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Alluvial Gravel Bars as an Example of Habitat of the Widest Ecological Spectrum in the Mountain Regions – A Case of Carpathians, Southern Poland

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Abstract. Alluvial stream-bank gravel bars are one of the most interesting types of habitat in montane regions. The flora of two streams representative of Eastern and Western Carpathians were analysed. Both valleys are similar in respect to geomorphological shape, length and the influence of anthropopressure, but different in respect to vegetation adjacent to stream-bed and flora richness. Both streams were divided into three sections. Ecological differentiation of analysed gravel flora in both stream valleys was expressed by the share of elements of different syntaxa (and EUNIS types of habitat) and in the other hand by species characterised with selected ecological indices (pH, humidity, trophism and soil dispersal). This paper is focused on proving that heterogeneity of gravel bars as habitat which is caused by frequent submergences makes the close coexistence of plants with extremely different habitat requirements possible. The results showed that the flora of gravel bars of both streams in spite of their floristic differences and regardless of floristic richness represents almost entire ecological spectrum.

Key words: alluvial habitats, gravel bars, montane flora of streambanks, ecological differentiation.

Introduction

Alluvial habitats, especially those located near the stream current that are flooded each season, are one of the most untypical and biologically differentiated habitats (NILSSON, 1987; BANÁSOVÁ *et al.*, 1994; EDWARDS *et al.*, 1999; UZIĘBŁO & CIAPAŁA, 2006; ELLENBERG, 2009). Periodically changeable surface of area available for plants, the changeable structure of the dispersal of the soil substratum that is dependent on time and the intensity of submergences, unstable species composition, distribution and its distinct dependence on both the quality of adjacent vegetation and floods, are the main characteristics of gravel bars. Many of species occur on gravels as single individuals, sometimes for a number of seasons and sometimes ephemerally for only one season. They are able to change their localities as well. A species may occur in the upper part of stream valley in one season and it may spread into lower sections of valley in the next, disappearing from primal location (UZIĘBŁO, 2001; 2011).

Alluvial areas located far from the stream, which are rarely and less intensively flooded, are ecologically more stable and covered by vegetation typical for the distant zone from the stream-bed (NILSSON *et al.*, 1989; VERVUREN *et al.*, 2003). The vegetation on analysed area (the lower montane forest zone) is formed by riparian forests, mainly Carpathian alder tree (*Alnetum incanae*). Near the stream current, the most common

unforested community is tall-herbs with glabrous butterbur (*Petasitetum kablikiani*) (UZIĘBŁO, 2011).

In this altitudinal zone there is no stable community on gravel bars nearest the stream current. They may be flooded several times during a vegetation season after each torrential rain. Therefore, in V-shaped valleys, the surface of gravels is very changeable and not very large. In the lower parts of valleys, where streams become montane rivers with wide bottoms, the vegetation reveals specific zonation ranging from alluvial meadows through thickets with *Calamagrostis pseudophragmites* or *Myricaria germanica* (*Epilobietalia fleischerii*), willow thickets and further riparian forests with willows (*Salicetum albo-fragilis*), (MATUSZKIEWICZ, 2012). The objects of this study were only gravels – open habitats nearest to the stream current in the altitudinal zone of mixed and deciduous forests – which were in different stages of initial succession and which were regularly flooded. The aim of the study was to show the ecological spectrum of flora (syntaxa analysis and ecological indices analysis) and in the same way to indicate important role of these habitats in maintaining local biodiversity in the natural environment of mountains.

Material and Methods

Floristic investigations were carried out (with appropriate permissions) in two national parks, biosphere reserves, in two streams which flow through valleys with similar geomorphological profiles and have springs located high in the mountain (subalpine zone). Studies were carried out in southern Poland on the gravels of the Terebowiec stream valley in the Bieszczady National Park, Eastern Carpathians in 2008-2012 and along Rybny stream and its environs in the Babia Góra National Park, Western Carpathians in 2009-2013. The highest located parts of V-shaped valleys were omitted because of lack of habitats of our interest. Places, where in low water stage exposed gravels, were included into the study. Both streams are formed in Carpathian flysch and their valleys are similarly shaped. The stream-beds were arbitrarily divided into three sections according to combination between types of vegetation and synanthropisation sources – upper, middle and lower. The first and last sections are, or were some time ago, influenced by human activity that indicated the possibility of the occurrence of synanthropic flora (Table 1).

Both streams are mainly surrounded by forest vegetation (Carpathian alder forest *Alnetum incanae*, Carpathian beech forest *Dentario glandulosae-Fagetum* in the case of Terebowiec stream and mainly spruce monocultures in the case of Rybny stream) in the middle sections. The anthropopression occurs in the upper and terminal sections of the stream-beds. In these parts of the valleys, adjacent to the stream-bed, vegetation is formed by meadows and bushes (open habitats).

Each year lists of gravel flora were complemented at the turn of spring and summer seasons because this is the period between the two highest water stages, thus the composition of species is relatively stable and it makes it possible to obtain a full list of the species growing on gravels.

Ecological differentiation was expressed by variety of noted flora in aspect of ecological requirements (ecological indices; ZARZYCKI *et al.*, 2002) and connections with different types of phytocoenoses (syntaxa). Widely used Ellenberg's ecological indices (ELLENBERG *et al.*, 2001) do not take into account some Carpathian species (especially Eastern Carpathian's flora which occurs in the Terebowiec valley) therefore Zarzycki's scale (which is compatible with Ellenberg's) was used in this paper not to avoid any representatives of Polish flora. In the case of a wide tolerance to a given environmental factor and a given range of index values, the average of extreme values was taken into the analysis. Statistical relationships between particular sections in respect to the share of diagnostic species, number of syntaxa and the share of species with extreme habitat requirements (indices 1-2 and 4-6) were determined by principal components analysis (PCA) using statistical package Statistica Software version 10 (STATSOFT INC., 2011). The syntaxonomical

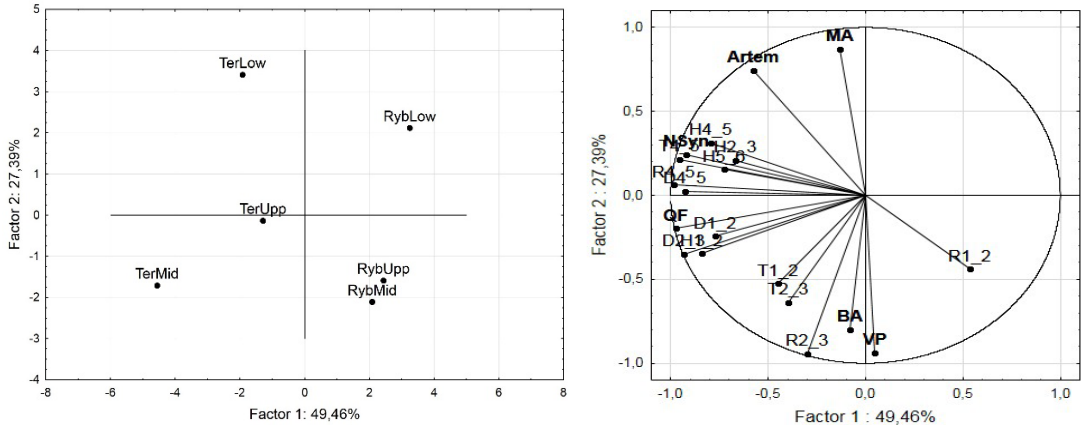
affinity of the vascular plants noted was adopted after DAVIES *et al.* (2004) and MATUSZKIEWICZ (2012), and nomenclature of species after MIREK *et al.* (2003). In the list of syntaxa represented in analysed type of habitat both EUNIS classification (DAVIES *et al.*, 2004) and classification commonly used in Poland (MATUSZKIEWICZ, 2012) were used in this paper. For comparison analysis of habitats published phytosociological data were used (KASPROWICZ, 1996; MICHALIK & SZARY, 1997; DENISIUK & KORZENIAK, 1999; ZEMANEK & WINNICKI, 1999; UZIĘBŁO, 2011). The length of the sections and the altitude above sea level was calculated using a GPS receiver.

Table 1. Characteristics of streams analysed.

Name of the stream	National Park	Locality of springs	Section	Altitude [m a.s.l.]	Section lenght [km]	Streambank vegetation	Sources of synanthropisation in vicinity of stream bed
Terebowiec The highest point of area studied 49°05'50.19"N 22°42'57.93"E	Bieszczady	N slope of the Tarniczka Mt. about 1200 m a.s.l.	Upper	840-805	1	Tall-herbs with glabrous butterbur (*Petasitetum kablikiani*), fertile beech forest (*Dentario glandulosae-Fagetum*)	Inactive timber storage area and quarry, strict reserve
			Middle	805-720	2.5	Tall-herbs with glabrous butterbur (*Petasitetum kablikiani*), fertile beech forest (*Dentario glandulosae-Fagetum*), riparian forest (*Alnetum incanae*)	Lack Strict reserve
			Lower	720-655	2	Tall-herbs with glabrous butterbur (*Petasitetum kablikiani*), meadow communities (*Arrhenatherion*)	Buildings
Rybny The highest point of area studied 49°35'52.99" N 19°32'34.59" E	Babia Gora	N slope of the Babia Gora Mt. 1445 m a.s.l	Upper	828-785	1	Tall-herbs with glabrous butterbur (*Petasitetum kablikiani*), meadow communities (*Arrhenatherion*)	Active timber storage area
			Middle	785-705	2.5	Tall-herbs with glabrous butterbur (*Petasitetum kablikiani*), riparian forest (*Alnetum incanae*), spruce monocultures	Lack
			Lower	705-670	1.5	Tall-herbs with glabrous butterbur (*Petasitetum kablikiani*), riparian forest (*Alnetum incanae*)	Buildings, road, streambank regulation

Results

Analysis of relationships between particular sections of both streams in respect to analysed parameters (PCA) revealed that they differ in respect to the share of species diagnostic for particular syntaxa, the degree of species variation expressed as a number of represented syntaxa, as well as the share of species with extremely different ecological requirements (Fig. 1a,b). The Rybny stream is distinguished with species of very acid habitats. Both streams weakly differ in respect to the contribution of tall-herbs species (*Betulo-Adenostyletea*) and species from *Vaccinio-Piceetea* class, but more significantly in respect to other classes. Lower sections of both streams are characterized mostly by greater share of meadow and synanthropic species (*Molinio-Arrhenatheretea* and *Artemisietea vulgaris*), and also by larger heterogeneity of flora (number of syntaxa) and the share of species of fertile, alkaline, heavy loams and claim habitats with entire spectrum of humidity as well. Upper and middle sections, particularly of Terebowiec stream, are dominated by the species of deciduous forests (*Querco-Fagetea*), and species of rocky and gravel habitats, rather poor and lightly acidic soils (Fig. 1a, b).



a) b)

Fig.1. a,b. Differentiation of particular sections of streams analysed in respect to vegetation parameters (PCA)

Abbreviations: RybUpp – Rybny stream upper section, RybMid – Rybny stream middle section, RybLow – Rybny stream lower section; TerUpp – Terebowiec stream upper section, TerMid – Terebowiec stream middle section, TerLow – Terebowiec stream lower section; Q-F – *Querco-Fagetea*, V-P – *Vaccinio-Piceetea*, B-A – *Betulo-Adenostyletea* (syn. *Mulgedio-Aconitetea)*, M-A – *Molinio-Arrhenatheretea*, Artem – *Artemisietea vulgaris,* NSyn – number of syntaxa; ranges of ecological indices: T – trophism, D – soil dispersion, H – soil humidity, R – pH

The flora of the entire part of Terebowiec stream that was analysed amounts 211 species, and amounts to only 154 on Rybny stream. The differences are less clear when division into particular sections is taken into account. 117 species were noted on gravels in the upper section of Terebowiec stream, 153 in middle section, and 142 in the lower section. In the case of Rybny stream, the number amounts to 110, 111 and 100 species, respectively (Appendix 1).

The gravel bars, thanks to a very heterogeneous structure, form microhabitats for species representing numerous extremely different ecological groups. In this study they were qualified as elements of miscellaneous syntaxa. Flora of both streams represents following types/classes: riparian woodland, mostly with dominant alder;

Fagus woodland; broadleaved swamp woodland (*Querco-Fagetea, Salicetea purpureae, Alnetea glutinosae* - EUNIS codes G1.1, G1.4, G1.6); conifer scrub close to the tree limit; coniferous woodland; mixed *Abies - Picea - Fagus* woodland; small coniferous anthropogenic woodlands (*Vaccinio-Piceetea* - F2.4, G3.1, G4.6, G5.4); swamps and marshes dominated by *Juncus effusus*; permanent mesotrophic pastures and aftermath-grazed meadows; low and medium altitude hay meadows; mountain hay meadows; moist or wet eutrophic and mesotrophic grasslands; moist or wet tall-herb meadows; (*Molinio-Arrhenatheretea, Agropyretea intermedio-repentis* - D5.3, E2.1, E2.2, E2.3, E3.4, E5.4), vegetated snow-patch; subalpine moist or wet tall-herb; subalpine deciduous scrub (*Betulo-Adenostyletea* syn. *Mulgedio-Aconitetea*) - E4.1, E5.5, F2.3); constructed, industrial and other artificial habitats (*Artemisietea vulgaris* - J); dry acid and neutral closed grassland (*Nardo-Callunetea* - E1.7); springs, spring brooks, *etc*. (*Montio-Cardaminetea* - C2.1), recently felled areas mostly clearings (*Epilobietea angustifolii* - G5.8); recently abandoned cultivated areas (*Stellarietea mediae* – I2.3); water-fringing reed beds and tall helophytes (*Phragmitetea* - C3.1); poor fens and soft-water spring mires (*Scheuchzerio-Caricetea nigrae* - D2.2); scree vegetation (*Asplenietea rupestria* - H2); periodically inundated shores with pioneer and ephemeral vegetation; (*Bidentetea tripartiti* - C3.5), perennial calcareous grassland (*Festuco-Brometea* - E1.2) and unvegetated or sparsely vegetated shores with non-mobile substrates or almost bare rock pavements, including limestone pavements (*Koelerio-Corynephoretea* - C3.7, H 3.5). In total 18 syntaxa and 28 EUNIS types of habitat are represented by flora of Terebowiec stream banks. In spite of floristic poverty of Rybny stream, elements of all vegetation classes mentioned above (with the exception of the last four) were noted on its gravels, and additionally *Quercetea robori-petraeae* (in total 15 syntaxa; 23 types). The middle section of Terebowiec stream had the highest number of represented syntaxa (15), while the number amounted to 11 occurred on the gravels of Rybny stream.

In total, the vegetation adjacent the stream-bed comprises more than half of the gravel flora of both streams (Fig. 2). Species of deciduous forests (*Querco-Fagetea)* are more numerous in the Terebowiec stream valley which in general more afforested than Rybny stream. The share of acidophilus species from coniferous forests (*Vaccinio-Piceetea*) is small and almost constant for both streams. Tall-herb species (*Betulo-Adenostyletea*) which gather mostly subalpine species, what is connected with locality of stream springs in the subalpine zone (Table 1), occurred in the upper and middle sections of Rybny stream (14%), and then their percentage decreases to 3%. These species occur in upper section of the Terebowiec stream valley (6%), but mostly in the middle part (8%), (springs of this stream are located 245 m lower than Rybny springs (Table 1). The contribution of meadow species (*Molinio-Arrhenatheretea)* in both streams increases in the lower sections from 22 through 19 to 31 in Rybny and from 18 through 17 to 25 in Terebowiec stream. The species from the *Artemisietea vulgaris* class (including synanthropic species) are almost double numerous in the Terebowiec stream valley than in the Rybny valley (Fig. 2) although Terebowiec valley has been taken under strict protection (since 1973), but in its case the history of anthropogenic influence is much longer and more diversified (timber storage, quarry, narrow railway and touristic route).

In analysed flora of gravel bars the most numerously represented syntaxa are simultaneously the communities adjacent to stream current or its springs (Fig. 2). It could be evidence that mentioned communities are external source of diaspores for gravel habitats by a seed rain and distant transportation. Quantitative differences in contribution of particular groups of species mentioned above reflect distribution and in certain sense also the area occupied by particular communities adjacent to stream current in both valleys.

The comparison of flora of gravel bars with flora of forest, meadow and tall-herb

habitats revealed dominance of species representing adequate classes and smaller differentiation of flora on comparative habitats (Fig. 2). Number of syntaxa on gravels of Terebowiec ranges respectively from 12 to 15, and on gravels of Rybny - from 10 to 11. The number of syntaxa on forest habitats of Terebowiec and Rybny amounts respectively five and seven, while in meadow habitats in both cases amounts eight. Flora of tall-herb habitats adjacent to Rybny springs contains species from seven syntaxa. The vegetation surrounding springs of Terebowiec is incomparable with subalpine tall-herbs from Western Carpathians because of completely different character of timberline and subalpine zone (beech forest and grasslands).

The analysis of the species composition of flora occurring on gravels of particular sections of both streams revealed that the flora represents almost entire ecological spectrum in respect to such habitat parameters as: acidity (Fig. 3a), humidity (Fig. 3b), trophism (Fig. 3c) and soil dispersal (Fig. 3d).

In reference to both streams analysed, each particular index value is represented with the exception of highest values of humidity in the Rybny stream (Fig. 3b). Therefore results of the analysis revealed that species from extremely acidic habitats and species from alkaline habitats, xerothermic and hydrophilous, extremely oligo- and eutrophic species, plants characteristic to rock slits near plants typical to heavy clay and loam coexist on one habitat and this is the main feature of ecological importance of gravel bars (Fig. 3a-d). It is obvious that the most numerous are species typical to a habitat that is moderately acidic, eutrophic, and with sandy-clay soils (the range of particular ecological indices for them amounts to 3-4).

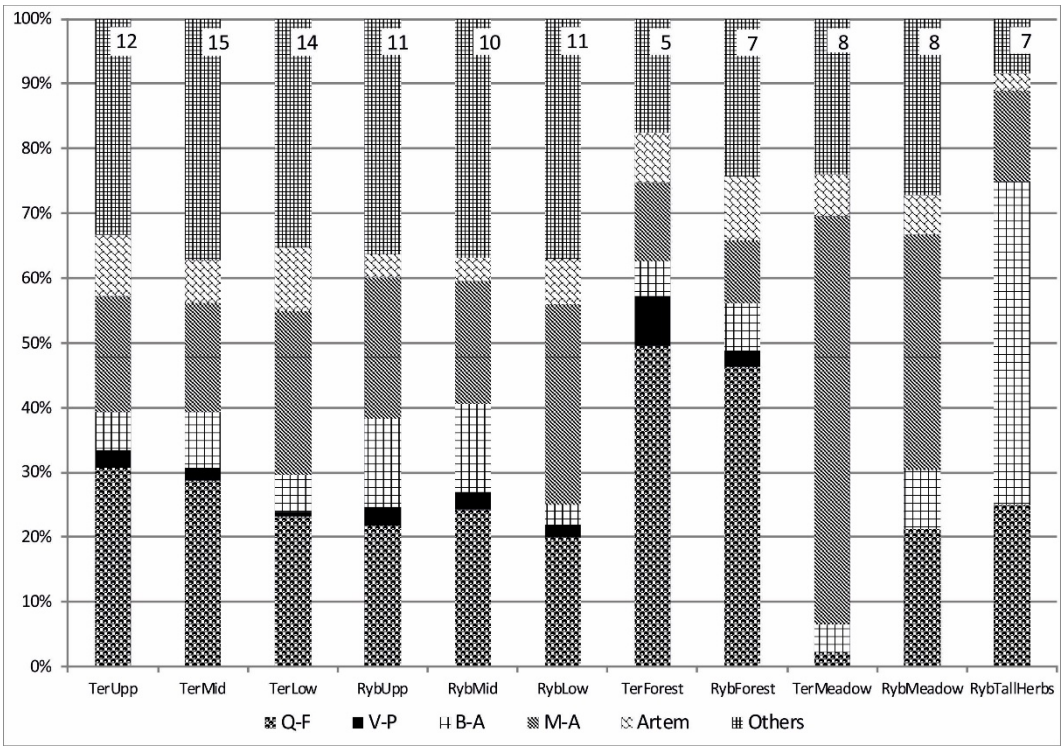


Fig.2. Comparison of differentiation of flora from gravel bars and adjacent habitats (only the most numerously represented syntaxa are distinguished; tall-herbs habitat from Terebowiec (TerTallHerbs) is excluded from the analysis – see comment in the text). Numbers in squares mean number of syntaxa.

Abbreviations: TerUpp – Terebowiec stream upper section, TerMid – Terebowiec stream middle section, TerLow – Terebowiec stream lower section; RybUpp – Rybny stream upper section, RybMid – Rybny stream middle section, RybLow – Rybny stream lower section; Q-F – *Querco-Fagetea*, V-P – *Vaccinio-Piceetea*, B-A – *Betulo-Adenostyletea* (syn. *Mulgedio-Aconitetea)*, M-A – *Molinio-Arrhenatheretea*, Artem – *Artemisietea vulgaris,* Others – syntaxa represented by a few species

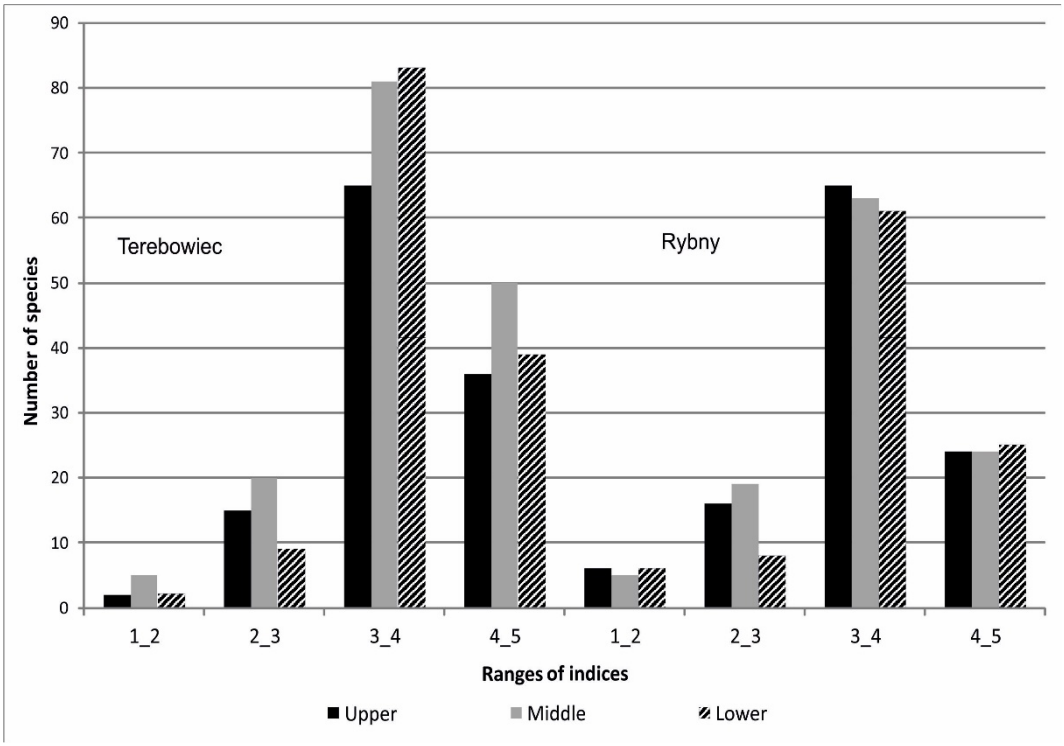


Fig.3a. Differentiation of species with various requirements to soil acidity (pH) in particular sections of streams analysed. Ranges of indices: 1_2 – strongly acid to acid, 2_3 – acid to middle acid, 3_4 – middle acid to neutral, 4_5 – neutral to alkaline

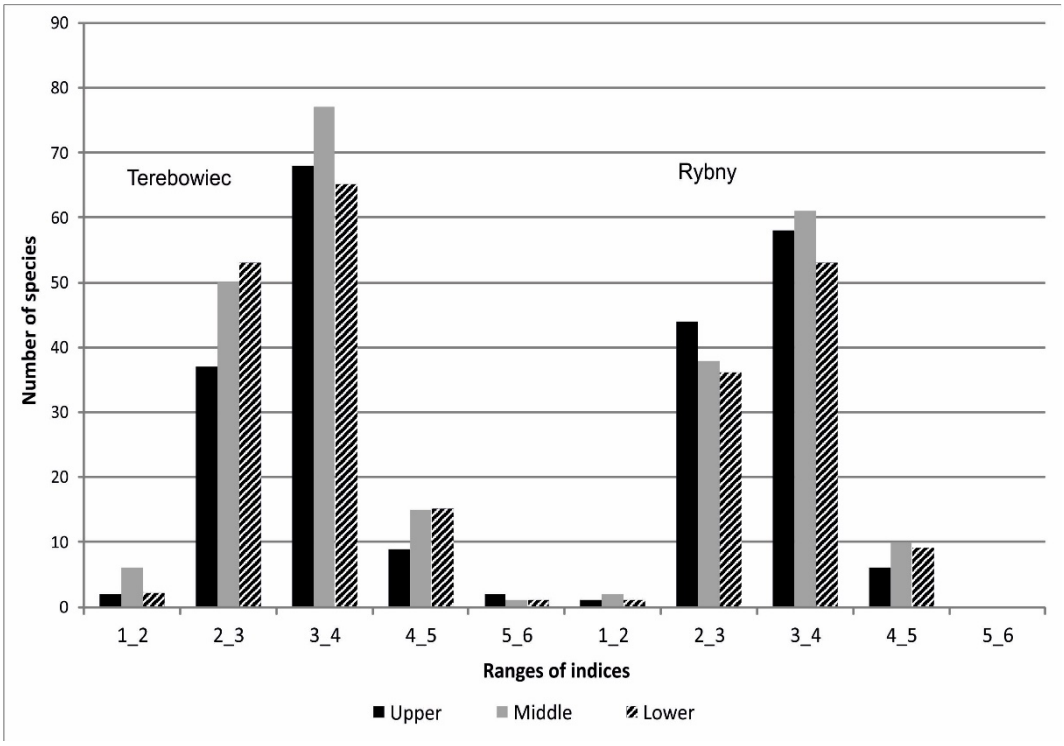


Fig.3b. Differentiation of species with various requirements to soil humidity in particular sections of streams analysed. Ranges of indices: 1_2 – dry to moderately dry, 2_3 – moderately dry to moderately moist, 3_4 – moderately moist to moist, 4_5 – moist to wet, 5_6 – wet to water

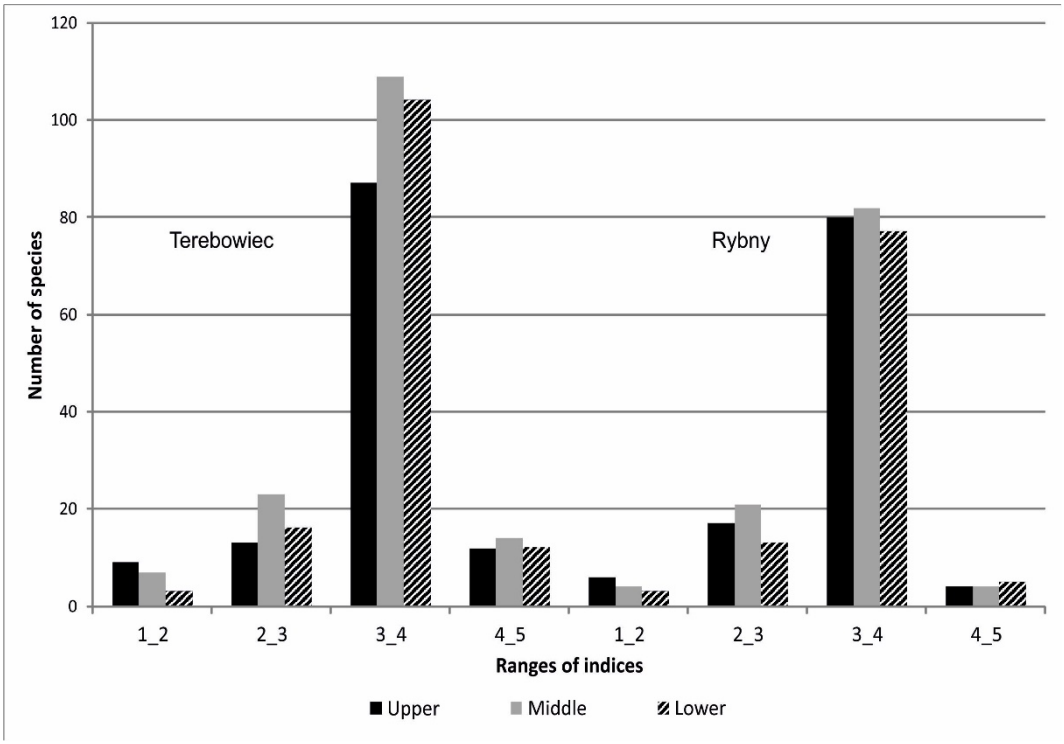


Fig.3c. Differentiation of species with various requirements to soil fertility in particular sections of streams analysed. Ranges of indices: 1_2 – extremely poor to poor , oligotrophic, 2_3 – poor to moderately poor; mesotrophic, 3_4 – moderately poor to fertile; eutrophic, 4_5 – fertile to extremely fertile

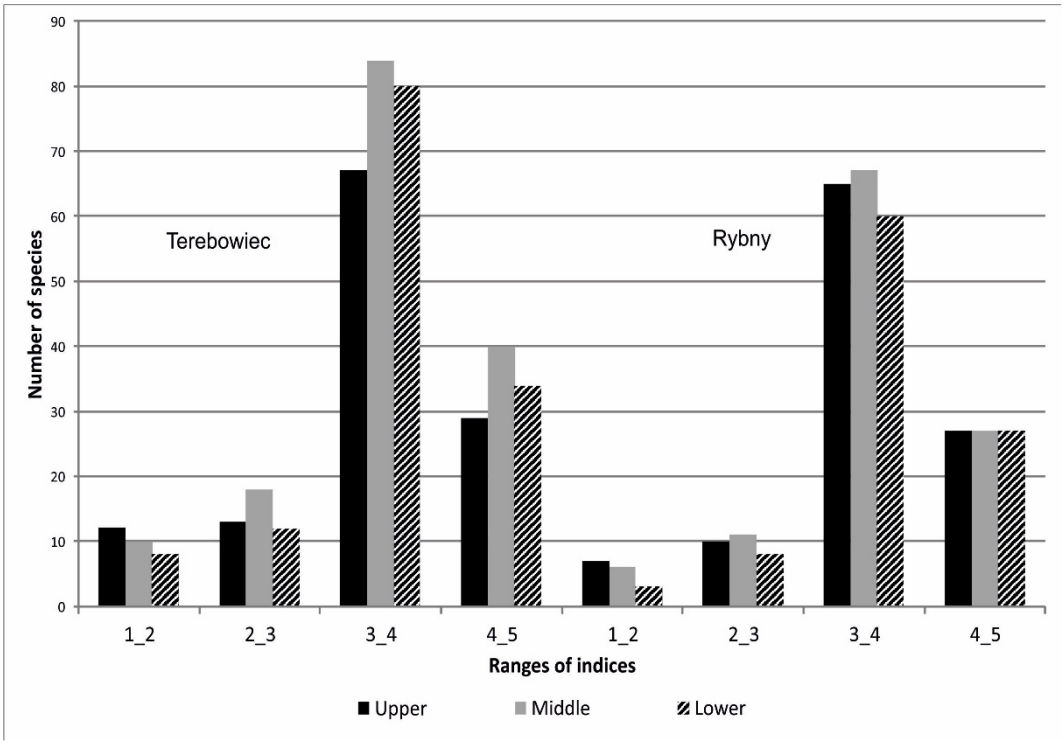


Fig.3d. Differentiation of species with various requirements to soil dispersion in particular sections of streams analysed. Ranges of indices: 1_2 – rocks, slits, debris, gravels, 2_3 – fine gravels to sand, 3_4 – sandy-loams, 4_5 – heavy loams to clays.

Summarising, it should be said that in spite of the quality and quantity differences between flora of both streams related to: (i) species richness, (ii) the adjacent communities, (iii) occurrence of species from higher altitudinal zones (tall-herbs), (Table 1, Fig. 2) the flora represents almost entire ecological spectrum that is connected with the specific character, structure and heterogeneity of the habitat.

Discussion

Alluvial gravels are one of the most interesting types of habitats in the natural environment of a montane area. Their structure resembles loose heaps formed by stones and gravels of various degrees of dispersal. In periods after floods they temporarily create a mosaic of microhabitats from small fens and silts suitable to be colonised by hydrophytes, through wet sandbanks to stony heaps with xerothermic conditions similar to those which are characteristic to rocks or dunes (BANÁSOVÁ *et al.*, 1994; EDWARDS *et al.*, 1999; DENISIUK, 2002; FRANCIS & GURNELL, 2006; UZIĘBŁO & CIAPAŁA, 2006; UZIĘBŁO, 2011).

These habitat extremes assembled over a relatively small area is conducive to forming short-lived plant configurations with unique compositions, where species of screes and xerothermic meadows are accompanied by species of deciduous forests, rushes or wet meadows (HOWARD-WILLIAMS & PICKMERE, 1994; HOLANDA *et al.*, 2005; UZIĘBŁO & CIAPAŁA, 2006). The specific conditions of a stream valley ecosystems cause that even if species appear only ephemerally for one vegetative season, they are able to undergo all life cycles and produce propagules which allow them to spread inside or outside of the valley thanks to temporal submergences or winds blowing within the interior of the valley (FRANCIS & GURNELL, 2006; UZIĘBŁO, 2011).

Flood, according to the vegetation period and the force of the flow, is an ecological factor of great importance (DENISIUK, 2002; VERVUREN *et al.*, 2003). On the one hand it washes away and deposits the sediments from silts, through sands, gravels to large stones, constantly modeling the structure of habitats of the bank zone (KRZEMIEŃ, 1976; EDWARDS *et al.*, 1999; DENISIUK, 2002; HOLANDA *et al.*, 2005); while on the other hand it transports diaspores, fragments of plants or all of the clumps of vegetation that were carried away from the upper parts of the stream valley (UZIĘBŁO, 2011). Moreover, the water that has subsided enriches poor, stony habitat with organic matter, and influences the temporal improvement of the humidity of soil from which water evaporates quickly into atmosphere (JAROLÍMEK *et al.*, 2000; FRANCIS & GURNELL, 2006). The relatively frequent influence of submergences limits interspecific competition as well, which particularly favors plants that are less resistant to this factor (BANÁSOVÁ *et al.*, 1994; EDWARDS *et al.*, 1999).

All of these factors create the possibilities for plants of extremely different habitat requirements to grow. This is very uncommon in a natural environment because most frequently species of similar requirements gather to form characteristic types of plant communities. This specific and mostly accidental species composition on gravels does not form any stable structure which could be classified as any plant association. Even *Petasitetum kablikiani* phytocoenoses, which occurred in this altitudinal zone as a dominant unforested association on alluvia, were distinguished by a very unstable species composition with over 90% sporadic species (UZIĘBŁO, 2011).

Relatively small number of species characteristic to poor and especially rocky, gravel habitats is rather surprising (Fig. 3d; indices 1-2), because we could expect that the type of habitat should have a larger group of specialized, stenotypic species. With such a range of abundance of particular ecological group of species which was noted we could state, that gravel bars can play a role of habitat for wide ecological spectrum of plants, but mainly as a vicarious or supportive habitat for forest and meadow plants in the event of any disturbances. There is an interaction between adjacent phytocoenoses and gravel bars because both of them can be a reservoir for diaspores for each other. Spreading of plants can occur in

two directions – along the valley or across it (seed bank, seed rain, distant transportation). These movements can also be a source of diaspores coming from communities other than the nearest to the stream.

Therefore the main characteristics of gravel vegetation could be defined as: significant ecological heterogeneity, unstable qualitative composition, distinct dependence on adjacent vegetation.

Both analysed streams are within the area of national parks which are biosphere reserves, thus the habitats and all natural processes are under protection. Particular attention in environmental management should be paid to naturally shaped gravel bars, especially in conditions of anthropogenic pressure outside the national parks and reserves. Legal base for protection of this kind can be found in EU Habitat Directive.

Conclusions

1. The gravel bars of montane streams create suitable conditions for plant of extremely different habitat requirements (or tolerance).

2. On alluvial gravels the highest share of flora representing adjacent vegetation is evidence that communities surrounding the streams are an important source of diaspores. Quantitative differences between the contributions of species coming from particular syntaxa reflect the local differentiation of ecosystems surrounding the streams.

3. The abundance of particular ecological groups of species indicates on a fact that in some circumstances gravel bars can play in environment a role of vicarious/supportive habitat for forest and meadow vegetation.

4. The gravel bars reveal the highest biodiversity on species level in comparison with habitats typical for mountain stream valleys (forest, meadow and tall-herb).

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Distribution and Assessment of the Nature Conservational Status of the Nature Habitat 91Z0 „Moesian Silver Lime Forests" in SCI „Svishtovska Gora" (BG0000576), Bulgaria

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Abstract. The protected zones or Site of community importance (SCI) are Natura 2000 areas which aim is to assure conditions for protection and survival of the most valuable and threatened species and habitats in Europe. Protected area BG0000576 „Svishtovska gora" is a part of Natura 2000 network in Bulgaria. It is declared mainly for the protection of habitat 91Z0 „Moesian silver lime forests" which is 5.7% from its area. The habitat includes forests with domination or co-domination of silver lime (*Tilia tomentosa* Moench.). In Bulgaria the silver lime forests are distributed mainly in Danube Hilly Plane and Northeast Bulgaria (Ludogorie). The main threats for this type of habitat are illegal cuttings, after the restitution of the forests. The aim of the present investigation is to assess the status of the habitat and to make a map of its location in the zone. The methodology for mapping and assessment of the nature conservation status of the natural habitats developed under the project: „Mapping and determining of the natural conservational status of the nature habitats and species - phase I" were used in the present research. As a result of the study the habitat distribution map (112.36 ha) was produced and its conservational status was assessed as unfavorable-unsatisfactory.

Key words: protected zone, SCI, natural habitat, mapping, assessment, state, Natura 2000.

Introduction

The Natura 2000 sites are established according to two basic for the biodiversity conservation European Council Directives - Directive 92/43/EEC for the conservation of natural habitats, wild fauna and flora and Directive 79/409/EEC for the conservation of wild birds.

The „Environment 2007 - 2013" operational program is the document, which sets the country strategic objectives and priorities in environment sector that will be financed by the European funds. It is directed to the implementation of commitments taken in the negotiation process in the sector and achievement of compliance with the EU requirements in the field of environment (EU/EDRF/CF, 2007). One of the program's priorities is axis 3: „Conservation and restoration of the biodiversity". The assessment of state, geographical distribution and mapping of Natura 2000 habitats as well as report preparation for conservation status of species and habitats included into Directive 92/43/EEC are some aims of the mentioned axis. The project „Mapping and determining of the environment conservational status of natural habitats and species - phase I", funded by Bulgarian Ministry of

Environment and Water (MOEW), covered the same targets. The present research is conducted in the frame of this project.

The aim of the current study is to assess the status and to make a map of Natura 2000 habitat 91Z0 „Moesian silver lime forests" distributed in Bulgarian protected zone BG0000576 „Svishtovska gora".

The silver lime communities in Bulgaria were subject of investigation of different authors, such as: BONDEV (1991), who determines three types lime communities – *Tilia tomentosa* forest; mixed forest of *Tilia tomentosa*, *Carpinus betulus* or *Quercus cerris*; mixed forest of *Tilia tomentosa*, *Carpinus betulus* and *Fraxinus ornus*; KALMUKOV (1987, Sofia, pers. comm.), who made investigation over breeding of the species and TZONEV (2003), who presents the syntaxonomy of silver lime communities in Bulgaria.

The results of present research are going to help National directorate for nature conservation in MOEW for continuing of the process of setting up and management of the Natura 2000 network. Habitat's distribution area and state ensure information for the aims of the assessment of the compatibility of the investment proposals, plans, programs and projects with the aims of conservation in the protected zones. Also they ensure basic data for monitoring of the biodiversity and for developing of plans for management of the protected zones.

Materials and Methods

The object of investigation is the natural habitat 91Z0 „Moesian silver lime forests" in BG0000576 "Svishtovska gora" protected zone (Fig. 1). The studied area (1917.2 ha) has a relation with BG0002083 "Svishtovsko belenska nizina" protected zone. The site is located south from the town of Svishtov and its geographical coordinates are: E 25° 35' 25" longitude and N 43°35' 29" latitude. The average altitude of the site is 141 m. The altitude of the lowest point is 33 m and of the highest - 232 m. The site is related to continental biogeographic region and it is important "stepping stone" of broadleaf forest in Danube Hilly Plane. The protected zone also falls into a developing agricultural region.

The site is declared to protect unique for the region more compact well preserved lime forests – habitat 91Z0, situated between the rivers of Osam and Yantra, which is a bridge towards the bigger massifs found in the Ludogorie. The site includes also loess steppe grasslands – habitat 6250 „Panonic loess steppe grasslands" which takes 2.3% of the site and habitat 91H0 „Panonic forests with *Quercus pubescens"*, occupying 0.05% of the site (Standard data form, MOEW).

The habitat includes forests with domination or co-domination of silver lime (*Tilia tomentosa* Moench.). In Bulgaria the silver lime forests are distributed mainly in Danube Hilly Plane and Northeast Bulgaria (Ludogorie) as well as more restricted in East Balkan at 50-60 to 800-1000 m a.s.l. They can be found in the hilly regions over loess or limestone. They occupy mainly the hills with north and east exposition with slope of 5 to 45°. The soils are Kastanik chernozems, Phaeozems and Luvisols. They have a well-developed humus horizon with high moisture. There is not real summer drought in these forests because of the lower evaporation and lower summer temperatures. Their high coverage does not allow forming of rich herb layer which could increase the transpiration and the soil drying additionally. Depending on local conditions these plant communities can be mesoxerophytic to xerophytic. The lime forests are mainly mono dominant. The main species in the tree layer is *Tilia tomentosa* and relatively often *Acer campestre*, *Fraxinus ornus*, *Quercus cerris*, *Q. robur* can be observed. The shrub layer isn't formed, only *Staphylea pinnata* as shade tolerant species can be seen more often at the slopes of the humid ravines. The other shrub species which participate in the composition of the lime communities are: *Berberis vulgaris*, *Cornus mas*, *C. sanguinea*, *Corylus avellana*, *Crataegus monogyna*, *Ligustrum vulgare*, *Viburnum lantana*. The herb layer is not developed, except some shade tolerant species as: *Arum maculatum*, *Buglossoides purpurocaerulea*, *Dactylis glomerata*, *Hedera helix*, *Geum urbanum*, *Melica uniflora*, *Melittis*

melissophyllum, *Ruscus aculeatus*, *Ruscus hypoglossum*. The mass development of spring ephemerides as *Anemone ranunculoides*, *Convallaria majalis*, *Corydalis bulbosa*, *Ranunculus ficaria* and others is very typical. Some geophytes of this group are developed at the end of the spring, as: *Cephalanthera damassonium*, *Lilium martagon*, *Limodorum abortivum*, *Platanthera chlorantha*. The silver lime communities in Middle Danube Plane are represented by endemic association *Staphyleo-Tilietum tomentosae*. They are relicts of the time of maximum distribution of the mesophytic forest vegetation (during Atlantic period of Holocene) in the Danube plane (KAVRAKOVA *et al.*, 2009).

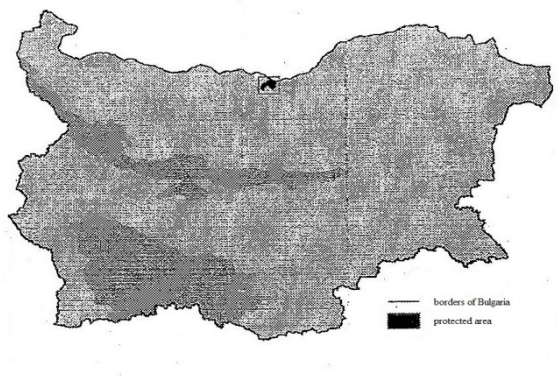

Fig. 1. Indicative map of Protected zone "Svishtovska gora" in Bulgaria.

Field work

The methodology of mapping and assessment of the nature conservation status of the habitats in Bulgaria is developed under the project: „Mapping and determining of the nature conservational status of the natural habitats and species - phase I" (GANEVA, 2013). The field work is carried out during 2011. Data of specific points and tracks were gathered at field and then polygons were built in GIS environment. The routes for field work were determined in advance because of the specification of distribution of the forest habitat in the zone. The habitat 91Z0 was ranged over the investigation of whole area located between villages of Oresh and Tzarevetc and Svishtov Town. The GPS coordinates were taken after checking 20 presented on the forest deductive model map sections. Relevés were carried out in selected typical parts of the habitat as well as some parameters for assessment of the nature conservational status were taken in the field work. The Braun - Blanquet (MAAREL, 1979) abundance assessment scale was used.

Methods of mapping

The preliminary created deduction model for the habitat distribution regarding the ecological criteria including: distribution (through the whole country), origin of the stand (seed or coppice stand), tree species (silver lime, first tree layer, density more than 40%) was used. The data is taken from the electronic database of Sylvicultural projects of Bulgarian forest territory, forest areas - level section, scale 1:10000. The field tracking around the zone was carried out to verify some areas of the deductive model. A map of the verified territory was made. The inductive model of the habitat distribution was generated on the base of taken during the field work information and also by using the obtained deductive model. For this purpose the following layers were used: GPS points where the habitat was registered – the centers of sample plots, where the plant communities' relevés were made or centers of verified forest sections, which confirm deduction model; all the habitat deduction models, which were done in the zone, without the checked polygons, which do not confirm the habitat presence. Free Maxent software for species habitat modeling, (PHILLIPS *et al.*, 2015) as well as: ESRI – ArcGIS Desktop 10 – ArcEditor, Spatial Analyst and Geostatistical Analyst (ESRI, 2011) were used for the created inductive model. The map of 91Z0 habitat distribution in the zone is created after topologic verification and joining the graphic polygons, built after the verification of polygons from deduction model during the field tracking. The mapped polygons on field and the received by the inductive model polygons are presented with different colour on the final habitat distribution map.

Methods of assessment of the nature conservational status

The assessment of the nature conservational status (NCS) was made on the base of specific criteria, presented by parameters with corresponding values. These values can be quantified or to be based on principle of "the best expert assessment". The obligatory criteria for determining of NCS at protected zone level are: habitat area in the zone, structure and functions, and future perspectives. If all parameters have favorable assessment than the assessment of the criterion is favorable. If one of the parameters is assessed as unfavorable – unsatisfactory or unfavorable – bad state than total assessment of the criterion is unfavorable – unsatisfactory or unfavorable – bad state respectively. And this determines the total assessment of the nature conservational status of the habitat in the zone.

Results and Discussion

The area of the 91Z0 nature habitat in the SCI "Svishtovska gora" is 112.43 ha according to the deduction model. The verified territory is 67.14 ha and 66.18 ha are confirmed as the area of 91Z0 natural habitat. The inductive model for distribution of the habitat is generated. There are not cases of fire recorded on the territory occupied by habitat (according to the information of GD "Fire Safety and Protection of Population", Bulgarian Ministry of Interior: Database of fires in Bulgaria, 2009 – 2011).

The created habitat distribution map in the borders of the zone is presented on Fig. 2. The total habitat area in the zone is determined of 112.36 ha. The areas generated after field investigations are shown with dark green color and they represent 59% from the total habitat territory. The territories obtained through inductive model which are 46.25 ha or 41% from the total habitat territory are given with light green color.

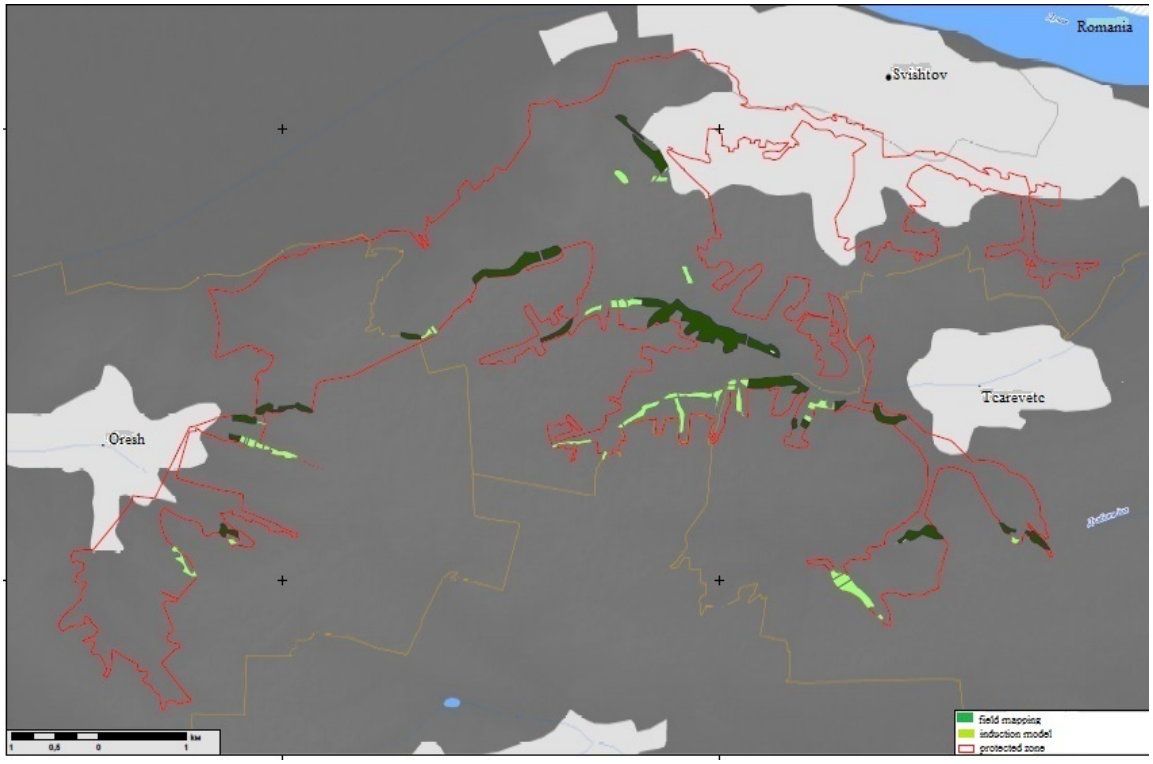

Fig. 2. Distribution of 91Z0 habitat in SCI "Svishtovska gora".

Assessment of the nature conservational status

Criterion 1. Area in the zone

Parameter 1.1. Habitat area in the zone

The present mapping showed that habitat 91Z0 is located on the area of 112.36 ha, which coincides with the referent value. The referent value for the distribution of 91Z0 in the zone according to the Standard data form is 112.4 ha (5.8% from 1917.2 ha). The assessment of this parameter shows favorable status as the area of the habitat is constant or not less than the referent area.

Criterion 2. Structure and functions

Parameter 2.1. Cover of the first tree layer

The coverage of tree layer is about 80%. It shows favorable state of the stands according to this parameter because the coverage is more than 60%. The assessment is favorable state.

Parameter 2.2. Composition of tree layer

The silver lime (*Tilia tomentosa* Moench.) is dominant in the tree layer composition with participation more than 5. In the layer composition take part also *Acer platanoides* L., *Acer campestre* L., *Fraxinus ornus* L. and *Acer tataricum* L. with lower than the dominant participation. The presence of typical species in opportune proportions is a base for the favorable assessment of parameter.

Parameter 2.3. Average age of tree layer

The average age of tree species in the first layer is around 60 years. It leads to assessment of their nature conservation status as unfavorable-unsatisfactory according to the recommendation of more than 60 years stand age for this type of forest.

Parameter 2.4. Old age forests

The old forests on the habitat's territory in the protected area were not observed that is not positive for the nature conservation status of studied habitat. The state of parameter can be modified in future by changing the land use management. This formulation enables us to assess the parameter as unfavorable-unsatisfactory state.

Parameter 2.5. Dead wood quantity

The age of the main tree species in the habitat is not enough for forming of essential quantities of dead wood. Around 6 m^3 of such wood were registered at some of visited polygons and about 6 numbers per ha were stand dead trees. These quantities however are not enough for giving the positive assessment. Regarding this parameter, assessment is unfavorable-unsatisfactory state.

Parameter 2.6. Presence of old trees (at least 20 years older than average stand age)

In the past incorrect high intensity cuttings had been carried on, which has determined to low age of the forest and lack of old trees. This parameter also can be changed in future through appropriate sylvicultural management that allows assessing the parameter as unfavorable-unsatisfactory state.

Parameter 2.7. Herb layer

The shrub layer is not formed. Only *Staphylea pinnata* L. and *Ligustrum vulgare* L. can be found more often at the humid slopes. The herb layer is not formed except some shade like species as: *Hedera helix* L., *Carex pilosa* Scop., *Polygonatum latifolium* (Jacq.) Desf., *Pulmonaria officinalis* L. These species are typical for this habitat. The given assessment in regards of this parameter is favorable state.

On the base of the assessed parameters the total assessment of criterion 2 is unfavorable-unsatisfactory state.

Criterion 3. Future perspectives (threats and influences)

Parameter 3.1. Incorrect cuttings; disturbances; poaching

Incorrect planned and done cuttings with high intensity were obtained in the habitat territory. The private forests occupy the most parts of area and the clear cuttings have been done at some places. This parameter is assessed as unfavorable-unsatisfactory state.

Parameter 3.2. Taking out of dead wood

The protected zone is poor of forest resources, very close to settlements that lead to utilizing of any inessential fall from the trees. The assessment of parameter is unfavorable-unsatisfactory state.

Parameter 3.3. Afforestation with exotic and alien species

Afforestation with exotic and alien species on the habitat territory was not observed. The experiments about changing of tree species with *Robinia pseudoacacia* L. were conducted at some places in the near past. These experiments were unsuccessful and *Robinia pseudoacacia* L. trees were withered. The habitat restored its typical species as *Tilia tomentosa* Moench. and *Fraxus ornus* L. The parameter assessment is favorable state.

Parameter 3.4. Fires

The territory of habitat is very close to settlements and is in a region with traditionally high summer temperatures, which can be theoretical prerequisite for rising of fires. However during the field visits the traces from fires were not observed. The assessment of NCS of 91Z0 regarding this parameter is favorable state.

Parameter 3.5. Recreation and tourism

The 91Z0 areas at protected zone are small parceled out forests located between agricultural lands and are not suitable for utilization for recreation and tourism. Because of this it can be reported absence of this threat for the habitat territory. So, the assessment of parameter is favorable state.

Parameter 3.6. Building and infrastructure

The habitat territory is not appropriate for building. There have not infrastructure objects on it. The state of 91Z0 regarding this parameter is assessed as favorable.

Parameter 3.7. Pasture

It was not established the usage of the habitat for pasture during the period of NCS assessment of 91Z0 habitat as well as traces from pasture at near past. Because of the lack of this threat, the assessment of parameter is favorable state.

Parameter 3.8. Nature disturbances and trends

Traces from windfalls, snowfalls, calamities and other nature disturbances were not observed on the territory of the habitat. The assessment of parameter is favorable state.

Parameter 3.9. Non regulated and incorrect gathering of non-wood forest resources (lime flowers)

Incorrect gathering of lime flowers on the habitat territory was not observed. The assessment of parameter is favorable state.

The total assessment according criterion 3 is unfavorable-unsatisfactory state on the base of the assessed parameters.

The total assessment of the status of the natural 91Z0 habitat in zone BG0000576 „Svishtovska gora" regarding the all three criteria is "unfavorable-unsatisfactory" state. The final assessment is due to the fact that criteria 2 and 3 are assessed as "unfavorable-unsatisfactory" and only criteria 1 is assessed as „favorable".

Conclusions

In regards of discussed parameters 91Z0 habitat in SCI "Svishtovska gora" is in unfavorable state. The observations during the field mapping have shown typical species composition of tree and herb layers. Not regulated gathering of non-wood forest resources, nature disturbances and using of the habitat for pasture have not been observed. The habitat area in the zone was not been threat by building and recreation pressure, and also by fires and afforestation with alien species. The coincidence of parameter "area of distribution" with reference value was observed. The protected area is poor of forest resources and is located very close to settlements. There were incorrect cuttings with high intensity in the new past. Because of that, the insufficient average stand age, lack of old forest, separate old trees, as well as insufficient quantity of dead wood were observed. All these negative status assessments are the result from the inappropriate management. To improve the natural conservational status of 91Z0 habitat in the zone the management of habitat territory has to be directed not only to wood utilization but also to maintaining of biodiversity, for example through leaving of old trees in quantity 10 numbers per ha and leaving of withered and fall trees after the relevant cuttings in quantity 8% of the stand volume.

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Blood Cells Morphology and Erythrocytes Count of Two Freshwater Turtles, Emys orbicularis and Mauremys rivulata, from Turkey

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Abstract. In the present study, the peripheral blood cells (erythrocytes, leucocytes and thrombocytes) morphology of *Emys orbicularis* and *Mauremys rivulata* were examined in the blood smears prepared using Wright's stain and the erythrocyte number was conducted as well. In *E. orbicularis,* the average of erythrocyte length was measured as 20.1µm, width 12.7µm and size 200.8µm^2; while in *M. rivulata* these were measured as 19.3µm, 12.3µm, and 186.7µm^2 respectively. The fact that agranulocytes (lymphocytes and monocytes) were dominant cells; nucleus cannot be distinguished in eosinophiles and basophiles due to intensive granulation in cytoplasm; thrombocytes are flat, ellipsoid cells were observed in the blood smears. In 1mm^3 blood, the average erythrocyte count in *E. orbicularis* was 430,666 and 467,500 in *M. rivulata,* and the erythrocyte count was established to be higher in males.

