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VERA ALEXEEVNA PODDUBNAIA-ARNOLDI (22.05.1902 – 18.03.1985) – COMMEMORATION OF THE 115 YEARS SINCE THE BIRTH OF THE GREAT EMBRYOLOGIST

A. CIUBOTARU[1], A. TONIUC[2], C. TOMA[2]

This article celebrates the personality of Russian Academician Vera Poddubnaia-Arnoldi on the occasion of 115 years since her birth. The great plant embryologist Vera Poddubnaia-Arnoldi was born on May 22 1902, in Kursk where she finished her secondary training in 1918. She continued her studies at the Moscow University - Faculty of Biology, graduating in 1925. Starting with 1929, when she publicly defended her PhD thesis, she activated at the Institute of Gummiferous Plants, where she organized the Laboratory of Cytology and Embryology whose coordinator/manager she was until 1934. Later she worked in the Laboratory of Cytology in the Institute of Phytotechnics (Leningrad), at the Institute of Genetics of the Academy of Sciences of the U.S.S.R (Moscow), at the Sugar Beet Institute (Tashkent), at the Agricultural Institute for Cereals (Moscow). Since 1950 until her death (1985) she worked at the Central Botanical Gardens of the Academy of Sciences of the U.S.S.R. (Moscow) in the Laboratory of Physiology of Plant Development. Professor Poddubnaia dedicated her whole life to science. In many of her works and monographic studies, published during several years, she demonstrated the importance of cytoembryological investigations not only for the systematics and phylogeny of plants but also for plant genetics and selection. The cytoembryological investigations performed by Prof. Poddubnaia-Arnoldi in the second half of the 20th century opened a new stage in comparative and experimental embryology of angiosperms.

Keywords: Vera Poddubnaia-Arnoldi, plant embryologist, 115 years since the birth.

The Russian embryologist Vera Poddubnaia-Arnoldi was born on May 22 1902, in Kursk, in the family of a legal expert. She spent her childhood and adolescence in Harkov, where she finished her secondary training in the year 1918. In 1920, the family moved to Krasnodar, where she attended the Faculty of Physics and Mathematics at the University of Kuban, while earning her living as an accountant. In 1921, she became a student of the Agricultural Institute, in the same town, until the year 1923, when she left for Moscow, continuing her studies at the University – Faculty of Biology, graduating in 1925. She was very active as a PhD student in the Department of Morphology and Systematics of Vascular Plants, led

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by M. I. Galenkin, where she specialized herself in cytology and embryology, under the guidance of Prof. C. I. Meier.

Starting with 1929, when she publicly defended her PhD thesis, she activated at the Institute of Gummiferous Plants, where she organized the Laboratory of Cytology and Embryology, which she coordinated until 1934. From family reasons, she moved to Leningrad in the same year. There she was employed as senior researcher at the Laboratory of Cytology in the Institute of Phytotechnics (guided by the famous cytologist G. Levițki). In 1936 she was conferred the scientific position of candidate in biological sciences. She returned to Moscow in 1939, where she worked as a senior collaborator at the Institute of Genetics of the Academy of Sciences of the U.S.S.R., under the guidance of Academician N. I. Vavilov. There she made research on the cytology and embryology of some hybrid and polyploid plants.

In 1942, the Poddubnaia-Arnoldi family moved to Tashkent, the Professor activating in the Department of Botanics as senior collaborator at the Sugar Beet Institute. Back to Moscow in 1943, she was employed at the Agricultural Institute for Cereals, in the Laboratory of Cytology and Embryology. In 1945 Vera Poddubnaia-Arnoldi defended her doctor habilitatus thesis entitled: "*Embryology of Angiosperms and its importance for systematics, selection and genetics*", a study awarded with the "V. L. Komarov" Prize in 1947. Since 1950 and until her death (1985) she worked at the Central Botanical Gardens of the Academy of Sciences of U.S.S.R. (Moscow), in the Laboratory of Physiology of Plant Development.

A. Ciubotaru (by that time a PhD student) made an especially useful internship in the laboratory of Prof. V. Poddubnaia-Arnoldi. On the very first day of his arrival (July 14, 1960), the plan of activities was elaborated and he received a desk, a microscope and a few boxes with permanent preparations, on which he could observe/study the embryology of various plant species (corn, kok-saghys, tau-saghys, etc.), apomixis, the influence of low temperatures upon the embryonic processes, etc.

At the same time, A. Ciubotaru received from Vera Poddubnaia-Arnoldi all preparations arranged in the order of their embryonic ontogeny (micro- and macrosporogenesis, gametogenesis, fecundation, embryogenesis) and he was told: "*Draw on paper what you consider important in the order of evolution of embryonic processes; in the end of the day we shall meet and discuss the aspects you observed*". Then she added: "*You should know that embryologists are not trained in universities, these institutions form specialists, while authentic embryologists are simply born*".

First, Prof. Vera Poddubnaia-Arnoldi suggested the young researcher that he should study the embryology of *Scorzonera tau-saghyz*, the gummiferous plant she

had analyzed herself, in parallel with the microsporogenesis of simultaneous type at *Carduus acanthoides* and then the macrosporogenesis, formation of the embryo sac, fecundation and embryogenesis of different other species.

In the notebooks of the year 1960 one may still find the drawings presenting the main stages of gametogenesis, formation of the embryo sac, of endospermogenesis and embryogenesis, as evidenced in the preparations of Vera Alexeevna, the differentiation of the morphology of chromosomes in metaphase included. Appreciating the quality of permanent preparations (fixation, colouring, differentiation, sectioning, selection of floral and embryogenic structures, etc.), prepared by Prof. Vera Poddubnaia-Arnoldi herself, it was extremely pleasant to evidence the distinctive morphological moments involved in the formation of some structure or another, such as, for example, the cells of macrosporal dyad, obviously differing among them, as well as the two cells of the bicellular proembryo. Such extremely important aspects (as to self-embryonic differentiation) may be analyzed only on a high quality experimental material, as the one prepared by Prof. Vera Poddubnaia-Arnoldi. At the same time, the formation and growth phases of the pollen tube from the pistil tissue, division of the generative cell and formation of sperms were drawn and evidenced. It was for the first time that A. Ciubotaru could follow, step by step, all embryonic phases, including the complete development of the seed in the above-mentioned species, as well as in *Triticum timopheevii* Zhuk. (tetraploide corn cultivated both as such and in the wild form).

An extremely valuable source of information had been for A. Ciubotaru the preparations of Prof. Poddubnaia-Arnoldi analyzed in chapter: “Anomalies in the process of sexual reproduction of *Scorzonera tau-saghyz*, induced by low temperatures”, devoted to the meiosis-megasporogenesis and the three consecutive divisions of the haploid nuclei in the embryo sac. Special stress was laid by Poddubnaia-Arnoldi on the modifications, deviations from the normal condition during meiosis, on the movements and attachments of chromosomes in meiosis and mitosis, on the occurrence of micronuclei in macrosporal cells, etc.

Prof. Vera Poddubnaia-Arnoldi introduced her PhD student to the formation of apomictic structures. If the normal embryonic development, namely: gametogenesis, fecundation and embryogenesis had been studied to a certain extent, at *Zea mays* L., apomixis as a phenomenon, the formation of the so-called restitutional nuclei in the pollen grain and the pre- and post-apomictic processes were wholly unknown. Once again, in the Laboratory of Vera Poddubnaia-Arnoldi he studied, for the first time, the non-reductional parthenogenesis in *Chondrilla pauciflora*. The PhD student A. Ciubotaru appreciated that the time of probation under the guidance of prof. Vera Poddubnaia-Arnoldi was also a very serious exam in cytoembryology, while never forgetting the example of high scientific stature of his mentor, who shaped the hard-working professional he was to become.

Along several years, Prof. Vera Poddubnaia-Arnoldi strived for applying her knowledge on embryological characteristics for solving various issues of the

systematics and phylogeny of the plant world. All these ideas, conceived by the renown embryologist K. Schnarf and analyzed in his work "*Comparative Embryology*" (1929), have been developed by Vera Poddubnaia-Arnoldi, who formulated a series of theoretical concepts in her monographic studies: "*Cytoembryology of Angiosperms*" (1976) and "*Characterization of angiosperm plant families on the basis of cytoembryological features*" (1982).

In this last monograph, Prof. Vera Poddubnaia-Arnoldi classified various taxonomic units starting from some distinct embryological characteristics, on considering the modifications of adaptable evolution, and showing that the duration of the changes observed in time and the character of the embryogenic processes are related to environmental factors, that different exogenous factors may modify the normal development of embryogenic processes. All these information had a great impact on the development of ecological and general cytoembryology, arousing a special interest in the beginning of the '80-ies.

Vera Poddubnaia-Arnoldi's contribution to the evolutional cytoembryology of gymnosperms, namely her theory that the development of embryonary ontogenetic characters in vascular plants should be considered exclusively in relation with their phylogenetic (and of habitat) evolution, was also highly appreciated.

In her works, Prof. Poddubnaia-Arnoldi demonstrated the importance of cytoembryological investigations not only for systematics, but also for genetics and selection. These ideas had been inspired by Academician N.I. Vavilov, in the years when Vera Poddubnaia-Arnoldi was working at the Institute of Phytotechnics.

