monuments and enjoys a high level of bio-diversity resources, making it the ideal candidate to be transformed into an exemplary tourist attraction pole, it is faced with major obstacles to reach this goal. In order to remove some of the problems of the region, strategic factors were analyzed and priorities delineated. The findings indicated that a focused attention and description of the values of the region is alleviated through the holdings of seminars or meeting and conferences as well as paving the way to give cultural knowledge and understanding to prevent inflicting widespread damages to the environment; to establish hotels; to provide welfare facilities and introduce natural resources as well as historical sites and their ancient heritage.

Keywords: Ecotourism, SWOT, Strategic factors, Alamut, weighted score.

Introduction

Today the subject of tourism and eco-tourism, due to the high income-generating feature, has encouraged many countries to allocate a sizeable amount of investment to this sector (TREMBLAY, 2006). Eco-tourism being only one part of the whole structure in the industry of tourism is regarded as a relatively recent venue (NYAUPANE & THAPA, 2004). This form of tourism is basically tuned to the leisure activities made possible in the open nature at large from the visiting of the natural attractions of the host communities, studding their myriad wonders and impacts and drawing enjoyment from the diversified presentations of the nature, be coupled with collateral cultural and spiritual benefits which is made possible if it is based on a streamlined and targeted host destination (JIANG, 2008).

That which is of vital importance in the considerations of eco-tourism is the subject of sustainability (HONEY, 2008). In this approach, the development of tourism with the utilization of the existing resources is

such that while responding to the economic and socio-cultural needs of the tourists as well as due consideration of their legal and conventional rights and prevailed on expectations, the unity, consolidation, cultural identity, environmental health, economic balance and welfare of the host communities are secured (ALVANI, 1993). Therefore, a pre-planned, deliberated tourism system, with the least flaws possible, will lead toward a healthy and suitable use of the environment and diverse peripheral, cultural, historical resources and the like in the region (EDGELL *et al*., 2008).

For this purpose, an action is to be taken to devise and draw up comprehensive plans and programmers for the said specified areas and whiten the framework of these working plans move toward strategic elimination and mitigation of the problems in the area. The removal on eradication of such problems for the good pends as sound reformation of the management and budgeting system in the economic, social and infrastructural fields related to the tourism enterprise and activities.

Iran, being among the first five countries in the world benefitting from territorial diversity, is one of the first ten countries in world with regards to historical and cultural attractions and is one of the first three countries enjoying a range of diversified handicrafts and a cache of biological diversity on earth. Compared to other branches of tourism, Iran enjoys a relatively advantageous position in the filed of eco-tourism (ZANGIABADI *et al*., 2006; TAVANGAR, 2010). Iran also ranks second in the south region of Asia in attracting the greatest number of tourists and the revenues in this section are only second to attract India (EBRAHIMZADEH & AGHASIZADE, 2009).

Alamut as one of the regions in the Qazvin province, enjoys an exceptionally unique status among its counterparts in the province. Qazvin is one of oldest civilization in Iran which was the capital of the Safavids dynasty for about 100 years. Alamut too as an area characterized for its various tourism attractions (the high diversity of flora and fauna, extensive historical and cultural sites as well as climate variability) possesses great potentials for tourism and conservation as well as research values but viable programming to make use of these favorable conditions is still in its toddler stages. No serious or significant steps have, as yet, been taken to benefit from the natural attractions of this region and it is vitally crucial to undertake a more precise study with regards to making use of these attractions and also the construction of appropriate infrastructures for the development of this novice industry. Thus, the present study tries to introduce the potentials and viabilities of tourism in the Alamut region as well as the difficulties and obstacles in the way of tourism ventures in the region. To this end, the study makes use of the SWOT method of analysis - an acronym for the words: strengths, weaknesses, opportunities and threats (WHEELEN *et al*., 2004), to provide an objective point of view based on the existing facts and realities for the purpose of identification of intervening factors in the development of Alamut tourism as well as finding the strengths; weaknesses; opportunities and threats thus engage in the development of strategies to promote tourism in the region and adopt appropriate managerial decisions to enhance and upgrade the attraction of tourism.

Study area

Alamut region is located in the northern east part of Qazvin province on a longitude of 50 ° 02 '54 "to 50 ° 52' 55" to the east and a latitude 36 ° 17 '11 "to 36° 41' 01" the north (Fig. 1). Alamut is spread over an area of 170461 hectares, housing 203 villages within a totally mountainous territory. The highest and lowest summits in this area are 4175m and 658m above sea-level respectively (ABTAHI, 2001). On the basis of the statistics and recordings released by the "Baghe Kelayeh" synoptic weather station, the annual average rainfall in the area is 404 mm. The dry period starts from almost late May and extends to early October. Most of atmospheric falls are in the form of rain and in the form of snow above the height of 3000 meters. The absolute minimum and

maximum temperature are -19°C and 42°C respectively. The greater Alamut area has been divided into the two regions of Roudbar Alamut (East Alamut) and Rudbar city (West Alamut) in the last geographical divisions each with three rural districts.

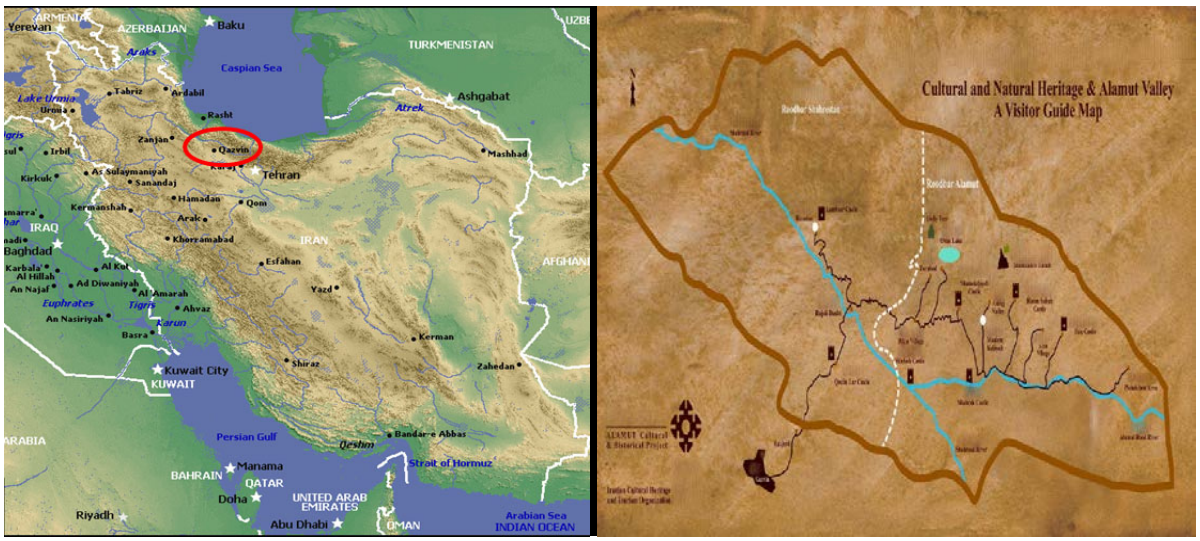

Fig. 1. Ecotourist map of Alamut (Qazvin province) in Iran.

Alamut region has a variety of vegetation due to the diversity of its topographical and climate conditions. A study of the rich flora of the region indicates the high bio-diversity existence of 782 species of plants belonging to 86 families and 452 genera (CHARKHCHIAN *et al*., 2009). Out of 1420 plant species identified in the Qazvin province, about 60 percent are distributed in the Alamut region (CHARKHCHIAN, 2000). A number of 7576 plant species have been identified in Iran. Alamut region with an area of 170461 hectares, houses 10 percent of the flora of the whole country. Therefore, the Alamut region as an indicative case for the study of its vegetation is representative (CHARKHCHIAN *et al*., 2009).

Due to its historical heritage, vast expanse and diversified ecosystem, there are 23 species of mammals, 47 species of birds and more than 20 species of reptiles and amphibians in the Alamut region, giving it the potential for tourism in general and drawing more attention for conservation efforts and research values. It can even be claimed that the region enjoys a particularly unique character as compared to its counterpart regions in the province (KARIMI GHASR, 2001). On the basis of the IUCN classification, three species of birds are in danger of extinction and one species is vulnerable to go the same way. The results gained by the IUCN classification system appears the same trend for the mammals of the region. The exists numerous natural caves in the Alamut region, some of which have been natural habitats to the human species throughout history such as Delvkan, Andaj, Akvjan and Sefid Abb cave to be between 250 to 290 million years old (KARIMI GHASR, 2001).

Valley cave is located at an altitude of 1620 meters belonging to the Permian period in the late part of the first geological period. The outstanding characteristic of the Alamut region in the old days is the existence of its various castles and fortresses. Hassan Sabah's Fortress (the ruling centre of Alamut Despot); Navizar; Meymoon; Lomisar (deputy governor and winter quarter of rulers in Razmian); Shirkooh; Ghestin Laar castles are some of the more important ones. Avan lake which is a beautiful natural pond giving this historical cultural region a great value, is located in the Rudbar Alamut region with an attitude of 1780 m above sea-level. It is 500 meters in length and also width. It has an area of more than 70000 square meters and an average depth of 5.70

meters. The only source of inflow of water into lake is the springs at bottom of the lake. 270 permanent springs and 106 seasonal springs have been spotted in the Roudbar Alamut region. The area conserved in which shooting and hunting are prohibited in the Alamut region is approximately 111118 hectares. The area replete with diversity of animal life begs for conservation measure taken (KARIMI GHASR, 2001).

Material and methods

With due consideration of the nature of the subject matter and its components under study, an analytical-descriptive approach is adopted. For the collection of data, procedures such as documental surveys, library reviews, field studies and a questionnaire were implemented. The information obtained was used to examine the attractions, possibilities and the general paradigm of tourism in the region. To determine the sample size for the distribution of questionnaires among the eco-tourists visiting the Alamut region, Cochran statistical formula was used:

$$n = \frac{(Z_\alpha)^2 P(1-P)}{(d)^2},$$

where: n = Sample number; Zα = Normalized variable (the confidence quotient is derived from the table); P = The numerator divided from division of the correct choices to incorrect ones (Respondents are allowed to select an option); d = Sampling error.

164 numbers (n) were obtained. Results were analyzed by using the SWOT model.

SWOT technique or matrix is a factor for the determination and understanding of threats and opportunities in an external environment and recognition of internal threats and opportunities for the assessment of the existing situation to guide and control the system (MORADI MASIHI, 2002; WHEELEN & HUNGER, 2004). With the information gathered and the results obtained from the tourist polling surveys as the host community and evaluating the relative parts accordingly, the SWOT matrix was completed and suitable strategies and guidelines were recommended for the development of tourism activities in the Alamut region.

Eight steps to build this matrix were considered. Preparation of 1) a list of the major opportunities that exist in the external environment; 2) a list of major threats in the environment; 3) a list of internal strengths; 4) a list of major internal weakness; 5) the internal weaknesses and the existing opportunities were compared and strategies result registered in WO; 6) strengths of internal and external opportunities were compared and the result registered in SO; 7) comparative strengths of internal and external threats were done and strategies result registered in ST; 8) comparison of internal weaknesses with external threats and the result registered in WT strategy group.

For the evaluation of strategic internal and external factors IFE and EFE matrices were used. IFE matrix is indicative of internal strengths and weaknesses and EFE matrix is representative of external threats and opportunities. Matrix was formed to evaluate each separate factor. To each factor was assigned a weighting score between zero (insignificant) to one (very important). As a result, the total point in each matrix is equal to one. Then, the present situation of each agent was determined with a number between 1 and 5 (poor = 1; lower than the average= 2; median = 3; above average = 4; good = 5) and weighted score was calculated for each factor. Rate of each row of internal and external factor playing an effect in the region was multiplied by the weight of the normalized factor and placed in a new column as a weighted score. After completion of both the IFE and EFE matrices, the table strategy is completed. This table compares the internal and external factors, and devises strategies to eliminate weaknesses and threats, and strategies to promote strengths and opportunities are developed. Finally, to set priorities for implementing these strategies, the data are entered into the matrix QSPM. Stages to from the QSPM matrix are as follows. In the first column in this matrix, the list of the external factors, strategic

factors including all threats and opportunities as well as internal factors, strategic factors including all weaknesses and strengths are recorded. In the second column the weighted score of each strategic factor is copied directly from the IFE and EFE matrices. The following columns contain a variety of strategies resulted from the SWOT matrices incudes the quadruple strategies of WO, ST, WT and SO. Each of the corresponding columns related to the strategies is divided to sub-categories (namely that of AS and TAS). Under the column of AS, attraction score is given; it such that each strategy is compared and measured with the relative strategy in question to see whether this factor does have an effect in the corresponding strategy? The attractiveness scores are distributed as such: 1 = not attractive; 2= relatively attractive; 3 = possible acceptable attractiveness; 4 = highly attractive.

The scores for the second column (weighted or balanced score) are multiplied by the rated attractiveness and the total points of interest or attractiveness are interested in the TAS column which represents the relative attractiveness of each factor on the strategy. The total point of TAS are calculated at the bottom row of each strategy column and the different options of strategy for each organization shall be determined through its numerical value and thus the basis for a comparison provided.

Results

A review of the questionnaires completed by tourists visiting the area showed that 56 percent of the visitors were men and 44 percent of them were women. 58 percent of visitors were married. 51 percent of them were between the ages of 20 to 40 years. Based on the polls conducted more than 80 percent of people are only familiar with the tourism concept very little. 15 percent of the people asked had no knowledge of the tourism concept which is indicative of weak information giving at advertisement on this industry in our country. This weakness may be regarded as one of the loopholes in the region. 48 percent of tourists are of belief that the promotion of ecotourism industry in the Alamut region can greatly contribute to employment and economic prosperity of the region in general.

38 percent of the tourists believe the high impact of eco-tourism on the environmental culture. Tourists believe that the Alamut region enjoys a great potential to attract tourism. This is one of the strengths of the region in their view. 90 percent of the people visiting the area rate information giving and advertisement as medium to very weak. 68 percent of the visitors to the region have an acceptance of the local culture. 63 percent of them have a moderate tendency to gain any familiarity of the culture and local, ethnic customs of the indigenous people. These are no common beliefs or understanding among tourists and visitors as the role and the responsibility of General Department of Environmental Protection of the province concerning the development of eco-tourism in Alamut region. This clearly shows lack of knowledge of the people about the role duties on responsibilities of such a department. More than 80 percent of tourists to the region evaluate the high potential of the region in the attraction of tourist. Also, 80 percent of the visitors to the region, evaluate as very weak the current facilities in the region quantitatively and qualitatively. 48 percent of tourists to the region are dissatisfied with the facilities in the region, they emphasize the need to upgrade and promote the quality of the facilities as a major requirement for the promotion of eco-tourism of the Alamut region.

The results of the Analysis of internal factors affecting and their role on Alamut ecotourism

As shown in Table 1, 10 points of strength and 11 points of internal weaknesses have been identified in this area. The organization and categorization of the internal factors in the two sub-categories of points of strengths and weaknesses move to evaluate the method of management and response of the system to these factors and with due consideration of the importance of

the Alamut region in general. The above factors were analyzed and their proportional influence index or coefficients were separately determined in Table 1. From the viewpoints of those tourists polled, the major points of strengths of the Alamut region from an eco-tourism perspective were the historical sites and attractions as well as the Avan Lake which were ranked first with a weighted score of 0.268. The weakest point in their evaluations was recorded as non-reconstruction of the cultural and historical sites or monuments and an evident lake of welfare facilities suitable for tourism which hit a low score of 0.132.

Table 1. Internal Factors Evaluation Matrix (IFE) (strengths and weaknesses) in Alamut

Strengths	**Weight**	**Score**	**Weighted Score**
S1: There are ancient and religious monuments in Alamut	0.067	4	0.268
S2: "Avan" Lake area in order to attract ecotourism	0.067	4	0.268
S3: High biological diversity in the region	0.068	3	0.204
S4: Topography of the area(658 to 4175 meters)	0.05	4	0.2
S5: Historical castle of Alamut	0.067	2	0.134
S6: There are hot springs in the area for therapeutic use	0.067	2	0.134
S7: The presence of rare animal and plant species	0.034	3	0.102
S8: Shahrood river is the economic importance	0.05	2	0.1
S9: Select the region as a hunting ban	0.033	3	0.099
S10: Salt resources in the region	0.016	1	0.016
Weaknesses			
W1: Ignorance of the reconstruction of ancient monuments	0.066	2	0. 132
W2: The lack of hotels and other tourist facilities	0.066	2	0. 132
W3: Reduction of density of vegetation in the area	0.05	2	0.1
W4: Failure to perform required activities to introduce the national and international level	0.05	2	0.1
W5: Lack of adequate funding for conservation and scientific research and education in the region	0.034	2	0.068
W6: Neglect to clean and beautify Avan lake and other rivers	0.033	2	0.066
W7: Change user the surrounding area	0.05	1	0.05
W8: Environmental pollution caused by tourists because of lack of culture required	0.05	1	0.05
W9: Lack of understanding and adequate information on the ecological characteristics	0.05	1	0.05
W10: Lack of scientific and technical relations with the global network of protected areas, including UNESCO	0.016	1	0.016
W11: Disregard of the plaster as the natural wealth	0.016	1	0.016
Total	1		2.305

Analysis of external factors affecting Alamut ecotourism

According to Table 2, 10 opportunities against 7 external threats have been detected in this region. For organization of external factors in categories in terms of opportunities and threats, given the importance of these factors were ranked and was calculated for each level of their influence on the functions of Alamut then shown in Table 2. Pay attention to the result of this table the most important opportunities in this area about tourism development include the economic importance of the region with a weighted score of 0.201 as the first opportunity and exposure in the central region of the country and reduce the distance of this region by Mazandaran-

Qazvin highway construction with a weighted score of 0.198 are ranks next in importance. On the other hand environmental pollution caused by road construction with a weighted score of 0.414 and regional ecosystems collide and its destruction due to highway construction with a weighted score of 0.33 are the main threats.

Table 2. External factors evaluation matrix (EFE) (opportunities and threats) in Alamut

Opportunities	Weight	Score	Weighted Score
O1: Important economic region in the country as the region's tourism	0.067	3	0.201
O2: Located in the central part of the country	0.066	3	0.198
O3: Reduction of 200 km from Qazvin–Mazandaran by highway construction	0.066	3	0.198
O4: Easy access to area the opportunities for ecotourism	0.05	2	0.1
O5: There are fruit orchards in the slope domain in order to erosion control	0.05	2	0.1
O6: Transfer Alamut river water to Qazvin	0.033	3	0.099
O7: Employment in the area by highway construction	0.033	2	0.066
O8: Feasibility ecological studies in the region	0.033	1	0.033
O9: Expand the use of water springs in order to provide drinking water for the region	0.033	1	0.033
O10: Introduced the region as a priority research area for students and researchers interested in	0.018	1	0.018
Threats			
T1: Environmental pollution caused by road construction	0. 138	3	0.414
T2: Highway Construction Qazvin–Mazandaran	0.11	3	0.33
T3: Flood flow in the region due to the reduced density of vegetation	0.068	3	0.204
T4: Poaching in the hunting ban	0.05	3	0.15
T5: Excessive livestock grazing in the pastures	0.05	3	0.15
T6: Agricultural waste pouring into the area of internal waters	0.068	2	0.136
T7: Failure to introduce the region as one of the four areas of environmental protection	0.067	1	0.067
Total	1		2.497

Analysis of strategic factors

In this model, using the analysis of internal and external factors and their combinations, the most important strategic factors in Alamut tourism are presented in Table 3. In fact, by analysis of strategic factors planners that strategic decisions are taken, they can limit strengths, weaknesses, threats and opportunities to the less number of factors. Strategy Objective (SO) is taking advantage of the capabilities and maximum use of opportunities. Establishment of welfare hotels and places for ecotourism in the region, introducing Evan lake as a national natural monument are among the strategies that have executive priority. Increase the existing capacity and reduce threats done by design ST strategy. The most important strategies are meetings and conferences at local and national levels to express the value, raising public culture through education to prevent the destruction of the environment and obligation on assessment of development effects of executive projects on environment of Alamut. WO strategy aim is to reduce weaknesses and enhance opportunities. Increased research funding for scientific

Table 3. Matrix of tourism development strategy of Alamut (Numbers in parentheses are based on the guidelines for each of the executive order).

Internal Factors / **External factors**	**Strengths** 1.There are ancient and religious monument 2. Avan Lake area in order to attract ecotourism 3. High biological diversity in the region 4. Topography of the area 5. Historical castle of Alamut 6. There are hot springs in the area for therapeutic use 7. The presence of rare animal and plant species 8. Shahrood river is the economic importance 9. Select the region as a hunting ban 10. Salt resources in the region	**Weaknesses** 1. Ignorance of the of reconstruction ancient monuments 2. The lack of hotels and other tourist facilities for tourism 3. Reduction of density of vegetation in the area 4. Failure to perform required activities to introduce the national and international level 5. Lack of adequate funding for conservation and scientific research and education in the region 6. Neglect to clean and beautify Avan lake and other rivers 7. Change user the surrounding area 8. Environmental pollution caused by tourists because of lack of culture required 9. Lack of understanding and adequate information on the ecological characteristics 10. Lack of scientific and technical relations with the global network of protected areas, including UNESCO 11. Disregard of the plaster as the natural wealth
Opportunities 1. Important economic region in the country as the region's tourism 2. Located in the central part of the country 3. Reduction of 200 km from Qazvin – Mazandaran by highway construction 4. Easy access to area the opportunities for ecotourism 5. There are fruit orchards in the slope domain in order to erosion control 6. Transfer Alamut river water to Qazvin 7. Employment in the area by highway construction 8. Feasibility ecological studies 9. Expand the use of water springs in order to provide drinking water 10. Introduced the region as a priority research area for students and researchers interested in	SO: Establishment of welfare places and hotels for ecotourism in the region(3) SO2: Introducing Evan Lake as the impact of nature (4) SO3: Income as an ecotourism area to protect the rare species(7) SO4:Increasing demand for research funding and funding by the Department of Environmental Protection(8) SO5: The use of regional potentials for conservation of endangered species(11) SO6: Efforts to reach the castle of Alamut in the UNESCO as one of the Monuments(14)	WO1: Increased research funding for scientific studies and research in the area (20) WO2: Introducing region to the UNESCO to obtain financial facilities (21) WO3: Spread the density of trees to prevent erosion (22) WO4: Creation of opportunities for peacekeepers to patrol the area (23) WO5: Change user area with the permission of the Environment (24) WO6: The use of plaster in the region as an economic resource (25)
Threats Environmental pollution caused by road construction Highway Construction Qazvin – Mazandaran Flood flow in the region due to the reduced to the density of vegetation Poaching in the hunting ban Excessive livestock grazing in the pastures Agricultural waste pouring into the area of internal waters Failure to introduce the region as one of the four areas of environmental protection	ST1: Meetings and conferences to express the value of Alamut in local and national levels (1) ST2: Raising public culture by train to prevent the destruction of the environment (2) ST3: assessment of development effects of executive projects on environment of Alamut (5) ST4: Promote tourism tours to the region to visit the area and create prosperity (6) ST5: Manage the use of agricultural pesticides and herbicide and the use of organic farming and sustainable in the region (10) ST6: Law Enforcement for the illegal hunters and impose heavy financial penalties for noncompliance (12) ST7: Prevent to damage of salt resources if road construction in its path (13)	WT1: Introduction to natural and economic values through the media to increase public awareness (9) WT2: Prevent the reduction of biodiversity during construction and operation of highways (15) WT3: Prevent environmental pollution during construction and operation of highways (16) WT4: Aware of the staffing in the region as a guide (17) WT5: Identification of flood areas to create barriers and strengthen the density of vegetation (18) WT6: Serious and persistent surveillance on user changes of surrounding land (19)

studies and research in the area and introducing region to the UNESCO to obtain financial facilities are the most important of the this strategy. Finally, WT strategy aim is to reduce weaknesses and threats. Introduction to natural and economic values through the media to increase public awareness, prevent the reduction of biodiversity during construction and operation of highways and prevent environmental pollution during construction and operation of highways to reduce the weaknesses and threats are more important than other strategies.

In this study in order to avoid duplication of research data total points of TAS columns from QSPM matrix is given in Table 4. Finally, among the four strategies (SO; WO; ST; WT) recommended 26 strategies to the priorities for developing ecotourism in the region of Alamut. In the strategies table (Table 3), the number is listed. According to the results of Table 4, meetings and conferences to express the value of Alamut in local and national levels are the first priority of the executive; raising public culture by train to prevent the destruction of the environment is the second priority and the establishment of welfare places and hotels for ecotourism in the region is the third priority. In order to promote the region to attract tourists can pay to them. Among the strategies, the first 10 strategies are more applied and it is better at first is considered.

Table 4. Sum of the scores TAS from the matrix QSPM to determine the priority of the executive of these strategies

	SO6	**SO5**	**SO4**	**SO3**	**SO2**	**SO1**
	3.991	4.625	5.793	8.822	7.028	8.845
ST7	**ST6**	**ST5**	**ST4**	**ST1**	**ST2**	**ST1**
4.09	4.271	4.968	6.757	6.837	7.914	8.08
	WO6	**WO5**	**WO4**	**WO3**	**WO2**	**WO1**
	1.925	2.394	2.427	2.527	2.726	2.895
	WT6	**WT5**	**WT4**	**WT3**	**WT2**	**WT1**
	3.574	3.702	3.935	3.946	3.966	5.462

Discussion

Most visitors are from the younger age group. Motivation for their journey to the revolving nature is divided in two age groups: Group that seeks adventure and has sports activities are less than 40 years of age. Another group is traveling to enjoy nature, recreation, entertainment and visiting wildlife. They are at the age of 35 to 54 years (BRANDON, 1996). Visitors are more men than women; this could indicate that because of the mountainous area of this region, more men than women tend to visit this region. Due to the higher percentage of married visitors, families must have a priority in planning the development of ecotourism in the region.

Increased productivity and upgrading of facilities and attractions for tourism, optimal use of resources and reduction in accident rates in environment are productivity results from the assessment by SWOT method. View of this model, an appropriate strategy can provide to maximize strengths, opportunities, weaknesses and threats to a minimum. For this purpose, strengths, weaknesses, opportunities and threats were linked to the four general ST, WO, SO and WT and strategy options selected between them (BABU & SUBRAMONIAM, 2009). By completing the SWOT matrix, developing various strategies will be done for conduction system in the future (GOLKAR, 2006). According to research of RAKHSHANI NASAB & ZERABI (2010), failure to understand the real value of natural resources, lack of understand attractive places for the tourism, lack of facilities and shortage of skilled managers in ecotourism

are including the main challenges for ecotourism in Iran. Alamut region despite having the capacity to become an example of regional tourism is in front of the main obstacles in the way of achieving this goal. For example, management issues, lack of accommodation and tourist facilities, and weak advertising. EBRAHIMZADE & AGHASIZADE (2009) by analyzing factors affecting the development of tourism in the Chabahar coastal areas in Iran by using of SWOT model analysis found similar to our results.

Height difference and high slope region are highly effective on environmental and human factors (KARRIMI GHASR, 2001). If disturb the ecological balance of mountain ecosystems due to the limited ability to repair, their potential reversibility to the initial conditions will very difficult. Protection of these ecosystems is not mean to their exploit, but the rational use commensurate with their capacities and capabilities are stability guarantee and ecological stability. According to research of JIANG (2008), between different types of tourism, ecotourism attracts tourists that travel to the region to see the landscapes with minimum investments than other types are possible. According to studies in line to optimal utilization of the land and establishing certain support to preserve natural lands with valuable biological resources. The most basic way is land preparation program and implementation of projects development on the environment that are fundamental solution to prevent environmental horrible consequences. Existing habitat of Alamut due to the diverse habitat conditions, the presence of diverse and valuable species that sometimes they are also vulnerable to threat and risk and cultural and historical heritage is the first priority to protect.

Pay attention to dissatisfaction of tourists from facilities of this area, thus the creation of accommodation and suits leisure not only adds capabilities in this area to attract tourists but also for tourists and indigenous people is very important in terms of job creation and recreational. The other hand, satisfaction of tourists will attract the participation (EPLER, 2002). Tourists attracting to visit places of interest and national monuments and ancient need to much invest for preparation of sites visited, construction of access roads, multi-star hotels, suitable vehicles, etc.

Alamut region is composed of many villages. With the development of rural tourism, can be minimized environmental and cultural damage, provide visitor satisfaction and help to region's economic growth. BAHRAMI (2010) reported that this fact indicates that arrival of tourists to rural areas make connections between indigenous people and the tourists have a significant impact on growth and promote social and cultural, higher education, and increase participation levels. The rural tourism as a tool for generating employment and economic development throughout the region can be reduced poverty and increased income. On the other hand the use of public information databases, information networks, distributes posters about the attractions of Alamut. The use of experts in the field of tourism and using local guides can be very effective. What is certain, ancient culture, valuable cultural and natural resources of this region can have many roles in attracting tourists. This development requires the cooperation of industry, government agencies such as the cultural heritage and tourism organization, the population is indigenous and foreign investors.

Conclusions

Pay attention to the failure and problems expressed tourism development in the region of Alamut, to achieve growth and prosperity of this industry in the region, improve the functions of tourism in regional tourism development and business income and more interests in tourism sector, we can raise public culture in this background by participation in decision making and tourism program and using of local guides. By comprehensive management plan for the region be invested to identify potential areas, building amenities and sanitary and the multi-residential services for nature tourists with the residential camps for

different purposes (recreation, sport, etc…). Try to introduce Alamut tourism attractions in level of national and international by the internet with international languages to attract tourists from other countries, articles published in scientific journals and press and holding conferences and seminars. Pay attention to biodiversity and the ecological status important of Alamut is necessary budget which is allocated to improve the level of protection, reduce the negative effects and protection of natural ecosystems area.

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Received: 14.11.2011
Accepted: 30.01.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 21-30

Epigeal and Hypogeal Macroinvertebrate Diversity in Different Microhabitats of the Yusmarg Hill Resort (Kashmir, India)

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Abstract. Soil macroinvertebrate communities are important within the soil system and contribute to a wide variety of soil processes. A soil study was conducted to assess the composition and diversity of soil macroinvertebrates in Yusmarg hill resort of Kashmir valley at four sites characterised by different types of vegetation and interferences like grazing or fencing, during the months of May, June, November and December 2010. During the study, it was observed that different sites exhibited variations in diversity of both epigeal as well as hypogeal soil macroinvertebrates. For epigeal macroinvertebrates, highest diversity was recorded in forest edge (2.089) and inner forest (2.058) and relatively low diversity in grazed (1.61) and fenced areas (1.09). For hypogeal macroinvertebrates, diversity was recorded highest for inner forest site (2.216) than forest edge (1.9) and relatively lower in fenced (1.22) and grazed (1.21) sites. The physical disturbance in the form of grazing and fencing probably reduce the diversity of the soil macro fauna as is inferred from the present study.

Key words: soil macroinvertebrates, diversity, physical disturbance, Kashmir, India.

Introduction

Soil, a still, porous medium within which temperature and moisture conditions are highly buffered were among the first terrestrial environments to be colonized because they possess environmental conditions that are intermediate between aquatic and aerial media (LAVELLE & SPAIN, 2001). Soil organisms are an integral part of terrestrial ecosystems and soil biodiversity is comprised of the organisms that spend all or a portion of their life cycles within the soil or on its immediate surface (including surface litter and decaying logs). Soil communities are among the most species-rich compartments of terrestrial ecosystems (ANDERSON, 1975; USHER *et al.*, 1979; GILLER, 1996), which carry out a range of processes that are important for soil health and fertility and thus, there are functional connections between soil biodiversity, especially soil macroinvertebrates with crop production (SUGIYARTO, 2004). The easiest and most widely used system for classifying soil organisms is to group them by size into three main groups: macro, meso and micro-fauna (SWIFT *et al.*, 1979). Micro-fauna comprises of microorganisms and the very small invertebrates (small soil mites, for example). Microorganisms are the smallest of the soil animals ranging from 20 to 200 µm in length (< 0.1 mm in diameter). The mesofauna is the next largest group and the animals range in size from 200µm to 10mm in length (0.1–2mm in diameter). These include mainly micro arthropods, such as

Pseudoscorpiones, Protura, Diplura, Acari, small Myriapoda and others. The macro fauna contains the largest soil invertebrates. A soil macro fauna taxon (group) is an invertebrate group found within terrestrial soil samples which has more than 90 percent of its specimens (individuals) in such samples visible to the naked eye (IBOY, 2000). The Soil macro fauna consists of a large number of different animals that live on the soil surface, in the soil pores and in the soil area near tree roots. These include organisms like earthworms, millipedes, centipedes, ants, coleoptera (adults and larvae), isopoda, spiders, slugs, snails, termites, dermaptera, lepidoptera larvae and diptera larvae. Their way of living, their feeding habits, their movements into the soil, their excretions and their death have direct and indirect impacts on their habitat. Soil macro fauna is involved in – degrading organic matter and mineralizing nutrients; controlling pathogen populations; improving and maintaining soil structure; mixing organic matter through the soil. These processes are regulated by a number of abiotic and biotic factors (LAVELLE *et al.*, 1993). These comprise (1) microclimate, mainly temperature and humidity (MEENTEMEYER, 1995), (2) litter quality (WOOD, 1995; ANANTHAKRISHNAN, 1996; AERTS, 1997; HEAL *et al.*, 1997; SARIYILDIZ & ANDERSON, 2003), (3) soil nutrient content (VERHOEVEN & TOTH, 1995), and (4) the qualitative and quantitative composition of decomposer communities, including bacteria, fungi and invertebrates (SWIFT *et al.*, 1979; KNOEPP *et al.*, 2000).

This paper is intended to study: (i) the epigeal and hypogeal macroinvertebrate diversity in different microhabitats of the Yusmarg hill resort (Kashmir, India), and (ii) its influence on the soil characteristics as a living entity.