Key words: Emydidae, Geoemydidae, blood cell morphology, blood smears, erythrocyte count.

Introduction

European pond turtle, *Emys orbicularis* (L., 1758), is distributed from Northern Africa, Southern, Central, and Eastern Europe, Caucasus to Western Asia (VAN DIJK *et al.,* 2014). Western Caspian turtle, *Mauremys rivulata* (Valenciennes, 1833), spread over from Balkan Peninsula, Middle East, and some Aegean and Mediterranean islands (UETZ & HOŠEK, 2014; VAN DIJK *et al.,* 2014). IUCN/SSC Tortoise and Freshwater Turtle Specialist Group listed *E. orbicularis* as lower risk/near threatened, while *M. rivulata* is listed in the least concern category (TFTSG, 1996; VAN DIJK *et al.,* 2004, 2014).

Even though there are numerous studies on the haematology of freshwater turtles (TAYLOR & KAPLAN, 1961; METIN *et al.,* 2006, 2008; HIDALGO-VILA *et al.,* 2007; PERPIÑÁN *et al.,* 2008; ARIZZA *et al.,* 2014), the haematology and blood biochemistry on the freshwater turtles living in Turkey is limited (UĞURTAŞ *et al.,* 2003; METIN *et al.,* 2006, 2008, YILMAZ & TOSUNOĞLU, 2010; TOSUNOĞLU *et al.,* 2011). In the present study, the blood cell morphology, size and erythrocyte counts of *E. orbicularis* and *M. rivulata* were evaluated comparatively and discussed with the literature.

Material and Methods

A total of 23 specimens, 15 *E. orbicularis* (10 male and 5 female) and 9 *M. rivulata* (4 male and 5 female), caught in İzmir (Western Anatolia, Turkey) in early April 2010 were used in the study. Peripheral blood samples were taken from the caudal veins of the caught individuals (SZARSKI & CZOPEK, 1966; ARIKAN & ÇIÇEK, 2014) and afterwards, the samples were released to the habitat they were caught from.

The blood smear preparations prepared using Wright's stain were utilized to measure the morphology and size of erythrocytes. The erythrocytes were measured using MOB-1-15x micrometric ocular. On each blood smear preparation, the length (L) and width (W) of 40 random erythrocytes and their nuclear length (NL) and nuclear width (NW) were measured (ARIKAN & ÇIÇEK, 2014). The erythrocyte sizes and their nuclear sizes were calculated according to the formulae (LWπ)/4 and (NLNWπ)/4. The cellular and nuclear shapes were compared using the L/W and NL/NW ratios, while the comparison of nucleus/cytoplasm ratio was made according to the N/C ratio. Besides, Leucocytes (lymphocytes, monocytes, heterophils, eosinophils, and basophils) and thrombocytes were also measured. The erythrocyte count was conducted as diluting solution with Neubauer hemocytometer by using standard Hayem solution (ARIKAN & ÇIÇEK, 2014). The photographs of the blood cells were taken with Olympus CX21-Altra 20 Soft Imaging system. PAST statistical program was used to perform statistical analyses (HAMMER *et al*., 2001).The means compared t-test and presented with standard deviations (SD).

Results

In the both freshwater turtles, typical shape of erythrocytes is oval, as is the case for fish, amphibians and other reptile species. Their nuclei are also oval and almost located at the centre of the cell. In Wright-stained preparations, cytoplasm are light yellowish pink and chromophilic nuclei are purplish black (Fig. 1A, B).

In *E. orbicularis,* the average length of erythrocytes was established as 20.1µm (1SD= 1.78), its width as 12.7µm (1.43) and its size as 200.8µm² (30.74), L/W ratio was identified as 1.6 (0.21); the average length of nucleus was established as 7.2µm (0.85), its width as 6.1µm (0.75), and its size as 34.7 µm² (7.04), the NL/NW) ratio was established as 1.2 (0.15) (Table 1). Whereas in *M. rivulata,* the average length of erythrocytes was calculated as 19.3µm (1.99), its width as 12.3µm (1.39), its size as 186.7µm² (34.53), L/W ratio was calculated as 1.6 (0.19); the average length of nucleus was calculated as 6.7µm (0.72), its width as 5.9µm (0.67), its size as 31.0µm² (5.82), NL/NW ratio was calculated as 1.1 (1.15) (Table 1).

Variation based on sex was observed in terms of values L (t= 2.33, P≤ 0.02) and L/W ratio (t= 1.99, P≤ 0.05) in *E. orbicularis,* and L (t= 7.78, P≤ 0.00), W (t= 4.46, P≤ 0.00), L/W (t= 2.04, P≤ 0.04), ES (t= 7.19, P≤ 0.00), NS (t= 1.99, P≤ 0.05), and ES/NS (t= 6.94, P≤ 0.00) in *M. rivulata.* Moreover, differences in terms of erythrocyte morphology between two species were observed [L (t= 6.69, P≤ 0.00), W (t= 4.17, P≤ 0.00), NL (t= 9.80, P≤ 0.00), NW (t= 2.98, P≤ 0.00), L/W (t= 1.74, P≤ 0.02), NL/NW (t= 4.10, P≤ 0.00), ES (t= 6.28, P≤ 0.00), NS (t= 6.31, P≤ 0.00), and ES/NS (t= 12.00, P≤ 0.00)].

Both small and large lymphocytes were observed in blood smears of two species examined. In small lymphocytes, almost the whole cell was filled with chromophilic nuclei. Cytoplasm was reduced to a small zone (Fig. 1C). In small lymphocytes, the average diameter was measured as 8.2 µm in *E. orbicularis* and as 8.5 µm in *M. rivulata* (Table 2). In large lymphocytes, spheric and chromophilic nucleus was localized in a specific area of the cell. Cytoplasmic zone was larger and was stained pale blue (Fig. 1D). The average diameter of large lymphocytes was measured as 12.1 µm in *E. orbicularis* and as 12.2 µm in *M. rivulata* (Table 2).

Monocytes were observed as kidney-shaped and as cells having dark purplish blue nuclei with Wright's stain. Cytoplasms are light grey (Fig. 1E). The average diameter of monocytes was measured as 12.6 µm in *E. orbicularis* and 11.9 µm in *M. rivulata* (Table 2). Agranulocytic cells (lymphocytes and monocytes) were observed to be dominant cells in both species. Heterophiles have usually 2-lobed nuclei; their cytoplasm was stained light blue and filled with numerous eosinophilic bright red granules (Fig. 1F). In heterophiles, the average diameter was measured as 10.7 µm for *E. orbicularis* and as 13.6 µm for *M. rivulata* (Table 2).

Table 1. The erythrocyte and their nuclei measurements established in the peripheral bloods of *E. orbicularis* (*E.o.*) and *M. rivulata* (*M.r.*). [L: Erythrocyte length, W: Erythrocyte width, ES: Erythrocyte size, NL: Nucleus length, NW: Nucleus width, NS: Nucleus size; NS/ES: Nucleocytoplasmic ratio].

Species		L (µm)	W (µm)	NL (µm)	NW (µm)	LW	NL/NW	ES (µm²)	NS (µm²)	ES/NS
E.o.	N	15	15	15	15	15	15	15	15	15
	Mean	20.1	12.7	7.2	6.1	1.6	1.2	200.8	34.7	1.4
	SE	0.08	0.06	0.04	0.03	0.01	0.01	1.30	0.30	0.10
	Min	14.0	9.3	4.7	4.7	1.1	0.8	115.1	17.0	0.1
	Max	24.5	16.3	9.3	8.2	2.3	1.8	298.3	52.2	9.9
	SD	1.78	1.43	0.85	0.75	0.21	0.15	30.74	7.04	2.38
M.r.	N	9	9	9	9	9	9	9	9	9
	Mean	19.3	12.3	6.7	5.9	1.6	1.1	186.7	31.0	0.2
	SE	0.10	0.07	0.04	0.04	0.01	0.01	1.82	0.31	0.00
	Min	14.0	9.3	5.8	4.7	1.1	1.0	115.1	21.3	0.1
	Max	23.3	16.3	9.3	7.0	2.3	1.5	298.3	51.1	0.3
	SD	1.99	1.39	0.72	0.67	0.19	0.15	34.53	5.82	0.04

Table 2. Leucocytes and thrombocytes measurements established in the peripheral bloods of *E.orbicularis* (*E.o.*) and *M. rivulata* (*M.r.*). [TL: thrombocyte length, TW: thrombocyte width].

Species		Lympho-cyte (small)	Lympho-cyte (Large)	Mono-cyte (µm)	Eosino-phil (µm)	Baso-phil (µm)	Hetero-phil (µm)	TL (µm)	TW (µm)
E.o.	N	15	15	15	15	15	15	15	15
	Mean	8.2	12.1	12.6	12.4	10.8	10.7	13.5	5.8
	SE	0.12	0.25	0.21	0.16	0.08	0.09	0.36	0.10
	Min	7.3	10.5	11.3	11.8	10.5	10.5	12.5	5.3
	Max	9.3	13.5	13.8	13.0	11.0	11.0	15.0	6.0
	SD	0.48	0.99	0.78	0.43	0.19	0.21	0.95	0.27
M.r.	N	9	9	9	9	9	9	9	9
	Mean	8.5	12.2	11.9	11.2	10.9	13.6	14.5	6.8
	SE	0.15	0.18	0.48	0.17	0.27	0.12	0.16	0.14
	Min	7.8	11.0	9.5	10.8	10.3	13.3	13.8	5.5
	Max	9.0	13.0	13.0	11.8	11.8	13.8	15.8	7.5
	SD	0.47	0.60	1.28	0.41	0.60	0.24	0.56	0.50

The cytoplasms of eosinophiles were stained light yellowish with Wright's stained. As the nucleus is masked by the large and bright granules in the cytoplasm, its shape cannot be distinguished properly (Fig. 1G). The average diameter in eosinophiles was measured as 12.4 µm for *E. orbicularis* and as 11.2 µm for *M. rivulata* (Table 2).

The cytoplasms of basophiles were filled with purplish black granules. As is the case with eosinophiles, the granules were observed to be masking the nucleus (Fig. 1H). The average diameter in basophiles was measured as 10.8 µm for *E. orbicularis* and as 10.9 µm for *M. rivulata* (Table 2).

Thrombocytes were observed as fusiform cells for both species. Almost the whole cell was filled with chromophilic nuclei (Fig. 1I). The average length of thrombocytes was established as 13.5 µm and width as 5.8 µm for *E. orbicularis,* the average length and width for *M. rivulata* were established as as 14.5 µm and 6.8 µm, respectively (Table 2).

The erythrocyte count in 1mm^3 blood (RBC) was calculated as 435714 (range= 380000 -580000) in males and as 426250 (330000-580000) in females for *E. orbicularis*. The erythrocyte count in 1mm^3 blood for *M. rivulata* was calculated as 475000 (450000-500000) and 460000 (440000-480000) respectively (Table 3). The erythrocyte count

Table 3. The erythrocyte count in 1mm^3 blood of *E. orbicularis* (*E.o.*) and *M. rivulata* (*M.r.*).

Species	Gender	N	Mean	Min	Max
E.o.	Males	10	435714	380000	580000
	Females	5	426250	330000	580000
	Total	15	430667	330000	580000
M.r.	Males	4	475000	450000	500000
	Females	5	460000	440000	480000
	Total	9	467500	440000	500000

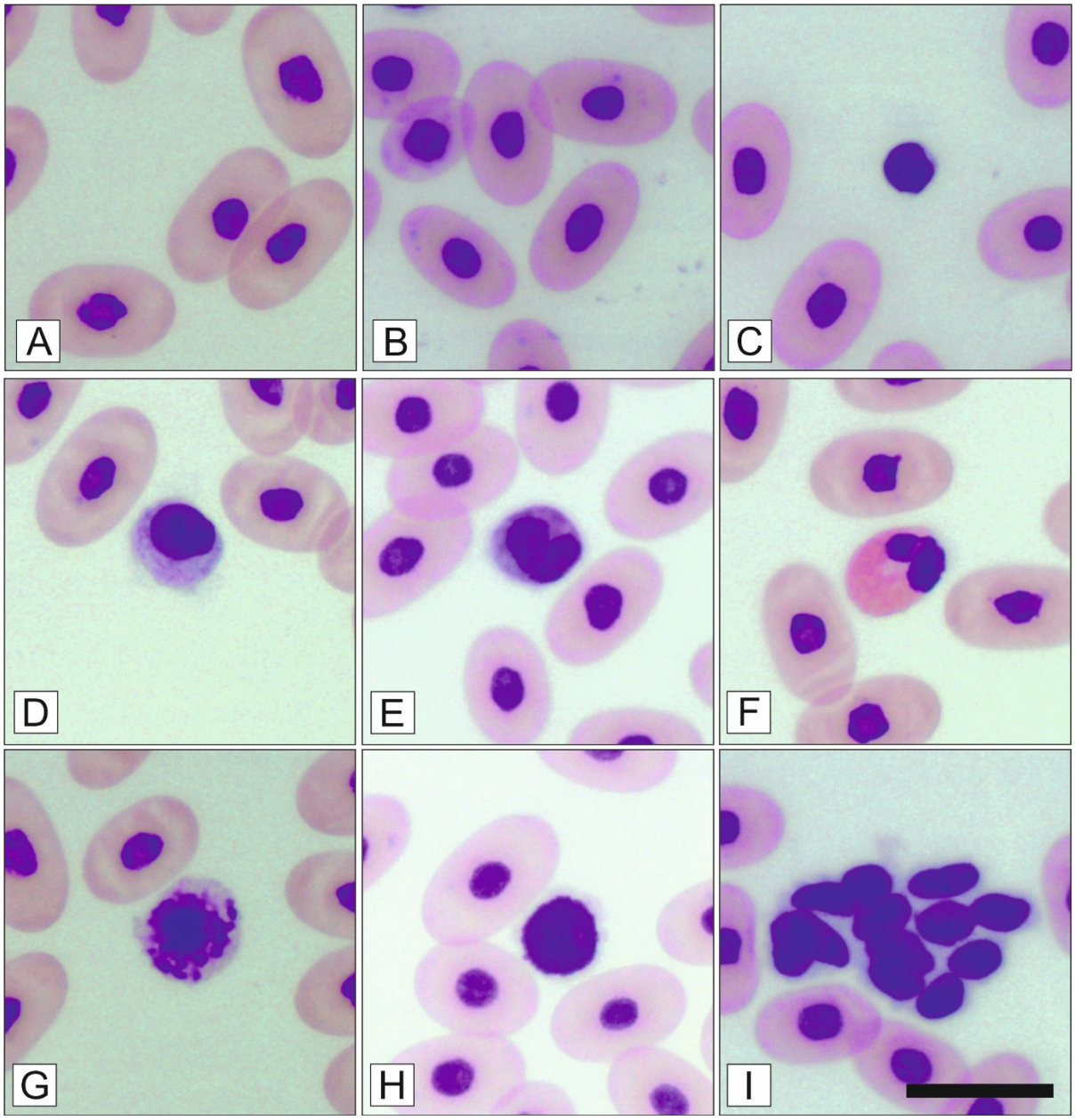

Fig. 1. Photomicrographs of peripheral blood cells in *E. orbicularis* and *M. rivulata*. A: erythrocytes (*E. orbicularis*), B: erythrocytes (*M. rivulata*), C: small lymphocyte (*M. rivulata*), D: large lymphocyte (*E. orbicularis*), E: monocyte (*M. rivulata*), F: heterophil (*E. orbicularis*), G: eosinophil (*E. orbicularis*), H: basophil (*M. rivulata*), I: a cluster of thrombocytes (*M. rivulata*). Scale (horizontal bar): 20µm.

was found to be higher in male than females for both species.

Discussion

Many researchers (e.g. HARTMAN & LESSLER, 1964; SZARSKI & CZOPEK, 1966; SAINT GIRONS, 1970; ARIKAN *et al.*, 2015) have reported that reptiles constitute a heterogeneous group among vertebrates in terms of blood cell morphology, and show significant variations between orders and even between species in the same family. In reptiles, the largest erythrocytes are found in *Sphenodon punctatus*, tortoises and alligators and the smallest in Lacertid lizards (HARTMAN & LESSLER, 1964; SAINT GIRONS, 1970).

In aquatic terrapins, the erythrocyte length was reported as 22.5µm in *Chelydra serpentine* as 22.2µm in *Trionyx spinifer*, as 21.0µm in *Emys blandingi* (SZARSKI & CZOPEK, 1966), as 20.5µm in *Pseudoemys scripta elegans* (TAYLOR & KAPLAN, 1961), as 18.6µm in *Pseudoemys ornata* (HARTAMAN & LESSLER, 1964), as 18.5µm in *Pseudoemys elegans* (HEADY & ROGERS, 1963).

The average erythrocyte length was established as 21.73 µm; width as 12.53 µm and size as 214 µm² in *E. orbicularis*; as 20.16µm, 11.64 µm, 184.30 µm² respectively in *M. rivulata* by UĞURTAŞ *et al.* (2003). In captivity conditions, 19.07µm, the values were 11.68µm, 174.95µm² for *Mauremys caspica* and 19.76µm, 11.44µm, 177.72µm² for *M. rivulata* (METIN *et al.*, 2008). In *Trachemys scripta elegans,* values of L 19.2µm, W 13.6µm, ES 204.9µm² and in *E. trinacris* values of L 22.5µm, W 14.1µm, ES 249.4µm² were recorded (ARIZZA *et al.*, 2014). In the present study, variations were observed interspecies and even in the blood smears of same species in terms of erythrocyte morphology. The average erythrocyte length was established as 20µm, erythrocyte width as 13µm and size as 201µm² for *E.orbicularis*; for *M. rivulata* these were 20µm, 12µm, 187µm², respectively.

The erythrocyte count in 1mm³ of blood was reported to be 370000 (250000-1160000) in Pascagoula Map Turtle (*Graptemys gibbonsi*); 420000 (200000 - 840000) in Southeast Asian Box turtle (*Cuora amboinensis*) (PERPIÑÁN *et al.*, 2008); 503000 (260000 – 680000 (DUGUY, 1970); in different populations of *E. orbicularis* (ALDER & HUBER, 1923); 401310 in summer and 540280 in autumn in *M. leprosa* (PAGES *et al.*, 1992), 330000 in females and 420000 in males (HIDALGO-VILA *et al.*, 2007). The average erythrocyte count in *E. trinacris* was measured as 422500 in males and 379400 in females (ARIZZA *et al.*, 2014). There is negative correlation between erythrocyte number and size (RYERSON, 1949). Our results support this hypothesis.

The erythrocyte count in *E. orbicularis* was stated to be 471594 (range= 360000 - 620000) during the breeding period and 433859 (273333 - 573333) at the end of the breeding period by YILMAZ & TOSUNOĞLU (2010). Same researchers established the erythrocyte count in *M. rivulata* as 399487 (226666-700000) during the breeding period and 391944 (165000-586666) at the end of the breeding period. The erythrocyte count, depending on the quality of water, was reported to be between 414333 – 455555 in *E. orbicularis,* and 197333–449333 in *M. rivulata* by TOSUNOĞLU *et al.* (2011).

Various researchers (e.g. HADEN, 1940; ALTMAN & DITTMER, 1961) have drawn attention to the important role various environmental factors play on the size of erythrocyte in amphibians. ATATÜR et al. (1999) stated that entirely aquatic species have bigger erythrocytes whereas terrestrial species have smaller erythrocytes. In this study, erythrocyte and nucleus sizes of aquatic terrapins, *E. orbicularis* and *M. rivulata* (Table 2) are larger than Testudinidae (ARIKAN & ÇIÇEK, 2014; ARIKAN *et al.*, 2015). Therefore, this finding confirms ATATÜR et *al.* (1999), UĞURTAŞ *et al.* (2003).

SAINT GIRONS (1970) have drawn attention to the fact that small and large lymphocytes are dominant cells in blood smears of different reptile species, in both eosinophiles and basophiles the nucleus cannot be seen clearly due to intensive granulation in cytoplasm. ARIKAN & ÇIÇEK (2014) have reached similar results in different reptile species from Turkey. In addition, ARIZZA *et al.* (2014) have stated that eosinophiles are found more in *E. trinacris*

males while lymphocytes are more present in *E. trinacris* females. In the present study, agranulocytes were observed to be dominant cells in both turtles. As in previous studies, the shape of the nucleus could not be distinguished in blood smears due to intensive granulation in eosinophiles and basophiles cytoplasm and the neutrophils were identified as rarely seen leucocytes. The thrombocyte nucleus was observed as considerably chromophilic, centrally-located and flat oval-shaped cells.

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Impacts of Climate Change on Droughts in Gilan Province, Iran

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Abstract. Drought as a complex natural hazard is best characterized by multiple climatological and hydrological parameters and its assessment is important for planning and managing water resources. So understanding the history of drought in an area is essential like investigating the effects of drought. In this study at first climate parameters affecting the drought have downscaled by LARS-WG stochastic weather generator over Gilan province in Iran. After choosing a suitable model, the outputs were used for assessing the drought situation in the period of 2011-2030. Assessing the drought was done by TOPSIS method during 2 periods (present and future). After validation of the method, zoning the drought was performed by IDW method in GIS. Results showed that the expanse of situations with lower drought index will increase. Also we will expect more droughts in these regions for the future.

Keywords: Climate change, Drought, GCM model, LARS-WG, TOPSIS method, Gilan province.

Introduction

Observed changes in the climate due to increasing greenhouse-gas concentrations have made it essential to investigate these changes. The application of General Circulation Model (GCM) results is the most current method in use in climate change studies. Although GCMs are imperfect and uncertain, they are a key to understanding changes in climate. Output from GCMs requires application of various downscaling techniques (BARROW *et al*., 1996; BARDOSSY, 1997; WILBY *et al*., 1998; MEARNS *et al*., 1999; MURPHY, 1999; SALON *et al*., 2009). One of the downscaling techniques to create daily site-specific climate scenarios makes use of a stochastic weather generator (WILKS, 1992; BARROW & SEMENOV, 1995; WILKS & WILBY, 1999; SEMENOV, 2007). Recently, weather generators have been used in climate change studies to produce daily site-specific scenarios of future climate (WILKS, 1992; MEARNS *et al*., 1997; SEMENOV & BARROW, 1997). Two important reasons for using LARS-WG model include the provision of a means of simulating synthetic weather time-series with certain statistical properties which are long enough to be used in an assessment of risk in hydrological or agricultural applications and providing the means of extending the simulation of weather time-series to unobserved locations. It has been used in various studies, including the assessment of the impacts of climate change (BARROW & SEMENOV, 1995; SEMENOV & BARROW, 1997; WEISS *et al*., 2003; LAWLESS & SEMENOV, 2005; KHAN *et al*., 2006; SCIBEK & ALLEN, 2006; SEMENOV, 2007; SEMENOV & DOBLAS, 2007; DUBROVSKY, 1996).

Owing to the rise in water demand and looming climate change, recent years have

witnessed much focus on global drought scenarios. Droughts are recognized as an environmental disaster and occur in virtually all climatic zones. They are mostly related to the reduction in the amount of precipitation received over an extended period of time, such as a season or a year (WILHITE, 1992). They impact both surface and groundwater resources and can lead to reduced water supply, deteriorated water quality, crop failure, reduced range productivity, diminished power generation, disturbed riparian habitats, and suspended recreation activities, as well as affect a host of economic and social activities (RIEBSAME *et al.*, 1991). They also affect water quality, as moderate climate fluctuations alter hydrologic regimes that have substantial effects on the lake chemistry (WEBSTER *et al.*, 1996).

Generally, drought is a phenomenon which occurs in every area or country, with either arid or humid climate and in our country. In fact, Iran's natural conditions and its geographical location are so that we have always witnessed droughts and it can be said that some of the regions are often faced with the phenomenon (KARDAVANI, 2001). However there are definitions and models for measuring the qualitative and quantitative of this phenomenon but there is no real comprehensive model to have all climatic, hydrological, agricultural, social and so on conditions and be responsive to the needs.

MADM is a practical tool for selecting and ranking of a number of alternatives, its applications are numerous. In recent years, TOPSIS has been successfully applied to the areas of human resources management (CHEN & TZENG, 2004), transportation (JANIC, 2003), product design (KWONG & TAM, 2002), manufacturing (MILANI *et al.*, 2005), water management (SRDJEVIC *et al.*, 2004), quality control (YANG & CHOU, 2005), and location analysis (YOON & HWANG, 1985). In addition, the concept of TOPSIS has also been connected to multi-objective decision making (LAI, 1994) and group decision making (SHIH *et al.*, 2001). The high flexibility of this concept is able to accommodate further extension to make better choices in various situations. This is the motivation of our study.

In this study at first changes in the climate variables are studied in Gilan, a Province in north of Iran. The output from two GCM models was compared with a stochastic weather generator, LARS-WG (Long Ashton Research Station Weather Generator) and suitable model used to produce a climate change scenario. Then TOPSIS method is used for determining and ranking of drought in the study area for 2 periods (present and future).

Materials and Methods

The study area is one of the 31 provinces of Iran. There is in the north of Iran and located in the South of Caspian Sea and has about 14000 kilometers in extent. Location of longitude is between 48 degrees 53 minutes and 50 degrees 34 minutes and latitude is between 36 degrees 34 minutes and 38 degrees 27 minutes, as shown in Fig.1. This area was selected to enable the researchers to collect data from a variety of climatic zones in the north of Iran (near the Caspian Sea). It has the best type of weather in Iran with a moderate and humid climate that is known as the moderate Caspian climate. The effective factors behind such a climate include the Alborz mountain range, direction of the mountains, the height of the area, and the Caspian Sea, vegetation surface, local winds, as well as the altitude and weather fronts. As a result of the above factors, three different climates exist in the region:

1. Plain moderate climate with an average annual rainfall amounts to 1200 or 1300 mm, decreasing to the east.

2. Mountainous climate which covers the high mountains and northern parts of the Alborz range. In the heights, the weather is cold mountainous and most of the precipitation is in the form of snow.

3. Semi-arid climate with the average annual rainfall stands at 500 mm and the average annual temperature is 18.2°C.

Firstly, the performance of the LARS-WG stochastic weather generator model was statistically evaluated by comparing the synthesized data with climatology period at

8 selected synoptic stations, based on 2 GCMs models (MPEH5, HADCM3) and 2 scenarios (A_2, B_1).

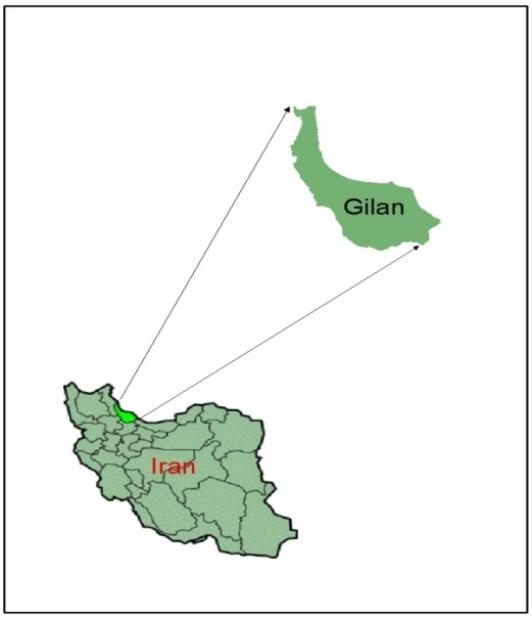

Fig. 1. Study area location

Name, latitude and longitude coordinates, as well as the elevation of the synoptic stations are shown in Table 1. After assessing LARS-WG ability in each station, it was performed for all 4 states (2 GCMs models based on 2 scenarios). Then the results were compared and the best model was chosen for predicting climatic parameters in the study area.

Table 1. Synoptic stations utilized in the study

Stations	Latitude (°N)	Longitude (°E)	Elevation (m)
Anzali	37° 29'	49° 27'	-23.6
Ardebil	38° 15'	48° 17'	1332
Astara	38° 22'	48° 51'	-21.1
Ghazvin	36° 15'	50° 3'	1279.2
Manjil	36° 44'	49° 25'	338.3
Ramsar	36° 54'	50° 4'	-20
Rasht	37° 19'	49° 37'	-8.6
Zanjan	36° 41'	48° 29'	1663

TOPSIS method is used for determining and ranking of drought in the study area. 7 Climatic Parameters consisting Precipitation in mm, Maximum and Minimum temperature in ^oC, Days with precipitation more than 0.1 mm (Number of wet days), Days with precipitation less than 0.1 mm (Number of dry days), Days with maximum temperature more than 30^oC (Number of hot days), Days with minimum temperature equal or less than 0^oC (Number of frost days) that are influencing on drought are used. Missing data are estimated by regression method and homogeneity of data is determined by Run-Test method. By using TOPSIS method and Excel software, droughts are identified and ranked in the study area for 2 periods (present and future). Then output data were compared with SIAP method. Finally, by using the interpolation method (IDW) in ArcGIS 9.3 software, zoning drought of study area is classified for 2 periods (present and future). Steps of operations can be expressed as followed:

1. Obtain performance data for 19 alternatives (Number of statistical years) over 7 criteria (Climatic Parameters). Raw measurements are usually standardized,

$$X = \left(X_{ij}\right)n \times m \tag{1}$$

2. Develop a set of importance weights wj, for each of the criteria.

$$\sum_{j=1}^{m} w_j = 1, \qquad j = 1,2,\ldots,m. \tag{2}$$

Doing this section has 4 steps:

Step 1: Determining distribution of each climatic parameter.

$$p_{ij} = \frac{r_{ij}}{\sum_{i=1}^{m} r} : \forall_{i,j} \tag{3}$$

Step 2: Calculating Anthropy for expressing amount of uncertainty in this distribution.

(4)

$$E_j = -k\sum_{i=1}^{m}\left[p_{ij}.Ln\left(p_{ij}\right)\right] : \forall_j \qquad k = \frac{1}{Ln \quad m}$$

Step 3: Calculating uncertainty for each climatic parameter.

$$d = 1 - E_j : \forall_j \tag{5}$$

Step 4: Calculating weight of climatic parameters.

$$W_j = \frac{d_j}{\sum_{j=1}^{n} d} : \forall_j \qquad (6)$$

3. Multiplying matrix X (consisting 7 climatic parameters and 19 years) in the vector Wj (weight of each climatic parameter).

4. Assimilating climatic parameters: Increasing in 4 Climatic Parameters consisting maximum and minimum temperature, Number of dry days, Number of hot days and Number of frost days and also decreasing in 2 other factors consisting precipitation and Number of Wet days are causing drought. They are respectively negative and positive index. In positive indexes, data of each year is divided on maximum amount of parameter and they are divided on minimum amount of parameter in negative indexes.

5. Identify the ideal and nadir alternative A^+, A^-: (7) and (8)

$$A^+ = \left(v_1^+, v_2^+, \ldots, v_n^+\right) = \left\{\left(\max_i \{v_{ij}\} \mid j \in B\right), \left(\min_i \{v_{ij}\} \mid j \in C\right)\right\},$$

$$A^- = \left(v_1^-, v_2^-, \ldots, v_n^-\right) = \left\{\left(\min_i \{v_{ij}\} \mid j \in B\right), \left(\max_i \{v_{ij}\} \mid j \in C\right)\right\}.$$

6. Develop a distance measure over each criterion to both ideal (S_i^+) and nadir (S_i^-).

(9) and (10)

$$S_i^+ = \left\{\sum_{j=1}^{m} \left(v_{ij} - v_j^+\right)^2\right\}^{0.5}; \qquad i = 1, \ldots, n,$$

$$S_i^- = \left\{\sum_{j=1}^{m} \left(v_{ij} - v_j^-\right)^2\right\}^{0.5}; \qquad i = 1, \ldots, n$$

7. For each alternative, determine a ratio Ti equal to the distance to the nadir divided by the sum of the distance to the nadir and the distance to the ideal,

$$T_i = \frac{S_i^-}{\left(S_i^+ + S_i^-\right)}; \qquad i = 1, \ldots, n. \qquad (11)$$

8. Rank order alternatives by maximizing the ratio in Step 7. Ti =1 is shown maximum rank and Ti = 0 is shown minimum rank. Higher Ti represents more humid conditions and lower Ti represents less humid conditions.

9. Using Standard Index Annual precipitation (SIAP) method for comparison.

$$SIAP = \frac{P_i - \overline{P}}{SD} \qquad (12)$$

Where SIAP is drought index, Pi is annual precipitation, $\overline{P}$ is mean of precipitation in period, and SD is standard deviation index of period.

Results and Discussion

Model validation is one of the most important steps of the entire process. The objective was to assess the performance of the model in simulating climate at the chosen site to determinate whether or not it is suitable for use. Firstly, LARS-WG model was performed based on the historical climate data obtained from 1992-2010 for verification of the model. A large number of years of simulated daily weather data were generated and were compared with observed data by using the t-test. The mean monthly correlation of the precipitation, minimum and maximum temperature and solar radiation were acceptable in 0.05 level of confidence.

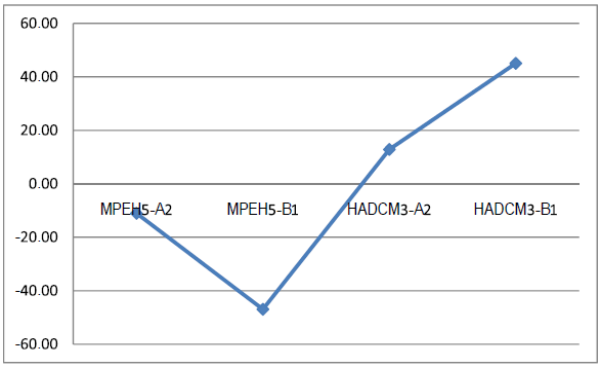

Fig. 2. Comparison between 2 models based on 2 scenarios

Then for selecting the suitable GCM model, LARS-WG stochastic weather generator model was performed for MPEH5 and HADCM3 models in A_2 and B_1 scenarios. As the Fig. 2 shows, between

these 4 states, MPEH5 model based on A_2 scenario that has the least difference with the mean of models has selected and used for predicting the future climate.

The contribution of each climatic parameter in drought is different. So at first, it needs to determine weights for each of the criteria. Sum of the climatic parameters weight is equal to 1. Results are shown respectively in Table 2 and 3 for present and future period.

According to Table 4 and 5, distance of each year from ideal and nadir are determined for present and future period.

Results of calculating ratio Ti respectively are shown in Table 6 and 7 for present and future period.

At the end, by using the interpolation method (IDW) in ArcGIS 9.3 software, zoning drought of study area is done for 2 periods (present and future) that are shown in Fig. 3.

Table 2. Weight of climatic parameters in stations of study area (1992-2010).

Station	Precipitation	T min	T max	Wet days	Dry days	Hot days	Frost days
Anzali	0.0141	0.0015	0.0011	0.0045	0.0013	0.3616	0.6158
Ardebil	0.0705	0.2790	0.0126	0.0159	0.0018	0.5776	0.0426
Astara	0.0535	0.0056	0.0034	0.0197	0.0058	0.3737	0.5383
Ghazvin	0.4430	0.0580	0.0137	0.1902	0.0106	0.1019	0.1828
Manjil	0.1462	0.0034	0.0047	0.0972	0.0062	0.1265	0.6157
Ramsar	0.0450	0.0021	0.0010	0.0046	0.0009	0.3058	0.6406
Rasht	0.0865	0.0047	0.0026	0.0273	0.0079	0.1581	0.7131
Zanjan	0.3093	0.2806	0.0243	0.1126	0.0085	0.1596	0.1052

Table 3. Weight of climatic parameters in stations of study area (2011-2030).

Station	Precipitation	T min	T max	Wet days	Dry days	Hot days	Frost days
Anzali	0.0658	0.0060	0.0047	0.0361	0.0081	0.2360	0.6433
Ardebil	0.1683	0.4246	0.0220	0.0255	0.0018	0.3476	0.0101
Astara	0.2091	0.0176	0.0110	0.0685	0.0162	0.1274	0.5503
Ghazvin	0.6350	0.0558	0.0145	0.2122	0.0108	0.0096	0.0620
Manjil	0.1906	0.0050	0.0071	0.0453	0.0016	0.0182	0.7321
Ramsar	0.1708	0.0081	0.0040	0.0359	0.0062	0.0603	0.7148
Rasht	0.1421	0.0072	0.0040	0.0568	0.0097	0.0359	0.7444
Zanjan	0.4407	0.2958	0.0302	0.1132	0.0080	0.0775	0.0345

Table 4. Distance measure over each criterion to both ideal (S_i^+) and nadir (S_i^-)-(1992-2010).

	Anzali		Ardebil		Astara		Ghazvin		Manjil		Ramsar		Rasht		Zanjan	
S_i^+, S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-
1992	0.04	0.14	0.01	0.07	0.03	0.04	0.01	0.02	0.03	0.05	0.14	0.04	0.07	0.01	0.01	0.02
1993	0.02	0.17	0.02	0.06	0.04	0.04	0.01	0.02	0.05	0.03	0.06	0.09	0.05	0.02	0.01	0.02
1994	0.03	0.16	0.01	0.08	0.04	0.04	0.00	0.02	0.04	0.04	0.04	0.11	0.03	0.04	0.01	0.02
1995	0.01	0.19	0.01	0.07	0.02	0.06	0.02	0.00	0.02	0.06	0.01	0.13	0.01	0.07	0.01	0.01
1996	0.01	0.19	0.03	0.05	0.02	0.05	0.00	0.02	0.03	0.05	0.02	0.13	0.03	0.04	0.01	0.01
1997	0.03	0.16	0.03	0.05	0.03	0.04	0.02	0.00	0.05	0.03	0.05	0.11	0.07	0.01	0.01	0.01
1998	0.04	0.16	0.03	0.05	0.02	0.05	0.01	0.01	0.03	0.05	0.04	0.11	0.05	0.02	0.01	0.01
1999	0.02	0.18	0.03	0.06	0.01	0.06	0.02	0.01	0.01	0.08	0.02	0.14	0.01	0.07	0.02	0.01
2000	0.03	0.18	0.04	0.04	0.02	0.05	0.01	0.01	0.02	0.06	0.02	0.13	0.02	0.05	0.01	0.01
2001	0.04	0.15	0.04	0.04	0.04	0.03	0.02	0.01	0.02	0.07	0.03	0.11	0.02	0.05	0.02	0.01
2002	0.03	0.18	0.04	0.04	0.02	0.05	0.01	0.01	0.03	0.05	0.01	0.14	0.04	0.03	0.01	0.01
2003	0.00	0.19	0.03	0.06	0.01	0.06	0.01	0.02	0.02	0.06	0.01	0.14	0.02	0.05	0.01	0.01
2004	0.01	0.19	0.03	0.06	0.02	0.05	0.01	0.01	0.01	0.07	0.02	0.14	0.01	0.06	0.01	0.01
2005	0.03	0.16	0.04	0.04	0.03	0.04	0.01	0.01	0.03	0.05	0.03	0.14	0.04	0.03	0.01	0.01
2006	0.03	0.17	0.06	0.02	0.03	0.05	0.01	0.02	0.05	0.03	0.03	0.13	0.03	0.04	0.01	0.01
2007	0.03	0.18	0.04	0.04	0.06	0.01	0.01	0.01	0.03	0.05	0.02	0.14	0.01	0.06	0.01	0.01
2008	0.18	0.03	0.03	0.05	0.02	0.06	0.02	0.00	0.08	0.00	0.11	0.05	0.07	0.01	0.02	0.01
2009	0.02	0.17	0.02	0.06	0.01	0.06	0.01	0.01	0.00	0.08	0.04	0.14	0.01	0.06	0.01	0.01
2010	0.06	0.18	0.08	0.00	0.04	0.04	0.01	0.02	0.01	0.07	0.02	0.14	0.01	0.07	0.02	0.01

Table 5. Distance measure over each criterion to both ideal (S_i^+) and nadir (S_i^-)-(2011-2030).

	Anzali		Ardebil		Astara		Ghazvin		Manjil		Ramsar		Rasht		Zanjan	
S_i^+, S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-	S_i^+	S_i^-
2011	0.01	0.07	0.02	0.02	0.01	0.03	0.01	0.03	0.01	0.07	0.01	0.08	0.02	0.04	0.01	0.02
2012	0.01	0.07	0.01	0.03	0.01	0.03	0.00	0.03	0.01	0.06	0.02	0.06	0.00	0.06	0.02	0.01
2013	0.03	0.05	0.03	0.01	0.01	0.03	0.02	0.01	0.01	0.07	0.02	0.06	0.04	0.02	0.01	0.01
2014	0.02	0.05	0.02	0.02	0.02	0.02	0.03	0.01	0.05	0.03	0.01	0.08	0.06	0.00	0.01	0.01
2015	0.00	0.07	0.02	0.02	0.01	0.03	0.00	0.03	0.01	0.07	0.02	0.06	0.00	0.06	0.01	0.01
2016	0.01	0.07	0.02	0.02	0.01	0.03	0.02	0.01	0.04	0.03	0.06	0.02	0.03	0.03	0.01	0.01
2017	0.01	0.07	0.02	0.02	0.01	0.03	0.02	0.02	0.04	0.03	0.00	0.08	0.02	0.04	0.02	0.01
2018	0.01	0.07	0.03	0.01	0.01	0.03	0.01	0.02	0.03	0.05	0.03	0.05	0.03	0.03	0.02	0.01
2019	0.00	0.07	0.03	0.01	0.01	0.03	0.02	0.01	0.02	0.06	0.02	0.06	0.02	0.04	0.02	0.01
2020	0.02	0.05	0.02	0.02	0.03	0.00	0.02	0.02	0.01	0.06	0.02	0.06	0.02	0.04	0.01	0.02
2021	0.02	0.05	0.03	0.01	0.01	0.03	0.01	0.03	0.04	0.03	0.00	0.08	0.02	0.05	0.01	0.01
2022	0.00	0.07	0.03	0.01	0.00	0.03	0.02	0.01	0.01	0.06	0.02	0.06	0.03	0.04	0.01	0.02
2023	0.00	0.07	0.03	0.02	0.00	0.03	0.01	0.02	0.01	0.07	0.03	0.05	0.03	0.04	0.02	0.01
2024	0.07	0.01	0.04	0.01	0.01	0.03	0.01	0.03	0.04	0.03	0.02	0.06	0.05	0.02	0.01	0.01
2025	0.02	0.05	0.01	0.03	0.01	0.02	0.01	0.02	0.03	0.05	0.02	0.06	0.04	0.02	0.02	0.01
2026	0.01	0.07	0.01	0.03	0.02	0.02	0.03	0.01	0.01	0.07	0.02	0.06	0.03	0.03	0.00	0.02
2027	0.01	0.07	0.02	0.02	0.03	0.01	0.03	0.01	0.00	0.07	0.08	0.00	0.02	0.04	0.01	0.02
2028	0.01	0.07	0.02	0.02	0.02	0.02	0.01	0.02	0.01	0.07	0.02	0.06	0.00	0.06	0.02	0.01
2029	0.01	0.07	0.02	0.02	0.01	0.03	0.01	0.03	0.07	0.01	0.05	0.03	0.06	0.00	0.01	0.01
2030	0.01	0.07	0.02	0.02	0.01	0.03	0.03	0.00	0.05	0.02	0.02	0.06	0.03	0.04	0.02	0.01

Table 6. Ratio Ti for each alternative (1992-2010).

	Anzali	Ardebil	Astara	Ghazvin	Manjil	Ramsar	Rasht	Zanjan
1992	0.763	0.914	0.584	0.654	0.642	0.223	0.110	0.733
1993	0.881	0.753	0.463	0.653	0.375	0.572	0.324	0.708
1994	0.822	0.930	0.500	0.851	0.489	0.716	0.620	0.691
1995	0.955	0.895	0.752	0.200	0.786	0.903	0.905	0.465
1996	0.955	0.641	0.734	0.846	0.572	0.866	0.614	0.484
1997	0.866	0.574	0.558	0.193	0.419	0.693	0.121	0.485
1998	0.812	0.589	0.655	0.565	0.624	0.734	0.309	0.391
1999	0.890	0.675	0.837	0.315	0.878	0.869	0.904	0.263
2000	0.878	0.530	0.705	0.475	0.741	0.850	0.726	0.476
2001	0.792	0.493	0.449	0.287	0.815	0.769	0.765	0.227
2002	0.873	0.503	0.756	0.571	0.569	0.954	0.422	0.388
2003	0.985	0.692	0.819	0.753	0.780	0.933	0.693	0.507
2004	0.960	0.689	0.756	0.552	0.852	0.893	0.838	0.480
2005	0.822	0.548	0.596	0.414	0.657	0.831	0.465	0.412
2006	0.833	0.283	0.650	0.618	0.421	0.819	0.608	0.463
2007	0.878	0.552	0.136	0.554	0.573	0.879	0.824	0.626
2008	0.155	0.589	0.746	0.128	0.030	0.305	0.089	0.308
2009	0.904	0.752	0.863	0.484	0.944	0.768	0.878	0.481
2010	0.763	0.016	0.499	0.654	0.862	0.898	0.879	0.252

Table 7. Ratio Ti for each alternative (2011-2030).

	Anzali	Ardebil	Astara	Ghazvin	Manjil	Ramsar	Rasht	Zanjan
2011	0.925	0.562	0.717	0.775	0.884	0.923	0.665	0.594
2012	0.932	0.781	0.743	0.952	0.800	0.793	0.953	0.273
2013	0.635	0.296	0.716	0.293	0.907	0.792	0.336	0.529
2014	0.659	0.541	0.487	0.233	0.357	0.924	0.032	0.424
2015	0.975	0.520	0.797	0.968	0.881	0.795	0.936	0.477
2016	0.874	0.519	0.738	0.350	0.457	0.201	0.500	0.552
2017	0.925	0.389	0.735	0.455	0.460	0.951	0.667	0.302
2018	0.903	0.269	0.740	0.619	0.636	0.593	0.502	0.345
2019	0.996	0.336	0.849	0.339	0.786	0.801	0.663	0.354
2020	0.659	0.499	0.124	0.456	0.813	0.798	0.661	0.677
2021	0.668	0.208	0.749	0.732	0.451	0.988	0.752	0.444
2022	0.956	0.302	0.891	0.376	0.807	0.795	0.579	0.684
2023	0.946	0.452	0.883	0.670	0.932	0.597	0.584	0.274
2024	0.105	0.220	0.736	0.760	0.451	0.783	0.248	0.400
2025	0.665	0.727	0.588	0.675	0.643	0.776	0.334	0.329
2026	0.895	0.689	0.500	0.240	0.882	0.785	0.497	0.949
2027	0.840	0.532	0.236	0.203	0.954	0.031	0.665	0.683
2028	0.925	0.389	0.499	0.634	0.864	0.792	0.981	0.255
2029	0.930	0.470	0.853	0.854	0.082	0.401	0.044	0.525
2030	0.867	0.403	0.729	0.032	0.279	0.793	0.578	0.363

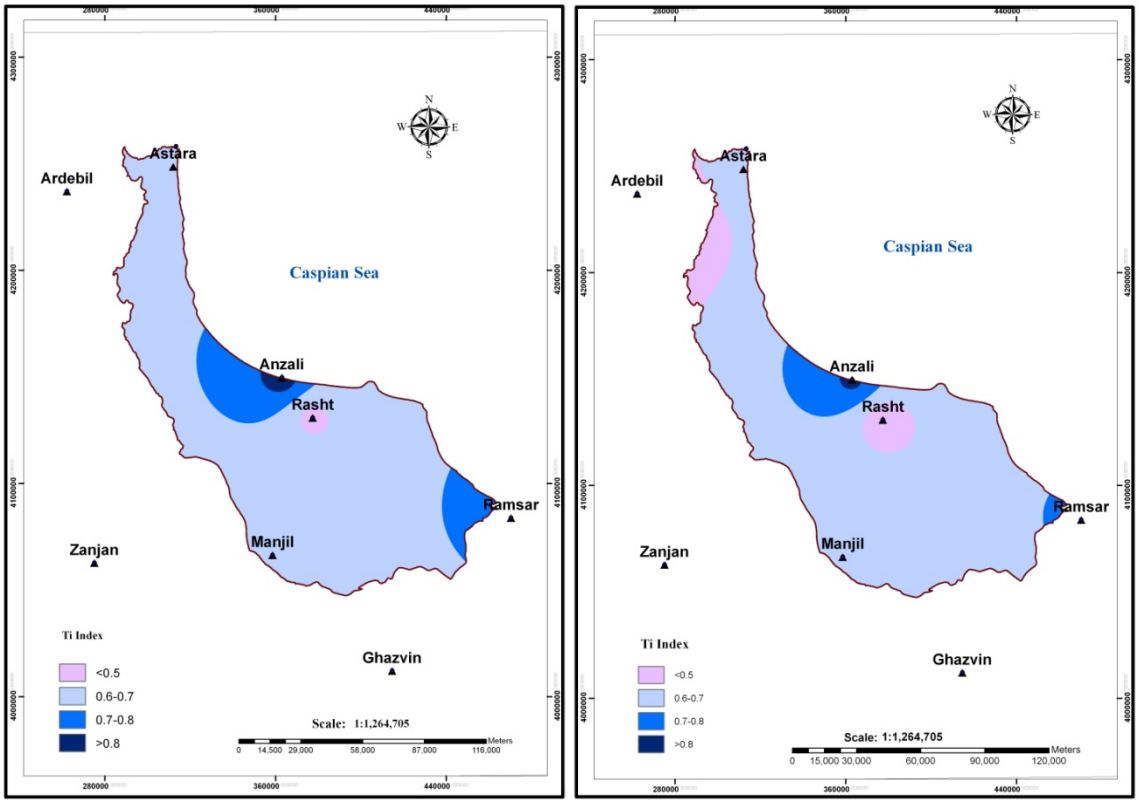

Fig. 3. Drought zoning by TOPSIS method for present (left) and future (right).

Conclusion

After all calculations, by t-test, the results of TOPSIS methods are compared with Standard Index Annual precipitation (SIAP) method. Results show that there is no significant differences between these two methods ($p \leq 0.05$). In the proposed method, systematic relationships between amounts of climatic parameters in different years is influence to determine drought and ranking it. In this method, we apply 7 climatic parameters, so it is more effective than other simple methods that only use one or two variables. Other ability of this method is ranking the drought. This method has more advantages than the SIAP and other methods. It minimizes the distance to the ideal alternative while maximizing the distance to the nadir. A relative advantage of this method is the ability to identify the best alternative quickly. It was found to perform almost as well as multiplicative additive weights and better than analytic hierarchy process in matching a base prediction model.

Comparison predicting period (2011-2030) with base period (1992-2010) show Ti index will decreased in the study area. Maps show the area of lower Ti index (<0.5) will increased in central parts of Rasht and western parts of Astara and Talesh in 2011 to 2030 period. It is expected that drought will increased in this period. In addition, area of higher Ti index (>0.7) will decreased in Anzali, Rezvanshahr, Masal, Somehsara and Roodsar. Though it is not expected drought, but wetness will decreased and it is alarm for these areas. In other areas, it is no changes in Ti index and it is from 0.6 to 0.7 in each period.

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Susceptibility of Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae) and Sitophilus oryzae (L.) (Coleoptera: Curculionidae) to Spinosad (Tracer®) as a Eco-friendly Biopesticide

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Abstract. *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.) are internal feeder of various stored grains and introducted as major stored product insect pests. Due to the resistance by insect pests and negative effects of synthetic pesticides to the environment, it is necessary to use novel and suitable compounds in insect pest's management. Spinosad is a bio-insecticide that is derived from fermentation of a bacterium *Saccharopolyspora spinosa* Mertz and Yao. In the present study, the toxicity of Tracer® as a spinosad based insecticidise was evaluated against *R. dominica* and *S. oryzae*. Insect species were kept in stored-products insects rearing room in Agriculture Faculty of Tehran University at 27 ± 2ºC, 65 ± 5% relative humidity. Adult insects were exposed to different concentrations of Tracer® by oral trials for 10 and 20 days exposure periods. The mortality data were subjected to probit analysis using SPSS software to estimate LC (lethal concentration) values and their related information. Tracer® showed strong toxicity against the adults of *R. dominica* and *S. oryzae*. Maximum mortality was occurred in the concentration of 250 and 80 ppm after 20 days exposure for *R. dominica* and *S. oryzae*, respectively. Direct relationship between mortality of insects with concentration and exposure period was found. Probit analysis displayed *R. dominica* (10-days LC_{50} = 49.89 ppm) was more susceptible than *S. oryzae* (10-days LC_{50} = 50.75 ppm) to Tracer®. Results of present study stimulated the utilization of Tracer® as an eco-friendly and safe agent for insect pests' management.

Key words: Bioassay, *Rhyzopertha dominica*, *Sitophilus oryzae*, Toxicity, Tracer®.

Introduction

Cereal grains such as wheat and rice are the main sources of human diets. These grains are highly susceptible to infestation by stored product insects such as the lesser grain borer [*Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)] and the rice weevil [*Sitophilus oryzae* (L.) (Coleoptera: Curculionidae)]. *R. dominica*, is a destructive insect pest of stored grains. Both larvae and adults of this insect feed on whole, sound grains and cause extensive damage (JOOD *et al*., 1996; REES, 2007). *S. oryzae* is one of the most widespread and destructive insect pest of stored cereals. These pests are internal feeders and cause considerable loss to cereals affecting the quantity as well as quality of the grains (KUCEROVA *et al*., 2003; REES, 2007).

Synthetic pesticides have been considered the most effective means to control insect pests of stored products.

Indiscriminate use of these chemicals have given increase to many serious problems, including resistance of pest species, toxic residues, and environmental and human health concerns (SANNA *et al.*, 2004; TAPONDJOU *et al.*, 2005; KOUL *et al.*, 2008). For example, the use of organophosphate was decreased, because of increasing resistance incidence in stored-product insects against these chemicals (FANG *et al.*, 2002). Fumigants such as phosphine and methyl bromide have been applied to control stored-products insects for a long time. Development of insect resistance to phosphine has been repoted recently (BENHALIMA *et al.*, 2004; COLLINS *et al.*, 2005) and application of methyl bromide has been largely scaled down because of its carcinogenicity and effect on the depleting ozone layer (MBTOC, 2002). There is, therefore, an urgent need to develop eco-friendly materials and methods with slight adverse effects on the environment and on consumers.

Spinosad is a secondary metabolite from the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yoa. The parent strain was originally isolated from an abandoned rum still in the Caribbean by Elanco in 1982 and introduced commercially in 1997 by Dow AgroScience (KIRST *et al.*, 1992). This is a mixture of spinosyns A ($C_{41}H_{65}NO_{10}$) and D ($C_{42}H_{67}NO_{10}$) and through contact and ingestion is highly toxic on a number of insect pests (COPPING & DUKE, 2007). It affects on target insects through the activation of the nicotinic acetylcholine receptor, but at a different site than nicotine or the neonicotinoids. It also affects on GABA receptors, but their role in the overall activity is unclear. There is currently no known cross-resistance to other insecticide classes. The mode of action causes rapid death of target phytophagous insects. Its moderate activity reduces the possibility of the onset of resistance (THOMPSON *et al.*, 2000). Spinosad is sold as a water-based suspension concentrate (SC) formulation under the trade names Tracer®, Conserve, Success, SpinTor (Dow AgroSciences) and etc. Spinosad with high efficacy such as a broad insect pest spectrum, low mammalian toxicity, and minimal environmental profile is unique among existing products currently used for stored-grain protection (HERTLEIN *et al.*, 2011). Spinosad is considered to be practically non-toxic to birds but slightly to moderately toxic to fish. It is highly toxic to honey bees, with less than 1 mg/bee of technical material applied topically resulting in mortality. Once residues are dry, however, they are non-toxic (CISNROS *et al.*, 2002). Spinosad is rapidly degraded on soil surfaces by photolysis and, below the soil surface, by soil microorganisms (SAUNDERS & BRET, 1997; TOEWS *et al.*, 2003). Moreover, spinosad does not have carcinogenic, mutagenic and tumorigenic effects (SCHOONOVER & LARSON, 1995). Spinosad is classified by the U.S. Environmental Protection Agency as an environmentally and toxicologically reduced risk material (CLEVELAND *et al.*, 2002).

Therefore, the objective of this research was to investigate the toxicity of Tracer® as a biological control agent against two major stored-grain insects: *R. dominica* and *S. oryzae*.

Materials and Methods

Tracer®. The Tracer® (48% SC) used in this study was purchased from American Dow AgroSciences Company (Zionsville Road, Indianapolis, IN 46268). Cytowet oil as a 100% pure liquid was used to distribute the insecticides evently on glasses (5 cm diameter and 15 cm height). Distilled water was used for dilution of Tracer®.

Insect rearing. Both insect species were kept in stored-products insects rearing room in Agriculture Faculty of Tehran University at 27 ± 2°C, 65 ± 5% relative humidity and 14 D: 10 L photoperiod. The rearing medium was wheat for *R. dominica* and soft wheat for rearing *S. oryzae*.

Bioassay. After preliminary tests with different concentrations and concentration fixing (ROBERTSON *et al.*, 2007), the main tests were performed using 5 concentrations of Tracer® - 60, 95, 150, 250 and 400 ppm for *R. dominica* and 15, 23, 36, 52 and 80 ppm for *S. oryzae*. The concentrations were prepared by diluting of insecticide in distilled water. A drop of cytowet oil which decreased the

surface tension was used for even distribution of insecticide on the foodstuff surface (one drop of cytowet oil was used for a 50 milliliters of solution). All the surfaces of foodstuff (40 grams of wheat) were impregnated by 9 milliliters of a given concentration. After treatment of the wheat, their were dried under ambient conditions. Fifteen adult insects were introduced to each glass and glasses were covered with muslin cloth and kept under rearing conditions. All stages were done for control group without insecticide adding. Each concentration was replicated four times and for each concentration totally 60 adult insects were used.