It is not at all exaggerated to assert that the cytoembryological investigations performed by Prof. Poddubnaia-Arnoldi in the second half of the last century opened a new stage in comparative and experimental embryology of angiosperms. Starting from these analyses, developed for longer than half a century, she opened and developed new directions in contemporary plant embryology.

Mention will be made in the following of only some of her directions of research:

(1) She was the first scientist to launch thorough researches on the biology of flowering and multiplication of gummiferous plants growing on the territory of the former U.S.S.R., the information she provided being included in various monographs and university handbooks.

(2) She pointed out the causes of non-interbreeding (non-fecundation) in the process of distant hybridization; of interbreeding of plants with a different polyploid level; of sterility in hybrids and polyploids. She also elaborated a method for avoiding the cases on non-interbreeding (incompatibility) of distant species and genera, such as the one between the species of the *Triticum, Agropyron*, *Taraxacum,* and *Nicotiana* genera.

(3) Prof. Poddubnaia-Arnoldi, together with the reputed cytologist G. Levițski, were the first to investigate and describe the morphology of chromosomes in *Triticum* species, thus laying the bases for some new concepts concerning the formation of species and their evolution within the *Triticum* genus.

(4) Prof. Poddubnaia-Arnoldi and Acad. N. Tzitzin performed ample investigations devoted to the biology of corn and wheatgrass hybrids development, as well as to perennial corn species. The main aspects considered for analysis were: gametogenesis, embryogenesis and endospermogenesis of *Triticum* x *Agropyron* and *Secale* x *Agropyron* hybrids, which played an important role in obtaining *Triticale* forms.

(5) Vera Poddubnaia-Arnoldi analyzed in detail non-reductional parthenogenesis (embryo development in diploid egg cell) of *Chondrilla* and *Taraxacum* species, described the meiotic disorders in apomictic, polyploid and hybrid plants belonging to the above-mentioned species and provided important explanations concerning the classification of apomixis types which, in her opinion, are derived from amphimixis.

(6) In relation with the concept of *fertility* and *sterility*, Vera Alexeevna approached aspects of sexuality, thus enabling plants' classification by their sex.

(7) Vera Poddubnaia-Arnoldi is a pionieer in the application of biotechnological methods for obtaining *Orchidaceae* plants from seeds; as known, these seeds have practically no endosperm, so that between 5 to 7 years are necessary for their germination in the soil.

Many other scientific successes – during more than 5 decades of intense work – may be also mentioned, remarkable results which brought to Professor Vera Poddubnaia-Arnoldi the deserved position – recognized at worldwide level – of the most reputed plant embryologist of the XXth century.

Vera Poddubnaia-Arnoldi published over 180 scientific papers, among which three monographic studies of special scientific value: "*General Embryology of Angiosperms*" (1964), "*Cytoembryology of Angiosperms*" (1976), "*Characterization of angiosperm plant families on the basis of cytoembryological features*" (1982).

Apart from her scientific researches, Vera Poddubnaia-Arnoldi dedicated much of her time to pedagogic and social activities. She delivered lectures of "*General botanics and embryology*" to the PhD students of the Institute of Phytotechnics (Leningrad), of the Institute of Genetics (Moscow) and of Lomonosov University (Moscow). In this way, under her direct guidance, tens of young researchers from both her country and various other countries of the world acquired high scientific qualification and prepared valuable PhD theses.

For several years, Vera Poddubnaia-Arnoldi was the president of the Scientific Section of the Soviet-Indian Society for cultural relations; she participated in the organization of several congresses, conferences, symposia, in the U.S.S.R. and abroad, where she delivered important scientific papers.

It was in the Central Botanical Gardens of the Academy of Sciences of the U.S.S.R. that colleagues organized a special birthday celebration on April 25, 1982 to Vera Alexeevna Podubnaia-Arnoldi, a most active embryologist, scientist, biologist-botanist, well-known beyond the borders of U.S.S.R., a university professor, PhD, emeritus woman of science, winner of several scientific and civil prizes.

Having no children, she dedicated her whole life to science, an activity always full of satisfactions. She was a kind and good-humoured character, always friendly with all those surrounding her: friends, pupils, collaborators, co-workers, always modest in appreciating her capacities and achievements; she used to say that all her achievements were simply the result of systematic, sustained daily work. She loved life and valued friendship, she liked to joke and she was always smiling. However, during any discussion on concrete scientific issues she became serious, demonstrating her rich knowledge; during all meetings she used to have with her subordinates she laid stress on the fact that cytoembryology is necessary for solving punctual, concrete problems, such as practical selection and genetics of plants, systematics or phylogeny.

A year before her death, Professor Vera Poddubnaia-Arnoldi offered her impressive scientific library (numerous books and scientific papers received from embryologists all over the world) to the reputed embryologist A. Ciubotaru, from Kishinew, as a token of affection, but also for his contribution to the further development of plant embryology in the Laboratory of the Botanical Gardens (Institute) at the Academy of Sciences of the Republic of Moldova. Mention should be made of the fact that numerous other embryologists (not only from Iaşi) were to benefit from the valuable documents of this donation.

Professor Vera Alexeevna Poddubnaia-Arnoldi passed away on March 18, 1985, in a hospital of Moscow, and was buried near her husband, C. A. Arnoldi.

All her disciples, collaborators and admirers will never forget her formidable personality who instilled in the hearts of several generations and developed their genuine passion for such a complex domain, whose importance is still to be recognized: cytoembryology of plants.

PROFESSOR MASAHIRO KAMEKURA AT HIS 70TH ANNIVERSARY. A LIFE DEDICATED TO RESEARCH OF HALOPHILIC AND HALOALKALIPHILIC BACTERIAL AND ARCHAEAL MICROORGANISMS

MĂDĂLIN ENACHE[1]

The present paper is dedicated to Professor Masahiro Kamekura's 70th anniversary. The international scientific personality of Professor Kamekura is reflected in this paper. On the other hand, his warm support and co-operation with Romanian researchers from the Institute of Biology Bucharest of the Romanian Academy are mentioned.

Keywords: halophiles, archaea, salt, Masahiro Kamekura.

PREAMBLE

More than 20 years ago, at the Microbiology laboratory of the Institute of Biology Bucharest of the Romanian Academy, an enthusiastic research team decided to approach a research field, namely the biology of microorganisms living in hypersaline lakes, microorganisms called halophiles, a category of extremophiles. The primary step of the investigations was the choosing of the area of study, and the researches started on Techirghiol Lake and one year later, in 1997, the year when I started my activity in the Institute of Biology, the hypersaline lakes from Slanic Prahova were added as study area. Another step was dedicated to literature investigation in this field. In this frame I discovered that membrane lipid composition was representing a valuable taxonomic marker for differentiating between various genera of halophilic microorganisms from the family *Halobacteriaceae*. The highly valuable scientific personality in the field of membrane lipids from halophilic archaea I found to be Masahiro Kamekura PhD from Noda Institute for Scientific Research, Noda-shi, Chiba-ken in Japan. Considering the status of communications at that time at the Institute of Biology Bucharest, namely the absence of e-mail correspondence or the limited internet access, the best way to contact Masahiro Kamekura PhD was a letter in which I asked a copy of the scientific paper representing the research field started in my laboratory. Few weeks later, an impressive big envelope containing a lot of reprints

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from dr. Kamekura's valuable work (Fig. 1) in the field of halophilic microorganisms arrived in my laboratory and marked the starting point of the most important international co-operation of my laboratory and a big change in my scientific activity.

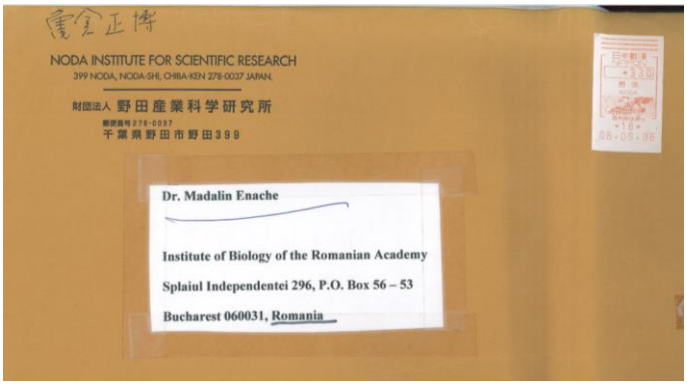


Fig 1. An example of envelope received along of years at the Institute of Biology Bucharest.