Material and methods

Study area

The study was conducted at Yusmarg (Fig. 1), a cluster of meadows bounded by magnificent trees in the lap of Pir Panjal mountain range, which is approximately 47 km from the Srinagar and lies in the district Budgam of Kashmir valley (Indian O). The study sites selected had relatively different vegetation and anthropogenic impacts. Site-1 represented the fenced area with geographical coordinates of N33°50′0.665″, E74°40′1.653″, and an elevation of 2418±6m. The site, dominated by herbaceous vegetation, was fenced by 6 ft wire mesh and as such was free of grazing and other anthropogenic activities. Site-2 (N33°50′1.768″, E74°39′57.555″; Elevation 2411±6m) was also dominated by herbaceous vegetation but witnessed grazing and anthropogenic activities. The third site (N33°50′0.034″, E74°39′57.506″; Elevation 2446±6m) was located in between the grazing area and the forest, having vegetation of conifers (*Picea smithiana* Wall. Boiss, *Pinus wallichiana* A.B. Jacks, and *Abies pindrow* Royle ex.D.Don), with an understory of shrubs (chiefly *Viburnum* sp.) and herbaceous vegetation (as *Fragaria nubicola*, *Cynodon dactylon,* etc.). Few marks of human interference in the form of lopped burnt stumps and logged wood were visible at this site. Site-4 (N33°49′55.747″, E74°39′56.262″ E; Elevation 2451±6m) comprised the forested area, with a dense cover of conifers dominated by *Abies* sp., interspersed with *Picea smithiana* and *Pinus wallichiana* trees.

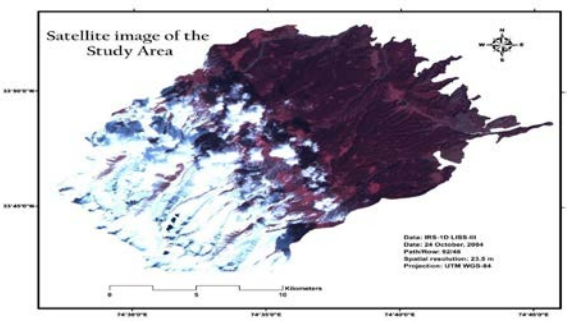

Fig. 1. Satellite image of the study area – Yusmarg.

Methods

The sampling methods used for the epigeal and hypogeal macroinvertebrates followed the recommended methods by the Tropical Biology and Soil Fertility Program (TSBF) (ANDERSON & INGRAM, 1993;

LAVELLE & PASHANASI, 1989) with fewer modifications. At each site, five samplings were performed for duration of 50 minutes on each sampling occasion. For sampling of epigeal macroinvertebrates, quadrat sizes of 25cmx25cm were used, with hand picking of the organisms using entomological forceps. Soil samples of the dimensions of 25cmx25cmx30cm were taken, after removing litter layer, for the collection of hypogeal macroinvertebrates. The soil sample obtained was then carefully hand-sorted on a large white cloth. The macroinvertebrates were collected, killed in a bottle containing cotton balls saturated with 40% formaldehyde at the bottom and a covering of filter paper above, and counted. The samples were preserved in 75% alcohol.

Due to the continuous unrest and prolonged curfew imposed by the Indian Security Forces stationed in Kashmir, the study could not be carried out during July to October 2010.

Data analysis

No single index encompasses all characteristics of an ideal index, i.e., high discriminate ability, low sensitivity to a sample size, and ease in calculation (MARGURAN, 1988). Therefore an observation of the different indices reflecting species evenness, dominance and diversity heterogeneity provide some valid viewpoints. Shannon's index of diversity (PRICE, 1997) reflects both evenness and richness (COLWELL & HUSTON, 1991) and is commonly used in diversity studies (KREBS, 1989). It is calculated as $H = -\Sigma P_i \ln P_i$; $i = 1-n$; where n is the number of species and P_i is the proportion of the i^{th} species in the total. Index of dominance is calculated as $\Sigma(ni/N)^2$ where *ni* is the number of individuals of a species and *N* is the total number of individuals of all species. Evenness indicates the degree of homogeneity in abundance between species and is based on the Shannon index of diversity. Shannon evenness [$E = H/H_{max} = H/\ln S$; where H is the Shannon diversity index and S the number of species in the community] ranges from 0 to 1.

Results

Taxonomical diversity

Epigeal macroinvertebrate fauna of the study area was found to be comprising of 25 genera (2 classes), and was represented by six orders –Araneida (6 genera), Orthoptera (2 genera), Hemiptera (5 genera), Coleoptera (8 genera), Hymenoptera (3 genera) and Diptera (1 genera) (Table 1). Hypogeal macroinvertebrate fauna was found to be comprised of 15 genera (four classes), representing six orders – Opisthopora (1), Scorpionida (1), Araneida (2), Scolopendromorpha (3), Coleoptera (5), and Hymenoptera (3 genera) (Table 2).

The inner forest and the forest edge exhibited nearly similar species diversity of epigeal macroinvertebrates with forest edge showing slightly higher richness (Table 3). The grazing site showed comparatively lower species diversity compared to the forest and the transition zone. However, the species richness was found to be significantly lower at the fenced site. Accordingly, higher uniform dominance was found to be exercised by the less diverse species at the fenced site (Table 3). The dominance was scattered among the more diverse species at the forest edge followed by the forest, and thus exhibited less dominance. Similarly, the dominance was higher at the grazing site compared to the forest edge and the forest; however, it was lesser than that at the fenced site (Fig. 2).

In case of hypogeal macroinvertebrates, the inner forest site showed higher diversity than the forest edge, while it was lower at the grazing and the fenced site (Table 4). Similarly the dominance was found to be high among the taxa at the grazing site followed by the fenced site. However, the dominance was scattered among the more taxa at the forest edge, and still much higher scatter or low dominance was found at the inner forest site (Table 4, Fig. 3).

Table 1. Epigeal macroinvertebrate fauna encountered at the different sites of the study area.

Taxa	Month (Year 2010)				Average	Relative Abundance
	May	June	Nov.	Dec.		
Site-1						
Elymana sp.	4	8	5	21	9.50	20.28
Harpalus sp.	3	1	0	0	1.00	11.39
Araneus sp.	0	1	2	0	0.75	8.54
Pyrrhocorris sp.	2	0	0	0	0.50	17.08
Lycosa sp.	0	1	0	0	0.25	8.54
Pardosa sp.	1	0	0	0	0.25	8.54
Endomychus sp.	0	0	1	0	0.25	8.54
Geotrupes sp.	1	0	0	0	0.25	8.54
Phytodecta sp.	0	0	1	0	0.25	8.54
Total	11	11	9	21	13.00	100.00
Site-2						
Elymana sp.	2	18	2	5	6.75	1.41
Messor sp.	2	3	2	0	1.75	4.23
Araneus sp.	1	2	1	0	1.00	4.23
Harpalus sp.	3	1	0	0	1.00	5.63
Pyrrhocorris sp.	3	0	0	0	0.75	8.45
Amphimallus sp.	1	1	0	0	0.50	8.45
Satacid sp.	0	1	0	0	0.25	16.90
Xysticus sp.	0	1	0	0	0.25	16.90
Onthophagus sp.	0	1	0	0	0.25	16.90
Unidentified beetle	0	1	0	0	0.25	16.90
Total	12	29	5	5	12.75	100.00
Site-3						
Araneus sp.	1	0	1	1	0.75	8.00
Elymana sp.	0	2	0	1	0.75	12.00
Messor sp.	0	0	3	0	0.75	24.00
Pholcus sp.	0	0	1	1	0.50	8.00
Lasius sp.	0	2	0	0	0.50	16.00
Harpalus sp.	1	0	0	0	0.25	8.00
Magdalis sp.	0	0	0	1	0.25	8.00
Asilius sp.	0	0	0	1	0.25	8.00
Vespa vulgaris	0	0	1	0	0.25	8.00
Total	2	4	6	5	4.25	100.00
Site -4						
Messor sp.	0	0	4	1	1.25	14.71
Cicada sp.	0	3	0	0	0.75	17.65
Lasius sp.	0	0	3	0	0.75	17.65
Leva sp.	0	0	3	0	0.75	8.82
Eurydema oleraceum	0	2	0	0	0.50	11.76
Empicoris sp.	0	0	0	2	0.50	11.76
Araneus sp.	0	0	0	1	0.25	5.88
Pardosa sp.	1	0	0	0	0.25	5.88
Aulachobothrus sp.	0	0	0	1	0.25	5.88
Total	1	5	10	5	5.25	100.00

Table 2. Hypogeal macroinvertebrate fauna encountered at the different sites of the study area.

Taxa	Month				Average	Relative Abundance
	May	June	Nov.	Dec.		
Site-1 (Fenced area)						
Eutypheous sp.	3	2	0	0	1.25	25.00
June bug larva	1	1	0	0	0.5	25.00
Unidentified larva	0	0	1	1	0.5	25.00
Amphimallus sp.	1	0	0	0	0.25	25.00
Total	5	3	1	1	2.5	100.00
Site-2 (Grazing area)						
Messor sp.	23	1	0	2	6.5	53.28
Eutyphoeus sp.	3	5	0	0	2	9.84
June bug larva	2	1	0	0	0.75	6.15
Lasius sp.	2	0	0	0	0.5	12.30
Amphimallus sp.	1	0	0	0	0.25	6.15
Unidentified larva	0	0	1	0	0.25	6.15
Unidentified moth	0	0	0	1	0.25	6.15
Total	31	7	1	3	10.5	100.00
Site-3 (Forest edge)						
Lasius sp.	2	13	0	0	3.75	33.04
Eutyphoeus sp.	2	3	1	0	1.5	5.29
Scolopendra sp.	2	2	1	0	1.25	4.41
Messor sp.	0	0	5	0	1.25	22.03
Monomorium sp.	0	2	0	0	0.5	8.81
Scolopendra morsitans	0	1	0	0	0.25	4.41
Amphimallus sp.	1	0	0	0	0.25	4.41
Harpalus sp.	1	0	0	0	0.25	4.41
Lympyrus sp.	1	0	0	0	0.25	4.41
Vespa vulgaris	0	0	0	1	0.25	4.41
June bug larva	1	0	0	0	0.25	4.41
Total	10	21	7	1	9.75	100.00
Site -4 (Inner Forest)						
Messor sp.	0	0	2	10	3	20.76
Unidentified weevils	0	0	8	0	2	27.68
Scolopendra sp.	2	3	2	0	1.75	4.84
Lasius sp.	0	0	6	0	1.5	10.38
Eutyphoeus sp.	0	1	2	0	0.75	3.46
Scorpiops sp.	0	2	1	0	0.75	3.46
Scolopendra morsitans	3	0	0	0	0.75	5.19
Archaeolithobius sp.	0	0	0	2	0.5	6.92
Araneus sp.	1	0	0	0	0.25	3.46
Lycosa sp.	0	0	1	0	0.25	3.46
Lamycetes sp.	0	1	0	0	0.25	3.46
Lympyrus sp.	1	0	0	0	0.25	3.46
Elm bark beetle	0	0	0	1	0.25	3.46
Total	7	7	22	13	12.25	100.00

Table 3. Diversity, dominance and evenness of epigeal macro invertebrates at different study sites.

Selected Sites	Shannon Diversity Index	Simpson's Index	Shannon Evenness Index
Site-1 (Fenced area)	1.096	0.547	0.50
Site-2 (Grazing area)	1.61	0.318	0.70
Site-3 (Forest edge)	2.089	0.134	0.95
Site-4 (Inner forest)	2.058	0.143	0.94

Table 4. Diversity, dominance and evenness of hypogeal macro invertebrates at the four different study sites.

Selected Sites	Shannon Diversity Index	Simpson's Index	Shannon Evenness Index
Site-1 (Fenced area)	1.22	0.34	0.90
Site-2 (Grazing area)	1.21	0.43	0.52
Site-3 (Forest edge)	1.9	0.21	0.80
Site-4 (Inner forest)	2.21	0.14	0.90

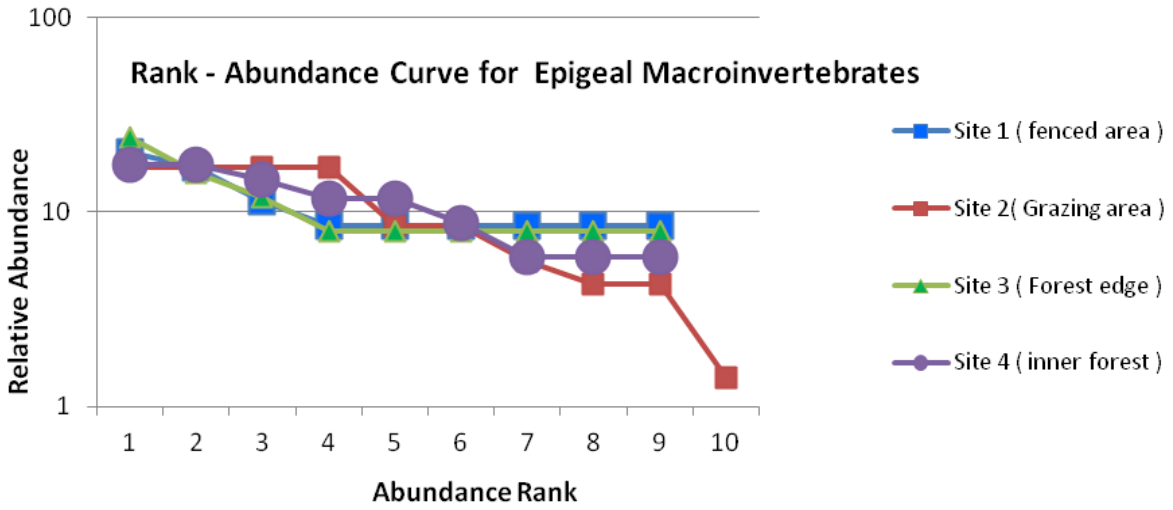

Fig. 2. Rank abundance curve for epigeal macroinvertebrates at the respective sites.

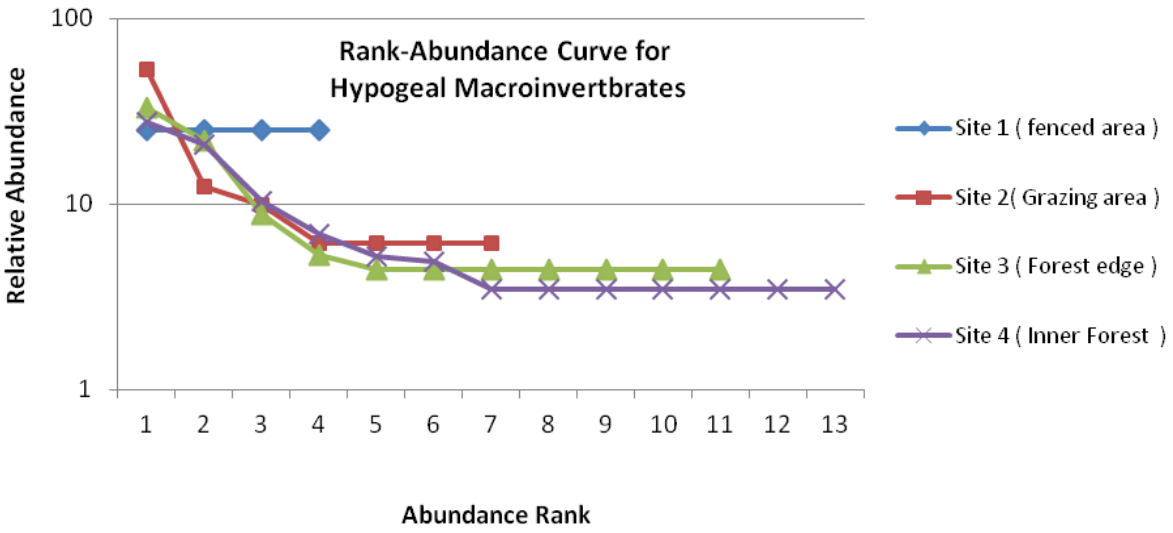

Fig. 3. Rank abundance curve for the hypogeal macroinvertebrates at the respective sites.

Discussion

The main groups of soil macrofauna in terms of their abundance and the importance of their activities in soil are earthworms, termites, ants, myriapoda, diptera and coleoptera (LAVELLE & SPAIN, 2001). The epigeal and hypogeal macroinvertebrate community of the study area was found to be more diverse in terms of genera belonging to order coleoptera (epigeal macroinvertebrates – 25 genera: Araneida {6}, Orthoptera {2}, Hemiptera {5},

Coleoptera {8}, Hymenoptera {3}, and Diptera {1}; hypogeal macroinvertebrates–15 genera: Opisthopora {1}, Scorpionida {1}, Araneida {2}, Scolopendromorpha {3}, Coleoptera {5}, and Hymenoptera {3}). To better understand the comparatively higher diversity of beetles, several explanations have been forwarded by various workers. One of the important factors for explanation of the overall establishment of the Coleoptera order was proposed to be the development of the forewings into sclerotized elytra (LAWRENCE & BRITTON, 1994) which cover the membranous flight wings and the abdomen. In this way, the elytra are thought to protect beetles against environmental stresses and predation (HAMMOND, 1979). With more than 350,000 species and approximately 40% of all described insects, Coleoptera has a high diversity of food habits (LAWRENCE & BRITTON, 1991). The effect of photoperiod, temperature and relation to the quality and availability of host-plants or possibly the asymmetric competition (LINZMEIER & RIBEIRO-COSTA, 2008), seasonality (WERNER & RAFFA, 2003; RINTOUL *et al.,* 2005), niche partitioning on the basis of habitat, or other factors such as soil type, grassland topography or landuse practices (RINTOUL *et al.,* 2005) have been documented as the factors for the relatively higher diversity of the Coleoptera.Forest edge (Site-3) exhibited highest diversity of epigeal macro-invertebrates. The most common explanation for this trend is that there is a mixing of distinct fragment and matrix faunas at habitat edges, giving rise to a zone of overlap with greater overall species richness (INGHAM & SAMWAYS, 1996; MAGURA, 2002). The transition zones offer the habitat features which are representative of both the transient habitats and as such species of both the habitats are found in this zone to a varying degree and thus an overall greater diversity of species in this zone, the so called "edge effect".

The Grazing area (Site-2) showed a relatively higher species richness of the epigeal macroinvertebrates compared to the fenced area (Site-1). The reason for this richness might be the return of the nutrients by the natural manuring of the grazing animals. Return of nutrients, defoliation and trampling appears to be the major components of grazing that could affect soil organisms. Return of nutrients in dung and urine can also influence the abundance and activity of decomposers (GRIFFITHS *et al.*, 1992). Defoliation of plants is known to affect soil organisms by changing the quality and quantity of carbon that enters the soil (PATERSON & SIM, 2000; SIROTNAK & HUNTLY, 2000; PATERSON *et al.*, 2003; HAMILTON *et al.*, 2008).

At Site-1 (fenced area), epigeal macroinvertebrates showed less diversity. This decline could be ascribed to habitat isolation as isolation disrupts species distribution patterns because species differ in their willingness to disperse through matrix environments (LAURANCE & YENSEN, 1991; COLLINGE, 2000), and forces dispersing individuals to traverse a matrix habitat that separates suitable habitat fragments from each other. An extreme example of this was highlighted by BHATTACHARYA *et al.* (2003), who found that two species of Bombus bumblebees would rarely cross roads or railways despite the presence of suitable habitat that was within easy flying range.

In case of hypogeal macroinvertebrates, Site-4 (inner forest) was showing relatively higher species richness, followed by forest edge site and grazing area. Soil and litter of forests generally contain highly diverse communities with a large number of organisms (DE RUITER *et al.*, 2002; SETALA, 2005; FITTER *et al.*, 2005) as higher habitat diversity, may in turn increase species diversity (LAVELLE & SPAIN, 2001). Since plant diversity has often been found to affect structural and biotic properties of ecosystems (e.g. GARTNER & CARDON, 2004; HOOPER *et al.*, 2005; SCHERER-LORENZEN *et al.*, 2005, UNSICKER *et al.*, 2006), it might also positively or negatively influence macroinvertebrate communities either directly or indirectly by modifying important habitat features for forest floor species (e.g. spatial and temporal changes in litter structure and microclimate). The high diversity of hypogeal macroinvertebrates at Site 4 (forest) compared to all other three

sites, could possibly be attributed to the reason that hypogeal macroinvertebrates apparently avoid light and open space. SUGIYARTO *et al.* (2007) showed that most of soil macroinvertebrates tend to avoid risk of open space or high light intensity. Another possible reason could be the less compact soils and availability of comparatively more moisture and more decaying organic matter on the forest floor. Trampling seems to reduce the abundance and diversity of hypogeal macroinvertebrates. These negative effects have been ascribed to soil compaction and reduction of pore spaces (DREWRY *et al.*, 2001).

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Received: 15.02.2012
Accepted: 16.04.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 31-38

Physico-Chemical Characteristics of the Grassland Soils of Yusmarg Hill Resort (Kashmir, India)

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Abstract. Physico-chemical analysis was carried out on the grassland soils of Yusmarg Hill Resort, Kashmir during the months of May, June, November and December 2010, at four micro sites with some minor variations in the abiotic and biotic factors including anthropogenic pressures. The following soil characteristics were examined: temperature, texture, moisture, organic matter, pH, and conductivity, content of calcium, magnesium, sodium, potassium, total phosphorus, and organic carbon. Soil texture analysis revealed the soils at all the study sites with major proportion being comprised by the sand fraction and having a sandy silt character. The moisture content was found to be directly related to the herbaceous vegetation cover with the highest value at Site 3 (fenced meadow area). The moisture content showed low percentage at Site 2, which was more affected by grazing and thus resulted in less cover of grasses and probably more evaporation of soil moisture from the exposed site. The soils at all sites were from acidic to mildly acidic in character. The amount of organic matter was fairly good except at Site 2 (non-fenced grazing area) probably due to overgrazing during which much of herbage vegetation was picked up by the grazing animals like sheep and cattle. The values of important cations, such as Ca^{2+} and Mg^{2+}, showed a gradual decrease from May to December except at Site 4 (transition between a coniferous forest and a meadow) which may be attributed to a good cover of vegetation and good amount of organic matter.

Key words: soil chemistry, microhabitat variations, anthropogenic activities, Kashmir, India.

Introduction

Soil may be defined as a natural body, synthesized in profile form from a variable mixture of broken and weathered minerals and decayed organic matter, which covers the earth in a thin layer and which supplies, when containing the amounts of air and water, mechanical support and imparts sustenance for plants (BRADY & WEIL, 2000). Partial heterogeneity in nutrient availability affects not only the spatial patterning of vegetative cover but overall community structure and productivity (BRADY & WEIL, 1990). The importance of the soil as a reservoir of nutrients and moisture for the production of forage and plant species has been recognized since the beginning of the forest management as a science (SCHLESINGER *et al.*, 1990). Grasslands have deep soils that are very nutrient rich because of the large amount of plant tissue (biomass) that dies off and is added to the soils through decomposition every year. Vegetation distribution and development largely depends on the soil conditions (DE DEYN *et al.*, 2004; KARDOL *et al.*, 2006). Nutrient limitation occurring in the soils is one of the most important factors affecting

the structure of plant communities (GRIME *et al.*, 1997). On the other hand, the changes in vegetation can cause shifts in the soil properties (WARDLE, 2006) because individual plants concentrate biomass in soils beneath their canopies and modify biogeochemical processes occurring in the soils (BURKE *et al.*, 1989; SCHLESINGER *et al.*, 1990). Chemistry of soil covers chemical reaction and process in the soil pertaining to plant and animal growth and human development. Soil chemical processes are fundamental to the evolution of geoderma, the biosphere and the human environment. Therefore, understanding of soil chemical reactions and processes is essential for developing innovative resource management strategies, and understanding and regulating the behavior of the terrestrial ecosystem at regional and global scales (SCHNITZER, 1986).

The main aim of the present study was to assess the physico-chemical characteristics of the grassland soils of the Yusmarg Hill Resort (Kashmir) subjected to different treatments.

Material and methods

Study area

The present study was conducted on the grassland soils of the Yusmarg (N33°49′42′′; E74° 39′59″; Elevation 2743±6 m), Kashmir, located at 47 km south-west from the Srinagar City (Fig. 1), during the months of May, June, November and December, 2010. Four study sites were chosen as is shown on Fig. 2. Site 1 (N33°49′51.5″; E74°39′51.5′′; Elevation 2395±6 m) was situated in a commercial/ tourist site. Site 2 (N33°49′58.3″; E74°39′58.4″; Elevation 2405±6 m) was selected in the non-fenced area highly influenced by anthropogenic activities. Site 3 (N33°49′58.0″; E74°40′02.2″; Elevation 2418±6 m) was a fenced meadow area which remained dominated by grasses, and the Site 4 (N33°50′00.5″; E74°40′04.6″; Elevation 2428±6 m) was located at the edge transitioned between a coniferous forest and a meadow land; few shrubs and grasses occupied this area, and immediately above the site coniferous trees predominated.

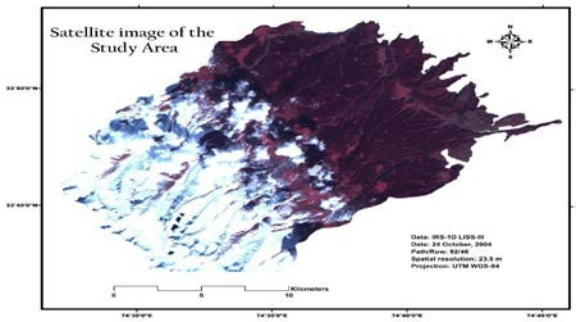

Fig. 1. Satellite image of the study area – Yusmarg Hill Resort, Kashmir (India).

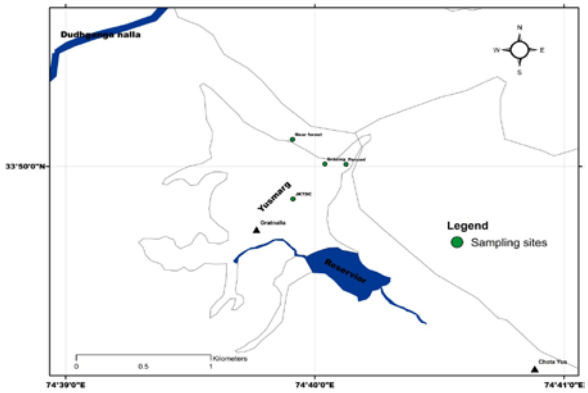

Fig. 2. Location of examined sites in the study area.

Methods

Soil physico-chemical characteristics were determined by collecting composite surface (0-10 cm depth) soil samples with the help of a soil corer from the four sites in order to give due representation to the micro-environment at each site. Soil samples were stored in air tight polythene bags for subsequent laboratory investigations. The samples were air-dried, mashed using a pestle and mortar and passed through 2mm sieve before analysis (GHOSH & KUNDUN, 1991).

Soil temperature was measured under shade usually in between 1:00 and 2:00 PM IST by a soil thermometer (Model RT 0124; Raj Thermometers; India) at a depth of 10-15 cm. Soil texture was determined through modified Udden-Wentworth Grade scale (sieve method) as demonstrated by LINDHOLM (1987). 100g of dry soil sample

was placed on the uppermost sieve in a set of stacked sieves of different mesh sizes ranging from 2mm to 0.037mm size. The stack of the sieves was arranged in order so that the coarsest sieve was at the top with finer ones below and the pan at the bottom. The stack of sieves was then placed on the shaking machine. After ten minutes of shaking, the sample collected on each of the sieves and the pan was removed and weighed to determine the textural class of each sample. Conductivity and pH were determined by electrometric method (Conductivity Meter, model: CD 601, Company: Milwaukee; pH Meter, Model: 101 E, Deluxe, Company: MS Electronics India) using 1:2 soil-water (w/v) suspension. Moisture content was determined following MICHAEL (1984). 50 g of soil sample was dried in an oven at 105 °C for 24 hours till constant weight was obtained. The soil was then allowed to cool in a desiccator and weighed again to find out the loss in weight. The loss in weight corresponds to the amount of water present in the soil sample. Percent moisture content of soil was calculated by the following formula: (loss in weight/initial weight) X 100. The organic carbon and organic matter percentage were determined by rapid titration method (WALKLEY & BLACK, 1934). Loss on ignition (in %) was determined by the muffle furnace method (HANNA, 1964), the Muffle Furnace (Model: Instron IN 301, Company: Jindal; India) temperature was maintained at 700 °C for half an hour. Exchangeable calcium and magnesium were estimated in 1N ammonium acetate – shaking and filtration method (SCHOLLENBERGER & SIMON, 1945) followed by EDTA-titration. Sodium and potassium were determined by using Flame Photometer (Systronics Flame Photometer 128; Ahmedabad, India). Total phosphorous was estimated by Tri-acid digestion method (PIPER, 1966) and the measurements of absorbance were carried out at 690 nm using a spectrophotometer (Model: Elico SL 171 Mini Spec; Hyderabad, India).

Means and Standard Deviation was computed through Microsoft Office Excel (2007) program (ETHERIDGE, 2007).

Results and Discussion

During the present study, the soil temperature depicted a progressively decreasing trend as the season shifted towards colder weather with a maximum value of 20°C recorded at Site 2 in June and the minimum temperature of 1.2°C found at Site 4 in December (Table 1). The cover, normally plants and litter, shades the soil; that is, it intercepts some of the incoming radiation, heating the cover itself instead of the soil below (SINGER & MUNNS, 1991). The maximum value of soil moisture content i.e. 21.6% was recorded at Site 3 in May and the minimum value of 12.2% was found at Site 2 in June. The highest value of moisture content, recorded at Site 3 in May, could be attributed to the high precipitation both with the dense vegetation at the site, resulting in lesser amount of radiation being received at the surface and consequently less evaporation of the soil moisture. The lowest value of moisture content at Site 2 in June could be due to the reason that lot of evaporation takes place from the exposed site in the hot summer days.

Soil texture analyses revealed the soils at all the study sites with major proportion being comprised by the sand fraction and having a sandy silt character.

The soil pH values averaged between 5.9±0.59 and 6.23±0.23. Based on pH values, the soils of all examined sites were found to be slightly acidic to acidic, the low pH values at Site 4 can be attributed to the presence of pine needles which contribute in increasing the soil acidity when they being decomposed. The presence of higher content of organic matter in the soil can be another plausible reason for lowering of the pH (HODGES, 1996). Higher levels of organic matter result in a greater number of cation exchange sites which tend to decrease the pH (NAIMAN *et al.*, 1994).

Table 1. Physico-chemical characteristics of the grassland soils of Yusmarg, Kashmir.

Parameter	*↓Site**	*Month, 2010*				*Average*	*SD*
		May	*June*	*Nov.*	*Dec.*		
Soil temperature, °C	*1*	15	17.5	11.5	1.5	11.37	± 7.02
	2	15.2	20.2	11.5	2.8	12.42	± 7.34
	3	15.5	19.2	12.5	2.5	12.42	± 7.16
	4	12.5	16.1	10	1.2	9.95	± 6.34
	Average	14.55	18.25	11.37	2		
	SD	± 1.38	± 1.81	± 1.03	± 0.77		
Moisture, %	*1*	20.3	13.6	19.5	17.2	17.65	± 3
	2	17.4	12.2	15.8	14.8	15.05	± 2.18
	3	21.6	14.1	20.6	14.6	17.72	± 3.92
	4	18.2	13.3	17.1	10.8	14.85	±3.42
	Average	19.37	13.3	18.25	14.35		
	SD	± 1.92	± 0.80	± 2.19	± 2.64		
pH	*1*	6.19	6.39	6.63	6.71	6.48	± 0.23
	2	6.52	5.9	6.6	5.9	6.23	± 0.38
	3	6.41	6.3	6.26	6.48	6.36	± 0.10
	4	5.82	5.7	5.25	4.51	5.32	± 0.59
	Average	6.23	6.07	6.18	5.90		
	SD	± 0.3	± 0.32	± 0.64	± 0.98		
Conductivity, *µS/cm*	*1*	270	290	250	245	263.75	± 20.56
	2	181	188	185	150	176	± 17.56
	3	258	210	230	243	235.25	± 20.35
	4	246	225	195	190	214	± 26.34
	Average	239	228	215	207		
	SD	± 39.72	± 43.88	± 30.27	± 45.74		
Loss on ignition, %	*1*	10	10.85	11.57	10.24	10.66	± 0.70
	2	6.2	5.82	5.8	5.64	5.86	± 0.23
	3	13.86	11.93	11.6	11.15	12.13	± 1.19
	4	13.4	16.99	15.3	11.5	14.30	± 2.37
	Average	10.86	11.39	11.06	9.63		
	SD	± 3.55	± 4.58	± 3.92	± 2.71		
Organic carbon, %	*1*	3.7	3.12	3.11	3.13	3.26	± 0.29
	2	1.83	1.55	1.43	0.97	1.44	± 0.35
	3	2.19	2.08	2.05	2.39	2.17	± 0.15
	4	3.01	4.47	4.06	4.02	3.89	± 0.62
	Average	2.68	2.80	2.66	2.62		
	SD	± 0.83	± 1.28	± 1.16	± 1.29		
Organic matter, %	*1*	6.37	5.37	5.36	5.39	5.62	± 0.49
	2	3.15	2.67	2.46	1.67	2.48	± 0.61
	3	3.77	3.58	3.53	4.12	3.75	± 0.26
	4	6.39	7.7	7.59	6.93	7.15	± 0.61
	Average	4.92	4.83	4.73	4.52		
	SD	± 1.70	± 2.21	± 2.24	± 2.22		

Calcium, *me/100 g*	*1*	3.65	3.45	2.49	2.45	3.01	± 0.62
	2	3.12	2.25	1.89	1.77	2.25	± 0.61
	3	3.1	2.88	2.31	2.23	2.63	± 0.42
	4	3.43	3.42	2.56	2.74	3.03	± 0.45
	Average	3.25	3	2.31	2.29		
	SD	± 0.26	± 0.56	± 0.30	± 0.40		
Magnesium, *me/100 g*	*1*	1.55	0.84	0.42	0.41	0.80	± 0.53
	2	0.72	0.69	0.41	0.35	0.54	± 0.18
	3	0.88	0.63	0.48	0.45	0.61	± 0.19
	4	1.06	1.21	0.55	0.41	0.80	± 0.38
	Average	1.05	0.84	0.46	0.40		
	SD	± 0.35	± 0.26	± 0.06	± 0.04		
Sodium, *mg/100 g*	*1*	8.4	7.8	9.3	8.5	8.5	± 0.61
	2	5.9	6.2	5.3	4.5	5.47	± 0.75
	3	9.7	7.7	6.6	7.8	7.95	± 1.28
	4	9.7	8.4	11.7	9.3	9.77	± 1.39
	Average	8.42	7.52	8.22	7.52		
	SD	± 1.79	± 0.93	± 2.85	± 2.10		
Potassium, *mg/100 g*	*1*	6.3	5.6	5.5	5.2	5.65	± 0.46
	2	6.9	5.1	5.8	5.6	5.85	± 0.75
	3	8	9.3	7.3	8.2	8.2	± 0.82
	4	5.5	5.6	5.2	5	5.32	± 0.27
	Average	6.67	6.40	5.95	6.00		
	SD	± 1.05	± 1.94	± 0.93	± 1.48		
Total phosphorus, *µg/g*	*1*	22	24	20	18	21	± 2.58
	2	12	18	16	18	16	± 2.82
	3	30	28	24	24	26.5	± 3
	4	32	36	30	27	31.25	± 3.77
	Average	24	26.5	22.5	21.75		
	SD	± 9.09	± 7.54	± 5.97	± 4.5		

**Site 1 – Commercial/ tourist site; Site 2 –Non-fenced grazing area; Site 3 –Fenced meadow area; Site 4 –Transition zone /edge between a coniferous forest and a meadow.*

Electrical conductivity showed variation between 150µS/cm at Site 2 in December to that of 290µS/cm at Site 1 in June. Generally it is believed that higher the concentration of ions in the soil solution more is its electrical conductance. Therefore, the higher value at Site 1 in June could possibly be attributed to the presence of higher amounts of calcium, magnesium and potassium ions at the site.