Statistical analysis. The data were corrected using Abbott's formula (ABBOT, 1925) for the mortalities in the controls. Experiments were arranged in a completely randomized design and a two way analysis of variance was used to analyze the effect of varying concentrations and exposure periods on insect mortality. The means were separated by the Tukey test at the 5% level. The lethal concentration LC_{50}, chi-square, and 95% confidence intervals for each regression coefficient were calculated by using probit analysis (FINNEY, 1971). All statistical analyses were performed using the statistical software SPSS version 16.0 (SPSS, 2007).

Results and Discussion

Present study showed strong toxicity of Tracer® on the adults of *R. dominica* and *S. oryzae*. Results indicated that Tracer® with concentration of 250 ppm caused 100% mortality on *R. dominica* at 20 days' exposure time (Fig. 1). Figure 1 also displays the 88% mortality in *S. oryzae* adults with concentration of 80 ppm after 20 days. On the other hand, maximum mortality was occurred in the concentration of 250 and 80 ppm after 20 days exposure for *R. dominica* and *S. oryzae*, respectively. Compare means of mortalities revealed direct relationship between mortality of both insects with concentration and exposure times (Fig. 1). Concentration-mortality response lines for both insects exposed to different concentrations of Tracer® have shown in Fig. 2, too.

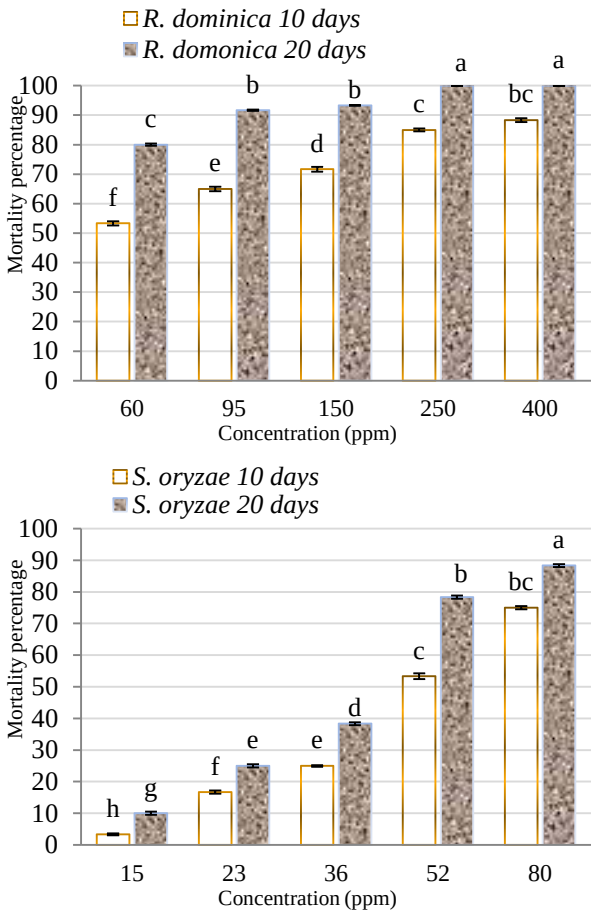

Fig. 1. Mean mortality (%) of *Rhyzopertha dominica* and *Sitophilus oryzae* adults exposed to different concentrations of Tracer® after 10 and 20 days of exposure times. Different letters on top of columns indicate significant differences according to Tukey's test at p = 0.05. Columns with the same letter are not significantly different.

Probit analysis demonstrated that *R. dominica* (10-days LC_{50} = 49.89 and 20-days LC_{50} = 31.39 ppm) was susceptible than *S. oryzae* (10-days LC_{50} = 50.75 and 20-days LC_{50} = 36.53 ppm) (Table 1). From the probit analyses, the calculated regression line equations of the first and second days' data were Y = 1.99X - 2.37 and Y = 2.85X - 4.27 for *R. dominica* and Y = 3.31X – 5.65 and Y = 3.57X – 5.58 for *S. oryzae*, respectively (Table 1). The concentrations were used as followed: 60, 95, 150, 250 and 400 ppm for *R. dominica* and 15, 23, 36, 52 and 80 ppm for *S. oryzae*. Number of insects for each time was 360 (15 insects × 4 replications × 6 concentration).

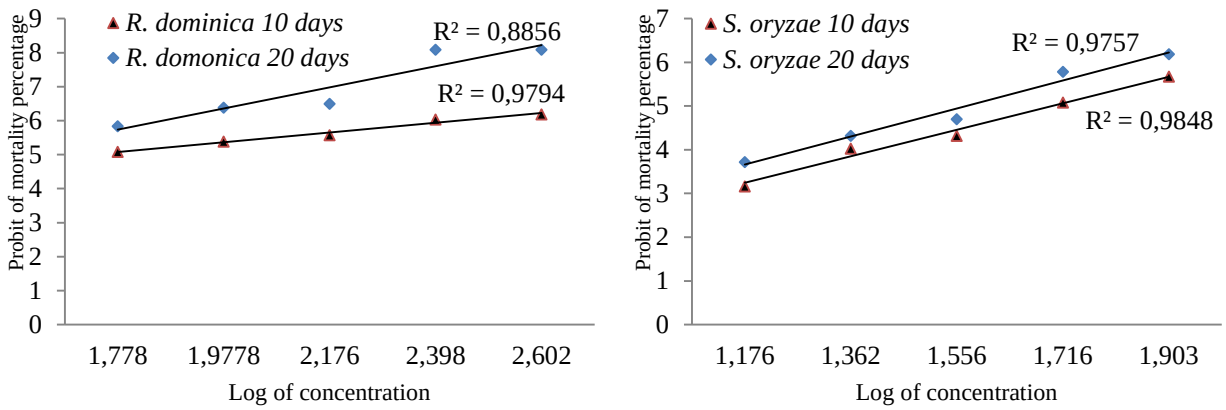

Fig. 2. Concentration-mortality response lines for adult of *Rhyzopertha dominica* and *Sitophilus oryzae* exposed to different concentrations of Tracer®.

Table 1. Probit analysis of diffirent treatments of Tracer® against two experimented insects in 10 and 20 days exposure times.

Insect	Time (day)	LC_{50} [a]	LC_{90} [a]	Intercept ± SE	Slope ± SE	χ^2 (df=3)	ρ [b]
R. dominica	10	49.89 (24.46 ± 70.87)	411.72 (275.59 ± 962.62)	-2.37 ± 0.61	1.99 ± 0.29	0.29	0.96
	20	31.39 (10.54 ± 45.63)	88.31 (71.20 ± 112.92)	-4.27 ± 1.45	2.85 ± 0.75	*0.84*	0.84
S. oryzae	10	50.75 (45.16 ± 58.30)	123.74 (86.62 ± 174.45)	-5.65 ± 0.62	3.31 ± 0.38	2.03	0.57
	20	36.53 (32.84 ± 40.71)	83.46 (70.27 ± 106.47)	-5.58 ± 0.57	3.57 ± 0.57	*4.72*	0.19

[a] 95% lower and upper fiducial limits are shown in parenthesis.
[b] Since goodness−of−fit Chi square is not significant (P > 0.15), no heterogeneity factor is used.

These results is similar to the results of FANG *et al.* (2002), MCLEOD *et al.* (2002) and TOEWS *et al.* (2003) that they have worked on some stored-products insect pests.

Spinosad is purposed for the control of a very wide range of important pests. For example, AHMED (2004 Cyro, Egypt, pers comm.) reported that the spinosad was the most effective compound against the newly hatched larvae of both pink and spiny bollworms after 12 days for laboratory and field strain, respectively. He added that spinosad contacts and affects the receptors of acetylcholine in different place contacts of acetylcholine, which caused hyper-activity in nervous system for a long time. SADAT & ASGHAR (2006) indicated that liquid formulation of spinosad (Tracer® 22.8%) had considerable contact toxicity against adults of *Callosobrochus maculatus* (F.). In that study, spinosad caused 75–100% mortality in 4 concentration rates: 400, 300, 185 and 150 ppm. In the study of SEMIZ *et al.* (2006), insecticidal effects of spinosad were expressed against the pine processionary moth, *Thaumatopoea wilkinsoni* Tams. In the other study, toxicity of Tracer® was evaluated to larve of common green lacewing, *Chrysoperla carnea* Stephens. In contact bioassay tests, a direct relationship was detected between the concentration of spinosad and mortality rate of first instar larvae so that the employing of 250 and 2500 ppm of Spinosad caused 33 and 67 percent mortality, respectively (MAROUFPOOR *et al.*, 2010). Toxicity of spinosad (Tracer® 24% SC) were evaluated against cotton leaf worm, *Spodoptera littoralis* (Boisd.) by KORRAT *et al.* (2012). Along with toxicity, pupation, fecundity, hatchability and sterility rates and adult emergence percentages of insect were affected by spinosad. Effectiveness of spinosad was evaluated against adults *C. maculatus* on four commodities: chickpea, split pea, cowpea and lentil by KHASHAVEH *et al.* (2011). Mortality of exposed individuals in all treated commodities was low at 1-day exposure even at 0.3 g/kg and did not exceed 20%. As expected, mortality increased with the increase of exposure interval and dose rates. After 10 days of exposure, mortality reached 100% in all

commodities except for split pea. The application of spinosad significantly reduced progeny production in four commodities tested in comparison with the untreated ones. Recently PIRI *et al*. (2014) displayed sublethal effects of spinosad including reduction in glutathione S-transferase activity, percentage of larval pupation and female fecundity against lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae).

Results of the present and mentioned studies adequately expressed the toxicity of spinosad against a wide variety of insect pest along with stored product insect pests. With retrospect, it could be concluded that spinosad and Tracer® as one of the spinosad based traditional formulation is merit to be considered as a potential compound in controlling the insects in question.

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Influence of Pheromone Trap Color and Placement on Catch of Male Potato Tuber Moth, Phthorimaea operculella (Zeller, 1873)

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Abstract. Potato Tuber Moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is one of the damaging pests of potatoes in both field crops and storage worldwide. Larvae develop in the foliage and tubers of potatoes and cause direct losses of product. Mass trapping application of synthetic pheromone has been found to be effective to control *P. operculella*; however, several factors have to be optimized for improving its efficiency. This experiment was carried out during the 2012 season, Ardabil province, Iran to evaluate the effectiveness of pheromone trap to capture males for future development of a mass trapping technique, and a subsequent decrease in insect reproduction. Particularly, in this experiment the influence of color (yellow, red and green), and height (ground level, 0.3 and 0.6 m) of water-pan trap on males captures was tested. The results showed that green traps captured significantly ($P < 0.05$) more males than red and yellow traps. Water-pan traps placed at 0.6 above plant canopy captured significantly ($P < 0.05$) the highest number of the moths in comparison to traps placed at ground level and 0.3 m.

Keywords: Monitoring system, pheromone baited traps, Potato Tuber Moth.

Introduction

The Potato Tuber Moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a major agricultural pest of solanaceous crops in tropical–subtropical countries around the world. In potatoes (*Solanum tuberosum* L.), the moth larvae feed on leaves at the beginning of the growing season, and migrate into the tubers towards harvest (DANGLERS *et al.*, 2008). Often, more than 10% of the harvested tubers are infested and unmarketable (SILESHI & TERIESSA, 2001). It is a highly adaptable insect, found in locations with very different climatic conditions (KROSCHEL & KOCH, 1994). Chemical management of *P. operculella* is challenging because of the cryptic behavior of larvae and because this insect has developed resistance to many traditional organophosphate, carbamate, and pyrethroid insecticides (GODFREY & HAVILAND, 2003; DOĞRAMACI & TINGEY, 2008; CLOUGH *et al.*, 2010). These results suggest that other control methods than pesticide use alone should be examined. In the case of *P. operculella*, there are been used different monitoring systems: random and selected leaf samples, pheromone traps (HORNE, 1993). Sexual pheromones are species-specific and highly selective, and since they are not toxic and do not represent health risks to humans and animals, they are valuable tools in integrated pest control management. Pheromone trap systems are used for monitoring pest dynamics and evaluate efficiency of pesticides (OMAR *et al.*, 2011; DEBANO *et al.*, 2010; VANEVA-GANCHEVA & DIMITROV, 2013).The use of pheromone traps for mass trapping is an insect control method that has been sufficiently researched (EL-SAYED *et al.*, 2006). It interferes with insect mating,

reducing the future larvae population and subsequent damage (ATHANASSIOU *et al.*, 2004, 2007).

Two chemicals have been identified as the components of the PTM sex pheromone, (E4, Z7)-tridecadienyl acetate (PTM 1) (ROELOFS *et al.*, 1975) and (E4, Z7, Z10)-tridecatrienyl acetate (PTM 2) (PERSOONS *et al.*, 1976). These two chemicals were synthesized (VOERMAN & ROTHSCHILD, 1978) and blends ranging from 9:1 to 1:9 tested (VOERMAN & ROTHSCHILD, 1978; RAMAN 1988). Though, there are some control studies of mass trapping and mating disruption of *P. operculella* (RAMAN, 1982, 1984; SALAS *et al.*, 1985; ORTU & FLORIS, 1989; LARRAÍN *et al.*, 2007, 2009; HERMAN *et al.*, 2005; SUBCHEV *et al.*, 2013; GIRI *et al.*, 2014).

The purpose of the present study was to evaluate the effect of color and placement of water pan trap on catch of the Potato Tuber Moth.

Materials and Methods

Experimental plot. The experiment was carried out during the 2012 season, in Saghs-e-lu village (38º 21´19˝ N, 48º 23´30˝ E, 1389 masl) belonging to the Ardabil Province, Iran. The crop (Agria cultivar) was planted on approximately 20 ha; the distance between plants and rows was of 0.3 x 0.70 m.

Agronomic management of the host crop. The planting date was 1 June 2012. The crop was fertilized with N, P2O5, and K2O in doses of 150, 120, and 60 kg ha^{-1}, respectively. Metribuzin 1 kg a.i. ha^{-1} (Sencor 70 WP) was initially applied to control weeds, but these were later controlled manually. Furrows were irrigated every 11 days. Harvest took time was between 20- 26 September 2012.

Evaluations. The efficacy of water-pan traps of different colors (yellow, red and green), placed at three heights (ground level, 0.3 and 0.6 m) on the capture of male moths was evaluated in this experiment. The treatments were replicated three times in a completely randomized design.

Pheromone traps. Rubber dispensers containing a mixture of the compounds E4-Z7 Tridecadienil acetate, and E4-Z7-Z10 Tridecatrienil acetate in a 1: 1 rate (Agrisense BCS Ltd., South Wales, UK) were used during the experiments. The rubber dispensers were placed on wires and suspended above water traps, made up by 5 liter plastic containing 2 liters water and 0.2% detergent to break the surface tension of the water, in order this to kill the captured males by drowning. Traps were placed at 50 m from each other, to avoid interference between traps (RODRIGUEZ *et al.*, 1991; NIETO-HERNANDEZ *et al.*, 1989; LARRAÍN *et al.*, 2007). The traps were placed within the plants at ground level, 0.3 and 0.6 m. Traps were checked weekly from 1 June - 26 September 2012.

Data analysis. The experiments were arranged by completely randomize design and the data were subjected to General Linear Model (GLM) by SPSS software version 16.0 (SPSS, 1999). Comparison of means was done through Tukey (HSD) test at 5 % level.

Results

Green traps had the greatest captures of the total moths captured and differed significantly ($P < 0.05$) from red and yellow trap colors (Table 1). Green traps were 43.19% of all moths captured and was more efficient than other traps (Table 1).

Green water pan traps at heights 0.6 had the greatest captures of the total moths captured and differed significantly ($P < 0.05$) from red and yellow trap colors (Table 1). Green water pan traps at heights ground level and 0.3 m captured 0.83, 0.53 times more male moths than red traps and 0.95, 0.78 times than yellow traps, respectively (Table 1). Similar pattern of captured to that of traps at 0.6 m was observed. Green water pan traps captured 1.17 times more male moths than red water pan traps and 2.11 times than yellow traps (Table 1). An issue with the water trap is that it took more time to service, mainly because there were more moths to count. The time taken to service the traps was measured once during this experiment. It took 7 minutes to count and remove 100 moths caught in a water pan trap.

Table 1. Average Potato Tuber Moth captures in response to pheromone baited pan traps at varying color and height trap disposition.

Factor	June	July	August	September
Trap color				
Green	120.11 a	162.77 a	284.88 a	418.11 a
Red	68.11 b	106.55 b	148.00 b	156.88 b
Yellow	37.55 b	41.11 b	71.88 b	122.44 b
Trap height				
Ground level	24.22 a	68.77 a	132.77 a	188.22 a
0.3 m	31.00 ab	91.22 ab	170.88 ab	280.66 ab
0.6 m	53.22 b	139.88 b	199.21 b	357.21 b

Different letters over columns indicate significant differences according to Tukey test at α= 0.05. Columns with the same letter are not significantly different.

Discussion

In this study, it was found that trap color affected the captures of PTM males. Trap color had a great impact on PTM catch (Table 1). Trap color has been reported to be a significant factor affecting catches of several other moth species (ATHANASSIOU *et al.*, 2004, 2007; KNIGHT & FISHER, 2006; TAHA *et al.*, 2012; BRAHAM, 2014). These are nocturnal species, so we expect that *P. operculella* moths may respond similarly. Physiologically, the spectra reflection of specific color can affect the discrimination and direction of the insect to that color. Results of CRAIG & OSCAR (2008) closed with our results where they found that green traps caught more Grab Root Borer (GRB) moths than other traps (white and blue) and the males prefer green pheromone-baited traps. BRAHAM (2014) reported influence of colored pan water traps on the capture of the tomato leafminer, *Tuta absoluta* males in open field tomatoes. For spring cultivation, green colored traps captured respectively, more than 7-fold, more than 4-fold, 4-fold and more than 2-fold for red, orange, yellow and white colored traps. In contrast, red traps were most effective in trapping moths of *Helicoverpa armigra*, *Earis insulana, Plutella xylostella* and *T. absoluta* while yellow pheromone traps attracted maximum number of *Spodoptera littoralis* moths (KUMAR et al., 2009; TAHA *et al.*, 2012). Although the above results contrast sharply, they demonstrate the impotence of considering the visual stimuli of lepidopterous moths in the design of pheromone traps and further study is required however, to answer the question as to why *P. operculella* moths are more attracted to green traps than to the other traps. According to the present results, green traps were selected for further studies.

In this study, it was found that water-pan traps placed at height 0.6 m captured significantly more PTM males than traps placed at ground level and 0.3 m (Table 1). HERMAN *et al.* (2005) reported that both trap heights (0.3 and 1.0 m) were equally effective in capturing PTM male moths. However, there was no significant difference in moth catch between the two trap heights. KENNEDY (1975) found that traps at 0.3 m caught more males than traps at 1.0 m, but also reported wide variation in the data. RAMAN (1988) found no differences between 3 heights, ground level, 0.4 or 0.8 m. In contrast, more significantly adults of codling moth (Lepidoptera: Tortricidae) were caught in traps placed high versus low on the tree (KNIGHT & LIGHT, 2005). EDI *et al.* (2004) reported that traps baited with Chemtica lures and placed at 1.5 m above the ground caught more significantly males of *Spodoptera frugipedra* (Lep.: Noctuidae) than traps placed at a height of 2 m. AHMAD & KHADHUM (1986) found that significantly more moths of *Aarsia lineatella* were caught in traps hung at a height of 3.0 m above the ground than at any other level. On the other hand, the trap height is one of the most

important aspects of trap development, along with trap density and trap position with respect to vegetation (WALL, 1989).

In conclusion, use of green pan traps at 0.6 m height, would allow for a greater efficacy in the captures of *P. operculella*. Since there are many factors that can affect the effectiveness of these control methods, it is important to conduct studies that confirm the effectiveness of pheromone use as a control technique for specific crop conditions where the moth constitutes an economically important pest. This study conducted that Potato Tuber Moth pheromones could be an extremely useful tool in a Potato Tuber Moth IPM program.

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The Impact of Different Habitat Conditions on the Variability of Wild Populations of a Medicinal Plant Betonica officinalis L.

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Abstract. Plants are important source of beneficial bioactive compounds which may find various applications as functional ingredients, such as components of food supplements, cosmetics, and pharmaceuticals. One such medicinal plant is *Betonica officinalis*, populations of which were investigated in 2012–13. The studies were conducted in patches of *Molinietum caeruleae* dominated by: small meadow taxa (patch I); the shrub willow *Salix repens* ssp. *rosmarinifolia* (patch II); large tussock grasses *Deschampsia caespitosa* and *Molinia caerulaea* (patch III); tall-growing macroforbs *Filipendula ulmaria* and *Solidago canadensis* (patch IV). Over successive patches, the average height of plant cover increased, as did soil moisture, while light availability at ground level decreased. Much greater abundance and density of the *Betonica officinalis* population were found in patches I, III and IV, while lower values for these parameters were noted in patch II. Individuals in pre-reproductive stages were absent during whole study period in all study plots, vegetative ramet clusters were observed in plots situated in patches I and III in the first year of observations, while only generative ramet clusters occurred in plots set in patches II and IV. The number of rosettes per ramet cluster, number and dimensions of rosette leaves, height of flowering stems, number of cauline leaves, length of inflorescences, as well as number and length of flowers increased gradually over successive patches, whereas the number of generative stems per ramet cluster did not differ remarkably among populations. On the basis of the performed studies it might be concluded that the condition of populations deteriorated from patches overgrown by large-tussock grasses and characterized by considerable share of native and alien tall-growing macroforbs, *via* patch dominated by small meadow taxa, to patch prevailed by shrub willows.

Key words: *Betonica officinalis*, individual, *Molinietum caeruleae*, morphological variability, light availability, population, soil moisture.

Introduction

Plants are important sources of beneficial bioactive compounds such as phenolic and nitrogen compounds, vitamins, and terpenoids, all of which may have various applications as functional ingredients. Some of them are natural antioxidants whose intake is associated with lower risks of cancer and cardiovascular disease (CHU *et al.*, 2002; DE CARVALHO-SILVA *et al.*, 2014; GUAJARDO-FLORES *et al.*, 2013; STANKOVIC *et al.*, 2011; SUN *et al.*, 2002; YANG *et al.*, 2004, 2009). Moreover, several of these secondary metabolites possess antifungal, antimicrobial or antiviral properties (ALZOREKY & NAKAHARA, 2003; BONJAR, 2004; KUMAR *et al.*, 2006; LOPEZ *et al.*, 2001). One such medicinal plant is *Betonica officinalis*, which has been used for centuries for treatment of disorders of the respiratory and gastrointestinal tracts, nervous system, skin ulcers and infected wounds, as well as gynecological problems (VOGL *et al.*, 2013). To date, biochemical compounds of the aforementioned taxa have been studied intensively by DUŠEK *et al.*,

(2009, 2010a), CZIGLE *et al.*, (2007), HAJDARI *et al.*, (2010), LAZAREVIĆ *et al.*, (2013) and ŠLIUMPAITĖ *et al.*, (2013). As opposed to a very large body of studies on the biochemical properties of *B. officinalis*, investigations of population and individual traits are still very scarce. Such observations were performed by DUŠEK *et al.*, (2009, 2010b), who found remarkable variability *inter alia* in in the height and width of individuals, dimensions of leaves and inflorescences, and seed production in natural localities and in field cultivation. It should be pointed out that further observations carried out *in situ* are still highly desirable. Such investigations conducted in different habitat conditions are very important for the assessment of favourable and unfavourable factors influencing the condition of populations and they might provide a basis for effective management programs.

In response to the current insufficiency of knowledge about *B. officinalis* populations, the presented investigations were performed. The main aim was to evaluate the effect of progressive increases in plant cover height and soil moisture, coincident with a gradual decrease in light availability, on population and individual traits in *Molinietum caeruleae* meadows. The specific goals concentrated on the assessment of: (1) abundance and density of populations, (2) developmental stages of individuals/ramet clusters, number and dimensions of leaf rosettes, number and height of generative stems, length of inflorescences, number of flowers per inflorescence, and flower length.

The hypotheses were following: (1) the abundance and density of populations would diminish over successive patches, (2) individuals in pre-reproductive stages would be absent in all patches, (3) dimensions of individuals and ramet clusters would increase over consecutive sites.

Materials and Methods

Study species. Betonica officinalis syn. *Stachys officinalis* (L.) Trevis is a perennial herb occurring in dry grassland, lightly grazed pastures, meadows and open woods in Europe, western Asia and North Africa. It is classified among rhizomatous species creating rosettes of leaves and upright, generative stems growing to 60 cm tall. Its cauline leaves are narrowly oval, with a heart-shaped base. The generative stems bear dense spikes of bright purple-red or occasionally white flowers, with the corolla attaining lengths of 1.0–1.5 cm. Flowering lasts from July to September. This taxon spreads notably by means of seeds (requiring a period of chilling to break dormancy) and by slow and steady vegetative growth leading to the division of senile parts of rhizomes into independent parts (FITTER & PEAT, 1994; KLIMEŠOVÁ & KLIMEŠ, 2006; KLOTZ *et al.*, 2002; SAMMUL *et al.*, 2003)

Because *B. officinalis* is a clonal species, the terms 'individual' and 'ramet cluster' were adopted as basic demographic units. An individual (genet) is a plant emerging from a single zygote. This designation might be applied in association with seedling and juvenile stages (plants with one leaf rosette), as only at these stages can it be established with certainty that it has developed from a zygote. The adult vegetative ramet cluster is understood as an integral group of leaf rosettes, the adult generative ramet clusters as an integral group of leaf rosettes and a generative stem or stems.

Field studies. The studies were carried out in the Kostrze district, on the western border of Cracow (southern Poland). The research area is located on the low flood terrace of the Vistula River, where peaty or clay- and silt-laden soils with fluctuating water tables are covered mainly by *Molinietum caeruleae* patches (DUBIEL, 2005). The abandonment of traditional land use for at least the past dozen years has favoured the encroachment of *Phragmites* swamps and willow brushwood, leading to the fragmentation of meadows (DUBIEL, 1991; 1996).

The studies were carried out in a study area consisting of three adjacent abandoned patches of *Molinietum caeruleae* sensu MATUSZKIEWICZ (2001) characterised by the presence of the following taxa: *Betonica officinalis, Dianthus superbus, Gentiana pneumonanthe, Gladiolus imbricatus, Iris*

sibirica, Molinia caerulaea, Potentilla erecta, Sanguisorba officinalis, Selinum carvifolia, Succisa pratensis. The studied patches (study sites) differed as to dominant species: patch I was dominated by small meadow taxa, patch II by shrub willows; patch III was overgrown by large-tussock grasses and sedges; patch IV was characterised by a considerable share of native and alien tall-growing macroforbs. In each patch, the site conditions were studied. The average height of vascular plants in particular patches was evaluated on the basis of measurements of 20 randomly chosen stems of different species, performed using a folding tape measure on 10 July 2012. Light intensity at soil level and ground humidity were evaluated on the basis of 20 measurements taken randomly in each patch on 20 July 2012 between 10.00 and 12.00 a.m. Light intensity was examined with a Voltcraft MS-1300 digital light meter (accuracy ±5% + 10 digits; range 0.01–50,000 lx). Soil humidity was measured using an Omega HSM50 handheld digital soil moisture sensor (accuracy ±5% + 5 digits; range 0% to 50% moisture content of soil). Site conditions in individual patches are described in Table 1.

Table 1. The characteristics of habitat conditions in study sites.

	Patch I	**Patch II**	**Patch III**	**Patch IV**
Area [m²]	2500	2900	3000	1800
Dominant species (with cover exceeding 20%)	*Potentilla erecta, Lotus corniculatus*	*Salix repens* var. *rosmarinifolia*	*Molinia caerulea, Deschampsia caespitosa*	*Filipendula ulmaria, Solidago canadensis*
Subdominant species (with cover level ranging 5-20%)	*Lychnis flos-cuculi Lathyrus pratensis*	*Salix caprea, Salix cinerea*	*Carex gracilis*	*Lysimachia vulgaris*
Average height of standing vegetation [cm]*	50.3	62.7	78.7	101.9
Average light intensity at soil level**	40 600	38 100	30 400	29 000
Average soil moisture***	5.1	6.3	7.4	7.8

The values of statistical significance of differences (the Kruskal-Wallis H test, df=3) among patches in: * height of standing vegetation achieved 22.9 *(P<0.001)*, ** light intensity at soil level achieved 50.5 *(P<0.001)*, *** soil moisture achieved 38.7 *(P<0.001)*.

In July 2012, in each study patch, the abundance of individuals and ramet clusters of *B. officinalis* and their mean number per m² were evaluated. Then, in each population, one 100-m² permanent plot was established and fenced in. In this plot, the abundance of individuals and ramet clusters and their developmental stages were investigated. Subsequently, in each individual/ramet cluster, the number of leaf rosettes, number of leaves per each rosette and the maximal length and width of the longest leaf blade were studied. In each generative ramet cluster the number of generative stems per ramet cluster and the height of generative stems (from the soil surface to the top of the highest inflorescence) were also noted. Furthermore, the number of cauline leaves and length of inflorescence, as well as number of flowers per inflorescence, were studied. Moreover, the length of 10 randomly chosen flowers per each studied inflorescence was investigated. For branched generative stems, only the main branch was examined. Statistical analyses based on the non- parametrical Kruskal-Wallis H test were performed using STATISTICA 10 software (STATSOFT INC., 2010).

Results

The abundance of *B. officinalis* populations in 2012 in successive patches achieved values of 354, 287, 310 and 338, while their density amounted to 0.14, 0.10, 0.10 and 0.19, respectively. In all permanent plots, only adult ramet clusters were found; their numbers were constant during the entire observation period, reaching 18, 12, 14 and 10, respectively. Vegetative ramet clusters were observed in plots situated in patches I

and III in the first year of observations, while only generative ramet clusters occurred in plots set in patches II and IV (Fig. 1).

The number of leaf rosettes increased from patch I, *via* patches II and III, to patch IV. In 2012 the mean number of rosettes per ramet cluster in patch I amounted to 1.3 (±0.6); in patch II, 4.0 (±2.6); in patch III, 4.3 (±1.8); in patch IV, 7.4 (±2.3). In 2013 this number mainly amounted to 2.3 (±1.1), 3.8 (±2.5), 5.1 (±1.9), and 6.6 (±2.8), respectively. The differences among populations were significantly different in both seasons (H_{2012}=34.1, H_{2013}=19.6; P<0.001). The smallest number of leaves per rosette was found in a plot located in patch I, while the largest number was observed in patch IV. The smallest length and width of rosette leaves were noted in a plot located in patch I; larger dimensions were exhibited by leaves found in patches II and IV; the largest dimensions were recorded in patch III (Fig. 2).

The number of flowering stems per generative ramet cluster did not differ remarkably among populations (Table 2). In both study seasons the height of generative stems, number of cauline leaves, inflorescence length and flower number were much lower in plots located in patches I and II than in patches III and IV (Fig. 3). The smallest flowers were found in patch I, the largest in patch IV (Table 3).

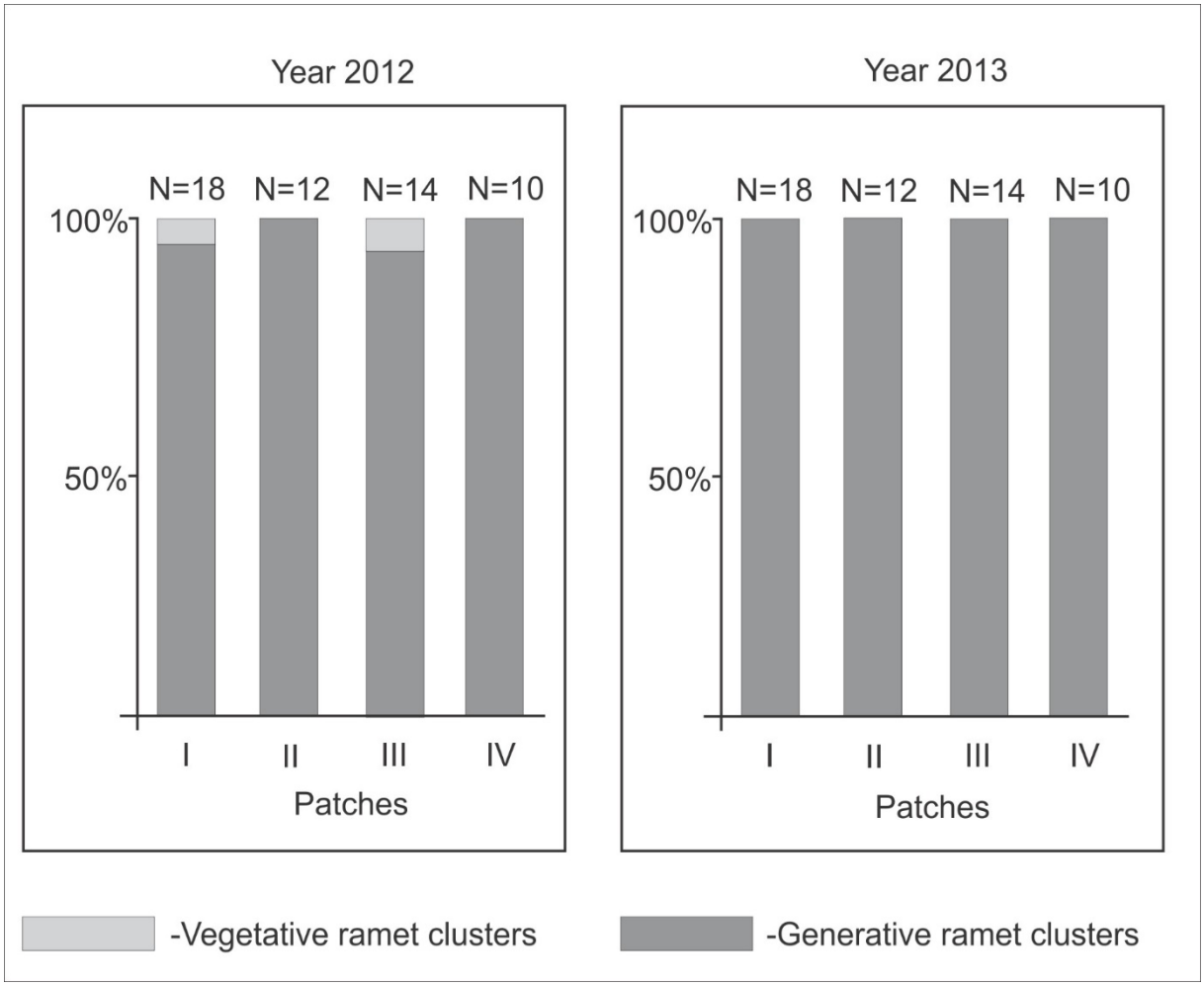

Fig. 1 The share of vegetative and generative ramet clusters of *Betonica officinalis* L. in patches of *Molinietum caeruleae* dominated by small meadow taxa (I); the shrub willow (patch II); large tussock grasses (patch III); tall-growing macroforbs (patch IV) in the years 2012-2013.

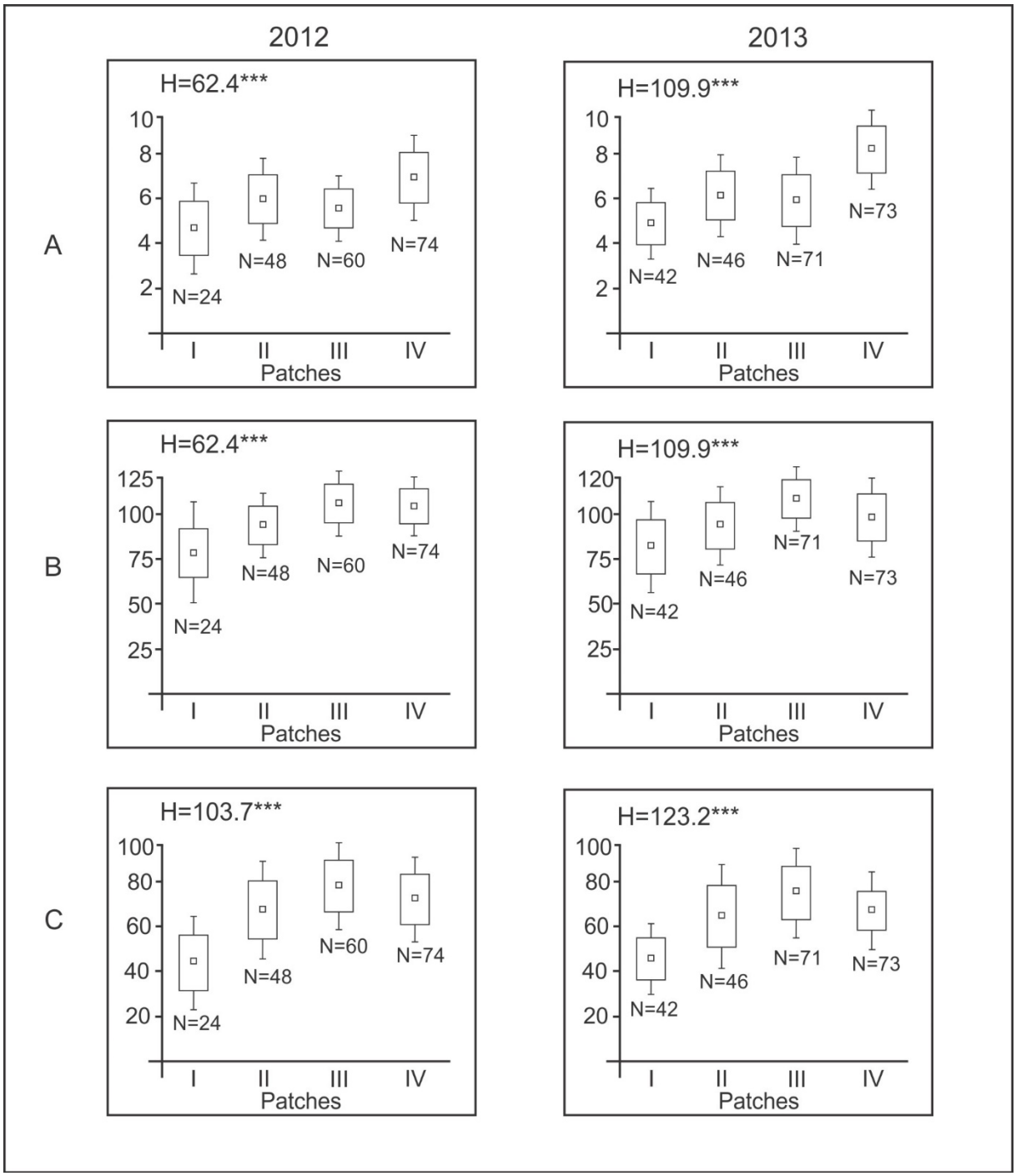

Fig. 2. The number of leaves per rosette (A), maximal length [mm] (B) and width [mm] (C) of the longest leaf blade of *Betonica officinalis* L. in patches of *Molinietum caeruleae* dominated by small meadow taxa (I); the shrub willow (patch II); large tussock grasses (patch III); tall-growing macroforbs (patch IV) in the years 2012-13. Box and whisker plots give the mean (square), SE (box) and SD (whiskers). Asterisks mean that there are significant differences among patches in each study year at the 0.001 level.

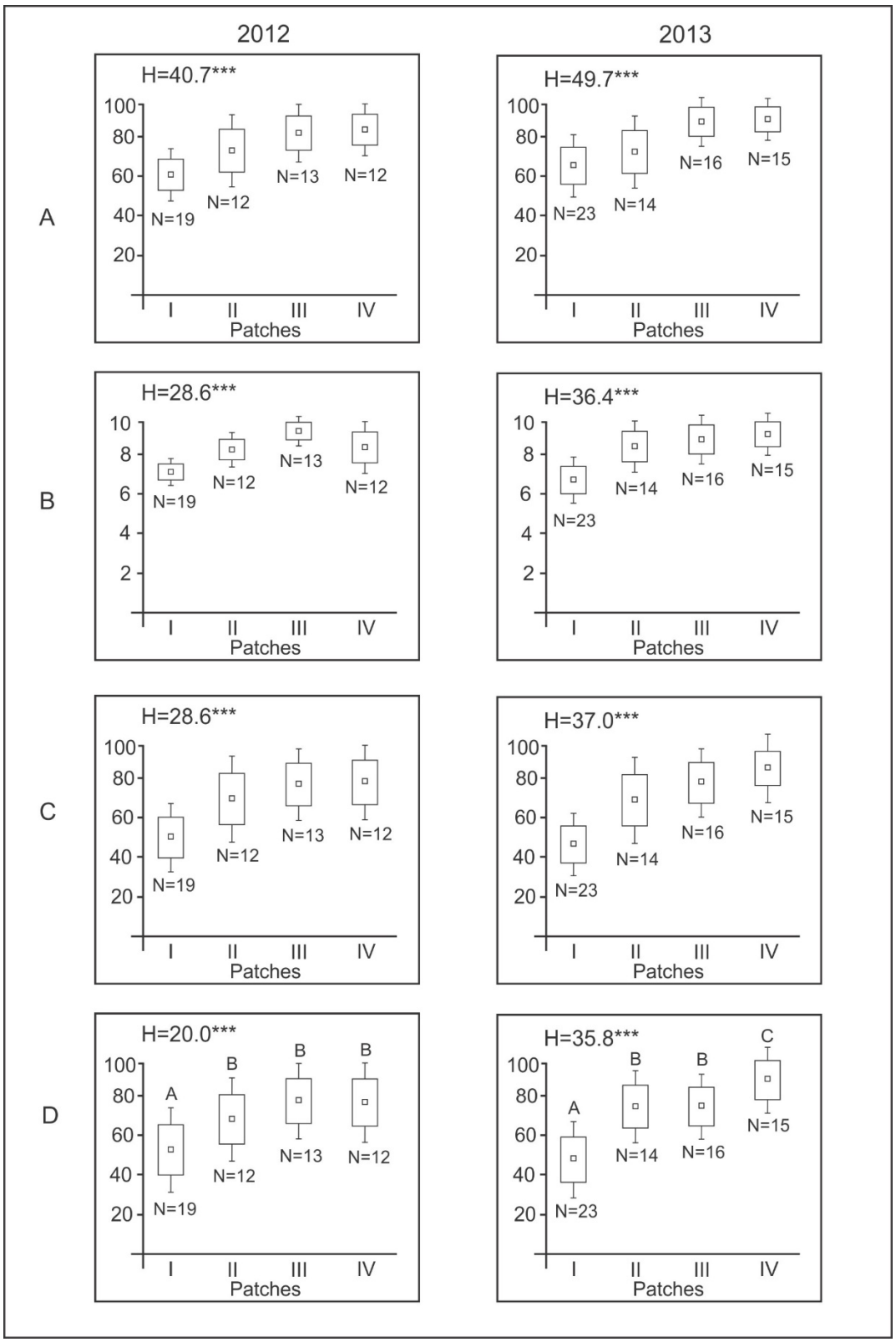

Fig. 3. The height of generative stems [cm] (A), number of cauline leaves (B), inflorescence length [mm] (C) and number of flowers per inflorescence (D) of *Betonica officinalis* L. in patches of *Molinietum caeruleae* dominated by small meadow taxa (I); the shrub willow (patch II); large tussock grasses (patch III); tall-growing macroforbs (patch IV) in the years 2012-13. Box and whisker plots give the mean (square), SE (box) and SD (whiskers). Asterisks mean that there are significant differences among patches in each study year at the 0.001 level.

Table 2. The number of generative ramet clusters per study plots and the mean number of generative stems per generative ramet cluster of *Betonica officinalis* within study plots set in patch dominated by small meadow taxa (patch I), prevailed by shrub willows (patch II), overgrown by large-tussock grasses (patch III) and characterized by considerable share of native and alien tall-growing macroforbs (patch IV) in the year 2012 and 2013.

	Patch	Number of generative ramet clusters	The mean number of generative stems per ramet cluster	The standard deviation	The statistical significance level (The Kruskal-Wallis H test, P value)
Year 2012	I	17	1.1	0.31	H=4.66, *P=0.12*
	II	12	1.0	0.0	
	III	13	1.0	0.0	
	IV	10	1.2	0.4	
Year 2013	I	18	1.3	0.6	H=4.48, *P=0.15*
	II	12	1.2	0.4	
	III	14	1.1	0.3	
	IV	10	1.5	0.5	

Table 3. The length of chosen 10 flowers in observed inflorescences of *Betonica officinalis* occurring within study plots set in patch dominated by small meadow taxa (patch I), prevailed by shrub willows (patch II), overgrown by large-tussock grasses (patch III) and characterized by considerable share of native and alien tall-growing macroforbs (patch IV) in the years 2012 and 2013.

	Patch	The mean length of flowers	The standard deviation	The statistical significance level (The Kruskal-Wallis H test, P value)
Year 2012	I	10.7	0.1	H=176.9, *P<0.001*
	II	11.9	0.7	
	III	11.6	1.2	
	IV	13.2	1.2	
Year 2013	I	10.6	0.9	H=30.4.7, *P<0.001*
	II	11.7	0.7	
	III	11.7	1.1	
	IV	12.3	1.1	

Discussion

The observations, showing, surprisingly, greater abundance and density of populations in patches I, III and IV than in patch II, might suggest that creeping and rooting stems of *Salix repens* ssp. *rosmarinifolia* mechanically limit the growth of *B. officinalis* individuals or even eliminate them from colonised sites. Additionally, the low abundance of *B. officinalis* populations might be a result of the activity of allochemicals produced by *Salix caprea*. The inhibition of the establishment and growth of genets of meadow taxa beneath *S. caprea* litter was observed by MUDRÁK & FROUZ (2012). The constant number of ramet clusters in both study seasons and lack of individuals in pre-reproductive stages in all permanent plots might suggest the absence of factors breaking seed dormancy and stimulating the germination process. The poor germinating ability of *B. officinalis* seeds not subjected to pre-treatment prior to sowing had already been proved in laboratory conditions (DUŠEK *et al.*, 2010a). A similar result was demonstrated in seeds of other species from the *Stachys* genera, in both laboratory (GÜLERYÜZA *et al.*, 2011) and

natural (KUPFERSCHMID *et al.*, 2000) conditions. Furthermore, the absence of seedlings and juveniles observed in the present study may be due to the lack of gaps in continuous plant cover considered 'safe sites for seedling recruitment'. A similar phenomenon had already been found in populations of several taxa inhabiting overgrown *Molinietum caeruleae* meadows such as *Dianthus superbus* (KOSTRAKIEWICZ-GIERAŁT, 2013a), *Gentiana pneumonanthe* (KOSTRAKIEWICZ-GIERAŁT, 2013b) and *Gladiolus imbricatus* (KOSTRAKIEWICZ-GIERAŁT, 2014).

The lowest number of rosette leaves of *B. officinalis* noted in patch I is in disagreement with investigations documenting that low density of adjacent plants and high light availability contribute to intense multiplication of rosette leaves (CALLAGHAN & PIGLIUCCI, 2002; ERIKSSON, 1985; MEEKINS & MCCARTHY, 2000; VAN KLEUNEN *et al.*, 2007; WINKLER & STÖKLIN, 2002). The existence of the lowest number of rosette leaves in a patch dominated by small meadow taxa might be result of the activity of herbivores, notably gastropods and grasshoppers (SCHEIDEL & BRUELHEIDE, 1999; STOLL *et al.*, 2006). The production of large rosette leaves in patches with lower light availability might enhance the chances for the effective use of photoperiod in a crowded environment. The existence of the largest dimensions of rosette leaves of *B. officinalis* in patch III is in accordance with the findings of DUŠEK *et al.* (2009), who observed the occurrence of large leaves in damp sites. At the same time, it is worth mentioning that the findings of GYÖRGY (2005, 2009) showed that the thickness of leaf blades of *B. officinalis* is much greater in open land than in shaded areas.

The observations showing that the number of generative stems per ramet cluster did not differ among populations are opposed to observations carried out in populations of other rhizomatous taxa such as *Iris sibirica* (KOSTRAKIEWICZ-GIERAŁT, 2013c) and *Doronicum austriacum* (STACHURSKA-SWAKOŃ & KUZ, 2011). Combining performed observations with published data (DUŠEK *et al.*, 2010b) enables the assumption that the average length of flowering stems of *B. officinalis* did not differ between patch I and an *ex situ* collection, where the competition from neighbouring plants is strongly limited. Furthermore, presented studies support the observations of GRIME & JEFFREY (1965), who noted that genets of *B. officinalis* growing in shade are strongly etiolated. The substantial height of flowering stalks in shaded stands had already been noted in other taxa occurring in moist meadows (KOSTRAKIEWICZ-GIERAŁT, 2013a; 2013b; 2013c; 2014). The considerable height of *B. officinalis* stems in patches III and IV might be also due to substantial ground humidity, as in populations of *Fritillaria meleagris* (CSERGÖ & FRINK, 2003). At the same time, it should be added that the findings presented here partially support observations conducted in populations of *D. austriacum* (STACHURSKA-SWAKOŃ & KUZ, 2011), supplying evidence that, in populations occurring in an overshadowed place with substantial ground humidity, generative stems achieve the greatest height and create the lowest number of cauline leaves.

The obtained results showing that the length of inflorescences increases along with the growing height of adjacent plants is contrary to the observations of DUŠEK *et al.*, (2009), who found longer inflorescences of *B. officinalis* in *ex situ* cultivation compared to those in crowded natural stands. However, the obtained results are in accordance with observations carried out on populations of many taxa inhabiting *M. caeruleae* patches, which showed that inflorescence length, as well as number and size of flowers, are greater in the neighbourhood of tall-growing plants than in the vicinity of small meadow taxa (KOSTRAKIEWICZ-GIERAŁT, 2013a; 2014).

Conclusions

The obtained results did not support hypothesis that abundance of populations would diminish in subsequent patches. At the same time performed studies confirmed assumption that individuals in prereproductive stages would be absent in all places, while presumption that

dimensions of genets and ramet clusters would increase in subsequent places was partially supported.

On the basis of performed studies the following conclusions were drawn:

1. The populations of *B. officinalis* occurring in patches III and IV show the most promising prospects for maintenance in colonised sites due to the large dimensions and substantial number of ramet clusters.
2. The population occurring in patch I presents an inferior state in spite of its considerable abundance.
3. The population from patch II is in the worst condition, as shown by the lowest abundance and the small size of ramet clusters.

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Morphology and Food Plants of Cuckoo Bees (Apidae: Hymenoptera) From Indian Himalayas

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Abstract. Cuckoo bees belong to the genus *Bombus* (Apidae: Hymenoptera) under **s**ub genus *Psythrus* Lepeletier and is widely distributed subgenus from the oriental region represented by 17 valid species. This subgenus is represented by eight valid species from Indian Himalayas viz. *B. ferganicus B. novus*, *B. morawatizianus*, *B. cornutus, B. branickii*, *B. skorikovi, B. tibetanus* and *B. turneri*. Due emphasis has been laid on their altitudional distribution, food plants, taxonomy, synonymy, and illustrations. Being their parasitic nature these species lack worker caste and has negligible role in pollination ecology although they have got preference to forage on different host plants. The species were observed feeding sluggishly on flower heads of *Rosa weibbiana*, *Cirsium* spp. and *Trifoium* spp. Many new food plants of these species have been recorded for the first time from the area under study. During the present studies six species of the cuckoo bees were collected and identified and one species *viz. B. turneri* which could not found during the present study were procured on exchanged from BMNH, London.

Key words: Morphology, food plants, Cuckoo bees, Indian Himalayas.

Abbreviations used in the text:
BMB – breadth mandible at its base, LF – length of flagellum, LHB – length of head breadth, LM – lamella, LOB – length of basitarsus, LS – length of scape, MBB – breadth of metabasitarsus, MF – median furrow, MS – length of malar space, OOL – ocello ocular length, POC – post-ocular line, POL – post-ocellar length, * – new record.

Introduction

The world bumblebee fauna consists of approximately 250 known species, and it is reasonable to assume that the majority of species have now been discovered (WILLIAMS, 1985; 1994; 1998; PEDERSEN, 1996). Recent classifications place all of the known species in a single genus *Bombus*. The majorities of these species are known as 'true' bumblebees, and have a social worker caste which is more or less sterile (they cannot mate but can lay unfertilized eggs that develop into males). The remaining 45 or so species are known as cuckoo bumblebees, and were formerly placed in a separate genus *Psithyrus*. These are inquilines that live within the nests of the true bumblebees. It is now clear that cuckoo bees have a monophyletic ancestry and belong within the genus *Bombus*, so that *Psithyrus* is now regarded as one of many *Bombus* subgenera (PLOWRIGHT & STEPHEN, 1973; PEKKARINEN *et al.*, 1979; ITO, 1985; PAMILO *et al.*, 1987; WILLIAMS, 1985, 1994; CAMERON *et al.*, 2007). All the species of Cuckoo bees have annual life cycles similar to those of typical temperate bumblebee species, except that instead of founding their own nest and rearing workers, they steal a nest from a 'true' bumblebee. The females are especially powerful, and force their way into the nests of their bumblebee hosts. They

kill or evict the queen and take over her workers as their own, using them to rear their own offspring. The *Psithyrus* female lays eggs that develop into either new breeding females or males. Mate location behaviour and hibernation are similar to other *Bombus* species. After emergence the female *Psithyrus* spends some time foraging on flowers while their ovaries develop. They then search for nests of their host species, probably at least in part using scent (FRISON, 1930). *Psithyrus* often resemble their hosts in coloration. Most authors agree that this is probably not to aid entry in to the nest, but that the *Psithyrus* and their hosts are members of Müllerian mimicry groups (ALFORD, 1975; PRYS-JONES & CORBET, 1991). Since Psithyrus do not have a worker caste, all of the offspring are males or future breeding females. The invading Psithyrus may eat host eggs and young larvae, but older ones are allowed to develop to add to the work force. Nests that have been invaded produce few or no host queens or males, although workers do lay eggs and a few of the resulting male off-spring may survive (FREHN & SCHWAMMBERGER, 2001). The frequency of invasion of bumblebee nests by *Psithyrus* (Cuckoo bees) is highly variable both between localities and years. In most other respects the life cycle of *Psithyrus* is rather similar to that of their hosts. Mating occurs in mid to late summer, and only females hibernate. Males are far more frequently seen than females, and they are very commonly observed feeding sluggishly on flower heads. The female *Psithyrus* also has a number of morphological adaptations, such as larger mandibles and a larger venom sac that increases her chances of taking over a nest. Upon hatching, the male and female Psithyrus disperse and mate. Like non-parasitic bumblebee queens, female *Psithyrus* finds suitable locations to spend the winter and enter diapause upon being mated.

Material and Methods

The research material for the present studies was collected from the last eight years (2007-2014) during different collection cum survey tours in various localities situated in the State's of Jammu and Kashmir and Himachal Pradesh falling in an altitudinal range of 1000-5500 m. These cuckoo bees were collected with sweeping hand net and the collected insect material was first sorted out in the field and latter brought to the laboratory for further identification and analysis. Distributional data including date of collection, number of specimens examined and locality with altitude was appended to each specimen to facilitate comparison. The microscopic examination of various morphological features was performed with the help of binocular microscope fitted with an ocular grid. The photographs of collected specimens were taken with Canon 18 MP LOS 5D. All the food plants of this species were collected side by side and got identified from the Centre of Plant Taxonomy, University of Kashmir. The following characters have been found trust worthy, stable and unambiguous while dealing with taxonomy of species. Shape and sculpturing of labrum and clypeus, length of malar space, mandibles, antennal segments, position of ocelli, Ocello-ocular areas of the vertex, Relative length of the pubescence and different parts of male genitalia. The terminology proposed by (WILLIAMS, 1991) for male genitalia have generally been adopted. Pictorial key for both males and females has been prepared. Temporary slides of different parts *viz.*, male genitalia, labrum, antenna, meso and meta basitarsal segments, 7th and 8th sternites etc were prepared in glycerin and alcohol. Some of its important features like penis valve, gonostylus, gonocoxites, gonobase, volsella and spatha hold a great taxonomic significance. The type specimens are deposited in the Museum of the Department of Zoology & Environmental Sciences, Punjabi University, Patiala, India for future references. The species were re described on the basis of the own material after confirmation /comparision with the type material at Natural History Musuem, London.

Results and Discussion

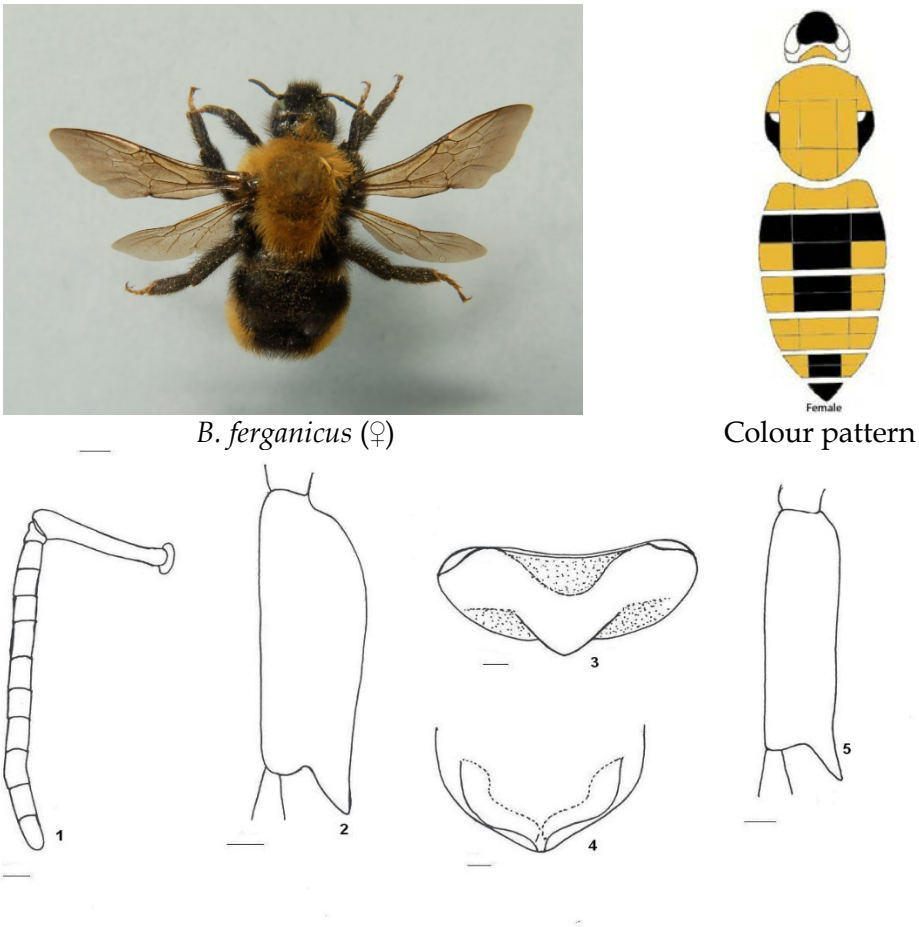
***Bombus* (*Psithyrus*) *ferganicus* RADOSZKOWSKI, 1893**

Bombus ferganicus RADOSZKOWSKI, 1893:241, Lectotype female USSR (MNHU) designated by TKALCU, 1969:206.