EDUCATION PERIOD OF DR. MASAHIRO KAMEKURA

Professor Masahiro Kamekura, currently in the 70th year of his life, was born in October, 7 1946 in Nagaoka, Niigata-ken, Japan. In 1965 he started the study at the Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Japan, one of the most important Universities in the world, known for the high standard in education and research. In 1969, the student Masahiro Kamekura became a graduate of the above mentioned Faculty and obtained license, a bachelor degree in the field of agriculture. Two years later, in 1971 he obtained Master's degree at the same prestigious University in Japan with his enzymological study on D-maltose oxidase from a plant pathogenic fungus, *Gloeosporium kaki* Hori. In the same year, he started his internationally valuable and appreciated scientific activity at Noda Institute for Scientific Research in Japan. Starting the research activity with a highly halophilic strain belonging to *Micrococcus* genus (presently *Nesterenkonia*), the young researcher Masahiro Kamekura performed a couple of studies on enzymes of *Micrococcus varians* subsp. halophilus and obtained the doctor's degree in agriculture at the University of Tokyo in 1980. In the period 1982-1985, Masahiro Kamekura PhD started his postdoctoral studies at the University of Ottawa in Canada, under the supervision of some distinguished scientists in the field of halophilic microorganisms, D.J. Kushner PhD and Morris Kates PhD. At this time, Masahiro Kamekura PhD firstly met some Romanian scientists, taking into account that Morris Kates PhD was born in Galati, Romania, and also Natalia Moldoveanu PhD working in his laboratory was a former employee of the Central Institute of Biology Bucharest.

INTERNATIONAL SCIENTIFIC PERSONALITY OF PROFESSOR MASAHIRO KAMEKURA

The impact of Prof. Kamekura's research activity into fields of halophilic microorganisms is reflected in more than 150 scientific papers published in prestigious international journals, complemented with a lot of reviews and materials in Japanese language dedicated to halophilic microbial world. On the other hand, Kamekura PhD gave more than 20 lectures at various highly recognized scientific meetings like FEMS congresses, IUMS congresses, ASM meetings and assembly, Halophiles – exploring life at high salinity conference, etc. His research interest into systematics and phylogeny of halophilic and haloalkaliphilic *archaea* and *bacteria* is reflected by proposing and contributing to propose a large number of new genera and new taxa, for example *Haloferax* and *Haloarcula* genera [24], *Halorubrobacterium* (today *Halorubrum*) and *Natrialba* genera [17], *Natronomonas* genus [19], *Halarchaeum* genus [22] etc. An important review related to the diversity of halophilic microorganisms pertaining to *Archaea* domain was also published in 1998 [20].


(a)

(b)

(c)

(d)

(e)

Fig 2. Pictures of Prof. Masahiro Kamekura:visiting the symbol building of the Romanian Parliament– (a); at the University of Bucharest, Faculty of Chemistry by courtesy of Prof. Elena Volanschi and Mirela Enache PhD – (b); inside salt mine Unirea, Slănic Prahova, together with Madalin Enache PhD (c);– giving a lecture at halophilic meetings in Slovenia (d); an enjoy to this meeting (e). Source for the picture d) and e) is: http://web.bf.uni-lj.si/bi/halophiles/gallery.html

The scientific interest of dr. Masahiro Kamekura in haloenzymes (enzymes obtained from halophilic microorganism) is reflected in a number of papers dedicated either to halolysin R4 [18], nuclease [10,11] or other proteases [13,15].

His passionate work in the fascinating field of membrane lipids of halophilic microorganisms could be discovered in numerous papers and review articles or book chapters representing important materials both for students or teachers involved into the field of extremophilic microorganisms [16,21].

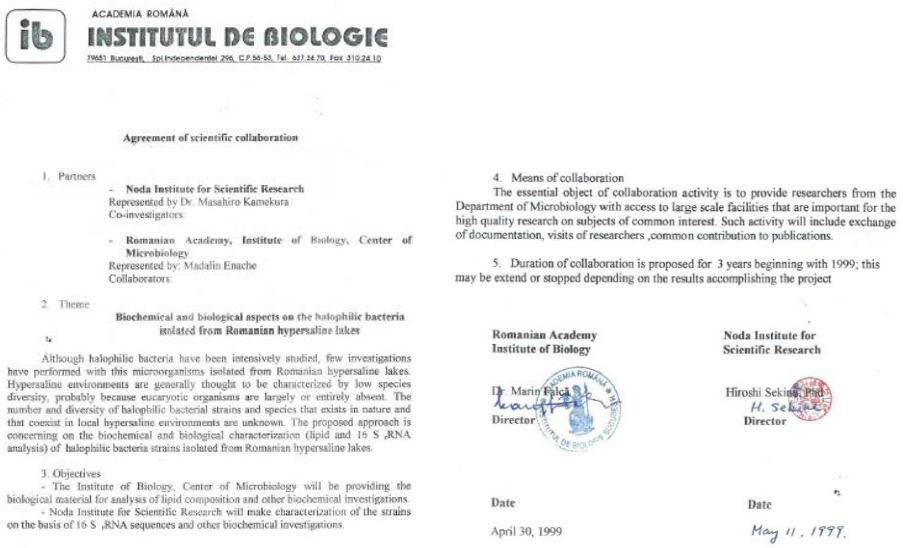
The effect of bacto-peptone composition of the culture medium, namely the bile acids content, for halophilic microorganisms investigations is reflected also in several papers [12,14], representing nowadays the basis for differentiating the domains *Bacteria* and *Archaea*.

As a leading scientist into the field of halophilic microorganisms, Prof. Kamekura is working as a member of the International Committee on Systematics of Prokaryotes, Subcommittee on the Taxonomy of *Halobacteriaceae* from 1996 and he is a founding member of the Japan Society for Archaea (1998) and Japanese Society for Extremophiles (1999). He is also a member in the scientific board of specific journals like Extremophiles, Romanian Journal of Biology – Plant Biology, etc. As a model for those who want to have a career into the field of halophilic microorganisms research, Prof. Kamekura shared his expertise with students from various universities in Japan like Tokyo University, Tsukuba University and others, giving lectures in different microbiology areas. He has been a visiting Professor of Toyo University since 2000.

THE SCIENTIFIC SUPPORT OF PROF. MASAHIRO KAMEKURA FOR ROMANIAN SCIENTISTS IN THE HALOPHILIC AREA

The warm and kind support and cooperation of Prof. Masahiro Kamekura with researchers from the Institute of Biology Bucharest of the Romanian Academy along the years, started from 1997 and is revealed by scientific papers published in various important Romanian scientific journals and book chapters in international prestigious publishing houses. Also, a scientific agreement of cooperation between the Institute of Biology Bucharest and Noda Institute for Scientific research was signed in 1999 revealing the warm support on behalf of Prof. Kamekura (Fig. 3).

On the other hand, Prof. Masahiro Kamekura visited the Institute of Biology Bucharest in 2006 (first trip in Romania) and gave an impressive lecture about the presence of halophilic microorganisms in environments apparently hostile for this halobacteria[2] and also in 2008, for attending the exploratory workshop "Extremophilic microorganisms: molecular adaptations and bionanotechnological applications" and gave the lecture "Enigmatic extreme halophiles and an enzyme nucleoside diphosphate kinase".



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Agreement of scientific collaboration

1. Partners
- **Noda Institute for Scientific Research**
Represented by Dr. Masahiro Kamekura
Co-investigators:
- **Romanian Academy, Institute of Biology, Center of Microbiology**
Represented by: Madalin Enache
Collaborators:

2. Theme
Biochemical and biological aspects on the halophilic bacteria isolated from Romanian hypersaline lakes

Although halophilic bacteria have been intensively studied, few investigations have performed with this microorganisms isolated from Romanian hypersaline lakes. Hypersaline environments are generally thought to be characterized by low species diversity, probably because eucaryotic organisms are largely or entirely absent. The number and diversity of halophilic bacterial strains and species that exists in nature and that coexist in local hypersaline environments are unknown. The proposed approach is concerning on the biochemical and biological characterization (lipid and 16 S ,RNA analysis) of halophilic bacteria strains isolated from Romanian hypersaline lakes.

3. Objectives
- The Institute of Biology, Center of Microbiology will be providing the biological material for analysis of lipid composition and other biochemical investigations.
- Noda Institute for Scientific Research will make characterization of the strains on the basis of 16 S ,RNA sequences and other biochemical investigations.

4. Means of collaboration
The essential object of collaboration activity is to provide researchers from the Department of Microbiology with access to large scale facilities that are important for the high quality research on subjects of common interest. Such activity will include exchange of documentation, visits of researchers ,common contribution to publications.

5. Duration of collaboration is proposed for 3 years beginning with 1999; this may be extend or stopped depending on the results accomplishing the project

Romanian Academy
Institute of Biology
Dr. Marin Falca
Director

Noda Institute for
Scientific Research
Hiroshi Sekiguchi, Phd
Director

Date
April 30, 1999

Date
May 11, 1999.

Fig 3. Scientific agreement of cooperation between the Institute of Biology Bucharest and Noda Institute for Scientific Research, signed in 1999.

The support of Prof. Kamekura for our research team resulted in publishing of the first holoarchaean taxon from Romania, namely *Haloferax prahovense* [4]. The cooperation is also reflected in a paper revealing the potential use of rpoB' gene and protein sequences for inferring relationships in family *Halobacteriaceae* [3]. The predominant presence of halophilic microorganisms in several saline environments from Romania is reflected also in various papers [5,6]. The biotechnological potential of halophilic microorganisms has been revealed towards degrading of pesticides [23] or by the economical values of halophilic enzymes [7]. The investigation of the presence of microorganisms in evaporate and rock salt was started in our laboratory following the recommendation of Prof. Masahiro Kamekura. The results were reflected in various papers published by Cojoc *et al.* 2009 [1], Enache *et al.* 2012 [8], Enache and Kamekura 2010 [7], Enache *et al.* 2015 [9].