Organic carbon and organic matter content was found to vary respectively between the minimum of 0.97% and 1.67% at Site 2 in December to the maximum value of 4.47% and 7.7% at Site 4 in June. Site 4 recorded high organic matter content with an average value of 7.7% as compared to the other sites which may be attributed to the rich litter deposition and due to the low mineralization caused by relatively lower temperature under the shade of dense trees and therefore to the slow rate of decomposition of organic matter (MOORE, 1981). It has been reported that the decomposition rates of organic matter has a tendency to increase as weather warms and to furnish maximum plant growth conditions (RUSSELL, 1950). The lower values of organic matter and organic carbon at Site 2 can possibly be a consequence of grazing and leaching.

The calcium content in the soil was found to vary between 1.77 me/100 g at Site 2 in December to 3.65 me/100 g at Site 1 in May. GUPTA *et al.* (1980) have reported the exchangeable calcium content in the soils of

Jammu & Kashmir (India) varying from 1.73 – 13.30 me/100 g and the presently observed results thus lie within this range. Magnesium showed lower concentrations at all sites during the study period. The magnesium was recorded with the minimum value of 0.35me/100 g at Site 2 in December to the maximum value of 1.55 me/100 g at Site 1 in May. The exchangeable calcium was found to be higher than exchangeable magnesium and potassium probably because calcium is more strongly bound to exchangeable sites than magnesium and potassium (BECKETT, 1965).

Sodium is generally regarded as essential nutrient for all higher plants. Sodium is involved in regeneration of phosphoenol pyruvate in C4 plants. Sodium is essential for animals, and herbivores depend on its content into the plants. Highest value of sodium (11.7 mg/100g) was recorded at Site 4 in November and the lowest value of 4.5mg/100g at Site 2 in December. In case of potassium, the highest value of 9.3mg/100g was estimated for the samples of Site 3 in June against the lowest value 5.0mg/100g at Site 4 in December.

Total phosphorus content was recorded highest (36 µg/g) for Site 4 in June which could be due to the high organic matter content. The soils with high organic matter content have better supplies of organic phosphates for plant uptake than have the soils with low organic content (MILLER & DONAHUE, 2001). Site 2 recorded the lowest value (12 µg/g) of phosphorous which might be due to the leaching. The soils with minimum leaching are known to contain high amount of phosphorous as compared to the soils with maximum leaching. Site 2 was found to be low in electrical conductivity also with a minimum value of 150µS/cm in December indicating low amount of soluble salts probably due to the leaching from surface to sub surface layers and the accumulation of these salts at the lower layers due to poor internal drainage (KATTI & RAO, 1979).

Among the human activities that degrade grasslands, overgrazing by livestock is perhaps one of the most significant (MAINGUET, 1994). The effects of overgrazing on the plant community and soils are considered destructive because of the reduction of canopy cover, the destruction of topsoil structure, and compaction of soil as a result of trampling (TAYLOR *et al*., 1993; MANZANO & NA´VAR, 2000). Loss of fine fractions in soils has major influences on such properties as moisture, soil consistence, and organic carbon and nutrient presence and availability (HENNESSY *et al*., 1986). These changes in turn, influence the kind and amount of vegetation the area would support.

Conclusions

The study focused on the impact of change in land-cover type on soil quality inferred by the changes in chemical and physical properties of relatively non-disturbed and disturbed soil systems. Continuous grazing at Site 2 (non-fenced grazing area) apparently has resulted in a decrease in ground cover which probably in turn leads to a further coarseness in surface soil, loss of moisture and soil organic carbon. Variations in these parameters infer that the grassland is in the stage of degradation.

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Received: 15.02.2012
Accepted: 28.03.2012

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 39-52

Natural Plant Essential Oils for Controlling the Grasshopper (Heteracris littoralis) and their Pathological Effects on the Alimentary Canal

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Abstract. In the present study, the toxic effect of three different natural essential oils of medicinal plants, namely Garlic (*Allium sativum)*, Mint (*Mintha pipereta)* and Eucalyptus (*Eucalyptus globulus)* were tested on 1st nymphal instar of the grasshopper (*Heteracris littoralis)*. The LC_{50} values of the tested oils were estimated after 14 days from feeding on treated diet mixed with different concentrations of the oil. The LC_{50} of the tested oils were arranged as follows: 0.067, 0.075 and 0.084ml. /100ml. diet for Garlic, Eucalyptus and Mint respectively. The effect of LC_{50} concentration of the oils on the biological aspects and histological changes that observed on the alimentary canal and fat bodies were recorded. The normal development of the grasshopper was exhibited. Results cleared that there was statistical variable numbers of increased the nymphal periods, life cycle, adults longevity and life span comparing with the control test. Garlic oil inhibited egg lying by the resulting females offspring of the treated1st instar nymphs. High reduction in the deposited eggs and egg fertility caused by Eucalyptus or Mint oil and marked malformation were observed. Histological changes on the alimentary canal and fat bodies of the remaining nymphs after treatment with Garlic oil (the most effective oil) were detected by the light microscope have been recorded. The results suggest that the natural plant essential oils of Garlic, Eucalyptus and Mint may be used in IPM control program against *H. littoralis* grasshopper.

Key words: grasshopper, *Heteracris littoralis,* medicinal plants, *Allium sativum*, *Eucalyptus globulus, Mintha pipereta,* toxicity, histopathological changes, alimentary canal.

Used abbreviations: Crop (co); Gastric caecae (gc); Midgut (md); Malpighian tubule (mp); Ovary (ov); Testes (ts); Intestine (int); Rectum (rc); Anus (an); Epithelial cells (ep); Columnar cells (cc); Goblet cells (gc); Nucleus (n); Goblet cavity (gv); Regenerative cells (rgc); Microvilli (mv); Peritrophic membrane (pr); Circular muscles (cm); Longitudinal muscles (Lm);; Trachea (Tc); Vacuoles (V); Gastric caeca (gc); Intiman (In); Muscular layer (Ml); Fat cells (fc); Fat droplets (Fa); Degenerated epithelial cells (dep);; Basement membrane (bm), hindgut (hg); Lumen (lu) . Food particles (fo).

Introduction

The grasshopper (*Heteracris littoralis*) considered one of the most harmful pests to different cultivated crops in Egypt. Its economic importance comes from attacking many vegetable cultivated areas even trees, feeding on it and causing great losses in quantity and quality of the attacked crops. In some cases thousands of cultivated hectares may be attacked by the swarms of grasshopper leaving it as a divested desert. The economic injury of *H. littoralis* in Egypt had been documented by MISTIKAWI (1929). Many grasshopper species could be found all over the year round and represent a pest status for many plants. One of these

grasshoppers is *H. littoralis* (IBRAHIM, 1983 & EL–SHAZLY, 1991). Essential oils are volatile, natural, complex compounds characterized by a strong odor and are formed by plants as secondary metabolites. In nature, essential oils play an important role in protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivorous by reducing their appetite for such plants. They also may, attract some insects to favor the dispersion of pollens and seeds or repel undesirable others (BAKKLI *et al*, 2008). The discovery and use of synthetic insecticides have reduced the interest in plant origin products. However, widespread use of these insecticides in public health and agriculture for the control of vector and pest species has created different problems, such as the development of physiological resistant in major vector species, environmental pollution and toxic hazards to human and other non- target organisms due to their broad spectrum of activity (WHO, 1992; 2005; HEMINGWAY & CRIAG, 2004; KOUL *et al*., 2008). As a result , there has been an increased interest in developing potential alternative or additional control methods or materials that are effective against the target vector species, environmentally safe, biodegradable, with low cost and can be used by individuals and communities in specific situation (REEDWANI *et al*., 2002). One of these potential alternatives or additional control methods or tools in the use of selective botanical derivatives against the target insect species (PERICH *et al*., 1995). Therefore, the use of essential oils extracted from aromatic plants to control insect pests has been investigated and is well documented (ISMAN, 2006; KOUL *et al*., 2008; RAJENDRAN & SRIEANJINI, 2008). For example, recently, the essential oil of Catnip (*Nepeta cataria* L.) was reported to have repellency against adult male cockroaches *Beriblanate germanica* L. (PETERSON *et al*., 2002). THAVARA *et al*. (2007) studied seven commercial essential oils for repellency against cockroaches and found *Citrus hystix* exhibited complete repellency against *P. americana* and *B. germanica*. TARE & SHARMA (2004) reported the larvicidal activity of essential oils of 11 plants against *Aedis aegypti* larvae. JANTAN *et al.* (2005) evaluated the leaf essential oils of eight *Cinnamomum* species for larvicidal activity against *A. aegypti* and *A. albopictus* they found five species of them to have significant larvicidal effects. MORIAS *et al.* (2006) evaluated the larvicidal activity of essential oil of four *Croton* species, they found three of them to be highly toxic against larvae of *A. aegypti.* Onion, Parsley and Cumin oils showed acceptable toxicity level against desert locust *Schestocerca gregaria* (ABDEL – HAMID, 2006). EZIAH *et al.* (2011) found that application of neem oil at 5 ml/l were effective dosage in preventing the development of *Ephestia cautell* larvae, mortality ranged from 32.5 – 55% after 96 hours of exposure period, the observed mortality can be attributed to the inherent properties of neem, mortality was dosage and time dependent.

The use of natural plant products is considered more sustainable, cheaper and safer. Natural pesticides are preferred for many reasons**:** 1 - They are cheaper compared with chemical pesticides. 2 - They are made from local resources and are readily available. 3 – They are safer to humans than chemical pesticides. The objective of the present work was mainly to evaluate in laboratory the toxicity and biological activity of three of plant essential oils Garlic (*Allium sativum*) , Eucalyptus (*Eucalyptus globulus*) and Mint (*Mintha pipreta*) for disrupting growth and development of *H. littoralis* and to evaluate the histological damages caused to the alimentary canal. The essential oils investigated in this study are used as pharmaceuticals and in flavoring and they are can use as safe bioinsecticides for controlling *H. littoralis*. However, the possibility of employing this natural insecticides in the management of the grasshopper is plausible, but is worthy of further investigation.

Materials and methods

Adults and nymphs of *H. littoralis* were collected from Giza governorate, Egypt. The colony was raised in laboratory stock and

reared in electrical heated wooden cages at constant temperature at 30 ± 1°C with fluctuating relative humidity (50 – 70%). Insects were fed on synthetic diet mentioned by SHARABY *et al.* (2010). For oviposition, cages were supplied with suitable oviposition pots. These pots were examined every day and, when laid in, were removed to glass jars (ca. 100 c.c.), hatched hoppers were transferred to large jars (ca. 7000 c.c.). After the fourth or fifth molt, hoppers were released in the larger cages. Biological notes were recorded including the developmental duration of each nymphal instar, number of instars, pre-Oviposition period, number of eggs per egg–pod, number of egg–pods per female, oviposition period and the duration of the post–oviposition period as well as effect of the treatments on development and reproduction. To study reproduction and longevity, ten pairs of newly emerged nymphs were used in pairs; each pair was placed in large glass jars. Each jar was provided with an oviposition pot and supplied with piece of synthetic diet for feeding replacing it every four days or when consumed. The experiment was conducted at 30°c and L.D. 12:12 and the relative humidity fluctuated between 50 - 60%.

Plant essential oils

Three essential oils: Garlic (*Allium sativum,* Liliaceae), Eucaluptus (*Eucalyptus globulus*, Myrataceae), Mint (*Mentha piperita*, Labiatae) were obtained from EL-Captain company (CAPPHARM), Alobour city (Cairo), Egypt. For determine the LC_{50} concentration of the different plant essential oils on the 1St nymphal instar of *H. littoralis*, five descending concentrations that permit the computation of LC_{50} was diluted on the basis of volume/volume (1, 0.5, 0.25, 0.13, 0.06%) from each plant oil were prepared by mixing known volume from the oil with 100 ml. diet during the diet preparation, one drop of Triton x100 was added as emulsifier for obtained the desired concentration. The treated diet poured into plastic box and kept in refrigerator till use. A piece of the treated diet was introduced into jars with containing 1St nymphal instar for feeding on it for seven days then remained treated diet replaced by untreated one, number of dead individuals were counted each day after treatment till 14 day (the end point) for calculating LC_{50} values. For each concentration, 25 individuals were tested in five replicates, 5 nymphs each. Control was fed on untreated diet. LC_{50} were determined according to (FINNEY, 1971) and mortality percent was corrected according to Abbott's formula (ABBOTT, 1925). After determined the LC_{50} value of each oil, different biological aspects of the resulted insects have been recorded. The newly emerged 1St nymphal instar was fed on diet mixed with the prepared concentration of the oil for seven days then the diet replaced by untreated one till reached to the adult stage. The remaining adults were noticed for egg oviposition and egg hatching. The different biological aspects were recorded for each plant oil, for each test, 200 insects were used. Statistically, all data were subjected to Analysis of Variance (ANOVA) through SPSS Computer Program, To differentiate between means, Duncan's multiple range test (DUNCAN, 1965) was used (P = 0.05).

Histological examination

The remaining treated nymphs after feeding for 7 days on the treated diet then on untreated diet for another seven days were used for morphological and histological examinations of the alimentary canal, control groups were also examined. Alimentary canal were dissected in 0.9 % NaCl solution and fixed in Bouin's solution for 24 hrs (HUMANSON, 1962) then dehydrated in ascending alcoholic series and cleared in xylen for few seconds then specimens were infiltrated in three changes of paraffin wax each lasted 20 minutes. Paraffin blocks were prepared and 6 u longitudinal or cross section were cut and stained with Ehrlich's acid haematoxylin and alcoholic eosin. The stained sections were dehydrated, cleared and mounted using D.P.X. for examination.

Results and Discussion

The toxicity of the tested three different essential oils on the 1st nymphal instar of *H.*

littoralis is shown in Table 1. Latent toxicity was observed, for this LC_{50} values were recorded after 14 days from the treatments. According to the LC_{50} values it could be arranged as follows: Garlic 0.067 > Eucalyptus 0.075 > Mint 0.084 ml./100ml of diet. A parallel course was obviously seen, a mostly between the mortality and the oil concentration levels. The variable toxicity may be due to the constituents of each oil and disturbance or the hormonal regulations (AL-SHAROOK *et al*., 1991), 200 species of plants, which produce chemicals substances able to act against insects, are known. The substances can have poisonous and repellent effects and can work as phagorestrainer ovicide and can affect the insect's hormonal system. Moreover, a great number of essential oils can reduce the reproduction system of several insects and they can also hinder the growth the development and the reproduction of some herbivore insects (HILL, 1990; PARTES *et al*., 2000). KOUL *et al.* (2008) Found that Cineol and Limonene (terpes) and essential oils of *Eucalyptus globulus* and *E. canaldulensis* have shown poisonous effects through the cuticle and in ingestion and fumigation, causing 100% of mortality of *S. zeamais* in dilution 2:8 (essential oil: acetone). DUKE (2005) proved that *E. globulus* species has in its composition, 71% Cineol, pinen, terpinen, anethol, benzaldehyde, estragol, eugenol, limonene, linalool, menthol, methilchavicol, methilicinamate, ocimen, rutin and thimol. The same author recorded that the essential oil of *E. globulus* seem as natural or botanical potential insecticide, once the secondary plant metabolic have been used as pesticides or as models for synthetic pesticides, thus thy can cause poisonous interference in the biochemical and physiologic functions of herbivore insects. KWON & SANG (2005) studded the effect of plant essential oils from 29 plant species for their insecticidal activities against the Japanes termite, *Reticukitermes speratus* kolabe, using fumigation bioassay, they found good insecticidal effects with essential oils of *E. globulus* and other *E.species* and Garlic oil between the other tested oils, Garlic gave 100% mortality within 2 days of treatment, three major compounds from garlic oil were identified as tri and disulfide. NAGANAWA *et al.* (1996) mentioned that Garlic (*Allium sativum*) essential oil containing sulfoxide sulfated terpenoids (Allicin, Ajoene), they recorded inhibition of microbial growth by Ajoenen, a sulfur–containing compounds that derived from garlic.

Table 1. Susceptibility of the grasshopper *H. littoralis* 1st instars nymph to the natural plant essential oils mixed with the artificial diet.

Plant Essential oils	**LC values mg,/100ml. diet**			**Fiducial limit**		**Slope**
	LC25	**LC 50**	**LC 90**	**Upper**	**Lower**	
Garlic *Allium sativum*	0.009	0.067	2.89	0.1038	0.031	0.7843
Eucalyptus *Eucalyptus globulus*	0.008	0.075	5.23	0.1187	0.0315	0.6951
Mint *Mintha pipreta*	0.001	0.084	215.62	0.1723	0.0053	0.376

The data presented in Table 2 cleared the biological aspects of the LC_{50} values of the tested essential on *H. littoralis* nymphs, there were statistical significance differences between all nymphs instars periods. The total nymphal period was 68.6, 63.4 and 57 days for Garlic, Eucalyptus and Mint oil respectively which reduced to 46 day of the control taste. Life cycle increased in case of Eucalyptus 84.0 and Mint 77.8 and Garlic oil 68.6 days comparing with the control 65.6 days. The adults that resulting from 1st nymphal instars that fed on diet mixed with Garlic oil could not deposited egg pods at all, Garlic oil inhibited egg laying or reproduction, this may be as a result of its inhibition effect on oogenesis. Preoviposition period lasted 34.8 and 35.4 days for

Mint and Eucalyptus oil comparing with 20.6 in control. Oviposition period greatly decreased from 39.8 days on control to 21.2-23.4 days for oils treatments. The mean numbers of the deposited eggs decreased from 152 eggs in control to 56.6 - 43.6 eggs for Mint and Eucalyptus oils respectively. Also there were great reduction in the eggs hatchability from 24 and 45.2 comparing with 95%. Reduced fertility was observed due to a failure of many oocytes to mature. Longevity of the resulted adults increased in case of Garlic oil. There were percentage of mortality reached 50–52% and malformation ranged from 45.6- 51.6% on the oils treatment. Life span was affected by the oil treatments they take 171-186 days comparing to 164 days in control. Inhibition of egg reproduction by Garlic oil may be related to the effect of oil on the digestive and enzymatic system that reflect on hormonal release. These results agree with the finding of ZUDAIRE *et al.* (1998), who cleared that, the gut of locust disturbed and showed disrupted in the endocrine cells as affected by food nutrition content, insect age and stage. HUSSEIN *et al.* (1994) mentioned that the effect of plant extracts on maggot of *Erias insulana* may due to digestion and absorption of plant oil. FREISEWINKEL & SCHMUTTERER (1991) showed that the treatment of fifth instar nymphs of *L. migratiria* by neem oil led to increase mortality mainly during molts, prolonged periods of development, and reduced fitness. GHZAWI (2005) recorded that lower doses of Azadirachtin treatment the 4th and 6th nymphal instars of *H. littoralis* caused prolongation of the insect duration, leading finally to death. MOHAMMED & EL-GAMMAL (2002) reported that Azadirachtin caused prolongation in duration of the last instar nymphs of *S.gregaria*.

Table 2. Biological parameters of *H. littoralis* treated as 1st instar nymph with the LC_{50} values of the tested natural plant essential oils that mixed with the artificial diet.

Biological parameters	Plant essential oils Mean ± S.E				F-value
	Garlic	Mint	Eucalyptus	Control (Artificial diet)	
1st instar nymph	12.00±0.32 a	9.00±0.63 b	10.80±0.58 a	8.20±0.37 b	12.082 **
2 nd instar nymph	12.60±1.08 a	9.20±0.73 b	11.80±0.86 a	8.80±0.37 b	5.499 **
3rd instar nymph	13.60±1.36 a	11.40±0.51 ab	12.60±1.03 a	9.20±0.37 b	4.321 *
4th instar nymph	14.00±0.84 a	12.60±1.08 a	13.00±1.14 a	9.60±0.40 b	4.321 *
5th instar nymph	16.40±1.03 a	14.80±1.24 a	15.20±0.86 a	10.20±0.37 b	8.502 **
Total nymphal period (in days)	68.60±1.69 a	57.00±2.70 b	63.40±2.29 a	46.00±0.45 c	24.265 **
Life Cycle(in days)	68.60±1.69 b	77.80±3.01 a	84.00±2.21 a	65.60±0.87 b	16.261 **
Pre-Oviposition period (in days)	0.00±0.00 c	34.80±1.62 a	35.40±2.23 a	20.60±1.08 b	125.936 **
Oviposition period (in days)	0.00±0.00 c	21.20±1.07 b	23.40±1.08 b	39.80±1.20 a	285.276 **
Post-Oviposition period (in days)	0.00±0.00 b	37.20±1.02 a	40.40±2.40 a	38.00±0.95 a	193.794 **
No. of deposited eggs/female	0.00±0.00 c	56.60±5.86 b	43.60±4.26 b	152.00±6.44 a	174.535 **
Percentage of Egg hatchability	0.00±0.0 d	45.20±6.13 b	24.00±3.67 c	95.00±1.41 a	123.269 **
Longevity (in days)	118.00±1.41 a	93.20±2.60 b	99.20±3.40 b	98.40±1.47 b	21.039 **
Total mortality percentage	52.40±1.29 a	50.00±3.16 a	51.60±1.17 a	0.00±0.00 b	202.695 **
Malformation percentage	51.60±0.93 a	45.60±2.23 b	50.40±0.98 a	0.00±0.00 c	360.991 **
Life span (in days)	186.60±2.73 a	171.00±0.84 b	183.20±4.72 a	164.00±1.34 b	13.790 **
Longevity (in days)	118.00±1.41 a	93.20±2.60 b	99.20±3.40 b	98.40±1.47 b	21.039 **
Total mortality percentage	52.40±1.29 a	50.00±3.16 a	51.60±1.17 a	0.00±0.00 b	202.695 **
Malformation percentage	51.60±0.93 a	45.60±2.23 b	50.40±0.98 a	0.00±0.00 c	360.991 **
Life span (in days)	186.60±2.73 a	171.00±0.84 b	183.20±4.72 a	164.00±1.34 b	13.790 **

Legend: **= Highly significant; *= Significant; Means followed with the same letter(s) within the horizontal column were not significantly different $p<0.05$.

Histological changes in the alimentary canal

The alimentary canal is a long straight tube running from the mouth to the anus, it fills most of the internal cavity of the grasshopper nymph, it is divided into the foregut, the midgut and the hindgut Fig. 1A. The foregut is considered to consist of four sections, the pharynx, the oesophagus, the crop and the proventriculus. The pharynx is the first part of the foregut and apart from being a tube that connect s the interior of the mouth area (Buccal cavity). The oesophagus is basically a tube leading to the midgut via the crop and the preventriculus. The Crop is simply a storage area, and the proventriculus is a muscular extension of the crop. This organ contains tooth-like denticles (known as the intima) that grind and pulverize food particles in addition to protect the foregut tissues from abrasion by food particles. The hard denticles inside the proventriculus are made from the chitin materials same of the external integument of the insects. Next is the stomach to which are attached six double finger–shaped digestive glands, the gastric carecae pouch–like structure arranged in a ring–like manner around the anterior end of the midgut Fig. 1A. The anterior lobe of each pair of the caecae extended over the crop while the posterior lobe extended over the midgut, gastric caecae produce enzymes that are secreted into the stomach to aid digestion. The gastric caeca serve to increase the surface area of the midgut, thus increase both its ability to secrete digestive enzymes and its ability to extract useful products from the partially digestive food. The useful proteins, vitamins and fats that are released by the digestive process pass across the wall of the midgut into the body cavity. The midgut runs from the gastric caeca to the Malpigahian tubules, a series of long thin tubes, in between these two the stomach or ventriculus or midgut which is the area of most active digestion (CHAPMAN, 1985). The digested food absorbed through the midgut tissue into the surrounding haemocoel then diffused to the different body parts. The hindgut comprises the intestine which compresses the undigested food and waste products, extracts more water before it is passed through the anus as faeces. The hindgut is lined by intima like the foregut. The digestive tract continues as the intestine, a thin tube without accessory structures. It leads to the short rectum which opens to the exterior via the anus. The hair–like tubules lying over the intestine are malpighian tubules, the excretory organs Fig. 1B.

Histological examination for the alimentary canal of the treated nymphs that remained after feeding on diet treated with the LC_{50} of the most effective essential oil (Garlic) were recorded. After desiccation the digestive tract became flabby, softness were observed, swollen in the gastric caeca and preventriculus, elongation of the midgut Fig. 2A&B.

Histopathological changes that observed through the alimentary canal were as follows: in the crop tissues were separation of the muscular layer, disorganization of the cells were observed and partial rupture in the intima that lined the lumen Fig. 10, 13 and 14 comparing with the control section Fig 8 and 9.

The midgut of the control last instar nymphs of *H. littoralis* consists of a unicellular layer (epithelium) resting upon a basement membrane. This membrane is surrounded externally by circular and then by longitudinal muscle fibers. The epithelium consists of columnar and goblet cells with clusters of small regenerative cells each of which contains a relatively large nucleus and strongly basophilic cytoplasm Fig. 3 and 4. The epithelium is, also, protected from the food particles by a detached sheath, peritrophic membrane, surrounding lumen Fig. 4. Also as seen in Fig. 4 is the control (normal) midgut appears with luminal surface of the epithelium which is provided with a striated border constituting long microvilli Fig. 4. Such microvilli protect inwards into the lumen to increase the absorption surface of the cells, as well as, the spaces between them act as a kind of sieve. The histopathological effects of Garlic oil on the epithelium of the treated nymphs of *H. littoralis* are demonstrated in Fig. 5 and 6). The microscopically studies show a destruction of the cell vacuolization

and a rupture of the cell wall, some signs of morbidity in both nuclei and cytoplasm of the epithelial cells, microvilli of the columnar cells were curled and ruptured, vacuolated cytoplasm with large area of necrosis Fig 6. Elongation of the columnar and goblet cells was observed in Fig 7, rupture of pritrophic membrane Fig 5, 6 and 7). CLARKE *et al*. (1977) mentioned that diflubenzuron blocked the production of peritrophic membrane in *Locusta migratoria*. TAPPOZADA *et al*. (1968) investigated the histological and cytological changes in the mid gut of *Spodoptera littoralis* by some insecticides such as elongation of the epithelial cells, fading of cell boundaries and degeneration of some cells, decamethrin, diflubenzuron and methomyl caused vacuolation, elongation and breakdown of the epithelium, separation and detachment of peritrophic membrane of the pink bollworm *Pectinophora gossypiella* (SAAD *et al*., 1985). Large vacuoles were observed in the epithelial layer of treated *chironomid* midges larvae (*Chironomous decorus* and *Tanypus gradhaus*) fed on diflubenzuron and triflumuron (PELSU, 1985). Similarly, in adult *Tribolium castaneum* fed on triflumuron, epithelium vacuoles were observed in the midgut (PARWEEN, 1997). The vacuolation, elongation and disintegration of the epithelial cells, as well as the disappearance of muscular and regenerative cells, detachment of the basement membranes, had been observed in the mid gut of *S. exigua* by the action of diflubenzuron, malathion and cypermethrin (YOUNES *et al*., 2000). Many investigations have been conducted on the antifeedant effects, growth inhibition and abnormal development in various insects caused by using natural insecticides including *Boxus chinensis* oil and precoceneII (NASSAR, 1995; BREAM *et al*., 2001). In the present studies we could postulate that Garlic oil may affect the neuroendocrine system of insects which affect the production of ecdysone and consequently disturb the molting process and finally cause insect death as mentioned by GUJAR & MEHROTRA (1983); MOSTAFA *et al.* (1995). NASIRUDDIN & MORDUE (1993) studied the histological and ultrastructure changes caused by azadirachtin on the midgut of locust. His findings revealed necrosis of epithelial cells, enlargement of cytoplasmic inclusions and small sized striated borders. Various aspects of histopathology in the midgut of locust *Heteracris arnulosa* caused by neem seed extract were discussed by NAQVI *et al. (*1994). ZUDAIRE *et al.* (1998) cleared that, the gut of locusts disturbed and showed disrupter in the endocrine cells as affected by food nutrient content, insect age and stage.

The gastric caeca presents notable morphological swollen Fig.2A, and histological modification Fig. 10, 12 and 13, there were elongation, hypertrophy and hyperplasia clearly observed in Fig 12. comparing to the control section Fig.11, there dilation showing increasing in to lumen surface accompanied by epithelial bursting and fall height of the epithelial folds. This modification would be probably due to the destruction occurred in the muscular layer surrounding the caeca. For changes on the hindgut a partial loss of cellular integrates which reaches even the muscular bases leading to loss of integrity of the basement membrane of the cells.

In the hindgut there were partial changes was observed including the intima layer that lined the cells was affected to some extents, a partial abortion was occur-ing Fig. 16 also degeneration of small number of their cell was noticed comparing with the control Fig. 15. These observations are to be brought closer the observation on *Schisticerca* reported by ARMAH *et al.* (1999).

The untreated nymph show a visceral fat body made up of cellular clusters which often take the form of cellular cords Fig. 17. The cells appear rectangular with a core and cytoplasm filled with many lipid droplets (not colored vacuoles) that noticed in Fig. 17. The treated fat cells became smaller and more colored, became less provided with a significant fall of the number of the fat cells Fig. 18. This tissue loss is often accompanied by cellular exudates. The change of the size is probably due to a change of permeability causing a loss of the cellular content. CHAIEB *et al.* (2007) mentioned that the destruction

of the cellular structure is probably due to the membrane destabilization followed by the bursting of the membrane and the discharge of the cellular contents. The same author revealed a cytotoxic effect of *Certum perqui* saponins on the fat body of *S. littoralis* larvae, the cells of this tissue decrease in size and became more colored by loss of their cytoplasmic content. They also noted a cell destruction of the foregut and the gastric caeca of *Schisticerca*, they concluded that saponins interact with membrane cholesterol, this causes a membrane destabilization and that provokes cell death. Finally the histological changes that observed and recorded in this studies revealed disturbances in the foregut, midgut, hindgut and gastric caeca caused disturbances in the metabolic process of digested food and decrease the distribution of the nutritive metabolites materials into the haenocoel and effect on the stored lipid contents in the fat bodies which recognized as a powerful of energy for all the physiological properties of the insects, accordingly metamorphosis, development and reproduction of the insects was greatly affected. Thus, it could be concluded that essential oil of Garlic plant and may be the other tested oils Eucalyptus and Mint oils could be used as an effective natural products to be included in the integrated pest management program of *H.littoralis* grasshopper and other pests as well.

Fig. 1. Untreated alimentary canal in normal nymph of *H. littoralis* (1A - anterior part and 1B - posterior part). **Fig. 2.** Treated alimentary canal (2A and 2B). Crop (co) white arrow

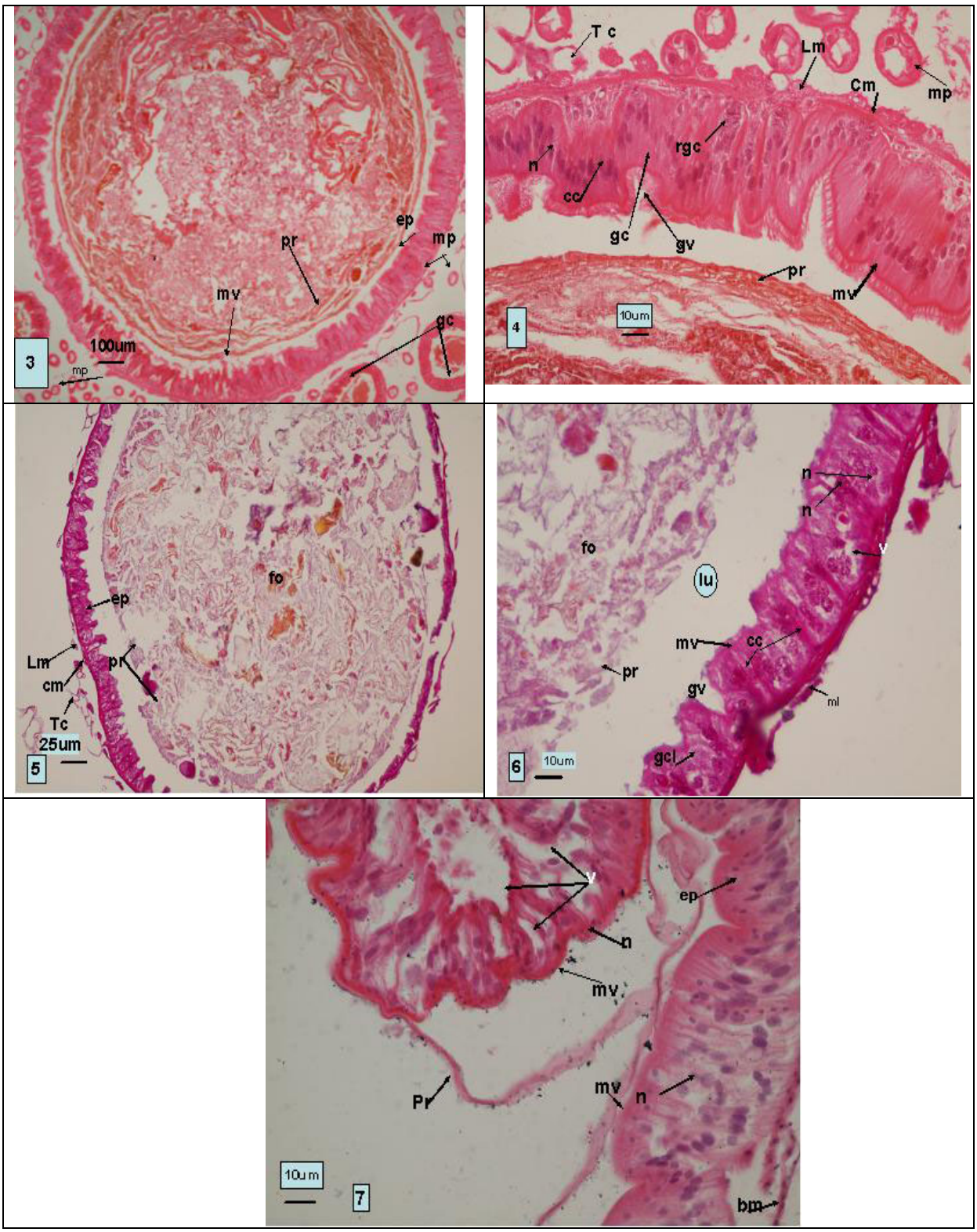

Fig. 3-7: Transverse section (T.S); Fig. 3. Normal midgut; Fig. 4. Magnified portion from Fig. 3; Fig. 5. Treated mid gut; Fig. 6 and 7. Magnified portion from Fig. 5. showing hypertrophy and hyperplasia of the epithelial cells of the treated midgut.