Synonymy: Apathus ochraceus MORAWITZ, 1894:5; *Psithyrus indicus* RICHARDS, 1929:139.

Diagnostic features

Female (Fig.1)*:* Pubescence on head, lateral aspects of mesonotum black; yellow are malar space, thorax, abdominal tergites 1 and 4; abdominal tergum 2 black with dirty yellow posterior corners; abdominal tergum 3 and 5 medially black with dirty yellow sides. Head covered with thick pubescence except malar space, clypeus, an area lateral to and in front of ocelli and narrow stripes on inner and post orbits. Labrum with basal transverse depression extending apically as a deep median furrow between pronounced lateral tubercles, displacing ridge between them to form a lamella that overhangs apical margins (Fig. 1-3). Clypeus nearly uniformly flat, only apico lateral corners curved back strongly towards occiput. MS: BMB=2:3. Antennal segments 3:4:5=1:1:1.0; LF: LS: LHB= 9:5:10.50 (Fig. 1-1). Band of punctures along eye margin in ocello-ocullar area opposite lateral ocellus, occupying half of distance between lateral ocellus and eye. The lateral ocelli just below POC. OOL: POL=2:2.5. The distoposterior corner of mesobasitarsus spinosely produced (Fig. 1-5). Basal depression shallow and with punctures, apex of lamella broadly rounded. Lateral keels of sternite 7 declining from near their mid points so that strongly swollen parts are separated by more than their own breadths (Fig. 1-4). Outer surface of meta-tibia convex, moderate to long hair throughout, but without a comb of stout spines along inner distal margin. Meta basitarsus (Fig. 1-2) with its posterior margin concave having a distoposterior corner spinosely produced and longer than distoanterior corner. Apex of tergite 7 triangularly notched, surface sculptured, but shining, no median furrow present.



B. ferganicus (♀) Colour pattern

Fig. 1. *B. ferganicus* (♀) – photograph, colour pattern and morphology. Legend: 1 – antenna, 2 – metabasitarsus, 3 – labrum, 4 – 7th sternite, 5 – mesobasitarsus, Scale bar = 0.5 mm.

B. ferganicus (♂)

Colour pattern

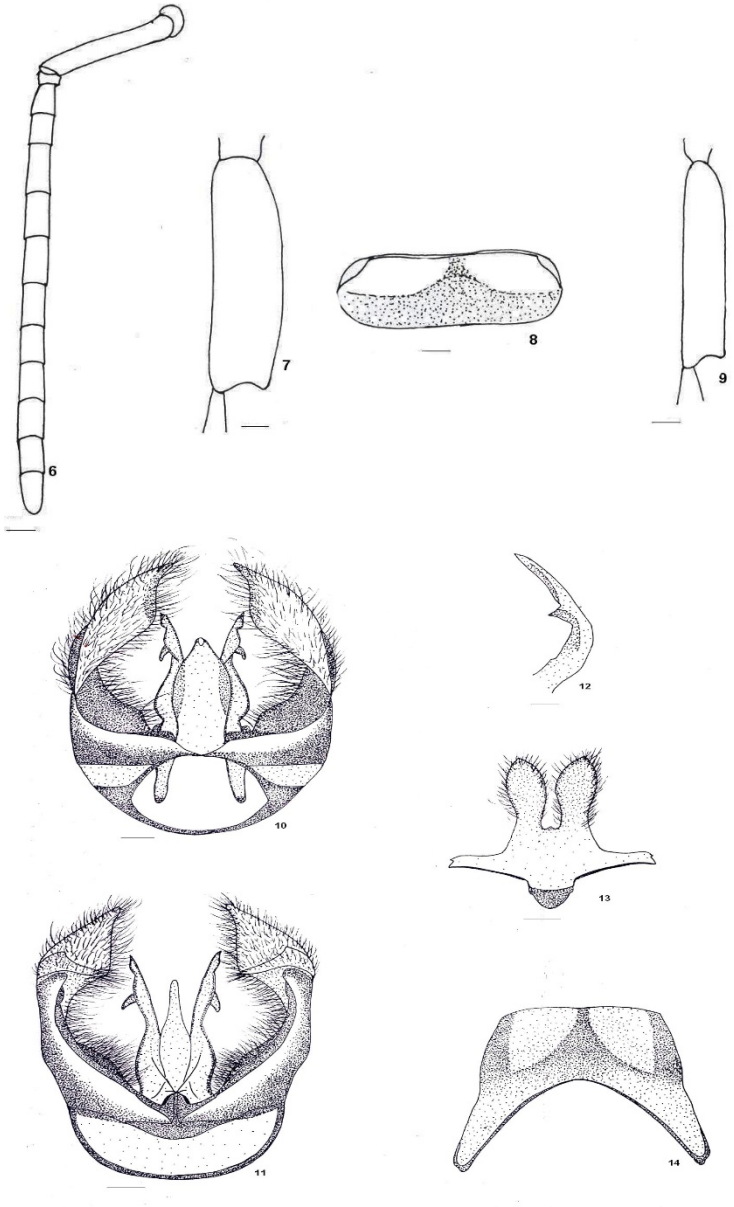


Fig 2. *B. ferganicus* (♂) – photograph, colour pattern and morphology. Legend: 6 – antenna, 7 – metabasitarsus, 8 – labrum, 9 – mesobasitarsus, Male genitalia: 10 – ventral aspect, 11 – dorsal aspect, 12 – penis valve, 13 – 8th sternite, 14 – 7th sternite. Scale bar = 0.5 mm.

Male: Head and mesonotum black, yellow are malar space, pronotum, metanotum and abdominal tergum 1; abdominal tergum 2 black with dirty yellow posterior corners; abdominal tergum 3-5 medially black with dirty yellow sides. Lateral tubercles raised, sloping inward, not meeting each other due to broad but shallow depression. Excepting a few large punctures close to top of tubercles, rest of the labrum with micropunctures. Anterior margin of labrum entire (Fig. 2-8). Area lateral to lateral ocellus in the ocello-ocular region unpunctured equal to the diameter of lateral ocellus. A band of punctures along eye margin covering half the area between lateral ocellus and eye margin. OOL: POL=2:3. The lateral ocelli are at the level of POC. Antennal segments 3:4:5=1:1.10:2.10; LF: LS: LHB=10:2.5:6.25 (Fig. 2-6). Head of penis valve nearly straight from dorsal aspect, not strongly curved but shaped like a slender arrow head from lateral apsect. Gonostylus broadly triangular with much long hair around interiobasal process. Inner corner of volsella well defined, strongly produced for some individuals without any inwardly directed hooks, nearly triangular in distal section, weakly sclerotised. Ventrobasal angle of penisvalve strongly and broadly produced ventrally and outwardly, so as to be clearly visible from dorsal aspect (Figs.2-10 to 2-14).

Material examined: Himachal Pradesh: Lahaul-Spiti, Gramphoo, 3800 m, 1 ♀, 2 ♂♂, 2.VIII.2003., 12.VIII.2013. Jammu and Kashmir: Bandipora, Achoora, 3300 m, 8 ♀♀ , 12 ♂♂, 28.VI.2009., 22.VI.2013.; Izmarg, 2500 m, 19 ♀♀, 20.VI.2007, 9.VII.2008, 25.VI.2012, 29.VII.2013, 14.VIII.2014 (Fig. 4); Baramulla: Affarwatt, 4000 m, 4 ♂♂, 21.VII.2009; Khilanmarg, 3300 m, 5 ♀♀, 9 ♂♂, 2.IX.2008, 3.VIII.2014.; Ganderbal: Baltal, 3350 m, 9 ♂♂, 11.VIII.2008, 23.VIII.2009, 13.VII.2012.; Kargil: Batalik, 3385 m, 2 ♀♀ (q), 7 ♂♂, 4.VIII.2008; Padam, 3650 m, 2 ♂♂, 31.VII.2008.; Leh: Hunder, 3600 m, 2 ♂♂, 5.VIII.2008. Budgam: Yousmarg, 2600 m; 16 ♀♀, 18♂♂, 21.V.2007, 22.VI.2008, 9.VIII.2014.

Distribution worldwide: India, Afghanistan, Pakistan and Kazakhstan, Kyrgyzstan and northwestern China (WILLIAMS, 2004).

Distribution within India: Kashmir, Himachal Pradesh, Uttarakhand and Sikkim (WILLIAMS, 1991; SAINI *et al.*, 2011).

Holotype depository: NHM, London.

Stratification: 2500-4000 m a.s.l.

Population variation: No variation.

Food plants: **Cirsium falconeri* (Hook.f.) Petrak, **C. wallichii* DC., *Cirsium* sp. (Fig. 3-2), **Echinops cornigerus* DC. (Asteraceae); **Brassica campestris* L. (Brassicaceae); *Dracocephalum heterophyllum* Benth. (Lamiaceae); **Trifolium pratense* L. (Fig. 3-1), *T. repens* L. (Papilionaceae); **Acantholimon lycopodioides* (Girard.) Bioss (Plumbaginaceae).

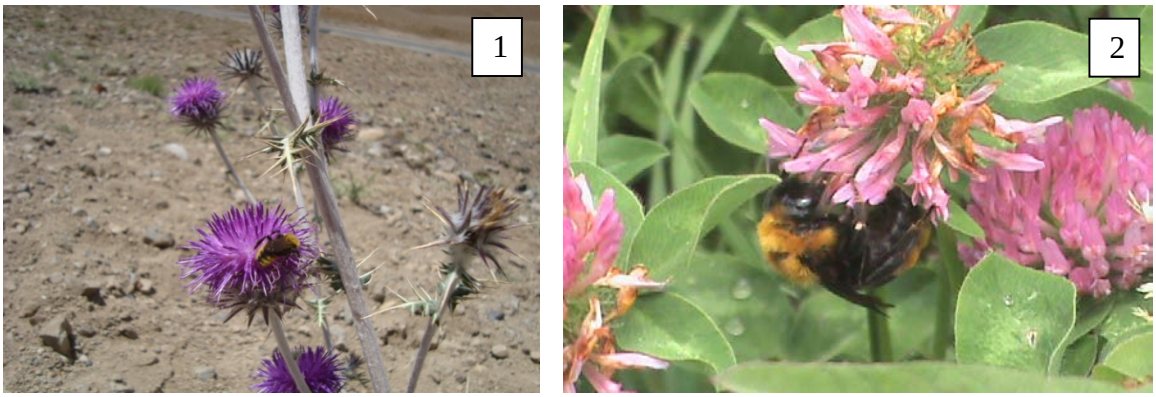


Fig. 3. 1 - *B. ferganicus* (♀) on *Trifolium pratense*. 2 - *B. ferganicus* (♂) on *Cirsium* sp.

Fig. 4. Collection locality (Izmarg), 2500 m.

***Bombus* (*Psithyrus*) *cornutus* FRISON, 1933**

Bombus Psithyrus cornutus FRISON, 1933: 338: Holotype female, India (ZSI); WILLIAMS, 1991: 45; YAO, 1995: 580; 1998: 404; YAO & LUO, 1997: 1694.

Synonymy: Psithyrus (*Psithyrus*) *pyramideus* MAA, 1948: 19; *Psithyrus acutisquameus* MAA, 1948: 21; *Psithyrus* (*Ceratopsithyrus*) *klapperichi* PITTIONI, 1949: 273; *Psithyrus* (*Eopsithyrus*) *cornutus* ssp. *canus* TKALCU, 1989: 42.

Diagnostic features

Female: Not recorded.

Male: Pubescence of head and abdominal tergum 4 black; lemon yellow are pronotum, metanotum, abdominal tergum 1 and 3; abdominal tergum 5 brick red; mesonotum lemon yellow with a median black patch; abdominal tergum 2 anteriorly black and posteriorly lemon yellow. Anterior margin of labrum weakly concave (Fig. 5-18). Tubercles meeting in centre at same level without any interruption. No median groove. Surface of tubercles covered with macropunctures. Rest of area with a mixture of macro and micropunctures. Area equal to the diameter of lateral ocellus situated in the ocello-ocular region, unpunctured. A broad band of punctures along eye margin covering two-third of the area between lateral ocellus and eye margin. OOL: POL=2:2.5. The lateral ocelli are just above the POC. Antennal segments 3:4:5=2:1.25:2.5; LF: LS: LHB=11.5:3.5:8 (Fig. 5-15); MS: BMB=2:1.75; MBB: LOB=2:9. Genitalia with the gonostylus and volsella moderately sclerotised, pale, and not thickened and the gonostylus densely hairy narrowing gradually from near its inner projection towards its outer margin, penis valve with the ventro-lateral angle strongly projecting and broad, forming almost a right angle distally, volsella near its midpoint marked only by a broad curve, not strongly produced inwards as an inner projection (Figs. 5-19 to 5-23).

Material examined: Himachal Pradesh: Kulu, Jalaori Pass, 3200 m, 8 ♂♂, 11.VIII.2004, 7.VIII.2013. Jammu and Kashmir: Leh, Hundur (Nobra valley), 3600 m, 1 ♂, 5.VIII.2008.

Distribution worldwide: India (Himalaya), Nepal, southwestern and central China. (WILLIAMS *et al.*, 2010).

Distribution within India: Himachal Pradesh, Uttarakhand and Kashmir (Ladakh) (WILLIAMS, 2004; SAINI *et al.*, 2011).

Holotype depository: ZSI, Kolkata.

Population variation: No variation.

Food plants: **Carduus nutans* L., **Cirsium falconeri* (Hook.f.) Petrak, **C. wallichii* DC., **Echinops cornigerus* DC., * *E. niveus* Wall., **Tanacetum* sp. (Asteraceae).

Stratification: 3600-4200 m a.s.l.

***Bombus* (*Psithyrus*) *novus* FRISON, 1933**

Bombus novus FRISON, 1933: 340, Holotype female, India: Kashmir, Nagaberan, (Bion) Kolkata (ZSI); BURGER *et al.*, 2009: 460.

Synonymy: *Psithyrus* (*Psithyrus*) *novus* subsp. *nepalensis* TKALCU, 1974: 318

Diagnostic features

Female: Pubescence of head, abdominal tergites 3-4 black; malar space white; yellow are pronotum, mesonotum, metanotum and abdominal tergites 1 and 2; abdominal tergum 5 with an anterior narrow black and posterior wide brick red bands. Outer surface of hind tibia convex, with moderate to long hair throughout, but without a comb of stout spines along inner distal margin; gastral sternum 6 with a pair of ventro-lateral keels, clypeus nearly uniformly flat, only apico-lateral corners curved back strongly towards occiput, crests of lateral keels of sternum 6, beyond projecting angle of mid-point, almost straight; sternum 2 with transverse ridge rounded and curved unevenly towards anterior margin in middle; labral furrow wide, about a third of total basal breadth of labrum, most of clypeus with scattered large punctures spaced more widely than their own widths, Mandible as illustrated (Fig. 6-27). Labrum with basal transverse depression extending apically as a deep median furrow between pronounced lateral tubercles, displacing ridge between them to form a lamella that overhangs apical margin; lateral furrow wide, about a third of total basal breadth of labrum (Fig. 6-28). MS: BMB=3:4. Antennal segments 3:4:5=1.50:1:1.25; LF: LS: LHB=8:4:10 (Fig. 6-24). The distoposterior corner of mesobasitarsus spinosely pointed (Fig. 6-25); and its length is longer than that of distoanterior corner. Metabasitarsus with its half of distoposterior margin concave (Fig. 6-25), outer surface of metatibia convex with moderate to long hair throughout but without a comb of stout spines along inner distal margin. Apex of tergite 7 having slightly raised boss, shallowly notched, with longitudinal median groove (Fig. 6-26).

Male: Pubescence black, white are: outer margin of pronotum, metanotum and abdominal tergite first and second, abdominal tergite third black whereas 4 to 5 are brick red. Anterior margin of labrum roundly, subtriangularly produced (Fig. 7-33); Lateral tubercles meeting in centre at same level, central area with some scattered punctures. Rest of the labrum with a mixture of macro and micropunctures. Area lateral to lateral ocellus in the ocello-ocular region unpunctured equal to the diameter of lateral ocellus. A broad band of punctures along eye margin covering two third of OOL; OOL: POL= 2:3. The lateral ocelli are just above the POC. Antennal segments 3:4:5= 1.25:1:1.35; LF:LS: LHB=16:5:12.5 (Fig. 7-31); MS:BMB=2:2.75; MBB:LOB=3:13. Gonostylus usually with many long hairs around interiobasal process. Distal section of volsella about as long as its maximum breadth from ventral aspect. Volsella weakly sclerotised yellowish in colour with a pronounced interiobasal process, associated with many long branched hairs. Ventrobasal angle of penisvalve strongly and broadly produced ventrally and outwardly so as to be clearly visible from dorsal aspect. Head of penisvalve, less than a quarter of total length, not strongly curved but shaped like a slender arrow head from lateral apsect. Head of penisvalve nearly straight from dorsal aspect. (Figs. 7-34 to 7-38).

Material examined: Jammu and Kashmir: Baramulla, Kongdori, 3000 m (Fig. 9); 1 ♀, 1 ♂, 10.VI.2007.; Razdan Pass, 3800 m; 16 ♂♂, 21.VIII.2011,06.IX.2011, 15.IX.2013, 4.IX.2014.

Distribution worldwide: India, Nepal and Pakistan (WILLIAMS *et al.*, 2010).

Distribution within India: Kashmir, Himachal Pradesh and Uttarakhand (WILLIAMS, 2004; SAINI *et al.*, 2011).

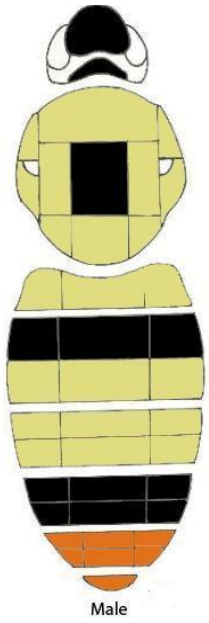
Holotype depository: ZSI, Kolkata.

Population variation: No variation.

Food plants: **Carduus edelbergii* (Fig. 8-1), *Circium* sp., **Saussurea lappa* C.B. Clarke, **Saussurea costus*, *Taraxacum officinale* (Asteraceae); *Swertia petiolata* D.Don (Gentianaceae); **Hyssopus officinalis* L., **Marrubium vulgare* (Lamiaceae); *Aconitum laeve* Royle, **Caltha alba* Camb., *Delphinium roylei* (Ranunculaceae) (Fig. 8-2); *Scrophularia*

pauciflora Benth. (Scrophulariaceae). *Stratification:* 2900-3900 m a.s.l.

B. cornutus (♂)



Colour pattern

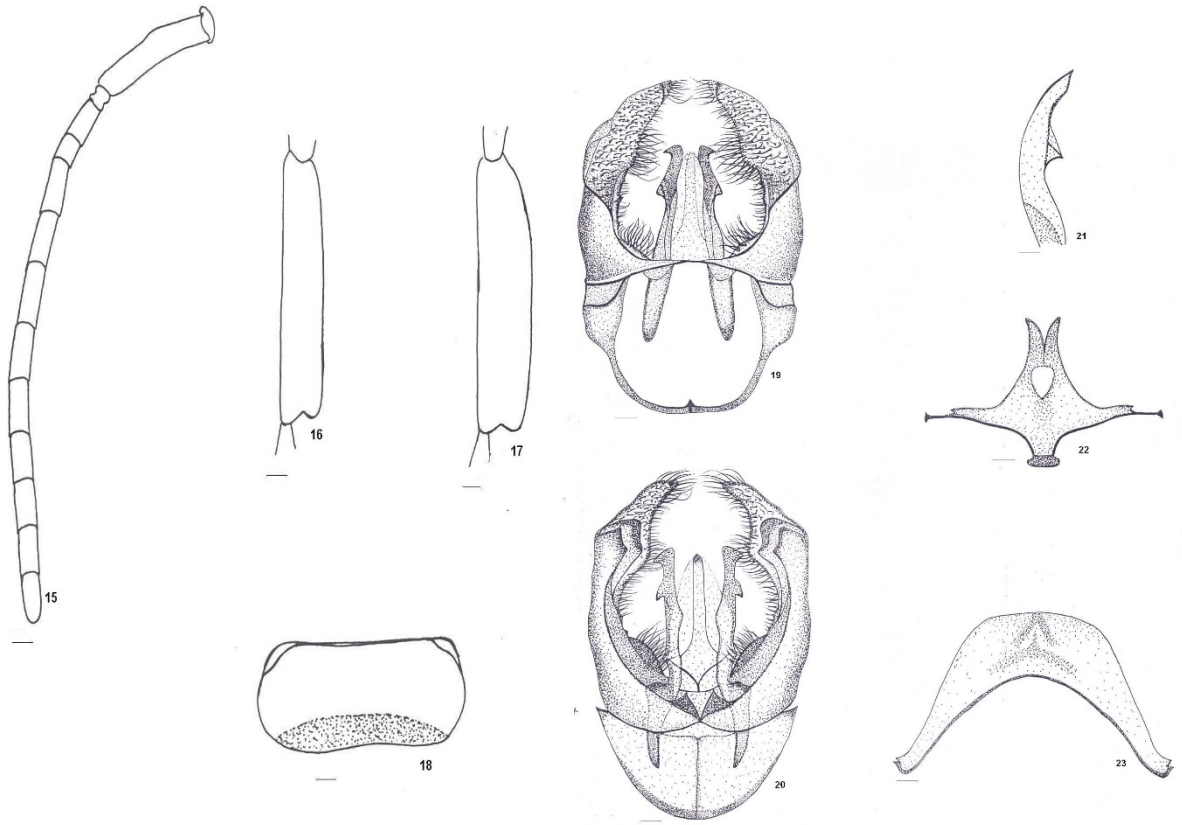
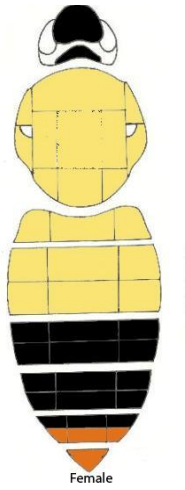


Fig. 5. *B. cornutus* (♂) – photograph, colour pattern and morphology. Legend: 15 – antenna, 16 – mesobasitarsus, 17 –metabasitarsus, 18 – labrum, Figs. 19-23. Male genitalia: 19 – ventral aspect, 20 – dorsal aspect, 21 – penis valve, 22 – 8th sternite, 23 – 7th sternite. Scale bar = 0.5 mm.

B. novus (♀)



Colour pattern

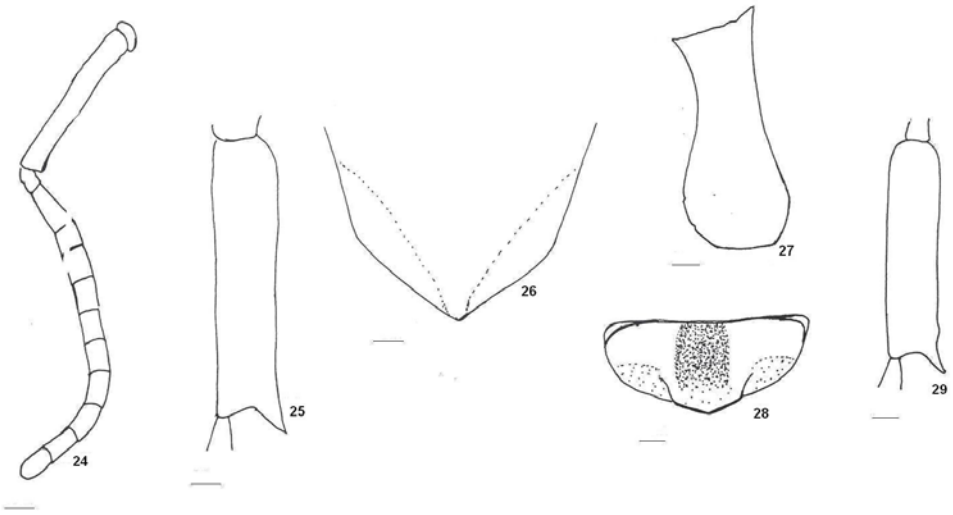
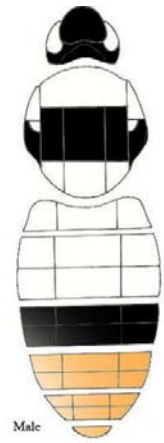


Fig 6. *B. novus* (♀) – photograph, colour pattern and morphology. Legend: 24 – antenna, 25 – metabasitarsus, 26 – 7th sternite, 27 – mandible, 28 – labrum, 29 – mesobasitarsus, Scale bar = 0.5 mm.

B. novus (♂)



Colour pattern

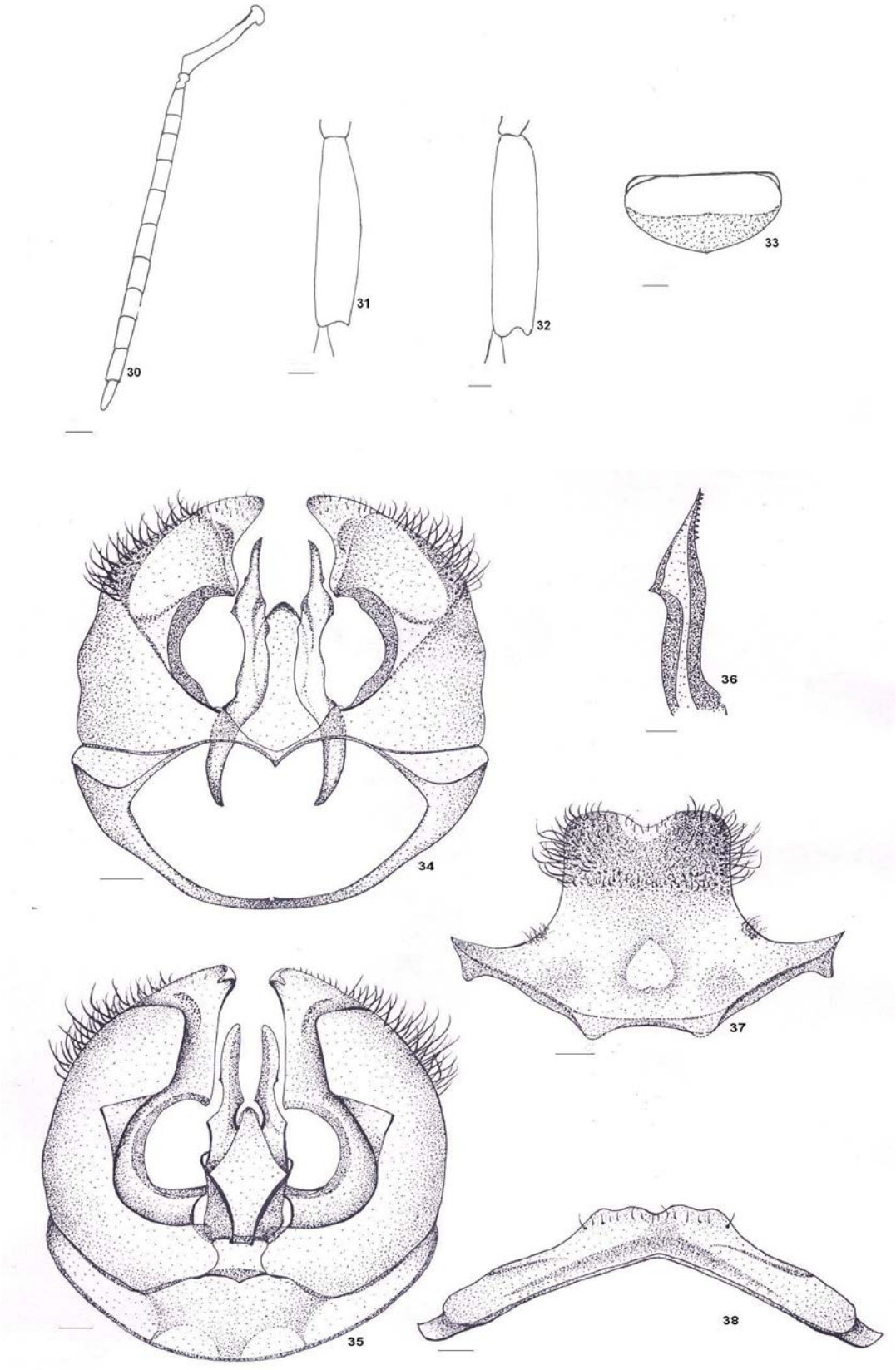


Fig 7. *B. novus* (♂) - photograph, colour pattern and morphology. Legend: 30 – antenna, 31 – mesobasitarsus, 32 –metabasitarsus, 33 – labrum, Figs. 34-38. Male genitalia: 34– ventral aspect, 35 – dorsal aspect, 36 – penis valve, 37 – 8th sternite, 38 – 7th sternite. Scale bar = 0.5 mm.

Fig. 8. 1 - *B. novus* (♀) on *Carduus edelbergii,* 2 - *B. novus* (♂) on *Delphinium roylei.*

Fig. 9. Collection locality - Kongdori, 3000 m.

***Bombus* (*Psithyrus*) *branickii* (RADOSZKOWSKI, 1893)**

Psithyrus branickii RADOSZKOWSKI, 1893: 241, Lectotype female, USSR, Kirgiziya (MNHU); TKALCU, 1969: 204; PESENKO, 2000: 8; WILLIAMS, 1991: 48.

Synonymy: Apathus chloronotus MORAWITZ, 1894: 6; *Psithyrus rupestris* var. *eriophoroides* REINIG, 1930: 110; *Psithyrus* (*Psithyrus*) *rupestris* subsp. *elisabethae* REINIG 1940: 231; *Psithyrus branichi* KIM & LTO, 1987.

Diagnostic features

Female: Pubescence of head and abdominal tergum 4 black; malar space and thorax yellow; abdominal tergum 5 brick red; abdominal tergites 2 and 3 black with yellow posterior corners; wings light brown. Mandible as illustrated (Fig. 10-43). Labrum with basal transverse depression extending apically as a deep median furrow between pronounced lateral tubercles, displacing ridge between them to form a lamella that overhangs apical margin (Fig. 10-41); labral furrow narrow, about fifth of total basal breadth of labrum; clypeus with many large punctures spaced more closely than their own widths, except in a well-defined narrowly unpunctured mid apical area; antennal segments 3:4:5=1.80:1:1.40; LF: LS: LHB=10:6:13 (Fig. 10-39). Outer surface of meta tibia convex with long hair throughout but without a comb of stout spines along inner distal margin. Crests of lateral keels of sternite 7, beyond projecting angle of mid-point, with a strongly concave margin (Fig. 10-42). Meta basitarsus with its posterior

margin nearly concave with a distoposterior corner spinosely produced and longer than distoanterior corner (Fig. 40). The distoposterior corner of mesobasitarsus spinosely produced. The length of this corner longer than the length of distoanterior corner (Fig. 10-44). Apex of tergite 7 slightly raised, with no boss and no median furrow.

Male: Pubescence of head, mesonotum and abdominal tergum 3 black; yellow are malar space, pronotum, metanotum, lateral aspects of mesonotum and abdominal tergites 1 and 2; abdominal tergites 4 and 5 are brick red. Anterior margin of labrum apically produced, lateral tubercles low lying meeting at centre at same level, entire area micropunctured (Fig. 48). Area lateral to lateral ocellus in the ocello-ocular area unpunctured equal to one and half times the diameter of lateral ocellus. A band of punctures along eye margin covering almost half of the area between lateral ocellus and eye margin. OOL: POL= 2:2.5. The lateral ocelli are above the POC. Antennal segments 3:4:5=1.5:1:1.75; LF: LS: LHB=9.5:3.25:7 (Fig. 45). Genitalia with the gonostylus and volsella weakly sclerotised and pale, the gonostylus densely hairy; penis valve with the ventro-lateral angle strongly projecting and distally narrowed so that it is almost spinose, volsella in the distal part extending inwards broadly towards the inner corner and almost twice as long as its maximum breadth from the ventral aspect (Figs. 49-53).

Material examined: Jammu and Kashmir: Kargil, Zanskar, 3600 m, 1 ♀, 1 ♂, 30.VII.2007.

Distribution worldwide: India, Afghanistan, Pakistan, Tibet, China, Kazakhstan, Kyrgyzstan, Mongolia, Russia and Tajikistan (WILLIAMS *et al.*, 2010).

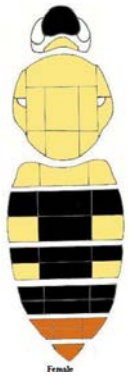
Distribution within India: Kashmir, Himachal Pradesh, Uttarakhand and Sikkim. (WILLIAMS, 2004; SAINI *et al.*, 2011).

Holotype depository: ZS, Munich.

Population Variation: No variation.

Food plants: Allium cepa (Amaryllidaceae); *Gypsophila cerastioides* (Caryophyllaceae); *Rhodiola crenulata* (Crassulaceae); **Hyssopus officinalis* L. (Lamiaceae); *Caragana versicolor* Wall., **Hedysarum* sp. (Papilionaceae); **Aconitum* sp. (Ranunculaceae).

B. branickii (♀)



Colour pattern

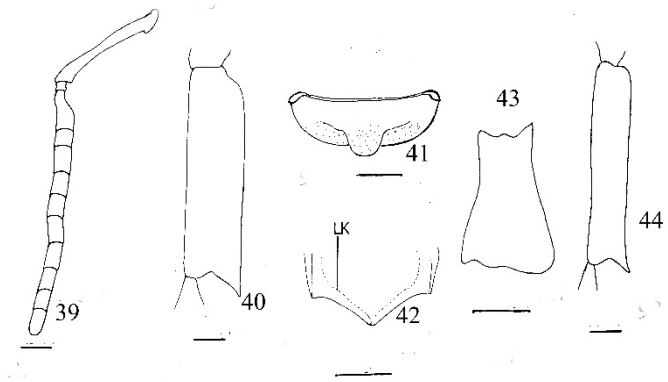
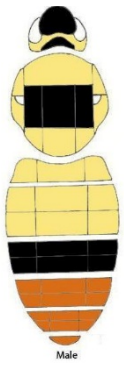


Fig. 10. *B. branickii* (♀) - photograph, colour pattern and morphology. Legend: 39 – antenna, 40 – metabasitarsus, 41 – labrum, 42 – 7th sternite, 43 – mandible, 44 – mesobasitarsus, Scale bar = 0.5 mm.

B. branickii (♂)



Colour pattern

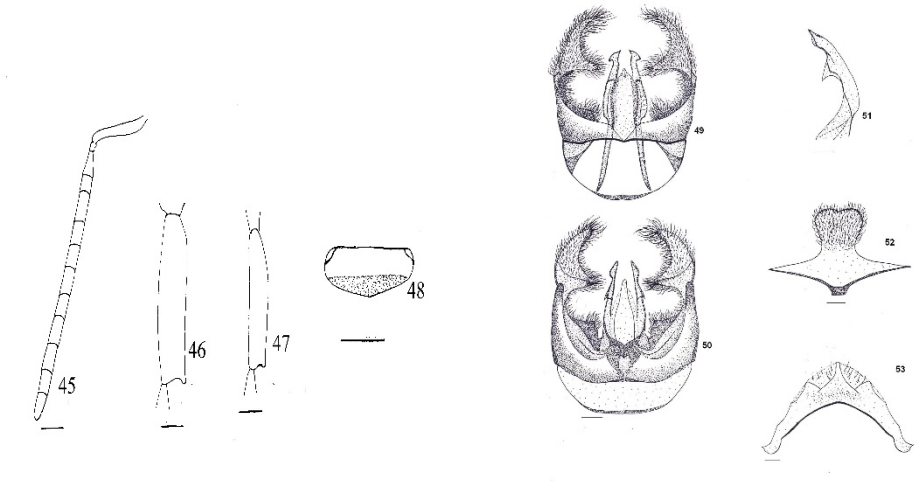


Fig. 11. *B. branickii* (♂) - photograph, colour pattern and morphology. Legend: 45 – antenna, 46 – metabasitarsus, 47–mesobasitarsus, 48 – labrum, Figs. 49-53. Male genitalia: 49– ventral aspect, 50 – dorsal aspect, 51– penis valve, 52 – 8th sternite, 53 – 7th sternite. Scale bar = 0.5 mm.

***Bombus* (*Psithyrus*) *skorikovi* POPOV, 1927**

Psithyrus skorikovi POPOV, 1927: 267, Holotype female, China: Gansu (ZI); (*Grum Grzhimailo*); WILLIAMS, 1991: 50, MACIOR & TANG, 1997: 3; BURGER *et al.*, 2009: 461.

Synonymy: Psithyrus skorikovi var. *mesoxanthus* RICHARDS, 1928: 360; *Psithyrus* (*Fernaldaepsithyrus*) *gansuensis* POPOV, 1931: 168, 202; *Psithyrus* (*Fernaldaepsithyrus*) *kuani* TKALCU, 1961: 362.

Diagnostic features

Female: Not recorded.

Male: Pubescence on head black; thorax, abdominal tergites 1 to 3 dark yellow; black are: last three abdominal tergites (Fig. 12). Genitalia with the gonostylus and volsella weakly sclerotised and pale, the gonostylus densely hairy; penis valve with the ventro-lateral angle strongly projecting and narrow, distally pointed; volsella in the distal half narrowed into an elongate finger.

Material examined: Razdan Pass, 3800 m (Fig. 14); 9 ♂♂, 06.IX.2011, 13.IX.2014 (very rare species).

Distribution worldwide: India, Pakistan, Nepal, China and Tibet (WILLIAMS *et al.*, 2010).

Distribution within India: Kashmir, Himachal Pradesh Uttarakhand and Sikkim (WILLIAMS, 2004; SAINI *et al.*, 2011).

Holotype depository: BMNH.

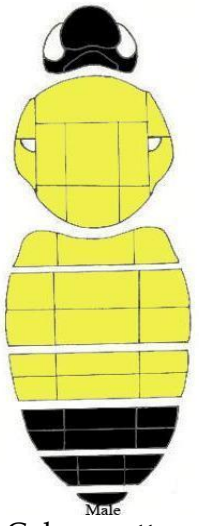
Population variation: No variation.

Food plants: *Aster* sp., *Cirsium* sp., **Saussurea costus* F. (Fig. 13-1), **Taraxacum officinale* (Asteraceae) (Fig. 13-2); **Swertia*

petiolata D.Don (Gentianaceae); *Prunella vulgaris* L. (Lamiaceae); **Trifolium pratense* L., **T. repens* L., (Papilionaceae); *Scrophularia pauciflora* Benth. (Scrophulariaceae).

Startification: 2700–4300 m a.s.l.

B. skorikovi (♂)



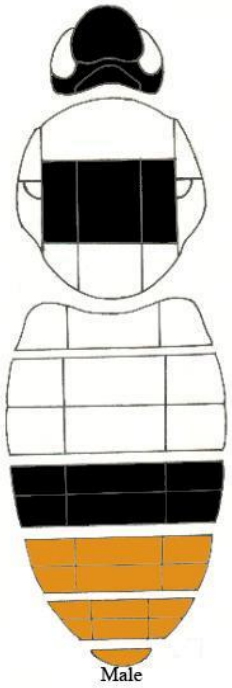
Colour pattern

Fig. 12. *B. skorikovi (♂)* – photograph and colour pattern.

Fig. 13. 1 - *B. skorikovi* (♂) on *Saussurea costus;* 2 - *B. skorikovi* (♂) on *Taraxacum officinale.*

Fig. 14. Collection locality: Razdan Pass, 3800 m.

B. turneri (♂)



Colour pattern

Fig. 15. *B. turneri* (♂) – photograph and colour pattern.

Bombus* (*Psithyrus*) *turneri RICHARDS, 1929

B. (*Psithyrus*) *turneri* RICHARDS, 1929:141, Holotype male BMNH India.; WILLIAMS, 1991:52; YAO & LUO, 1997: 1695.

Synonymy: Psithyrus monozonus FRIESE, 1931:304; *Psithyrus* (*Eopsithyrus*) *decoomani* MAA, 1948:26; *Psithyrus* (*Eopsithyrus*) *martensi* TKALCU, 1974:314.

Diagnostic features:

Female: not recorded

Male: (length 12–14 mm) with the hair of the thorax and tergum 3 black, sometimes with greyish bands on the anterior, posterior, and lateral aspects of the thorax, terga 1–2 white, 4–7 orange-red; genitalia with the gonostylus and volsella weakly sclerotised and pale, the gonostylus densely hairy, narrowing gradually from near its outer margin towards its inner projection; penis valve with the ventro-lateral angle strongly projecting and narrowed and pointed, like a shark's fin; volsella proximally with a large inwardly projecting swelling that is irregular but nearly rectangular, volsella near its mid-point marked only by a broad curve, not strongly produced inwards as an inner projection (Fig. 15).

Material examined: 1♂, procured on exchange basis from British Museum (Natural History), London, UK. The species was not found under the present study. Photograph of adult male supplied by Dr. Norton, Sr. Curator, British Museum (Natural History), London, UK.

Distribution worldwide: India, Himalaya, Nepal, Bhutan, eastern Tibetan plateau, Taiwan and south-western China (WILLIAMS *et al.*, 2010).

Distribution within India: Sikkim and Arunachal Pradesh (WILLIAMS, 2004).

Food plants (based on literature): Carduus sp., *Centaurea* sp., *Cirsium* sp. (Asteraceae); *Rosa* sp. (Rosaceae).

Stratification: 3300-3800 m a.s.l.

***Bombus* (*Psithyrus*) *morawitzianus* (POPOV, 1931)**

This description of this species has not been included in this paper as the same is published by the RAINA *et al.*, (2013) in Entomological News.

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The Influence of Cadmium on the Food Consumption and Utilization of the Cotton Leaf Worm Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae)

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Abstract. The third, fourth, fifth, and sixth instars of the cotton leaf worm *Spodoptera littoralis* (Boisduval, 1833) were reared on castor bean leaves *Ricinus communis (*Linnaeus, 1753*)* treated with cadmium 100 mg $CdCl_2$/kg, using the dipping method, to evaluate the effect of cadmium (Cd) on nutritional indices. It was observed that the consumption index was significantly decreased at all the studied instars except for the third instar. The absorptive capacity, in terms of approximate digestibility, was significantly decreased at the sixth instar. The food utilization efficiencies, in terms of the conversion of ingested and digested food (ECD) to biomass, were significantly decreased at both fourth and fifth instars. Moreover, the ECD was significantly decreased at the sixth instar. The growth rate decreased at the different studied instars except for the sixth instar.

Keywords: *Spodoptera littoralis,* Heavy metals, Cadmium treatment, Nutritional indices

Introduction

The cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae), is one of the most destructive agricultural lepidopteran pests in Egypt as well as Mediterranean and Middle East countries (ADHAM *et al.,* 2005a; TIESSEN, 2012).

Cadmium (Cd) is a non–essential toxic heavy metal and it is highly ranked on the EU list of hazardous substances (ALLWAY, 1995). It has received considerable attentions over the past years as a result of increased environmental burdens from industrial, agricultural, energy, and municipal sources (WAGNER, 1993). A significant amount of cadmium can be found near mines and zinc smelters, on fertilizer-treated lands, industrial sewage sludge, and disposal sites of batteries. Cadmium exerts a negative impact on living organisms and accumulates in food chains (ROBERTS *et al.,* 1979). At organism level, it affects food consumption and digestibility (MIGULA & BINKOWSKA, 1993; FOUNTAIN & HOPKIN, 2001; VAN OOIK *et al.,* 2007). Also, cadmium interferes with the important biological processes (BUCHWALTER *et al.,* 2008). It was also found that the ephemeropterid larvae, *Baetis thermicus* (Uéno, 1931), collected from a metal-contaminated river in Japan, accumulated cadmium heavily in the body as indicated by LINDQVIST & BLOCK (1994).

The relationships between food consumption and growth of different instars of *S. littoralis* caused by metal contamination were less studied. Hence, the aim of this research is to investigate the effect of Cd on food utilization efficiencies such as approximate digestibility (AD), the efficiency of conversion of ingested food (ECI), the efficiency of conversion of

digested food (ECD), growth rate (GR) and consumption index (CI) of the third, fourth, fifth, and sixth instars of *S. littoralis*.

Material and Methods

Insect rearing. The colony of *S. littoralis* was started with egg patches obtained from a standard laboratory colony maintained in the Department of Entomology, Faculty of Science, Cairo University. Newly hatched larvae were placed in plastic boxes (12 × 6 × 18 cm). Larvae were fed on fresh castor bean leaves, *Ricinus communis (*Linnaeus, 1753*)*, until pupation. The culture was kept at 25 ± 2°C, 65 ± 5%R. H. and 12:12 hours (D:L) photoperiod.

Cadmium treatment. The heavy metal Cd was obtained in the form of $CdCl_2$ (Sigma-Aldrich®). Newly hatched third, fourth, fifth and sixth instars of *S. littoralis* were freely fed on fresh *R. communis* leaves dipped in 100 mg $CdCl_2$/kg (BAGHBAN *et al.,* 2014) for 10 seconds, then air-dried at room temperature (KRISHNAPPA & ELUMALAI, 2012; MOADELI *et al.,* 2014). Each experiment was replicated three times of twenty larvae each. The remaining diet was replaced regularly with freshly treated one at every 24 hours. The experiment was started with larvae hatched in the same day and were offered daily Cd-contaminated food from hatching till pupation.

Effects of cadmium on nutritional indices. The fresh weight of larvae, faeces, and of leaf consumed, and weight gain of larvae were recorded regularly. All uneaten food and faeces excreted were collected and immediately frozen; these matters were later dried at 105°C in an oven (Thermo Scientific® Compact Oven) and weighed using analytical balance (Mettler® M22) according to ADHAM *et al.* (2005a,b) for calculating the food utilization. Exuviae were measured with faeces since they were considered as parts of the insect at the end of the experiment as suggested by REESE & BECK (1976).

Nutritional indices of the 3rd, 4th, 5th and 6th instars were calculated using standard gravimetric procedures described by WALDBAUER (1968) as follows:

- Consumption index (CI) = F/TA,
- Approximate digestibility (AD) = 100 × (F-E)/F,
- Efficiency of conversion of ingested food into biomass (ECI) = (G/F) × 100,
- Efficiency of conversion of digested food into biomass (ECD) = (P/F- E) × 100,
- Growth rate (GR) = P/TA,

where:

A = fresh weight of larvae during the feeding period

E = dry weight of produced feces

F = dry weight of food eaten

G = weight gain at the end of the feeding period

P = dry weight of the biomass of larvae

T = duration of feeding (days)

Statistical analysis. All data were presented as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple range test. All statistical computations were carried out using SAS program (SAS INSTITUTE, 2002). Significance was set at $P < 0.05$.

Results and Discussion

The Cd-treatment decreased significantly the CI ($P < 0.05$) compared to the control at all the instars, except for at the 1st instar where the change was insignificant (Table 1). The CI was steadily decreased through the studied instars, with a significant difference only between the 3rd and the other instars (Table 1). This may be due to the findings declared by BAGHBAN *et al.* (2014). Our results were in agreement with those declared by HARE (1992); who found that "non-essential" elements such as Cd, even at low concentrations, are toxic for organisms.

Approximate digestibility significantly decreased ($P < 0.05$) due to Cd treatment only at the 6th instar compared to the control (Table 1). With advancing age, significant difference ($P < 0.05$) was attained only between the 6th instar and the remaining instars (Table 1). Such decrease in AD could be due to the metal toxicity which impaired food absorption as suggested by YAZDANI *et al.* (2014).

Table 1. The mean values of nutritional indices of the 3rd, 4th, 5th and 6th instars of *Spodoptera littoralis* fed castor bean leaves treated with 100 mg $CdCl_2$/kg.

Instar \ Treatment	Control Mean ± SD	Cd Mean ± SD	(P) value
3rd instar			
CI	0.933 ± 0.092 a	0.609 ± 0.218 a	Ns
AD (%)	85.925 ± 2.001 a	89.414 ± 2.15 a	Ns
ECI (%)	20.828 ± 6.258 a	23.235 ± 1.964 a	Ns
ECD (%)	23.985 ± 6.35 5 a	28.93 ± 2.741 a	Ns
GR	0.185 ± 0.027 a	0.099 ± 0.018 b	0.013
4th instar			
CI	0.753 ± 0.002 a	0.406 ± 0.017 b	Hs
AD (%)	84.313 ± 1.012 a	84.607 ± 1.733 a	Ns
ECI (%)	24.205 ± 3.45 a	12.793 ± 1.848 b	0.004
ECD (%)	28.64 ± 3.5 a	12.747 ± 1.62 b	Hs
GR	0.182 ± 0.025 a	0.051 ± 0.006 b	Hs
5th instar			
CI	0.469 ± 0.011 a	0.278 ± 0.009 b	Hs
AD (%)	73.048 ± 2.851 a	65.278 ± 2.744 a	Ns
ECI (%)	32.118 ± 10.838 a	12.643 ± 1.031 b	0.008
ECD (%)	43.153 ± 7.11 a	22.548 ± 2.427 b	0.034
GR	0.153 ± 0.033 a	0.033 ± 0.004 b	0.012
6th instar			
CI	0.353 ± 0.033 a	0.233 ± 0.021 b	0.022
AD (%)	57.063 ± 2.33 a	32.938 ± 6.427 b	0.013
ECI (%)	20.865 ± 5.275 a	34.588 ± 1.059 a	Ns
ECD (%)	39.325 ± 5.742 a	19.153 ± 5.71 b	0.003
GR	0.245 ± 0.173 a	0.058 ± 0.007 a	Ns

- Means in rows followed by the same letters are significantly different ($P < 0.05$)
- Means in columns followed by the same letters are significantly different ($P < 0.05$)
- Ns: Not significant ($P > 0.05$), Hs: Highly significant ($P < 0.01$)

Similar to our results, BAGHBAN *et al.* (2014) found that under Cd-treatment, a probable damage to the epithelium of the gut of the noctuid moth; *Helicoverpa armigera* (Hübner, 1809) (Lepidoptera: Noctuidae) thereby; may hinder the absorption capacity.

Treatment with Cd significantly decreased ($P < 0.05$) the ECI compared the control at 4th and 5th instars (Table 1). On the contrary, significant increase in the values of ECI compared to the control was attained only at the 6th instar (Table 1). With advancing instars, there was a significant difference ($P < 0.05$) between 3rd and 4th, 3rd and 5th, 4th and 6th, and 5th and 6th instars (Table 1). EMRE *et al.* (2013) and BAGHBAN *et al.* (2014) attributed the increase in ECI under the stress of heavy metal treatment to the fact that insect requires a lot of energy to deal with the metal toxicity. This explanation may extend to our results.

The ECD at Cd-treatment significantly decreased ($P < 0.05$) at all the instars except the 3rd one (Table 1). Among the different instars, a significant difference ($P < 0.05$) was observed only between the 3rd and other instars (Table 1).

The growth rate was significantly decreased ($P < 0.05$) post-treatment with Cd at the 3rd, 4th, and 5th instars. Whereas, the change in GR was insignificant compared to the control at the 6th instar (Table 1). With advancing age, there was a significant difference ($P < 0.05$) between 6th and the remaining instars (Table 1). It has to be mentioned that high or moderate food consumption by insects is not always associated with high GR, as the latter might be affected by a low ECI value (WALDBAUER,

1968; MEHRKHOU, 2013). The decrease in GR in the present study may be accounted for by the decrease in CI and food utilization efficiencies expressed in terms of ECI and ECD. This suggestion is confirmed by the findings of WOODRING *et al.* (1978) who indicated that the amount of growth reduction was proportional in general to reduced food consumption. In agreement with our results, MIGULA *et al.* (1989) recorded reduced AD, ECI, and ECD in *Acheta domesticus* (Linnaeus, 1758) exposed to heavy metals.

In conclusion, it appears that exposure of *S. littoralis* larvae to Cd would reduce the weight gain. From practical standpoint, this finding may negatively impact the population dynamics of the next generation of this pest.

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Intercropping System for Protection the Potato Plant from Insect Infestation

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Abstract. The use of intercropping system provides an option for insect control for organic farmers that are limited in their chemical use. Additionally, intercropping systems can be attractive to conventional growers as a cost-effective insect control solution. A study was carried out for two seasons 2011-2012 and 2012-2013 to evaluate the effect of intercropping of potato (*Solanum tuberosum* L.) with onion (*Allium cepa* L.) on whitefly (*Bemicia tabasi* Gennadius) and aphids' *Myzus persicae* Sulz. and *Aphis gossypii* Glover infestation in potato fields. Results indicated that intercropping significantly reduced potato plant infestation with whitefly by 42.7, 51.3% while it was 62.69% reduction with aphids during the two successive winter seasons than when potato plants were cultivated alone. Therefore, intercropping could be recommended as a protection method of reducing pest population in the fields.

Key words: intercropping, whitefly, aphids, potato, onion, pest infestation.

Introduction

The use of chemical insecticides for the control of insect pests results to resistance of some pests to some conventional insecticides. Alternative methods of managing pests such as cultural control have to be employed to reduce pest infestation on crops. BIDOO *et al.* (2012) reported that onion was used as an intercrop to manage the pests of cabbage.

Intercropping is the planting of more than one crop in close proximity as part of the same farming system. The design of intercropping system can vary dramatically depending on the purpose of the intercrop for the farming operation. Intercropping produces the benefits of on-farm diversity, increased productivity, resource distribution balance, farm risk reduction, and weed and insect pest control. Intercropping systems for insect pest control includes the planting of a crop that has a repellent effect, an attractant effect, or a combination of both, on a target pest in close proximity to a crop that has the potential to be attacked by it (DEGRI *et al.*, 2014). Many spatial combinations are possible for intercropping, including mixed intercropping, in which different crops are planted in the same row or at alternating rows. Crop rotation means planting different crops on the same plot during different times of the year, and can include some of the benefits of intercropping, such as reducing insect pest populations, increasing beneficial insects, and weed suppression. In addition, non-crop plants such as weeds, cover crops, and habitat

plantings can be combined in space and time to influence numbers of pest and beneficial arthropods on the main crop (SMITH & LIBURD, 2015). Some plant combinations, for instance, with non-hosts reduce the spread of pests within crops (GAUTAN & KAUSHIK, 1980; WILLEY & GARVER, 1981; DEGRI *et al*., 2014). Non-host plants in such mixture may emit chemicals or odors that adversely affect the pests, thereby conferring some level of protection to the host plant (SING *et al*., 1986; REDDY, 2012). Studies indicate that crop diversification through intercropping, such as cereals with legumes, is effective in reducing insect pest damage (NWANZE, 1989). Also, the intercropping of groundnut with pearl millet (*Pennisetum glaucum* L.) increased the population of *Gonizous sp*., a parasitoid species that effectively manages leaf miner *Arachis hypogaea* L. population in groundnut (DHALIWAL & ARORA, 1966). Work by CHABI-OLAYE *et al*. (2005) found a considerably reduced amount of noctuid eggs laid by *Sesamia calamistis* Hampson and *Busseola fusca* Fuller due to lack of the host found by the ovipositing adult moths in maize intercropped with grain legumes or cassava than those in monocrop. The aim of the present experiment was to evaluate the effect of intercropping of potato plant with onion for protection the potato from insect infestation under field experiment.

Materials and Methods

Experiments were carried out at an experimental farm in Kalubia governorate, Egypt, during the successive two late winter seasons 2011-2012 and 2012-2013 in order to evaluate the effect of intercropping of onion, *Allium cepa* (L.), with potato plants *Solanum tuberosum* (variety Necola) on potato infestation with aphids and white fly which have been recorded as the major pests (MOAWAD, 1999, Ain Shams University, pers. comm.). Each plot contained 10 rows and was separated from other plots by uncultivated rows. Each row was cultivated with six potato tubers at distances of 25 cm (MICHEAL, 1995, Zagazig University – pers. comm.). Tubers were buried in soil at 10 cm depth. The planted area was kept free from any chemical insecticidal treatment. The transplanted onion was cultivated at the end of each potato row; which was surrounded from east and west by three onion transplants; while the north and south of potato rows were planted only by an onion row, contained 12 onion transplants. The check plots were cultivated by potato plants only and were separated from the experimental area by an unplanted plot (20 rows). The check and test plots were divided in an area of 300 m^2. Experiments were replicated three times for each of the two seasons. Sampling of potato (25 leaflets at 7 days intervals for three months duration from December, January and February) was carried out after 30 days of plantation for whitefly *Bemicia tabaci* and aphids *Myzus persica* and *Aphis gossypii* infestation. The collected samples were kept in paper bags in a refrigerator till examined by the use of a binocular microscope. They were separated, identified and counted. The data was statistically processed, using t-test.