The cooperation and warm support along the years on behalf of Prof. Masahiro Kamekura for our research team from the Institute of Biology Bucharest of the Romanian Academy is reflected as follows:

- book chapters:

1. Mădălin Enache, Gabriela Teodosiu, Takashi Itoh, Masahiro Kamekura, Helga Stan-Lotter, 2016, *Halophilic microorganisms from man-made and natural hypersaline environments: physiology, ecology and biotechnological potential*, In: Adaptation of Microbial Life to Environmental Extremes, Eds. Helga Stan-Lotter & Sergiu Fendrihan, Springer, Wien, New York, 2nd edition.

2. Mădălin Enache, Roxana Cojoc, Masahiro Kamekura, 2015, Halophilic Microorganisms and Their Biomolecules: Approaching into Frame of Bio(Nano) Technologies, In *Halophiles*, Sustainable Development and Biodiversity 6, D.K. Maheshwari, M. Saraf (eds.), Springer International Publishing Switzerland,161-172, ISBN 978-3-319-14595-2_5

3. Mădălin Enache, Gabriela Popescu, Takashi Itoh, Masahiro Kamekura, 2012, *Halophilic microorganisms from man-made and natural hypersaline environments: physiology, ecology and biotechnological potential*, In: Adaptation of Microbial Life to Environmental Extremes, Eds. Helga Stan-Lotter & Sergiu Fendrihan, Springer Wien New York, 173-197, ISBN 978-3-211-99690-4.

- scientific papers:

1. Mădălin Enache, Roxana Cojoc, Akinobu Echigo, Hiroaki Minegishi, Takashi Itoh, **Masahiro Kamekura**, 2014, *Taxonomy of a novel extremely halophilic archaeon belonging to the genus Haloarcula isolated from a low saline environment, Techirghiol Lake, Romania*, Oltenia. Studii şi Comunicări. Stiinţele Naturii, **30**, 192-197.

2. Mădălin Enache, **Masahiro Kamekura**, 2013, *Halophilic archaea in the Neogene salt massif from Slănic Prahova, Romania*, Oltenia. Studii şi Comunicări. Stiinţele Naturii, **29**, 247-253.

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CONCLUDING

Distinguished Professor, at this anniversary moment, I wish you "Many and very good years" from this moment on, together with your family, students and collaborators!

せんせい、ななじゅっさいをむかえられた、このよきひから、かぞく、がくせい、そして、きょうどうけんきゅうしゃといっしょに「さらにおおくの、そしてじゅうじつしたとしつきを」すごされるよう、おいのりいたします。

Distinse domnule Profesor, la acest moment aniversar vă dorim „La mulţi ani!”, multă sănătate şi împliniri alături de familie, studenţi, colaboratori.

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CHOROLOGY OF *STIGMIDIUM* GENUS IN ROMANIA

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The research activity based on identification of lichen species tabulated in *Stigmidium* genus was performed between 2009-2015 especially in forestry areas. Only one species of this genus, namely *Stigmidium microspilum* (Körb.) D. Hawksw was found in Botoșani and Suceava counties within Crujana and Tudora reserves. Moreover, future researches on chorology of *Stigmidium* genus are of a great importance for the lichen flora of Romania.

Keywords: chorology, *Stigmidium*, Romania.

INTRODUCTION

In Romania *Stigmidium* genus is represented by three species, namely *Stigmidium microspilum* (Körb.) D. Hawksw., *Stigmidium cerinae* Cl. Roux et Triebel and *Stigmidium rouxianum* Calatayud and Triebel. Species of this genus are lichenicolous fungi known as parasites on other lichen species (Alstrup and Olech, 1993; Calatayud and Triebel, 2003; Khodosovtsev *et al.*, 2013). The investigated genus comprises over 90 taxa based on a species type termed *Stigmidium schaereri* A. Massal. (Kocourková and Knudsen, 2012). *Stigmidium* genus is represented by lichenicolous ascomycetes and a lot of species belong to a distinctive phylogenetic group (Zhurbenko and Triebel, 2008).

The aim of this study is based on the knowledge of the *Stigmidium* genus chorology on the Romania territory. The objective of the study consists in the mapping of *Stigmidium* distribution in Romania.

MATERIALS AND METHODS

The researches regarding the distribution of *Stigmidium* genus on the Romania territory were performed from 2009 (March) till 2015 (December). The chorology of *Stigmidium* genus and taxonomy are based on Ciurchea (2004) work and informations from a database found to following link: www.mycobank.org.

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RESULTS AND DISCUSSION

General distribution of *Stigmidium microspilum*

In Europe *S. microspilum* is widely distributed as a parasite specimen (Kocourková and van den Boom, 2005; Motiejūnaitė *et al.*, 2012). Thus it was found within the following countries: **Czech Republic**, Bohemia region, on *Graphis scripta* whose host trees were *Pinus* and *Fraxinus excelsior* (Kocourková and van den Boom, 2005), **Denmark**, Northeast Zealand district, Jægerspris Nordskov, on *G. scripta* and East Jutland district, Gydelløkke, on *G. scripta* (Alstrup *et al.*, 2004), **France**, Ardennes Department, on *G. scripta* found on corticolous substrata (Diederich *et al.*, 2006), **Germany**, Lower Saxony, on *G. scipta* (Otte *et al.*, 2006), Swabia, on *G. scripta*, Upper Bavaria and Lower Bavaria, on *G. scripta* (Triebel and Scholz, 2001; von Brackel, 2009), **Estonia** without any indication of locality and its host (Motiejūnaitė *et al.*, 2012), **Lithuania**, Asveja Regional Park, on *G. scripta*, **Spain**, Navarra Province, on *G. scripta* (Etayo and Diederich, 1998), **Sweden**, Skåne Province, on *G. scripta* hosted by *Fraxinus* (Santesson, 1986), **Switzerland**, Swiss Alps, Jura Mountains, on *G. scripta* growing on beech (von Brackel, 2013).

Distribution of *Stigmidium microspilum* in Romania

Old Literature Data

Caraș-Severin County, the Banat Mountains, Domoglet Mountain, Băile Herculane (Ciurchea, 2004).

Original data

Suceava County, Crujana Forest Natural Reserve, on *G. scripta* sampled on *Fagus sylvatica* L., leg. Vicol Ioan, 27.06.2013, det. Vicol Ioana, 02.07.2013, [BUCM L2033].

Botoșani County, Tudora Forest Natural Reserve, on *G. scripta* sampled on *Quercus* sp., leg. Vicol Ioan, 19.08.2013, det Vicol Ioana, 16.09.2013 [BUCM L2122].

Taxonomy

Stigmidium microspilum syn. *Arthopyrenia microspila* Körb., *Pharcidia microspilum* Körb., *Pharcidia microspila* (Körb.) G. Winter, *Pyrenula rhyponta* Hepp non Ach., *Arthopyrenia rhyponta* Mass., *Arthopyrenia rhyponthella* Lojka, *Pyrenula rhyponta* Trevis. non Ach., *Verrucaria microspila* (Körb.) Harm. This genus is tabulated within Ascomycota Class, Pyrenocarpeae Series, Dothideales Order, Arthopyreniaceae Family, Fungi non-lichenized (Ciurchea, 2004).

Thallus morphology

Stigmidium microspilum is a parasitic species on *Graphis scripta* (L.) Ach. The thallus is recognized as grayish or blackish spots on host thallus. This species has 1-septate ascospores, with a median constriction, thin septa, thin mucous coating, ascospores contain oil drops. Ascospores have the following dimensions: (13) 14 – 19 (20) × (3) 4 – 5 μm (Ciurchea, 2004).

New records for Romania

Two other lichen species such as: *Stigmidium cerinae* Cl. Roux and Triebel identified on *Lecanora epibryon* (Ach.) Ach., in Hunedoara County, Retezat Mountains (Vondrák and Liška, 2013) and *Stigmidium rouxianum* Calatayud and Triebel found on *Acarospora cervina* A. Massal. in Caraș-Severin County, Banat Mountains, Domoglet Mountain, Băile Herculane (Vondrák and Šoun, 2008).

As distribution, on the one hand *S. cerinae* is found in Austria, Germany, Swizerland and Italy (Roux and Triebel, 1994), and one the other hand *S. rouxianum* was identified in several countries such as: France, Italy, Spain, Switzerland, Czech Republic, Ukraine, Russia (Calatayud and Triebel, 2003; Urbanavichus *et al.*, 2011; Vondrák and Šoun, 2008).

CONCLUSIONS

Although a lot of trees with smooth rhytidome such as: beech, cherry and hornbeam were sampled within natural and seminatural forest habitats (com. pers.), no chorological data about *Stigmidium* genus were obtained, with the exception of Crujana and Tudora forest natural reserves. *S. microspilum* is easy to see on its host, thereby it cannot be overlooked. A plausible explanation consists in that it is rather uncommon in Romania; therefore, further investigations are needed.