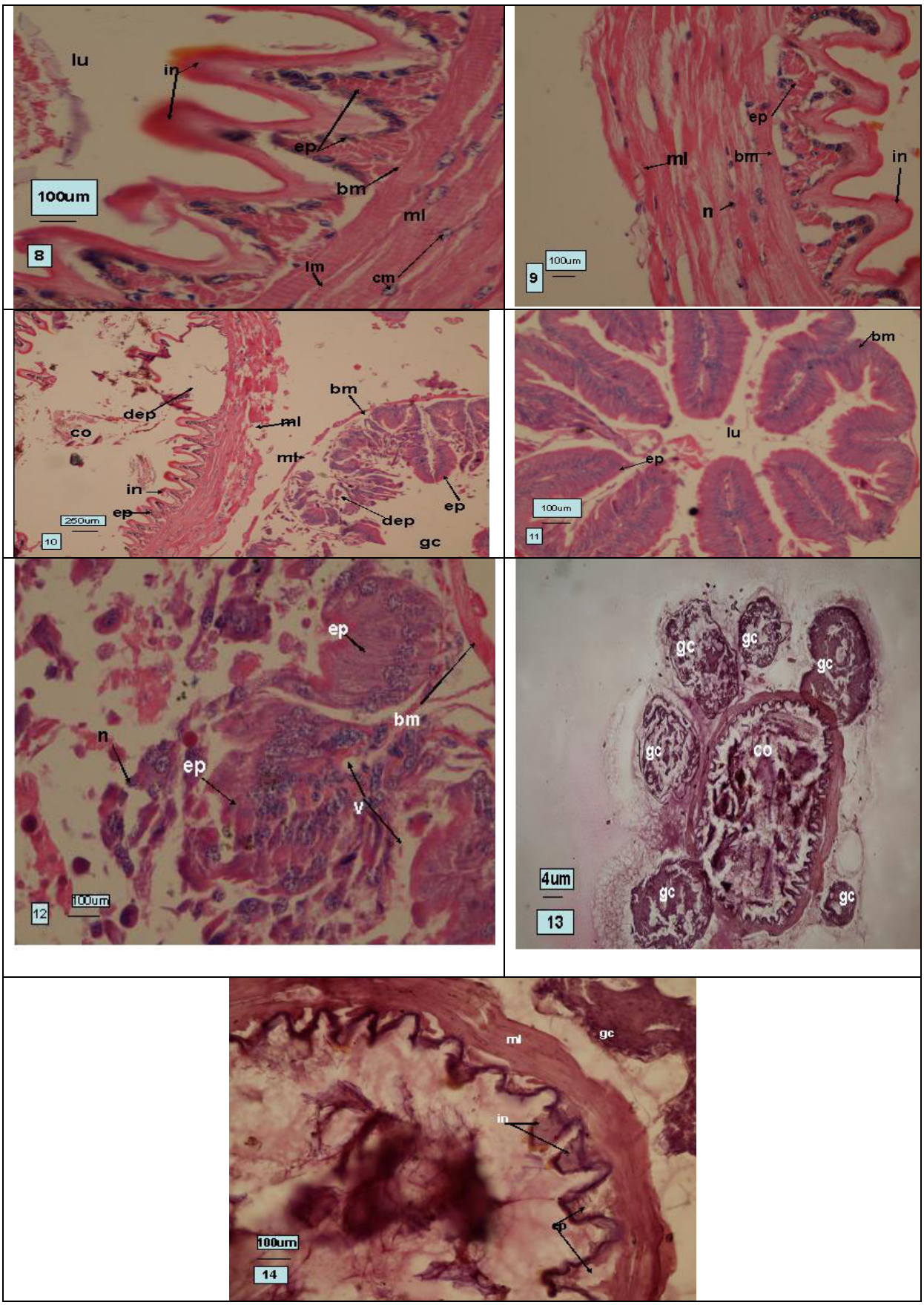

Fig. 8-14: Fig. 8 and 9. Normal crop; Fig. 11. Normal gastric caecae; Fig. 10, 13 and 14. Treated crop; Fig. 10 and 13. Treated crop and gastric caecae; Fig. 12 and 13. Treated gastric caecae.

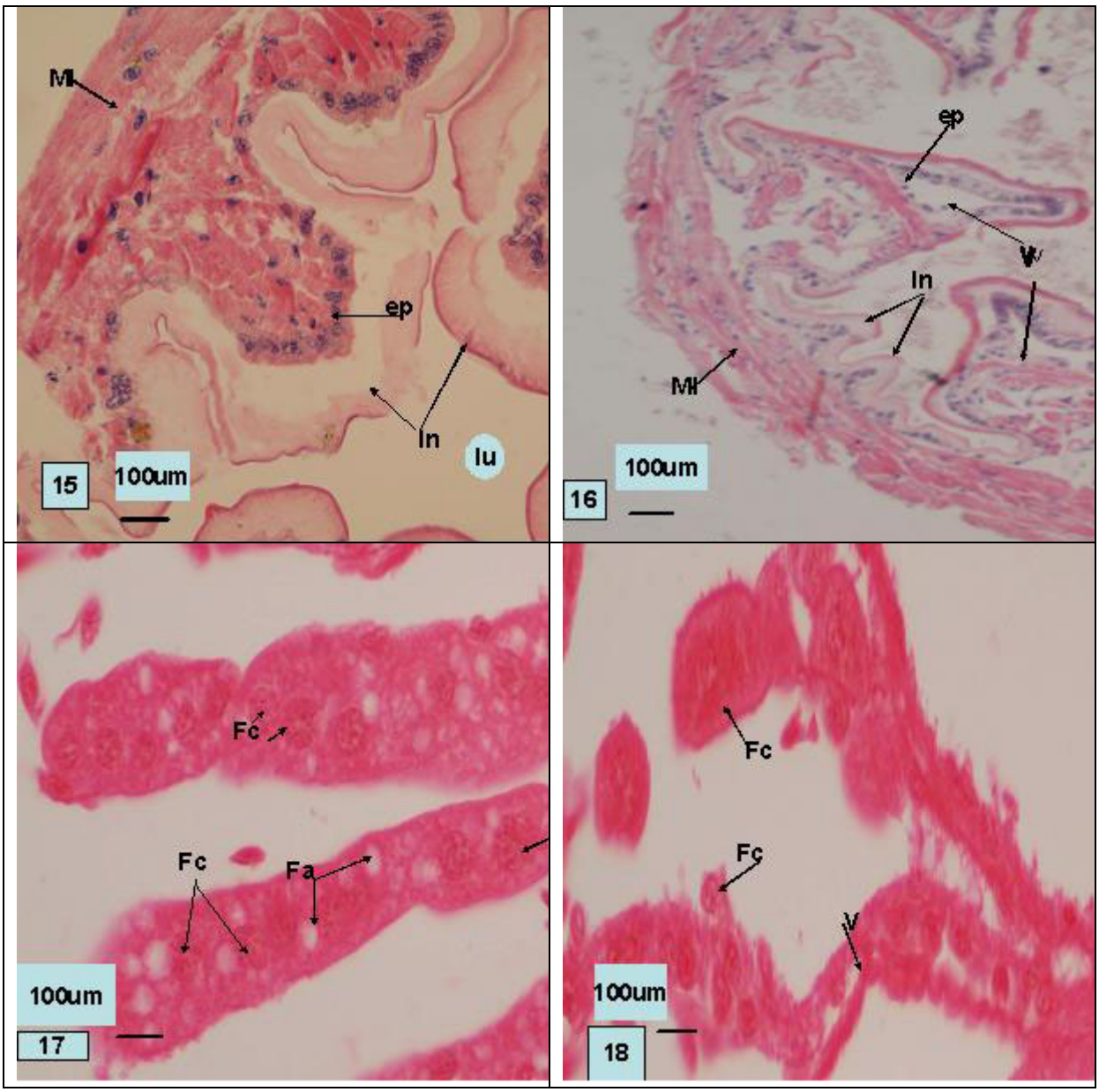

Fig. 15-18: Fig 15. Normal intestine; Fig. 16. Treated intestine; Fig. 17. Normal fat body; Fig. 18. Treated fat body.

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Received: 15.02.2012
Accepted: 28.04.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 53-62

Acute, Lethal and Synergistic Effects of Some Terpenes Against Tribolium castaneum Herbst (Coleoptera: Tenebrionidae)

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Abstract. Terpenes are found in abundance in all plant essential oils. In the present study two pure volatile compounds of terpene group, α-pinene and β-caryophyllene have been evaluated for their repellent, acute toxicity and developmental inhibitory activities alone and in binary combination against red flour beetle *Tribolium castaneum*. In repellency assay, α-pinene and β-caryophyllene repelled *T. castaneum* adults significantly even at 0.025% concentrations. Fumigation of larvae and adults of *T. castaneum* with these two compounds caused lethality in them. Median lethal concentrations (LC_{50}) of α-pinene and β-caryophyllene against adults were 0.998 and 1.624 μl/cm^3 and against larvae were 1.379 and 1.949 μl/cm^3 respectively. In binary combination, the LC_{50} values against adults and larvae were found 1.277 and 1.438 μl/cm^3 respectively. Fumigation with two sublethal concentrations viz. 40 and 80% of 24-h LC_{50} of these two compunds alone and in binary combination significantly reduced oviposition potential of adults and inhibited pupation and adult emergence in larvae. All the responses were found concentration-dependent. From the present study, it can be concluded that α-pinene is more active than β-caryophyllene and these two volatile compounds in binary combination shows synergism and thus, can used as efficient insecticidal tool against *T. castaneum* as fumigant either alone or in combination.

Keywords: α-Pinene, β-Caryophyllene, *Tribolium castaneum*, Fumigant toxicity, Terpenes.

Introduction

Stored-grain insect pests have been damaging food grains in granaries and store houses and accounts for 10-40% loss worldwide (MATTHEWS, 1993). In India, this damage approximates for 10% during farm level storage (LAL, 1988). Despite application of improved storage structures and traditional control techniques, 70-90% of food grain is still stored not more than six months to a year at farmer's level. Thus, to protect stored grains from insect infestation exploration of other alternatives becomes quite essential. In this regard, synthetic pesticides came into existence, but uncontrolled use of these chemicals causes great environmental hazards due to their persistent nature, increased risk of neurotoxic, carcinogenic, teratogenic and mutagenic effects in non-target animals (BAKKALI *et al*., 2008; AYAZ *et al*., 2010). Besides, efficacy of these chemicals against stored-grain insect pests varies greatly after treatment and induced resistance against such chemicals (ZETTLER & CUPERUS, 1990; JEMBERE *et al*., 1995; PINTO *et al*., 1997). In another approach, plants and its products have been used in insect pest management programme since time immemorial. Amongst such plant derived products, essential oils have got much attention as fumigants since last two decades (AGRAWAL *et al*., 2001, TRIPATHI *et al*., 2003; CHAUBEY, 2007; SHUKLA *et al*., 2008; ABDEL-SATTAR *et*

al., 2010; AYAZ *et al.,* 2010; ZAPATA & SMAGGHE, 2010; CHAUBEY, 2011; STEFANAZZI *et al.,* 2011). Essential oils are complex mixture of volatile compounds produced as secondary metabolites. Bioactivity of these essential oils depends on its chemical composition which varies with plant part used for extraction, harvesting time, plant age, nature of the soil and growth conditions (ANGIONI *et al.,* 2006; ISMAN *et al.,* 2007). Efficacy of these essential oils is also affected by proportion of chemical constituents and synergism or antagonism among them (HUMMELBRUNNER & ISMAN, 2001; SAMPSON *et al.,* 2005; ANGIONI *et al.,* 2006).

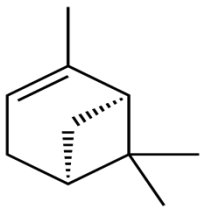
α-Pinene is a monoterpene containing a reactive four-membered ring. It is found as a important constituent in essential oils of *Nepta racemosa* (DABIRI & SEFIDAKON, 2003), *Ferulago* spp. (KHALIGHI-SIGAROODI *et al.,* 2005), *Syzygium aromaticum* (ALMA *et al.,* 2007), *Biden pilosa* (DEBA *et al.,* 2008), *Zingiber officinale* (KOROCH *et al.,* 2007; SASIDHARAN & MENON, 2010), *Eucalyptus* spp. (CHENG *et al.,* 2009; MACIEL *et al.,* 2010), *Citrus* spp. (KAMAL *et al.,* 2011) and *Vicia dadianorum* (KAHRIMAN *et al.,* 2012). β-Caryophyllene, a bicyclic sesquiterpene having cyclobutane ring, has been reported in *Piper cubeba* (LAWLESS, 1995), *Scutellaria pinnati* (GHANNADI & MEHREGAN, 2003), *Ferulago* spp. (KHALIGHI-SIGAROODI *et al.,* 2005), *Syzygium aromaticum* (ALMA *et al.,* 2007), *Biden pilosa* (DEBA *et al.,* 2008), *Eucalyptus* spp. (CHENG *et al.,* 2009; MACIEL *et al.,* 2010), *Citrus* spp. (KAMAL *et al.,* 2011) and *Pistacia lentiscus* essential oils (BURHAM *et al.,* 2011).

In the present study, two pure terpenes, α-pinene and β-caryophyllene have been evaluated for their repellent, acute toxicity and developmental inhibitory activities alone and in binary combination against red flour beetle *Tribolium castaneum*.

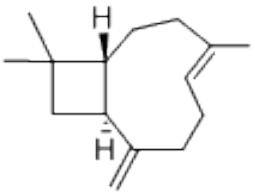
Materials and methods

Tested Terpenes

The two pure terpenes viz. α-pinene and β-caryophyllene were purchased from Sigma Chemicals, USA.



α-pinene



β-caryophyllene

Insects

Red Flour beetles *T. castaneum* (Coleoptera: Tenebrionidae) were used to determine insecticide activity of terpenes. The insects were reared on wheat flour in the laboratory at 30±2°C, 75±5% RH and 10:14h (L: D) photoperiod.

Bioassay Tests

1. *Repellent activity of terpenes.* Repellency assay was carried out in glass petri dishes (diameter 8.5 cm and height 1.2 cm). Test solutions of serial dilution, 0.2, 0.1, 0.05 and 0.025% of α-pinene and β-caryophyllene were prepared in acetone. Whatmann filter paper was cut into two equal halves and each test solution was applied to filter paper half as uniform as possible using micropipette. The other half of filter paper was treated with acetone only. The volatile terpenes treated and acetone treated halves were dried to evaporate solvent completely. Both treated and untreated halves were then attached with cellophane tape and placed at the bottom in each petri dish. Twenty adults of *T. castaneum* were released at the centre of filter paper disc and then petri dishes were covered and kept in dark. Four replicates were set for each concentration of compounds solution. Number of insects on both treated and untreated halves was recorded after 4 h of start of experiment in mild light.

2. *Fumigant toxicity of terpenes against T. castaneum adults/ larvae.* Fumigant toxicity of α-pinene and β-caryophyllene alone or in binary combination was tested against *T. castaneum* adults and larvae. Ten adults/larvae taken from the laboratory culture were placed with 2 g of wheat flour in petri dishes (diameter 8.5 cm and height 1.2 cm). Filter paper strips (4 cm^2) impregnated with test solutions of α-pinene

and β-caryophyllene alone or in binary combination prepared in acetone, was pasted on the under cover of petri dishes. All petri dishes were closed and kept under same environmental conditions described for rearing of insect. Six replicates were set for each test solution as well as control group. After 24 h of fumigation, mortality in adults/larvae was recorded. In binary combination, α-pinene and β-caryophyllene were mixed in equal ratio. In control group, filter paper strips were treated with acetone only.

3. *Oviposition inhibitory activities of terpenes.* Oviposition inhibitory activity of α-pinene and β-caryophylene alone or in binary combination was tested against *T. castaneum* by fumigation method. Adults of mixed sexes were fumigated with filter paper strip (4 cm^2) impregnated with 40 and 80% of 24-h LC_{50} of test solutions prepared in acetone as was done in toxicity assay. After 24 h fumigation, adults were transferred to clean petri dishes having wheat flour. After seven days of daily observation, adults were removed and discarded. Number of larvae hatched was counted for treated as well as control groups for ten days continuously. Six replicates were set for each concentration of compounds as well as control group. In binary combination, α-Pinene and β-Caryophylene were mixed in equal ratio. In control group, filter paper strips were treated with acetone only.

4. *Developmental inhibitory activities of terpenes.* Developmental inhibitory activities of α-pinene and β-caryophyllene alone or in binary combination were determined against 4^{th} instars larvae of *T. castaneum*. Larvae were fumigated with filter paper strip (4 cm^2) impregnated with 40 and 80% of 24-h LC_{50} of test solutions prepared in acetone as was done in toxicity assay. After 24 h of fumigation, treated larvae were transferred to petri dishes having wheat flour. Number of pupae transformed from treated larvae and adults emerged from transformed pupae were recorded. Six replicates were set for each concentration of compounds as well as control groups. In binary combination, α-pinene and β-caryophylene were mixed in equal ratio. In control group, filter paper strips were treated with acetone only.

Data analysis

Chi-square test was applied to establish the repellent activity of the tested terpenes (SOKAL & ROHLF, 1973). Median lethal concentration (LC_{50}) was calculated by POLO programme (RUSSEL *et al.*, 1977). Correlation and linear regression analysis were conducted to define all concentration-response relationships (SOKAL & ROHLF, 1973). Analysis of variance was performed to test the equality of regression coefficient (SOKAL & ROHLF, 1973).

Results

Repellency effect

In repellency assay, percentage of *T. castaneum* in treated filter paper disc half was 30%, 22.5%, 7.5% and 2.5 at 0.2%, 0.1%, 0.05% and 0.025% concentration of α-pinene (Table 1). Similarly, percentage of insects in treated filter paper disc half was 25%, 17.5%, 10% and 3.75% at 0.2%, 0.1%, 0.05% and 0.025% concentration of β-caryophyllene (Table 1). Chi-square analysis indicated that α-pinene and β-caryophyllene were repellent to *T. castaneum* adults. These two pure allelochemicals showed significant repellent activity even at 0.025%concentration as the hypothesis of ratio 1:1 was rejected (Table 1).

Larval and adult mortality

Fumigation of *T. castaneum* larva and adults with α-pinene and β-caryophyllene alone or in binary combination caused toxicity in them by vapour action. Median lethal concentrations (LC_{50}) of α-pinene and β-caryophyllene against larvae were 1.379 and 1.949 µl/cm^3 and against adults were 0.998 and 1.624 µl/cm^3 of air, respectively (Table 2). In binary combination of α-pinene and β-caryophyllene, LC_{50} values against larvae and adult were 1.438 and 1.277 µl/cm^3, respectively (Table 2). With regard to larval mortality, regression analysis showed a concentration dependent significant positive correlation of α-pinene

(F = 226.61), β-caryophylene (F = 137.84) and α-pinene and β-caryophyllene mixture (F = 144.12) (P<0.0001, Table 5). Similarly, with regard to adult mortality, regression analysis showed a concentration dependent significant positive correlation of α-pinene (F = 138.35) and β-caryophyllene (F = 72.25), and α-pinene and β-caryophyllene mixture (F = 70.99) (P<0.0001, Table 5).

Oviposition inhibition

Fumigation of *T. castaneum* adults with two sublethal concentrations of α-pinene, β-caryophyllene and its binary combination reduced oviposition potential of insect. Oviposition was reduced to 71.91% and 58.54%; and 68.20% and 48.09% of the control when *T. castaneum* adults were fumigated with 40 and 80% of 24-h LC_{50} of α-pinene and β-caryophyllene alone (Table 3). Similarly, oviposition was reduced to 56.4% and 36.52% of control when *T. castaneum* adults were fumigated with 40 and 80% of 24-h LC_{50} of α-pinene and β-caryophyllene binary combination (Table 3). Regression analysis indicates that oviposition potential of the *T. castaneum* showed significant negative correlation with concentration when fumigated with α-pinene (F = 24.13), β-caryophyllene (F = 39.20), and α-pinene and β-caryophyllene binary combination (F = 70.52) (P<0.0001; Table 5).

Developmental inhibition

The percentage of larvae transformed into the pupae and percentage of pupae transformed into adult were decreased when fumigated with two sublethal concentrations of α-pinene and β-caryophyllene alone or in binary combination. Pupation in treated larvae was reduced to 70.8% and 55%; 85% and 64.2%; and 64.2% and 34.2% of control when *T. castaneum* larvae were fumigated with 40 and 80% of 24-h LC_{50} of α-pinene and β-caryophyllene alone or in binary combination respectively (Table 4). Adult emergence was reduced to 50.8% and 39.2%; 66.7% and 45.8% and 47.5% and 15.8% of control when *T. castaneum* larvae were fumigated with 40 and 80% of 24-h LC_{50} of α-pinene and β-caryophyllene alone or in binary combination respectively (Table 4). Regression analysis showed a concentration-dependent significant negative correlation of α-pinene fumes with pupation (F = 68.16) and adult emergence (F = 94.44) (P<0.0001; Table 5). β-caryophyllene fumes showed concentration-dependent significant negative correlation with pupation (F = 78.6) and adult emergence (F = 89.55) (P<0.0001; Table 5). Binary combination of α-pinene and β-caryophyllene also showed negative correlation with pupation (F = 180.5) and adult emergence (F = 565.31) (P<0.0001; Table 5).

Table 1. Repellenct effect of α-Pinene and β-Caryophyllene against *Tribolium castaneum* adults

Concentration %(Vol:Vol)	α-Pinene			β-Caryophyllene		
	Treated Mean±SE	Untreated Mean±SE	χ^2-values	Treated Mean±SE	Untreated Mean±SE	χ^2-values
0.2	2.5±0.50	97.5±0.50	36.4[a]	3.75±0.48	96.25±0.48	34.5[a]
0.1	7.5±0.50	92.5±0.50	29.2[a]	10±0.85	90±0.85	28.1[a]
0.05	22.5±0.50	77.5±0.50	12.4[b]	17.5±0.29	82.5±0.29	17.0[a]
0.025	30±0.81	70±0.81	8.2[c]	25±0.41	75±0.41	10.2[b]

[a]Significant at P<0.001, [b]Significant at P<0.01, [c]Significant at P<0.05

Table 2. Fumigant toxicity of α-Pinene and β-Caryophyllene alone and in binary combination against *Tribolium castaneum* adults and larvae

Terpenes	Parameters	LC_{50} (µl/cm³)	LCL (µl/cm³)	UCL (µl/cm³)	g-value	t-ratio	Hetero geneity
α-Pinene	Adult mortality	0.998	0.895	1.072	0.29	3.21	0.34
	Larval mortality	1.379	1.292	1.468	0.31	3.33	0.30
β-Caryophyllene	Adult mortality	1.624	1.204	2.025	0.32	0.41	0.41
	Larval mortality	1.949	1.776	2.099	0.34	3.54	0.39
α-Pinene + β-Caryophyllene	Adult mortality	1.277	1.13	1.424	0.35	3.17	0.30
	Larval mortality	1.438	1.277	1.60	0.28	3.26	0.35

LC_{50} represents lethal concentration that causes 50% mortality
LCL and UCL represent lower confidence limit and upper confidence limit respectively
g-value, t-ratio and heterogeneity are significant at all probability levels (90, 95 and 99%)

Table 3. Oviposition behaviour of *Tribolium castaneum* adults when fumigated with α-Pinene and β-Caryophyllene alone and in binary combination

Concentration	Number of larvae emerged per adult treated (Mean±SD)		
	α-Pinene	β-Caryophyllene	α-Pinene + β-Caryophyllene
Control	8.90±0.96 (100)	8.90±0.96 (100)	8.90±0.96 (100)
40% of LC_{50}	6.40±0.84 (71.91)	6.07±1.03 (68.20)	5.02±0.42 (56.40)
80% of LC_{50}	5.21±0.99 (58.54)	4.28±0.69 (48.09)	3.25±0.37 (36.52)

Values in parentheses indicates per cent change control taken as 100%

Table 4. Developmental inhibitory activities of α-Pinene and β-Caryophyllene alone and in binary combination against *Tribolium castaneum* by fumigation method

Conc.	Number of pupa transformed (Mean±SD)			Number of adults emerged (Mean±SD)		
	α-Pinene	β-Caryophyllene	α-Pinene + β-Caryophyllene	α-Pinene	β-Caryophyllene	α-Pinene + β-Caryophyllene
Control	10.0±0.0 (100)	10.0±0.0 (100)	10.0±0.0 (100)	10.0±0.0 (100)	10.0±0.0 (100)	10.0±0.0 (100)
40% of LC_{50}	7.08±0.93 (70.8)	8.5±0.71 (85)	6.42±0.32 (64.2)	5.08±0.97 (50.8)	6.67±0.76 (66.7)	4.75±0.31 (47.5)
80% of LC_{50}	5.50±0.71 (55)	6.42±0.49 (64.2)	3.42±0.43 (34.2)	3.92±0.97 (39.2)	4.58±0.49 (45.8)	2.58±0.18 (25.8)

Values in parentheses indicates per cent change control taken as 100%

Table 5. Regression parameters of oviposition and developmental inhibitory effects on *Tribolium castaneum* treated with α-Pinene and β-Caryophyllene alone and in binary combination by fumigation method

Terpenes	Parameters	Intercept	Slope	Regression Equation	Regression coefficient	F-value
α-Pinene	% Adult mortality	- 10.06	0.93	Y = - 10.06 + 0.93X	0.965	138.35(P<0.0001)*
	% Larval mortality	- 11.08	0.66	Y= - 11.08 + 0.66X	0.947	226.61(P<0.0001)*
	% Oviposition	6.83	8.41	Y = 6.84 + 8.41X	- 0.979	24.13(P<0.0001)**
	% Pupal survival	6.83	2.05	Y = 6.83 + 2.05X	- 0.985	68.16(P<0.0001)**
	% Adult emergence	6.11	0.66	Y = 6.11 + 0.66X	- 0.942	94.44(P<0.0001)**
β-Caryophyllene	% Adult mortality	- 3.39	0.50	Y = - 3.39+ 0.50X	0.993	72.25(P<0.0001)*
	% Larval mortality	- 7.74	0.46	Y = - 7.74 + 0.46X	0.969	137.84(P<0.0001)*
	% Oviposition	6.0	1.25	Y = 6.0 + 1.25X	- 0.992	39.2(P<0.0001)**
	% Pupal survival	7.27	3.11	Y = 7.27 + 3.11X	-0.995	78.6(P<0.0001)**
	% Adult emergence	6.50	1.75	Y = 6.5 + 1.75X	-0.991	89.55(P<0.0001)**
α-Pinene + β-Caryophyllene	% Adult mortality	- 7.29	0.68	Y = - 7.29 + 0.68X	0.965	70.99(P<0.0001)*
	% Larval mortality	- 4.10	0.57	Y = - 4.1 + 0.57X	0.993	144.12(P<0.0001)*
	% Oviposition	5.71	0.04	Y = 5.71 + 0.04X	- 0.977	70.52(P<0.0001)**
	% Pupal survival	5.89	1.04	Y = 5.89 + 1.04X	-0.998	180.5(P<0.0001)**
	% Adult emergence	5.34	0.22	Y = 5.34 + 0.22X	-0.972	565.31(P<0.0001)**

*df = 4,25; **df = 2,15

Discussion

Use of plant oils and its components as fumigants has received much attention of the scientific communities in pest management programme (AGRAWAL *et al.*, 2001, TRIPATHI *et al.*, 2003; CHAUBEY, 2007; SHUKLA *et al.*, 2008; ABDEL-SATTR *et al.*, 2010; AYAZ *et al.*, 2010; ZAPATA & SMAGGHE, 2010; CHAUBEY, 2011; STEFANAZZI *et al.*, 2011). The volatile components of essential oils can be classified into four main groups viz. terpenes, benzene derivatives, hydrocarbons and other miscellaneous compounds (NGOH *et al.*, 1998). Terpenes and terpenoids are the most representative molecules constituting 90% of the essential oils and allow a great variety of structures with diverse functions (BAKKALI *et al.*, 2008).

Many of the essential oil components of various chemical groups have also been evaluated for their role in insect pest management programme. *Mentha citrata* oil containing linalool and linalyl acetate exhibits fumigant toxicity to rice weevils (SINGH *et al.*, 1989). Linalool has been demonstrated to act on the nervous system affecting ion transport and the release of acetylcholine esterase in insects (RE *et al.*, 2000). DON-PERDO (1996) has studied effect of citrus peel oils and its components against *Callosobruchus maculatus*. Several compounds including the major component of all citrus peel oils, limonene has been found to be insecticidal. A combined study has established that in artificial mixtures, several pure components of citrus peel oil potentiate their individual fumigant activity (DON-PERDO, 1996). Carvone and menthol are effective as fumigant while 1,8-cineole exhibits both contact and fumigant toxicity

against *Tribolium castaneum* and *Callosobruchus maculatus* (TRIPATHI *et al.*, 2001). LEE *et al.* (2001) have reported toxicity of menthol, methonene, limonene, α-pipene, β-pipene and linalool against *Sitophilus oryzae* and proved that these essential oil components exert its toxicity by inhibiting acetylcholine esterase enzyme. *l*-carvone has been reported to cause more fumigant toxicity than its contact toxicity to *Rhizopertha domestica* (TRIPATHI *et al.*, 2003). *Trans*-anethole, thymol, 1,8-cineole, carvacrol, terpineol and linalool have been evaluated as fumigants against *Tribolium castaneum* but only compound to show significant effect against this insect species is trans-anethole (KOUL *et al.*, 2007).

A comparative study has been conducted to assess contact and fumigant toxicities of monoterpenes viz. camphene, camphor, carvone, 1-8-cineole, cuminal-dehyde, fenchone, geraniol, limonene, linalool, menthol and myrcene on *Sitophilus oryzae* and *Tribolium castaneum*. In fumigant toxicity assays, 1-8-cineole has found most effective against *Sitophilus oryzae* and *Tribolium castaneum*. Structure-toxicity investigations reveal that carvone has the highest contact toxicity against the both insects. *In vitro* inhibition studies of acetylcholine esterase from adults of *Sitophilus oryzae* show that cuminaldehyde inhibits enzyme activity most effectively followed by 1-8-cineole, limonene, and fenchone. 1-8-Cineole is the most potent inhibitor of acetylcholine esterase activity from *Tribolium castaneum* larvae followed by carvone and limonene (ABDELGALEIL *et al.*, 2009).

In the present study, α-pinene and β-caryophyllene have been evaluated for their repellent, acute toxicity and developmental inhibitory activities alone and in binary combination against flour insect pest *Tribolium castaneum*. These two compounds alone caused fumigant toxicity in adults and larvae both. Fumigation with two sublethal concentrations viz. 40 and 80% of 24-h LC_{50} of these two compunds significantly reduced oviposition potential of adults and inhibited pupation and adult emergence in larvae. In reducing the dose of active compunds, target multiple site of action and resistance in insects, synergism can play an important role. Essential oil combinations such as thyme, anise and saffron have been demonstrated for synergistic activity (YOUSSEF, 1997). HUMMELBRUNNER & ISMAN (2001) reported that different combinations of monoterpenes produced synergistic insecticidal effects. Present study indicates that α-Pinene and β-Caryophylene in binary combination shows synergism and reduces the egg laying capacity and inhibits pupation and adult emergence in *T. castaneum*. These earlier reported findings clearly support the result of the present study. The mode of action of these essential oils is yet to be confirmed but it appears that death of the adults, larvae, oviposition inhibition and development inhibition may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism (DON-PERDO, 1989). Rapid action of essential oils or its constituents against insect pests is an indicative of neurotoxic actions (KOSTYUKOVSKY *et al.*, 2002; PRIESLEY *et al.*, 2003; ISMAN *et al.*, 2007). Recent researches have demonstrated the interference of monoterpenes with acetylcholinesterase enzyme activity in insects (ZAPATA & SNAGGHE, 2010). Thus, it can be suggested that fumigants from volatile oils of plant origin could have greater potential in future on the basis of their efficacy, economic value and use in large-scale storage.

Acknowledgements

The author is highly thankful to University Grants Commission, New Delhi for providing financial assistance under Minor Research Project Grant [8-2(136)/2011(MRP/NRCB].