Results and Discussion

Results on the effect of intercropping potato with onion *(Allium cepa* L.*)* on the population density of immature stages (eggs, nymphs and pupae) of the whitefly, *B. tabaci* and aphids, *M. persicae* and *A. gossypii*, in the two seasons of winter plantations are given in Tables 1 and 2.

Effect on the whitefly infestation

The data presented in Tables 1 and 2 indicated that the lowest number of immature stages of the whitefly was observed on potato leaves intercropped with onion plant. Thus, during the two seasons, the mean number of immature stages of the whitefly/25 leaflets were 59.02 and 51.7 during the first and the second winter seasons, compared with 154.95 and 168.3 immature stages of the whitefly/25 leaflets for the control (sole potato), respectively. Reduction in percent of whitefly infestation reached 62% and 69% in the first and the second winter seasons, respectively.

Statistical analysis (t-test) revealed that there was highly significant differences (p<0.01) between the population density of

the whitefly on potato leaves intercropped with onion and that on potato leaves cultivated alone in the two seasons of the study.

Table 1. Effect of intercropping potato with onion on the population density of the whitefly (*B. tabaci*) in the first winter season (2011-2012).

Inspection dates		Mean number of immature stages of *B. tabaci* /25 leaflets±SE.	
		Potato : onion	Sole potato
Dec.	10	11.3±0.11	20.7±0.08
	17	21.3±0.01	46.7±0.12
	24	61.0±0.14	130.6±0.15
	31	98.7±0.51	395.1±1.04
Jan.	7	146.0±0.22	386.0±1.11
	14	142.3±0.31	302.0±0.46
	21	94.3±0.25	281.3±0.33
	28	81.7±0.07	193.0±0.17
Feb.	4	44.7±0.13	62.4±0.22
	11	6.9±0.03	41.7±0.12
	18	0.0±0.00	0.0±0.00
	25	0.0±0.00	0.0±0.00
Total		708.2	1859.9
General mean		59.02*± 0.32	154.95 ±0.05

Table 2. Effect of intercropping potato with onion on the population density of the whitefly *(B. tabaci*), in the second winter season (2012-2013)

Inspection dates		Mean number of immature stages of *B. tabaci* /25 leaflets	
		Potato: onion	Sole potato
Dec	15	3.6±0.08	15.7±0.12
	22	7.3±0.14	67.7±1.03
	29	73.7±0.20	326.3±0.12
Jan.	5	151.7±0.22	459.0±1.06
	12	159.0±0.12	451.7±0.08
	19	110.0±0.20	253.7±0.11
	26	59.70.12	233.0±0.05
Feb.	1	33.3± 0.14	143.7±0.21
	8	21.7±0.04	55.3±0.04
	15	0.0±0.00	13.7± 0.12
	22	0.0±0.00	0.0±0.00
Mar.	1	0.0±0.00	0.0±0.00
Total		620.0	2019.8
General mean		51.7* ±0.01	168.3±0.12

Effect on aphids. Infestation

Results are presented in Tables 3 and 4, they cleared that the infestation was much lower in case of potato plants intercropped with onion than in case of potato plants cultivated alone. Thus, the rate of infestation of potato plants cultivated with onion was 64.5 and 81.2 aphids/25 leaflets, compared with 112.6 and 166.8 aphids/251eaflets for the potato crop alone, with reduction in percent of aphids infestation reached 42.7% and 51.4% in the two seasons' respectively. The difference was statistically highly significant (p<0.01).

The aforementioned results clearly showed that intercropping potato with onion plant exhibited considerable protection against the whitefly, but relative-

ly less protection against aphids. The effect may be attributed mostly to the odor of the non-host crop (onion). The obtained result agrees with those obtained by TAVAIENEN & ROOT (1974) and LAL (1991) who found that intercropping potato with onion and other crops exhibited a best protection of potato crop from the potato tuber moth *Phthorimaea operculilla* attack more than sole potato culture. It also agrees with POTTIS & GUNADI (1991) who found that intercropping potato crop with *Allium cepa* or *A. sativum* caused reduction in the population of *Myzus persicae, Aphis gossypii* and *Emposca* spp. LAWANI (1982) and KHAN *et al*. (1997) stated that intercropping has been reported to reduce insect pest populations due to colonization deterrence in crop field with increased plant diversity that creates unsuitable or unfavorable environment to some pest species.

Table 3. Effect of intercropping of potato with onion on the population density of aphids in first winter season (2011-2012).

Inspection dates		Mean number of aphids /25 leaflets.	
		Potato: onion	Sole potato
Dec.	10	0.0± 0.00	0.0 ±0.00
	17	0.0±0.00	28.6±1.12
	24	21.3±0.03	40.3± 0.11
	31	22.7± 0.12	57.0± 0.02
Jan.	7	32.0±0.19	110.3±1.17
	14	71.7±0.22	129.7±1.06
	21	90.3± 0.10	123.0±0.22
	28	90.3± 0.04	212.0±0.16
Feb.	4	173.7±1.01	253.0± 0.12
	11	168.7± 0.14	186.7±0.23
	18	59.0± 0.11	144.7±1.13
	25	44.3±0.02	66.0 ±0.12
Total		774.0	1351.3
General mean		64.5 *±0.20	112.6 ±0.32

Table 4. Effect of intercropping potato with onion on the population density of aphids in the second winter season (2012-2013).

Inspection dates		Mean number of aphids/25 leaflets.	
		Potato: onion	Sole potato
Dec.	15	0.0 ±0.00	0.0 ±0.00
	22	0.0±0.00	6.7±0.02
	29	2.7±1.02	26.7±0.14
Jan.	5	36.3±0.04	40.8 ±0.18
	12	44.0±0.12	63.3 ±0.22
	19	83.7±0.11	148.3± 0.36
	26	200.0±1.19	490.3±1.15
Feb.	1	313.7±1.32	416.0 ±0.16
	8	101.3±0.17	318.7±1.04
	15	112.6±0.52	285.0 ±1.06
	22	48.0± 0.33	131.3 ±0.44
Mar.	1	32.3±0.04	74.7 ±0.12
Total		974.6	2001.8
General mean		81.2* ±0.02	166.8 ±0.17

DHALIWAL & ARORA (1996) found increased number of parasitoid and predators in pearl millet under intercropping (UVA, 1985; AJAYI, 1990; KHAN *et al*., 1997). Up to 30% crop pest reductions due to natural enemy effect have been observed by BALIDDAWA (1985) in intercropping system. WSZELAKI (2014) reported that the use of intercropping can provide benefits to a management system, including decrease insect pest pressure, reduced need for external inputs, increases in biodiversity, enhanced production and lower economic risk. Separation susceptible plants with non-host species provides a physical barrier to insect pest movement, limiting spread and decreasing likelihood of damage to susceptible varieties. For example, separating planting of solanaceous crops, such as tomatoes and potatoes, that are susceptible to Colorado potato beetle, with a non-host crop, such as corn, can reduce the movement of Colorado potato beetle from one solanaceous crop to another. The additions of multiple species enhance biodiversity and encourage beneficial insect population. Resulting beneficial interactions between plants can confuse insects, lowering insect reducing the need for external inputs. Inclusion of multiple crops utilizing different environmental niches increases the productivity per unit of land, allowing for financial diversification, as well as a reduced financial risk in the event of crop failure. BAIDOO *et al*. (2012) mentioned that onion was used in an intercrops as a non-host crop to manage the pests of cabbage. SMITH & LIBURD (2015) stated that when an herbivore encounters a plant it cannot feed on, it must expend additional time and energy searching for an acceptable plant. This reduces the time and energy of the insect for crop damage or deposit offspring, and in some instances it encourages the insect to migrate from the area. Insects rely on visual, olfactory, and tactile cues to find host plants. The presence of non-host plant can interfere with an insect' s ability to detect host plant by physically masking the presence of the host plant or by producing volatiles that confuse the insect. In this sense, diverse habitat can reduce the appearance of host plants to pests.

Conclusions

The present research recommends that combination of potato as a major crop and onion plants as a cover crop during cultivation is an assistant factor to protect the potato plant from injury of the whitefly and aphids which are among harmful pests for the potato cultivations in the field.

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Estimation and Mapping Forest Attributes Using "k Nearest Neighbor" Method on IRS-P6 LISS III Satellite Image Data

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Abstract. In this study, we explored the utility of k Nearest Neighbor (kNN) algorithm to integrate IRS-P6 LISS III satellite imagery data and ground inventory data for application in forest attributes (DBH, trees height, volume, basal area, density and forest cover type) estimation and mapping. The ground inventory data was based on a systematic-random sampling grid and the numbers of sampling plots were 408 circular plots in a plantation in Guilan province, north of Iran. We concluded that kNN method was useful tool for mapping at a fine accuracy between 80% and 93.94%. Values of k between 5 and 8 seemed appropriate. The best distance metrics were found Euclidean, Fuzzy and Mahalanobis. Results showed that kNN was accurate enough for practical applicability for mapping forest areas.

Keywords: Forest attributes, IRS, k Nearest Neighbor (kNN).

Introduction

Monitoring forest attributes as an important tool to sustainable forest management need up-to-date information on forest resources (LABRECQUE *et al.*, 2006; MOHAMMADI *et al.*, 2011). Ground field inventory is the most frequently way to get forest cover information but it is not the optimal quick way (JIA *et al.*, 2014). Therefore, nowadays methods of estimations are used by remote sensing data (MOHAMMADI *et al.*, 2011). Remote sensing refers to indirect measurement of emitted electromagnetic energy using sensors (AHAMED *et al.*, 2011). Remote sensing has been efficient and effective means to obtain forest cover information, mapping and monitoring forest cover in recent decades due to its large scale information collection ability and synoptic and repeated coverage especially if combined with field data (REESE *et al.*, 2002; JIA *et al.*, 2014). There are a wide range of methods to integrate remote sensing data with ground inventory data (LATIFI *et al.*, 2010; CHERNETSKIY *et al.*, 2011).

Classification of satellite data for mapping forest attributes is the most frequent use of them (IVERSON *et al.*, 1989). A classification procedure may be supervised (training samples are labeled), semi supervised (only some of training samples are labeled) or non-supervised (the training samples are not labeled and these classes are not known a priori) (LIU *et al.*, 2011; SOUZA *et al.*, 2014). In recent years, hardwares and softwares have improved to process digital satellite data (IVERSON *et al.*, 1989), mapping forest species, illustrating distribution different vegetation species in a study area and providing essential indices for forest inventory and management. These maps are mostly generated with image classification methods. All of classification methods are object or pixel base, hard or soft classifier, parametric or non-parametric (TORABZADEH *et al.*, 2014).

One of the simplest and also more sophisticated nonparametric techniques that are used to link between field inventory data and remote sensing data is k nearest neighbor (kNN) classification method. kNN finds a group of k objects in the training set that are closest to the test object, and bases the assignment of a label on the predominance of a particular class in this neighborhood. Therefore, there are three key elements of this approach: 1) a set of labeled objects (stored records such as ground sampling plots data), 2) a distance or similarity metric to compute distance between objects and 3) the value of k (number of nearest neighbors). To classify an unlabeled object such as pixels of satellite image data, the distance of that to the labeled object is computed, its k nearest neighbors are identified and the dominant class of these nearest neighbors are used to determine the class label of the object (WU *et al.,* 2008). kNN expands terrestrial point observations to spatially explicit coverage by utilizing similarities in the spectral image space of remote sensing data (STUMER *et al.,* 2010). It is a simple classifier to use the k training samples that are closest to the test sample to classify it (XU *et al.,* 2013).

Satellite remote sensing data and kNN have played a key role in forest studies and have several applications in the field of forest inventory (MUINONEN *et al.,* 2001; OHMANN *et al.,* 2014). JUNG *et al.* (2013) improved the accuracy of estimation with moderate spatial resolution satellite imagery and the kNN algorithm for forest carbon stock estimation due to its simplicity and feasibility. ZHU & LIU (2014) studied the accurate mapping of forest types using kNN method. OHMAN *et al.* (2014) studied integrating forest inventory plot data and satellite image data for regional forest mapping using kNN method. WILSON *et al.* (2012) used kNN imputation method to mapping tree species over large areas using forest inventory plots and moderate resolution raster data. Also, kNN was used to estimate tree biomass (NYSTROM *et al.,* 2012), tree volume (CHIRICI *et al.,* 2008; KAJISA *et al.,* 2008), basal area and density (BAFFETTA *et al.*, 2009).

This study was investigated estimation forest attributes and accurate mapping using kNN method with IRS (Indian Remote-Sensing Satellite) imagery data. In this study, we evaluated the potential of the IRS-P6 LISS III satellite data and performance of kNN method as a non-parametric supervised classifier to create forest attributes mapping.

Material and Methods

The study area was a plantation that located in west of Guilan province in the north of Iran in 37˚ 32' to 37˚ 36' N latitude and 49˚ 2' to 49˚ 7' E longitude. It has total area of 1850.44 hectare and covered a smooth topography (Fig. 1.). This plantation characterized by even aged pure conifers stand (*Pinus taeda* (loblolly pine) as dominant coniferous species, pure deciduous stand (*Alnus subcordata* (caucasian alder), *Alnus glutinosa* (common alder) and *Populous* spp. (poplar)) and uneven aged mixed deciduous stand (*Acer velotonium* (persian maple), *Pterocarya fraxinifolia* (Caucasian wing nut), *Populus caspica* (caspian poplar) and *Carpinus betulus* (hornbeam)). Therefore, forest types were determined based on dominant tree species in sampling plots and five forest cover types were assigned to classification consist of 0: non-Forest, 1: *Pinus taeda*, 2: *Alnus glutinosa*, 3: *Populous* spp. and 4: mixed deciduous species.

408 circular sampling plots with 1000 m^2 (0.1 ha) area were distributed according to a systematic-random design and a network grid with 150m×200m spacing. Sampling plots were measured in the September to November of 2012. In each sampling plot diameter at the breast height (DBH) of all trees species with a DBH ≥ 7.5 cm was measured. Two trees height for one the nearest tree to the sampling plot center and another one that have the largest DBH in the sampling plot were recorded.

An IRS-P6-LISS satellite image data collected on July 18 2008 (Path 67; Row 43) was orthorectified with a digital elevation model (created from elevation of the 1:25000 national topographic database) and georeferenced by 20 ground control points (UTM, WGS 84, zone 39 N) (Table 1).

We used kNN imputation in the yaImpute package (CROOKSTON & FINLEY, 2008) in statistical software R (R DEVELOPMENT CORE TEAM, 2010) and the kNN-forest software (CHIRICI *et al.*, 2012) in the Idrisi Selva 17.0 to data analyses consist of forest inventory and satellite images data combination, search optimum number of k and distance metric calculation. The dependent variables were calculated from field inventory data including volume, basal area, density and forest cove types and independent variables were defined by satellite images band's average digital numbers (i.e. pixel value) in the center of sampling plots. Spectral distance was derived using three common different distance metrics Euclidean, Mahalanobis and Fuzzy distances (Fig. 2.).

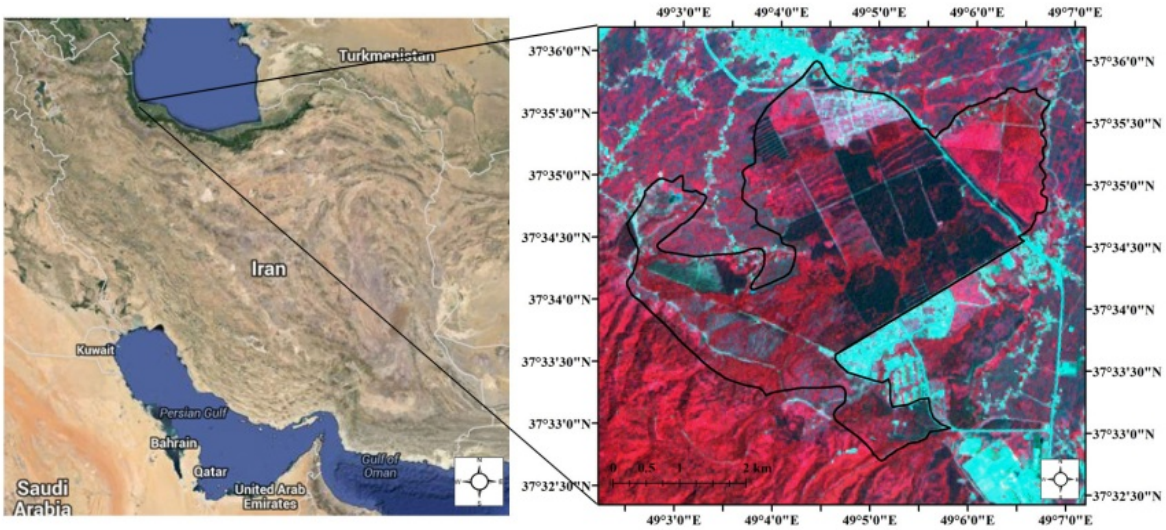

Fig. 1. Location of the study area.

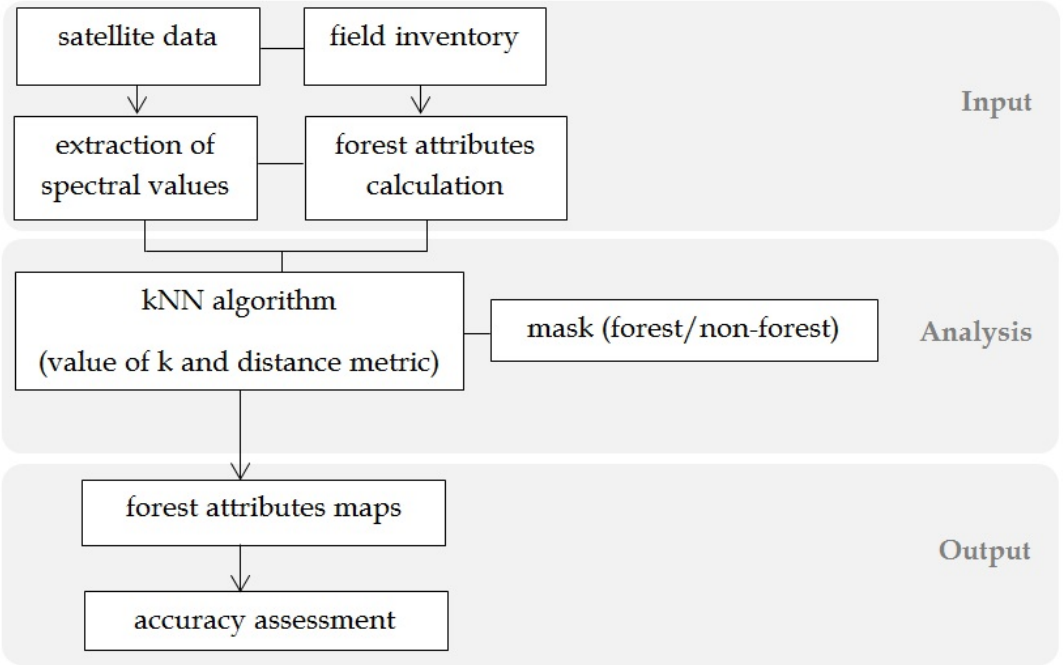

Fig. 2. Flowchart of the procedure used in kNN method.

Table 1. Detailed information of satellite image used in this study.

Satellite	Sensor	Bands	Wavelength (μm)	Spatial resolution (m)	Radiometric resolution (bit)
IRS-P6 (Resourcesat)	LISS-III (Linear Imaging Self-Scanning Sensor-III)	2 (G) 3 (R) 4 (NIR) 5 (mid IR)	0.520-0.590 0.620-0.680 0.770-0.860 1.550-1.700	23.5	7

The accuracy of the number of neighbors (k), distance metrics and forest attributes estimations were evaluated using Leave One Out Cross Validation that define as omitting training sample units one by one. For each omission, apply the kNN prediction to the remaining sample and summarize the error. In totality, it is applied the prediction rule n times and predicts the outcome for n units and estimate of error called Root Mean Square Error (RMSE) (Equation 1) (FRANCO-LOPEZ *et al.*, 2001). We used leave one out cross validation test for different values of k (the numbers of nearest neighbors) from 1 to 25.

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \qquad (1)$$

where $\hat{y}_i$ is the estimated forest attribute and y_i is the forest attribute value measured in field sampling plots.

All preliminary analysis was performed in PCI Geomatica 9.1 and ARCGIS 10 softwares.

Results

Results showed that DBH and tree height have strongly correlation (r=0.743) with each other, and DBH has moderate correlation with volume and basal area (0.594 and 0.531, respectively). Tree height has moderately correlation to volume and basal area (0.629 and 0.591, respectively) and volume has strongly correlation to basal area (r=0.805).

Correlation analysis among spectral bands information and forests attributes showed a weak correlation in all cases except band 2 (Green) and 4 (NIR). Green band has the highest correlation for height, volume and DBH, respectively. NIR band was the band that shows the highest correlation for DBH, height, volume and basal area, respectively. Table 2 illustrates the Pearson correlation between the various bands and forest attributes for all sampling plots data.

The results of the optimum number of neighbors (k) and distance metrics have showed in Fig. 3. (a to f). The lowest value of cross validation RMSE as the best obtained in the estimation of Euclidean distance metric was in k=6 for DBH (Fig. 3., a), Euclidean distance metric in k=8 for tree height (Fig. 3., b), Fuzzy distance metric in k=5 for volume (Fig. 3., c), Mahalanobi distance metric in k=6 for basal area (Fig. 3., d), Euclidean distance metric in k=6 for both density and forest cover type (Fig. 3. e and f, respectively).

In all cases, there was a steep decrease in RMSE in the first numbers of neighbors then fixed the value of error.

kNN classification map of DBH, tree height, volume, basal area, density and forest cover types was produced and showed in Fig. 4. (a to f). The results of accuracy assessments including overall accuracy and kappa coefficient have showed in Table 3. Results showed that the amount of accuracy was high in all maps (from 80% to 93.94%) and map of density have the highest amount of accuracy (93.94%).

Discussion

The purpose of this study was to investigate the possibility of kNN method to estimate main forest attributes including trees DBH, height, volume, basal area, density and forest cover type based on existing forest inventory field sampling data as reference data to produce highly accurate

Table 2. Pearson correlation coefficient between attributes and mean digital numbers of sampling plots.

Attributes	DBH	H	V	BA	D	T	Mean digital number by band			
							B2	B3	B4	B5
DBH	1									
H	0.743	1								
V	0.594	0.629	1							
BA	0.531	0.591	0.805	1						
D	-0.364	-0.076	0.205	0.384	1					
T	-0.323	-0.215	-0.187	-0.164	0.074	1				
B2	-0.374	-0.478	-0.389	-0.391	-0.076	0.088	1			
B3	-0.036	0.006	-0.020	-0.016	0.017	0.044	0.043	1		
B4	-0.503	-0.417	-0.391	-0.332	0.167	0.196	0.226	-0.006	1	
B5	-0.066	-0.106	-0.078	-0.085	-0.034	-0.030	0.024	0.000	-0.007	1

Note: DBH: diameter at the breast height (cm), H: tree height (m), V: volume (m^3/ha), BA: basal area (m^2/ha), D: density (tree count/ha), T: forest cover type, B2: band 2, B3: band 3, B4: band 4, B5: band 5.

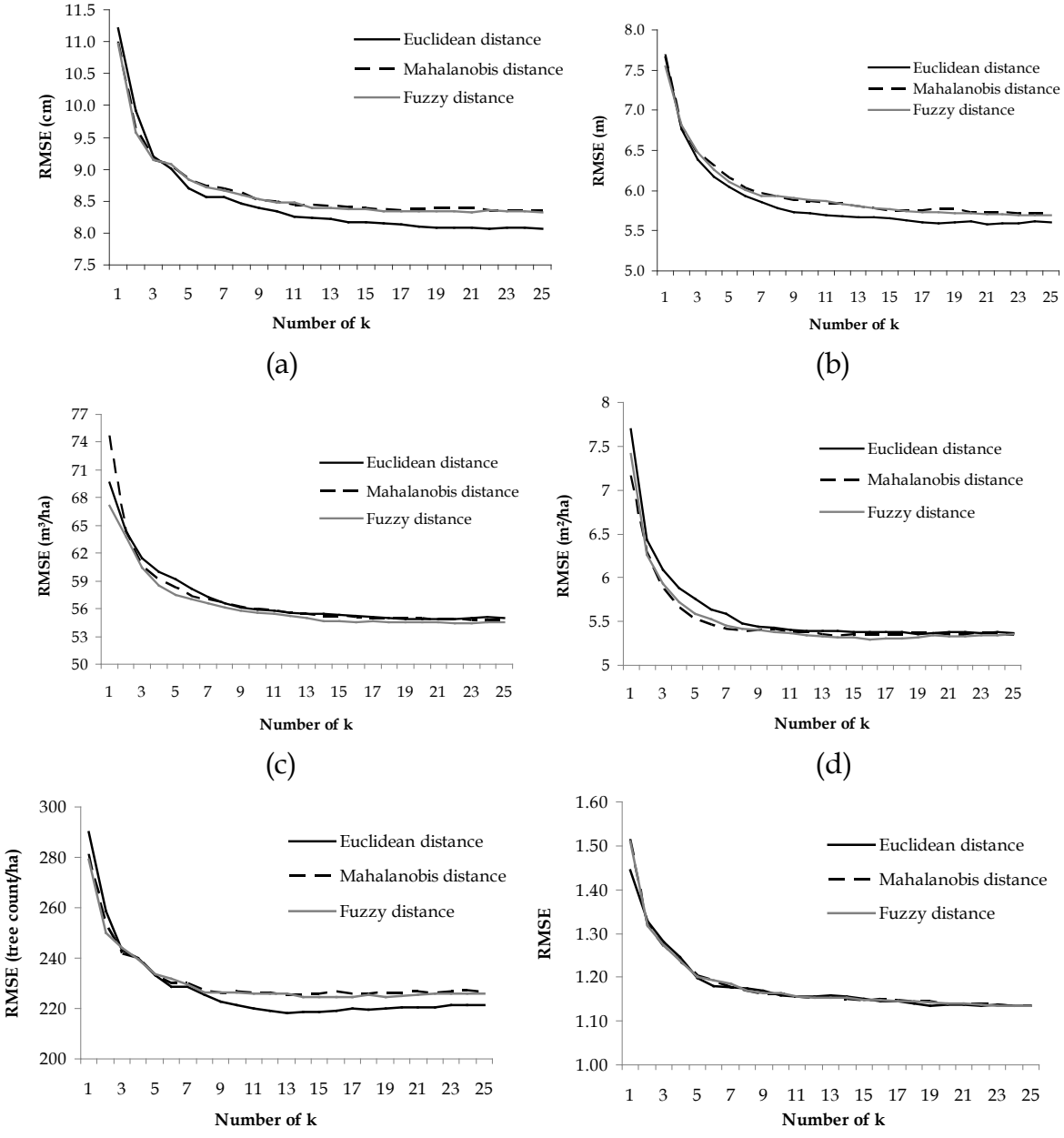

Fig. 3. Relation between k and RMSE for DBH (a), tree height (b), volume (c), basal area (d), density (e) and forest cover types (f) estimation.

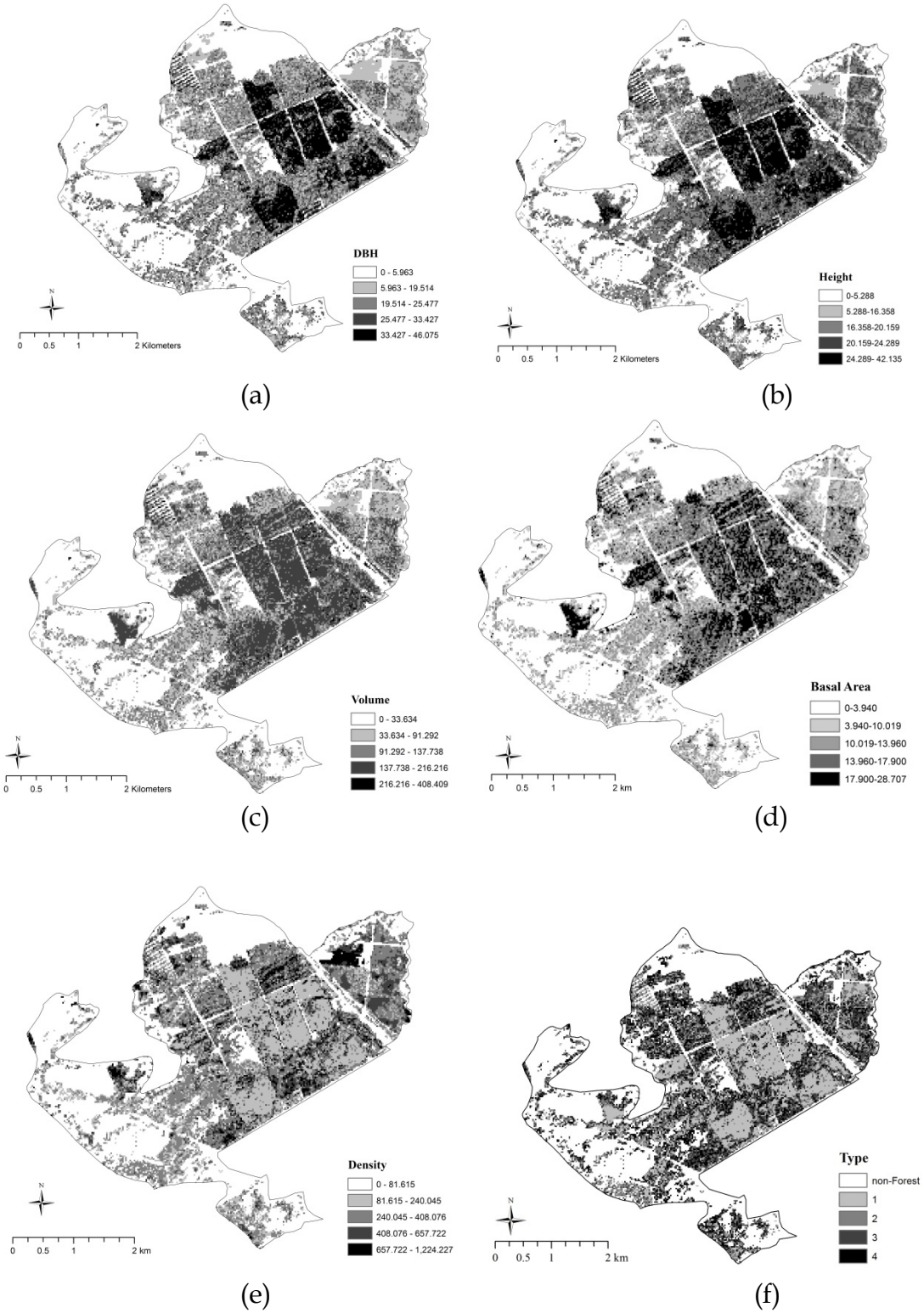

Fig. 4. kNN classification maps of DBH (a), tree height (b), volume (c), basal area (d), density (e) and forest cover types (f).

Table 3. Accuracy assessment of mapping forest attributes.

	DBH	Height	Volume	Basal area	Density	Type
Overall accuracy (%)	87.88	80.0	88.89	80.0	93.94	89.90
Kappa coefficient (%)	0.61	0.61	0.72	0.57	0.79	0.59

classification maps. The visualization of forest variables is an important tool and easy way for decision making (PEREIRA, 2006, Italy – pers. comm.). Land cover map as a visual tool has basic information to management of natural resources (TRIEPKE *et al.*, 2008).

The first step in kNN method application is the calibration of kNN parameters to obtain the most appropriated distance and number of neighbors (k) for each variable that to be predicted (PEREIRA, 2006, Italy – pers. comm.). Therefore, we computed the optimum numbers of neighbors (k) and optimum distance metric in the best set for estimating and mapping. Consequently, forest attributes map was prepared using optimum values for every attributes separately in our study area. According to the same author the application of kNN method to other forest areas should always be done with prior kNN parameters calibration (k, distance) to local data.

We found that k less than 10 was the best in our study area. This result is similar to other researches in different forest conditions (RAHMAN, 2006; WILSON *et al.*, 2012; JUNG *et al.*, 2013). Increasing the number of neighbors produces an undesirable reduction of variability in predicted values, especially if the goal of using kNN is map production. Clearly, this problem is reduced with use of fewer neighbors (FRANCO-LOPEZ *et al.*, 2001). In all cases, there was a steep decrease in RMSE in the first numbers of neighbors then fixed the value of error. This behavior was reported by several studies. OHMANN *et al.* (2014) reported that maps assessed with k=5 were much more accurate than when assessed with k=1. It is clear that there was a rapid early gain in overall precision with the addition of neighbors (FRANCO-LOPEZ *et al.*, 2001).

Another essential in kNN method is satellite image correctly rectified and coordinates of the field plots were accurately determined until satellite rectification error and sampling error have the lowest amount and accuracy of created maps have been acceptable (JUNG *et al.*, 2013; OHMANN *et al.*, 2014). The main criterion to classification by kNN is the minimum spectral distance between target pixels and reference pixels. Therefore, the integration of forest inventory plot data with remotely sensed data provide a powerful tool for mapping of forest attributes (OHMANN *et al.*, 2014). Suitable methods to combine remote sensing and terrestrial sample based inventory data is the main difficulties in the estimation procedure (RAHMAN, 2006). The use of different remotely sensed data and various inventory sampling plots procedure showed that kNN method is a feasible classification technique. This method has the potential for estimating various attributes by different image data. kNN method was well studied in recent years to integrate ground information and remote sensing data (SOUZA *et al.*, 2014; ZHU & LIU, 2014; KAJISA *et al.*, 2008; FRANCO-LOPEZ *et al.*, 2001).

Error of kNN estimation was determined by RMSE (root mean square error). Almost, all studies on kNN method were analyzed by this criterion. RMSE is a diagnostic measure of difference between observed and predicted values, which is most commonly utilized for model evaluation. This measure has a relatively high weight to large errors because the errors are squared before being averaged. Thus, RMSE is most useful when large errors are particularly undesirable. RMSE= {[Σ(P−O) 2]/N}0.5; P: predicted value; O: observed value (HAMADA *et al.*, 2011). But, some researchers believed that there was not a reliable and sufficient method of error evaluation in the kNN estimation and also, further studies should be carried out to overcome this limitation (RAHMAN, 2006). However, this algorithm is being improved in terms of accuracy and efficiency.

Results of correlation between spectral band information and forest variables showed weak correlation due to heterogeneous conditions of forest stands (PEREIRA, 2006, Italy – pers. comm.).

Our results showed that kNN has good results in pure stands of conifers and deciduous species and also the mixture of deciduous species in our study area. This

result is consistent with CAVARAVDICH (2007, Germany – pers. comm.). Therefore, high accuracy of our study maps may be due to the kNN approach strength and IRS-P6 LISS III image data strength. We concluded that forest attributes maps can be easily produced with kNN with accuracy estimation and level of prediction error (PEREIRA, 2006, Italy – pers. comm.). kNN method was well studied in recent years to integrate ground information and remote sensing data. In general, kNN classification method applied to estimate and mapping forest attributes in order to assess accurate estimations in this study. Results indicated that kNN method could estimate and mapping forest attributes by suitable accurate classification and produce feasible thematic maps.

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The Allelopathic Effect of the Exotic Tree Acacia saligna on the Germination of Wheat and Canola

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Abstract. This study was carried out to investigate the allelopathic effect of aqueous extracts derived from leaves and stems of *Acacia saligna* (Labill.) H.L.Wendl. upon two agricultural crops, wheat and canola. Seed germination (%), shoot and root elongation, fresh and dry weight, vigor index and phytotoxicity parameters were estimated. Leaf extract exhibits higher inhibitory effect than stem extract. Wheat seeds were more tolerant to the allelopathic action of *A. saligna* extracts than canola. Canola germination minimized to 8.33% at concentration 10% of leaf extract but the percent of germination was 60% in the case of stem extract. At 10% leaf extract, 76.67% of wheat seeds germinated; but at 10% stem extract, 93.33% of the seeds were germinated. The other growth parameters as shoot and root length, fresh and dry weight and vigor index also showed continued decrease with the increasing of allelopathic extract concentration. Leaf extract exhibits the stronger allelopathic effect. The phytotoxic effect was stronger on the germination of canola compared with wheat. It reached up to 91.76% inhibition at concentration 10%, but reached up only 23.33% in the case of wheat, respectively.

Key words: *Acacia saligna*, Allelopathy, Allelochemical, Phytotoxicity, Canola, Wheat.

Introduction

Allelopathy is one of the important phenomena used by plant to defense against competition with others. Many species use allelopathy as effective strategy to invade, stablish and increase their area of occupancy. MAY & ASH (1990) defined allelopathy as the interference mechanism, in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth. These chemical substances are called allelochemicals (intermediary metabolic products) and include: phenols, terpenes, glycosides, alkaloids, amino acids and sugars as preferably defense compounds (HARBORNE, 1989).

Acacia saligna (Labill.) H.L.Wendl. (subfamily Mimosoideae, family Fabaceae) is a very adaptable and fast growing tree native to Western Australia (MIDGELY & TURNBULL, 2003). In some areas, *A. saligna* has gone on to become an invasive species with a wide range of impacts. In areas where it has become invasive, *Acacia saligna* is known to form dense monospecific stands, excluding native species and preventing their regeneration (HOLMES & COWLING, 1997; HADJIKYRIAKOU & HADJISTERKOTIS, 2002). *Acacia saligna* has been planted extensively in Africa and the Mediterranean region including Egypt (MIDGELY & TURNBULL, 2003).

According to SCHWARTZ *et al.* (1996) and SHINWARI *et al.* (2012); the exotic invasive species are considered to be the second largest cause of biodiversity loss after habitat destruction. Most of the exotic plant

effects reported have been identified as caused by allelopathic interaction which resulted in interference with physiological and biochemical processes in plants, due to chemicals released by exotic plants (ALHAMMADI, 2008). RAFIQUL HOQUE *et al.* (2003) showed that trees of genus *Acacia* are known as a versatile source of components with bioactive properties. BARNES *et al.* (1996) suggested a large inhibitory potential in *Acacia* genus which dominates the dry south Saharan regions of Africa.

Many workers (BITENDE & LEDIN, 1996; CODER KIM, 1999; SHAYO & UDEN, 1999; ABDULRAZAK *et al.*, 2000; DUBE *et al.*, 2001; RUBANZA *et al.*, 2005 and NAKAFEERO *et al.*, 2007) found that tannins, phenols and phenolic compounds are the most common compounds in different *Acacia* organs (leaves, stem, pods, fruits, petiole, and root). DUKE (1983) reported that natal-grown bark of *Acacia saligna* contains up to 30.3% tannin.

The aim of the current work was to test the allelopathic effect of *Acacia saligna* leaf and stem extracts on the seed germination of canola and wheat.

Material and Methods

Preparation of extracts. Aerial parts (stems and leaves) of *Acacia saligna* plants at the vegetative stage were collected separately, air dried and ground to obtain fine powder. For extract preparation: 10 g powder (leaves or stem) was added to 100 ml distilled water and left at room temperature for 24 h to obtain 10% (w/v) extract. The extract was filtered through filter paper. This was served as original stock solution and stored at 4°C for further use (EL-KHATIB & ABD-ELAAH, 1998; EL-KHATIB & HEGAZY, 1999; EL-KHATIB, 2000). Different concentrations (2, 4, 6, 8 and 10%) of aqueous leaf and stem extracts were prepared.

Germination and seedling growth bioassay. Seeds of wheat (*Triticum aestivum* variety Giza 168) and canola (*Brassica napus* variety sero 4) were obtained from the Faculty of Agriculture, South Valley University. The healthy seeds were surface sterilized with 3% ethanol and thoroughly rinsed with sterilized distilled water. The comparative assessments of the influence of various concentrations of aqueous extracts on seed germination and early growth of seeds were carried out in 9 cm Petri dishes.

Five ml of the various concentrations of leaf and stem extracts (control (distilled water), 2, 4, 6, 8 and 10 %) was added to the dishes (4 replicates per treatment) containing 10 uniformly seeds of the tested species on two pieces of Whatman no. 1 filter papers. Dishes were incubated under the growth chamber conditions with 25/18˚C day and night temperature respectively, 13/11 hours light/dark period (WU *et al.*, 2007). Germination of seeds and early seedling growth were recorded after 7 days of germination. Germination percentage, shoot and root length, fresh and dry weight were estimated. Also vigor index (VI) and phytotoxicity percentage (reduction) was computed using the following equations:

% Germination = (no of germinated seeds / total no of seeds) × 100 (after SCOTT *et al.*, 1984).

VI = [seedling length (cm) × germination percentage] (after ABDUL-BAKI & ANDERSON, 1973).

% Phytotoxicity = [(Control - Extracts) ÷ Control] × 100 (after VOKOU, 1992).

Data were statistically analyzed using ANOVA and difference between means under different treatments were calculated by Least Significant Differences (LSD) test at $P<0.05$ and $P<0.001$using SPSS program v. 20 (IBM Corp., 2011).

Results

Data of the test species demonstrates significant degrees of suppression and a negative response to the increasing concentration of different extracts. There are significant differences between the different concentrations and control especially at higher concentrations.

Germination percentages of both canola and wheat seeds decreased under the effect of different concentrations of *A. saligna* extracts (Table 1). The treated seeds with leaf extract inhibited significantly (up to 92% inhibition in canola and 23% in wheat) compared with those treated with stem

extract (40% inhibition in canola 20% in wheat). The allelopathic effect was stronger upon canola seeds compared with wheat.

Recorded germination percentage of canola was 100% under the control treatment. The application of different concentrations of *A. saligna* leaf extract reduced germination significantly from 98.33% at concentration of 2% to 8.33% at concentration 10%. On the other hand, stem extracts showed lower effect on germination rate. At concentration 10%, the germinated seeds reached 60%. In case of wheat, seeds were more tolerant to the allelopathic effect of *A. saligna* extracts, whether extracted from leaves or stems. At concentration 10%; 76.67% of the seeds germinated under leaves extract. With the application of stem extracts, the percentage varied between increase and decrease with different concentrations. The lowest percentage was recorded at concentration 2% (80%) while other percntages increased with concentration (Table 1).

Table 2 explains the allelopathic effect of *A. saligna* extracts on canola fresh and dry weight. Canola exhibits a significant decrease in both fresh and dry weight under the different concentrations of leaf extract. The estimated fresh weight was 380 mg at control. It decreased down to 16 mg at concentration 10%. On the other hand, stem extract didn't exhibit any significant effect on the fresh or dry weight. On contrast, the lower concentrations of stem extract (2% and 4%) showed a simulating effect on the fresh and dry weight, but at concentrations 6% - 10% a slight decrease was noticed.

Table 1. Germination percentage (%) ± standard deviation of canola and wheat under the different concentrations of *Acacia saligna* leaf and stem extracts.

Treatment	Canola		Wheat	
	Leaf extract	Stem extract	Leaf extract	Stem extract
Control	100.0±0.00	100.00±0.00	100.0±0.00	100.00±0.00
2%	98.33±2.89	98.33±2.89	100.00±0.00	80.00±17.32
4%	83.33*±17.56	98.33±2.89	80.00**±17.32	93.33±11.54
6%	46.67**±7.63	93.33±2.88	76.67**±5.78	90.00±17.32
8%	15.00**±8.66	85.00*±5.24	73.33**±11.54	86.67±5.77
10%	8.33**±7.64	60.00**±6.55	76.67**±11.55	93.33±5.77

* at P<0.05 and ** P<0.01.

Table 2. Fresh and dry weight (mg/plant) ± standard deviation of canola seedlings under the different concentrations of *Acacia saligna* leaf and stem extracts.

Treatment	Leaf extract		Stem extract	
	Fresh wt., mg	Dry wt., mg	Fresh wt., mg	Dry wt., mg
Control	380 ±12	51±8	429±59	38±3
2%	310* ±53	38*±6	489±52	41±3
4%	129** ±26	34**±11	493±48	40±2
6%	46.0**±22	15**±4	406±123	35±9
8%	23.0**±14	7.0**±3	273±89	11±11
10%	16.0**±15	6.0**±5	143±47	5.0±8

* at P<0.05 and ** P<0.01.

Table 3. Fresh and dry weight (mg/plant) ± standard deviation of wheat seedlings under the different concentrations of *A. saligna* leaf and stem extracts.

Treatment	Leaf extract		Stem extract	
	Fresh wt., mg	Dry wt., mg	Fresh wt., mg	Dry wt., mg
Control	1204±15	302±17	1144±31	265±1
2%	1373±14	367±13	1223±25	280±65
4%	1019±28	308±6	1346±14	227±17
6%	745.0**±5	309±15	1278±35	291±87
8%	633.0**±9	266±52	1290±13	307±4
10%	596.0**±16	307±71	1344±16	331±41

* at P<0.05 and ** P<0.01.

The results of wheat seedlings were illustrated in Table 3. Significant differences appeared at concentrations 6%, 8% and 10% of leaf extract on the fresh weight only. Under the effect of stem extract there was a slight increase in both fresh and dry weight with increasing concentration compared with control.

Leaf extract caused a significant decrease in both shoot and root lengths of canola at concentrations 4% - 10% (Table 4). At concentration 2% there was a significant increase in the shoot length (3.66 cm) compared with control (2.92 cm). At concentrations 4% - 10%, the recorded shoot lengths were 1.21, 1.03, 1.58 and 0.42 cm respectively, while the recorded root lengths were 3.55, 1.74, 0.53, 0.57 and 0.25 cm respectively.

Canola shoot lengths exhibit different reactions toward stem extract. A stimulating effect appeared at concentrations 2%, 4% and 6% with significant values at the first two concentrations. At the higher concentrations, shoot lengths decreased compared with control. Root length decreased with the increasing concentration showing significant values starting from 6% to 10%. The lowest length was detected at 10% concentration. The reductions in both shoot and root length (2.92 and 1.90 cm) reach to about 60% at 10% concentration.

In case of wheat, the extracts of *A. saligna* leaves and stems caused a significant decrease in the shoot and root length (Table 5). The decrease in shoot and root length was significant at 4%, 6%, 8% and 10% concentrations of leaves extract. The highest decrease was recorded at concentration 10%. Stem extract decreased the length of wheat seedlings shoots significantly at concentration 10%, while the roots length decreased significantly at 8% and 10%. The stems extract at concentration 2% showed a stimulating effect where the length of both shoots and roots increased comparing with control.

Table 4. Shoot and root length (cm) ± standard deviation of canola under the different concentrations of *Acacia saligna* leaf and stem extracts.

Treatment	Leaf extract		Stem extract	
	Shoot length, cm	Root length, cm	Shoot length, cm	Root length, cm
Control	2.92±0.20	6.20±1.63	3.41±0.29	4.76±2.40
2 %	3.66**±0.21	3.55**±0.76	4.45*±0.50	4.35±0.85
4 %	1.21**±0.19	1.74**±0.42	4.47*±0.47	3.79±0.40
6 %	1.03**±0.27	0.53**±0.15	3.92±1.01	2.72*±0.47
8 %	1.58**±0.37	0.57**±0.08	2.80±0.56	2.49*±0.33
10 %	0.42**±0.38	0.25**±0.22	2.92±0.77	1.90**±0.54

* at P<0.05 and ** P<0.01.

Table 5. Shoot and root length (cm) ±standard deviation of wheat seedlings under the different concentrations of *Acacia saligna* leaf and stem extracts.

Treatment	Leaf extract		Stem extract	
	Shoot length, cm	Root length, cm	Shoot length, cm	Root length, cm
Control	8.58±0.33	11.12±0.71	8.87±0.63	11.30±0.40
2 %	7.56±0.33	10.53±0.69	9.73±0.44	11.89±1.27
4 %	6.08**±0.42	7.16**±1.05	8.80±0.53	10.69±0.56
6 %	3.39**±1.01	3.71**±0.62	9.33±0.44	10.96±1.21
8 %	3.00**±0.26	3.21**±0.13	8.56±0.31	8.54**±0.23
10 %	0.78**±0.59	2.25**±0.11	7.54**±0.60	7.43**±0.76

* at P<0.05 and ** P<0.01.

Subjecting canola and wheat seeds to different concentrations of *A. saligna* extracts reduced the vigor index of both species seedlings (Fig. 1A and B). Canola seedlings were affected more than wheat ones. The effect of leaves extract was stronger than stems extract. Vigor index of canola decreased quickly, under the effect of allelocompounds extracted from *A. saligna* leaves, from 912 to 2.89 at concentration 10%. On the other hand, vigor index of wheat was 2016 and dropped gradually to 236.9.

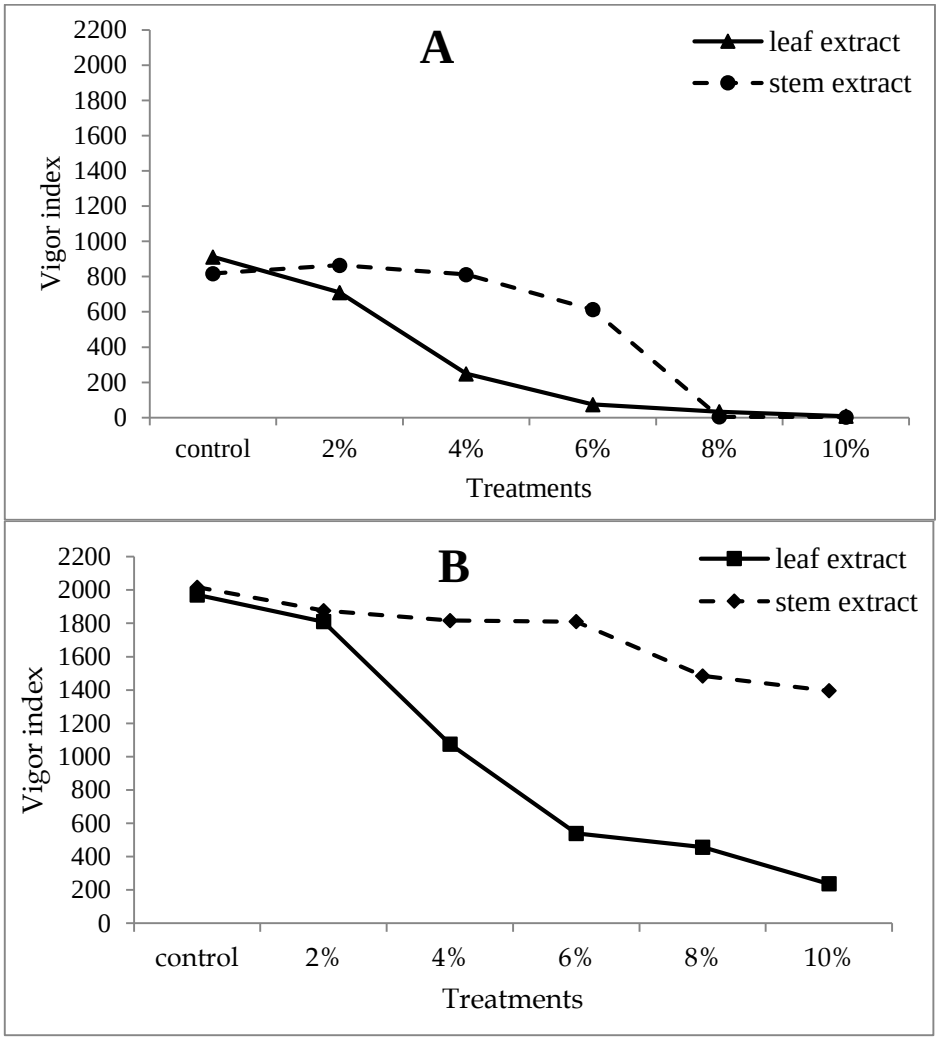

Fig. 1. Vigor index (VI) for canola (A) and wheat (B) seeds under different concentrations of *A. saligna* leaf and stem extracts.

Results represented in Fig. (2A and B) indicate the phytotoxic effect of *A. saligna* extracts on canola and wheat species. There was a remarkable reduction in the germination percentage for the two examined species comparing with control. The phytotoxic effect was stronger on canola compared with wheat. It reached up to 91.76% inhibition at concentration 10%, but reached up only 23.33% in the case of wheat. At lower concentration (2%), leaf extract exhibit neglected phytotoxic effect on canola. It cleared that the phytotoxicity increased paralleled with the increasing of extract concentration in the case of canola. In case of wheat, toxicity action was smaller and did not exceed 26% (leaf extract) and 13% (stem extract).

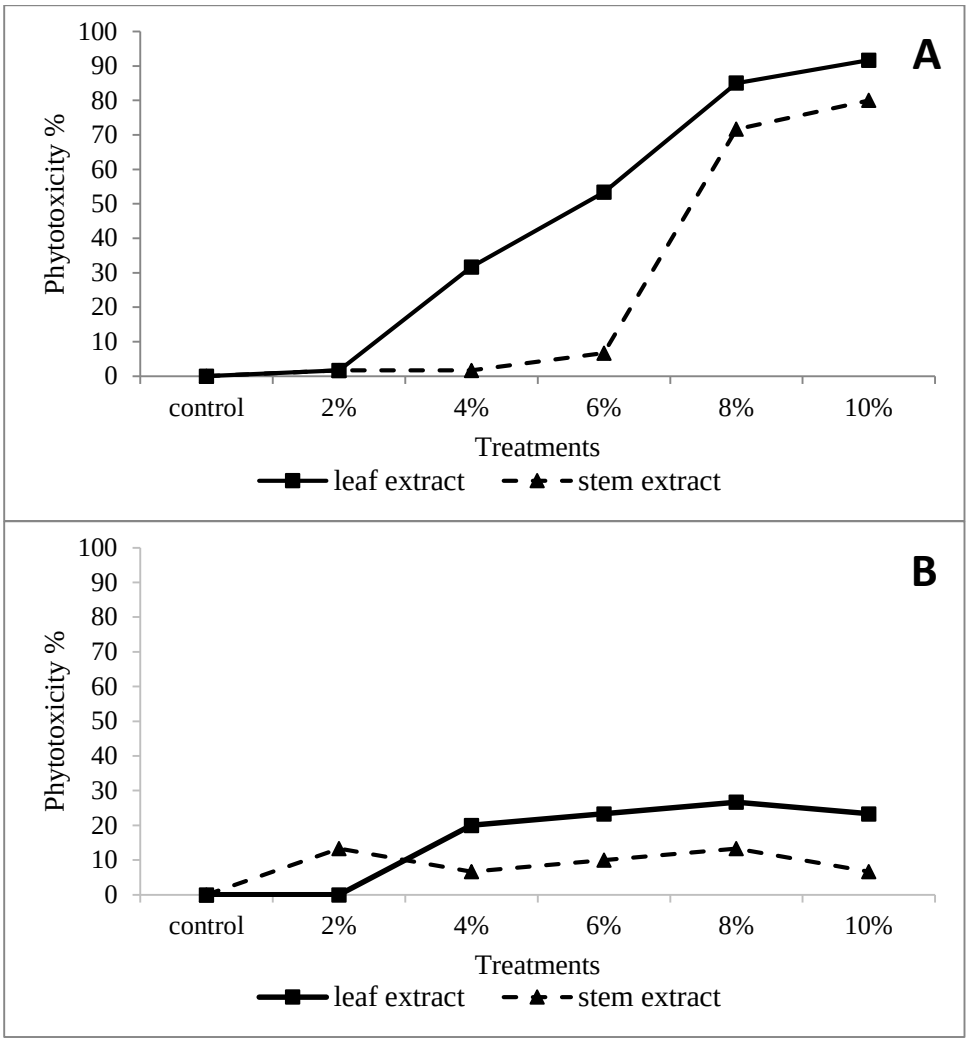

Fig. 2. Phytotoxic effects of the different concentrations of *A. saligna* leaf and stem extracts on the germination of canola (A) and wheat (B) seeds.

Discussion

Data presented in this study demonstrates the allelopathic effect of *A. saligna* leaf and stem extract on seed germination and seedling growth of two different seeds (canola and wheat). Data revealed that the inhibition in the germination percentage occurred in both canola and wheat may be owing to the alteration of enzyme activity, which affects the mobilization of storage compounds during germination (EINHELLIG, 1995) or inhibition of mitosis (ROSHCHINA, 2001; IRSHAD & CHEEMA, 2004; MOHAMADI & RAJAIE, 2009). Delaying or cessation of storage compounds transmission can reduce respiration substrates and metabolic energy in allelochemicals exposed seeds which decrease germination and seedling growth (YARNIA *et al.*, 2009).

The inhibition caused by leaf extract of *A. saligna* was more effective than by stem extract. SUMAN *et al.* (2002), TEFERA (2002), DEVI & DUTTA (2012), VERMA *et al.* (2012) reported that leaf extract of plants showed most prominent allelopathicity than root, stem and seed extract. PALANI & DASTHAGIR (1998) observed a significant yield reduction in cowpea, sesame, horse gram and sorghum by aqueous leaf extracts of *Acacia holosericea.*

Data also revealed that at lower concentrations of the extract it might have a positive effect or non-inhibitory effect on the germination or growth of the test seeds. These results were also obtained by Cheema in 1988 (after RANDHAWA *et al.*, 2002), who reported that lower concentration show promoting effect, while higher concentration had inhibitory effect.

The reduction in the fresh and dry weight of the studied seedlings under leaf extract was higher than stem extract, especially in canola. The same results were obtained by El-KHATIB *et al.* (2004) who suggests that the effect of allelopathic extracted substances on cell division causes a reduction in root growth. The root growth reduction affects the root efficiency. Consequently, this decreases mineral uptake, nutrient absorption and the transport of nutrients from the root to other plant parts. In summation, the dry matters become reduced.