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A REPORT ON ANTILEISHMANIAL (PROMASTIGOTE STAGE OF *Leishmania donovani*) ACTIVITY OF DEUTEROMYCOTA FUNGUS *Fusarium oxysporum* f. sp. *cubense*

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The leishmaniasis caused by 17 species of protozoan parasite *Leishmania*, is a major infectious disease in many parts of the world including Asia, Africa, Southern Europe and South and Central America. Nearly two million people are affected annually by this parasite and 350 million are vulnerable to attack by the pathogen . Visceral leishmaniasis is one of the four major clinical forms of leishmaniasis which frequently assumes epidemic proportion resulting in high mortality rate. India is also a major hot spot of visceral leishmaniasis called *Kala-azar* caused by *Leishmania donovani* .The parasite exists as flagellated promastigote in the female sandfly vector and as amastigote in the mammalian host. In the present paper, we report the efficacy of crude soluble extract (CSE) prepared from the fungus *Fusarium oxysporum* f.sp. *cubense* for its leishmanicidal (promastigote stage) activity. The CSE (crude soluble extract) of *F. oxysporum* f.sp. cubense isolated from rhizosphere of banana var. Malbhog, has been evaluated as an anti-leishmanial agent on promastigotes of *L. donovani* and compared with leishmanicidal effect of two well known anti-leishmanial drug SAG (Sodium Antimony Gluconate) and Amphotericin–B. *L. donovani* promastigotes were cultured (2 x 10^6 p/mL) in presence of fungal CSE as well as SAG and Amphotericin-B individually. Significantly, CSE (0.5 mg/ml) of *F. oxysporum* showed 100% inhibition of *Leishmania* promastigote proliferation in 48 hours while Amphotericin-B and SAG showed 100% and 44% parasite inhibition respectively.

Keywords: *Leishmania donovani*, visceral leishmaniasis, *Kala-azar*, *Fusarium oxysporum* CSE (crude soluble extract), Amphotericin-B, SAG (Sodium Antimony Gluconate).

INTRODUCTION

The leishmaniasis, caused by a protozoan parasite *Leishmania* belonging to family Trypanpsomatidae, is a major infectious health hazard in many parts of the world including Asia, Africa, Southern Europe and Latin America (WHO Expert Committee, 1984). Nearly two million people are affected annually by this parasite

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(Ashford *et al*., 1992). The disease affects people mostly belonging to rural areas or low socio-economic strata. In India the disease called visceral leishmaniasis or *kala-azar* is caused by *Leishmania donovani* . Since no vaccine has been reported so far, chemotherapy is the only effective method of treatment of *Kala-azar*. However, there are several reports indicating inefficacy of many anti-leishmanial drugs in different parts of the world (Faraut – Gambarelli *et al*., 1997; Lira *et al*., 1999) and also in State of Bihar, India (Thakur *et al*., 1998; Sunder *et al*., 2001) where the disease frequently assumes epidemic proportion. Sodium Antimony Gluconate (SAG) was considered to be the most preferred drug for leishmaniasis in the past that has shown clinical resistance in most of the patients of *Kala-azar* (Thakur *et al*., 1998; Sunder *et al*., 2001; Narayan, 2001; Singh, 2008). The other drugs are either toxic or too expensive for the poor patients. Miltefosine, an anti-neoplastic agent is being used in recent years for the treatment of 'Visceral Leishmaniasis', but extensive clinical trials and treatment need more validation before accepting it as an effective drug for *Kala-azar*. The most important drug is the Amphotericin-B which is highly effective but very costly. The situation seems aggravated as there have been several reports of co-infection with HIV in *Kala-azar* (Thakur *et al*., 1998; Sinha *et al*., 2001). The global demand for complementary and alternative medicine (CAM) has encouraged the screening of herbal plants for leishmainicidal activities (Wright *et al*., 1990; Iwu *et al*., 1994; Akendengue *et al*., 1999; Chenari and Nazer, 1999; Khalid *et al*., 2005; Luize *et al*., 2005; Ganguli *et al*., 2006; Khan *et al*., 2009; Singh *et al*., 2011). However, very few reports on bioprospecting of fungi as antileishmanial agent have been recorded (Martinez-Luis *et al*., 2008; Rosa *et al*., 2009; Rosa *et al*., 2010; Valadares *et al*., 2011; Valadares *et al*., 2012) . Keeping this in view, we have attempted to test the crude soluble extract (CSE) of *Fusarium oxysporum* f. sp. *cubense* (Deuteromycota) for its leishmanicidal (promastigote stage) activity.

MATERIALS AND METHODS

Isolation of *Fusarium oxysporum* f.sp. *cubense*

Rhizosphere and rhizoplane cultures were used to isolate *Fusarium oxysporum* following standard methods (Mahadevan and Sridhar, 1986). For the isolation of fungus infected Malbhog banana rhizomatous parts were collected and sealed in small polythene bags using sterile scissors and scalpels. The collected materials were brought to the laboratory for further investigation and experimentation. The infected rhizomatous explants were kept for thorough washing under tap water during 30 min for washing soil and removal of other dried parts. After washing in tap water, the explants were washed by using a liquid detergent soap (Teepol). It was followed by washing with sterilized distilled water

containing few drops of Savlon. The explants were again washed by sterilized distilled water 2-3 times. These explants were cut into pieces of desired size. The cut pieces were again washed by sterilized water and inoculated on PDA medium (Potato Dextrose Agar Medium containing Rose Bengal). The inoculated explants were cultured at 30 ± 2^0 C for a week. Pure cultures were obtained from the fungal colonies appearing in the culture tubes by subculturing. The identification of the fungal species was confirmed by their morphology and spore characteristic following Blanchard and Tattar (1981).

The protocols described by Singh (2008) and Singh *et al.* (2011) have been followed in the present experiment for culture of *L.donovani,* preparation of crude soluble extract of *Fusarium oxysporum* and evaluation of leishmanicidal activity fungal CSE.

Preparation of *Fusarium oxysporum* f. sp. *cubense* Crude Soluble Extract (CSE)

Fusarium cultures containing mycelium and spores were crushed and centrifuged at 10,000 X g for 30 min in refrigerated centrifuge (Kubota 20,000 Japan). The supernatant (Crude Soluble Extract) was poured in Petri dishes and evaporated under reduced pressure at 37^0 C to obtain a dry extract. The dried extract was weighed and dissolved in distilled water (100 mg/mL) and stored at -70^0 C for further experiment.

Culture of *Leishmania donovani*

L. donovani amastigotes aspirated from the spleen of SAG (Sodium Antimony Gluconate) unresponsive *Kala-azar* patients were cultured at 24^0 C in RPMI-1640 (Hi-Media) supplemented with 2000mg/L $NaHCO_3$, 20% heat inactivated FCS (Sigma,USA), 20 mg/L Gentamycin, 100 U/mL Streptomycin and 100 U/mL Penicillin.

Test of leishmanicidal activity of Crude Soluble Extract (CSE) prepared from *Fusarium oxysporum* f. sp. *cubense*

2×10^6 Promastigotes/mL of early phase (96 hour culture) *L. donovani* culture grown in monophasic medium as described above were dispensed into each well of 24 well culture plate in total volume of 1mL of RPMI complete medium. The minimum effective concentration of fungal CSE (0.5 mg/mL) was added in the culture well. Further, two well known leishmanicidal agents SAG (Albert Devid Ltd. Calcutta, India) and Amphotericin-B (E.R. Squibb and Sons, NJ, USA) were added in separate wells at 20 µg/mL and 1 µg/mL concentration respectively as positive control. Cultures supplemented with equal volume of normal saline (0.85% w/v NaCl, Merck, India) were set as negative control. Each experiment was set in duplicate and they were repeated five times. The treated promastigotes were incubated at 24^0 C $\pm$ 2^0 C and studied microscopically after 24 h and 48 h of incubation and the effect of treatment

was studied by counting the parasite using Neubauers Chamber (Jaffe *et al.*,1984). The data obtained have been presented as mean value and were used to analyze percentage inhibition of Leishmania replication using the formula:

$$\% \text{ inhibition} = \frac{A-B}{A} \times 100$$

where A = number of parasites in culture treated with normal saline,
B = number of parasites after CSE treatment.

RESULTS AND DISCUSSION

The present study evaluates the effect of CSE (crude soluble extract) prepared from the fungus *F. oxysporum f. sp. cubense* causing 'Panama Wilt' of banana has been evaluated for its anti-promastigote activity in *L. donovani*. Fig. 1 describes the % inhibition in replication of *L. donovani* promastigotes after 24 h and 48 h of culture in the presence or absence of 0.5 mg/mL of fungal CSE which was found to be the minimum effective concentration (Singh, 2008). In 24 hours of treatment with the fungal CSE at the concentration of 0.5 mg/mL showed 41 % inhibition, while after 48 hours of treatment the % inhibition induced by the fungal CSE was found to be 100 %. The corresponding values have been compared with % inhibition in replication of *L.donovani* promastigotes caused by two established anti-leishmanial drugs (SAG and Amphotericin-B) at their minimum effective concentration (Singh, 2008; Singh *et al.*, 2011). Leishmanicidal effect of two known anti-leishmanial drugs viz. SAG and Amphotericin-B showed different results. SAG induced 57% and 44% parasite inhibition after 24 h and 48 h of treatment respectively, while Amphotericin-B showed 100% inhibition of replication of *L.donovani* promastigotes in 24 h of treatment.