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Received: 25.02.2012
Accepted: 27.03.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 63-71

Crassulacean Acid Metabolism Permutation and Survival of Caralluma Species (Apocynaceae) in Arid Habitats

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Abstract. Several species of the stem succulent *Caralluma* (Apocynaceae) are abundant perennials in arid regions of the Arabian Peninsula. These arid regions have a short wet season with erratic rainfall and are characterized by harsh climatic conditions of high temperature, high evaporation and sand storms. Work presented in this paper aimed at investigating importance of Crassulacean Acid Metabolism (CAM) for survival of three *Caralluma* species in their natural habitat. Investigations involved studying stomatal characteristics, stomatal diffusive conductance, chlorophyll fluorescence, and CAM in three species of *Caralluma*, namely *C. acutangula* (Decne.) N.E.Br., *C. edulis* (Edgew.) Benth. ex Hook.f., and *C. subulata* (Forssk.) Decne. Microscopic examination revealed a pattern of stomatal characteristics typical of CAM plants in these three *Caralluma* species. Results showed that these three *Caralluma* species were obligate CAM plants exhibiting this mode of photosynthesis during both the wet and the dry seasons. Under protracted water stress during the long dry season very low values of stomatal diffusive conductance and dampening of CAM acidification-deacidification cycles denoted the tendency of these three *Caralluma* species to shift from the obligate CAM physiotype to CAM-idling mode. Chlorophyll fluorescence measurements indicated that protracted water stress induced a reduction in Photosystem II (PSII) antenna efficiency and quantum yield in the three studied *Caralluma* species. This reduction of PSII activity occurred in concomitance with a marked rise in non-photochemical quenching of chlorophyll fluorescence denoting operation of non-photochemical energy dissipating mechanisms known to be important for photoprotection of the photosynthetic apparatus.

Key words: Apocynaceae, Crassulacean Acid Metabolism (CAM), CAM-idling, *Caralluma*, chlorophyll fluorescence, diffusive conductance, stomata

Introduction

Plant survival in desert ecosystems is profoundly limited by water availability. Arid regions of the Arabian Peninsula are characterized by high temperature, high evaporation, scarce water, and erratic rainfall. In such arid habitats, leaf succulents reduce their transpirational surface area by seasonal partial leaf shedding (SAYED, 1996; SAYED, 1998; SAYED, 2001a; BOBICH & NORTH, 2009), whereas leafless stem succulents rely on ample water storage capacity, morphoanatomical features, and physiological adaptations (NOBEL, 1988; SAYED, 2001a; MASRAHI *et al*., 2012). Many desert succulents exhibit the CAM pathway of photosynthesis with its unique nocturnal carbon acquisition pattern and beneficial ecophysiological consequences including improved plant water economy (BORNALD *et al*., 2000; SAYED, 2001b; DODD *et al*., 2002; LÜTTGE, 2002). A large body of information exists in the literature on the importance of CAM in stem succulents of the Agavaceae and Cactaceae (NOBEL, 1988; LÜTTGE, 2004; LÜTTGE, 2008). However, very few studies

have dealt with these aspects as survival mechanisms that enable members of the Apocynaceae to survive months of protracted water stress in their natural arid habitats (LANGE *et al*., 1975; WINTER *et al.* 1976; LANGE & ZUBER, 1977). Moreover, many species of the stem succulent *Caralluma* (Apocynaceae) inhabit arid regions of the Arabian Peninsula, of which *C. acutangula*, *C. edulis*, and *C. subulata* are abundant (COLLENETTE, 1999). The present paper aimed at describing the existence and possible permutation of CAM in these three *Caralluma* species by studying stomatal characteristics, stomatal diffusive conductance, and chlorenchyma diurnal acidity changes.

Furthermore, plants with the CAM pathway exhibit different CAM physiotypes including obligate CAM, facultative CAM, CAM-cycling, and CAM-idling (LÜTTGE, 2004; HERRERA, 2009). Of these modes, CAM-idling has early been recognized (OSMOND, 1978; TING, 1985; TING & SIPES, 1985), and is considered to be a very strong permutation of CAM (CUSHMAN, 2001; SAYED, 2001b; CUSHMAN & BORLAND, 2002; DODD *et al*., 2002; OSMOND *et al*., 2008). The CAM-idling mode is characterized by stomatal closure during the entire day and night, no net CO_2 uptake, and acidification fed by internal recycling of nocturnally re-fixed respiratory CO_2 (LÜTTGE, 2004; HERRERA, 2009). During Phase III of CAM that takes place behind closed stomata under high irradiance and high temperature, PSII can become over-energized (NIEWIADOMSKA & BORLAND, 2008), and CAM-idling is thought to invoke reduction of oxidative stress caused by this over-energization (LÜTTGE, 2002). It is thought that under such conditions, CAM-idling plays a photoprotective role by non-radiative excess energy dissipation via the xanthophyll cycle (TALLMAN *et al*., 1997; TALLMAN, 2004; HERRERA, 2009). Earlier studies suggested that operation of CAM coupled to the xanthophyll cycle was at the heart of a photoprotective mechanism operating under severe drought (TALLMAN *et al*., 1997; TALLMAN, 2004). In this context, chlorophyll fluorescence is a subtle reflection of primary reactions of photosynthesis and a useful non-invasive tool that helps reveal stress-induced changes in photosynthetic biophysical processes (BOLHAR-NORDENKAMPF & ÖQUIST, 1993; SAYED, 2003). Therefore, our work also involved using pulse amplitude modulated chlorophyll fluorescence technique to investigate the involvement of CAM-idling in alleviating water stress-induced effects on the photosynthetic machinery.

Materials and methods

Study Site. The study site - southwest of Saudi Arabia (17°19'N; 42°48'E) is characterized by sand-loam soil, high temperature, high irradiance, scarce water, erratic rainfall, and a climate influenced by a tropical maritime air mass (BROWN & JACKSON, 1979; MULLER, 1984; FISHER & MEMBERY, 1998). The wet season is a short three month period (June–August) associated with spells of strong sand storms that add to the harshness of the environment, and the long dry season extends over a period of nine months (MIDDLETON, 1986).

Climatic Conditions. Records of the past 40 years (1970-2010) of mean monthly maximum air temperature, precipitation, and evaporation were obtained cortsey the Ministry of Electricity and Water (Riyadh, Saudi Arabia). Soil temperature was monitored at depth of 10cm using a field digital thermometer (Kestrel 2000, Boothwyn, Philadelphia, USA).

Plant Material. The plant material used in this field study included mature plants of the stem succulents *C. acutangula* (Decne.) N.E.Br., *C. edulis* (Edgew.) Benth. ex Hook.f., and *C. subulata* (Forssk.) Decne.

Measurements. Stomatal density and the percentage of stem area occupied by stomata were determined in stem epidermal strips using an ocular micrometer at 400x mounted on research microscope (Accu-scope 3025 Ergo Tilting Microscope, Nikon, Kingston-Upon-Thames, Surrey, UK). Stomata and stomatal pore were treated as ellipse shape, and hence stomatal area and stomatal pore area were determined using the following equations:

Stomatal Size = π . Ls . Ws

where: Ls, and Ws are stomatal length, and maximum stomatal width, respectively.

Stomatal Pore Size = π . Lp . Wp

where: Lp, and Wp are stomatal pore length, and maximum stomatal pore width, respectively.

Stomatal diffusive conductance and pulse amplitude modulated chlorophyll fluorescence were measured in intact stem using a porometer (AP4, Delta-T Devices, Cambridge, UK), and a chlorophyll fluorescence monitoring system (FMS2, Hansatech Instruments, Norfolk, UK), respectively. Measured chlorophyll fluorescence parameters included Fv/Fm, and Φ PSII, reflecting the efficiency of PSII antenna and the quantum yield of PSII, respectively (BOLHAR-NORDENKAMPF & ÖQUIST, 1993; SAYED, 2003). The value qNP reflecting non-photosynthetic quenching of chlorophyll fluorescence was calculated using standard fluorescence nomenclature (BUSCHMANN, 1999; SAYED, 2003) and the equation:

qNP = (Fm-Fm') / (Fm-Fo)

where:

Fo minimal fluorescence level emitted by antenna chlorophyll molecules,

Fm maximal fluorescence level emitted when all PSII traps become closed,

Fm' light-adapted fluorescence maximum.

Chlorenchyma was separated along parts of the stem length and cell sap was extracted by grinding a known weight of tissue. Cell sap was then expressed through two layers of muslin and diurnal changes in cell sap titratable acidity were determined (OSMOND *et al.*, 1991).

Statistical analyses. All experiments were routinely repeated in samples taken from ten different individuals and the standard deviation was calculated using SPSS v.11.5 software.

Results

Climatic records of the study site indicated that the dry season is a long nine months period and the wet season is a short three months period (Fig. 1).

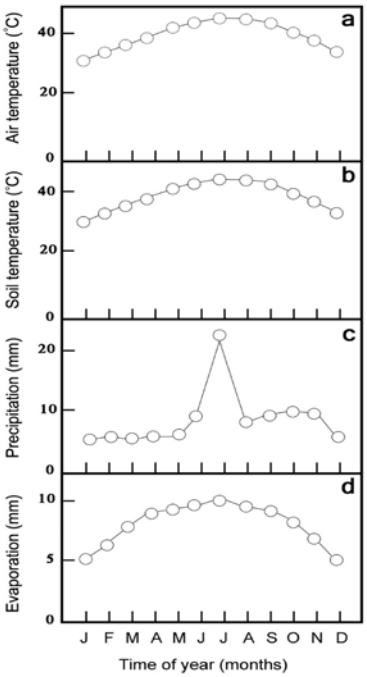

Fig. 1. Climatic records (1970-2010) of the study site.

During the wet season air temperature, soil temperature, and evaporation markedly increase (Fig. 1a, b, d). Climatic records also showed that the total annual rainfall at the study site was in the order of 100 mm occurring mainly during the period June–August (Fig. 1c). Microscopic examination of stomatal characteristics of *C. acutangula*, *C. edulis*, and *C. subulata* were performed on stem epidermal strips and stomatal density, stomatal size, stomatal pore size, and the area of stem occupied by stomata are given in Table 1.

Measurements of stomatal diffusive conductance during the wet season indicated that *C. acutangula*, *C. edulis*, and *C. subulata* exhibited high night-time values in the range of 40-50 mmol m^{-2} s^{-1}), and low day-time values in the range of 5-10 mmol m^{-2} s^{-1} (Fig. 2). On the other hand, measurements during the dry season indicated that *C. acutangula*, *C. edulis*, and *C. subulata* exhibited very low values of stomatal diffusive conductance in the range of 3-5 mmol m^{-2} s^{-1} during both night and day (Fig. 2).

Table 1. Stomatal characteristics of the three studied *Caralluma* species (mean ± standard deviation, n = 10).

Species	Stomatal Density (stomata mm^{-2})	Stomatal Size (µm^2)	Stomatal Pore Size (µm)	Area of Stem Occupied by Stomata (%)
C. acutangula	26 ± 3	829 ± 5	6.0 ± 2	1.4 ± 0.3
C. edulis	25 ± 5	818 ± 2	7.2 ± 1	1.6 ± 0.2
C. subulata	30 ± 8	887 ± 4	6.9 ± 1	1.9 ± 0.4

Table 2. Chlorophyll fluorescence parameters in the three studied *Caralluma* species measured during the wet and the dry seasons (mean ± standard deviation, n = 10).

Species	Chlorophyll Fluorescence Parameters					
	Fv/Fm		ΦPSII		qNP	
	Wet	Dry	Wet	Dry	Wet	Dry
C. acutangula	0.84 ± 0.1	0.78 ± 0.2	0.83 ± 0.1	0.69 ± 0.3	0.02 ± 0.005	0.06 ± 0.008
C. edulis	0.83 ± 0.3	0.74 ± 0.1	0.83 ± 0.4	0.75 ± 0.4	0.01 ± 0.003	0.05 ± 0.005
C. subulata	0.78 ± 0.5	0.67 ± 0.4	0.82 ± 0.5	0.69 ± 0.1	0.01 ± 0.001	0.04 ± 0.003

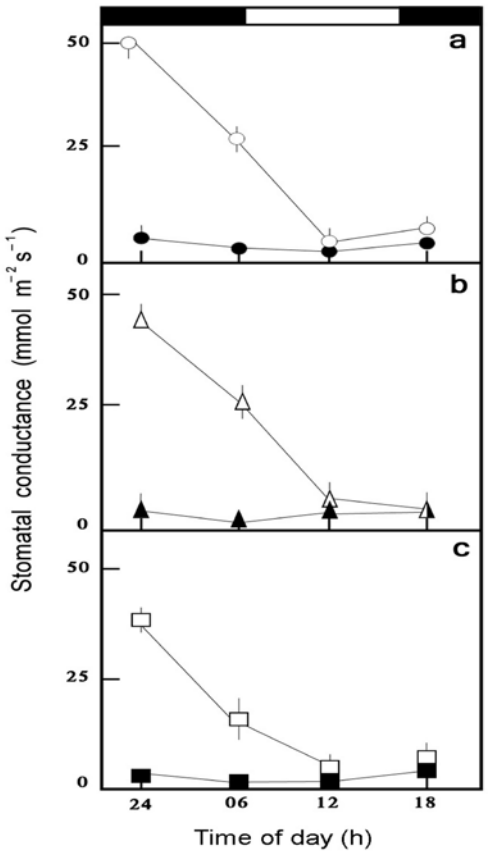

Fig. 2. Diurnal changes (white colour - wet season; black colour - dry season) in stomatal conductance of *C. acutangula* (a), *C. edulis* (b), and *C. subulata* (c). (mean ± standard deviation, n = 10).

Determinations of chlorenchyma cell sap titratable acidity during the wet season indicated that *C. acutangula*, *C. edulis*, and *C. subulata* exhibited diurnal acidity changes (Fig. 3). These changes in chlorenchyma cell sap titratable acidity were markedly dampened during the dry season (Fig. 3). Comparison of chlorophyll fluorescence parameters measured during the wet and the dry seasons indicated that water stress-induced effects in the three studied *Caralluma* species included 10-15% reduction in the value of the parameters Fv/Fm, and ΦPSII occurring in concomitance with a marked increase in the value of the parameter qNP (Table 2).

Discussion

Harsh environmental conditions represent a formidable challenge for plant survival in desert arid habitats. Climatic records reflected the extreme aridity prevailing at the study site manifested by a nine-month-long dry season and a short wet season characterized by scarce water availability, and increased temperature and evaporation (Fig. 1). In such arid habitats, arido-active stem succulents survive periods of protracted drought due to morpho-anatomical and physiological adaptations

that enable them to tolerate harsh environmental conditions (NOBEL, 1988; SAYED, 2001a; SAYED, 2001b; MASRAHI *et al*. 2011; MASRAHI *et al*. 2012). Microscopic studies indicated that *C. acutangula*, *C. edulis*, and *C. subulata* exhibit low values of stomatal density, stomatal size, stomatal pore size, and area of stem occupied by stomata (Table 1). These stomatal features have repeatedly been recognized as characteristic of plants exhibiting the CAM pathway (TING, 1987; HERRERA & CUBEROS, 1990, WILLMER & FRICKER, 1996; CROXDALE, 2000).

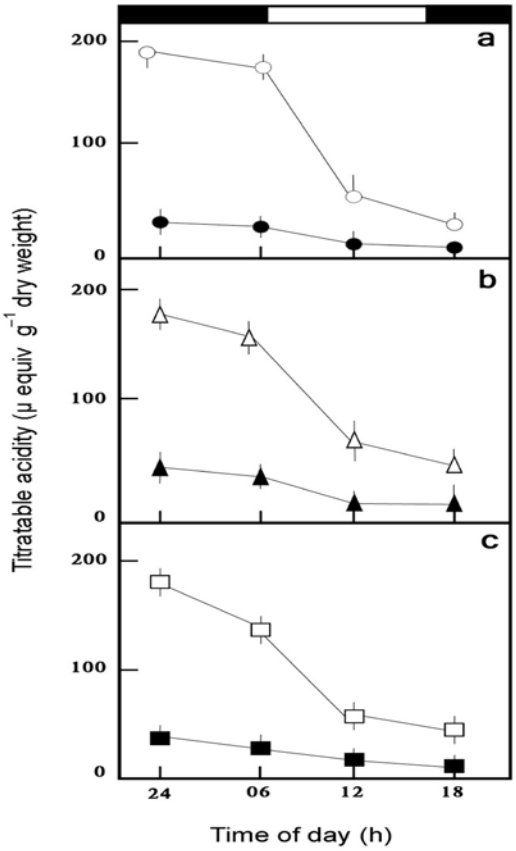

Fig. 3. Diurnal changes (white colour - wet season; black colour - dry season) in chlorenchyma cell sap titratable acidity of *C. acutangula* (a), *C. edulis* (b), and *C. subulata* (c). (mean ± standard deviation, n = 10).

During the wet season, *C. acutangula*, *C. edulis*, and *C. subulata* exhibited values of stomatal diffusive conductance that were high during the night and low during the day (Fig. 2) revealing stomatal behavior typical of plants with the CAM pathway (NOBEL, 1988; SAYED, 2001b; LÜTTGE, 2004; HERRERA, 2009). Determination of stem chlorenchyma cell sap titratable acidity during both the wet and the dry seasons indicated the occurrence of diurnal acidification-deacidification cycles denoting the operation of obligate CAM in the three investigated *Caralluma* species. Obligate CAM has previously been shown in some *Caralluma* species (LANGE *et al*., 1975; WINTER *et al.* 1976, LANGE & ZUBER, 1977; MASRAHI *et al*., 2011; MASRAHI *et al*., 2012). Nocturnal CO_2 uptake and daytime stomatal closure associated with CAM imply avoidance of gas exchange when environmental conditions favor transpirational water loss and enhanced plant water economy (WINTER & SMITH, 1996; BORLAND *et al*., 2000; SAYED, 2001b; LÜTTGE, 2002; LÜTTGE, 2004; SCHULZE *et al*., 2005; LÜTTGE, 2008; HERRERA, 2009). However, during the dry season, the three investigated *Caralluma* species exhibited very low values of stomatal diffusive conductance during the entire day and night (Fig. 2) and markedly dampened diurnal oscillation of chlorenchyma cell sap titratable acidity (Fig. 3). These results indicated that the three investigated *Caralluma* species shifted from the obligate CAM physiotype to CAM-idling in response to protracted water stress. The CAM-idling mode is a strong permutation of CAM that greatly enhances plant water economy by stomatal closure during the entire day and night and sustaining diurnal fluctuations in cell sap titratable acidity by nocturnally re-fixed respiratory CO_2 (SAYED, 2001b; DODD *et al*., 2002; LÜTTGE, 2002; NOBEL & DE LA BARRERA, 2002; LÜTTGE, 2004; LÜTTGE, 2007; HERRERA, 2009).

Comparison of chlorophyll fluorescence parameters measured during the wet and the dry seasons in the three studied *Caralluma* species indicated that drought resulted in reduction of Fv/Fm and ΦPSII (Table 2) denoting reduction of the efficiency of PSII antenna, and PSII quantum yield, respectively (BUSCHMANN, 1999; SAYED, 2003). Similar reduction of PSII activity manifested by reduction of Fv/Fm and ΦPSII has been shown for the CAM plant *Clusia minor* under protracted drought

(MATTOS *et al*., 1999). Reduction of PSII activity under such conditions was attributed to over-energization of PSII during Phase III of CAM that takes place behind closed stomata and under high irradiance and high temperature (MATTOS *et al*., 1999; NIEWIADOMSKA & BORLAND, 2008). However, our observed reduction in PSII activity occurred in concomitance with a markedly increased qNP (Table 2) denoting increased non-photochemical quenching of chlorophyll fluorescence and hence increased non-photochemical excess energy dissipation (BUSCHMANN, 1999; SAYED, 2003). Similar observation of increased qNP was reported in *Clusia minor* performing CAM-idling (LÜTTGE, 2007). Increased non-photochemical energy dissipation under protracted drought was attributed to a relationship between zeaxanthin, a precursor of abscisic acid, and CAM-idling. Inhibition of zeaxanthin accumulation in guard cells of CAM-performing *Mesembryanthemum crystallinum* was suggested to prevent stomatal opening in response to light (TALLMAN *et al*., 1997). Daytime decarboxylation and high rate of respiration due to high temperature implies high intercellular CO_2 concentration that ensures daytime stomatal closure by favouring Calvin cycle activity in guard cell chloroplasts, consumption of NADPH, and prevention of destruction of endogenous guard cell abscisic acid (TALLMAN, 2004). It was also suggested that CAM-idling plays a role in photoprotection by non-radiative excess energy dissipation via the xanthophyll cycle (ROBINSON & OSMOND, 1994; TALLMAN *et al*., 1997; TALLMAN, 2004; HERRERA, 2009). The CAM-idling mode is thought to result in reduction of oxidative stress by processing reactive oxygen species that appear when PSII becomes over-energized during Phase III and hence confer photoprotection under protracted drought (LÜTTGE, 2007; NIEWIADOMSKA & BORLAND, 2008). Similar views on photoprotection associated with CAM were shown upon comparing performance of *Mesembryanthemum crystallinum* plants in the C_3-mode of photosynthesis with those performing CAM. The CAM-performing *M. crystallinum* plants exposed to oxidative conditions of high ozone concentrations showed no signs of oxidative damage in contrast to plants in the C_3-mode which showed necrosis and reduction in Fv/Fm (HURST *et al*., 2004). Moreover, when the CAM-less *M. crystallinum* mutant and the wild type were subjected to salinity, the activities of several isoforms of the enzyme CuZn-superoxide dismutase, used as markers for the production of reactive oxygen species, increased in both genotypes. However, this increase was larger in the mutant indicating a smaller oxidative load in the wild type (BORLAND *et al*., 2006). More recent studies indicated that photoprotection in CAM plants under protracted drought can be attributed to up-regulation of the antioxidative response enzyme CuZn-superoxide dismutase (SILVERA *et al*., 2010).

Conclusions

It can be concluded that the three studied stem succulents *C. acutangula*, *C. edulis*, and *C. sublata* exhibited stomatal characteristics typical of CAM plants. Occurrence of CAM during both the wet and the dry seasons indicated that the three studied *Caralluma* species are obligate CAM plants. The observed low stomatal diffusive conductance during the entire day and night combined with dampened acidification-deacidification cycles indicated that these three species shift from the obligate CAM physiotype to CAM-idling in response to protracted drought during the long dry season. Moreover, under protracted drought, PSII activity in these three *Caralluma* species was slightly reduced due at least in part to oxidative stress during CAM-idling. However, this oxidative stress appears to be somewhat alleviated by operation of photoprotective non-radiative excess energy dissipation as reflected by increased non-photochemical quenching of chlorophyll fluorescence.

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Received: 01.03.2012
Accepted: 24.03.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 73-80

Composition and Structure of Testate Amoebae Fauna (Protozoa: Arcellinida and Euglyphida) in Durankulak Lake (Northeastern Bulgaria)

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Abstract. An ecological study of the testate amoebae fauna in Durankulak Lake (Northeastern Bulgaria) revealed 91 taxa, belonging to 20 genera. The genera *Difflugia* (34 species), *Centropyxis* (14), *Arcella* (6) and *Difflugiella* (6) had the highest species diversity. The species *Trinema enchelys* (31.2% relative abundance), *Euglypha rotunda* (15.1%) and *Microchlamys patella* (6.6%) were present with high population density and were dominants in the lake. The species richness and abundance of testaceans differed considerably among separate localities. By the mean of some basic indices for structure of communities, a characterization of the diversity of testate amoebae was made.

Keywords: Testate amoebae, Durankulak Lake, diversity, frequency, relative abundance

Introduction

Wetlands are one of the greatest natural resources on earth. The function of natural wetlands can be classified by their ecosystem benefits. The United Nations Millennium Ecosystem Assessment and Ramsar Convention found wetlands to be of biosphere significance and societal importance in the following areas: flood control, groundwater replenishment, shoreline stabilisation and storm protection, water purification, reservoirs of bio-diversity, wetland products, cultural values, recreation and tourism, climate change mitigation and adaptation. Wetlands as habitats provide unique living conditions for many plant and animal species. They are characterized by rich taxonomic diversity of organisms, high number of endemic species, and extremely high productivity.

On the other hand, wetlands are among the most endangered places on the planet. They are extremely vulnerable ecosystems - highly sensitive to pollution, changes in water currents, etc. A number of species, whose survival depends mainly on the wetlands, are in danger of extinction.

In the early 20th century wetlands covered about 2000 km² or 2% of the territory of Bulgaria. Most of them have already been drained, heavily modified or converted into fishponds and reservoirs. Today these ecosystems cover only 0.1% of the country and are among the highest priority conservation sites (WETLANDS, 2012).

Durankulak Lake is among the most important and best preserved coastal wetlands in Bulgaria. It is a coastal lake of the firth type, included in the list of the Ramsar Convention as a wetland of international importance for conservation of more than 260 species of endemic, rare and endangered plants and animals.

A number of scientific articles on the biodiversity of the lake have been published up to now (GEORGIEV, 1998), but its testate amoebae fauna have not been an object of

study. The purpose of this paper is to investigate the taxonomic diversity of testate amoebae in Durankulak Lake and to describe the distribution of testaceans at different localities.

Materials and Methods

Study Area. Durankulak Lake is situated in Northeastern Bulgaria to 4-9 km from the Romanian border and 12-17 km north of the town of Shabla (28° 33' 43'' E, 30° 40' 30'' N) (Fig. 1). It is a shallow, covered firth formed at the beginning of the Holocene (about 10 000 years ago). The main hydrographic parameters of Durankulak Lake are given in Table 1 (GEORGIEV, 1998). The lake widens to the north, and this part of it is locally known as the Kartaliysko swamp, separated from the rest of the lake by a narrow grass-covered mud-bank.

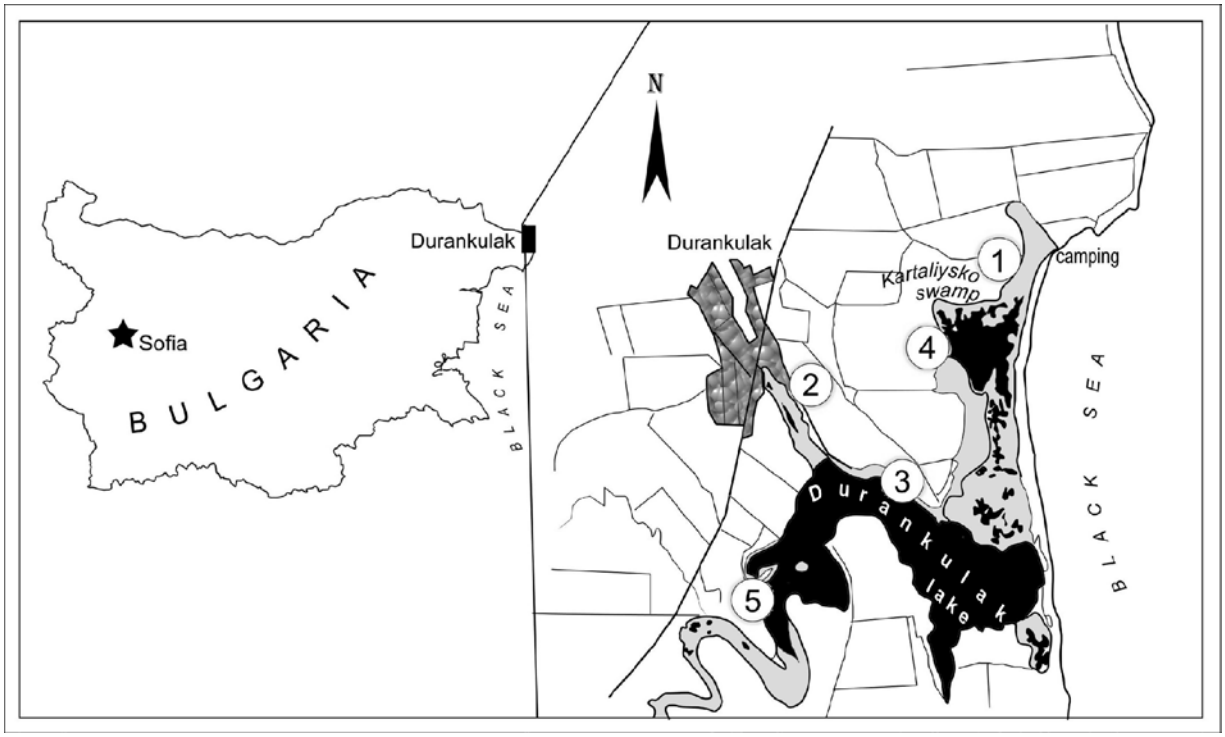

Fig. 1. Map of the study area.

Table 1. Main hydrographic parameters of Durankulak Lake

Parameters	Values
Total area, dec.	4037.4
Open water areas, dec.	2 533.2
Altitude of the water level, from… to…, m a.s.l.	+0.09-+0.60
Depth, m	max. 4.0, average 1.4
Water volume, $m^3 .10^3$	2 500
Main water supply	underground water (80%)
Catchment area, km^2	subterranean - 542

Despite their shared geodesic origin and the marshy area which separates them, both parts of the lake – Durankulak Lake proper and Kartaliysko swamp have a completely different nature and landscape (GEORGIEV, 1998). As for its mineral composition, the water of Durankulak Lake is fresh with high-grade mineralisation. The water in Kartaliysko swamp is brackish, with very high-grade mineralization. The water column in Durankulak Lake is saturated with oxygen from the surface to the bottom (average 86%), whereas oxygen saturation is lower in Kartaliysko swamp,

since it is shallow and closed (average 73%). Heavy ammonium concentration is registered in both parts of the lake, which is typical of hypertrophic water basins. The amount of dissolved and suspended organic matter in Durankulak Lake is normal for an eutrophic lake, whereas the concentration of organic matter in Kartaliysko swamp is considerably higher. Bottom sediments in both reservoirs are formed mainly of clay rich in organic substance – remains of plants and animals. In the northern and south-western parts of the lake there are vast areas of aquatic vegetation, dominated mainly by *Phragmites australis*, *Typha angustifolia*, *Typha latifolia* and *Shoenoplectus triqueter*.

Sampling and sample analysis. Thirty samples of bottom sediments from the littoral zone of the Durankulak Lake were collected in 2010. The lake was visited six times during the year (in January, February, March, August, November and December) and each time samples were collected from five permanent localities, including the open water area and the "branches" of the lake (Fig.1). Station 1 is located at a longer distance from the main open water area, in the northern part of Kartaliysko swamp. This part of the lake is covered with reed and other aquatic vegetation almost completely; its water level is low - less than 1 m, and often dries up. Station 2 is located at the so-called "Village branch", partially covered with aquatic vegetation, and its water level is 1-2 m. Station 3 is in the open water area, with little vegetation and water level of about 4 m. Station 4 is situated in Kartaliysko swamp, with an overgrowth of aquatic vegetation and the water level of 1-2 m. Station 5 is located near the Big Island, at the beginning of "Vaklinski branch" and is overgrown with aquatic vegetation in certain places. The water level in it is about 2m.

Half of each sample was fixed *in situ* with 4% formaldehyde. The other half was kept alive for investigation in vivo. For each sample, 0.1 cm^3 of the sediment was taken and studied after homogenization. The number of the species found in it was calculated in specimens/cm^3.

Data analysis. The frequency of occurrence of the particular species was calculated using the formula: pF = m/n x 100, where m is the number of samples in which one species was found and n is the total number of samples. Different species, depending on pF index, were divided into 3 categories as follows: constant - found in more than 50% of all samples; incidental - found in 25-50% of all samples; accidental - found in less than 25% of all samples (BODENHEIMER, 1955).

The relative abundance of each species was used to determine the dominant structure of testacean communities. The dominance was calculated by the formula: D = n/N x 100, where n is the number of the specimens of every species and N is the total number of all specimens. All species were divided into 4 groups, according to the 4-grade classification of TISCHLER (1955): subrecedent – with relative significance < 1%; recedent – with relative significance 1-2%; subdominant – with relative significance 2-5%; dominant – with relative significance > 5%.

The following indices were used to analyse the structure of the testacean communities and to asses the environmental conditions in the lake (BONNET, 1964; ODUM, 1981):

- Simpson's index (C) for concentration of domination, calculated by the formula: $C = \Sigma (n_i / N)^2$, where n_i is the assessment of significance of every species (number of specimens); N – total assessment of significance (total number of specimens).

- Margalef index (d) of species variety, calculated by the formula: $d = S - 1 / \ln N$, where S is the number of species; N – total number of specimens.

- Fisher-William´s index (α) for species diversity, calculated by the formula: $S = \alpha \ln (1 + N / \alpha)$, where S is the number of species; N – total number of specimens.

Statistical analysis was carried out using software package BIODIV 5.1 (BAEV & PENEV, 1995) and STATISTICA 6 (STATSOFT INC., 2000).

Results and Discussion

Altogether 91 species, varieties and forms from 20 genera were identified (Table 2). The highest species diversity was in the

genera *Difflugia* (34 species), *Centropyxis* (14), *Arcella* (6) and *Difflugiella* (6). About a half (9) of the 20 established genera were represented by 1 species only. The results showed that aquatic testacean fauna in Durankulak Lake is comparatively rich and varied. The species composition of the testate amoebae is typical to the freshwater reservoirs (SCHÖNBORN, 1962, 1965; MORACZEWSKI, 1965, 1967; GOLEMANSKY, 1968; TODOROV & GOLEMANSKY, 1998, 2000; NICHOLLS, 2005; MAZEY & TSYGANOV, 2006; DAVIDOVA, 2010).

Seventeen species or 18.7% were established in all investigated stations in the lake – *Arcella hemisphaerica, Centropyxis aculeata*, *C. ecornis, C. platystoma, Cyclopyxis eurystoma, Difflugia gramen, D. pristis, Difflugiella angusta, D. oviformis, Euglypha rotunda, Microchlamys patella, Phryganella hemisphaerica, Plagiopyxis declivis, Psammonobiotus linearis*, *Tracheleuglypha*

Table 2. List of taxa, their relative significance (D) and frequency of occurrence (pF) in different stations of Durankulak Lake.