Both shoot and root lengths of tested canola and wheat were more sensitive to the leaves extract of *A. saligna* compared with extract of stems. The inhibitory effect on root length was obvious than shoot length. The same results were obtained by AL HAMDI *et al.* (2001), INDERJIT & STREIBIG (2001) and BELZ & HURLE (2004) who reported that wheat root length had the highest sensitivity to allelochemicals and root length is more affected than shoot growth. Root length may be a key parameter to verify allelopathic strength. The higher inhibition of roots compared with shoots may be due to their more intimate contact with the treated filter paper.

The drop in germination percentage and growth parameters indicates the negative effect of the allelopathic action of different concentrations of the *A. saligna* extracts on the vigor status and viability of seed embryo and seedling growth. The decrease in the vigor of canola or wheat with the increase of extract concentration or vice versa is expected result that explains the allelopathic effect of *A. saligna*. Vigor index reflects the ability of seeds to produce normal seedlings under less than optimum or adverse growing conditions similar to those which may occur in the field. Reduced seed germination and seed vigor index of test crops under laboratory conditions indicated the accumulation of toxic allelopathic substances of the donor plant, which was harmful to the growth of seedlings of receptor plants (CHOU, 1992 and MANDAL *et al.*, 2012).

The inhibitory action on the test species reveals the phytotoxic effect of allelochemicals in *A. saligna* extracts. Wheat tended to prevent the toxicity may by increasing its tolerance or by produce substances which can play antagonistic effect against allelopathic substances. Inhibition does not affect seed germination, seedling growth and dry matter production only but can cover all the stages of plant growth. El-KHATIB *et al.* (2004) point out that allelopathic compounds do not cause an inhibition of plant development and growth for the individual plant only but can influence an ecosystem by changing the pattern of vegetation.

Conclusion

Wheat seeds are more tolerant than canola seeds to the allelopathic effect resulted from the treatment with different concentrations of *A. saligna* extracts. The extracts of the exotic tree *Acacia saligna* showed an inhibitory effect on the germination of seeds. The unique allelopathic effect of some exotic species on native, 'inexperienced' communities also contribute to invasive success. Allelopathy is expected to be an important mechanism in the plant invasion process.

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Flora of the Mediterranean Rivers in Bulgaria

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Abstract. Species composition and distribution of aquatic bryophytes and vascular plants assemblages in Mediteranean Rivers in Bulgaria are presented in this work. Aquatic macrophytes were studied at thirteen rivers in South Bulgaria during 2014, together with abiotic factors (flow velocity, shading, and substrate type), mean depth and altitude. In total, 73 species were registered, of them 13 bryophytes and 60 vascular plants were identified. Aquatic bryophytes included 10 mosses and 3 liverworts. The recorded bryophytes species refer to 7 families and 12 genera. The most frequently distributed species was *Leptodictyum riparium* (Hedw.) Warnst., followed by *Cratoneuron filicinum* (Hedw.) Spruce and *Platyhypnidium riparioides* (Hedw.) Dixon, *Brachythecium rivulare* Schimp. and *Hygroamblystegium tenax* (Hedw.) Jenn. The recorded 60 species of vascular plants refer to 25 families and 43 genera. The most common hydrophyte species was *Lemna minor* L., followed by *Ranunculus trichophyllus* Chaix, *Myriophyllum spicatum* L. and *Potamogeton nodosus* Poir. The most abundant species from the group of helophytes and amphiphytes was *Mentha aquatica* L., followed by *Agrostis stolonifera* L. *Mentha spicata* L., *Berula erecta* (Huds.) Coville, *Juncus effusus* L., *Lycopus europaeus* L., *Lythrum salicaria* L., *Phalaris arundinacea* L., *Ranunculus repens* L., *Sparganium erectum* L., *Typha latifolia* L., and *Veronica anagalis-aquatica* L. The majority of studied rivers sites were sunny, with moderate velocity, stony bottom, average depth up to 0.3 m and altitude between 100 and 500 m a.s.l.

Keywords: Flora, Mediterranean rivers, Bulgaria.

Introduction

River flora and vegetation comprise species which are a group of macroscopic photosynthetic organisms that colonize a multiplicity of habitats from submersed and emerged rocks to soft substrates, bankside edges tree trunks and roots, and that can also occur unattached on the water surface. These plants are represented by 7 plant divisions: Cyanobacteria, Chlorophyta, Rhodophyta, Xanthophyta, Bryophyta, Pteridophyta, and Spermatophyta. Species composition and distribution of aquatic bryophytes (Bryophyta) assemblages are less well-known than for the aquatic vascular plants (Pteridophyta and Spermatophyta), which are represented by 88 families with 2614 species belonging to 412 genera. These 2614 aquatic species of Pteridophyta and Spermatophyta evolved from land plants and represent only a small fraction (~1%) of the total number of vascular plants (CHAMBERS *et al.*, 2008). Local habitat characteristics determine species composition of aquatic bryophytes and vascular plants, particularly light availability, current velocity and sediment patterns (BIRK & WILLBY, 2010). Rocks and

hard, immobile substrates and variable flow regime are associated with bryophytes and the exclusion of vascular hydrophytes (SCARLETT & O'HARE, 2006).

Bryophytes are dominants in lotic ecosystems, especially in undisturbed conditions. River bryophyte flora and vegetation in Bulgaria comprise about 94 species growing in or beside the water. According to current data on the distribution of aquatic macrophytes in Bulgaria, vascular plants (Pteridophyta and Spermatophyta) count of 345 species, belonging to 158 genera of 66 families. They comprise only 9% of the total number of vascular plants, which are 4030 species for the country (PETROVA & VLADIMIROV, 2010). About 68 species of aquatic vascular plants are submerged (plants that grow completely submerged under the water, with roots or root-analogues in, attached to, or closely associated with the substrate), free-floating macrophytes (plants that typically float on or under the water surface) and 277 species are emergent organisms tolerant to seasonal drought (plants that are rooted in submerged soils or soils that are periodically inundated, with foliage extending into the air). Families of highest species richness are: *Cyperaceae* (66 species), *Poaceae* (22), *Ranunculaceae* (20), *Potamogetonaceae* (17). The most species are belonged to the genera: *Carex* (29), *Potamogeton* (15), *Ranunculus* (13), *Juncus* (13), *Cyperus* (11), *Equisetum* (8) and *Oenanthe* (7).

Studies related to aquatic macrophytes are part of floristic investigation in Bulgaria. The extent and level of knowledge on river flora and vegetation in the country by the end of the 20th century were generalized and analysed by VODENITCHAROV *et al.* (1993) and APOSTOLOVA (2007). In recent years numerous floristic reports were published identifying new fields of aquatic macrophytes in the country (PETROVA, 2008; 2010; PETROVA *et al.*, 2009; 2010; SOPOTLIEVA, 2006). Also a new "key" for determining the species of narrow-leaved group of representatives of the genus *Potamogeton*, as well as information about their distribution was updated and two new species for Bulgarian flora were reported – *P. obtusifolius* Mert. & W.D.J.Koch and *P. berchtoldii* Fieber (KIRYAKOV & CHESHMEDJIEV, 2007). The species composition and abundance of macrophytes in Natural Park "Vrachanski Balkan" were studied (VALCHEV & STOEVA, 2010), the diversity of aquatic vascular plants and conservation significance species of the macrophytes along the Danube (YURUKOVA, 2002; VALCHEV *et al.*, 2006), as well as the hygrophytes and hydrophytes vegetation in some protected areas near the Danube (TZONEV, 2009).

Mediterranean River typology is characterised by a predictable annual cycle of flood and drought that varies in intensity according to the levels and duration of annual and interannual rainfall (HUGHES *et al.*, 2009). This is the first study describing the aquatic macrophytes composition and distribution, relation to abiotic habitat factors (flow velocity, shading, substrate type), and relative abundance of species of Mediterranean Rivers in Bulgaria. Altitude and riparian vegetation were also discussed.

Materials and Methods

Aquatic macrophytes were studied in the beginning of growing season (mid May 2014) at 15 sampling sites along thirteen rivers in South Bulgaria (Fig. 1). All bryophytes and vascular plants were included in 3 groups: hydrophytes, amphiphytes (species capable of growing on land or in water), and helophytes (emergent plants, rooted under water). Species were recorded together with site descriptions (speed of the water flow, shading, substrate type, mean depth and altitude, riparian vegetation). The length of a survey site was approximately 100 m. Bryophyte samples were stored in collecting packets, determined in the laboratory according to PETROV (1975) and SMITH (1980, 2004). Nomenclature accepted in GROLLE & LONG (2000) for liverworts and HILL *et al.* (2006) for mosses was followed. The taxonomy of vascular plants followed Flora Europaea (TUTIN *et al.*, 1964–1980, 1993). Macrophyte relative abundance was quantified based on

percentage frequency of occurrence at 15 sampling sites.

Speed of the water flow, shading and mean depth were determined according to SCHAUMBURG *et al*. (2004, 2006) in a semi-quantitative way using class scales, to enable a fast and easy field application. Velocity of flow was recorded via a 6-point scale: I = not visible, II = barely visible, III = slowly running, IV = rapidly running (current with moderate turbulences), V = rapidly running (turbulently running), VI = torrential. The substratum conditions at the sampling site were classified in 5% steps according to an 8-point scale: % mud, % clay/loam (<0.063 mm), % sand (0.063–2.0 mm), % fine/medium gravel (2.0–6.3/6.3–20 mm), % coarse gravel (20–63 mm), % stones (63–200 mm), % boulders (>200 mm), and % organic/peat. Mean depth was noted on a 3-degree scale (I = 0–30 cm, II = 30–100 cm, III >100 cm). Shading was noted based on the 5-degree scale (1 = completely sunny, 2 = sunny, 3 = partly overcast, 4 = half-shaded, 5 = completely shaded) of WÖRLEIN (1992).

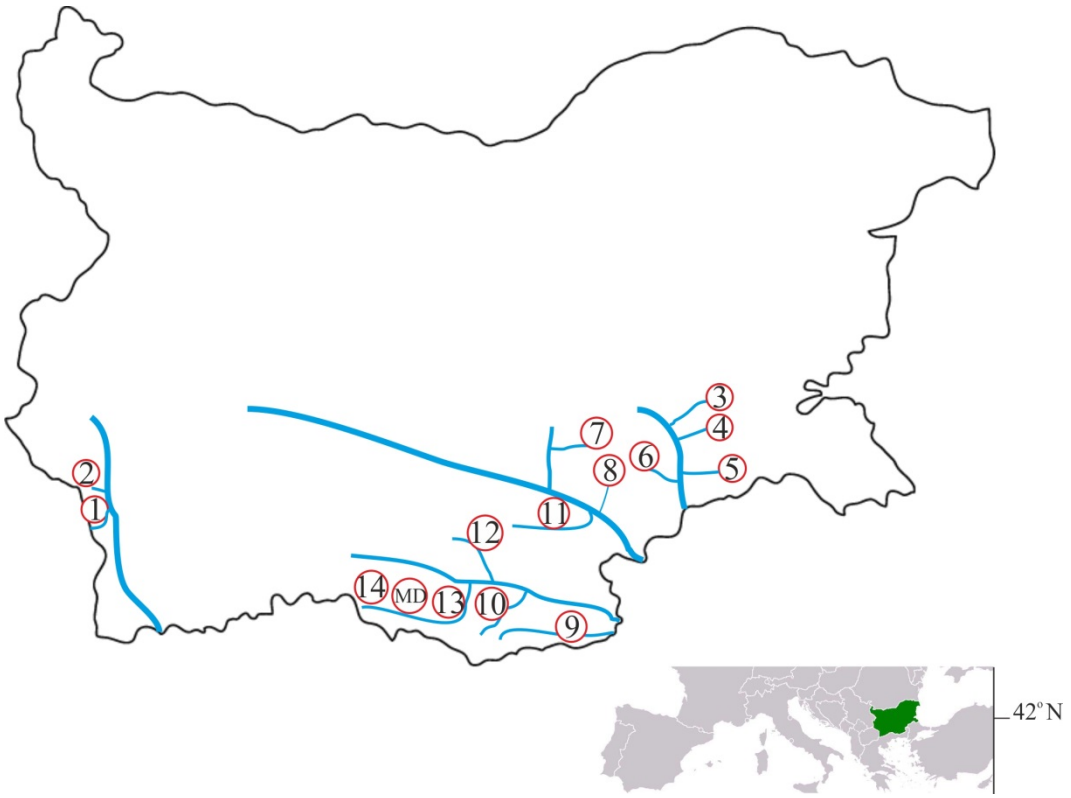

Fig 1. Location of the studied Mediterranean rivers in Bulgaria. Sites: 1-Brezhanska, 2-Stara, 3-Dereorman, 4-Popovska, 5-Melnishka, 6-Manastirska, 7-Sokolitsa, 8-Yerusalimovska, 9-Byala, 10-Krumovitsa, 11-Biserska, 12-Perperek, 13-Varbitsa before Studen Kladenets Dam, 14-Varbitsa before Zlatograd town; MD-Macrophyte depopulation: Varbitsa–Krilatitsa bridge.

Results and Discussion

Taxonomic composition

The list of aquatic bryophytes (Bryophyta) and vascular plants (Pteridophyta and Spermatophyta) were presented (Table 1). In total, 73 species were observed, of which 13 were bryophytes, registered at 9 sites. Aquatic vascular plant determined patterns of distribution at 14 sites, where 60 species were registered including 8 hydrophytes and 52 helophytes and amphiphytes. Macrophyte depopulation was assessed at 1 site. Aquatic bryophytes include 10 mosses and 3 liverworts. The recorded bryophytes species referred to 7 families and 12 genera. The most frequently distributed bryophyte was *Leptodictyum riparium* (at 7 sites, relative

abundance 47%), followed by *Cratoneuron filicinum*, *Platyhypnidium riparioides* (at 4 sites, relative abundance 27%), *Brachythecium rivulare* and *Hygroamblystegium tenax* (at 3 sites, relative abundance 20%). Vascular plants referred to 25 families and 43 genera. The most common hydrophyte was *Lemna minor* L. (at 5 site, relative abundance 33%), followed by *Ranunculus trichophyllus* (at 4 site, relative abundance near 27%), *Myriophyllum spicatum* and *Potamogeton nodosus* (at 3 site, relative abundance 20%). The most abundant species from the group of helophytes and amphiphytes was *Mentha aquatica* (at 8 site, relative abundance 53%), followed by *Agrostis stolonifera* and *Mentha spicata* (at 7 site, relative abundance near 47%), *Berula erecta*, *Juncus effusus*, *Lycopus europaeus*, *Lythrum salicaria*, *Phalaris arundinacea*, *Ranunculus repens*, *Sparganium erectum*, *Typha latifolia*, *Veronica anagalis-aquatica* (at 6 site, relative abundance 40%).

Two species were critically endangered: hydrophyte *Groenlandia densa* and helophyte *Myricaria germanica* (DIMITROVA, 2012; GUSSEV, 2012; PETROVA & VLADIMIROV, 2009).

Almost 65% of the 19 most frequent species for the common Mediterranean rivers type (AGUIAR *et al.*, 2014) were registered at studied Bulgarian rivers.

Table 1. List of registered bryophytes and vascular plants.

Species	Number of registered sites	Relative abundance, %
Bryophytes		
Amblystegium humile (P. Beauv.) Crundw.	1	6.7
Aneura pinguis (L.) Dumort.	1	6. 7
Brachythecium rivulare Schimp.	3	20
Brachythecium rutabulum (Hedw.) Schimp.	1	6.7
Bryum pallens Sw. ex anon.	1	6.7
Cratoneuron filicinum (Hedw.) Spruce	4	26.7
Eurhynchium swartzii (Turner) Curn.	1	6.7
Fontinalis hypnoides C.Hartm.	1	6. 7
Hygroamblystegium tenax (Hedw.) Jenn.	3	20
Leptodictyum riparium (Hedw.) Warnst.	7	46.7
Marchantia polymorpha L.	1	6. 7
Pellia endiviifolia (Dicks.) Dumort.	2	13.3
Platyhypnidium riparioides (Hedw.) Dixon	4	26.7
Vascular plants		
Hydrophytes		
Callitriche platycarpa Kütz.	1	6.7
Groenlandia densa (L.) Fourr.	1	6.7
Lemna minor L.	5	33.3
Myriophyllum spicatum L.	3	20
Potamogeton pusillus L.	1	6.7
Potamogeton crispus L.	2	13.3
Potamogeton nodosus Poir.	3	20
Ranunculus trichophyllus Chaix	4	26.7
Helophytes & Amphiphytes		
Agrostis stolonifera L.	7	46.7
Alisma lanceolatum With.	3	20
Berula erecta (Huds.) Coville	6	40
Cardamine amara L.	1	6.7

Carex pseudocyperus L.	2	13.3
Carex remota L.	1	6.7
Carex riparia Curtis	4	26.7
Carex vulpina L.	1	6.7
Cyperus longus L.	2	13.3
Echinochloa crus-galli (L.) P.Beauv.	2	13.3
Eleocharis palustris (L.) Roem. & Schult.	1	6.7
Equisetum arvense L.	4	26.7
Equisetum palustre L.	3	20
Galium uliginosum L.	2	13.3
Glyceria fluitans (L.) R. Br.	1	6.7
Glyceria maxima (Hartm.) Holmb.	1	6.7
Juncus articulatus L.	1	6.7
Juncus effusus L.	6	40
Lycopus europaeus L.	6	40
Lycopus exaltatus L.f.	2	13.3
Lysimachia nummularia L.	4	26.7
Lythrum salicaria L.	6	40
Mentha aquatica L.	8	53.3
Mentha spicata L.	7	46.7
Myosotis palustris (L.) Nath.	1	6.7
Myosoton aquaticum (L.) Moench	1	6.7
Myricaria germanica (L.) Desv.	1	6.7
Nasturtium officinale R.Br.	3	20
Oenanthe aquatica (L.) Poir.	1	6.7
Paspalum paspalodes (Michx.) Scribn.	3	20
Persicaria lapathifolia (L.) Delarbre	1	6.7
Persicaria maculosa Gray	1	6.7
Petasites hybridus (L.) P.Gaertn. B.Mey. & Scherb.	2	13.3
Phalaris arundinacea L.	6	40
Phragmites australis (Cav.) Trin. ex Steud.	1	6.7
Plantago lanceolata L.	1	6.7
Polygonum hydropiper L.	1	6.7
Polygonum mite Schrank	1	6.7
Ranunculus muricatus L.	1	6.7
Ranunculus repens L.	6	40
Rorippa sylvestris (L.) Besser	3	20
Rumex aquaticus L.	1	6.7
Rumex crispus L.	1	6.7
Scirpus lacustris L.	3	20
Solanum dulcamara L.	1	6.7
Sparganium erectum L.	6	40
Stellaria nemorum L.	1	6.7
Typha angustifolia L.	2	13.3
Typha latifolia L.	6	40
Veronica anagalis-aquatica L.	6	40
Veronica catenata Pennell	2	13.3
Veronica beccabunga L.	3	20

Site characteristics

The majority of the studied Mediterranean Rivers were sunny, with moderate flow velocity and coarse bottom and average depth up to 0.3 m. The altitude of the rivers was between 100 and 500 m and the vegetation along the riverside was dominated by forests and agricultural areas. The site along Varbitsa River, near Krilatitsa village, assessed in macrophyte depopulation was characterized with rapidly running water and substrate was presented by sand and gravel. Thus, it was assumed that natural site characteristics accounted for the depopulation.

Conclusions

Seventy-three species were registered in studied 13 highly seasonal rivers in Bulgaria during 2014; among them were recorded 65% of the most frequent species for the common Mediterranean rivers type. Macrophyte flora at these small and medium streams was represented by both bryophytes and vascular plants. The most distributed bryophyte was *Leptodictyum riparium*, while *Lemna minor* was the most common hydrophyte. The most frequent bank species was *Mentha aquatica*. Based on this first preliminary study of Mediterranean rivers in Bulgaria, it could be summarized that species-rich macrophyte communities were recorded.

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A Case Study of Allelopathic Effect on Weeds in Wheat

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Abstract. Most powerful and effective method of weed control is by chemical substances called herbicides. In recent years, they were published quite data on different side effects of herbicides on humans, animals, crops and the environment as a whole. Therefore, the increased interest for biological weed control lately is reasonable, since its improvement and expansion will contribute to limiting excessive use of herbicides, respectively their harmful effects and will support the successful implementation of complex weed control. The purpose of this study was to investigate the effect of selected plant species, containing allelopathic active substances, on germination, growth and biomass of some widespread weeds in wheat. Experiments were carried out at laboratory conditions using seeds of wheat (*Triticum aestivum* L., sort Sadovo 1) and most common weeds therein: Johnson grass (*Sorghum halepense* (L) Pers), white pigweed (*Chenopodium album* L.), twitch (*Cynodon dactylon* L.) and curly dock (*Rumex crispus* L.). Allelopathic substances were extracted with distilled water from flowers of lavender (*Lavandula angustifolia* Mill.), leaves of basil (*Ocimum basilicum* L.), leaves of spearmint (*Mentha longifolia* (L) Huds.), and leaves of peppermint (*Mentha piperita* L.). Of the tested active allelopathic plants, the most negative impact on germination of all weeds seeds (including wheat), as well as on the development of plants exhibited the water extract of lavender. Lavender and basil had a stronger negative effect on white pigweed and twitch compared with both mint species. A significant inhibitory effect of spearmint even at low concentrations was recorded on the germination of all weed species tested while the wheat was slightly affected, which manifests this plant as a potential effective species in strategies for weed control management.

Keywords: allelopathy, weed control, wheat, laboratory experiment.

Introduction

Weeds are one of the most serious problems in agricultural production. They are volunteer plants from the wild or semi culture species that are found in food crops despite the will of the people and harm reducing yields. Today, some 30 000 species of weeds, i.e. repeated more than crops and in quantity, size and distribution are second group after natural vegetation. According to the FAO, from the total losses worldwide caused by the crop pests, the weeds account for 35% of losses in wheat, 28% in vegetables, 29% in fruit species and vineyards, 37% in tobacco, etc.

In modern "organic farming" the problem of weed control is increasing and refusal of chemical resources of protection from them is usually accompanied by a sharp decrease in yields. Solution to this problem could be found in the development of integrated systems for weed control,

including the advantages of chemical, biological, mechanical and preventive methods to combat in minimizing their negative sides. Integrated weed control in most respects the principle of greening and environmental protection simultaneously with increased weed control and saving energy (BARNES & PUTNAM, 1986; STOIMENOVA *et al*., 2008).

In the last decades there were a huge number of publications concerning allelopathy (RICE, 1974, 1995; REIGOSA *et al*., 1999; KADIOGLU *et al*., 2005; DIMITROVA & SERAFIMOV, 2007), and recently it was included in the sustainable agriculture, which is defined as organic, alternative, restorative, biodynamic, low costing and preserving resources (DIMITROVA, 2008). Despite the attention paid to allelopathy by ecologists, biologists and herbologists, complicated relationship "competition - allelopathy" in the system "weed - crop plant" is not fully understood. Interaction between weeds and crops is simultaneously and/or sequentially, with direct or indirect effect of one plant species to another, through the synthesis of various chemical compounds – alelochemicals, that are released into the environment and affect (inhibit and/or stimulate) the germination of seeds and the development of a number of weeds and crop (REIGOSA *et al*., 1999; KOSTADINOVA *et al*., 2002; KADIOGLU *et al*., 2005). Results are contradictory, confirming in varying degrees the inhibitory or stimulatory effect of various weeds on germination of seeds (KADIOGLU *et al*., 2005; SERAFIMOV *et al*., 2005; ALEKSIEVA & SERAFIMOV, 2008). Difficulties in this regard, from one hand are due to the lack of specific unifying experimental methods to model the laboratory and field experiments, and from the other - to make a distinction between allelopathy and competition in plant communities (STEVEN *et al*., 1984; STOIMENOVA *et al*., 2004a,b; SERAFIMOV *et al*., 2008).

Due to the selective nature of allelopathy, it should not expect that it alone could destroy all weeds in a typical agricultural environment, so it could function as an element of an integrated strategy for weed control. Integrated control is recognized as a preferred strategy in the program of the United Nations Conference on Environment and Development. Its advantages are its complexity, in full destruction of weeds and in the lower risk of environmental pollution. This requires more detailed laboratory studies on allelochemical interactions aimed at demand and supply opportunities for practical application of allelopathy in weed control in order to reduce the use of chemicals.

Most commonly used method of proving allelopathic interference in plant communities or in the "weed - crop plant" is establishing stimulating or inhibitory effect of extracted plant material on the test plants or study the effect of plant residues and their application in quartz sand and/or soil made in the laboratory (STREIBIG *et al*., 1993; KALINOVA *et al*., 2012; KAWORU *et al*., 2001; PENEVA, 2006). So, in this study we aimed to investigate ex-situ the effect of selected plant species, containing allelopathic active substances, on germination, growth and biomass of some widespread weeds in wheat (*Triticum aestivum* L.).

Material and Methods

Allelopathic plant species. Based on a literature review performed as active allelopathic plant species were chosen:

- Lavender (*Lavandula angustifolia* Mill.) - Flowers
- Basil (*Ocimum basilicum* L.) - Leaves
- Spearmint (*Mentha longifolia* (L) Huds.) - Leaves
- Peppermint (*Mentha piperita* L.) - Leaves

Dried plant material of lavender, basil, spearmint and peppermint was purchased from a certified drug manufacturer.

Aqueous extracts of allelopathic plants were prepared using the following scheme: 50 g of dry plant material were crushed in a mortar with quartz sand and quantitatively transferred into a flask with 1000 ml of distilled water. After standing in the dark for 24 hours, the solution was filtered and a 5% aqueous extract was prepared (TENEVA *et al*., 2007). By dilution with distilled water thereof were prepared test solutions with concentrations 1.25%; 2.5% and 3.75% aqueous extract.

Test species. For the purpose of this study we used seeds of wheat (*Triticum aestivum* L. sort Sadovo 1) and most common weeds therein:

- Johnson grass (*Sorghum halepense* (L) Pers)
- White pigweed (*Chenopodium album* L.)
- Twitch (*Cynodon dactylon* L.)
- Curly dock (*Rumex crispus* L.)

Seeds of wheat were purchased from a certified manufacturer and these of weeds were collected on the field in the autumn of 2013, in the period after their maturation. Viability of the collected weed seeds was tested before starting the allelopathic experiment, and the percentage of germination for the four species was above 85%.

Experimental setup. For each experimental variant, 50 seeds of each weed (Johnson grass, white goose-foot, twitch, and curly dock) and wheat, respectively, were put into containers with quartz sand and 100 ml of different test solutions. Simultaneously with each essay were set control samples with 50 seeds of the same plant species and 100 ml of distilled water (VELCHEVA *et al.*, 2013).

Laboratory experiments were conducted in June-July 2014; the exposure time was 21 days at 24-28°C and 12/12 hours light period. Periodically was adding a quantity of the solution in containers with cultures in order to maintain a constant level. All test variants were carried out in three replications.

Effect assessment. For assessing the results of the experiments were used the following parameters: 1. Quantitative parameters: number of germinated seeds in each test variant; percent of germination in each test variant (%); percent of germination comparing to the control (%); 2. Biometric parameters: length of the young plants, cm; biomass of the young plants, g. Length was measured using graph paper and the weight was recorded on an analytical balance.

Statistical evaluation of the results obtained was performed by descriptive statistical analysis and t-test ($p<0.05$) using software Statistica 7.0 (STATSOFT INC., 2004).

Results and Discussion

From the four studies allelopathic active plants, the strongest negative effect on weeds and wheat exhibited the aqueous extracts of lavender flowers (Table 1). Even at the lowest test concentration (1.25%) we recorded strong inhibition of germination of seeds of white pigweed and Johnson grass, and at the next concentration (2.5%) it was completely suppressed. About 35% of the seeds of twitch and curly dock germinated at the concentration of 2.5%, but with worsened biometric parameters. At the concentrations 3.75% and 5% it has been observed no germination in all plant species. Significant reducing the length of the plant with increasing concentration of the aqueous extract of lavender was found in wheat and curly dock ($p<0.05$).

Aqueous extract of basil leaves had a strong depressing effect on germination of seeds of white pigweed, reaching up to 92% at the lowest test concentration (Table 2). Twitch also proved as highly sensitive - inhibition of germination was 77%. Germination of wheat was affected slightly from the lowest test concentration of aqueous extract of basil, but at the next one the germination of wheat was reduced by half. Relatively resistant to the effects of basil were the seeds of curly dock, which remained viable in the presence of 3 times larger quantities of extracts of basil in the grow medium. Statistical processing of the data showed that with increasing concentration of the basil extract the germination, growing up and biomass production in the Johnson grass, curly dock and wheat significantly decreased ($p<0.05$).

Impact of the aqueous extract of spearmint leaves on the test seeds was generally negative (Table 3), more pronounced in the seeds of white pigweed and Johnson grass. Almost complete inhibition of germination was recorded there even in low concentrations of aqueous extract, while for the twitch this effect was found when increasing the dose. Germination of seeds of curly dock and development of young plants proved to be less influenced at concentration 1.25%. They showed high values in both quantitative and biometric parameters and at a concentration of 2.5%, where the germination was above 50%.

Table 1. Effect of aqueous extract of the lavender flowers on quantitative and biometric parameters

Plant species	Concentration of aqueous extract, %	Germination of test seeds, %	Germination compared to the control, %	Length, cm	Weight, g
Wheat (*Triticum aestivum* L.)	**0%**	86%	-	19.7 cm	118.2 g
	1.25%	53%*	62%	7.9* cm	45.1* g
	2.5%	12%*	14%	2.3* cm	11.6* g
	3.75%	0%*	-	-	-
	5%	0%	-	-	-
Johnson grass (*Sorghum halepense* (L) Pers)	**0%**	88%	-	4.1 cm	35.7 g
	1.25%	22%	25%	1.9 cm	12.2 g
	2.5%	0%	-	-	-
	3.75%	0%	-	-	-
	5%	0%	-	-	-
Twitch (*Cynodon dactylon* L.)	**0%**	93%	-	3.6 cm	29.4 g
	1.25%	71%	76%	3.1 cm	22.2 g
	2.5%	34%	37%	2.4 cm	11.8 g
	3.75%	0%	-	-	-
	5%	0%	-	-	-
White pigweed (*Chenopodium album* L.)	**0%**	85%	-	3.5 cm	26.0 g
	1.25%	11%	13%	1.1 cm	8.3 g
	2.5%	0%	-	-	-
	3.75%	0%	-	-	-
	5%	0%	-	-	-
Curly dock (*Rumex crispus* L.)	**0%**	98%	-	6.8* cm	66.5 g
	1.25%	60%*	61%	4.4* cm	33.8* g
	2.5%	32%*	33%	2.7* cm	14.9* g
	3.75%	1%*	1%	1.4* cm	2.2* g
	5%	0%*	-	-	-

* Significant difference with the control at $p<0.05$

Table 2. Effect of aqueous extract of the basil leaves on quantitative and biometric parameters

Plant species	Concentration of aqueous extract, %	Germination of test seeds, %	Germination compared to the control, %	Length, cm	Weight, g
Wheat (*Triticum aestivum* L.)	**0%**	86%	-	19.7 cm	118.2 g
	1.25%	75%*	87%	11.3* cm	99.1* g
	2.5%	43%*	50%	5.5* cm	43.3* g
	3.75%	2%*	3%	1.4* cm	6.8* g
	5%	0%*	-	-	-
Johnson grass (*Sorghum halepense* (L) Pers)	**0%**	88%	-	4.1 cm	35.7 g
	1.25%	61%*	69%	3.5* cm	22.9* g
	2.5%	27%*	31%	2.2* cm	15.5* g
	3.75%	5%*	6%	1.4* cm	2.8* g
	5%	0%*	-	-	-
Twitch (*Cynodon dactylon* L.)	**0%**	93%	-	3.6 cm	29.4 g
	1.25%	21%	23%	2.0 cm	6.3 g
	2.5%	0%	-	-	-
	3.75%	0%	-	-	-
	5%	0%	-	-	-
White pigweed (*Chenopodium album* L.)	**0%**	85%	-	3.5 cm	26.0 g
	1.25%	7%	8%	0.4 cm	3.5 g
	2.5%	0%	-	-	-
	3.75%	0%	-	-	-
	5%	0%	-	-	-
Curly dock (*Rumex crispus* L.)	**0%**	98%	-	6.8 cm	66.5 g
	1.25%	91%	93%	5.5* cm	58.8 g
	2.5%	56%*	57%	4.2* cm	31.0* g
	3.75%	23%*	24%	2.5* cm	19.1* g
	5%	0%*	-	-	-

* Significant difference with the control at $p<0.05$

Significant negative effect with increasing concentration of the aqueous extract of spearmint leaves was found both on the germination of all the tested seeds, and the development of plants ($p<0.05$).

Wheat proved relatively resistant to the effects of spearmint and maintained good parameters for the three lower concentrations. This result is promising and it is necessary to carry out more detailed laboratory and field experiments to determine the potential of spearmint in integrated strategy for weed management.

Strongest negative impact of the aqueous extract of peppermint leaves we reported in Johnson grass seeds, followed by wheat seeds (Table 4). Concentration of 1.25% aqueous extract proved less active in terms of germination of all test seeds and stronger inhibitory effect occurs with increasing dose. It was also established that peppermint has a significant negative impact on growth. For wheat, twitch, Johnson grass and white pigweed the length of young plant under effect of 1.25% aqueous extract was reduced by half compared with the control.

Considering displayed strong sensitivity of seeds and young plants of wheat we can point out that peppermint is not suitable for allelopathic active species in wheat crops.

Chenopodium album L. is a common weed of wheat and other arable crops and orchards. Cases of resistance of *C. album* to herbicides like atrazine, metribuzin and linuron have been reported in many European countries and the USA since 1980 (ELEFTHEROHORINOS *et al.*, 2000). This has promoted research for alternative eco-friendly methods for its control. Preliminary studies of ANJUM & BAJWA (2007) have shown the susceptibility of broadleaved weeds like *C. album* to sunflower extracts. These authors tested the potential of sunflower leaf extract in weed management of *C. album*. In comparison with synthetic herbicides the crude extract failed to eradicate this weed completely, but the highest tested concentration successfully killed the weed and overcame weed crop competition and consequently increased wheat yield significantly. In our study, *C. album* seeds were found as very sensible to both basil and lavender aqueous extracts – the germination was almost fully suppressed at lowest test concentration of 1.25% extract. Extracts from spearmint and peppermint leaves also exhibited negative effect at seed germination but at concentrations above 3.75%.

Cynodon dactylon and *Sorghum halepense* are C4 perennial grasses that are considered to be among the world's worst weeds (HOLM *et al.*, 1977). *Cynodon dactylon* propagates mainly vegetatively, through stolon and rhizome fragmentation, but *Sorghum halepense* reproduces both by seed and by rhizomes (HOROWITZ, 1973). Rhizomes of both weeds are the main reserves of carbohydrates and dormant buds for over-wintering. A single plant of *Sorghum halepense* can produce 40 to 90 m of rhizomes per growing season while *Cynodon dactylon* fresh weight of subterranean parts down to 45 cm can range from 420 to 780 g m^{-2} during one year (HOROWITZ, 1972, 1973). Also, rhizomes are the primary means of *Cynodon dactylon* and *Sorghum halepense* dispersal in the field because mechanical tillage of weed-infested fields produces fragmentation and dispersal of rhizomes propagules, from which new ramets can be formed (FERNANDEZ, 2003; MITSKAS *et al.*, 2003).

Allelopathic potential of residues of some brassica species, which are round white radish (*Raphanus sativus* L.), garden radish (*R. sativus* L.), black radish (*R. sativus* L. var. niger), little radish (*R. sativus* L. var. radicula, turnip (*Brassica campestris* L. subsp. rapa) and rapeseed (*Brassica napus* L. oleifera DC.) on Johnson grass were investigated under both laboratory and field conditions by UREMIS *et al.* (2009). All these species suppressed Johnson grass in field and laboratory conditions. In our research, the most effective plant species against Johnson grass was found to be lavender and peppermint which reduce the germination of seeds up to four times even at 1.25% aqueous leaf extract, and completely suppressed it in higher doses. Basil and spearmint leaf extracts also inhibited seed

Table 3. Effect of aqueous extract of the spearmint leaves on quantitative and biometric parameters

Plant species	Concentration of aqueous extract, %	Germination of test seeds, %	Germination compared to the control, %	Length, cm	Weight, g
Wheat (*Triticum aestivum* L.)	**0%**	86%	-	19.7 cm	118.2 g
	1.25%	78%	91%	12.7* cm	98.3 g
	2.5%	54%*	63%	6.5* cm	43.4* g
	3.75%	19%*	22%	2.7* cm	23.2* g
	5%	0%*	-	-	-
Johnson grass (*Sorghum halepense* (L) Pers)	**0%**	88%	-	4.1 cm	35.7 g
	1.25%	46%*	52%	2.3* cm	22.9* g
	2.5%	8%*	9%	1.7* cm	6.5* g
	3.75%	1%*	1%	1.1* cm	2.3* g
	5%	0%*	-	-	-
Twitch (*Cynodon dactylon* L.)	**0%**	93%	-	3.6 cm	29.4 g
	1.25%	68%*	73%	2.6* cm	19.7* g
	2.5%	22%*	24%	1.9* cm	10.2* g
	3.75%	4%*	4%	1.1* cm	3.9* g
	5%	0%*	-	-	-
White pigweed (*Chenopodium album* L.)	**0%**	85%	-	3.5 cm	26.0 g
	1.25%	25%*	29%	1.2* cm	9.9* g
	2.5%	3%*	4%	0.4* cm	1.8* g
	3.75%	0%*	-	-	-
	5%	0%*	-	-	-
Curly dock (*Rumex crispus* L.)	**0%**	98%	-	6.8 cm	66.5 g
	1.25%	90%	92%	6.1 cm	60.9 g
	2.5%	55%*	56%	4.8* cm	42.4* g
	3.75%	15%*	15	2.2* cm	21.0* g
	5%	0%*	-	-	-

* Significant difference with the control at $p<0.05$

Table 4. Effect of aqueous extract of the peppermint leaves on quantitative and biometric parameters

Plant species	Concentration of aqueous extract, %	Germination of test seeds, %	Germination compared to the control, %	Length, cm	Weight, g
Wheat (*Triticum aestivum* L.)	**0%**	86%	-	19.7 cm	118.2 g
	1.25%	41%*	48%	9.4* cm	75.1* g
	2.5%	9%*	10%	1.8* cm	11.6* g
	3.75%	10%*	11%	1.6* cm	11.4* g
	5%	0%*	-	-	-
Johnson grass (*Sorghum halepense* (L) Pers)	**0%**	88%	-	4.1 cm	35.7 g
	1.25%	24%*	27%	1.8* cm	10.4* g
	2.5%	2%*	2%	0.9* cm	2.6* g
	3.75%	0%*	-	-	-
	5%	0%*	-	-	-
Twitch (*Cynodon dactylon* L.)	**0%**	93%	-	3.6 cm	29.4 g
	1.25%	72%*	77%	2.9* cm	20.5* g
	2.5%	31%*	33%	1.8* cm	11.2* g
	3.75%	1%*	1%	0.6* cm	1.6* g
	5%	0%*	-	-	-
White pigweed (*Chenopodium album* L.)	**0%**	85%	-	3.5 cm	26.0 g
	1.25%	66%*	78%	2.4* cm	19.1* g
	2.5%	18%*	21%	1.5* cm	9.2* g
	3.75%	3%*	4%	0.8* cm	2.7* g
	5%	0%*	-	-	-
Curly dock (*Rumex crispus* L.)	**0%**	98%	-	6.8 cm	66.5 g
	1.25%	80%*	82%	5.8* cm	51.3* g
	2.5%	52%*	53%	3.3* cm	29.9* g
	3.75%	19%*	20%	1.6* cm	17.3* g
	5%	0%*	-	-	-

* Significant difference with the control at $p<0.05$

germination but at concentrations above 3.75%.

Cynodon dactylon is the most damaging weed in world scale and takes the fourth place among weeds in production of allelopathic compounds (AL-SAADAWI *et al.*, 1990). Allelopathic effect is not just decreasing germination but also delaying the time of germination which could affect plant competition (ESCUDERO *et al.*, 2000; EL-KHATIB *et al.*, 2004). CHAVES & ESCUDERO (1997) found that the exudate secreted by *Cistus ladanifer* leaves inhibited the seed germination of *Cynodon dactylon* and *Rumex crispus*. Allelopathic species used in our study demonstrated significant negative effect on seed germination of *C. dactylon* on concentrations above 3.75% aqueous extract, most pronounced in basil and peppermint.

Rumex crispus L. (curled dock) and *Rumex obtusifolius* L. (broad-leaved dock) are among the most often studied weed species worldwide, the latter is also considered as one of the five most widely distributed non-cultivated plant species in the world (ALLARD, 1965). These *Rumex* species are of agricultural significance because they compete with sown or native pasture species or arable crops and occupy area which could be utilized by more palatable crop species. For Central Europe, it is estimated that more than 80% of all herbicides used in conventional grassland farming are used to control these species (GALLER, 1989). The fear of an infestation of grassland and arable land by *R. crispus* and *R. obtusifolius* is also among the most important obstacles for farmers to switch from conventional to organic farming in Central Europe (DIERAUER & STOPPLER-ZIMMER, 1994). Extracts or natural chemicals derived from various plant species have been shown to reduce germination and shoot and root development of *Rumex* seedlings (ZALLER, 2004). REIGOSA *et al.* (1999) have tested the effect of six phenolic compound derived from *Capsicum annuum* leaves on the germination and growth of *Rumex crispus* and other 5 weeds. They found that highest concentrations and combined mixture of phenolic substances inhibited the germination, but lower concentrations had no effect or were stimulatory. Our results were similar as we also found that low doses of tested allelopathic active plants could not influence the seed germination of this weed. Most pronounced was the effect of lavender flowers aqueous extract which suppressed germination at 3.75% concentration.

Conclusions

From the allelopathic active plant species used in this study, the aqueous extract of lavender flowers exhibited most negative impact on germination of all test seeds and on the development of young plants. Significant inhibitory effect of spearmint leaves even at low concentrations was recorded on the germination of weed species tested, while the wheat seeds were slightly affected.

So, we can point out that due to proven strong negative effect of lavender both on weed and on wheat, it is unsuitable for mixed plantings. In terms of spearmint, there was demonstrated inhibitory effect on weeds while the wheat was found to be resistible. These results indicate the potential for integration of spearmint in sustainable agriculture and organic farming, but for this purpose it is necessary to continue research in the field.

Potential allelochemicals must be characterized as they can provide new and cheap synthetic analogues of natural products having greater selectivity, stability and efficacy to control weeds and pests. They should also undergo toxicity testing to confirm their safety on non-target species. A logical progression in this research is required to develop agrochemicals based on natural products.

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Short note

Artificial Nesting of Common Goldeneye Bucephala clangula on the Park Lake in Puławy (Eastern Poland)

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Abstract. The number of breeding pairs of Common Goldeneye in Lublin region was estimated to approx. 15. The survey in 2012 was conducted to estimate the breeding and the non-breeding population of the species on the Vistula oxbow in Czartoryskis Park in Puławy revealed one breeding pair and up to seven non-breeding. In March 2013 two nest boxes for Common Goldeneye were hung on trees in the middle and in the end of the oxbow. In 2014 the nest box in the middle of the oxbow was unoccupied and the one in the end of the reservoir was occupied by the pair of Common Goldeneye. We noted the successful breeding with six nestlings. Same time the second pair of the species successfully brood in the natural tree hollow. More than one breeding pair of the species on the same reservoir is unusual in this part of Poland.

Key words: Common Goldeneye, nest box, city park, park lake, Poland.

The area of the Common Goldeneye *Bucephala clangula* occurrence spreads into Asia, Europe and North America (BIRDLIFE INTERNATIONAL, 2014). In Poland the species is rarely nesting, while the limit of its nesting range lies in the northern part of the country. In the rest of the country, the species is extremely rarely nesting, found only on a few water reservoirs (TOMIAŁOJĆ & STAWARCZYK, 2003). In Lublin ornithological region only about 15 nesting pairs were noted, on sites in Vistula river valley and in Polesie region, where cases of using nest boxes were noted (CIOS, 2014, Lublin, pers. comm.). The species was firstly noted nesting near small ponds in parks in the second half of the 20th century (SIKORA *et al.*, 2007).

The Czartoryskis Park in Puławy is a historical complex, founded around the Czartoryskis Palace. The important part of the park is the Vistula oxbow, turned into semi-natural reservoir, visited by tourists and local people, mostly in spring and summer. The occurrence of nesting Common Goldeneye makes this place highly important in the Lublin region and south-eastern part of Poland.

The aim of the study was to enlarge the nesting population of goldeneye on the park reservoir in Puławy, by providing safe nesting places in nest boxes.

The Vistula oxbow in Czartoryskis Park in Puławy is 1,2 km long. The width of the reservoir is about 50 meters, the depth is up to three meters. On the east side of the reservoir adjoins to the park and the urban area. The western side is adjacent to the field, in the first year of the survey, the field was covered with rape. Between the field

and the reservoir is the belt of willow thicket and some large poplars. Te part of the park adjacent to the oxbow is covered with old stand, mostly consisting of oak, maple and chestnut trees. Some trees has large, natural holes. After modernization in early 90', there was no further action carried out in the place. The reservoir is semi-natural, with a stable population of crucian carp *Carassius* spp., perch *Perca fluviatilis* and other fish species. By the oxbow lake flows the clean water from the rain sewage treatment plant placed nearby. The most important threads to birds occurring on the reservoir are the presence of American mink *Neovison vison* and anglers.

Mounting nest boxes was preceded by the survey of Common Goldeneye and other waterfowl species on the reservoir. It lasted over a year and revealed one breeding pair and up to seven non-breeding pairs of the species, found on the park lake since late winter (after the ice melt) to the third decade of April (STASIAK & PIEKARSKA, 2013). It also provided data about the avifauna of the oxbow and the threads, which may impact on the birds' breeding success, e.g. interrupting birds by tourists and anglers. Two nest boxes for the Common Goldeneye were made of pine wood. The entrance hole had 10 cm in diameter. The bottom was 18x18 cm, with small holes drilled for drainage. The depth from the entrance to the bottom was 24 cm. Nest boxes were mounted on selected trees, according to the guide (DU FEU, 2005). Each of them was placed about 5 m over the ground, with the entrance hole facing the water. Boxes were hidden from passers, but were placed easily for birds to fly into. Box no. 1 was placed in the middle of the reservoir, box no. 2 near the estuary. In the first year (2013), none of the nest boxes were occupied by birds. In late autumn, the control of nest boxes' inside revealed that no. 1 was occupied by squirrel *Sciurus vulgaris*, while in no. 2 we found the dormouse *Muscardinus avellanarius*. In 2013 on the reservoir one breeding pair of Common Goldeneye was found, with 13 nestlings. Birds were nesting in the natural tree hole. In 2014 from March to April 5 pairs of the species were observed on the oxbow. One of them was interested in nest box no. 1, but didn't start nesting. Nest box no. 2 was used by the other pair, especially by the female. During the control in early May 2014 we found the Common Goldeneye clutch of six eggs in the second nest box. Same time, the female and four nestlings were observed on the other part of the oxbow. Birds brood in the natural tree hole near the reservoir, about 8 meters above the ground. At the beginning of the 2015 breeding season, the nest box no. 2 was occupied by the pair of Common Goldeneyes, while the nest box no. 1 stayed unoccupied both in winter and spring.

The reservoir in Puławy is one of the breeding grounds of Common Goldeneye in Lublin region, but never more than one nesting pair was noted (SAFADER, 2009; personal observations). The number of breeding pairs of the species in southern part of Poland is many times lower, than in the north. As an example, in Augustowska forest, the species was present on every lake, nesting in natural tree holes, while in Lublin region, with many lakes, oxbows and rivers, only 15 breeding pairs were noted (BUCZEK, 2005; ZAWADZKA *et al.*, 2011). The fact of nesting of the Common Goldeneye in the city park is worth mentioning. The survey of cavity nesting species in parks of Poznań and Warsaw didn't reveal occurrence of this species, though those cities are settled far more in the north than Puławy (LUNIAK, 1992). The species nesting in city parks was noted by M. Stajszczyk only in the north part of Poland, in Łężany, Olsztyn, Mrągowo, Szczecin and Milicz, which is very advantageous for waterfowl to breed (SIKORA *et al.*, 2007). In suitable habitat, the number of birds is increasing, e.g. on Milickie ponds, where Common Goldeneyes started to breed in 70s of 20th Century, and the population raised up to 20 breeding pairs (WITKOWSKI & ORŁOWSKI, 2012). It is mentioned, that the number of birds breeding on city ponds is increasing, but still the total number remains quite low (SIKORA *et al.*, 2007). The species willingly inhabits nest boxes (EADIE & GAUTHIER, 1985; ZICUS & HENNES, 1989; CORRIGAN *et al.*, 2011), but there's not much information about size of those.

We used the D-type boxes with circular entrance, same as in the other research of Common Goldeneye in Lublin region. The size is smaller than in other studies (CORRIGAN *et al.*, 2011), but this construction cannot be occupied by the Tawny Owl *Strix aluco*, which is breeding in the park (SAFADER, 2009; personal observations) and in the whole region (CIOS & GRZYWACZEWSKI, 2013). There was only one case of this owl unsuccessfully trying to inhabit this sort of nest box (CIOS, 2014, Lublin, pers. comm.), while authors noted numerous cases of the owl nesting in bigger nest boxes, even placed almost over the water.

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Synopsis

Current Trends in the Studies of Allelochemicals for Their Application in Practice

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Abstract. The allelochemicals have been largely used in agriculture, forestry, landscape design and ornamental plant growing for many decades. However, there is a lack of the comprehensive studies, where existing publications are analyzed and synthesized with regards to the theoretic aspects for such usage. The objective of this paper was to systemize the advances in the research on allelochemicals' application in practice. Numerous novel methodological propositions have risen recently. We classified them into the physical, chemical, biological, biotechnological and crop-growing approaches. The allelochemicals consist of the wide diversity of the substances according to their chemical nature. Among these substances we outlined, firstly, the unidentified plant exudates and the products of green manuring, secondly, the chemically characterized or purified substances, which include alcohols, organic acids, aliphatic compounds, aromatic, alicyclic and nitrogen-contain organic compounds. Several groups of the biotic sources of allelochemicals were described: dicotyledonous and monocotyledonous plants, particularly under their colonization by non-pathogenic strains of *Fusarium oxysporum*, marine flora and fungi, which exhibit the herbicidal activity. Different targets of the allelochemical application were listed in the paper and they were categorized into several groups: higher flora, animals, unicellular and multi-cellular fungi. We concluded that there is lack of the modern multifaceted knowledge bases for the information about the allelochemical application. Those knowledge bases must be useful in order to choose the appropriate biological method for solving each particular problem of plant cultivation. To that end we systemized the results of current investigation about the usage of allelochemicals in practice.

Key words: allelochemical, crop-growing, crop protection, donor, mechanism of action, organic farming, pathogen control.

Introduction

The allelopathy, since its formation, has evolved from the pure theoretical science into the sphere of knowledge, which is practically beneficial for the humanity. Nowadays the allelopathical researches, directed on the solving of applied tasks, are progressing rapidly. The evaluation of the economical effects, which depend on the presence or the absence of the allelochemicals, is the significant aspect of these researches, but not unique one. The scientific basis is the indispensable prerequisite for such evaluation. It mostly has to do concern with the methodological principles for the management of the applied studies, the knowledge about the chemical properties and the origin, finally the targets and the effects for the impact of the allelochemicals. The comprehension of these effects will be rather incomplete, if the mechanisms of the realization of the allelopathical processes remain unknown. This review especially focuses on the procedures of crop protection. Here the benefit from the allelopathic processes is

proposed to be considered as the alternative for the pesticide application, with its harmful consequences.

Primary term 'allelochemicals' meant the out-organism regulators for the biological processes, which are formed with the direct or indirect participation of the plants (GRÜMMER, 1955). However in world scientific fiction the allelochemicals also have been traditionally considered as the compounds, which are synthesized by plants and associated microbiota in order to manage the various biological processes.

Novel methodological achievements in studies on allelochemicals

There exists the modern interpretation of the allelopathy – the multidisciplinary science, where ecologists, chemists, soil scientists, agronomists, biologists, plant physiologists and molecular biologists offer their skills to give an overall view of the complex interactions occurring in a certain ecosystem (MACÍAS *et al.*, 2007).

Jack bean *Canavalia ensiformis* (L.) DC. is known to be slightly vulnerable to diseases. This observation may be considered to be a significant methodological question. That is why this species was proposed to be used as the model object in the allelopathical investigations (SANTOS *et al.*, 2010).

In the following brief analysis of the recent methodological propositions for the allelopathy we grouped them into several directions: physical, chemical, biological and related with the manufacturing – the crop-growing one.

The novel methods, based on the physical principles:

- electrospray time-of-flight mass spectrometry – ESI-TOFMS (BONNINGTON *et al.*, 2003);

- methods of high resolution, such as the UV-analysis, the mass-spectrometry, and the NMR, which are applied to identify the chemical structure of the substance, e.g. myrigalone A (POPOVICI *et al.*, 2011).

The novel methods, based on the chemical principles:

- solid-phase microextraction, which provides the detection of the traces of substances, e.g. 1,8-cineole, camphor, coumarin, menthol, and carveol, also the last one is not up-taken by tomato *Lycopersicon esculentum* (LOI *et al.*, 2008);

- HPLC (LING *et al.*, 2013), and HPLC, combined with the collection of the extractions on the matrix XAD-4 (KURTZ & SHOUTEN, 2009);

- sequential two-steps cation-exchange chromatography for the extraction the protein from the latex (DE FREITAS *et al.*, 2011);

- capillary electrophoresis (SANTOS *et al.*, 2010);

- optimization of the concentration and the time of the exposition in the course of the extraction of aquatic macroflora with the ethyl-acetate (WU *et al.*, 2013).

The novel methods, based on the biological principles:

- combination of the following conditions: Hoagland nutrient medium (pH 6,0); 25°C, 12/12 h light/dark photoperiod, irradiance of 280 mcmol/(m² s) for 24 or 48 h for the examination of the plant responds on the exogenous treatment with the allelochemicals were tried (DOS SANTOS *et al.*, 2008);

- disc diffusion method (VU *et al.*, 2012).

The biotechnological approaches:

- *in vitro* conditions (WU *et al.*, 2009);

- cloning of the genomes of the investigated objects for their subsequent sequenation (YANG *et al.*, 2013);

- knock-out of genes, that control the biosynthesis of the certain plant-toxic metabolite: this technique was successfully demonstrated through the example of terpene synthases (OsCPS4 and OsKSL4), responding for the momilactone formation (XU *et al.*, 2012).

Methodological discoveries in crop-growing:

- demonstration of the amplification of the effect of certain allelochemicals and herbicides on plants due to their combination (FAROOQ *et al.*, 2011);

- induction of the resistance for the wilt by means of non-pathogenic form of *Fusarium oxysporum*; here it was confirmed that the diagnostic attributes, important for the detection of this effect, were: a) rapid spore germination; b) orientation in

response to root exudate; c) active penetration in roots; d) passive conidia transport in stem; e) enough lag period between induction and challenge inoculation (MANDEEL, 2006);

- prevention of the wilt during the Japanese bunching onion *Allium fistulosum* seeds germination by means of the treatment with the freeze-dried root exudates from shallot *Allium cepa* Aggregatum group (VU *et al*., 2012);

- suppression of the algae blooms due to the application of the plant extracts without increasing the release of cyanotoxin (WU *et al*., 2013);

- recirculating hydroponic culture system, developed for continuous trapping of the root exudates (LING *et al*., 2013).

Chemical nature of allelochemicals

The range of allelochemicals is very diverse in sense of their chemical nature. Many of them are applied on practice. It is reasonable to divide them into two groups of the agents: 1) the materials without accurate chemical identification; 2) the substances or the groups of substances, which are more or less chemically characterized or purified at least.

The first of these groups, particularly, includes the volatile and gaseous exudates of the belowground part of the young stone fruit trees (AFIFI *et al*., 1977), and the gaseous exudates from the germinating seeds of corn *Zea mays* L. and lentil *Lens culinaris* Medicus (CASTKA & VANCURA, 1980). This group must be also enlarged by the products from the plant decomposing residues, leachates and crude homogenates, which come in the root-containing soil horizons. Listed materials may serve for the nematode control. Elaborated crop-rotation system, intercropping, usage the rapid-growing crops with the responding allelopathic properties provide the enrichment of the rhizosphere with the mentioned products. Same effect may be achieved under the green manuring with such plants (HALBRENDT, 1996). It sounds interesting, but unordinary that the plant pathogenic fungi are allelopathically affected by the pollen and in greater measure by the propolis, originated from five Turkish regions (OZCAN *et al*., 2004). The allelopathic analyses involve volatiles from the crushed leaves (ZHANG *et al*., 2008). The resins from the sweet potato *Ipomoea batatas* (L.) Lam., and the residues of the semi-tropical leguminous plants have been tested as insecticides (DUKE *et al*., 2003). The metabolites from the marine flora have been several times reported to act both as antifeedants and as allelochemicals within recent years. Nevertheless there are few studies on the role of these substances in the suppression of the competitive plants (see the final part of this article) (SIEG & KUBANEK, 2013).

In the second group there exist the compounds, which are made by explorers with a certain purposes. Some of them have completely uncharacterized chemical nature. Thus BARAZANI & FRIEDMAN (2001) proposed to yield two herbicidal preparations (phosphinothricin and bialaphos) on the basis of three active ingredients, which were the phytotoxins: geldanamycin, nigericin and hydanthocidin. These substances were produced by the plant pathogenic fungi.