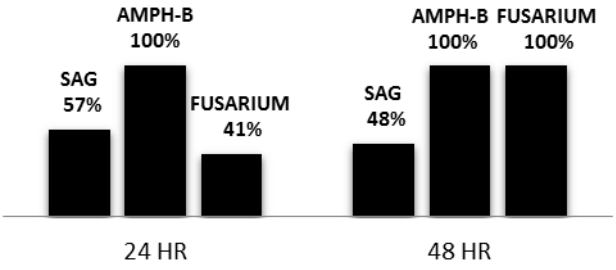

Fig. 1. Comparative efficacy and Leishmanicidal effect of *Fusarium oxysporum* crude soluble extract and existing antileishmanial drugs SAG (Sodium Antimony Gluconate) & Amphotericin-B in terms of percentage inhibition of replication of *L. donovani* promastigotes after 24 h and 48 h of treatment.

In recent years, search for new and effective agents for the treatment of leishmaniasis has gained momentum (Wright *et al.*, 1990; Iwu *et al.*, 1994; Akendengue *et al.*, 1999, Chenari and Nazer,1999; Khalid *et al.*, 2005, Luize *et al.*,2005; Ganguly *et al.*, 2006) as the disease is yet to find a satisfactory cure in the absence of effective chemotherapy. Clinical resistance of anti-leishmanial drugs especially SAG has been frequently reported in patients of kala-azar (Thakur *et al*., 1998; Sunder *et al*., 2001). While other drugs Pentamidine, Amphotericin-B or Miltefosine are either very toxic or too costly for the affected people. Some other drugs Paromycin, Sitamaquin etc. are still under clinical trial. Thus, attempts are being made globally to find some novel, cost effective alternative for the disease. The screening for leishmanicidal effect is usually done in promastigote culture by determining inhibition (in %) by counting after 24 h of incubation with or without the test chemical (Plock *et al.*, 2001). While most of the studies are based on observations made within few hours or within 24 h of promastigotes culture with drug. In our experiment, the observations were also taken after 48 h of culture in order to study whether the fungal CSE had any time dependent interventions on the growth of *Leishmania* promastigotes *in vitro*. Although, there are reports on anti-leishmanial properties of plants belonging to family Asteraceae, Meliaceae, Papilionaceae, Mimosaceae, Liliaceae and Bignoniaceae (Guru, 1992; Chenari and Nazer, 1999; Plock *et al.*, 2001; Khalid *et al.*, 2005; Luize *et al.*, 2005; Ganguly *et al.*, 2006; Singh *et al.*, 2011) but except few, most of these have been studied in cutaneous form of leishmaniasis. Recently, the extracts of fermentation broth and mycelium of the endophytic fungus *Edenia* sp. (Ascomycota) have been reported to cause significant inhibition in the growth of amastigote form of *L.donovani* (Martinez-Luis *et al.*, 2008). In another report, the basidiomes and fermentation broth extracts from *Gymnophilus areolatus, Irpex lacteus, Lentinus strigosus, Nothopanus hygrophanus, Pleurotus flabellatus* and some unidentified Basidiomycetes were found to be toxic to amastigotes of *L. amazonensis* (Rosa *et al.*, 2009). *F. oxysporum* , isolated from the host *Palicourea tetraphylla* Cham. & Schltdl. which was identified on the basis of sequencing of ITS region, have been found to produce bioactive metabolites showing 90% killing of amastigotes of *L. amazonensis* (Rosa *et al.*, 2010). Valadares *et al*. (2011, 2012) have found the extracts of *Agaricus blazei* Murill highly effective in killing promastigotes and amastigotes of *L. amazonensis, L. chagasi, L. major*. Fusaric acid (5- butyl picolinic acid) which is an important constituent of the fungus *F. oxysporum* has been orally administered for treatment of human squamosa cell carcinoma of the head and neck (Ruda, 2006). It has also been granted US patent (No. 4124715) for the treatment of craving and withdrawal syndrome associated with person addicted to narcotics and amphetamines (Jose, 1978). In the present work, we have used the fungus *F. oxysporum* f. sp. cubense belonging to Deuteromycota isolated from Malbhog banana, for the first time and very significantly it has been found to possess leishmanicidal activity (promastigote stage) nearly comparable to Amphotericin-B. Thus, keeping in view the cost and effect, the fungal CSE seems to be a potential source with considerable pharmaceutical implication in kala-azar.

CONCLUSIONS

In conclusion, this study has shown that *F. oxysporum* crude soluble extract (CSE) possesses bioactive metabolite(s) showing 100% inhibition of replication in *L.donovani* promastigotes in 48 h of treatment and may be explored as a potential source of valuable drug for use in anti-leishmanial therapy.

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SURVEY OF AFFECTED ECOLOGICAL FACTORS TO *JUNIPERUS EXCELSA* REGENERATION IN GHASEMLOU VALLEY BY EMPLOYING THE ECO-PHYTOSOCIOLOGICAL METHOD

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Physiographic maps of *Juniperus excelsa* distribution including hypsometric, slope and direction maps have been carried out at the scale of 1:8854 in the studied area by ArcView 3.2a. To study the field, Braun-Blanquet method was used. Sample plots located as a randomly systematic method used quadrate minimum area ($40\times40m^2$). The relationship between qualitative and quantitative species features and ecological properties were determined using CCA ordination method through PC-ORD Ver.4. The establishment of species had positive correlation with slope degree and direction, soil texture, pH and altitude. Sorensen similarity index surveyed between communities which contained *Juniperus excelsa* species using NTSYS-pc Ver.2.02e.

Keywords: *Juniperus excelsa*, phytosociology, ecological factors, ordination, Ghasemlou valley.

INTRODUCTION

Junipers are evergreen shrubs or trees belonging to the family *Cupressaceae*. *Juniperus excelsa*, with the Persian name of "Arduj" (Sabeti, 1975), include the upright-standing species (Karouri *et al.*, 2000; Lavina 1984, Negussie, A. 1991, 1995) with needle-shaped leaves in Iran. *J. excelsa* subsp. *excelsa* is a medicinal plant (Hooper and Field, 1937; Yesilada *et al.*, 1995; Muhammad *et al.*, 1992; Singh, 1995; Sadeghi-aliabadi *et al.*, 2009). *J. excelsa* is included in the IUCN list of threatened conifers. It has been stated recently that the natural regeneration rate of juniper depends on several ecological factors including both biotic and abiotic, pests and diseases being the major negative factor (Vardanyan 1993).

The main objectives of this investigation are: (I) Phytosociological study of *Juniperus excelsa*, (II) survey of affected ecological factors of regeneration of *Juniperus excelsa* in this area, (III) Biometrical study and species healthiness degree (IV) study of distribution of *Juniperus excelsa* in the field.

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MATERIALS & METHODS

Site Description and Techniques

Ghasemlou valley forest reserve and its adjacent region with a surface area of 577 ha and defined by the coordinates latitude 37° 15' to 37° 20' North and longitude 45° 5' to 45° 10' East, lies in the south of Urmia province. At the outset, physiographic mapping (hypsometric map, slope facing and direction map) of the regional distribution of *J. excelsa* species has been carried out at the scale of 1:8854 at the studied area using GPS data, and then the regional boundary along with the interior regional scale have been calculated by Arc View 3.2a. (Figure 1). To study the field, the Braun-Blanquet method was used. Sampling was done by using quadrate minimum area within the plots. Sample plots located as a randomly systematic method within *Juniperus excelsa* community in preserved and non-preserved regions. In order to do an ecological analysis of the targeted species, the plots of $40\times40m^2$ have been positioned in the area and all the other qualitative, quantitative properties of the trees within the plots have been registered. In these plant units, sampling parameters like the number of the trees in the area, the trees heights, the stem diameter, the trees canopy diameters, the small and large canopy diameter, the productivity trend, the habitat properties, tree community and their shrub community, the vitality and health of the trees and the other qualitative, quantitative parameters have been measured and recorded (Table 3). The relationship between these ecological variables and species traits were determined using CCA ordination method through PC-ORD Ver.4. Phytosociological studies were surveyed by Sorensen similarity index between *Juniperus* community with other communities using NTSYS-pc Ver.2.02e. In order to determine the coherence and non-coherence of the plant coverage of *Juniperus* species, frequency classes were used. In this calculation 5 frequency classes were taken into consideration.

RESULTS

Physiographic Maps

Physiographic mapping (hypsometric map, slope facing and direction map) of the regional distribution of *J. excelsa* species has been carried out at the scale of 1:8854 at the studied area based on GPS data, and then the regional boundary along with the interior regional scale have been calculated by Arc View 3.2a (Figure 1A, B & C).

Climatic Factors

With regard to meteorological data of the region and the annual humidity conditions of the earth, the xeric humidity and mesothermal patterns are dominant in this region (Banai, 1998). Considering the climatic divisions, the region of study is

located in mid-dry-cold climate. The average annual precipitation is 367.5 mm and the maximum and minimum absolute temperature is 33.1°C and -15.5°C, respectively. February and August are considered the coldest and respectively the hottest month at the region and the average frost days equal 119 (Regional Water Organization, West Azerbaijan, 1991-2004) (Figure 2). Considering the fact that about 61.28% of the species growing in the relevant region is related to the growing elements of Irano-Turanian, it could be concluded that this region belongs to Irano-Turanian region.