Stations / Taxa	1		2		3		4		5	
	D	pF	D	pF	D	pF	D	pF	D	pF
Arcella *discoides* EHRENBERG, 1843	–	–	0.1	50.0	0.1	16.7	–	–	–	–
A disc. v. scutelliformis PLAYFAIR, 1917	–	–	0.4	50.0	–	–	–	–	1.7	16.7
A. gibbosa v. mitriformis DEFLANDRE, 1928	–	–	0.2	33.3	–	–	–	–	–	–
A. hemisphaerica PERTY, 1852	0.4	33.3	1.1	33.3	4.4	33.3	0.1	16.7	0.1	33.3
A. hem. f. undulata DEFLANDRE, 1928	–	–	–	–	–	–	0.1	16.7	–	–
A. rotundata PLAYFAIR, 1917	–	–	0.1	16.7	–	–	–	–	–	–
Centropyxis *aculeata* (EHRENBERG, 1830) STEIN, 1857	0.5	33.3	0.4	50.0	0.6	33.3	2.7	66.7	1.1	50.0
C. aerophila DEFLANDRE, 1929	0.9	50.0	–	–	0.1	16.7	1.4	66.7	0.1	16.7
C. aerophila v. sphagnicola DEFLANDRE, 1929	0.7	16.7	0.1	16.7	–	–	0.1	16.7	0.1	16.7
C. cassis (WALLICH, 1864) DEFLANDRE, 1929	0.7	16.7	–	–	0.2	33.3	0.2	16.7	–	–
C. cassis v. spinifera PLAYFAIR, 1917	–	–	–	–	1.1	33.3	0.4	33.3	–	–
C. constricta (EHRENBERG, 1841) DEFLANDRE, 1929	0.4	16.7	–	–	–	–	0.2	16.7	–	–
C. delicatula PENARD, 1902	–	–	0.9	33.3	–	–	0.2	33.3	0.2	33.3
C. ecornis (EHRENBERG, 1841) LEIDY, 1879	0.2	16.7	0.2	50.0	0.2	33.3	0.2	33.3	0.1	16.7
C. elongata (PENARD, 1890) THOMAS, 1959	0.2	16.7	–	–	–	–	–	–	–	–
C. hirsuta DEFLANDRE, 1929	–	–	–	–	0.3	33.3	0.2	33.3	0.7	33.3
C. laevigata PENARD, 1890	0.2	16.7	–	–	0.1	16.7	–	–	–	–
C. minuta DEFLANDRE, 1929	–	–	–	–	0.1	16.7	–	–	0.1	16.7
C. platystoma (PENARD, 1890) DEFLANDRE, 1929	0.2	16.7	0.9	50.0	0.7	33.3	4.0	16.7	1.0	33.3
C. sylvatica (DEFL., 1929) BONNET & THOMAS, 1955	–	–	0.1	16.7	–	–	0.1	16.7	–	–
Corythionella *georgiana* NICHOLLS, 2005	–	–	0.1	16.7	5.3	16.7	9.5	50.0	1.2	33.3
Cryptodifflugia *compressa* PENARD, 1902	–	–	0.1	16.7	–	–	–	–	–	–
Cyclopyxis *eurystoma* DEFLANDRE, 1929	1.3	50.0	0.7	66.7	0.2	33.3	1.5	66.7	0.2	16.7
C. kahli DEFLANDRE, 1929	–	–	0.1	16.7	–	–	–	–	–	–
Cyphoderia *ampulla* (EHRENBERG, 1841) LEIDY, 1870	–	–	–	–	0.1	16.7	22.0	33.3	–	–
Difflugia *acuminata* EHRENBERG, 1838	–	–	0.1	16.7	–	–	–	–	–	–
D. acutissima DEFLANDRE, 1931	–	–	–	–	0.1	16.7	–	–	–	–
D. ampullula PLAYFAIR, 1918	0.2	16.7	0.1	16.7	–	–	0.2	16.7	0.1	16.7
D. brevicola CASH, 1909	–	–	0.1	16.7	–	–	–	–	–	–
D. cylindrus (THOMAS, 1954) OGDEN, 1983	–	–	–	–	–	–	0.1	16.7	–	–
D. decloitrei GODEANU, 1972	–	–	–	–	–	–	–	–	0.1	16.7
D. difficilis THOMAS, 1954	0.2	16.7	–	–	–	–	0.1	16.7	0.1	16.7
D. dragana OGDEN & ZIVKOVIC, 1983	–	–	0.1	16.7	–	–	–	–	–	–
D. elegans PENARD, 1890	–	–	–	–	0.1	16.7	–	–	–	–
D. glans PENARD, 1902	–	–	0.1	16.7	–	–	0.1	16.7	10.4	16.7
D. globularis (WALLICH, 1864) LEIDY, 1877	0.4	16.7	0.2	33.3	–	–	0.2	16.7	–	–

D. globulosa DUJARDIN, 1837	–	–	0.1	33.3	–	–	–	–	–	–
D. gramen PENARD, 1902	0.5	16.7	0.2	33.3	0.3	16.7	0.3	33.3	4.5	33.3
D. lacustris (PENARD, 1899) OGDEN, 1983	0.7	16.7	1.0	33.3	0.3	33.3	–	–	–	–
D. lanceolata PENARD, 1890	0.5	16.7	0.5	33.3	–	–	–	–	–	–
D. levanderi PLAYFAIR, 1918	0.5	16.7	0.6	50.0	–	–	–	–	–	–
D. lithophilla (PENARD, 1902) G.-L. & THOMAS, 1958	–	–	–	–	0.2	16.7	0.1	16.7	–	–
D. lobostoma LEIDY, 1879	–	–	–	–	–	–	0.1	16.7	0.1	16.7
D. lucida PENARD, 1890	–	–	0.2	16.7	–	–	0.5	33.3	–	–
D. manicata PENARD, 1902	0.4	33.3	0.3	33.3	0.1	16.7	–	–	0.6	50.0
D. microstoma (THOMAS, 1954) OGDEN, 1983	–	–	–	–	–	–	0.3	16.7	1.3	33.3
D. minuta RAMPI, 1950	–	–	0.1	16.7	11.0	33.3	–	–	1.7	33.3
D. minuta v. grandis (RAMPI, 1950) G.-L. & THOMAS, 1958	–	–	0.1	16.7	–	–	–	–	–	–
D. oblonga EHRENBERG, 1831	–	–	0.1	16.7	–	–	–	–	–	–
D. parva (THOMAS, 1954) OGDEN, 1983	–	–	–	–	–	–	–	–	0.1	16.7
D. pauli OGDEN, 1983	–	–	0.1	33.3	–	–	–	–	–	–
D. petricola CASH, 1909	0.2	16.7	0.1	16.7	–	–	–	–	–	–
D. pristis PENARD, 1902	0.9	33.3	0.1	33.3	3.6	33.3	0.4	16.7	1.9	50.0
D. pulex PENARD, 1902	–	–	0.3	33.3	4.8	66.7	4.8	50.0	3.0	50.0
D. sarissa LI SUN TAÏ, 1931	–	–	0.1	16.7	–	–	–	–	–	–
D. stoutii OGDEN, 1983	–	–	0.1	16.7	–	–	–	–	0.1	16.7
D. szczepanskii SCHÖNBORN, 1965	0.5	33.3	–	–	–	–	–	–	–	–
D. ventricosa DEFLANDRE, 1926	–	–	0.2	16.7	0.1	16.7	–	–	0.1	16.7
D. venusta (PENARD, 1902) OGDEN, 1983	–	–	0.1	16.7	–	–	–	–	–	–
Difflugiella *angusta* SCHÖNBORN, 1965	1.1	16.7	0.2	16.7	0.5	33.3	0.1	16.7	2.0	33.3
D. horrida SCHÖNBORN, 1965	–	–	0.2	33.3	1.0	33.3	1.5	16.7	0.2	16.7
D. oviformis BONNET & THOMAS, 1955	1.3	16.7	0.8	50.0	1.3	33.3	3.8	50.0	0.9	16.7
D. ovif. v. fusca (PENARD, 1890) BONNET &THOMAS, 1955	2.4	16.7	–	–	–	–	–	–	–	–
D. patinata SCHÖNBORN, 1965	–	–	–	–	0.4	16.7	–	–	–	–
D. pusilla PLAYFAIR, 1918	–	–	0.2	33.3	1.0	50.0	1.0	33.3	–	–
Euglypha *acanthophora* (EHRENBERG, 1841) PERTY, 1849	–	–	8.8	16.7	0.3	16.7	0.1	16.7	–	–
E. filifera PENARD, 1890	–	–	–	–	–	–	1.2	16.7	–	–
E. rotunda WAILES & PENARD, 1911	5.5	66.7	27.8	83.3	8.0	50.0	6.4	66.7	12.1	50.0
E. tuberculata DUJARDIN, 1841	–	–	5.0	66.7	4.6	50.0	2.2	66.7	4.5	33.3
E. tuberculata v. minor TARANEK, 1881	3.6	16.7	–	–	–	–	–	–	–	–
Euglyphella *delicatula* VALKANOV, 1962	–	–	0.1	16.7	–	–	–	–	–	–
Microchlamys *patella* (CLAP. & LACH., 1885) COCKERELL, 1911	5.1	83.3	7.4	100.0	8.2	66.7	5.0	83.3	5.8	100.0
Paraquadrula *irregularis* (ARCHER, 1877) DEFLANDRE, 1932	–	–	0.1	16.7	–	–	–	–	–	–
Phryganella *hemisphaerica* PENARD, 1902	1.3	33.3	0.2	33.3	0.6	50.0	1.3	50.0	0.5	66.7
Phr. paradoxa PENARD, 1902	–	–	0.5	33.3	–	–	–	–	–	–
Plagiopyxis *declivis* THOMAS, 1955	3.1	50.0	1.4	50.0	1.7	33.3	0.3	33.3	0.7	66.7
Pl. minuta BONNET, 1959	0.9	33.3	0.5	33.3	0.1	16.7	–	–	0.1	16.7
Psammonobiotus *linearis* GOLEMANSKY, 1970	0.9	16.7	0.3	33.3	7.9	50.0	2.5	33.3	2.9	33.3
Pseudodifflugia *compressa* SCHULZE, 1874	0.2	16.7	–	–	0.1	16.7	0.2	33.3	–	–
Ps. fascicularis PENARD, 1902	–	–	0.1	16.7	–	–	–	–	–	–
Ps. fulva ARCHER, 1870	–	–	0.1	16.7	–	–	–	–	–	–
Ps. gracilis SCHLUMBERGER, 1845	–	–	–	–	0.1	16.7	–	–	–	–
Schaudinnula *arcelloides* AWERINTZEW, 1907	–	–	–	–	–	–	0.1	16.7	–	–
Schonbornia viscicula SCHÖNBORN, 1964	–	–	–	–	–	–	–	–	0.1	16.7
Tracheleuglypha *acolla* BONNET & THOMAS, 1955	0.2	16.7	0.8	66.7	1.7	50.0	3.0	83.3	3.0	50.0
Tracheleuglypha dentata DEFLANDRE, 1938	–	–	0.9	50.0	1.4	66.7	–	–	1.2	66.7
Trinema *complanatum* PENARD, 1890	0.5	33.3	–	–	–	–	–	–	–	–
Tr. enchelys (EHRENBERG, 1838) LEIDY, 1878	60.1	100	32.0	100	23.8	100	15.5	83.3	35.2	83.3
Tr. lineare PENARD, 1890	1.6	83.3	2.0	50.0	2.6	50.0	5.0	50.0	–	–
Tr. lineare v. truncatum CHARDEZ, 1964	1.3	16.7	0.1	16.7	0.6	16.7	0.7	33.3	0.1	16.7
Tr. penardi THOMAS & CHARDEZ, 1958	0.2	16.7	–	–	–	–	–	–	–	–
91	**39**		**61**		**44**		**46**		**41**	

acolla, Trinema enchelys and *Tr. lineare v. truncatum*. Of them three species were present with high population density and were dominants in all stations. These are *Trinema enchelys* (31.2% relative abundance), *Euglypha rotunda* (15.1%) and *Microchlamys patella* (6.6%), together made up 52.9% of the total count. One species - *Psammonobiotus linearis* appeared as dominant only in one of the studied stations. The other widespread species were established with a small number of specimens and in terms of their relative abundance, belong to the group of the subrecedent, recedent and subdominant respectively. Thirty-five or 38.5% of the species were found in only one of studied stations, as the most of them (91.2%) had relative significance below 1%. Almost a half of them - 47.1% belong to the genus *Difflugia*.

The species richness and abundance of testaceans differed considerably among separate stations. The number of species per sample varied from eight to thirty-one. The lowest number of species – 39 was observed in station 1 (from 9 to 14 species per sample, on average 11.8 species per sample). In the stations 3, 4 and 5 were established the similar species diversity – in them were found 44, 46 and 41 species respectively, on average 13.7, 14.3 and 13 species per sample, but in station 4 the number of species varies greatly - from 8 to 24 species per sample and in the other stations this number remains relatively constant - from 8 to 18 and from 9 to 15 species per sample. The highest number of species and the greatest variability in species richness is typical for station 2 – 61 (from 13 to 31 species per sample, on average 18.8 species per sample) (Fig. 2A).

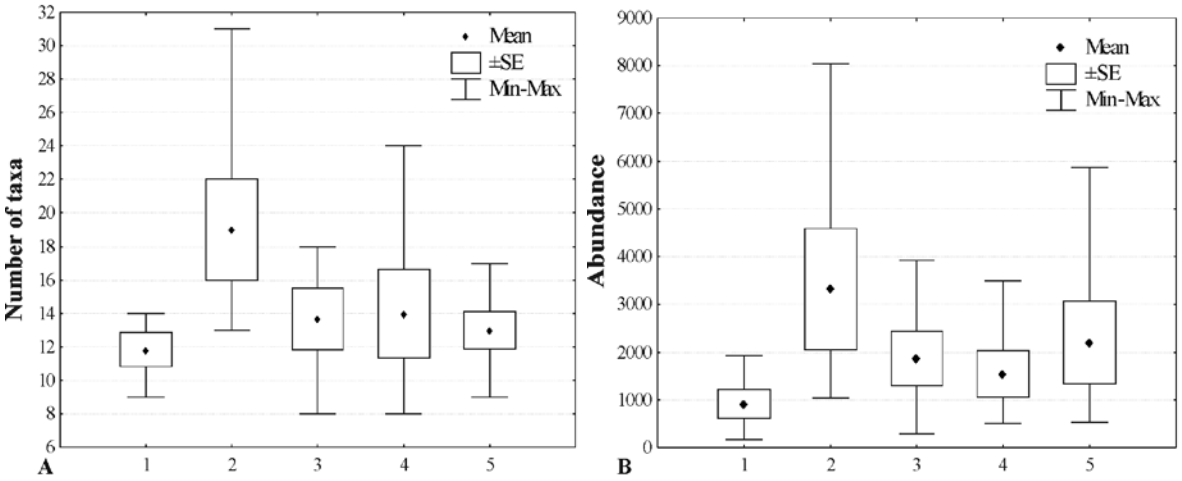

Fig. 2. Box & Whiskers plots comparing the species richness (A) and abundance (B) in studied stations in Durankulak Lake

The lowest testacean abundance was found in station 1 with average 915 specimens/cm^3 (minimum 170 and maximum 1930 specimens/cm^3). The highest value for testacean abundance is characteristic for station 2 – 3323.6 specimens/cm^3 on average (minimum 1030 and maximum of 8040 specimens/cm^3). The communities from the other stations are of medium abundance (average values of specimens per cm^3 are 1541 for station 4, 1868 for station 3 and 2200 for station 5) (Fig. 2B).

The results of the study show that both species diversity and abundance are lowest in station 1. The possible reason for this is that station is located in the utmost northern part of the lake (Kartaliysko swamp, see Study area), which is characterized by relatively unfavorable conditions.

The analysis of the frequency of occurrence and relative abundance revealed that there are essential differences also in the occurrence and dominance of the species in studied stations (Table 2).

In station 1 three species - *Euglypha rotunda* (5.5% relative abundance, 66.7% frequency of occurrence), *Microchlamys patella* (5.1%, 83.3%) and *Trinema enchelys* (60.1%, 100%) were both dominants and constants. One of them - *Tr. enchelys* had an exclusively high relative abundance of 60.1%. Other four species were established with high frequency, but were found in single specimens and have low relative significance.These are *Centropyxis aerophila* (0.9%, 50.0%), *Cyclopyxis eurystoma* (1.3%, 50.0%), *Plagiopyxis declivis* (3.1%, 50.0%) and *Trinema lineare* (1.6%, 83.3%).

Five of the species in station 2 were dominants with a relative abundance of more than 5%. According to their abundance they are: *Trinema enchelys* (32.0%), *Euglypha rotunda* (27.8%), *Euglypha acanthophora* (8.8%), *Microchlamys patella* (7.4%) and *E. tuberculata* (5.0%). Sixteen testaceans are widespread and appear as constant species here. These are the dominants *Tr. enchelys* (100% frequency), *M. patella* (100%), *E. rotunda* (83.3%) and *E. tuberculata* (66.7%), as well as the species *Cyclopyxis eurystoma* (66.7%), *Tracheleuglypha acolla* (66.7%), *Arcella discoides* (50.0%), *A. discoides v. scutelliformis* (50.0%), *Centropyxis aculeata* (50.0%), *C. ecornis* (50.0%), *C. platystoma* (50.0%), *Difflugia levanderi* (50.0%), *Difflugiella oviformis* (50.0%), *Plagiopyxis declivis* (50.0%), *Tracheleuglypha dentata* (50.0%) and *Trinema lineare* (50.0%).

Table 3. Indices for structure of the communities in different stations in the Durankulak Lake.

Index	Stations				
	1	2	3	4	5
d	6.03	7.90	6.12	6.59	5.57
α	9.62	11.9	9.13	10.2	8.03
C	0.38	0.19	0.10	0.09	0.16

Six of the established in station 3 testate amoebae were dominants - *Tr. enchelys* (23.8%), *Difflugia minuta* (11.0%), *M. patella* (8.2%), *E. rotunda* (8.0%), *Psammonobiotus linearis* (7.9%) and *Corythionella georgiana* (5.3%). Their relative share is 64.2% of all the established individuals. According to the occurrence one can see that four of the dominants namely *Tr. enchelys*, *M. patella, E. rotunda* and *Ps. linearis*, as well as other seven species – *Difflugia pulex*, *Difflugiella pusilla*, *E. tuberculata*, *Phryganella hemisphaerica*, *Tracheleuglypha acolla*, *Tr. dentata* and *Tr. lineare*, are the most frequently occurring species.

Six dominant species were found in station 4: *Cyphoderia ampulla* (22.0%), *Tr. enchelys* (15.5%), *Corythionella georgiana* (9.5%), *E. rotunda* (6.4%), *M. patella* (5.0%) and *Tr. lineare* (5.0%). Thirteen of the species found here have frequency of occurrence higher than 50% and belong to the group of the constant ones. These are: *M. patella* (83.3%), *Tr. acolla* (83.3%), *Tr. enchelys* (83.3%), *Centropyxis aculeata* (66.7%), *C. aerophila* (66.7%), *Cyclopyxis eurystoma* (66.7%), *E. rotunda* (66.7%), *E. tuberculata* (66.7%), *Corythionella georgiana* (50.0%), *Difflugia pulex* (50.0%), *Difflugiella oviformis* (50.0%), *Phr. hemisphaerica* (50.0%) and *Tr. lineare* (50.0%).

Only 4 species were dominants in station 5. Three of them namely *Tr. enchelys* (35.2%, 83.3%), *M. patella* (5.8%, 100%) and *E. rotunda* (12.1%, 50.0%) were frequently occurring and appear as constant species too. It should be noted that these species have high relative abundance and frequency in all other stations. The dominant *Difflugia glans* (10.4%, 16.7%) had a comparatively high relative abundance here, but it was established in only one of the studied samples and belong to the group of the accidental species. In the other stations of the lake it is not established or found with small number of individuals. The relative share of the four dominant species is 63.6% of all individuals. Other eight species were established with high frequency – *Phr. hemisphaerica* (66.7%), *Pl. declivis* (66.7%), *Tr. dentata* (66.7%), *C. aculeata* (50.0%), *Difflugia manicata* (50.0%), *D. pristis* (50.0%), *D. pulex* (50.0%) and *Tr. acolla* (50.0%).

The received results about distribution of testate amoebae in the lake are confirmed of the values for indices used to analyse the structure of testacean communities, presented on Table 3. Maximal species

diversity estimated by Margalef (d) and Fisher`s (α) diversity index was measured in station 2. In the other stations, the values of diversity indices are lower. The index for concentration of domination (C) have the lowest value in stations 3 and 4, where dominance is shared between more species. The index C is considerably higher for station 1, where only one species were presented with great number of specimens – *Trinema enchelys*, while the rest are with considerably less abundance (Table 2).

Acknowledgements. This study was carried out under a project № RD-07-336 / 2011 funded by the University of Shumen.

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Received: 03.03.2012
Accepted: 14.04.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 81-85

Preliminary Data on Age Estimation and Body Size of the Dwarf Lizard, Parvilacerta parva (Boulenger, 1887) (Reptilia: Lacertilia) from Akşehir, Konya (Turkey)

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Abstract. In this study, age determination was done by using the skeletochronology method on Akşehir, Konya (Turkey), 14 (5♂♂; 9♀♀) *Parvilacerta parva* specimens. Cross-sections of femurs were examined in total 14 individuals, the lowest number of LAGs was seen in one female and one male individuals as 4, the highest number of LAGs were seen in two female individuals as 8. Average SVL was found 50.8 mm (SD=2.27) in male individuals, and 53.1 mm (SD = 3.27) in females. For all the samples, the age-length equation was calculated as SVL (mm) = 37.82 + (2.47 * age). As a result of Pearson correlation analysis, a significant positive correlation (r=0.93, P<0.01) between age and SVL. Pileus length does not increase constantly with age (r=0.007, P=0.98), while pileus width increases normally together with age (r=0.212, P=0.46).

Key words: Skletochronology, *Parvilacerta parva*, lacertidae, LAGs, Turkey.

Introduction

Parvilacerta parva, which was defined as *Lacerta parva* in 1887 by BOULENGER; HARRIS *et al.* (1998) was added to a new genus different from *Lacerta*, MÜLLER (2002) as referred to the name *Parvilacerta* used as a subgenus. *Parvilacerta parva* is distributed in a large part of Anatolia as well as Armenia and the north-west of Iran in the east (IUCN, 2009). These species is named 'dwarf lizard' as its total length is shorter than the other Lacertid lizards.

Various methods are used for the age estimation of animals. The most useful methods among these are nature tracking and mark-recapture (DURHAM & BENETT, 1963). But this method has disadvantages such as requiring too much effort and long time to reach the results. In many other methods, growth frequency, lens thickness, tooth abrasion, gonad formation, isotropic rate and morphometric data of phased-developed bone tissue and other hard tissues are being used (CASTANET *et al.*, 1993).

The skeletochronology method is based on counting the lines of growth (LAGs) in cross-sections of the long bone diaphysis such as femur, humerus (CASTANET *et al.*, 1993). In this method, growth marks (GM) are shaped by various internal and environmental factors. According to these factors, growth marks in bones can be examined in three parts as opaque zone, translucent zone (annuli) and the lines of arrested growth (LAGs). LAGs show that growth in bone has stopped temporarily (SMIRINA *et al.*, 1986).

Skeletochronolgy method is commonly used for age estimation of amphibians and reptiles (SMIRINA, 1974, 1986; CASTANET & SMIRINA, 1990; CASTANET *et al.*, 1993;

CASTANET, 1994; SNOVER & RHODIN; 2008; AVENS *et al.*, 2009; GUARINO *et al.*, 2010). By using this technique, it is possible to gather information about the age of individuals, longevity, sexual maturity age and activity period of the species (KLEINENBERG & SMIRINA, 1969; CASTANET & SMIRINA, 1990; CASTANET *et al.*, 1993; CASTANET, 1994; SMIRINA, 1994; MIAUD *et al.*, 1999).

In Turkey, skeletochronology studies are done generally on amphibians (OLGUN *et al.*, 2001; OLGUN *et al.*, 2005; GUARINO & ERIŞMIŞ, 2008; ÜZÜM, 2009; ÜZÜM & OLGUN, 2009; ÜZÜM *et al.*, 2011). Studies of the age structure of lizard populations are very rare. So far there are no studies on the age structure of *P. parva*. This study aims to calculate the age of *P. parva* specimens collected from Akşehir (Konya) by using the skeletochronology and then examine the relationship between the age and the total body length.

Material and methods

In the current study, 14 *P. parva* (5 ♂♂; 9 ♀♀) samples were used, which were collected from Akşehir (Konya). The material deposited in the collection of Faculty of Sciences and Arts, Çanakkale Onsekiz Mart University and incorporated into the collection of ZDEU-ÇOMU (Zoology Department Ege University-Çanakkale Onsekiz Mart University), Turkey. Total body length, pileus length and pileus width were measured by using a digital caliper (Mitutoyo, CD-20 CPX) with an accuracy of 0.01 mm. For the age estimation, right femurs of the individuals were removed and they were cleaned of muscles. Then the bones were decalcified in 5% nitric acid for 3-5 hours depending on bone thickness. Decalcified femurs were embedded in paraffin; cross-sections of 10 µm were stained with Ehrlich's hematoxylin and examined under a light microscope.

Kruskal Wallis test was used to understand the relationship between age and total body length. Furthermore, age-SVL relationships were tested by linear regression and Pearson rank order correlation coefficient. Statistical analyses were performed; SPSS (vers. 16.0) and alpha set 0.05.

Results and Discussion

According to morphological measurements, SVL of males varies between 46.92-52.51 mm, and the average SVL was calculated as 50.81 mm (SD=2.27). Regarding females, SVL is between 47.98-58.62 and average SVL is 53.11 mm (SD=3.27) (Table 1). MÜLAYIM *et al.* (2001), reported that there is a statistically important difference in SVL, pileus length, pileus width between female and male individuals of *P. parva* samples collected from Beyşehir in 2001. In our study, it is observed that the male individuals have bigger average pileus length and width than females, while female individuals were bigger in average SVL than males.

Age estimation was done for 14 (5♂♂; 9♀♀) individuals from Akşehir (Konya) population samples. In cross-sections of the femur diaphysis of *P. parva*, 4-8 LAGs were counted (Fig. 1). When females and males are evaluated together, it is seen that number of LAGs varies between 4 and 8. 6 LAGs were seen among 8 individuals in total (Fig.2).

Table 1. Snout-vent length, pileus length and pileus width of males and females *P. parva* (SVL: Snout-Vent Lenght; PL: Pileus Length; PW: Pileus Width; SE: Standard Error; SD: Standard Deviation).

	n	Min	Max	Mean	SE	SD
SVL ♂♂	5	46.92	52.51	50.81	1.016	2.272
SVL ♀♀	9	47.98	58.62	53.11	1.091	3.274
PL ♂♂	5	10.64	11.70	11.28	0.181	0.404
PL ♀♀	9	10.18	12.15	11.05	0.212	0.636
PW ♂♂	5	5.30	5.90	5.66	0.106	0.237
PW ♀♀	9	5.31	6.20	5.59	0.094	0.282

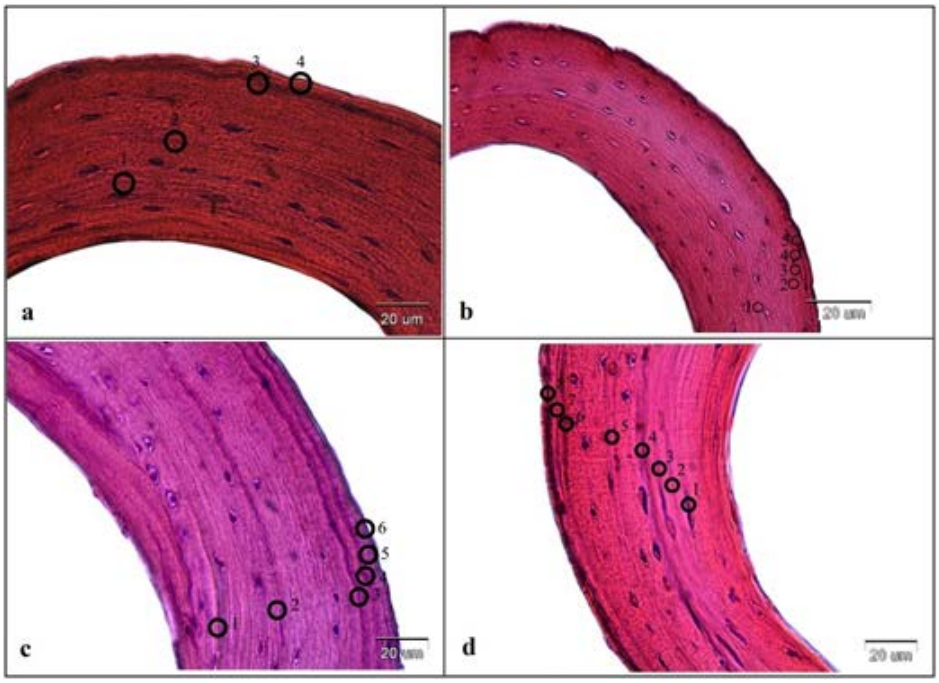

Fig. 1. Cross-sections of the femur diaphysis of adult *P. parva* specimens (a: Male 46.92 mm SVL with 4 LAGs; b: Female 50.15 mm SVL with 5 LAGs; c: Female 51.75 mm SVL with 6 LAGs; d: Female 57.12 mm SVL with 8 LAGs).

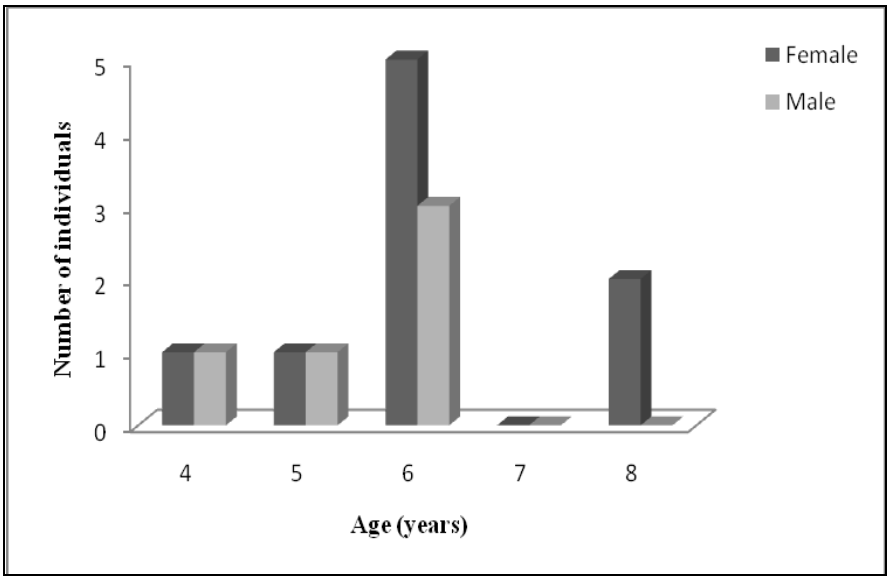

Fig. 2. Frequencies (number of individuals) by age class of *P. parva* in males and females.

Age and SVL relationships are examined in the samples which were used in the study. As a result of Kruskal-Wallis test, the difference between age and SVL was significant (P=0.032). Age-SVL equation is calculated as SVL=37.82 + (2.47 * age). Pearson correlation coefficient is calculated as r=0.93 between age (years)-SVL. In addition, as a result of linear regression analysis, a strong positive relationship between age and SVL is observed (ANOVA: F=84.77; df=1; P<0.00) (Fig. 3).

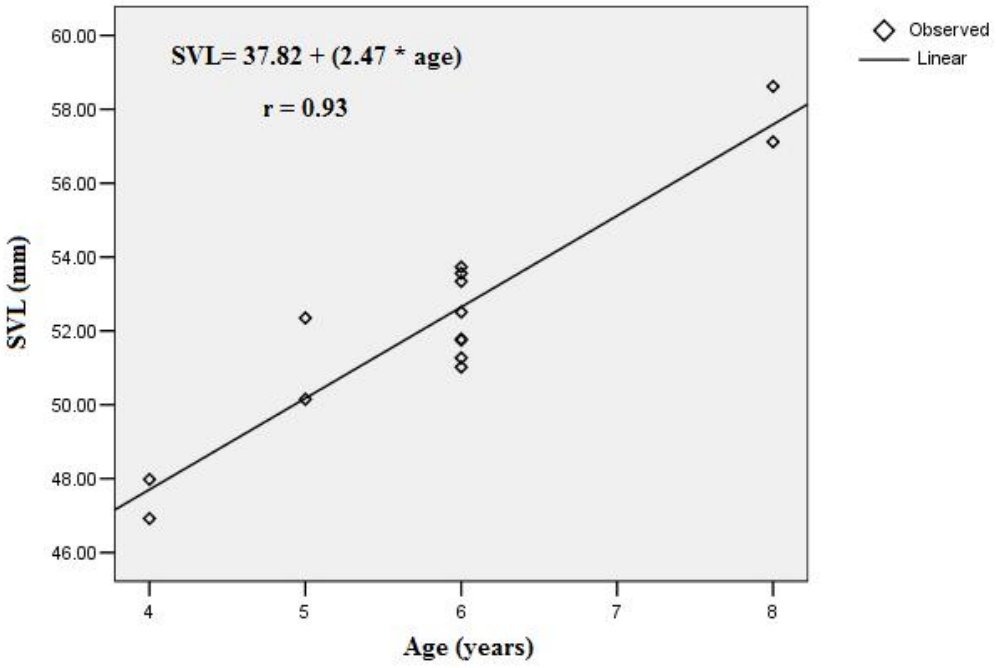

Fig. 3. Age (years) – SVL relationship of *P. parva*

In conclusion, a positive correlation (r=0.93, P<0.01) is seen between the age and the SVL is observed. The increase in SVL value and age for the current material is directly proportional.

Similarly, considering the relationship between the length and the width of pileus, it is observed that pileus length does not increase constantly with age (r=0.007, P=0.98), while width increases normally together with age (r=0.212, P=0.46).

The age formula of SVL=37.82 + (2.47*age) is seen suitable for *P. parva* samples living in this locality. Thus, it is thought that individual age estimation can be done without harming the species gathered from the aforementioned locality whose SVL value is calculated.