The researches on the allelochemicals have been related with the identification of the organic substances with the different structural complexity. These could be, particularly, the mixture of ethanol and methanol with acetaldehyde (CASTKA & VANCURA, 1980); organic acids (their effect has been tried together with saccharides) (KRAVCHENKO *et al*., 2003); aliphatic organic substances and carboxylic acids (TADDEI *et al*., 2002), including propanedioic acid (JU *et al*., 2002). The above-mentioned agents have been examined mostly within the root exudations.

The number of substances, being in the solutions, act as low-molecular carboxylic acids. These are saccharides (TADDEI *et al*., 2002; KRAVCHENKO *et al*., 2003); saponins from ginseng *Panax ginseng* C.A.Meyer – ginsenosides (YOUSEF & BERNARDS, 2006); saponin chitosan and also 2-deoxy-*D*-glucose (DUKE *et al*., 2003). Alkanes, fatty acids, and esters (LI *et al*., 2009; 2013a)

present the allelochemicals with more remote properties.

Traditionally the experts in the allelopathy have shown the significant interest in substances, which include the phenolic fragments, aryl groups. The studies on the phenolic derivates provide the advantages for the human practical activities. The list of the most investigated simple aryl substances includes: resorcinol, derived from the seedlings of the aquatic macrophytes (SÜTFELD *et al.*, 1996), acting as an important regulator of the relations between macrophytes and other aquatic inhabitants; soybean-produced phthalic (JU *et al.*, 2002) and benzoic (WU *et al.*, 2009) acids. The last one served for the artificial simulation of the allelopathic impact. The larger number of the researches deals with more complex aryl derivates – the aromatic structures, bound with the aliphatic tails. The aromatic substances in the root exudates from cornflag *Gladiolus communis* L. (TADDEI *et al.*, 2002); caffeic, cinnamic, and chlorogenic acids from the cucurbits crops (LING *et al.*, 2013) have been examined. Also the mechanisms of the action of ferulic acid (DOS SANTOS *et al.*, 2008) and coumarin (LOI *et al.*, 2008) on the plant organisms, myrigalone A from the fruits and leaves of myrtle *Myrica gale* L. on knotweed *Fallopia* × *bohemica* (Chrtek & Chrtková) J. P. Bailey, which had invaded in the North American habitats (POPOVICI *et al.*, 2011), as well as cinnamic, *p*-coumaric, ferulic, syringic, and vanilic acids on the pathogens of wilt (WU *et al.*, 2010) have been diligently clarified.

Sorgoleone has been known as a major component of the hydrophobic root exudates from *Sorghum bicolour* (Moench) L. It can serve as a demonstrative example of the aryl groups, coupled with bulky linear aliphatic structures. In South Korea this substance was utilized for the formulation of the preparation, which is a wettable powder (4.6 WP) (UDDIN *et al.*, 2014).

Among the traditionally depicted allelochemicals there are not only the compounds, containing the aromatic cycles, but along with them there are also alicyclic substances. The latter may be classified into several groups. The non-terpenic structure resides in coffein (ASHIHARA *et al.*, 2008), camphor, carveol, and menthol (LOI *et al.*, 2008), myrigalone A (additionally to its aromatic residue) (POPOVICI *et al.*, 2011), as well as in momilactones A and B, which are common for the roots of rice *Oryza sativa* L. (KATO-NOGUCHI, 2011; XU *et al.*, 2012). These are the agents form the first group. Monoterpenes (e.g. 1,8-cineol), diterpenes (labdane- and casbane-type (SHMELZ *et al.*, 2014)), and sesquiterpenes (eremorhilane-, eudesmane- (GARSIA *et al.*, 2003), and β-macrocarpene-derived (SHMELZ *et al.*, 2014)) can be believed the following three groups of the alicyclic allelochemicals. It is supposed that the metabolites, listed in the second and third groups, could act as phytoalexins.

The great number of the allelochemicals is presented by the nitrogen-containing organic substances. Non-polymeric ones include 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one (DIBOA), 2(3H)-benzoxazolinone (BOA), and 2,2'-oxo-1,1'-azobenzene (AZOB), which were found in rye *Secale cereale* L. (CHASE *et al.*, 1991). 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) was discovered to support the defence of the live organisms against aluminium (POSCHENRIEDER *et al.*, 2005). The polymeric nitrogen-containing compounds include reported about the number of proteins, particularly containing in the plant latex: CpLP from Sodom apple *Calotropis procera* (Aiton) R.Br, PrLP from templetree (Pagoda tree; frangipani) *Plumeria rubra* L., P1G10 from mountain papaya *Carica candamarcensis* Hook.f., and also EtLP from aveloz *Euphorbia tirucalli* L. Their antifungal activity was compared with the same property of cysteine proteinase CMS2MS2 from *Carica candamarcensis*, papain (EC 3.4.22.2) and cysteine proteinase from papaya *Carica papaya* L., trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1), two serine proteases (SOUZA *et al.*, 2011). The similar action against plant pathogenic organisms was described for osmotin- and thaumatin-like proteins from *Calotropis procera* latex (DE FREITAS *et al.*, 2011).

Biotic sources of allelochemicals

It is reasonable to consider the sources of allelochemicals, which are mentioned in the publications. First of all it needs to be said, that the allelochimicals mean not only the metabolites themselves, but also the diverse preparations, which are applied for the artificial simulation of the physiological effects, taking place in the interspecific relationships.

Wishing to present better the knowledge about the allelochemicals' donors in this article, we divided them in following way: firstly, into crops and uncultivated plants, secondly, into monocotyledons, dicotyledons and the plants of other taxonomic groups.

The most investigated allelopathic donors among the monocotyledonous crops have been *Secale cereale* (CHASE *et al.*, 1991), corn (CASTKA & VANCURA, 1980; POSCHENRIEDER *et al.*, 2005), barley *Hordeum vulgare* (LANOUE *et al.*, 2010a; 2010b), *Oryza sativa* (KATO-NOGUCHI, 2011; XU *et al.*, 2012), and also several vegetable crops, such as garlic *Allium sativum* L. (cv. Caijiapo та Cangshan) (ZHOU *et al.*, 2011), *Allium cepa* group Aggregatum (VU *et al.*, 2012), Chinese anion *Allium chinense* G.Don (YANG *et al.*, 2013), which excrete biological active substances through the root system.

The non-pathogenic strain *Fusarium oxysporum* Fo162 endophytically colonizes the bananas *Musa* × *paradisiaca* L. It evokes the plants' the system responds. Particularly the chemical content of the root exudates alters (KURTZ & SCHOUTEN, 2009).

Momilactones A and B have previously been reported to be found out in *Oryza sativa* and moss *Hypnum plumaeforme* Wilson only. Meanwhile these specific metabolites have been known to act as the allelochemicals and the phytoalexins. H. KATO-NOGUCHI (2011) proposed to spread taxonomic screening of the plant diversity for presence of the momilactones and for analysis of their functional role. The flora of the Earth is well-known to be not restricted by two abovementioned species. The author dwelt on the analysis of the evolutional processes in the plant world by means of the mentioned biosynthesis products.

The dicotyledonous crops were studied as well. It was tested the potential of the green manure with rapeseed for the nematode control (HALBRENDT, 1996) and the herbicidal abilities of the sorghum root exudates (UDDIN *et al.*, 2014). The new possibilities of the natural control of pests and pathogenic fungi were discovered concerning chili pepper *Capsicum frutescens* L., *Ipomoea batatas*, and the residues of the semi-tropical leguminous plants (DUKE *et al.*, 2003). The anti-fungal properties of *Lens culinaris* (CASTKA & VANCURA, 1980), *Lycopersicon esculentum* (ZHANG *et al.*, 2008) (particularly under fungal microsymbiont colonization (SCHEFFKECHT *et al.*, 2006)), ginseng *Panax ginseng* C.A.Meyer (YOUSEF & BERNARDS, 2006), the germinating seeds and the roots of the seedlings of apricot *Prunus armeniaca* L. (AFIFI, 1977), the root exudates of *Lycopersicon esculentum* (KRAVCHENKO *et al.*, 2003), soybean *Glycine max* Moench (HAN *et al.*, 2005), seven year melon *Cucurbita ficifolia* Bouche (HUANG *et al.*, 2007) were found out. Also the natural mechanisms of the chemical defense and allelopathic properties of some species of *Camellia* L., *Coffea* L., *Theobroma* L., and *Ilex* L. genera were described (ASHIHARA *et al.*, 2008). The biochemical distinctions of genetically modified (GM) insect-resistant cotton *Gosypium hirsutum* L. comparing to the parental lines were characterized. In the both cases the similar types of substances were revealed, but the unsimilar content of the metabolites in separate types and the unsimilar quantitative ratios between the different types of compounds were observed. E.g., the type and content of the fatty acids and esters were significantly reduced, whereas some alkanes were increased in the root exudates of the GM *Gosypium hirsutum*. By the way some specific components were revealed among these esters (LI *et al.*, 2009; 2013a).

It was assumed, that the specifics in the content of the root exudates of the susceptible (Ganhua-5) and mid-resistant (Quanhua-7) cultivars of peanut *Arachis hypogaea* L. took part in the formation of the

mechanisms of the plant resistance for infection with plant pathogens from the rhizosphere soil (LI *et al.*, 2013b).

LING *et al.* (2013) revealed the discrepancy in the content of root exudates of watermelon *Cucurbita citrullus* L., grafting onto bottle gourd *Lagenaria siceraria* (Molina) Standl., comparing with the own-watermelon and own-bottle gourd exudates. Salicylic acid was identified in all three root exudates, but chlorogenic, caffeic and cinnamic acids were abundant only in separate variant of exudates.

Speaking about the uncultivated plants, first of all the studies, conducted on the aquatic plants: on the marine flora (authors didn't define its specific content) (SIEG & KUBANEK, 2013); on the fresh-water macophytes (namely on the exudates of yellow water-lily *Nuphar lutea* (L.) SM.) (SÜTFELD *et al.*, 1996); on water lettuce *Pistia stratioles* L. (WU *et al.*, 2013) as well as on fresh-water and marine cyanobacteria (BERRY *et al.*, 2008) must be mentioned.

Meanwhile the vast range of the cases of the allelopathy, displayed by the terrestrial uncultivated plants, was studied.

Studying allelopathic properties of neem *Azadirachta indica* A.Juss, the researchers obtained azadirachtin. To this end they used the alcohol extraction of the seeds from this plant. The researchers (GOPAL *et al.*, 2007) observed the series of the indices of the functioning of the micro- and macroflora, related with plants and soil, under impact of the granulated 10 % azadirachtin. The other works pertained the testing of the properties of the laticifer plants (*Calotropis procera*, *Plumeria rubra*, *Carica candamarcensis*, *Euphorbia tirucalli*) to control plant pathogens as well as the possibilities of the fruit and leaves of *Myrica gale* to control weeds (POPOVICI *et al.*, 2011).

Whitetop weed *Parthenium hysterophorus* L. drastically reduces harvest of crops in America, Asia, Africa, and Australia. However PATEL (2011) described the range of the benefits from the utilization of this species. The plant may serve for the removal of heavy metals and dyes from the environment, the eradication of aquatic weeds, the usage as substrate for commercial enzyme production, as additives in cattle manure for biogas production, as biopesticide, as green manure and compost. The compounds, responsible for hazardous properties, were reported to be present in this species.

A number of researches investigate the anti-fungal potential of the plants. Contrastingly some of the studies focused on the herbicidal activity of the fungi: *Streptomyces hygroscopicus* and *S. viridochromogenes* (BARAZANI & FRIEDMAN, 2001).

Target objects for practical application of allelochemicals

The sense of allelopathy can be formulated as one of the forms of the interactions between the organisms, mediated by means of substances, commonly called chemical interactions. Allelopathy is distinguished from the inter-organism interactions by the participation of plants. The latest can act as donors, acceptors, sometimes even as modifiers of BAS. This opinion makes us consider the targets and the effects of the action of the allelochemicals to be one of the most important issues for the applied studies in allelopathy in the last part of the article.

For the purposes of discussion these targets can be divided into plants and other representatives of biota: microbiota, mycobiota and animals (mostly invertebrates). There are the favorable and antagonistic, the beneficial and deleterious organisms among these objects. The pathogenic fungi occupy the lion part of the unfavorable organisms.

For example, the Agricultural Research Service of the USDA set the goal of searching the molecular mechanisms of the action of the insecticides and the fungicides, which are obtained from the natural sources (DUKE *et al.*, 2003). In this research authors revealed several novel molecular sites for the action of the tested substances. The sites responded the asparagine synthetase and the fructose-1,6-bisphosphate aldolase. The genes for polyketide synthases involved in production of pesticide polyketide

compounds in fungi were discovered. It may serve as the new clue for the pesticide application.

The novel way of the utilization of bacterial BAS to control algae, noxious plants and pests, particularly in the aquatic environments, was proposed in the similar, but later work (BERRY *et al.*, 2008).

Higher flora as targets

Substances with the allelopathic properties act on the vast range of the higher plants, as it follows from the investigations. Some of them were proposed to be used as the model objects. Particularly we have already mentioned *Canavalia ensiformis* as one of the models (SANTOS *et al.*, 2010). The seeds of lettuce *Lactuca sativa* L. cv Blackseeded Simpson and barnyardgrass *Echinochloa crus-galli* (L.) Beauv cv Kudiraivali were ascertained to serve as indicators in the studying of the physiological properties of momilactone (XU *et al.*, 2012). At the same time the allelopathic properties of the mature plant: *Allium chinense* were indicated with *Cucumis sativa* (YANG *et al.*, 2013). The latest study treated the stimulation of test seedlings by the root exudates of donor plants.

Cress *Lepidium sativum* L., *Echinochloa crus-galli*, cucumber *Cucumis sativus* L. and snap bean *Phaseolus vulgaris* L. were used as indicators in the examinations of the action of the hydroxamic allelochemicals on the test plants. AZOB, BOA and DIBOA were all applied singly at 50, 100, and 200 ppm and in two- and three-way combinations, where the total concentrations reached 200 ppm. The treatment with 100 and 200 ppm AZOB inhibited plants worse (38-49 %) comparing with the treatment with DIBOA. The three-way combination, contained 100 ppm AZOB + 50 ppm BOA + 50 ppm DIBOA, depressed plants more considerably (54-90%), than BOA+DIBOA mixture. Under the examination of the joint action of the hydroxamic acids allelochemicals on the germination and growth barnyard grass plants exhibited the slight antagonistic response, cress – the synergetic ones, while snap beans and cucumber – the both types of the response (CHASE *et al.*, 1991).

Two species from the Cryptophytaceae completely eliminate the presence of resorcinol in the aquatic ecosystems with the concomitant increase of the size of starch granule enclosures. Futhermore, this substance depresses the model object, which belongs to animals – daphnia. However, SÜTFELD *et al.* (1996) observed no effects on Cyanophyceae and Chlorophyceae.

BARAZANI & FRIEDMAN (2001) demonstrated that sometimes the higher plants under the allelopathic influence of bacteria exhibited the distinctions even on the levels of subspecies and cultivar. This was proved on *Lactuca sativa* and wheat.

LOI *et al.* (2008) showed the fact of the uptake of the BAS by common radweed *Artemisia annuifolia* L., *Lycopersicon esculentum*, and purslane *Portulaca oleracea* L. These scholars emphasize on the pivotal importance of this phenomena in the allelopathical studies. There is a lack of methods to measure allelochemical uptake in the natural conditions. So the idea about the activity of these substances may be judged on the basis of their observed toxicity. The authors stated that the elaboration of the solid-phase microextraction (see above: the part 'Novel methodological achievements in studies with allelochemicals') provided the new possibilities for the extraction for the observation on the transport of compounds inside the target plants.

Myrigalone A is considered to be effective environmental unthreatening herbicide with the potential to mitigate the *Fallopia* × *bohemica* invasion (POPOVICI *et al.*, 2011).

The treatment of the lettuce seeds with the root extractions of *Allium sativum* in 10- and 5-fold dilutions improved the germination and the growth of the lettuce. Vice versa, these processes were suppressed by the higher (40% and 60%) concentrations of the extractions, especially obtained from cv. Caijapo. Thus, the lettuce exhibited the two-phase respond, depending on the concentration of the allelochemical (ZHOU *et al.*, 2011).

The broadleaf weeds were more susceptible than grass species to sorgoleone according with the seed germination and shoot growth. At the concentration 0.2 g/l (a.i.) sorgoleone the inhibition of the germination and the growth of the broadleaf species were achieved in a growth chamber study reached 100%. The growth of the weeds was supressed 20-25 % greater under the post-emergence application of sorgoleone comparing to the pre-emergence one in the greenhouse experiments. The developed preparation inhibited the growth more than 90 % of the analysed broadleaf species in all experimental conditions. Japanese sorrel *Rumex japonicus* Houtt. as well as Chinese plantain *Plantago asiatica* L. were completely suppressed under 0.4 kg(a.i.)/ha sorgoleone. The crops proved to be less susceptible to sorgoleone even under 0.4 kg(a.i.)/ha, that was the highest of the tested dose, exhibiting only 30 % diminishing of the growth. Thus, the impact of wettable powder (4,6WP) of sorgoleone on the weeds specifically distincted from the same impact on the crops (UDDIN *et al.*, 2014).

Unicellular organisms and multi-cellular fungi as targets

The Canadian scientists focused on the range of the allelopathic effects of *Panax ginseng* on the soil microbiota (YOUSEF & BERNARDS, 2006). The article of BERRY *et al.* (2008) was devoted to the influence of the BAS from cyanobacteria on the microorganisms and the water macrophytes.

GOPAL *et al.* (2007) quantified the amount of the various representatives of microbocenosis, including fungi, as well as the dehydrogenase, phosphatase activity and respiration of the soil under the impact of azadirachtin. The listed indices were estimated fifthly: simultaneously and on the 15th, 30th, 60th and 90th day after the treatment of the sandy loam soil, collected from the field, with azadirachtin. It was found that the preparation inhibited actinomycetes, fungi and bacteria, including nitrifiers, and the intensity of the soil respiration, but didn't acted on *Azotobacter* sp. The listed observations were the most severe in the first 15 days. The populations of bacteria, actinomycetes, the respiration, and the phosphatase activity were recovered after the 60th day, in contrast to the fungi and the nitrifiers. Very high biocidal effects were achieved at the two- and five-times doses of the tested substance.

The anti-algal effect of water lettuce root exudations on the algae *Microcystis aeruginosa* and the excretion of microcystin-LR by the cells of this alga were registered. The lowest concentration of this allelochemical, enabling the strongest anti-algal half-effect, equaled 65 mg/l. The allelochemicals, extracted with the solvent – ethyl acetate, exhibited the strongest inhibitory effect on the growth of algae when used within a dose range of 60-100 mg/l. The relative inhibitory ratio reached 50-90%, the reduction of chlorophyll a content – 50-70%. However the intensity of the microcystin-LR excretion into the environment left stable (WU *et al.*, 2013).

The root exudates of *Allium cepa* increased the population and improved some species of bacteria, actinomycetes, and improved the structure of their communities in the rhizosphere soils, on the one hand, but decreased the fungal community, including the plant pathogen *Fusarium* sp., on the other hand. These effects were most significantly caused by onion cv. L-06 with the high allelopathic activity (YANG *et al.*, 2013).

The root exudates of *Lycopersicon esculentum* influence on the growth and anti-fungal activity of the pseudomonade strains, stimulating the plant growth. KRAVCHENKO *et al.* (2003) believed saccharides and organic acids to enhance this effect.

We find it reasonable to dwell on the numerous researches, which study how the plant metabolites of the various origin and chemical nature influence on the plant pathogenic fungi. First of all the *Fusarium* sp. are to be mentioned (e. g. VU *et al.*, 2012).

Some plant metabolites of the germinating seeds and the roots of the apricot became known to provide the mechanism of the virulence of *F. solani*

through the enhancing the mycelium growth and the conidia germination of the plant pathogen (AFIFI, 1977). Likewise the volatile exudates from the lentil cultivar, which is susceptible to the series of the agent of the root diseases, promoted the germination of the *Botrytis cinerea, Mucor racemosus, Trichoderma viride, Virticillium dahliae*, and especially *Fusarium oxysporum* spores. However the analogous exudates of the less susceptible cultivar restrain this process (CASTKA & VANCURA, 1980).

The continuous cropping of *Glycine max* leads to the accumulation of the infectious agents in soil. The laboratory experiments, the sand and water cultures were used to investigate this problem. The promotion of the growth of *F. oxysporum*, *Gliocladium roseum*, and especially *F. semitectum* were registered under the continuous cropping, but not under the control variant – the rotation system. The root exudates in lower concentrations stimulated the growth rather than in higher ones. Moreover, the continuous *Glycine max* cropping exhibited this effect stronger comparing with the control variant. The significant and the especially significant levels of the suppression of the three above-mentioned pathogens were reached under the high concentration of phthalic acid and propanedioic acids. *F. semitectum* exhibited the greatest decline. Nevertheless the low concentration of these acids led to the considerable stimulations of all three pathogens (JU *et al.*, 2002).

Gladiolus communis cv. White Prosperity is more resistant to *F. oxysporum* f. sp. *gladioli* than susceptible cv. Spic Span. Probably the increased content of the aromatic compounds within the root exudates induces the exhibition of anti-fungal properties of gladiolus cv. White Prosperity (TADDEI *et al.*, 2002).

The extract from the propolis inhibit *F. oxysporum* f. sp. *meloni* stronger comparing with *Alternaria alternata* (OZCAN *et al.*, 2004).

HAN *et al.* (2005) reported about the following distinctions, which were exhibited by plant pathogens, tested for the interaction with *Glycine max* roots (Table 1).

Table 1. Effects of the different fractions of root exudation on the plant pathogens

Infestant	Fraction of root exudations and genotype of *Glycine max*			
	water-soluble carbohydrates	water-soluble amino acids		organic acids
	both genotypes	9536	Jilin 30	both genotypes
Fusarium oxysporum	stimulation at low and suppression at high concentrations	significant suppression at mid and high con-centrations	mostly stimulation at mid and high con-centrations	significant suppression (concentrations aren't pointed)
Fusarium semitectum				
Gliocladium roseum	no effect			

Consequently the various genotypes of *Glycine max* can have different potential to suppress the agents of root rots.

The properties of the arbuscular mycorrhiza are suggested to be inconstant. The increment of the level of root colonization with symbiotic fungus results in the promotion of microconidia germination. The investigation of the mutualistic pair of *Lycopersicon esculentum*–*Glomus mossea* testifies that the exudates of mycorrhiza facilitated the germination of the *F. oxysporum* f. sp. *lycopersici* conidia. The tests with the different phosphorus levels allow concluding, that the improving of the phosphorus status of plants doesn't cause the enhancing of microconidia germination (SCHEFFKNECHT *et al.*, 2006).

The volatiles, emitted from *Lycopersicon esculentum*, decrease their inhibitory efficiency on the spore germination and mycelia growth of *F. oxysporum* and *Botrytis cinerea* in the following order: plants in anthesis stage>10-leaf>2-leaf plants. It was noted that the first above-mentioned pathogen was more susceptible to the

exudates of *Lycopersicon esculentum*, than the second one (ZHANG *et al.*, 2008).

Exogenously supplied ferulic acid induced premature cessation of root growth, with disintegration of the root cap, compression of cells in the quiescent centre, increase of the vascular cylinder diameter, earlier lignification of the metaxylem, as well as the reduction of the activity of cinnamyl alcohol dehydrogenase (EC 1.1.1.195) with the concomitant increased peroxide level, activity of the anionic isoform of peroxidase (EC 1.11.1.7) and lignin content in roots. Piperonylic (heliotropic) acid (an inhibitor of the cinnamate 4-hydroxylase), being applied jointly with the feruloyl-CoA, promoted the lignin content (DOS SANTOS *et al.*, 2008).

Benzoic acid stimulates some factors of the virulence of *F. oxysporum* f. sp. *niveum*, although it suppresses the growth of mycelium (83-96 %), the sporulation, and the conidia germination under the application in 0.2 ‰ concentration (WU *et al.*, 2009).

Gosypium hirsutum can acquire the chemical properties, which enhance its resistance to *F. oxysporum* due to the genetic modification (LI *et al.*, 2009; 2013a).

The inoculation of barley *Hordeum vulgare* with *F. graminearum* led to the significant increase of the pools of *trans*-cinnamic, *p*-coumaric, ferulic, syringic and vanilic acids in the root exudates within 2 days. The biosynthesis of *trans*-cinnamic acid occurred simultaneously. The biochemical alternations, caused by the inoculation, inhibited the germination of the *F. graminearum* macroconidia. The exudation of this metabolite from the roots accompanied with its accumulation in roots. The natural mechanism of the barley resistance to plant pathogens may be the result of the de novo biosynthesis and exudation of the substances with anti-pathogenic properties (LANOUE *et al.*, 2010a). It was supposed that wild or less domesticated varieties exhibited the described mechanism more strongly, than the domesticated cultivars (LANOUE *et al.*, 2010b).

Gallic acid has been known as allelochemical and its application at 0.08 % concentration (1250-fold dilution) inhibited the growth and the conidia germination of *F. oxysporum* f. sp. *niveum* on 9.5 % and 52.3 % respectively. This treatment furthered the sporulation and the synthesis of mycotoxin by this fungus. The gradual increment of gallic acid level was accompanied with the alternations of pectinase and protease activities. The both activities initially rose and then declined. Thus gallic acid has potential for the usage as environment friendly remedy, which enables to control the mentioned plant pathogens (WU *et al.*, 2010).

The proteins from the plant latex can suppress *F. solani*. IC_{50} for CpLP was quantified as 2.07×10^{-2} ‰ and for P1G10 – 2.53×10^{-2} ‰. Both the thermal inactivation and the proteolysis eliminated this suppression, suggesting, that these are proteins, which respond for the inhibition. The reducing agent dithiothreitol improved the antifungal properties of CpLP and P1G10. However, the pre-treatment with iodoacetamide drastically reduced endogenous proteolytic activities and partially abrogated antifungal activity. The antifungal property could be directly regulated by the proteases, presenting in plant latex, because the vast range of the purified enzymes from the respective plants adversely influenced on the spores. The numerous proteases induced the effects, which were quite similar to those, observed for CpLP and P1G10. Papain, CpLP and CMS2MS2 enhanced the ROS generation and consequently oxidative stress in the spores of *F. solani*. The latex proteins seemed to be one of the factors of plant defense from the fungal disease (SOUZA *et al.*, 2011). Likewise DE FREITAS *et al.* (2011) quantified IC_{50} for osmotin (the protein, purified from latex) as 6.7×10^{-2} ‰ regarding *F. solani*, 3.21×10^{-2} ‰ regarding *Colleotrichum gloesporoides*, and 5.75×10^{-2} ‰ regarding *Neurispora* sp.

The suppression of the spore germination and the mycelial growth of *F. oxysporum* directly depended on the concentration of the root exudates of *Allium sativum* with different resistance for the plant pathogen. *F. oxysporum* f. sp. *cucumerinum* exhibited greater susceptibility

for the impact of these preparation, than *F. oxysporum* f. sp. *niveum* (ZHOU *et al*., 2011).

Caffeic and chlorogenic acids were suggested to enhance the wilt-resistance of watermelon onto bottle gourd rootstock. The inverse dependence of the spore germination and the conidial growth intensities on the doses of the both phenolic acids was determined with stronger inhibitory effect, was observed for chlorogenic acid (LING *et al*., 2013).

The processes of spore germination, sporulation, mycelia growth of such soil-borne plant pathogens as *F. oxysporum* and *F. solani* directly depend on the concentration of the root exudates from two cultivars of *Arachis hypogaea* L. One of them is susceptible and the other is mid-resistant for the wilt (LI *et al*., 2013b).

Animals as targets

The possibility to control the population of American dagger nematode *Xiphinema americanum sensu lato* by means of the green manuring was discovered due to the experiments in temperate orchards (HALBRENDT, 1996).

Sesquiterpenes are often considered as the allelochemicals. Their administration to red flour beetle *Tribolium castaneum* larvae evoked the morphological, chemical alternations and great lengthening in the duration of the pupal stage (GARSIA *et al*., 2003).

The plant nematodes slighter colonize the bananas roots under the symbiosis with one of the non-pathogenic strains of *F. oxysporum*. KURTZ & SHOUTEN (2009) ascertain that the chemical content of the extractions slightly modifies the inoculation of the bananas with this strain, excepting few compounds.

The relationship between the presence of the allelochemicals in plants and the behavior of their herbivores was critically reviewed (GLINWOOD *et al*., 2011).

The shredding and the chemical degradation of the detritus by animals and microorganisms, which belong to the detritivores, can be avoided due to the presence of the allelochemicals. Currently this phenomenon is considered to be a mechanism for the chemical defence of the marine flora. It results in the delaying the time before the plant matter enters the microbial loop (SIEG & KUBANEK, 2013).

Conclusion

The recent research trends reflect the present state of the allelopathy. Today this subject field adopts the approaches and the methods from the diverse branches of science extensively, increases the diversity of the examined objects (both in the taxonomical and ecological senses), as well as involves additional hierarchical levels for the observation of the allelopathic phenomena. These all don't only promote a better cognitive understanding, but also serve for the application of allelochemicals in the human practical activity. Particularly the results of the modern allelopathic studies meet the demands of crop protection, including the withstanding the pathogens – this can be concluded on the basis of the reviewed sources. It is arguable to predict, that the enhancement of the management of the wide spectrum of the natural phenomena will get better. It is important for the durable relationships between the human and the biosphere.

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Synopsis

Fish in Ecotoxicological Studies

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Abstract. Water contamination (heavy metals, pesticides, POPs, etc.) is a serious environmental issue which has been raising lots of attention in the last decades because it can destroy aquatic ecosystems and hence, reduce biodiversity. In the field of ecotoxicology it is of main interest to investigate what the effects of organic and inorganic toxicants on different biological organization (cell, tissue, organism, population) are. Thus, many authors use different test organisms and particularly, fish. In the current study we aimed to present collected data from the last years which describe why fish is an appropriate species in terms of ecotoxicological research.

Key words: fish, water contamination, ecotoxicology, toxic effects, biological organization.

Introduction

Water pollution is the burning issue nowadays all over the world. Aquatic ecosystems are frequently contaminated with different toxicants through anthropogenic activities, and some of them such as metals may be naturally present and essential in low but toxic in higher concentrations (AJMAL *et al.*, 1988; EKPO & IBOK, 1999; PANDEY *et al.*, 2008; SEKABIRA *et al.*, 2010; CARASSCO *et al.*, 2011; LUSHCHAK, 2011; ONDARZA *et al.*, 2012; PEREIRA *et al.*, 2013; JÖRUNDSDÓTTIR *et al.*, 2014).

Since not all chemical forms of pollutants are equally bioavailable and some pollutants can be accumulated in living organisms to a greater extent than others, we need to study the levels of pollutants in the organisms to be able to predict the environmental risk (RAINBOW & PHILLIPS, 1993; CONNELL *et al.*, 1999). Thus, chemical analyses of the tissues of aquatic organisms are used as a routine approach in studies of aquatic pollution, providing a temporal integration of the levels of pollutants with biological relevance at higher concentrations than those present in water or sediment, and facilitating their quantification (RAINBOW & PHILLIPS, 1993).

In ecotoxicology, fish have become the major vertebrate model, and a tremendous body of information has been accumulated (STEINBERG *et al.*, 1995; BRAUNBECK *et al.*, 1998; MOISEENKO, 2005; RAISUDDIN & LEE, 2008; SCARDI *et al.*, 2008; HERMOSO *et al.*, 2010; SOUZA *et al.*, 2013; MURTHY *et al.*, 2013; CZÉDLI *et al.*, 2014). Fish are among the group of aquatic organisms which represent the largest and most diverse group of vertebrates. A number of characteristics make them excellent experimental models for toxicological research, especially for the contaminants which are likely to exert their impact on aquatic systems (LAW, 2003; DE LA TORRE *et al.*, 2010). Thus, according to DE LA TORRE *et al.* (2005) monitoring sentinel fish species is widely used to assess the degree of

accumulation of pollutants and the effects on health status. Furthermore, toxicant accumulation in water suggests that fish may serve as useful indicators for contamination in aquatic systems because they respond with a greater sensitivity to changes in the aquatic environment than invertebrates and tend to accumulate some poisons often in concentrations several times higher than in the ambient media (PAPAGIANNIS *et al.*, 2004; HAS-SCHÖN *et al.*, 2006; VELCHEVA, 2006; MOISEENKO *et al.*, 2008; HUANG *et al.*, 2013; EAGLES-SMITH & ACKERMAN, 2014; DHANAKUMAR *et al.*, 2015; ZHAO *et al.*, 2015). In this sense, bioaccumulation is a process in which a toxicant is absorbed in a fish organism by all routes as it occurs in the natural environment, i.e., dietary and ambient environment sources (ARNOT & GOBAS, 2006). Hence, bioaccumulation occurs primarily due to the inability to excrete necessary levels of contaminants and its degree is the result of imbalance between the input rate and the rate of toxicant elimination. Under certain environmental conditions (e.g., season, water temperature, pH, hardness, and river flow) and biotic factors (e.g., fish species, age, tissue, organism life-history traits) toxicants can accumulate to toxic concentrations and cause ecological damage (URAL *et al.*, 2012; ANTAL *et al.*, 2013; CHAHID *et al.*, 2014).

Fish have also been found to be good indicators of water contamination in aquatic systems because they occupy different trophic levels; they are of different sizes and ages and in comparison with invertebrates, are also more sensitive to many toxicants (DALLINGER *et al.*, 1987; BARAK & MASON, 1990; BURGER *et al.*, 2002). They are preferred in toxicological research because of their well-developed osmoregulatory, endocrine, nervous, and immune systems (SONG *et al.*, 2012). In addition, fish may absorb toxicants directly from the surrounding water and sediments (waterborne exposure), or ingest them through contaminated food in the food chain (dietary exposure), enabling the assessment of pollutant transfer through the tropic web (PÉREZ CID *et al.*, 2001; FISK *et al.*, 2001; MOISEENKO & KUDRYAVTSEVA, 2001; RASHED, 2001b; MONDON *et al.*, 2001; MANSOUR & SIDKY, 2002; USERO *et al.*, 2004; MENDIL & ULUÖZLÜ, 2007; ÖZTÜRK *et al.* 2009; SOUNDERAJAN *et al.*, 2010; ROWAN, 2013). In general, as RAYMENT & BARRY (2000) state fish have been popular targets of monitoring programs in marine environments because sampling, sample preparation and chemical analysis are usually simpler, more rapid and less expensive than alternative choices such as water and sediments. Last but not least, fish are the final chain of aquatic food web and an important food source for human. Therefore, some toxicants in aquatic environments can be transferred through food chain into humans (UYSAL *et al.*, 2008; 2009; METIAN *et al.*, 2013). Water pollution leads to contamination of fish which may pose a health risk (KARADEDE *et al.*, 2004; MENDIL *et al.*, 2005; ULUOZLU *et al.*, 2007; TÜRKMEN *et al.*, 2008; MENDIL *et al.*, 2010). Thus, in view of the quality of public food supplies, TÜRKMEN & CIMINLI (2007); YILMAZ *et al.* (2007b) and BILANDŽIĆ *et al.* (2011) suggest that toxicant levels in aquatic environment should be monitored regularly to check water quality and animal health.

Fish and biomarkers

The presence of xenobiotics in the marine environment exerts well-known biological effects on marine organisms. Occasionally, when properly evaluated in selected sentinel species, these effects may be considered as biomarkers, a sort of early warning signaling useful in assessing the quality of marine habitats (BAYNE *et al.*, 1985; CAJARAVILLE *et al.*, 2000; LAM & GRAY, 2003; MOORE *et al.*, 2004). Biomarkers have been proposed as sensitive tools for the early detection of environmental exposure to pollutants and their adverse effects on aquatic organisms (VAN DER OOST *et al.*, 2003; DE LA TORRE *et al.*, 2005). They also serve as links between the environmental contamination (cause) and its effects, providing unique information on the ecosystem health (MARIA *et al.*, 2009). In the past 25 years, numerous biomarkers have been developed with the objective to apply them for environmental biomonitoring (MCCARTHY & SHUGART, 1990; PEAKALL, 1994; SHUGART & THEODORAKIS, 1998;

SCHMITT *et al*., 2007; MEDGELA *et al*., 2006; MUÑOZ *et al*., 2015).

Toxicant effects can be studied at different levels of biological organization. BERNET *et al*. (1999), MONTEIRO *et al*. (2005), CAZENAVE *et al*. (2009) and MARCHAND *et al*. (2009) consider that the different changes in many biochemical and morphological parameters of fish may be used as successful biomarkers for toxic effects of xenobiotics. Initial effects of toxicant pollution may be evident only at cellular or tissue levels before significant changes are identified in fish behavior or external appearance. Histological alterations for example have been examined for decades in fish tissues and organs in order to assess the effects of pollutants (JOHNSON *et al*., 1993; STENTIFORD *et al*., 2003; AU, 2004). As an indicator of exposure to chemicals, histology represents a useful tool to assess the degree of pollution (PERRY & LAURENT, 1993). According to WESTER & CANTON (1991), histopathological changes have been widely used as biomarkers for the evaluation of the health of fish, exposed to contaminants in laboratory conditions. One of the important advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examining specific target organs. According to RABITTO *et al*. (2005) and OLIVEIRA RIBEIRO *et al*. (2006) the exposure to chemical contaminants can induce a number of lesions and injuries to different fish organs but the gills and liver represent important target organs suitable for histopathological examination in searching for damages to tissues and cells According to HINTON & LAUREN (1990) for field assessments, histopathology is often the easiest method of assessing both short and long-term toxic effects. On the other hand, WESTER & CANTON (1991) state that histology is relatively labor-intensive and requires some experience, but after all it has the considerable advantage that pathological alterations in different tissues (e.g., gills, liver) can be observed individually, thus creating a direct link with physiological functions such as growth, reproduction, respiration and nutrition.

Antioxidant enzymes are considered to be sensitive biomarkers, and they are important parameters for testing water quality and the negative effects of metals on fish (OLSVIK *et al*., 2005; HANSEN *et al*., 2006; VELMURUGAN *et al*., 2008; 2009; BANEE *et al*., 2011; YOUSAFZAI & SHAKOORI, 2011; GABRIEL *et al*., 2012). Therefore, various responses of enzymes have been also observed in fish exposed to metallic and persistent organic contaminants which indicate an increase or a decrease in the activity depending on the dose, species and route of exposure (WONG & WONG, 2000; LOPES *et al*., 2001; HARIKRISHNAN *et al*., 2003; CAO *et al*., 2010; KOENIG *et al*., 2012; LU *et al*., 2013). For example, the biochemical parameters in fish liver are sensitive for detecting potential adverse effects and relatively early events of pollutant damage (STENTIFORD *et al*., 2003). Changes in the activity of liver enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) serve as an indicator for a normal liver function, and they also can be used as biomarkers for tissue damage (ALMEIDA *et al*., 2002). Thus, it can be concluded that these enzymes are sensitive biomarkers for the determining stress in the fish subjected to various pollutants present in the waters (ADHIKARI *et al*., 2004).

Fish organs most commonly used in ecotoxicological studies

Fish can be exposed to toxicants via two exposure routes, waterborne: gills and derma and dietary (SLOMAN, 2007). Toxicants are taken up through different organs of the fish because of the affinity between them. In this process, many of them are concentrated at different levels in different organs of the fish body (RAO & PADMAJA, 2000). Therefore, in teleost fish, the gills, liver, kidney and muscles are the tissues most frequently utilized in ecological, toxicological and pathological studies (SAUER & WATABE, 1989; VELCHEVA, 2002; HEIER *et al*., 2009) because they are metabolically active tissues and accumulate toxicants at higher levels (ANDRES *et al*., 2000; KARADEDE & ÜNLÜ, 2000; MARCOVECCHIO, 2004). TERRA *et al*. (2008) consider that toxicants enter the body

mainly through the gills and consequently, with the blood they reach the parenchymal organs where they retain for a longer time. In addition, according to KROGLUND *et al.* (2008) toxicant concentrations, particularly in the gills reflect the toxicant concentrations in the water where the fish live; whereas, concentrations in other organs such as the liver and kidney represent storage of toxicants. According to JOVIČIĆ *et al.* (2014) studies of metal accumulation in fish are mainly focused on the muscle tissue, while the metal accumulation patterns in other tissues have been largely neglected. Elemental accumulation in many fish tissues and organs and their potential use in monitoring programs have not received proper attention. Therefore, the authors measured the metal concentrations in 14 tissues of the wels catfish (*Silurus glanis*) from the Danube River. Some of them are not very common but they also could provide valuable information in terms of ecotoxicology − muscle, gills, spleen, liver, kidneys, intestine, stomach, heart, brain, gallbladder, swim-bladder, vertebra, operculum, and gonads.

Gills

The fish gills are multifunctional organs involved in ion transport, gas exchange, acid–base regulation and waste excretion (DANG *et al.*, 2001). Given that the gills accounts for well over 50% of the surface area of a fish it is not surprising that one of the major target organs for waterborne toxicants is the gill (PLAYLE, 1998). The gills are regarded as the important site for direct uptake from the water, whereas the body surface is generally assumed to play a minor role in xenobiotics uptake of fish (POURANG, 1995). Thus, in teleost fish the gills are most frequently utilized in bioaccumulation studies and the pathological damage produced allows the toxicity of the environment to be defined, making fish highly suitable for evaluating the health of aquatic systems (MALLATT, 1985; OLIVEIRA RIBERIO *et al.*, 2000; OLSVIK *et al.*, 2000; MOISEENKO, 2005; OGUNDIRAN *et al.*, 2009). Fish metabolism, acting principally through the gills can be seriously damaged since toxicant incorporation occurs mainly through this respiratory organ (BERVOETS & BLUST 2003; SLOMAN 2007; TERRA *et al.*, 2008). Furthermore, the fish gills are very sensitive to physical and chemical alterations of the aquatic medium such as: temperature, acidification of the water supply due to acid rain, salts and heavy metals, and to any change in the composition of the environment which is an important indicator of waterborne toxicants (SABER, 2011). According to CARPENE & VAŠAK (1989); PERRY & LAURENT (1993); TKACHEVA *et al.* (2004) and ROSSELAND *et al.* (2007) the fish gills are the main route of penetration of toxicants into the fish organism, thus they are the first organs which come in contact with environmental pollutants, and are also sensitive subjects for identifying the effects of water toxicants on fish organisms. The fish gills can accumulate bioavailable pollutants, and their measurement on gills can reflect the speciation of pollutants, and in particular metals in water, therefore, they are a useful tool for assessing bioavailability of elements in water (HEIER *et al.*, 2009). Moreover, gill surface serves as metal-binding ligands and metal bioaccumulation in particular can occur due to positively charged metal species in the water to negatively charged sites on the gills (TEIEN *et al.*, 2006; TERRA *et al.*, 2008; PLAYLE *et al.*, 2011). ROSSELAND *et al.* (2007) state that the gills are considered to be important site for direct toxic effects to metals in high concentrations, for sub-lethal effects at lower metal concentrations, and, along with uptake from food, an important point of entry into the organism for both essential trace elements (Cu, Zn, Se, Fe) and non-essential elements (Al, As, Cd, Cr, Ni, Pb). There is also a close relationship between the gill morphological alterations and chemical induced stress. Thus, one of the methods that proved toxic effects is to study the morphology of gills (PETERS & HONG, 1985; OLOJO *et al.*, 2005; GEORGIEVA *et al.*, 2014). Histological changes in the gills are recognized as a valid and fast method to determine the damage caused in fish by the exposure to different pollutants (ARELLANO *et al.*, 1999). There are reports on various histological changes in gills under the effects

of different toxicants in water both in field and laboratory conditions (CENGIZ, 2006; FIGUEIREDO-FERNANDES *et al.*, 2007; FONTAÍNHAS-FERNANDES *et al.*, 2008; MOHAMED, 2009), but it is often difficult to decide whether morphological alterations are adaptive or destructive (TKACHEVA *et al.*, 2004).

Liver and kidney

Once the toxicants cross the biological barriers and enter the bloodstream, they will reach and accumulate in the internal organs of fish. Numerous studies have quantified contaminants in fish organs to evaluate environmental quality, seeking causal relationships with fish health, and, based on these, the liver is likely to be the best choice, followed by the kidney and gills (HANSON, 1997; BEGUM *et al.*, 2004; POKORSKA *et al.*, 2012; MAJNONI *et al.*, 2014). The liver is reported to be the primary organ for bioaccumulation and thus, has been extensively studied in regards to the toxic effects of xenobiotics (HINTON & LAURÉN, 1990; DE BOECK *et al.*, 2003; YILMAZ *et al.*, 2007a; VAN DYK *et al.*, 2007; SIMONATO *et al.*, 2008; MADUREIRA *et al.*, 2012; NUNES *et al.*, 2015). According to MOHAMED (2009) the liver is also a target organ due to its large blood supply which causes noticeable toxicant exposure. In addition, according to HINTON & LAURÉN (1990) it is a detoxification organ and it is essential for both, the metabolism and the excretion of toxic substances in the body. The vertebrate kidney is the main organ involved in the maintenance of body fluid homeostasis. The morphology and function of the kidney have been modified through evolution to fulfill different physiological requirement and the widest range of kidney types is found in fishes (HENTSCHEL & ELGER, 1989). In teleosts, the kidney, together with the gills and intestine, are responsible for excretion and the maintenance of the homeostasis of the body fluids (HINTON *et al.* 1992; OJEDA *et al.*, 2003) and, besides producing urine, act as an excretory route for the metabolites of a variety of xenobiotics to which the fish may be exposed (WHO, 1991; HINTON *et al.*, 1992; EISLER, 1998). The kidney also excretes other nitrogen-containing waste products from the metabolism such as ammonia and creatinine. In addition, in fish as in higher vertebrates, the kidney performs an important function related to electrolyte and water balance and the maintenance of a stable internal environment (CENGIZ, 2006). Thus, many studies showed that different toxicants accumulate mainly in metabolic organs such as the liver and kidney (KARADEDE *et al.*, 2004; OLIVEIRA RIBEIRO *et al.*, 2005; JABEEN *et al.*, 2011; YANCHEVA *et al.*, 2014a, b; DE JONGE *et al.*, 2015; VASEEM & BANERJEE, 2013) which can lead to many histological alterations (HINTON & COUCH, 1998; CENGIZ, 2006; POLEKSIĆ *et al.*, 2010; STENTIFORD *et al.*, 2003). Levels of heavy metals such as lead, copper, cadmium, and zinc in marine fish have been extensively documented in the primary literature (e.g., ROMÉO *et al.*, 1999; ZAUKE *et al.*, 1999; JUREŠA & BLANUŠA, 2003). These metals tend to distribute differentially between the liver and kidney and other organs, most likely because of metal-binding proteins such as metallothioneins in the metabolic organs (HAMILTON & MEHRLE, 1986; ROESIJADI, 1992; DE SMET *et al.*, 2001; ATLI & CANLI, 2003). These proteins bind copper (Cu), cadmium (Cd), and zinc (Zn), but not lead (Pb), allowing organs such as the liver to accumulate higher levels of metals than other organs such as muscle (PLOETZ *et al.*, 2007). FALFUSHYNSKA & STOLIAR (2009); SHINN *et al.* (2009); POLEKSIĆ *et al.* (2010); BARONE *et al.* (2013) and SISCAR *et al.* (2014) think that pollutant accumulation in the internal organs is associated not only with organ function such as haematopoiesis, antioxidant defense, detoxification, and excretion, but also with metallothionein synthesis which is directly related to the increase in some metal concentrations (MONTEIRO *et al.*, 2013; SISCAR *et al.*, 2014). Another reason for higher toxicant levels in the internal organs may be gastrointestinal route of exposure (SLOMAN, 2007), rendering the liver and the kidney additionally vulnerable to chronic toxicant exposure (OLSVIK *et al.*, 2000). Furthermore, according to OLSVIK *et al.* (2000) and SHARMA *et al.* (2009) liver and kidney are vulnerable organs during prolonged toxicant exposures, both

from waterborne and dietary sources. Livers for example are also examinated for histopathological alterations since several studies carried out in coastal waters have shown correlation between environmental contaminants and the occurrence of toxicopathic liver lesions in fish (VETHAAK & JOL, 1996; STENTIFORD *et al*., 2003; FEIST *et al*., 2004). In recent years, fish diseases and liver histopathological alterations have been used as indicators of pollution effects and have been implemented in monitoring programs (LANG, 2002; FEIST *et al*., 2004). The presence of inflammatory lesions, hepatocellular fibrillar inclusions, and preneoplastic and neoplastic lesions is higher in fish captured in polluted environments than in fish from reference sites (STENTIFORD *et al*., 2003). Overall, KARADEDE *et al*. (2004) state that the liver stores xenobiotics which eventually will be detoxicated and kidneys are involved in the process of excretion.

Muscles

The fish flesh (muscle) is a very important, valuable and recommended food in the human nutrition due to low content of fat and high content of proteins and mineral substances as well as optimal ratio of unsaturated fatty acids with cardio-protective effect. On the other hand, fish muscle may be the depositary for different contaminants, which occur in the water ecosystem (ANDREJI *et al*., 2012). Such environmental pollutants are dioxins and PCBs, heavy metals, and organochlorine pesticides are a global threat to food safety, thus muscles could lose these properties due to environmental contamination (BAJC *et al*., 2005). Hydrobionts can bioaccumulate many of these contaminants potentially making seafood of concern for chronic exposure to humans (NØSTBAKKEN *et al*., 2015). According to SVOBODOVA *et al*. (1996) the metal concentrations in the water are positively correlated with the concentrations in fish tissues, but WIDINARKO *et al*. (2000) state that the metal concentrations in the sediments are the most important factor for their levels in the aquatic biota. Consumption of fish contaminated with heavy metals have deleterious effects on human health which was widely acknowledged after a series of events in the period from 1953 to 1960 when several thousand people died in Minamata Bay in Japan as a result of poisoning caused by the consumption of mercury contaminated fish (HARADA, 1995). Among the metals, mercury (Hg) is of the most widespread concern in connection with fish consumption and advisories related to fish consumption are issued by health authorities in many countries (UNEP, 2002). Therefore, concern regarding the presence of this metal and other contaminants in seafood has arisen during the last decades (FRANCESCONI & LENANTON, 1992; OLIVEIRA RIBERIO *et al*., 2000; VAZQUEZ *et al*., 2001; HITES *et al*., 2004; COHEN *et al*., 2005; WILLETT, 2005; FORAN *et al*., 2006; MOZAFFARIAN & RIMM, 2006; USYDUS *et al*., 2009; IBRAHIM *et al*., 2011). In order to evaluate the risk to consumers, there is a continuous need for data on contaminant levels such as mercury in fish as highlighted by the European Food Safety Authority (EFSA, 2012). Even though, the observed toxic effects raised public safety and human health concerns repeatedly since Minamata, prompting legislators to set limits on the lead (Pb), cadmium (Cd) and mercury (Hg) levels detected in the fish muscle, but other heavy metals and fish tissues were not included in the European Union (EU) regulations as explained by JOVANOVIĆ *et al*. (2011).

Fish muscles are tissues which are important to analyze in terms of human health and this is the reason to be included in many monitoring and risk assessment programs (OLSSON *et al*., 1978; SCHMITT & BRUMBAUGH, 1990; DUŠEK *et al*., 2005; CHALMERS *et al*., 2010). However, according to RASHED (2001a); SHINN *et al*. (2009); POLEKSIĆ *et al*. (2010); VISNJIC-JEFTIC *et al*. (2010); JARIĆ *et al*. (2011) and TAWEEL *et al*. (2013) toxicant concentrations, particularly heavy metals are usually lower in the muscles than in the other studied organs and the muscles are not always a good indicator of the whole fish body contamination. This can be explained by the very fast rate of decontamination in this tissue. However, fish is considered as one of the main protein sources of food for humans and water contamination

consequently leads to contamination of fish (DURAL *et al.*, 2007; GONZÁLEZ-MILLE *et al.*, 2010; DE LA TORRE *et al.*, 2010; REJOMON *et al.*, 2010; ROSE *et al.*, 2015). That is why, in recent decades much attention has been paid to the investigation of xenobiotic concentrations in fish as a result of the increasing concern of food poisoning (NAGATA & OKA, 1996; SENTHILKUMAR *et al.*, 1999; HENRY *et al.*, 2004; SAPOZHNIKOVA *et al.*, 2005; ANDREJI *et al.*, 2006; CHEUNG *et al.*, 2007; TUZEN & SOYLAK, 2007; TÜRKMEN *et al.*, 2009; GUÉRIN *et al.*, 2011; MERCIAI *et al.*, 2014).

Table 1. Maximum permissible levels for some toxic elements in fish muscle according to international regulations and recommendations (mg kg^{-1}).

Regulation/Recommendation	Al	As	Cd	Cu	Cr	Ni	Pb	Zn	Hg	Tin
Norm 31 (Bulgaria)	30	1	0.05	10	0.3	0.5	0.2	50	0.5	-
EC (2008)	-	-	0.1	-	-	-	0.3	-	1	200
FAO/WHO(2011)	-	0.1	-	0.4	-	-	0.3	-	0.5-1	250
Food Safety Authority of Ireland (2009)	-	-	0.05	-	-	-	0.3	-	-	200
Australia/New Zealand Food Standards Code (2013)	-	2	-	-	-	-	0.5	-	0.5-1	250

**additional information regarding other non-metal and organic contaminants in fish can be found in the presented regulations/recommendations.*

Conclusions

Overall, on the basis of the studied literature we can conclude that fish are very good indicators for impaired water quality as they have different size, occupy different tropic levels and are long-living and mobile. Hence, they have been successfully applied in ecotoxicological research in the last few decades and many researchers prefer them instead of invertebrates. Depending on the main purposes of research, i.e. bioaccumulation, histological and biochemical analyses or other investigated biomarkers, different fish organs can be applied. The most commonly used are the respiratory organs - the gills and parenchymal organs – liver and kidney, but in terms of human health the most appropriate tissue are the muscles. However, we have to add that there other organs with important functions which can be more thoroughly studied such as the spleen or otoliths. Therefore, we suggest that further investigation should be carried out in this particular are in order to better understand the negative effects of pollution on the fish biology.

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Synopsis

Factors Affecting Mitigation of Methane Emission from Ruminants: Management Strategies

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Abstract. Nowadays, greenhouse gas emission which results in elevating global temperature is an important subject of worldwide ecological and environmental concern. Among greenhouse gases, methane is considered a potent greenhouse gas with 21 times more global warming potential than carbon dioxide. Worldwide, ruminant livestock produce about 80 million metric tons of methane each year, accounting for about 28% of global emissions from human related activities. Therefore it is impelling animal scientists to finding solutions to mitigate methane emission from ruminants. It seems that solutions can be discussed in four topics including: nutrition (feeding), biotechnology, microbiology and management strategies. We have already published the first review article on feeding strategies. In the current review, management strategies such as emphasizing on animals - type and individual variability, reducing livestock numbers, improving animal productivity and longevity as well as pasture management; that can be leads to decreasing methane production from ruminant animal production are discussed.

Key words: global warming, methane, enteric fermentation, ruminant, management.

Abbreviations: GHG - greenhouse gas; WSC - water soluble carbohydrate; MCR - methane conversion rate; F:C ratio - forage to concentrate ratio; CT - condensed tannins; DMI - dry matter intake; PEG - polyethylene glycol; VFA - volatile fatty acids; FA - fatty acid; bST - bovine somatotrophin; OSHF - Overseas Holstein; FCCC - framework convention on climate change.

Introduction

Climate change is a subject of global environmental concern. Increased anthropogenic Greenhouse Gas (GHG) emissions have increased the global temperature the last 100 to 200 years (MIRZAEI-AGHSAGHALI & MAHERI-SIS, 2011). Methane is considered a potent greenhouse with capability of trapping 21 times more heat (Global Warming Potential) than carbon dioxide also its life time in the atmosphere is 9-15 years and over the last two centuries, methane atmospheric concentrations have more than doubled arising 1% yearly in comparison with 0.5% of carbon dioxide. Worldwide, ruminant livestock produce about 80 million metric tons of methane each year (representing 11% sheep and goat), accounting for about 28% of global emissions from human related activities (MURO-REYES *et al.*, 2011; UMEGHALU & OKONKWO, 2012; SHRESTHA *et*

al., 2013). Under the Climate Change, the UK Government is legally required to reduce greenhouse gas (GHG) emissions across the UK economy by 80% of 1990 levels, by 2050. The agriculture sector is committed to playing its part in meeting this national goal and will need to demonstrate an 11% reduction on 2008 levels, by 2020. To support the industry's position and efforts, better data are required on the carbon footprint of milk production from dairy farms. Focusing on more efficient use of inputs will also help reduce costs of production, as well as enhance the environmental credentials of the dairy industry (DAIRY CO, 2012).

Ruminant animals (particularly cattle, buffalo, sheep, goat and camels) produce significant amount of methane under the anaerobic conditions of the digestive processes (SEJIAN *et al.,* 2011a; ASSAN, 2014; ASSAN, 2015). Methane produced during anaerobic fermentation in the rumen represents 2-12% gross energy loss and emission from livestock contributes approximately 15% of the total atmospheric methane flux (ZHI-HUA *et al.,* 2012; MAHESH *et al.,* 2013). CH_4 is considered a 'greenhouse gas' and emission of the global cattle population of 1-3 billion are estimated to be 58 million tonnes/year, or 73% of the emissions from all livestock species according to the US Environmental Protection Agency (1994) (TIEMANN *et al.,* 2008; KURIHARA *et al.,* 1999). As indicated before, dietary changes are a promising means to reduce CH_4 losses. Such changes may well affect the composition of the products (WAGHORN & WOODWARD, 2004).