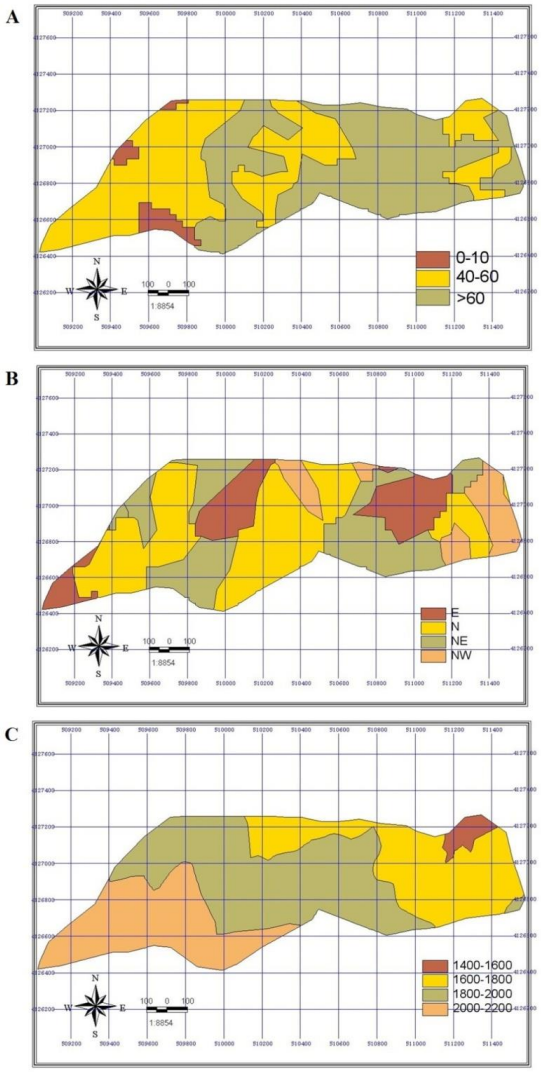

Figure 1. Map of distribution gradient, Geographic direction and height stories of *Juniperus excelsa* in studied region.

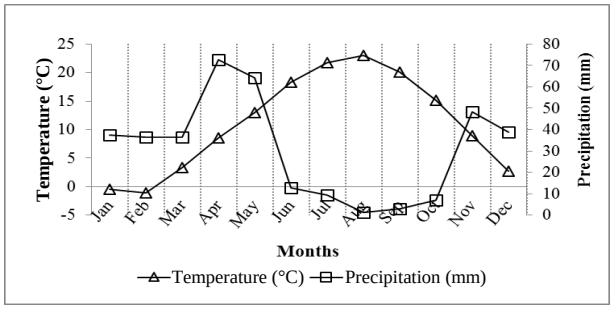

Figure 2. Embrothermic diagram of studied area since 1991-2004 (Annual Reports of Meteorological State of Ghasemloo).

Altitude

In the studied region, the dispersion of *Juniperus excelsa* is within the heights of 1650-2200 m A.S.L (Figure 1C). Other studies in Iran have announced the growth heights of *Juniperus excelsa* from 1400-2800 m (Edishow, 1998; Nasseri *et al.*, 1999; Ezeddin *et al.*, 1999; Ramezani and Shirdel, 1997; Amirabadizadeh, 2000, Naseri *et al.*, 2002 and Abolghasemi, 2004). It seems that in the regions with warmer climate, the dispersion area of *Juniperus* expands to higher altitudes and shows more harmony in the regions under study for this purpose in comparison with the regions with closer climate from this aspect. As the results of ordination analysis (Figure 6), altitude as an ecological factor has the important role in formation of community of plots 3, 7, 4 and 6, respectively in terms of significance degree.

Slope Direction

The direction of the land slope is part of the factors that are very effective on the amount of received light by the ecosystems and the ability to produce bio-copper. On a bigger scale, one of the important factors of creating growth regions in the mountains is the direction of the slopes. Due to receiving less light on northern slopes, the temperature is low, but the moisture is high. *Juniperus excelsa* has dispersion in the northern, northeastern and northwestern slopes (Figure 1B). Ghelichnia (1999) in Nardin region and Dianatnejad and Islami Jadidi (1996) in Andokhtgah Zisepehr Touran showed the impact of the direction factor on the dispersion of plant centers. According to the results (Figure 1B, Table 2), *J. excelsa* commonly grows on north and northeast slope facing the Ghasemlou valley.

Slope Degree

The slope angle has impact on the amount of rain and the stability of the soil surface. The abrupt slopes generally create conditions whereby the groups have more resistance against shortage of water. In mountainous regions, many existing slopes are usually unstable. Fall of the soil surface materials in inferior areas usually prevents the

growth and development of the group; therefore, it keeps the ecosystem always at the initial stages of continuity. In the regions under study, *Juniperus excelsa* always exists in the areas with slopes higher than 60% (Table 2). Other studies in Iran have shown the scope of the growing slope of *Juniperus excelsa* over 15% up to 100% (Ramezani and Shirdel, 1997; Amirabadizadeh and Saghafi Khadem, 2000; Abolghasemi et al., 2004). Based on obtained results from ordination analysis, slope percentage has the important role in formation of community of plots 1, 4, 5, 6, 9 and 2, respectively in terms of significance degree (Figure 6).

Soil Texture & Ph

One of the important ecologic factors in creating plants is soil that has direct impact on the combination of growing plants. In the studied region, *Juniperus excelsa* has dispersion in the sand-clay soil. The reddish brown surface soil with sandy-clay texture is mainly 35cm depth and consists of granular structure with a soil PH of 7.9 - 8 (Table 1). Findings of Masoumi (1993), Batouli (1997), Jafari Kookhandan (2002), Nazarian (2003) and Jafarpour (2004) represent the significance of the edaphic factor in variety and expansion of the plants. As the results of ordination analysis, soil depth has the important role in formation of community of plots 1, 5, 3 and 7, respectively in terms of significance degree. The bare soil is only affected in formation of plots 8 and 9, while soil pH and gravel have the important role in formation of all plots except plots 8 and 9 (Figure 6). According to the results from foraging of arenas at the studied region, *J. excelsa* commonly tend to sand-clay soil (Table 1).

Biometrical Assessment & Healthiness

Based on the results from Table 3, the numerous quantities of *Juniperus excelsa* reproduction accomplish under sprout regeneration (72.1% of sum) and remainder is related to seed germination (27.9% of sum). Furthermore, the great amount of healthy individuals is related to sprout regenerated form (51.8% of sum). The dense stands vary from 40-60 trees per hectare. According to the results of the ecological investigations of the targeted species, the standard and maximum heights of the trees are 2.35 m and 3.5 m, respectively. The standard tree trunk height is 1.02 m. The normal canopy height is 2.01 m. The standard height of the small and large canopy is 3.04 and 2.16 m in diameter, respectively. The normal diameter at the breast height equals 7.4 cm. 70.9% of *Juniperus excelsa* individuals are including sprout regenerated in Ghasemlou valley (Table 3).

Phytosociological Study

The canopy cover is about 50%, sand and gravels about 40%, leaf debris (leafy twigs) about 3%, bare soil about 7% with positive succession. In the vegetation composition of these species, 11% Phanerophytes, 4% Chamaephytes, 30% Hemi-cryptophytes, 33% Therophytes and 13% Cryptophytes control the plants ecological range (Figure 4).

The main species of *Juniperus* communities may include: *Juniperus excelsa*, *Acer monspessulanum* L. subsp. *cinerascens*, *Amygdalus pabotii*, *Rhamnus pallasii*, *Pistacia atlantica* Desf. subsp. Kurdica, *Ferula communis*, *Thymus kotschyanus*, *Thymus migricus* and *Astragalus kabristanicus* (Table 4). Sorensen similarity index was analyzed using NTSYS-pc Ver.2.02e. The results indicated that *Juniperus* community had the highest (26.78%) similarity with the *Amygdalus elaeagnifolia* spach.- *Acer monspessulanum* L.ssp. cinerascens (Boiss.) Yaltirik. community (Figure 5). These two communities are subscriber at the possession of *Juniperus excelsa*, *Acer monspessulanum* L. subsp. *cinerascens*, *Amygdalus pabotii* and *Amygdalus elaeagnifolia* that *Acer monspessulanum* L. subsp. *cinerascens* and *Amygdalus pabotii* had the highest participation in the formation of both communities (Figure 5).