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Received: 23.03.2012
Accepted: 18.04.2012

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 87-93

Climate Change Assessments for Lakes Region of Turkey

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Abstract. Climate change is one of the most important challenges for forestry. Forests are known to be most efficient natural tools to ensure availability and quality of water in many regions. Besides, planning of forest resources towards water quality and quantity is essential in countries that are expected to face with more frequent drought periods in the next decades due to climate change. Watershed management concept has been supposed as the primary tool to plan natural resources in a more efficient and sustainable way by both academicians and practitioners to mitigate and adapt climate change. Forest cover among other land use types provides the best regulating mechanism to mitigate erosion, sedimentation, desertification, and pollution. In addition, climate change can potentially affect forest stand dynamics by influencing the availability of water resources. Therefore, the amount of forest cover in a watershed is an indicator of climate change mitigation and adaptation. Climate change is a concern and risk for the sustainability of water resources in Lakes Region of Turkey. The objective of this study is to make a comprehensive assessment in lake watersheds of the Lakes region considering the forest cover. For this purpose, the study gives a general view of trends in climatic parameters using Mann Kendall trend test. The results showed that Mann Kendall trend test for temperature and precipitation data is not enough to evaluate the magnitude of potential changes of climate in terms of forest cover. Understanding impacts of changes in temperature and precipitation on forest cover, runoff data should be evaluated with temperature and precipitation for watersheds of forest areas in Lakes Region.

Key words: Climate change, Mann Kendall test, Watershed management, Watersheds forest cover.

Introduction

Ecological systems are intrinsically dynamic and are constantly influenced by climatic variability. The water resources sector in many regions and countries, therefore, is vulnerable to changes in climate (WATSON *et al.*, 1998). The outcomes of different climate change scenarios have showed regional variability of climate change. Forests are particularly sensitive to climate change, because the long life-span of trees does not allow for rapid adaptation to environmental changes. The changes in average temperatures that forests will have to face over the next 100 years range, according to latest projections, is between 4 - 5°C increase in some parts of the Mediterranean region (LINDNER *et al.*, 2008). Climate is expected to become more variable with greater risk of extreme weather events, such as prolonged drought, storms and floods. Forests will have to adapt to changes in mean climate variables but also to increased variability (UNFCCC, 2011). Temperature is predicted to increase and this will have a positive impact on forest growth and wood production in northern and western parts (i.e. less water limited) and a negative impact on southern and eastern parts of the Mediterranean region (i.e. water limited). Adaptive capacity is also robustly limited in the Mediterranean region where large forest areas are only extensively managed or unmanaged. The extreme forest

fire risk is the major threat in the Mediterranean region (LINDNER *et al.*, 2008). Forest ecosystems play an important role in the global biogeochemical cycles (MAGNANI *et al.*, 2007). Forests can contribute to the mitigation of climate change, but under the existing global climate policy frame this alone will not be enough to halt climate change. Mediterranean forest growth is constrained by drought and high temperatures during summer. Effects of climate change on these forests depend on how changes in water availability and temperature will take place. Temperature and rainfall may constrain growth during certain periods but if rainfall increases in the future, a positive effect on growth is likely to happen (SÁNCHEZ *et al.*, 2002).

Forest ecosystems are being rapidly and directly transformed by the land uses of the expanding human populations and economies. Currently less evident are the impacts of ongoing climate change on the world's forests. The common implicated causal factor in these examples is elevated temperatures and/or water stress, raising the possibility that the world's forests are increasingly responding to ongoing warming and drying (ALLEN *et al.*, 2010). The main characteristic of plants is to response to water scarcity. Water use efficiency, on the other hand, can have profound effects on forests in possible decreases in precipitation attending changes in climate.

This study uses Mann Kendall trend test to asses to climate change for Lakes Region of Turkey in the Mediterranean Region. Major objectives of the study is (i) to assess spatial distribution of forests in Lakes Region, and (ii) the potential changes in climate depending on temperature and precipitation in the watershed ecosystems of Lakes Region.

Background

Basins in Turkey and Forest Cover. Turkey is divided into 26 river basins. Around 50 % of the forested areas in all basins are coniferous forests (HIZAL *et al.*, 2008). The Mediterranean river basin is one of them and includes Lakes Region in Turkey.

Lakes Region of Turkey. In Lakes Region, many natural lakes provide drinking and irrigation water to the settlements in the region. Besides, some of the lakes are declared as Ramsar site providing habitat to wildlife. The water use from the lakes has been increasing in the recent years due to increasing population and urbanization. Climate change is another concern and risk for the sustainability of water resources of the region.

Lakes Region, covering Antalya, Isparta, and Burdur provinces is located in the western Mediterranean Region of Turkey and has an area of 36.672 km². The significant lakes in the region are Egirdir, Burdur, Golcuk, and Kovada. Lake Egirdir is the fourth largest lake of the country and also is the second according to irrigation potential after Lake Beysehir. Lake Kovada is located below Lake Egirdir and also has a linkage with it. A portion of Lake Egirdir waters drains into Lake Kovada, which also provides water for hydroelectric power unit. Lake Golcuk is a crater one at 1300 m altitude. Among these Lake Burdur is a protected, and Egirdir Lake is an important wetland due to biodiversity they acquired according to Ramsar Convention. Besides, Lake Kovada and its watershed have a National Park, Lake Golcuk and its watershed has a Nature Park status given by Ministry of Environment and Forestry.

The significant wetlands located in this region make it a region of priority for watershed planning purposes. The area of the region makes 4.5 % of total country area. The area is estimated to face with drier conditions during the next century according to climate model outputs. Therefore, forest cover in the watersheds of these lakes and evaluating their conditions and impacts from water production point of view is essential. Egirdir watershed is a semi-closed watershed while Burdur and Golcuk are closed ones, which means not drained outside the watershed with any fluvial system. Lake Kovada watershed is an open and drains into Mediterranean Sea.

Lake Egirdir Watershed. Lake Egirdir is a tectonic formation on a karstic parent material. The water flowing out of the lake

flows into Lake Kovada and then to the Mediterranean Sea. The drainage area of the lake is 282.368 ha (BASAYIGIT, 2002). It is the largest lake of the region. The mean elevation of the lake is 918.8 m. The lake is listed in Ramsar Convention as "International significant wetland" (ERDEM, 2004). It provides water for Kovada I and II hydroelectric power plants (KARAGUZEL, 1995). The outflow of the lake is also used for irrigation, and drinking water supply for Isparta city. The annual water inflow and outflow rates of the lake are 817.85 hm^3, and 848.37 hm^3, respectively (ESENDAL, 2007). There are 22 towns inside the watershed and the tree species are Black pine, cedar, and junipers. There is also deciduous coppice inside the watershed. Agriculture and livestock are the major means of living in the watershed. Afforestable land area in the watershed is 68.000 ha. The total forested area (100.024 ha) is composed of productive high forests (32.5%), degraded forests (36.5%), productive coppices (0.5%), degraded coppices (12.5), and shrublands (17.5%). Over 80% of the forests are composed of coniferous species (KARATEPE, 2004) due to be the mountain area.

Lake Kovada Watershed. Lake Kovada is a natural extension of Lake Egirdir. It is also a tectonic formation. It has been assigned as a National Park in 1970 because of biodiversity its environment posses. The area of the lake is 6763.5 ha on an elevation of 900 m. The streams that drain into the lake are intermittent (TABUR, 2002). The wide variety of tree and shrub species include red pines, black pines, junipers, cedars, oaks, blackberry, laurel, and cistus.

Lake Golcuk Watershed. Lake Golcuk had been formed by filling of a crater with water at an elevation of 1300 m. The Lake has been providing water to Isparta city for a long time. The lake surface has an area of 94 ha (DAVRAZ & KARAGUZEL, 2001). It has been assigned as Nature Park in 1991. Therefore, it is used as a recreation area. The tree species of the area are Black pine, Red pine, Black locust, Oak and Cedar. The larger part of the plantations inside the watershed was done by State Hydraulic Works and General Directorate of Forestry in 1956. Vegetation cover was poor before that. Any treatment inside the watershed has a potential to affect water quantity and quality (EROL, 2005) because of coniferous species near the lake and a grove of apple trees.

Lake Golcuk is in a distance of 12 km to Isparta and 25 km to Burdur cities. The lake watershed is inside the Natural Park of 5842 ha. The mean annual precipitation is 702.5 mm.

*Lake Burdur Watershed***.** This lake one of the largest lakes of Turkey is also a tectonic formation. Due to degradation of ecosystems in its watershed, water pollution is a major problem. 38.125 ha of the lake watershed was assigned as Wildlife Protection area in 1993 and 50 % of the lake area has been one of Ramsar areas of Turkey since 1994. The elevation of the lake is 845 m. The lake watershed is a closed one drained by small streams. Only 6.1 % of the lake watershed is covered with productive forest. There are wide degraded Maquis formations (KIRIZOGLU *et al.*, 1995). The forested areas of the watershed (688.700 ha) is 320.338 ha.

The study area is in the Mediterranean region of Turkey. In Figure, 1 Lake Egirdir, Kovada, Burdur and Golcuk are shown by numbers 1, 2, 3 and 4 respectively.

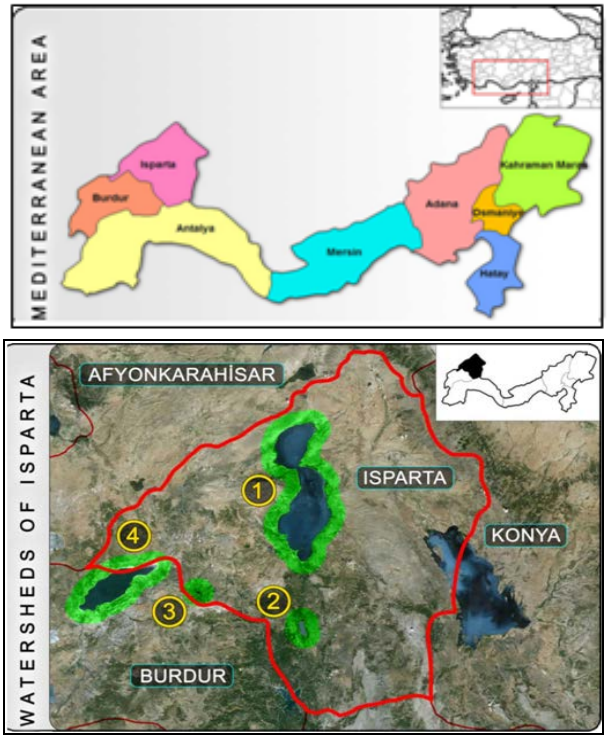

Fig.1. Location of the study area

Material and Methods

The data used in this study include annual maximum precipitation (mm), monthly mean temperature (°C), and monthly total precipitation (mm). All data used in the study belong to Turkish State Meteorological Service. The data have been completed for the period of 1975-2009 from 7 stations in only one part of Lake Region, which include Lake Egirdir, Lake Kovada, a part of Burdur and Lakes Beyşehir. To identify statistically significant trends, the original data series were tested with Regional Mann Kendall statistic, assuming 95% significance level (DENNIS *et al*., 2006a). The study performes time series analyses on long term monthly precipitation, temperature, and maximum rainfall data to assess the risks that may emerge due to climate change. The forest cover in the watersheds of Lake Golcuk, Burdur, Kovada, and Egirdir has been determined based on the data provided by General Directorate of Forestry.

*The Mann-Kendall trend test***.** The Mann-Kendall trend test (MANN, 1945; KENDALL, 1975) is based on the correlation between the ranks of a time series and their time order. In the test trend, the test statistic is given for a time series.

A Mann-Kendall test is computed for individual locations and results are combined into one overall test for consistent regional trend (HELSEL & FRANS, 2006). The Regional Kendall test performs the Mann-Kendall (MK) trend test for individual locations in area (DENNIS *et al*., 2006b). In the test, trends at numerous locations within a region are tested to determine whether the direction of trend is consistent across the entire region.

Regional Kendall test. The Regional Kendall test extends to spatial locations rather than seasons and looks for consistency in the direction of trend at each location, and tests whether there is evidence for a general trend in a consistent direction throughout the region. Patterns at an individual location occurring in the same direction as the regional trend provide some evidence toward a significant regional trend, even if there is insufficient evidence of trend for that one location (HELSEL & FRANS, 2006). In the study the Lakes region data were used as the basis for the Regional Kendall test for trend. In the test trend analysis has been extensively used to assess the potential impacts of climate change using temperature and precipitation data in the region to assess the spatial distribution of forests in the Lakes region including lake watersheds.

QIAN & LIN (2004) and KARL *et al.* (1993) have reported in their studies that a statistically significant decreasing trend in the precipitations and temperatures is to be expected as an indicator of a global warming signal. Meteorological data which such as temperature and precipitation are indicators of climate change (DENNIS, *et al*., 2006). In this study, meteorological data which such as temperature and precipitation are used as some parameters of the climate change.

Table 1. Mann-Kendall trend results for annual max. precipitation (mm)

Well ID	*Number of Data Points*	*Minimum value*	*Maximum value*	*Mann Kendall Statistic (S)*	*Normalized Test Statistic (Z)*	*Probability*	**Trend (At 95% level of significance)**
Burdur		0.0	91.0				
Isparta		0.0	100.7				
Senirkent		0.0	94.5				
Yalvaç	35	0.0	71.6	315.0	1.686	0.0918	No Trend
Uluborlu		0.1	76.0				
Egirdir		0.0	141.0				
Beyşehir		0.1	90.5				

Table 2. Mann-Kendall trend results for monthly mean temperature (°C)

Well ID	*Number of Data Points*	*Minimum value*	*Maximum value*	*Mann Kendall Statistic (S)*	*Normalized Test Statistic (Z)*	*Probability*	**Trend (At 95% level of significance)**
Burdur		-1.3	27.4				
Isparta		-2.8	26.0				
Senirkent		-5.0	27.1				
Yalvaç	35	-5.7	25.8	1149	6.173	0.0000	No Trend
Uluborlu		-4.0	25.7				
Egirdir		-3.1	25.7				
Beyşehir		-7.1	24.9				

Table 3. Mann-Kendall trend results for monthly total precipitation (mm)

Well ID	*Number of Data Points*	*Minimum value*	*Maximum value*	*Mann Kendall Statistic (S)*	*Normalized Test Statistic (Z)*	*Probability*	**Trend (At 95% level of significance)**
Burdur Isparta Senirkent Yalvaç Uluborlu Egirdir Beyşehir	35	0.0	195.1	66.0	0.349	0.7272	No Trend

Results and Discussion

This study highlighted 1975–2009 trends by using the Regional Mann Kendall test, which performs the Mann-Kendall (MK) trend test, for climate change assessment for Lakes Region. The results of the study show that the region has not a likely threaten to the climate change. Concluding this study shows that assessments for changes in the climate should be taken account of runoff with the precipitation and temperature.

Results of the Regional Mann Kendall test for annual and monthly time series of precipitation and monthly temperature indicate that 7 stations data, which have 245 component of well ID, exhibited no trend during the past 35 years statistically. The study results show that (Table 1, 2, 3) the precipitations and temperatures exhibites to have no trend for the 35 years. The main point is that the environmental conditions in Lakes Region have a significant role for the conservation of water resources. It is thought that the presence of water resources, especially in watersheds of Lake Egirdir and Burdur have a tendency to be affected to changes in the climate, even though the trend of precipitation and temperature detected in this study. In the Lakes Region, in addition, some forest sites including watersheds of Lake Egirdir and Lake Burdur have degraded. In watersheds of Lake Egirdir and Burdur there are degraded forested area, 59% and 93% of total forested watershed areas, respectively.

It is clear that there is need further research to verify the results of this study. Some studies focused on climate change show that precipitation and runoff should be determined together because precipitation is the main driver of runoff (BLAKE *et al.*, 2000; GREGORY *et al.*, 1997; CHIEW *et al.*, 2009). In this case, more reliable results can be likely to occur to assess effects of climate change on soil and water resources for the region.

There is need more monitoring data and further research on watersheds in Lake

Regions to assess soil and water conservation functions of these forested areas using Mann Kendall trend test. It is known that the watershed areas are very mountainous and their soils are prone to erosion. This reason alone is enough to say that the region would be likely sensitive to climate change so it is important to understand effects of climate change on runoff from precipitation and by drainage from water stored in soils.

Conclusions

The forested area inside the watersheds of lakes in Lakes region of Turkey has been evaluated with this study. All watersheds mentioned in the study have a special conservation status. The forested areas are generally composed of coniferous stands and also degraded deciduous. The magnitude of climate change cannot be detected with the Mann Kendall trend test results. The existing forest cover should be conserved to keep the lakes from sedimentation even if not any change of climate in the present conditions.

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Received: 16.04.2012
Accepted: 19.05.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 95-97

Vertical Distribution of Species of the Subfamily Aphidiinae (Hymenoptera: Bracondae) from the Southwestern Bulgaria

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Abstract. The vertical spreading of some species of the subfamily of Aphidiinae (Braconidae) from Southwest Bulgaria was investigated during the period 2002 – 2005. The research was carried out for four out of the six vegetation zones which are typical for this region: Xerothermic oak woods, Mesophyll oak woods, Beech woods, Coniferous woods, Subalpine sparse woods.

Key words: Aphidiinae, Braconidae, Hymenoptera, aphid, biological control, parasitoid.

Introduction

Until 2002, 43 species have been reported from Bulgaria, which parasitise 12 families (ATANASOVA, 1997; KOLAROV, 1997; STARÝ, 1962) and until the present moment 63 species from 21 families have been reported (TODOROV, 2008). A large number of species from different vegetation zones have been included in the research.

The recent research shows the distribution of the parasitoids for the Southwest of Bulgaria. The explored territory was chosen because of its high level of diversity of different types of habitats which is a premise for a larger range of species that can be included in the analysis.

Material and Methods

The material for the research was collected on the territory of Suthwestern Bulgaria (Slavyanka Mt., Belasitza Mt., Ograzhden Mt., Maleshevska planina Mt., Rila Mt., Pirin Mt. and trom the riverside of Struma) in the period 2002 – 2005. The parasitoids were collected with Malaise traps, fall-pit traps, entomological nets and the material brought out from between 200 and 1980 meters altitude. Because the definite tables are formed only on the basis of female representatives, from all the 3656 only the female individuals were singled out. From the defined 1213 specimen 50 species were determined from 14 genera.

The mountainous nature of the most of the explored territory determines the vertical climate changes and the respective changes in the vegetation communities on different altitudes. On the territory of Southwest Bulgaria there are six clearly differentiated vegetation zones: xerothermic oak woods belt (100- 300 m.), mesophyll oak woods belt (300- 900 m.), beech woods belt (900- 1600 m.), coniferous woods belt (1600- 2300 m.), subalpine and alpine belt (2300 - 3000 m.).

The terminology used in description follows STARY (1970). The studied and identified material is deposited in the collection of the author.

Results

From the six examined vegetation zones in the study there are not any detected species in the alpine belt from the studied group, in the subalpine sparse woods belt there have been found only 3 species. That is why these two mentioned zones are not included in the analysis. Each one of the vegetation communities, developed in different climatic conditions, determines the distribution of animal communities, in particular, of the representatives of the examined group. The results of studying the vertical distribution are presented in Table 1 and Fig. 1.

Table 1. Distribution of species defined by vegetation zone in Southwestern Bulgaria during the period 2002-2004.

Belts	Number of species
Xerothermic oak woods	23
Mesophyll oak woods	22
Beech woods	30
Coniferous woods	11
Subalpine sparse woods	3

The analysis shows that with the increasing of the altitude the species diversity is decreasing. This result demonstrates the connection between the optimum conditions for the development of the species from the examined group and the ecological factors exerting influence on them in the different vegetation zones of the explored region. The beech belt is an exception, where the number of species is the highest (30 species or 34% of total amount) because of its highest level of humidity in comparison with the other belts. The number of *Aphidiidae* is a direct result of the dependence of aphids on the humidity of the environment.

Twenty three species or 26% of the total amount are registered in the xerothermic oak woods belt which is characterized by presence of drought-resistant and heat-resistant vegetation.

In the mesophyll oak woods belt are registered 22 species (25%), in the coniferous woods belt 11 species or 12% of the total amount are to be found. In the subalpine sparse woods only 3 species are registered.

The presented data confirm the fact that *Aphidiinae* find a bigger number of host organisms (polyphags) as a whole within the borders of the deciduous and mixed woods, in comparison with the other vegetation zones.

Depending on the distribution of the vegetation belts the *Aphidiinae* are classified in three zonal groups (Table 2). Stenozonal (found only in one of the zones), Mesozonal (found in 2 or 3 zones) and Evryzonal (found in all vegetation belts).

Table 2. Number of species registered in the period 2002-2004 in Southwest Bulgaria classified in zonal groups.

Zone groups	Number of species	% of total amount
Stenozonal	33	62
Mesozonal	8	15
Evryzonal	12	23

The distribution of species by zonal groups in the area is as it follows: stenozonal – 33 species or 62% of the total amount; mesozonal– 8 species or 15%; evryzonal – 12 species or 23% of the total amount.

The stenozonal species present the biggest percentage of distribution in the zonal groups. They are presented in each of the vegetation zones (Fig. 3.) as it follows: in the xerotherm oak belt – 8 species or 8,29 % of the total amount; in the mesophyll oak belt – 9 species or 9.32%, in the beech belt – 9 species or 9,32 %; in the coniferous belt – 2 species or 2,7 % of the total amount. No stenozonal species have been found in the subalpine belt. The majority of these species find their host organisms in the corresponding vegetation zones and this is an after-effect of their overall distribution in the explored region.

Forty-one of the registered *Aphidiinae* species from the region participate in the formation of the mesozonal group (15 %) and evryzonal group (23 %). Representatives

with a larger number of host organisms (polyphagus) or having as host organism's species with a larger scale of vertical spread are also related to this group. Migrations and exchange of fauna between the belts are not excluded as well.

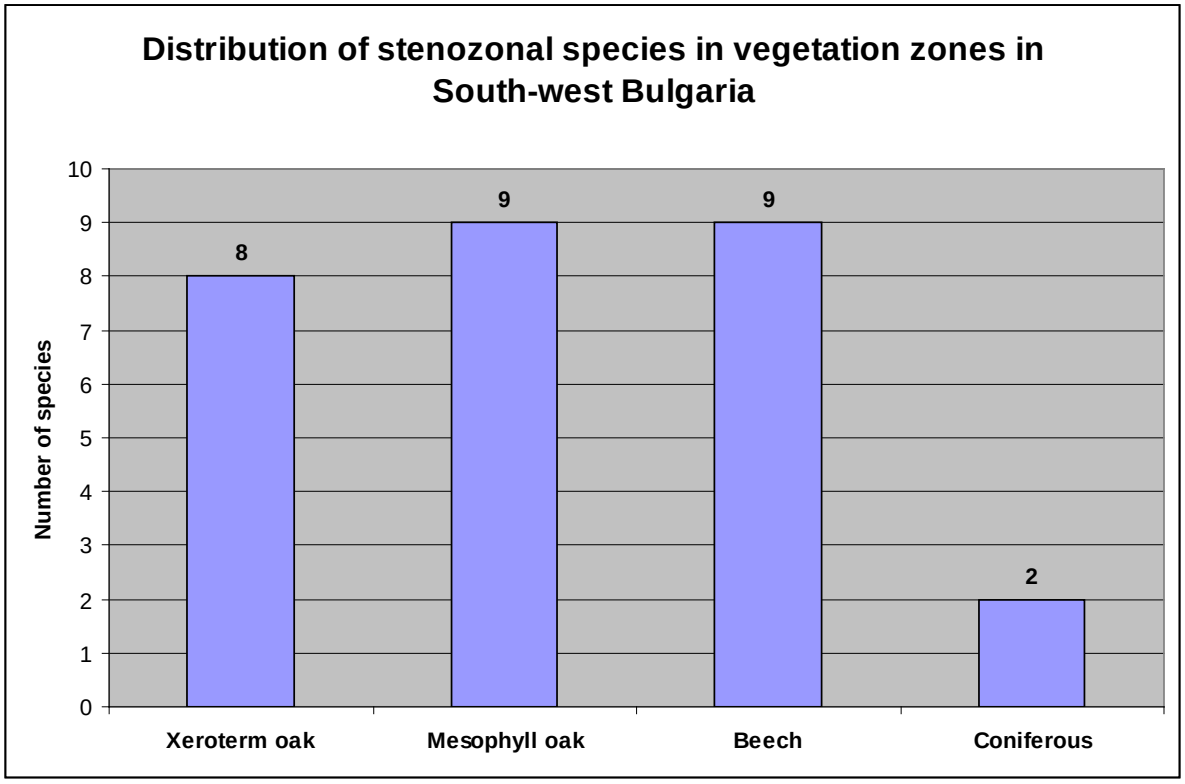

Fig. 3. Distribution of stenozonal species in vegetation zones in Southwest Bulgaria.

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Received: 20.04.2012
Accepted: 03.05.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 99-105

Implication upon Herpetofauna of a Road and its Reconstruction in Carei Plain Natural Protected Area (Romania)

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Abstract. In autumn 2011 we monitored a 5 km long road, paved with cobblestone, situated in Carei Plain Natural Protected Area, a road that is due to be modernized and continued across the border into Hungary. Dead bodies from eight different animal groups were observed on the road, amphibians presenting the greatest amount. The most frequent were the *Triturus dobrogicus* corps, a species with conservation importance. The amphibians were affected in the areas where the road is neighboring the wetlands, while on the opposite pole sits the area with acacia plantations. The high number of mortalities recorded on the road, despite the low traffic speed, is alarming. It is likely that the modernization of the road that will surely increase its traffic and the speed of the vehicles, will make the situation even worse. However, the rebuilding could contribute to the reduction in the impact on amphibians, if certain measures are considered while planning the action. Thus, in the areas near the wetlands, there should be undercrossings, fences and speed limits. In this way, the modernization would at least represent an experiment regarding the diminution of the road's impact on amphibians.

Key words: road mortality, amphibians, protected area, conservative measures, Romania

Introduction

The negative impact of roads and traffic on biodiversity is generally acknowledged (e.g. RIFFELL, 1999; KUITUNEN *et al*. 2003; CIESIOŁKIEWICZ *et al*. 2006; ROE *et al*. 2006; RAO & GIRISH, 2007; HARTEL *et al*. 2009; GEORGE *et al*. 2011; KAMBOUROVA-IVANOVA *et al*. 2012). The development of road networks put a lot of pressure on biodiversity, identifying and conserving areas without or with very few roads becoming extremely important (see in: SELVA *et al*. 2011). This fact is even more crucial in protected areas, which should be situated in areas without roads or localities (see in: RAYN & SUTHERLAND, 2011). The network of protected areas expanded greatly in Romania in recent years (e.g. IOJĂ *et al*. 2010), but many areas were assigned without taking into consideration the reality in the field. As such they comprise in both natural and affected areas and encompass some human localities which ultimately only make the anthropogenic pressure grow constantly. Although some authors consider that there are no major contradictions between economic growth and biodiversity (FUENTES, 2011), economic development, expressed also through the expansion and modernization of the road networks does affect protected areas, Romanian being a part of this trend. In this situation we find the Carei Plain Natural Protected Area which, being on the border between

Romania and Hungary, fully feels the pressure of building roads between the two countries. Thus, our study was started by the authorities` wish of modernizing a road inside the protected area. The aim of the study was to quantify the impact of the road on the herpetofauna during the fall of 2011, by establishing the affected species, the exposed sectors and the potential measures for conservation.

Material and Methods

The analyzed road is situated in the northern part of the Carei Plain Natura 2000's Habitats Directive Site (Campia Careiului – ROSCI0020) (Fig. 1). The area is located in north-western Romania, representing a stretch of approximately 10km wide and 50 km long situated between the border with Hungary and the Oradea - Satu-Mare rail road. The road is perpendicular with the area and the border, linking the Sanislau and Horea villages, from where it is continued until the border. Nowadays the road, of about 5 km in length, is paved with cobblestone from Sanislau to Horea and then, its last 500m until the border; it's paved with simple quarry stone. The cobblestone sector is degraded, the travelling speed being very low (about 30 km/h) and the traffic is very low. Our research took place in autumn 2011, consisting in more field trips in September and October. The methods were similar to those used in other studies (e.g. CIESIOŁKIEWICZ *et al*. 2006; ELZANOWSKI *et al*. 2009). We used the direct observation of the dead bodies from the road, counting them and making notes on their location and the aspect of the surrounding areas. The road was covered by foot, in order to accurately spot all the bodies. There were three observers that walked in parallel, two on the sides of the road and on the middle.

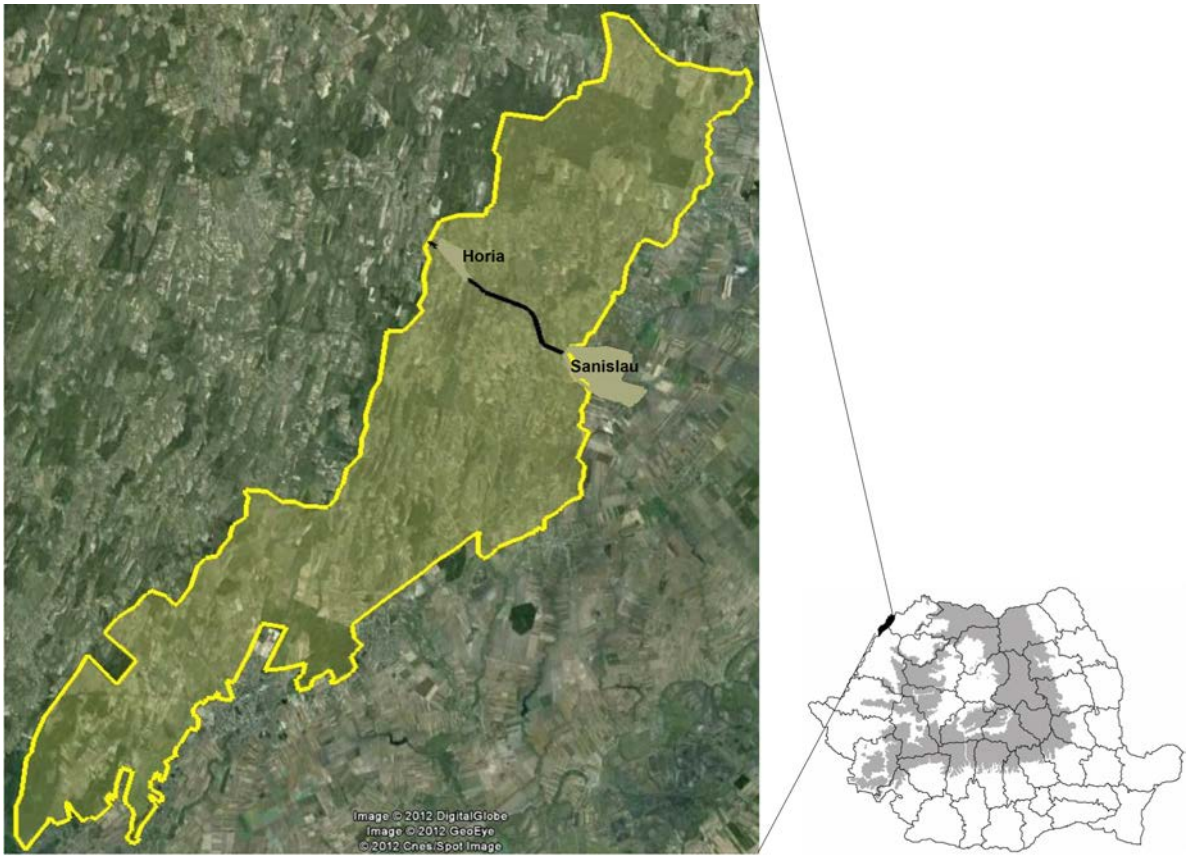

Fig. 1. Map showing the geographical position of the Carei Plain Habitat Directive Site (ROSCI0020) and the studied road between the localities Horia and Sanislau (after GoogleEarth).

Results

Not all the dead bodies identified on the road from the Carei Plain Natural Protected Area were amphibians. We identified dead bodies belonging to eight different groups of animals, both vertebrates (amphibians, reptiles, birds and mammals) and invertebrates (Diplopoda, Odonata, Coleoptera and Lepidoptera). Amphibians represented the majority of the bodies identified on the road between Sanislau and Horea, both as number of individuals and as number of species. All amphibian bodies were determined to a species level. Thus, we came across 64 dead bodies belonging to 5 species (*Triturus dobrogicus*, *Bombina bombina*, *Pelobates fuscus*, *Bufo bufo*, *Pelophylax esculentus*) (Fig. 2). From the reptiles we only identified bodies of snakes belonging to *Natrix natrix*. Due to the slow speed of the vehicles, birds and mammals had low amounts. On the other side, amphibians – slow animals – were the most frequent victims.

The magnitude of the road killings all along the entire length is variable. There are sections where one can count more than one dead body on a meter long stretch of the road, but also areas where they occur several tens of meters apart. Generally, amphibian bodies were recorded in three main areas, all with vast wetlands next to the road. The magnitude of amphibian road killings is increased by the fact that the road is right next to all three swamps. Aside for amphibians, only dragonflies and damselflies present a distribution tied to wetlands. All the other groups (which have very low amounts from the total number of dead bodies) are spread relatively uniformly along the road. The areas least affected by road kills are the sections near the acacia plantations.

The amphibians that are affected by the road have a high conservative value (Table 1 also see Council Directive 92/43/EEC, O.U.G. 57/2007). *T. dobrogicus* is a species with high conservative value, which is rare in Romania and is distributed in the southern and western lowlands of the country (e.g. COGĂLNICEANU *et al*. 2000; IFTIME, 2005).