With appropriate strategy and potential future technologies and management practices could reduce CH_4 emissions per unit of animal product by 25–75% (MOSIER *et al.,* 1998). However, except for the improved feeding management, the present technologies to control CH_4 emission from ruminants are seen with pessimism (JOHNSON *et al.,* 1996; SEJIAN *et al.,* 2011b).

Important manure management factors affecting CH_4 formation during storage are the dry matter (DM) content of manure and its storage duration, and also the ambient temperature (STEINFELD *et al.,* 2006; MIRZAEI-AGHSAGHALI & MAHERI-SIS, 2008).

This review looks more closely at the reasons for, and the consequences of, methane production from ruminant livestock which in turn is dependent on management strategies.

To discuss factors relation to emissions of GHG's (specific methane gas) from ruminants, we divided them in four groups, nutrition, management, biotechnology and microbiology. In this article, we will discuss factors relation management strategies and factors relation to biotechnology and microbiology will discuss in further article.

1. Animals - type and individual variability

The decrease in emissions through low CH_4 producing animals has been debated in the last few years. It has been established by several research groups that between-animal variability, at the same level of performance and using similar diets, is high.

Methane production from individual animals may vary over time, even when animals are fed a constant amount of the same quality feed each day. Within animal variation in absolute CH_4production from day-to-day in sheep and cattle has been reported to be approximately 7% (coefficient of variation, CV) when animals were fed a constant amount of consistent quality feed. One group of researchers reported that the CV for day-to-day variation in CH_4 production was approximately 27% whether animals were fed ad libitum or on a restricted diet (JOHANNES, 2008, New Zealand, pers. comm.).

Intensification of livestock production through better breeding and/or feeding to decrease global greenhouse gas emissions needs to be carefully assessed and will remain a hot debate in the foreseeable future (MARTIN *et al.,* 2010). Calorimetric studies have reported between-animal differences (CV) in daily CH_4 production of 7-8% and 11.7% when animals were fed a constant diet and 17.8% for lactating daily cattle fed ad libitum (GRAINGER *et al.,* 2007).

DADO & ALLEN (1994), investigate the variation in and relationships among

feeding, chewing, and drinking variables for lactating dairy cows. In this experiment twelve Holstein cows (63 DIM; 6 primiparous) were offered a common diet and monitored for 21 d (11 d of adaptation, 10 d of collection) with a data acquisition system to measure continuously feed and water intakes and chewing behavior and reported coefficients of variation across cows ranged from 5 to 41% for the variables studied as coefficient of variation in their eating time of 17% (mean 301 min/day), 16% for ruminating time (mean 457 min/day) and 24% for their water intake (mean 78 L /day) (DADO & ALLEN, 1994).

BLAXTER & CLAPPERTON (1965) reported a 7.2% CV for day-to-day variation based on 989 24-h determinations of CH_4 for sheep and cattle. They also reported a CV between animals of 5.0 to 7.5% for sheep given a fixed amount of feed. The CV between animals, however, appears to be larger in chamber studies when intake is not restricted (BLAXTER & CLAPPERTON, 1965).

Further, grazing animals may differ in the diet eaten by selectively grazing certain parts of the sward (BRAND, 2000). Salivation rates also differ, with typical quantities of saliva produced per day of 150 liters in cattle and 10 liters in sheep, although estimates vary from 38 to 190 L/day for non-lactating dairy cattle (JACQUES *et al.*, 1989). Saliva is essentially a bicarbonate-phosphate buffer with a pH around 8, and the large volumes secreted provide an aqueous medium for the rumen organisms and help to jeep the rumen contents at near neutrality (HOBSON & STEWART, 1997). Feeding rate, drinking rate, and quantity of produced will affect the time spent in the rumen of both fluid and particulate matter. The CH_4 yield was negatively correlated to the particulate with the quantity of rumen organic matter and rumen fill. The latter author reported that the rumen particulate outflow rate accounted for approximately 57 % of the between-sheep variation (JOHANNES, 2008, New Zealand, pers. comm.).

WAGHORN & WOODWARD (2004) showed mean methane production from four highest and four lowest producing sheep (selected from a random group of 20 animals) over a four month period was 3.75 vs. 5.15% of gross energy intake. Earlier reports found 86% of variation in methane production by sheep consuming 900-1700 g DM day-1 was due to animal variation and only 14% was attributable to diet.

Also, ULYATT *et al.* (2002a), investigate the effect of seasonal variation in methane emission from dairy cows and breeding ewes grazing ryegrass/white clover pasture in New Zealand and this experiment Daily methane emission from 12 Romney-cross-bred ewes and 10 lactating Friesian dairy cows, rotationally grazed on perennial ryegrass/white clover dominant pastures, was measured during four seasons of a year (September, November, March, and June/ July).the result of this experiment suggested that 71 – 95% of variation between days was attributable to animals even though intakes and composition of each diet were relatively constant (ULYATT *et al.*, 2002b).

The impact of genotype was highlighted in a trial involving New Zealand Friesian (NZHF) and Overseas Holstein (OSHF) cows fed either pasture or total mixed rations produced 8-11% less methane, as a percentage of gross energy (GE) intake, compared to New Zealand genotypes. The OSHF genotypes produced 8-11% less methane, as a percentage of gross energy (GE) intake, compared to New Zealand genotypes at both 60 and 150 days of lactation (ROBERTSON & WAGHORN, 2002).

WAGHORN & WOODWARD (2004) showed sheep with high CH_4 yields had larger rumen volumes, a slower particulate outflow rate, higher fibre digestibility and longer retention times than sheep with low CH_4 kg^{-1} DM intake. Methane yield was best predicted as a function of particulate fractional outflow rate, organic matter intake (g kg $LW^{-0.75}$) and molar proportion of butyrate (r^2 = 0.88). Differences between animals may be affected by salivation, feed comminution (or eating rate) as well as rumen pool size, turnover and outflow.

Trials conducted at the University of Manitoba suggest that as much as 27 % of the variation in CH_4 emission for cattle consuming forage diets is related to animal-

to-animal variation (BOADI & WITTENBERG, 2002). Work has not been done to determine whether these differences are related to intake behavior, or to potential anatomical and physiological differences in the gastrointestinal tract of cattle or the heritability of this trait. However, the degree of variability suggests that there is potential to select for low methane emitting animals.

Differences in intake explain only a part of the variability: in sheep consuming the same amount of DM, LASSEY *et al.* (1997) noted extreme daily CH_4 emissions of 14.6 and 23.8 g between animals (MARTIN *et al.*, 2010).

The factors responsible for animal-to-animal variation in CH_4 emission by ruminants fed fresh forages are scarce. In contrast to lactating cows, animals in this study (non-lactating, non-pregnant) lacked the feeding drive to maximize feed intake, and previous large differences in CH_4 yields were much reduced. Absolute emissions were strongly associated with feed intake (especially of digestible fiber) but the implication of salivation on animal differences warrants further investigation. The absence of anticipated differences between cows in CH_4 yield per unit of feed intake limited the establishment of relationships with rumen pool size and rumen digesta retention time observed previously with sheep. The data support previous conclusions that effects of animal-related factors are most apparent at high intake levels, for example during lactation. The ranking of animals in CH_4 production per kg DM intake differs between physiological stages with a change in diet (PINARES-PATINO *et al.*, 2007).

These latter authors evaluated the repeatability (i.e. between animals/total variations) as 47% and 73% according to the diets. Collectively, these results suggest that the genetic component of CH_4 production is low. However, data obtained on fattening cattle show that animals having a high feed efficiency, measured as the residual feed intake, produced, 20% less CH_4 than the less efficient ones (NKRUMAH *et al.*, 2006; HEGARTY *et al.*, 2007). Differences between these animals could be due to individual differences in rumen microorganisms associated to the rate of degradation processes and fermentation parameters and/or to intrinsic animal characteristics such as retention time of particles in the rumen (MARTIN *et al.*, 2010).

GUAN *et al.* (2008) reported that the bacterial profiles were more likely clustered within a certain breed, suggesting that host genetics may play an important role in rumen microbial structure. The correlations between the concentrations of volatile fatty acids and feed efficiency traits were also observed. Significantly higher concentrations of butyrate and valerate were detected in the efficient steers. The results of this experiment show that link between the diversity of the rumen bacteria and VFA pattern with the feed efficiency in cattle.

In dairy cows, body weight, milk yield, and type of roughage influence CH_4 production.

An equation between CH_4 production and milk yield has been calculated from numerous measurements of CH_4 production in dairy cows of different milk yields and fed according to their requirements. Calculations by KIRCHGESSNER *et al.* (1994) showed that a considerable amount of CH_4 (216 g/d) seems to be released independently of milk production.

Using today's current calculation practices, it can be concluded that the increase in cow productivity results in a decrease in CH_4 emission per kg milk, due to cow nutrition in present dairy systems. However, it should be noted that CH_4 emissions during a cow career should be split between milk and meat productions. The meat produced should take into account not just the cow but also that from the (male) offspring (MARTIN *et al.*, 2010).

Between-sheep variation in CH_4 emission has long been recognized from measurements in respiration chambers and in vitro, and recently confirmed under grazing conditions. The latter authors reported that about 85% of the variation in daily CH_4 production (g/day) from sheep grazing temperate pastures was due to variation between animals. If such between-

animal variability is persistent in the long term, and the animal trait(s) that account for such variation is (are) inherited, breeding of animals for low CH_4 emission might be viable (PINARES-PATINO *et al.*, 2003).

2. Reducing livestock numbers

As methane emissions from livestock are the predominant source of greenhouse gases reducing livestock numbers would be one way of meetingframework convention on climate Change FCCC commitments. However, such countries are heavily dependent on their livestock industries for generating national income and imposition of regulations aimed at reducing livestock numbers would be politically unacceptable. Reducing livestock numbers through normal market processes can be effective. For example, in New Zealand sheep farming has become less profitable over the past ten years and farmers have reduced sheep numbers and used the land for alternative enterprises, such as forestry. Sheep numbers have reduced from 57.9 million in 1990 to 45.2 million in 2000, while dairy cattle and beef cattle numbers have increased slightly. The net outcome has been a decline in ruminant methane emission from 1.45 to 1.31 Tg/year from 1990 to 2000. Livestock numbers will respond positively to improved economic conditions and if sheep farming becomes more profitable an increase in stock numbers and thus CH_4 emission is a possibility (ULYATT & LASSEY, 2001).

Total GHG emissions from livestock are positively related to the numbers of livestock. It is likely that our systems will be under political and social pressure to reduce livestock numbers to reduce the levels of emissions. Additionally, lower numbers of more productive animals will also contribute to more efficiency of production relative to emissions. Globally, one of the main issues relates to numbers of livestock, in particular numbers of livestock for a given level of off take (animal product). There are large differences between developed and developing countries in this respect. Taking beef cattle as the example, developing countries have twice the numbers of cattle of the developed countries (858 versus 410 million) yet the annual meat off take is only half (15.2 vs. 34.6 million tones) giving in excess of a 4 fold difference in efficiency. However, it is important to remember, that in developing countries livestock at often about more than just production they have a multi- purpose role. Globally, we are likely see differences in adaptation between developing and developed countries with developed countries perhaps seeing fewer, more productive animals producing quality products for niche local markets. However there remains the requirement to meet the ever increasing global demand for livestock products associated with the combination of increased human population and growing affluence fueling the Livestock Revolution (ROWLINSON, 2008).

The more lambs born and raised per ewe, the less methane is produced per lamb by the ewe. Therefore, the number of lambs per ewe is an important factor to consider when calculating emissions from sheep farms. In Sweden, breed differences in this aspect exist, but this alone should not be the reason for choosing a specific breed in a herd, the type of breed should instead primarily be adapted to the production system used. See Figure 9 for the number of lambs born and raised per ewe depending on the breed (ALLARD *et al.*, 2009).

3. Animal productivity

The improvement of animal productivity was suggested by FAO (2010) as an efficient way to increase world production of animal products and meet the increasing world demand, without increasing the use of land or the emission of globalgreenhouse gas (DOURMAD *et al.*, 2008).

The concept of increasing animal productivity to reduce methane emissions from ruminants is based on the maintenance of overall production output and as a result, increased production of useful product would mean methane production per unit product would decline. A reduction in total emissions of methane would only result if total output levels (e.g. total milk or beef produced) remained constant and livestock

numbers were reduced. Possible options for increasing ruminant productivity are discussed in the following sections (ANGELA *et al.*, 2000).

The primary method for reducing methane emissions from enteric fermentation is to improve production efficiency, which reduces methane emissions per unit of product (e.g., methane emissions per kilogram of milk produced). As part of the improvement in production efficiency, a greater portion of the energy in the animal feed is directed towards the creation of useful products (milk, meat, power) so that methane emissions per unit product are reduced. This increase in production efficiency also leads to a reduction in the size of the herd required to produce a given level of product. Because many countries are striving to increase production from ruminant animals (primarily milk and meat) improvements in production efficiency will help these goals to be realized while simultaneously avoiding increases in methane emissions (FAO, 2010; GWORGWOR *et al.*, 2006).

Increasing animal productivity will generally reduce methane emissions per kg of product (milk or meat) because the emissions associated with maintenance are spread over a larger amount of product. However, daily emissions and thus emissions per animal per year are usually increased because the higher productivity is usually associated with higher intake. Methane production is closely related to dry matter (DM) intake (O'MARA, 2004).

KIRCHGESSER *et al.* (1995) suggest an annual methane production rate of 110 kg from a dairy cow producing 5000 kg milk/year; doubling milk production only adds 5 kg to the methane production, as increasing milk yield from 4000 to 5000 kg/year increases annual methane emissions, but will decrease emissions per kg of milk by 0.16 for a 600 kg cow. A further increase to 6000 kg/year would decrease emissions per kg of milk by a further 0.128. Thus, there are quite significant reductions in methane emissions to be made by improved productivity in dairy cows as long as the number of cows is reduced to compensate for the increased milk yield. It should also be noted that the decline in methane emissions per kg of milk in response to increasing milk yield is curvilinear because the maintenance cost becomes increasingly diluted. Thus in high yielding herds, the reduction in methane emissions from further increases in milk yield will be relatively small (KIRCHGESSNER *et al.*, 1995).

JOHNSON *et al.* (2002) reported that for Wisconsin and New Zealand dairy herds demonstrate that there is still a reduction in total farm emissions from higher animal productivity after all these factors (Manure CH_4, CO_2, N_2O, Enteric CH_4) have been taken into account (JOHNSON *et al.*, 2002).

Management systems designed for high milk output per cow will tend to result in lower emissions per unit of milk produced. In contrast, more extensive systems require more animals to produce a given quantity of milk-- resulting in higher methane output per litre. The opportunities to reduce methane emissions by increased animal productivity are larger in the extensive systems compared to the intensive systems with already high milk production levels per cow (FAO, 2010).

4. Longevity

The longer that cows stay in a herd, the lower number of replacements required, and thus the lower the total farm methane emissions. An example of a 100 cow farm is presented in Figure 1, where the average number of lactations varies from 2.5 to 5. It is assumed that dairy cow emissions are 118 kg/yr while the rearing of a replacement heifer to calve at 2 years old results in methane emissions of 100 kg. Figure 1 shows that total farm emissions of CH_4 from enteric fermentation decline from 15,800 kg/yr to 13,800 kg/yr (0.127 less) as the average number of lactations increases from 2.5 to 5. This does not factor in the higher yield of the older cows which would further reduce emissions per kg of milk. Thus any measures which reduce involuntary culling should be encouraged. There will also be corresponding reductions in methane and nitrous oxide emissions from manure, and

in nitrous oxide emissions from soil (less fertilizer N usage due to lower stocking rate). Reducing the replacement rate will also leave more calves available for beef production (instead of cull cows) (O'MARA, 2004).

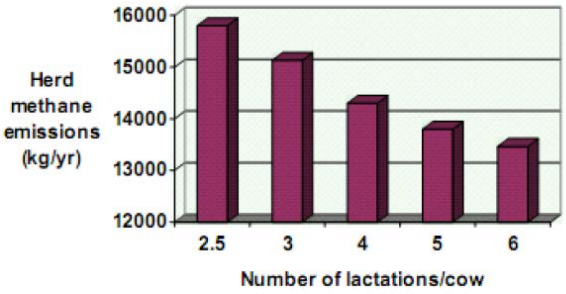

Fig. 1. The effect of number of lactations per cow on annual total herd (100 cows plus replacements) emissions of methane from enteric fermentation.

Similarly if improved animal performance leads to animals reaching target slaughter weight at a younger age, then total lifetime CH_4 emissions are reduced. On the other hand, going for increased performance may reduce longevity and thus even increase total lifetime emissions when accounting for rearing for replacement (O'MARA *et al.*, 2008).

Longevity will lower greenhouse gases /product because growing and maintenance are non productive periods of a life cycle. Improved production will not necessarily lower total emissions but more food could be produced whilst retaining profitability (WAGHORN, 2008).

Cow longevity might also be improved from the current level of 25% replacement rate, meaning fewer replacements would need to be reared. Replacements are a necessity to maintain production but each heifer consumes feed and emits methane for at least two years prior to producing any milk. As such, an increase in the average number of lactations the cows achieve is more carbon efficient, when set against an increase in the number of heifers reared each year. A conscious effort is made to keep on top of herd health. The herd has been managed as a predominantly closed system and the key areas of mastitis and foot health have been tackled to the benefit of longevity and milk production. When cattle are bought, they are typically yearling heifers to allow them time to adapt to the conditions and disease challenges specific to the farm, prior to bulling and calving (DAIRY CO, 2012).

5. Strategies to Reduce Greenhouse Gas Emissions through pasture management

Improving pasture quality is often cited as a means of reducing emissions, especially in less developed regions, because of improvements in animal productivity, as well as a reduction in the proportion of energy lost as CH_4 due to a reduction in dietary fibre. However, there is evidence that the impact of pasture quality on CH_4 emissions per kg of pasture consumed is small in temperate, well-managed swards (O'MARA *et al.*, 2008).

MOLANO & CLARK (2008) investigate the effect of level of intake and forage quality on methane production by sheep and reported no difference in CH_4 emissions per kg of grass dry matter intake (DMI) between lambs fed pasture with OM digestibility of 666 or 766 g/kg (MOLANO & CLARK, 2008).

The effect of pasture improvement in Australian sheep farms was recently modelled by ALCOCK & HEGARTY (2006), who reported only a small reduction in CH_4 output per kg live weight. But in their case, the assumed individual sheep productivity was already quite high, and the pasture improvement was calculated to lead mainly to an increase in stock numbers. In addition, the simulation showed little effect on digestibility of the forage, but rather gave an increase in the quantity of forage available. One group of researchers modelled dairy production systems in contrasting soil types (wet and impermeable vs dry and free-draining) and reported that the drier soils with a substantially longer grazing season supported milk production with significantly lower GHG emissions per kg of milk produced (O'MARA *et al.*, 2008).

WAGHORN *et al.* (2002) fed sheep a wide range of fresh cut, good quality forages and observed a two-fold range in emissions from

11.5g CH_4/kg dry matter intake (DMI) with lotus to 25.7g CH_4/kg DMI with pasture and a 16% reduction in methane production due to the Condensed tannins in lotus. This range in emissions from good quality forages represents a loss of about 7-11% of metabolisable energy and presents a clear direction for future research to better utilise the feeding value of pastures and reduce greenhouse gas (GHG) emissions from agriculture. All forages were delivered to the animal daily and had a DM digestibility of 70 % or greater. Animals grazing on pasture have the ability to be more selective than animals in this feeding trial, therefore the possibility exists that differences between forage species is even greater for pastured animals. Condensed tannins, a constituent of some legumes, have been associated with reduced enteric CH_4 emissions. Also these researchers reported that the impact of Condensed tannins on methanogenesis is small but significant (a 16% reduction). In addition to the impact on methanogenesis, Condensed tannins have beneficial effects on ruminant nutrition and production such as the reductions in the incidence of bloat and lowered intestinal worm burdens (WAGHORN *et al.*, 2002).

SEJIAN *et al.* (2011a,b) investigate forage and flax seed impact on enteric methane emission in dairy cows. The result of the experiment showed that both high proportion forage feeding and flax seed supplement reduced the enteric methane emission.

Legumes have higher nutritive value and voluntary intake than grasses, and steer gains are higher on legume-grass mixtures than on N-fertilized grass monocultures. However, most legumes can cause bloat. In a uniform stand, a maximum of 50% bloat-causing legume is considered bloat-safe, but bloat has been reported in mixtures with less than 15% bloat-causing legume where selective grazing could occur. The low digestibility of tropical legumes has been attributed to their high tannin content. Well-managed temperate grass-legume pastures, however, can have excessive CP and therefore animal performance can benefit from the presence of moderate concentrations of condensed tannins that control bloat and decrease ammonia and methane production in the rumen while increasing rumen undegradable protein (MACADAM *et al.*, 2006; MAHERI-SIS *et al.*, 2007).

MIRZAEI-AGHSAGHALI *et al.* (2008) reported that methane (g per day, g per kg BW and gr per kg $BW^{0.75}$) were similar in legume (two Iranian alfalfa varieties) hay, whereas methane production (g per day, g per kg BW and gr per kg $BW^{0.75}$) in grass hay were significantly higher than that of legume hay.

OLSON & WALLANDER (2002) compared five forage stands on foothill rangeland in Utah. Treatments included native rangeland and pastures seeded to Hycrest crested wheat grass (*Agropyron desertorum* x *A. cristatum*), Nordan crested wheat grass (*A. desertorum*), Vinall Russian wild rye (*Psathrostachys junceus*) and Syn-A Russian wild rye. There were three replicates each forage, pastures being established in a complete block design. When non lactating beef cows grazed these pastures in October, the native mixture compared favorably with improved species. Methane emissions by lactating cows on these same pastures in the following spring again showed that native pasture resulted in the highest CH_4 emissions and was the least productive. Olsen's work, although preliminary, suggests that variation does exist among grass species and that the choice of species may depend on the season of pasture use. Whether using rotational or continuous grazing strategies, there is tremendous fluctuation in for age quality during the grazing season which affects fermentation efficiency and enteric methane emissions. Grain supplementation has been recommended as a means of improving the efficiency of fermentation for cattle when forage quality is poor.

Results of a study recently completed by BOADI & WITTENBERG (2002) found grain supplementation for pastured yearling steers did result in increased DM intake and rates of gain, but there was no benefit relative to enteric emissions. That study clearly demonstrated forage quality to be the

major factor influencing enteric emissions for pastured cattle. For example, the lowered quality and availability of forage from the time cattle entered a paddock to the time they were removed from that paddock in a rotational grazing system resulted in a 58 % reduction in forage DMI, but daily methane emissions remained the same (WITTENBERG, 2008, Winnipeg, pers. comm.).

Emissions from grazing livestock can be hard to predict, the exact feed intake is hard to estimate and the nutritional value of the pasture differs within the season. Several studies with grazing sheep have though been conducted (ALLARD *et al.*, 2009).

5.1. Management to Mitigate Methane in Grazing Animals

Effective management to mitigate methane could be viewed in terms of animal productivity vs. animal methane emissions. Expression could be on an annual basis to avoid short term bias, for example cows grazing ryegrass pastures produced 11.7, 19.4 and 24.3 g CH_4 kg^{-1} milk at day 60, 150 and 240 of lactation. The difference in emissions was largely due to a live weight loss contributing energy to milk synthesis in early lactation and use of dietary energy to restore live weight in late lactation. A similar scenario applies to sheep, with very high CH_4 emissions associated with wool growth (typically 10-12 g day^{-1}) in adult animals, but a lesser emission cost associated with growing lambs and reproduction. Mitigation can be achieved by minimizing maintenance costs as a proportion of feed intake and maximizing the productive worth of livestock. High intakes of high producing animals dilute their maintenance cost and lower the methane emissions per unit of production. This will be best achieved by offering high quality diets to animals of high genetic merit and imposing good livestock and pasture management practices. These effects are illustrated for 30 kg lambs growing at 100, 200 and 300 g day^{-1} with methane emissions of 166, 115 and 98 g kg^{-1} live weight gain respectively. Comparative values for 450 kg grazing dairy cows producing 12, 20 or 24 kg milk day^{-1} were 17.2, 13.6 and 12.7 g CH_4 kg^{-1} milk. The methane emissions associated with production increased from 49 to 61 and 66% for the respective treatments. Animal performance can be improved by selection for a high metabolic efficiency or by using rumen modifiers to alter products of digestion. Any factor able to improve feed conversion efficiency will lower CH_4 emissions $unit^{-1}$ production. However farmers need to achieve a balance between increasing efficiency of feed utilisation and the efficiency of pasture utilization (WAGHORN & WOODWARD, 2004).

Several Canadian research studies have examined the impact that pasture and grazing management has on enteric CH_4 emissions. A study by MCCAUGHEY *et al.* (1997) reported that CH_4 production was greatest for steers continuously grazing at low stocking rates (1.1 steer ha-1; 307 L d^{-1}) and least for steers grazing continuously at high stocking rates (2.2 steers ha-1; 242 L d-1). A possible explanation for these observed results for the higher stocking rate may be due to lower forage availability and intake for the grazing animal. When pastures were rotationally grazed, stocking rates had no effect on CH_4 production. At low stocking rates, CH_4 production was 9% lower on rotational grazing than continuous grazing. Measurements of CH_4 production from grazing beef cows found a 25% reduction in CH_4 losses with alfalfa + grass pastures (7.1% of gross energy intake) compared to grass-only pastures (9.5% of gross energy intake) (MCCAUGHEY *et al.*, 1997). Other researchers observed early grazing of alfalfa+grass pastures reduced CH_4 production by 29 to 45% in steers compared to grazing at mid and late seasons. Pasture quality is the critical factor in ensuring lower CH_4 emissions from grazing animals in any particular grazing system (IWAASA, 2007).

LASSEY *et al.* (1997) investigate the methane emissions measured directly from grazing livestock in New Zealand with the ERUCT technique. The pasture was a typical improved one with mostly perennial ryegrass and white clover (LASSEY *et al.*, 1997).

ULYATT *et al.* (2002a) was measured methaneemission from 10 dairycows and 12 wethersheep grazing kikuyu grass in New Zealand two different years, 1997 and 1999. In 1997, the same CH_4 yield could be found for both cattle and sheep. The pasture in year 1999 had a better nutritional value compared to the one in 1997 and the emissions were lowered for both species of animals, but the reduction was clearer marked in the sheep. The authors suggested that the extra low values in 1999 could be a result of the pasture containing compounds that could inhibit methanogenic bacteria, and not only of the pasture's better quality. Kikuyu grass (subtropical C4-plant) have a lower digestibility than C3-plants, resulting in higher CH_4 emissions from rumen fermentation, thereby also the lower digestibilities compared to the other pastures (ULYATT *et al.*, 2002a).

ULYATT *et al.* (2005), compared four groups of sheep were grazed on four late summer/autumn pastures: southern North Island summer moist hill country (Ballantrae); good quality perennial ryegrass/white clover dominant pasture in the Manawatu (Aorangi); severe late summer drought pasture in Hawke's Bay (Poukawa sheep); and after drought conditions in Canterbury (Springston). Mature ewes were used at Springston, while young wethers were used at all the other sites. The study was conducted over the years 1997-1999 and variations in the weather sometimes made the conditions somewhat unusual for the season for all cases except Poukawa sheep, resulting in higher feeding values than normal in some cases. The mature ewes grazing in Springston had the highest emissions, which could be explained by the fact that mature sheep probably cause higher CH_4 emissions than younger sheep. But the method used for measurements was not the same as for the other sheep in the study and therefore the results should not be directly compared to each other. The higher emissions for the Poukawa sheep than the Aorangi and Ballantrae sheep could be explained by the lower digestibility of the dead matter grazed at Poukawa (ULYATT *et al.*, 2005).

ULYATT *et al.* (2002b) investigate the impact of seasonal variations on methane emissions using a perennial ryegrass/white clover pasture in New Zealand and found the highest emissions of methane from sheep grazing in November. In this experiment, Daily methaneemission from 12 Romney-cross-bred ewes and 10 lactating Friesian dairycows, rotationally grazed on perennial ryegrass/whiteclover dominant pastures, was measured during four seasons of a year (September, November, March, and June/July).Methaneemission was measured from each animal for 5 consecutive days in each measurement period using the sulphur hexafluoride tracer gas technique. This is in accord with the low feeding value of pastures in New Zealand at this time of the year, but the authors found no explanation to the low values in July. They concluded that seasonal variation in the chemical composition of pastures had little importance in this study for the rate of methane emitted. They could also see that cows and sheep had about the same efficiency of utilizing the feed. They saw that the emissions from grazing dairy cows and grazing ewes were about the same expressed in g CH_4/kg digestible dry matter intake with values of 26.6 and 25.2 for cows and sheep respectively (ULYATT *et al.*, 2002b).

A cow-calf study at Brandon, Manitoba compared performance and enteric emissions of alfalfa-grass and grass only pastures over the course of a grazing season. Dry matter intake was greater for cows grazing alfalfa-grass pastures than for grass-only pastures (11.4 vs. 9.7 kg d^{-1}), however, methane production, adjusted for differences in body weight, was the opposite (0.53 vs. 0.58 g kg BW d^{-1}, respectively). Energy lost as enteric methane emissions were 7.1 % of gross energy intake for alfalfa-grass vs. 9.5 % of gross energy intake for grass-only pastures. An 11 % increase for calf growth rates on the legume-grass pasture would serve as further incentive to consider legume incorporation as a mitigation strategy. The lowered methane loss observed with legumes is attributed to the lower proportion of structural carbohydrates and faster rate of passage of

legumes, which will shift the fermentation pathway towards higher propionate production. The extent to which forage species can influence enteric methane emissions of pastured ruminants is not known under Canadian conditions (MCCAUGHEY *et al.*, 1999).

LASSEY *et al.* (1997) measured emission in March in cows from the same herd fed similar pasture as in the present work at 262.8 g/day at an estimated DM intake of 12.9 kg/day (20.4 g CH_4/kg DMI; MY 6.2%), compared with 181.5 g/day and 14.9 kg/day (12.3 g CH_4/kg DMI; MY 3.7%) respectively in the present work.

MURRAY *et al.* (2001) could see that sheep grazing on a pasture with both clover and perennial ryegrass had significantly higher emissions of methane, than sheep grazing only grass which received fertilizer. But as the digestibility of the feeds were not included in the calculations this could mean that per unit of production such as growth or lactation, the emissions measured from the sheep grazing clover could be of another value. Clover pastures often have better digestibility than grass pastures. Therefore the total amount methane emitted may be higher for a certain intake of gross energy but not of digestible energy intake (ALLARD *et al.*, 2009).

Several Canadian research studies have examined the impact that pasture and grazing management has on enteric CH_4 emissions. BOADI & WITTENBERG (2002) observed early grazing of alfalfa+grass pastures reduced CH_4 production by 29 to 45% in steers compared to grazing at mid and late seasons. Pasture quality is the critical factor in ensuring lower CH_4 emissions from grazing animals in any particular grazing system (IWAASA, 2007).

BOADI & WITTENBERG (2002) reported methane production as a percent of gross energy intake (GEI) was not influenced by diet, as CH_4 emissions of 6.0, 7.1 and 6.9% of gross energy intake (GEI) from beef and dairy heifers fed ad libitum legume and grass hays containing 41.8, 58.1 and 68.8% NDF in the DM, respectively methanogenesis was not related to feed quality.

The associated basal feedstuffs used may also influence this value. With dry forages, methane losses are a little higher (0.01%/GE) than with grass silages. No statistical difference appeared for peas, faba beans, sugar beet pulp and sorghum when tested with either gramineae hays or with maize silage. Kinds of concentrates do not seem to statistically modify the methane losses of the basal feed. In conclusion, reciprocal influence of feeds sometimes exists, but generally it might be smaller than the incertitude of measurements. Methane variation losses were significantly decreased with the increase of ether extract or of one of the cell wall constituents (NDF, ADF or ADL). Lignin (ADL) was the best chemical predictor in methane variation losses and explained 61% of the variations of gross energy losses as methane (GIGER-REVERDIN & SAUVANT, 2000).

5.2. Grassland management and grazing

Lucerne is another component of the shorter-term grass leys. As a legume, it is a nitrogen fixer and the deep root network means lucerne will remain productive in dry spells. The forage containing the lucerne/clover/ grass mix is high in dietary protein and fibre. The dry periods in summer often mean fertilizer application offers less than it might do in less dry regions; this is reflected in below-average fertilizer use.

Evolution Farming consultant, Oliver Hall, identifies grassland management as a key area. The aim is always to produce quality over quantity and the M.E. average across all cuts should exceed 11.5MJ/ kg. High energy forage will result in smaller volumes of methane being emitted by each cow. Also, by producing quality grass silage, a greater proportion of the milk can be generated from this grass, reducing the need for high volumes of purchased feeds to push yields up. Fertilizer use is above average Ammonium Nitrate is the only product used on the dairy supporting area. The tonnage used indicates an average of 160kg of N per Ha (64kg per acre) is supplied from artificial fertilizer across the total land allocated to

the dairy. Fertilizer is both an economic and carbon costly product and so can have a big effect on the carbon footprint of a business, therefore reinforcing the importance of using manures and slurry as efficiently as possible. The slurry lagoon is allowed to crust which reduces the greenhouse gas emissions to air. The slurry is spread by contractors using a splash plate. It may be worth considering other methods of slurry application so that the slurry is applied at the base of the plant. If the slurry is applied according to crop demand, it may be possible to reduce the amount of bagged fertilizer required (DAIRY CO, 2012).

5.3. The Impact of Different Pasture Species

Most species of pasture grasses and legumes the predominant pastures plants are perennial. Perennials offer several advantages over annual crops grown for feed, such as corn, soybeans, and sorghum. For example, well managed perennials provide groundcover throughout the year, reducing pollution runoff and soil erosion (GURIAN-SHERMAN, 2011).

Grass species may differ in their effect on methane emissions. However, these differences are usually narrower than those between grasses and legumes. Feeding forage legumes to ruminants grazing grass-dominant pastures will improve animal performance and lessen the reliance on a single species to meet all nutritional requirements (WAGHORN & CLARK, 2004).

MOURINO *et al*. (2003) investigate the animal performance and pasture composition from 1998 to 2000. Some legume species may improve pasture quality by competing better with grasses. The lower NDF of kura clover–grass indicates that steers on this pasture had greater intake potential than those on red clover grass pasture. Neutral detergent fiber concentration was fairly constant for the kura clover–grass pasture while it increased (P0.08) 64 g kg^{-1} from1998 to 2000 in red clover grass and the average daily weight gain of steers on pastures with mixed grass and kura clover (*Trifolium ambiguum*) in Wisconsin, for example, was 22 percent higher than that of steers on pastures with grass and red clover (another legume) - the latter a common mixture in the United States. The analysts attributed this difference to the fact that the percentage of kura clover was higher than that of red clover in the pastures. In addition, kura clover–grass pasture had lower levels of fiber, higher protein concentration, and better digestibility than the red clover grass pasture. As a result, kura clover–grass pastures displayed greater average daily gain and gain per hectare than red clover grass pastures (MOURINO *et al.*, 2003).

BURNS & STANDAERT (1985) reported that average daily gain and gain per hectare are usually greater on legume–grass systems until N clover mono application rates on N–grass systems exceed 200 kg ha_1.

MIN *et al.* (2006) investigate the Effects of condensed tannins supplementation level on weight gain and in vitro and in vivo bloat precursors in steers grazing winter wheat and reported that daily supplementing quebracho condensed tannins to steers grazing wheat forage improved animal performance and minimized bloat frequency without deleterious effects to the animals. Quebracho condensed tannins supplemen-ted ruminal fluid incubated with minced wheat forage led to less *in vitro* gas and methane production. Quebracho condensed tannins supplementation is a potentially effective feed additive for decreasing bloat impacts and increasing ADG in stocker cattle-wheat systems common to the Southern Great Plains (MIN *et al.*, 2006).

PUCHALA *et al.*, (2005) investigate the effect of condensed tannin-containing forage on methane emission by goats and reported that Methane emission expressed as both quantity per day or relative to DMI was lower ($P< 0.001$) for Sericea lespedeza than for crabgrass/tall fescue (7.4 vs. 10.6 g/d and 6.9 vs. 16.2 g/kg DMI). Substantial differences between the forages in condensed tannins concentration and methane emission by Angora goats suggest that condensed tannins decreased methane emission (PUCHALA *et al.*, 2005).

WOODWARD *et al.* (2001) reported that in sheep, methane emission relative to

digestible DMI was decreased by 24 to 29% when the CT-containing forage Lotus pedunculatus was fed compared with ryegrass or lucerne.

Legumes tend to be less resilient in the face of trampling by cattle, and grass species often out-compete legumes over several years, reducing their percentage in the pasture. Good management is therefore critical to maintaining legumes in pastures. Different legumes also grow best in different types of soil and climates. For example, alfalfa grows best in neutral, well-drained soil, while birdsfoot trefoil can tolerate more flooding (GURIAN-SHERMAN, 2011).

Legumes are important components of pastures. Legumes not only fix atmospheric nitrogen (N_2) for their own use when properly inoculated, they provide nitrogen (N) for associated grasses and forbs. A range from 150 to 240 lb N per acre is needed to equal the contribution of legume N in legume-grass mixtures. Using a legume reduces the purchase and application costs of N fertilizer and may reduce soil acidification and N losses to the environment. Many legumes are deep-rooted and therefore more drought-tolerant than grasses. Under grazing, legumes are more commonly used as a component of mixtures with grasses than as monocultures. This is because fibrous-rooted grasses are valuable sources of soil organic matter, they provide better protection from soil erosion, are more resistant to grazing and treading damage than legumes, and well-managed grass-legume mixtures provide more than adequate levels of crude protein (CP) for highly productive livestock (MACADAM *et al.*, 2006).

Management of soil acidity for temperate and tropical regions has often differed but increasingly depends on acid-tolerant legume cultivars and rhizobia, with soil liming only to a pH at which Al and Mn are no longer toxic. Legumes are often grown after corn or rice and are seeded toward the end of the growing season. They may have short growing seasons and may be subject to intermittent or terminal drought. Progressive soil chemical and physical degradation and acid soil conditions may also limit their productivity (GRAHAM & VANCE, 2003).

5.4. Using Harvested Forages: Silage and Pelleting

Harvested forages silage and hay are an important component of pasture beef farms in many parts of the country. Cattle may eat harvested forages when pastures are dormant or have matured, and are growing slowly or not at all. BENCHAAR *et al.* (2001), suggested that intake of NDF was lower (–11%) with alfalfa silage than with alfalfa hay. Ruminal digestion of OM and NDF (% of intake) were also reduced (–21 and –9%, respectively) when alfalfa was preserved as silage rather than hay. Ruminal microbial efficiency was slightly enhanced (+9%) by the utilization of alfalfa silage. Ruminal pH was higher for alfalfa silage. The intensity of ruminal fermentation was quantitatively influenced by the method of preservation of alfalfa; total and individual VFA productions were lower with alfalfa silage compared to alfalfa hay. Total methane production (Mcal d^{-1}) was depressed (–33%) by the utilization of alfalfa silage instead of alfalfa hay. Fractions of GE intake and DE lost as methane were also lower (–32 and –28%, respectively) with alfalfa silage than with alfalfa hay (BENCHAAR *et al.*, 2011).

VARGA *et al.* (1985) reported a decrease in methane production from cattle consuming alfalfa silage compared to orchardgrass silage. The utilization of less-mature herbage has been shown to lower methane yields.

Methane production, dry matter digestibility and urinary energy loss were reduced when first-cut alfalfa was pelleted but pelleting had no influence on these parameters with second-cut alfalfa. Methane and CO_2 production and O_2 utilization increased sharply after feeding. There were interactions between type of feed (chopped vs. pelleted) and cut of alfalfa in CH_4 (1 kg^{-1} feed DM) and CO_2 production and O_2 utilization. Energy digestibility and CH_4 losses were similar at maintenance and 1.6 times maintenance level of feeding. Although methane production was lower in

cattle fed pellets in three out of four comparisons of pelleted and chopped hay diets, the decline in energy loss as CH_4 due to pelleting was not sufficient to justify the extra energy expended to pellet diets from an environmental or economic point of view (HIRONAKA *et al.*, 1996).

5.5. Better pasture management: rotational grazing

Managed rotational grazing (MRG) also known as managed intensive rotational grazing—boosts the productivity of pasture, and can improve the nutritional quality of pasture forages. In MRG, beef producers rotate grazing cattle often among several fenced paddocks within a pasture. MRG prevents cattle from overgrazing, which curbs the ability of pasture plants to grow, and allows paddocks to recover between grazing periods. MRG also promotes more uniform grazing, so pasture plants can grow at optimal rates. Under continuous grazing, in contrast, cattle graze anywhere on a pasture at will. However, the data on the effect of MRG on methane emissions are ambiguous, and insufficient to draw clear conclusions about the impact on climate change (GURIAN-SHERMAN, 2011).

DERAMUS *et al.* (2003) investigate methane emissions of beef cattle on forages and reported that CH_4 annual emissions in cows reflect a 22% reduction from best management practices when compared with continuous grazing in this study. With the best management practices application of management-intensive grazing, less methane was produced per kilogram of beef gain.

MCCAUGHEY *et al.*, (1997) reported that voluntary intake and CH_4 production, adjusted for differences in body weight, were unaffected by grazing management, sampling period or by monensin controlled release capsule administration and averaged 0.69 ± 0.1 L kg BW^{-1} d^{-1} across all grazing management treatments. The results obtained in this study indicate that it will not be easy to manipulate CH_4 production of steers grazing alfalfa/grass pastures through changes in grazing management. On the improved pastures used in this experiment, CH_4 production and voluntary intake remained relatively constant regardless of variations in diet quality.

6. Vaccine

One technology to reduce methane emissions currently patented and being investigated by CSIRO is a methanogen vaccine. The vaccine stimulates antibodies, which are active against the methanogens. Preliminary results of this work have found a significant reduction of in vitro methane emissions along with increased animal production. It may also be possible to develop vaccines against rumen protozoa. The major advantage of this sort of technology is the potential for use in extensive grazing systems due to the expected long-term efficacy of the treatments. There is also little likelihood of consumer resistance to this technology as the approach uses the immune system of the animal to inhibit the methanogens (REYENGA & HOWDEN, 1999).

There is also research being conducted to develop a vaccine, which stimulates antibodies in the animal that are active in the rumen against methanogens. The problems with some of these mitigation strategies to reduce CH_4 are potential toxicity to the rumen microbes and the animal, short-lived effects due to microbial adaptation, volatility, expense, and a delivery system of these additives to cows on pasture (ISHLER, 2008).

WRIGHT *et al.* (2004), investigate the reducing methane emissions in sheep by immunization against rumen methanogens and this experiment thirty mature wether sheep were randomly allocated to three treatment groups (n=10). One group received an immunization of adjuvant only on days 0 and 153 (control), a second group received an immunization with a 3-methanogen mix on days 0 and 153 (VF3+3), and a third group received an immunization of a 7-methanogen mix on day 0 followed by a 3-methanogen mix on day 153 (VF7+3). Four weeks post-secondary immunization, there was a significant 7.7% reduction in methane production per kg dry matter intake in the VF3+3 group compared to the

controls. However, methane emissions from sheep immunized with VF7+3 were not significantly different when compared to the sheep in the control group.

WRIGHT *et al.* (2007) expressed, it is difficult to draw any conclusions about whether the geographical isolation between these two herds of cattle or differences between the two diets directly influenced community structure in the rumen. However, if there were a geographical effect, then there should be unique phylogenetic groupings of methanogens that have been identified from sheep in Australia, Canada, Japan, New Zealand, Scotland, and Venezuela; rather, they are scattered throughout the tree.

CALLAWAY *et al.* (1997) reported that some bacteriocins (nisin and monensin) are able to inhibit ruminal methane, decrease acetate to propionate ratios and prevent amino acid deamination. Nisin and monensin have similar effects on carbohydrate fermentation, but nisin is a more potent inhibitor of obligate amino acid fermenting ruminal bacteria. LEE *et al.* (2002) reported that the bacteriocins may provide an alternative strategy for decreasing ruminal methane production. The compound(s) in question reduced numbers of methanogens, but, like many other inhibitors that are efficient in vitro, the effect was lost in sheep after continuous administration for a few days (NOLLET *et al.*, 1998). And other researchers also suggested the use of archaeal viruses to decrease the population of methanogens, but, to our knowledge, no bacteriophages active against rumen methanogens have been isolated so far (MARTIN *et al.*, 2010).

A vaccine developed from a three-methanogen mixture produced a 7.7% reduction (kg^{-1}DM) in methane emissions from sheep (P=0.051) despite only one antigen being effective against the methanogenic species in the sheep. The vaccine was much more effective than the seven methanogen mix tested previously and was able to increase saliva and plasma antibody titres by 4 – 9 folds over the seven methanogen mixture. Successful elevation of antibody titres in saliva and a significant reduction in methane emissions offers real potential for a widespread application to ruminants in all environments. At present vaccines do not have sufficient efficacy for commercial use and funding has recently been curtailed. Opportunities through rumen additives, defaunation and specific compounds targeting methanogens provide several routes for reducing methane production. However these agents have not addressed the inevitable production of hydrogen from fermentation of fibre. Ruminants are able to utilize fibre because of their microflora and hydrogen production is an unavoidable consequence. Excess hydrogen accumulation will inhibit microbial growth, but acetogens offer an opportunity for production of acetate as well as removing accumulated hydrogen. Acetogens are present in moderate concentrations in the digestive tract of horses, llamas and buffalo (104 – 105 ml^{-1}) but values for sheep and cattle have been very low (WAGHORN & WOODWARD, 2004).

Conclusion

Significant mitigation of greenhouse gas emission is a critical subject of biological, ecological and environmental research area in the world. Due to higher global warming potential of the methane, it is subjected to many studies in recent years. From the view point of methane emission, ruminant animals are consequential than that of other animal species owing to higher fermentation activities. Integral management strategies in ruminant production such as consideration of animals -type and individual variability, reducing livestock numbers, improving animal productivity and longevity, valid pasture management as well as vaccination against methanogenic microbes; can be results in mitigating methane production. It is notable that, other than management related strategies, three important strategies including nutritional, biotechnological and microbiological strategies are required for controling and decreasing methane emission.

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ECOLOGIA BALKANICA - INSTRUCTIONS FOR AUTHORS - 2015

General information

Submissions to "*Ecologia Balkanica*" can be original studies dealing with all fields of ecology, including ecology and conservation of microorganisms, plants, aquatic and terrestrial animals, physiological ecology, behavioral ecology, population ecology, population genetics, community ecology, plant-animal interactions, ecosystem ecology, parasitology, animal evolution, ecological monitoring and bioindication, landscape and urban ecology, conservation ecology, as well as new methodical contributions in ecology. Studies conducted on the Balkans are a priority, but studies conducted in Europe or anywhere else in the world are also accepted, after a consideration of the Editorial Board. *The Editorial Board of "Ecologia Balkanica" reserves its right to reject publication of any manuscript that does not fit the aim and scope or does not comply with these instructions.*

Manuscript submission

The following types of manuscripts are accepted: *short research notes* (up to 4 pages), *research articles* (4 to 10 pages) and *review papers* (10 to 20 pages). *Short research notes* are shorter submissions of a preliminary nature or those including new records or observed phenomenon, etc. *Research articles* should present significant original research in the various fields of ecology, mentioned above. *Review papers* should deal with topics of general interest or of contemporary importance, being synthetic rather than comprehensive in emphasis. Authors of Review papers should consult with the Editor before submission. The Editor may also invite review articles concerning recent developments in particular areas of interest. The Editor reserves the right to decide if a manuscript should be treated as a Short note or Research article. In general, studies that are purely descriptive, mathematical, documentary, and/or natural history will not be considered.

Manuscripts must conform strictly with the instructions to authors and sent to the Editor. **All manuscripts must be accompanied with a cover letter,**

which can be downloaded from here. All fields from the cover letter form must be filled out and the cover letter must be sent along with the full text of the manuscript to the journal's e-mail. Incoming manuscripts are initially judged by the Editor. *Manuscripts may be rejected without peer review if they do not comply with the instructions to authors or are beyond the scope of the journal.* If the manuscript is acceptable, it will be forwarded to referees for evaluation. All manuscripts are peer-reviewed by 2 or 3 independent reviewers. After final edition and approval by the Editorial Board, the manuscript will be accepted for publication. The Editor reserves the right to make editorial changes. Authors agree, after the manuscript's acceptance, with the transfer of copyright to the publisher.

Legal requirements

Submission of a manuscript implies: that the work described has not been published previously (except in the form of an abstract, or as part of a published lecture, or thesis); that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities - tacitly or explicitly - at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

Manuscript preparation

Language

The manuscripts must be prepared in English. *Contributors who are not native English speakers are strongly advised to ensure that a colleague fluent in the English language*, if none of the authors is so, *has reviewed their manuscript.* Spelling should be British or American English and should be consistent throughout. All abbreviations and acronyms should be defined at first mention. To facilitate reader comprehension, abbreviations should be used sparingly.

Technical information

Manuscripts must be submitted in **electronic version only**, as well as the original figures and tables, implemented in the text. The manuscript text should be **MS-Word** processed (all versions are acceptable), justified, font size 11, font "Book Antiqua", without footnotes, column or page breaks, single spaced (about 60 lines per page), on A4 (210 x 297

mm) paper, with margins of exactly 2.5 cm on each side. Pages should not be numbered.

The manuscripts should conform to the following format:
Title: Provide a title that is concise, but also an informative synthesis of the study. Where appropriate, include mention of the family or higher taxon.
Author(s): Full first name(s), middle initials and surname(s) in ***bold italic.*** The corresponding author should be marked with the *-symbol.
Address(es): As complete as possible. E-mail address is given only for the corresponding author.
Abstract: Maximum length 300 words. The abstract should state briefly the objective of the research, the primary results and major conclusions, with no description of methods, discussions, references and abbreviations.
Key words: Usually 3–10 words suitable for information-retrieval system.

The standard order of sections should be: Abstract, Key words, Introduction, Material and Methods, Results, Discussion (or Results and Discussion), Conclusions (optional), Acknowledgements (optional) and References.

The *Introduction* has to explain the actuality of the researched problem and give the aim of the study.
Materials and Methods have to provide sufficient information to permit repetition of the experiment and/or fieldwork. The technical description of study methods should be given only if such methods are new; otherwise a short presentation is enough.
The *Results* section must be a concise presentation of the finding of the study. Avoid presentation of the same information as text and/or figure and/or table.
Discussion section should be separate from the results section at full-length papers and should deal with the significance of the results and their relationship to the aims of the paper. Also include how the findings of the paper will change or influence the state of our knowledge about the topic at hand. In separate cases a joint section "Results and Discussion" is allowed, but not preferable.
Conclusions should shortly describe the main contributions and recommendations of the study without including citations and statistics.

In the *Acknowledgements* section all persons and organizations that helped during the study in various ways, as well as the organization that financed the study must be listed.

Short Notes (generally less than four-five manuscript pages) should be produced as continuous text, preceded by an abstract of no more than 150 words.

Tables: The tables must not repeat information already presented in the figures or in the text. Each table must be self-explanatory and as simple as possible. No fold-outs are accepted. Tables must be numbered consecutively. **They should be placed within the text at the desired position by the author(s).** An explanatory caption, located on the top of the table, should be provided.

Example:

Table 1. Shannon-Wiener indexes in the burned ($\mathbf{H}_{burned}$) and control ($\mathbf{H}_{control}$) territory for the total duration of the study (2004–2006).

Figures: They must not repeat information already presented in the tables or in the text. Lines and letters in figures must be able to be enlarged or reduced without reduction in quality. They should conform to the size of the type area (16 × 24 cm) which is the limit for all illustrations. Magnification should be shown by scale bars. Colour illustrations are accepted, but will appear only in the electronic version of the journal (PDF). The illustrations in the hardcopy printed version will be greyscale. All illustrations must be sharp, of high quality with at least 300 dpi. The following formats are acceptable: JPEG, GIF, TIFF, EPS. Figures must be numbered consecutively and should be provided with an explanatory legend below them. **They must be placed within the text at the desired position by the author(s).**

Example:

Fig. 1. Indicative map of the study area.

All tables and figures must be referred to in the text!

Citations and references

Literature citations in the text should indicate the author's surname in SMALL CAPITALS (for more information on SMALL CAPITALS and how to

make them in Word) with the year of publication in parentheses, e.g. CARLIN (1992); BROOKS & CARLIN (1992); SHAPIRO *et al.* (1968). Citations in brackets should be divided with semicolons and the author's name and the year of publication with comma (*example:* CARLIN, 1992; BROOKS & CARLIN, 1992; SHAPIRO *et al.*, 1968). If there are more than two authors, only the first should be named, followed by "*et al.*" in *italic*. References at the end of the paper should be listed in alphabetical order by the first author's family name and chronologically. If there is more than one work by the same author or team of authors in the same year, a, b, etc. is added to the year both in the text and in the list of references. Each citation in the text must be accompanied by a full reference in the list of references and vice versa.

Examples:

A journal article:

AUTHOR A. 1990. Title of the article. - *Full title of the journal*, 56(3): 35-105.

AUTHOR A., B. AUTHOR. 1990. Title of the article. - *Full title of the journal*, 56(2): 35-105.

AUTHOR A., B. AUTHOR, C. AUTHOR. 1990. Title of the article. - *Full title of the journal*, 56(1): 35-105.

A book:

AUTHOR A. 2000. *Title of the book.* Vol. I. Place of publication. Publishing house.

Proceedings or book chapter:

AUTHOR A., B. AUTHOR 1990. Title of the contribution. - In: Author A. (Ed.): *Title of the book or proceedings.* Place of publication. Publishing house, pp. 235-265.

Software:

STATSOFT INC. 2004. *STATISTICA (Data analysis software system)*, Vers. 7. Computer software. Available at: [http://www.statsoft.com].

GARMIN LTD. 2007. *MapSource*, Vers. 6.12. Computer software. Available at: [http://www.garmin.com]

Website:

FAUNA EUROPAEA. 2007. *Invertebrates. Fauna Europaea.* Vers. 1.1. Available at: [http://www.faunaeur.org]. Accessed: 12.10.2009.

In case of papers written in other than Latin letters, if there is an English (or German, or French) title in the summary, it is recommended to be used. If there is not such a summary, the author's names must be transcribed and the title of the paper must be translated into English and put in square brackets. If the name of the journal is also not in Latin letters it also should be transcribed (not translated). This should be noted in round brackets at the end of the paragraph, for instance: (In Bulgarian, English summary).

Example:

ANGELOV P. 1960. Communications entomologiques. I. Recherches sur la nourriture de certaines espèces de grenouilles. – *Godishnik na muzeite v grad Plovdiv*, 3: 333-337. (In Bulgarian, Russian and French summary).

KOROVIN V. 2004. [Golden Eagle (*Aquila heliaca*). Birds in agricultural landscapes of the Ural]. Ekaterinburg, Published by Ural University, 57 p. (In Russian).

Names of persons who provided unpublished information should be cited as follows: "(ANDERSSON, 2005, Stockholm, pers. comm.)".

Unpublished theses (BSc, MSc, PhD, DSc) are not considered officially published scientific literary sources, therefore from January 2015, "*Ecologia Balkanica*" no longer allows citations of such references.

Citing references that are still "in press" is also considered frown upon, but not forbidden. If possible, please avoid using such references.

For your effortlessness, from January 2015, "*Ecologia Balkanica*" provides an EndNote™ style template based on the Harvard reference style (with modifications) available at our site.

Additional requirements

For special symbols (Greek letters, symbols for male and female etc.) use the Symbol list on the Insert menu in Microsoft Word with the following preferable fonts: Symbol, Webdings, Wingdings, Wingdings 2 and Wingdings 3. Degree symbols (°) must be used (from the Symbol list)

and not superscript letter "o" or number "0". Multiplication symbols must be used (×) and not small "x" letters. Spaces must be inserted between numbers and units (e.g., 3 kg) and between numbers and mathematical symbols (+, –, ×, =, <, >), but not between numbers and percent symbols (e.g., 45%).

Nomenclature and units. Follow internationally accepted rules and conventions: use the International system of units (SI). If other quantities are mentioned, give their equivalent in SI. Please use a standard format for the units and be uniform in the text, tables and figures. Please prefer one of two possible styles: "m/s" or "m s-1". When using "m s-1" leave a space between symbols.

Small capitals and italic letters. The Latin genus and species names must be cited completely once in the text and should be typed in *italic*. Family names of the authors in the citations and the publications listed in the reference list must be in SMALL CAPITALS, but never for collectors, preparators, acknowledgements, etc.

Statistics

Mean values should always be accompanied by some measure of variation. If the goal is to describe variation among individuals that contribute to the mean standard deviation (SD) must be used. When the aim is to illustrate the precision of the mean standard errors (SE) should be given. The last paragraph of Materials and Methods section should briefly present the significance test used. Quote when possible the used software. Real p values must be quoted both at significance or non-significance. The use of the sign is acceptable only at low values of p (e.g. $p<0.0001$).

Ethics

The authors of articles that are based on experiments that caused injuries or death of animals should explain and justify the grounds of the study and state that the scientific results of the study is at least in trade-off with the sufferings caused. In the Materials and Methods section of the manuscript, the authors should detail as precisely the conditions of maintenance, transport, anaesthesia, and marking of animals. When available, references should be added to justify that the techniques used were not invasive. When alternative non-harming

techniques exist, but were not used, the manuscripts may not be considered for publication.

Proofs and Reprints:

Proof will be sent to the **first (or corresponding) author** for checking (a PDF file) only once and it should be returned without delay. Corrections should be limited to typographical errors. No additional changes of the manuscript are allowed. Following publication, the first (or corresponding) author will be provided with electronic copy (PDF) of the article. Hardcopy reprints are no longer sent to the authors, since 2011.

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