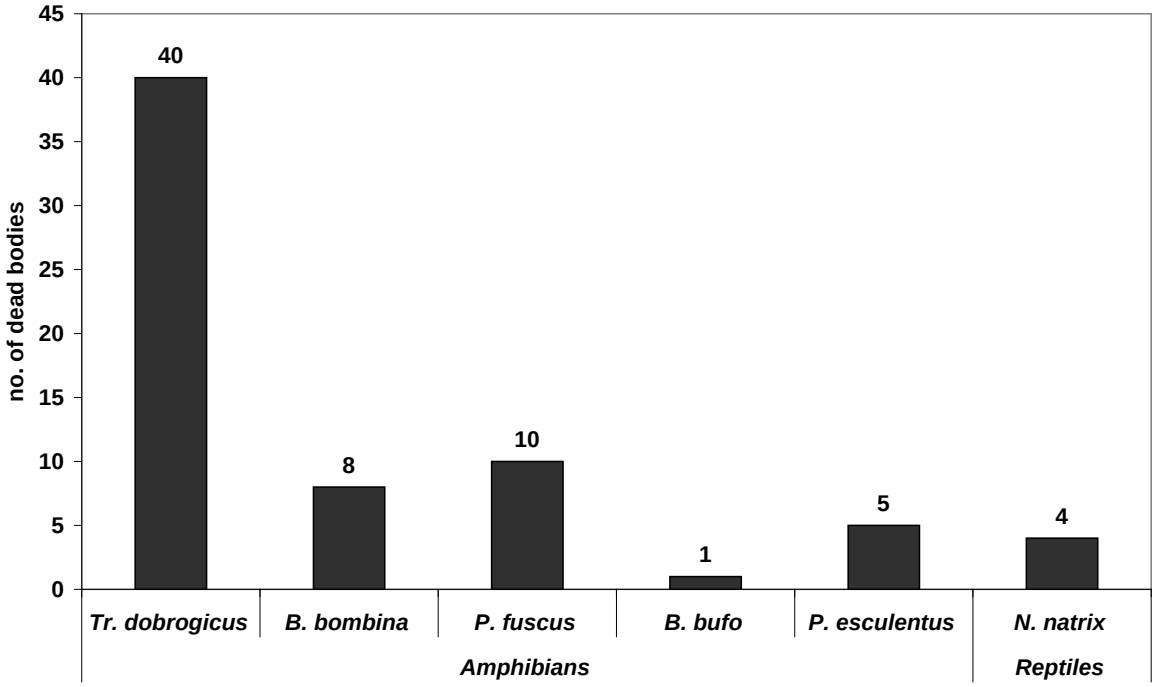

Fig. 2. The number of amphibian and reptile mortalities along the studied road.

Table 1. The road mortality affected amphibian and reptile species Natura 2000's status at the European and Romanian level. Legend: N2K=Natura 2000 status; SpCA=species needed special conservation areas; CI-SP=species with community interest – strictly protected; NI-SP= species with nationally interest – strictly protected; CI-M= species with community interest with exploitation management.

	N2K status	
Species	Council Directive 92/43/EEC	O.U.G. nr. 57 / 2007
Triturus dobrogicus	SpCA	SpCA
Bombina bombina	SpCA, CI-SP	SpCA, CI-SP
Pelobates fuscus	CI-SP	SpCA, CI-SP
Bufo bufo	-	NI-SP
Pelophylax esculentus	CI-M	CI-M
Natrix natrix	-	-

Discussion

The negative impact of the road is great even nowadays, fact proven not so much by the number of dead bodies but by their taxonomic diversity. Thus, even in its current form, it is necessary to take measures to limit the effect of the road on the wildlife in the protected area. The presence of localities and roads which lead to them in the area are realities that can't be changed, but their impact should be limited. The analyzed road is perpendicular on the protected area, basically dividing it. Consequently as the number of roads will increase or will be upgraded, the fragmentation of the area will be higher.

The high frequency of *T. dobrogicus* mortalities is probably a consequence of the distribution of the species in the region and the season. Thus, in the Carei Plain the species`s populations seems to be abundant (COVACIU-MARCOV *et al*. 2008 a, b, 2009), which increases the probability of mortalities of some specimens on the roads. Also, during this season there is increased road mortality with this species, as shown in other cases the newts, which became victims of traffic more often in the autumn (GRYZ & KRAUZE, 2008). Probably in the days prior to the study the newts began to withdraw from terrestrial habitats into the water for hibernation. This fact is substantiated by the identification of a live specimen that passed the road to get to the wetland. The negative effect on the newts was probably amplified by their slow movement in the terrestrial environment.

Road mortality indicates that *T. dobrogicus* uses a vast terrestrial habitat, some bodies being found 500 m away from the swamp. The newts cross the road and hunt even in affected areas like the acacia plantations, crossing the road twice. This underline the necessity of conserving the terrestrial habitat neighboring the aquatic habitat used by amphibians (e.g. DODD JR. & CADE, 1998; POREJ *et al*. 2004). Also, it seems that the territory used by this species during its terrestrial phase is bigger than that of other crested newts, which after reproduction stay near the aquatic habitat (e.g. JEHLE & ARNTZEN, 2000; MULLNER, 2001). This may be a consequence of *T. dobrogicus*' adaptation to the large wetland areas (NECAS *et al*. 1997; IFTIMIE, 2005), as in Carei Plain, where the traveling between habitats is made through favorable wet areas, while for other crested newts the aquatic habitat can be surrounded by hostile terrestrial areas. As well as *T. dobrogicus*, the other amphibian mortalities were also found near the wetlands

On the road, among reptiles we only identified *Natrix natrix*. Snakes become victims of road traffic frequently (e.g. CIESIOLKIEWICZ *et al*. 2006; SANTOS & LLORENTE, 2009; TOK *et al*. 2011). The small number of observed specimens was probably a consequence of the reduced speed of the vehicles, which will likely

increase once the road is modernized. Although they are faster than amphibians, snakes are disadvantaged by their length. Lizards have not been recorded dead on this road, although in other cases, they are frequent victims of the road traffic (e.g. BOGDAN *et al*. 2011; TOK *et al*. 2011). There are *Podacris tauricus* individuals found at the road`s edges, a species important in this area (COVACIU-MARCOV *et al*. 2009), however, they don't fall victim to the traffic, due to their faster mobility.

The road mortality for amphibians is concentrated in the area of the three wetlands that are crossed by the road. Generally, for reptiles, the phenomenon reaches high values in the regions where the roads are situated at less than 100 m from the wet areas (LANGREN *et al*. 2009). The least affected by road mortality are the acacia plantations. The areas where the road adjoins agricultural fields are without high road impact and conservative value. These data confirm the fact that the frequency in road mortality is influenced by the type of roadside habitats (GLISTA *et al*. 2007). The most important wetland is located in the neighboring area of Sanislau, as demonstrated by the large number of mortalities in its vicinity.

Our results show a negative effect of road on the amphibians from the natural area of Carei Plain and argue against its modernization. The degradation of the road will require repairs, but these should be made on a small scale and the road shouldn't be continued to Hungary. Upgrading and connecting the road to Hungary will increase a lot the impact of the road, whereas in the protected areas the constructions of roads and settlements should be avoided (RAYN & SUTHERLAND, 2011). If the construction of new roads is inevitable, they should be built in a manner that they won't reduce the conservation value of the habitats (UNDERHILL & ANGOLD, 1999). Based on our results we suggest the following conservation measures in the area, some undercrossings and fences on the side of the road should be made which could reduce, at least in the mortality of newts. Undercrossings should ensure the movement of the fauna on both sides of the road, and fences should stop the access of the amphibians and reptiles to the road. Such measures have proved to be useful for most species of amphibians, helping to reduce road mortality (DODD JR. *et al*. 2004). In the wetland areas the speed limit should be set to a maximum of 40 km/h, as up to these speeds the butterflies and dragonflies are not affected (RAO & GIRISH, 2007). The above measures will hopefully reduce the impact of the road on the wetlands' wildlife. However, these measures are not efficient for some species like *Hyla arborea* or for some larger snakes which can climb the barriers.

If the reconstruction of the road is done according to these recommendations, as it was shown in previous studies (see in: DODD JR. *et al*. 2004), it should reduce its impact on the herpetofauna. However as to our knowledge, in Romania there are no roads built that take into consideration the conservation imperative. Thus, the effect of the measures will be checked after time. In conclusion, it remains to be seen if the measures meant to protect the herpetofauna will globally reduce the pressure of the road on all the wildlife of the protected area from the Carei Plain. Last but not least we hope that this will be at least an experiment, which will be useful in other case studies.

Acknowledgements

The study was done with the help of the Freies Europa Weltanschauung Foundation, custodian of the Carei Plain Natural Protected Area, whom we thank. The study is part of a program that supports the inventory of the biodiversity of the Carei Plain Natural Protected Area, the result serving to the protection of the area. Some of the recommendations and interpretations from this paper are present in the point of view, referring to the modernization of the road, which the authors have submitted to the custodian of the natural protected area.

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Received: 05.05.2012
Accepted: 01.06.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 107-110

Short note

Updated Information on the Habitat Distribution and Diversity of the Freshwater Malacofauna of Sarnena Gora Mountain (Bulgaria)

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Abstract: According to the recent research and the critical overview of the literature it can be summarized that a total of 13 species of freshwater snails and 6 species of mussels are known to occur in Sarnena Gora Mountain. The highest richness of freshwater molluscs species in the surveyed area were found in the medium sized rivers having microhabitats favorable for the aquatic molluscs such as fast flowing oligotrophic stretches combined with slow moving eutrophic parts and small floods on the banks. The species community of the freshwater molluscs known to live in the mountain studied was consisted mainly by some widely distributed species with Holarctic and Palearctic distributions, followed by the European species. Two local endemics were also registered in the mountain.

Key words: aquatic molluscs, Gastropoda, Bivalvia, habitat distribution, zoogeography, Bulgaria.

Introduction

The first information on the freshwater malacofauna of Sarnena Gora Mountain (a part of Sredna Gora Mountain ridge) was given by GEORGIEV (2005) who reported 12 species of aquatic snails and 4 species of mussels. Later on, some additional information was given by GEORGIEV & STOYCHEVA (2009) reporting of *Anodonta anatina* (Linnaeus, 1758) from a pond in the Starozagorski Bani resort. Two new species of Hydrobiidae (Gastropoda) were described as new from the Sarnena Gora Mountain by GLÖER & GEORGIEV (2009), i.e., *Belgrandiella zagoraensis* Glöer & Georgiev, 2009 and *Radomaniola bulgarica* Glöer & Georgiev, 2009 (wrongly reported by GEORGIEV (2005) as *Bythinella austriaca* (Frauenfeld, 1857), and *Pseudamnicola consociella euxina* Wagner, 1927, respectively).

The aim of this study was: (i) to revise, summarize and update the information on the freshwater malacofauna of Sarnena Gora Mountain according to its species diversity and habitat distribution, and (ii) to provide some additional analysis of its zoogeographical structure.

Material and methods

The massif of Sarnena Gora Mt (size around 5950 km^2) is situated between 25° and 26° meridians, and south of the central parts of Stara Planina Mt, from which it is separated by the Kazanlashka valley. From south and east it borders with the Upper Thracian Lowland, and from west with the Sashtinska Sredna Gora Mt (Fig. 1).

Revision of all the literature published and materials collected concerning the area was done (GEORGIEV, 2005; GEORGIEV & STOYCHEVA, 2009; GLÖER & GEORGIEV, 2009). Some additional collections of molluscs were also carried out during 2010-2011 at Bedechka River near Stara Zagora city. The living snails were preserved in 75%

ethanol. The shells were collected by sieving river deposits by 1x1 and 2x2 mm mesh width sieves. The dissections and measurements were carried out by means of CETI stereo microscope and an eye-piece micrometre for the needs of species determination. The nomenclature, species determinations and zoogeographical categories followed GLÖER (2002), GLÖER & MEIER-BROOK (2003) and ANGELOV (2000).

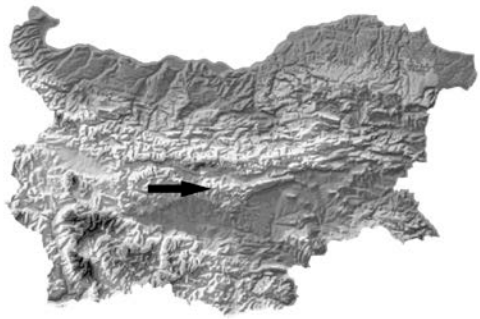

Fig. 1. Position of Sarnena Gora Mountain, pointed by an arrow.

Results and Discussion

1. Species diversity

According to the synopsis and the critical overview of the literature it can be summarized that a total of 13 species of freshwater snails and 6 species of mussels are known to occur in Sarnena Gora Mountain (Table 1).

Misidentified species. The following species were considered as wrongly reported for the area during previous studies: *Bythinella austriaca*, *Pseudamnicola consociella euxina*, *Radix ovata* (Draparnaud, 1801), and *Planorbis carinatus* O. F. Müller, 1774, and according to our revision refer to: *Belgrandiella zagoraensis*, *Radomaniola bulgarica* and/or *Grossuana* sp., *Radix auricularia* (Linnaeus, 1758), and *Planorbis planorbis* (Linnaeus, 1758), respectively.

New records for the study area. The species *Gyraulus crista* (Linnaeus, 1758) and *Pisidium personatum* Malm, 1855 were reported for the first time at the Sarnena Gora Mountain during present study. These species were collected as empty shells in Bedechka river near Stara Zagora city. Also an unidentified species of *Grossuana* Radoman, 1973 was found to live together with *Belgrandiella zagoraensis* at the mountain foothills from springs were the latter species was described (GLÖER & GEORGIEV, 2009).

2. Habitat distribution

In the paper of GEORGIEV (2005) some spring-dwelling species as the representatives of the Rissooidea superfamily, were erroneously reported from rivers, where they were found by empty shells in the alluvium. Same unsure records were reported and for the empty shells of other snails found in rivers, springs, and canals (which could live in other habitats but were transported there by floods as empty shells), which was corrected during present synopsis.

Most rich on species in the mountain were the medium sized rivers (14 species). The micro dams and the springs had 6 species each. The canals reported in GEORGIEV (2005) were omitted in this study because were considered as a temporary habitat with uncertain living mollusc fauna.

The species *Acroloxus lacustris* (Linnaeus, 1758) prefers stagnant waters (ANGELOV, 2000) but was not found in the micro dams during present study, so its presence in this habitat could be supposed. Also the euribiotic wide spread in Bulgaria (ANGELOV, 2000) species as *Physella acuta* (Draparnaud, 1801), *Galba truncatula* (O. F. Müller, 1774), and *Radix auricularia* could be expected in springs or other habitats in the area, where they were not registered. The river mussel *Unio pictorum* (Linnaeus, 1758) is known to live and in clean standing waters (ANGELOV, 2000) so its finding in the small dams of Sarnena Gora is possible.

3. Zoogeography

The species community of the freshwater molluscs known to live in the mountain studied was mainly consisted by some widely distributed species with Holarctic and Palearctic distributions (40%), followed by the European species (25%). The other categories were represented only by 1 or 2 species from which two local endemics were registered (Table 1, Fig. 2).

Table 1. Species diversity, habitat distribution and zoogeographical categories of the freshwater malacofauna of Sarnena Gora Mountain.

Species	Zoogeographycal category	Rivers	Dams	Springs
Gastropoda				
Valvata piscinalis (O. F. Müller, 1774)	Palearctic	*		
Belgrandiella zagoraensis Glöer & Georgiev, 2009	Local endemic			*
Radomaniola bulgarica Glöer & Georgiev, 2009	Local endemic			*
Grossuana sp.	Not known			*
Acroloxus lacustris (Linnaeus, 1758)	Euro-Siberian	*		
Radix auricularia (Linnaeus, 1758)	Palearctic	*	*	
Galba truncatula (O. F. Müller, 1774)	Palearctic	*		
Planorbarius corneus (Linnaeus, 1758)	Euro-Siberian		*	
Planorbis planorbis (Linnaeus, 1758)	Holarctic	*	*	
Gyraulus albus (O. F. Müller, 1774)	Holarctic	*		
Gyraulus crista (Linnaeus, 1758)	European	*		
Physella acuta (Draparnaud, 1801)	Mediterranean	*	*	
Ancylus fluviatilis O. F. Müller, 1774	European	*		*
Bivalvia				
Unio pictorum (Linnaeus, 1758)	European	*		
Anodonta cygnaea (Linnaeus, 1758)	European	*	*	
Anodonta anatina (Linnaeus, 1758)	European		*	
Musculium lacustre (O. F. Müller, 1774)	Palearctic	*		*
Pisidium personatum Malm, 1855	Holarctic	*		*
Pisidium casertanum (Poli, 1791)	Holarctic	*		

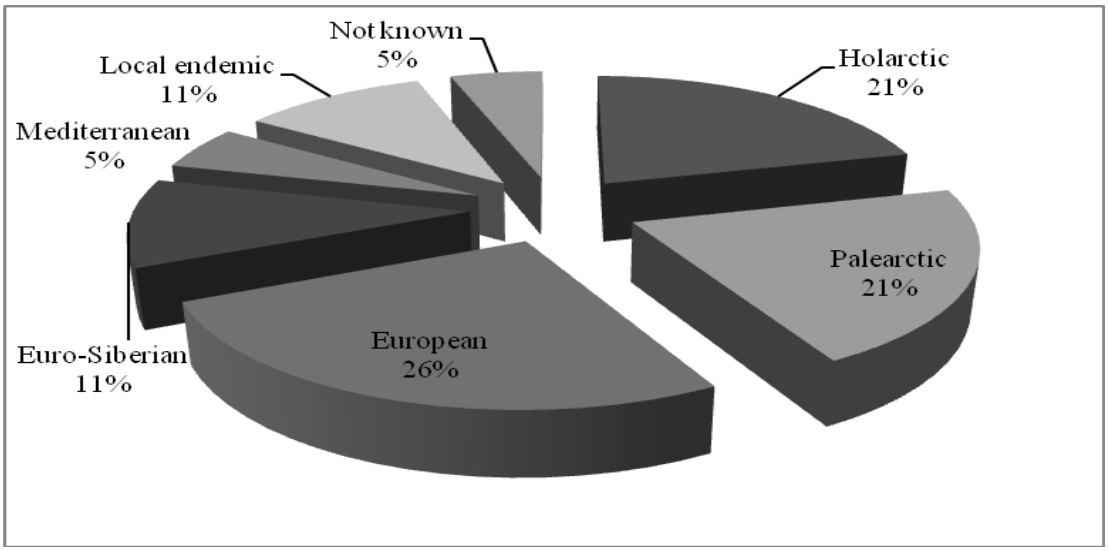

Fig. 2. Zoogeographical structure of the malacofauna of Sarnena Gora Mountain.

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Received: 25.02.2012
Accepted: 11.03.2012

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp. 111-115

Short note

Shell Size of the Freshwater Snail Radix auricularia (Linnaeus, 1758) Collected from Water Vegetation: A Case Study from South-East Bulgaria

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Abstract. The specimens of the freshwater snail *Radix auricularia* collected from Southeastern part of Bulgaria during the cold period as a whole were with mean shell height of 3.7 mm. During spring and summer it was similar, 3.3 mm. The ratio of the size groups was more equally spread during cold seasons rather than in warm ones. The variation index during cold seasons is about seven times higher than in the warm ones (Var = 13.1 and 2.14, respectively). On the five plant species the gastropods had different mean shell heights. For *C. demersum* it was 3.4 mm (min-max = 1.4-15.8 mm), and for *E. canadensis* 7.9 mm (min-max = 2.4-14.6 mm), *M. spicatum* it was 2.8 (min-max = 2.4-3.4 mm), *P. natans* 3.9 (min-max = 1.1-9.0), *P. pusillus* 4.8 (min-max = 3.1-8.2).

Key words: freshwater, quantity, gastropods, size, vegetation.

Introduction

The studies on the Bulgarian freshwater snails have started from the work of MOUSSON (1859), and continued with many others mainly focused on the taxonomy and diversity of species in various regions of the country (for example: BOURGUIGNAT, 1870, 1880; WAGNER, 1927; URBAŃSKI, 1960; ANGELOV, 1959, 1965, 1967, 1972, 1976).

Some ecological notes on the freshwater snails were given as a result of hydrobiological works (as RUSSEV *et al.*, 1998; KIRIN *et al.,* 2003, and many others) or synopses (ANGELOV, 2000; HUBENOV, 2005, 2006). Recently the first data on the habitats (GEORGIEV, 2005a, 2005b, 2006, 2008; GEORGIEV & STOYCHEVA, 2009), and species diversity, especially this one of the family Hydrobiidae Troschel, 1857 was intensively studied (GLÖER & PEŠIĆ, 2006; ZETTLER, 2008; IRIKOV & GEORGIEV, 2008; GEORGIEV & STOYCHEVA, 2008, 2011; GLÖER & GEORGIEV, 2009; 2011; GEORGIEV, 2009, 2011a, 2011b, 2011c, 2011d; GEORGIEV & GLÖER, 2011).

In Bulgaria there is a lack of detailed investigations regarding the ecology of the freshwater molluscs, while in the same time the foreign literature is quite rich on such kind of researches. Some of the most significant aspects of the ecology of freshwater gastropods are their relations with the aquatic plants. Both are quite sensitive to water pollution, and are often used as bio-indicators (GECHEVA & YURUKOVA, 2008). Focus on this question was made by the works of VASILEVA *et al.* (2009, 2011) but not considering the size of the gastropods and their age groups.

The aim of this study is to investigate the size characteristic of the populations of *Radix auricularia* (Linnaeus, 1758) dwelling on different water macrophytes during the cold and warm seasons in South-East Bulgaria.

Material and methods

The research was conducted through the period 2008 - 2009 in the Upper Thracian Lowland: Maritsa River in the city of Plovdiv, flood area near the bridge at UFT, N42°09` E24°43`; Eastern Rhodopes: Varbitsa River at around 3 km south of the town of Kardzhali, N41°34` E25°23`; Eastern Rhodopes: Perperek River, within the village of Perperek, N 41 ˚ 45` E 25 ˚ 21`; Eastern Rhodopes: Chernoochene dam in the village, N 41 ˚ 40 `E 25 ˚ 32'. The field trips were made from 19.02.2009 until 12.11.2009.

The mollusks were collected by hand or with a sack, along with the aquatic vegetation and were transported to the laboratory. The material was collected from total of 3119 g herbage biomass from the plant species: *Ceratophyllum demersum* L. – Rigid Hornwort (Varbitsa River: 150 g, and Maritsa River: 575 g, Chernoochene: 300 g), and *Elodea canadensis* Michx. – Pondweed (Maritsa River: 809 g), *Myriophyllum spicatum* L.- Eurasian watermilfoil (Chernoochene: 350 g), *Potamogeton natans* L.- floating pondweed (Varbitsa River: 685 g), *Potamogeton pusillus* L.- small pondweed (Perperek River: 250 g). The analysis of the results was made according to the plant species and season (cold – autumn and winter, and warm – spring and summer).

The material (total of 335 specimens) was separated from the plants by hand and by running water. The shells of the molluscs were measured (only the shell height was considered) and determined by GLÖER & MEIER-BROOK (2003) and a reference collection. The size groups were considered according to 1 mm. The index of variation was calculated using the program MS Excel.

Results and Discussion

The specimens collected during the cold period as a whole (number of specimens N = 28) were with mean shell height of 8.8 mm (min-max = 4.4-15.8 mm). During spring and summer (number of specimens N = 307) it was more than twice lower, 3.3 mm (min-max = 1.1-9.7 mm).

During the warm season specimens (in the following, in parentheses % of the total number of collected specimens) with shell height of 1-4 mm dominated (78.15%), and the most numerous was the group size 2.1-3 mm (35.50%l). Lowest percentage had the snail with shell height of 9.1-10 mm (0.33%). Specimens with shells higher than 10 mm were not registered. Such we found during the cold seasons, those with shell height between 9 and 16 mm (42.84%). Higher percent had the group 4-9 mm (57.16%), and specimens shorter than 4 mm were not collected. Accepting the maximal sizes of the species pointed by GLÖER & MEIER-BROOK (2003), of 8-12 mm shell height we consider that during warm seasons on the water vegetation studied the juvenile specimens dominate, and during the cold period subadults are as frequent as the juvenile, and some adults could also be found. The ratio of the size groups was more equally spread during cold seasons rather than in warm ones (Table 1, 2).

Table 1. Number and percent of the size groups of *Radix auricularia* on the freshwater macrophytes during spring and summer.

Size group	Number of specimens	%
1-2 mm	48	15.65
2.1-3 mm	109	35.50
3.1-4 mm	83	27.00
4.1-5 mm	33	10.76
5.1-6 mm	17	5.54
6.1-7 mm	6	1.96
7.1-8 mm	5	1.63
8.1-9 mm	5	1.63
9.1-10 mm	1	0.33
Total	307	100.00

The variation index during cold seasons is about seven times higher than in the warm ones (Var = 13.1 and 2.14, respectively).

On the five plant species the gastropods had similar mean shell heights. For *Ceratophyllum demersum* it was 4.8 mm (min-max = 2.5-8.9 mm), *Elodea canadensis* 7.2 mm (min-max = 3.6-10.0 mm) *Myriophyllum spicatum* it was 2.8 (min-max = 2.4-3.4 mm),

Potamogeton natans 3.9 (min-max = 1.1-9.0), *Potamogeton pusillus* 4.8 (min-max = 3.1-8.2).

Table 2. Number and percent of the size groups of *Radix auricularia* on the freshwater macrophytes during autumn and winter.

Size group	Number of specimens	%
4.1-5 mm	4	14.29
5.1-6 mm	4	14.29
6.1-7 mm	5	17.86
7.1-8 mm	3	10.71
8.1-9 mm	0	0
9.1-10 mm	3	10.71
10.1-11 mm	1	3.57
11.1-12 mm	1	3.57
12.1-13 mm	3	10.71
13.1-14 mm	3	10.71
14.1-15 mm	0	0
15.1-16 mm	1	3.57
Total	28	100.00

Acknowledgements

I would like to tank to Dr Dilian Georgiev and Dr Gana Gecheva (Department of Ecology and Environmental Conservation, Plovdiv University) who helped me a lot during the work on this study.

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Received: 12.03.2012
Accepted: 02.04.2012

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

ECOLOGIA BALKANICA

2012, Vol. 4, Issue 1 June 2012 pp.117-120

Short note

Some Data on the Terrestrial Isopods (Isopoda, Oniscidea) from a Wet Meadow near an Artificial Canal in North-Western Romania

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Abstract. We studied the terrestrial isopod fauna in a wet grassy area near an artificial canal in the northern part of the Western Plain, Romania, between April and September 2009. We identified five terrestrial isopod species: *Hyloniscus riparius, Trachelipus arcuatus*, *T. rathkii, T. nodulosus* and *Armadillidium vulgare*. The dominant species were *T. rathkii*, *A. vulgare* and *T. nodulosus*. Despite of the unforested character of the habitat, a sylvan species, *T. arcuatus,* was also present. This species could probably survive in the wet areas surrounding the canal following deforestation. The highest abundance was observed during the summer months, corresponding to the main activity period of females with marsupium.

Key words: wetland, praticulous and sylvan isopods, surface activity, pitfall trapping.

In the north-western part of Romania, terrestrial isopods have been observed both in opened habitats, poor in vegetation, as well as in forested areas, the most numerous populations being identified in the natural areas with dense vegetation (TOMESCU *et al*., 2008). However, in opened habitats of wet areas there are favourable conditions for many terrestrial isopod species (WIJNHOVEN, 2000). In the north-western part of Romania, many natural swamps were eliminated (see in: ARDELEAN & KARACSONYI, 2005) or fragmented, reduced to areas neighbouring canals. Despite their decreased dimensions, these wet areas still shelter species of the initial swamps (COVACIU-MARCOV *et al*., 2008). Thus, we have proposed to verify if this situation is also valid in the case of isopods, analysing assemblages from a wet area that border an artificial canal from north-western Romania. Moreover, the fact that the samples were taken monthly, allowed analysing the dynamics of the isopod assemblages.

The investigated habitat is located in north-western Romania, in Somes Plain, near Caraseu locality (47º43`41.33``N / 23º06`24.41``E) at 131 m a.s.l. A wet area formed along an artificial canal represents it. The vegetation is represented by a dense grassland (coverage almost 100%). The habitat is situated near a road, at approximately 500 m from the locality, presenting a high degree of anthropogenic disturbance. The neighbouring areas were used as pastures and agricultural fields. The study took place in 2009, from April to September. The samples were taken by

pitfall traps, three of them being placed in each study month. These were positioned near the water, at approximately 7 m from each other, in areas with rich vegetation and high humidity.Traps were emptied monthly (at the beginning of each month), from May to October, reflecting the isopods' surface activity during the previous month. The method allowed collecting only the terrestrial isopod species with high mobility (e.g. TOMESCU *et al*., 2008). Species were identified in the laboratory using the keys (e.g. RADU, 1983, 1985) in accordance with the present nomenclature (SCHMALFUSS, 2003). The study aimed to get data on the dynamics of the terrestrial isopod assemblages. We calculated the relative abundance of the species and their frequency in the traps during the study period. The diversity was estimated in different months, using Shannon-Wiever diversity index (SHANNON & WIEVER, 1949). The Kruskal-Wallis test (ZAR, 1999) was used in order to compare the differences between the periods.

We identified five terrestrial isopod species at Caraseu, belonging to several ecological categories (Radu 1983, 1985): paludicolous (*Hyloniscus riparius*), sylvan (*Trachelipus arcuatus*), euritope (*Trachelipus rathkii*) and praticolous species (*Trachelipus nodulosus* and *Armadillidium vulgare*). On a whole, we identified 194 individuals. *T. rathkii*, *A. vulgare* and *T. nodulosus* were the most abundant species (Table 1).

Table 1. The terrestrial isopod species' relative abundance (A%) and frequency (f%)

Species		***H. riparius***	***T. arcuatus***	***T. rathkii***	***T. nodulosus***	***A. vulgare***
Males	A%	0.94	7.54	48.11	11.32	32.08
	f%	7.14	7.14	85.71	42.86	64.29
Total females	A%	1.13	3.40	46.59	12.50	36.36
	f%	7.14	7.14	78.57	50.00	57.14
Females with marsupium	A%	1.13	2.27	23.86	2.27	10.23
	f%	7.14	7.14	35.71	14.29	7.14
Total	A%	0.94	5.18	46.23	12.26	35.38
	f%	7.14	7.14	92.86	57.14	64.29

Table 2. The terrestrial isopod species' relative abundance (A%) and diversity (H) depending on the study periods (A – % of all individuals from a species collected during the whole season)

Season		**April**	**May**	**June**	**July**	**August**	**September**
Total	A%	12.74	4.24	24.53	40.57	6.60	11.32
	H	0.74	0.31	0.91	1.09	0.52	0.68
H. riparius	A%	100.00	-	-	-	-	-
T. arcuatus	A%	-	-	100.00	-	-	-
T. rathkii	A%	17.35	5.10	28.57	41.84	5.10	2.04
T. nodulosus	A%	23.08	-	19.23	42.31	3.84	11.54
A. vulgare	A%	2.667	5.33	10.67	45.33	10.67	25.33

Differences in the species' relative abundance and frequency can be observed depending on time period. The values were slightly high during the first sampling period, after which they decreased, and then rose again during July and August. The highest relative abundance of individuals was observed in July. Regarding the species' diversity, the highest values were registered in June and July (Table 2). Kruskal-Wallis test ($H=4.38$, $df=5$, $p>0.05$) proved that seasonal differences are not significant.

The number of identified species in the area was lower than in other studies from Romania (e.g. TOMESCU *et al*., 2001, 2005, 2008), where also pitfall traps were used.

This fact is probably a consequence of the limited, homogenous and partially affected habitat. The presence of most of the isopod species identified at Caraseu is a consequence of the habitat's particularities. *H. riparius* is a common species in Romania (RADU, 1983), occuring in very wet areas (WIJNHOVEN, 2000). Thus, the habitat from Caraseu corresponds with its ecological requirements. *T. rathkii*, an euritope species, is found in very diverse habitats (TOMESCU *et al*., 2011), even in ones without a wooden vegetation, where the number of isopod species is lower (SPUŢGIS, 2008). The ecological preferences of *H. riparius* and *T. rathkii* are similar and explain the frequent co-occurrence of these species (JASS & KLAUSMEIER, 2003). *T. nodulosus* is considered to be rather common in the Hungarian plains (FARKAS, 2010). It was also mentioned from the western part of Romania (TOMESCU *et al*., 2008). The presence of the praticulous species, *A. vulgare* is also predictable from this habitat (RADU, 1985). Moreover, *A. vulgare* is a disturbance tolerant species and occurs regularly along roads (present case) and in antropogenic areas (Radu 1985).

The presence of *T. arcuatus* is especially remarkable. It is considered to be a sylvan species (TOMESCU *et al*., 2005), recorded often in the forests from western Romania (TOMESCU *et al*., 2008). However, the area from Caraseu lacks forests, the nearest one being at about 20 km far in Codru Hills. The occurrence of *T. arcuatus* might be explained by the phenomena experienced also in other groups of living organisms in the Western Plain: namely mountainous species are present in atypical habitats, they have been mentioned from the region in the past (COVACIU-MARCOV *et al*., 2008, 2009). The situation also seems to be in the case of terrestrial isopods. Recently, some sylvan species (*Protracheoniscus politus* and *T. arcuatus*) were recorded in a nonforested swamp in north-western Romania (TOMESCU *et al*., 2010). The explanation of *T. arcuatus*`s presence at Caraseu might have probably similar with the one previously described (TOMESCU *et al*., 2010). The region was covered by forests in the past and sylvan isopods could survive after deforestations in wet areas along the canals.

The surface activity of the mature terrestrial isopods fluctuates in time. It has been generally observed that after reproductive periods a high mortality may occur in terrestrial isopod populations (see in: WARBURG *et al*., 1984). In our case, a slight decrease could be observed in the frequency and relative abundance of all species after the first sampled month. An increase of the abundance was observed at the common species (*T. rathkii, T. nodulosus* and *A. vulgare*), starting with June, which varies afterwards until the end of the study period depending on species. High abundance and species diversity was registered in the summer months (June, July) when females with marsupium were present. Females surface activity increases during reproduction when they are looking for shelters for their (marsupial) progeny (DANGERFIELD & HASSALL 1994). A higher density and species' richness was observed in the summer months also in other studies (SFENTHOURAKIS *et al*., 2005). In case of *T. rathkii* and *T. nodulosus,* the relative abundance was high at the beginning of the study. On the contrary, the relative abundance increased at *A. vulgare* towards the end and was maintained high until the end of the study. This could be a result of the species` thermophilic character (RADU, 1983), having an increased tolerance towards high temperatures (TOMESCU & RADU, 1971).

Acknowledgements

In this way we would like to express our gratitude to Dr. Nicolae Tomescu for his valuable comments on this manuscript.

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Received: 12.03.2012
Accepted: 02.04.2012

Union of Scientists in Bulgaria – Plovdiv
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