Table 1. Determination of the orientation using retrogression symptoms in plants and soil types of *Juniperus excelsa*

Emergence of retrogression symptoms	Points	
	-	+
A. In plants		
1. Different types of trees up to a certain height are related to grazing domestic animals and upper than that could re-grow.	2	0
2. In short bushes as a result of abundant grazing, the dense branches and loss of young branches out of the bush crown are created.	1	0
3. As a result of excessive grazing, the plants dry out and cannot re-grow.	1	0
4. The reproduction of different good farm plants is not observed during current year.	0	3
5. The general weakness of the plant growth as a result of excessive grazing is noticed.	1	0
6. There is no balance of age classes in the good farm plants (existence of young shrubs and average old plants).	0	3
7. Some of the non-eupeptic plant is also grazed.	1	0
B. In the soil		
1. As a result of excessive grazing or special ecologic conditions of the region, some areas lacking plant coverage have been created.	0	0
2. Relatively deep waterways exist with sloppy walls lacking plant coverage.	1	0
3. The emergence of stone pieces at the soil level is because of soft soils washing surface.	0	2
4. The formation of new sediments lacking plant coverage is to the extent that there is no chance for the plants to be reinstated.	0	2
5. Appearance of the light color of the layers under ground in comparison with the surface layers is the result of soil to be washed away.	0	0
6. Dust is created due to non-preservation of soil as a result of wind breezing, movement of domestic animals and dust.	3	3
7. Concentration of sand and fine soil under the bushes is the result of wind erosion.	0	3
8. The surrounding soil or the soil between the bushes has different heights as a result of erosion.	0	3
9. Behind the permanent plants and obstacles located in the sloppy mountain slopes, a compilation of soil is observed.	0	3
10. Root and curb of different old plants emerge.	1	0
11. The marked lines of the surfaces of old soils exist on the stones of the region.	1	0
12. The water of rivers is muddy.	0	0
13. As a result of continuation of movement of domestic animals in the sloppy regions, slender and micro terrace lines have been created.	0	0
Total:	12	22

Table 2. Slope degree and geographical directions of *Juniperus excelsa* in the studied region

	Slope (%)			Geographical directions			
	0-10	40-60	>60	N	NE	E	NW
Area (h)	4.18	52.8	56.26	47.99	32.68	20	12.57
Total (%)	3.7	46.6	49.7	42.4	28.9	17.7	11

Table 3. Biometrical study (qualitative and quantitative properties) of *Juniperus excelsa* species in sampled plots at the conservative area

Species form	Canopy condition	Trunk quality	Total samples (%)	Average Total height (m)	Average Trunk height (m)	Average Canopy height (m)	Average Big canopy diameter (m)	Average Small canopy diameter (m)	Average DBH (cm)
Sprout regenerated	Healthy	Healthy	51.8%	2.1	0.84	1.86	3	2.7	7.72
	Unhealthy	Healthy	11.4%	3.5	1.3	1.6	4	1.5	9
		Unhealthy	8.9%	1.5	1	2.5	2	1	7
Seed germinated	Healthy	Healthy	20.3%	2.5	1.5	2.5	2.5	2	5.85
		Unhealthy	7.6%	3	0.75	2.2	4	1.5	8

Table 4. The main species of *Juniperus excelsa* community at the conservative area

Releve No.	1	2	3	4	5	6	7	8	9
Area (h)	15.5	12.57	25.49	20	22.5	13	4.18	7.2	6.24
Canopy cover (%)	45	45	50	45	50	50	50	75	75
Altitude (m)	1660	1850	2120	2000	1860	1970	2160	1720	1870
PH	7.9	7.9	8	8	7.9	7.9	8	7.5	7.4
Soil depth (cm)	35	32	34	31	35	33	34	31	31
Gravel (%)	36	38	40	39	37	37	40	14	14
Bare (%)	7	7.2	6.7	7.1	6.8	7	6.9	8	8
Inclination in °	60	57	45	62	63	65	10	43	58
Aspect	NE	NW	N	E	N	NE	NE	NE	NE
Sampling area in sqm									
Tree layer cover (E3)									
Acer monspessulanum L. subsp. cinerascens (Boiss.)Yaltirik	2	2	1	1	2	2	1	2	2
Amygdalus elaeagnifolia	.	.	.	.	.	.	.	3	3
Amygdalus pabotii	1	1	1	1	1	1	+	2	2
Cerasus microcarpa	+	+	.	.	.	.	.	1	1
Festuca ovina	+	.	.	.	+	.	.	1	1
Pistacia atlantica	1	1	1	2	1	1	1	1	1
Shrub layer cover (E2)									
Juniperus excelsa	2	2	2	2	2	2	2	1	1
Rhamnus pallasii	1	1	1	1	1	1	1	.	.
Herbaceus layer cover (E1)									
Astragalus effusus Bge.	+	.	.	.	+	.	.	1	1
Astragalus kabristanicus	1	1	+	+	1	1	+	.	.
Ferula communis	1	1	+	+	1	1	+	.	.
Thymus kotschyanus	1	1	1	+	1	1	.	.	.
Thymus migricus	+	+	+	+	1	1	.	.	.
Trifolium hybridum	+	+	.	.	+	.	.	1	1
Pyrus glabra Boiss	+	.	.	.	.	.	.	+	+
Rosa canina L.	.	.	+	.	+	+	+	+	+
Poa bulbosa L.	+	.	.	+	+	+	.	+	+
Bromus tectorum L	+	+	+	+	+	+	.	+	.
Bromus tomentellus Boiss	+	.	+	.	+	+	.	+	+
Agropyron repens (L.)P	+	.	.	.	+	.	.	+	.
Triticum aestivum L	+	+	+	.	+	+	+	+	+
Daphne mucronata Royle.	+	+	.	.	.	.	.	+	+
Festuca ovina L	+	.	.	.	+	.	.	+	+
Aegilops cylindrica Host	.	+	+	.	.	.	.	+	+

DISCUSSION

Abiotic Factors

The results from the slope facing and direction map demonstrate that the overriding distribution of this species is located on the slopes of greater than 60% which encompasses an area equal to 56.26 h. The slope steepness of 40-60% with an area of 52.8 h is of secondary importance. Furthermore, a slope steepness of 0-10% encompasses 4.18 h (Figure 1A & B; Table 2).

Regarding the geographical direction map, the maximum distribution area of this species belongs to the north geographical direction which embraces an area of 47.99 h. Also, it covers 32.68 ha facing east-north direction, 20 ha facing east direction, and 12.57 h facing west-north direction (Figure 1B; Table 2).

Considering that *J. excelsa* has developed the vegetation cover on the north facing slope and owns the maximum distribution in this geographical direction, we can conclude that the soil condition is more favorable and is of greater depth on the north facing slopes. Soil profile is ABC in this region. Currently, the soil density, reduction of suitable porosity for root respiration, and mycorrhiza behavior are of threatening factors to *J. excelsa* habitation. This condition has made the soil impermeable, hence the osmosis is deactivated and saplings experience drought condition and water shortage during the dry season. Due to being severely washed away and its erosion on the south facing slopes, the soil profile of AIC type loses its depth and *J. excelsa* has been constantly ruined (Table 1).

Overall, the results from ordination analysis demonstrate that the main effectiveness ecologic factors affecting the variety of *Juniperus* community are altitude, the slope direction and degree, and the soil texture (Figure 6).

Biotic Factors

Since the region under study includes the preserved territory and the unpreserved zones around it, comparing these two territories, it was found out that in the preserved zone, the percentage of eupeptic plants of class I & II is high; whereas in the non-protected region, the percentage of the plants of eupeptic plant of class III has increased (Figure 3). Also in the preserved zone, the variety of the plants coverage is high and the density of *Juniperus* and small trees with phyllode has increased. In the preserved zone, soil erosion and gold-washing is prevented and there is a continuous coverage in the crown area and the coverage percentage is also observed to be 100% and this is one of the most effective factors to maintain the balance of the ecosystem of the natural territories (Table 1). The invading species such as *Peganum harmala* and *Cousinia grandis* are seen abundantly in the non-preserved zones. The abundance of the plants of chicory species could be because of the destruction process in some of the zones of the region and it should be considered a warning for the preserved zone and one should try to find a reason for it. Experience has shown that when the percentage of

destruction of the plant coverage in a region goes up, some of the plant species such as chicory species will appear more in the region flora. Researches have shown that in the critical regions, the number of invading species is higher than in the region of reference and the number of invading species in these regions includes *Euphorbia* and *Cousinia* (Ghelichnia, 1996). The results from study of protected plant coverage against grazing have shown that more protection time of the farms against grazing is less than plant coverage will be and if this period is short and the amount of grazing is reasonable, the variety of plant coverage will increase (Somodi *et al.*, 2004). Our results from foraging from Ghasemlou valley related to *Juniperus* communities are to more extent in agreement with the results of Ghelichnia in 1996 and Somodi in 2004.

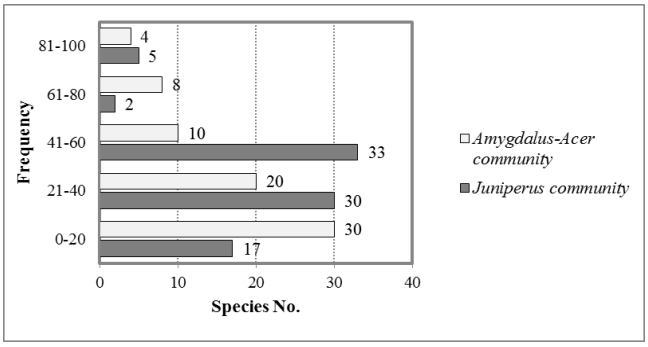

Figure 3. Frequency of *Juniperus excelsa* class within *Juniperus* and *Acer- Amygdalus* communities.

Phytosociological Study

According to the results from analysis of Sorensen similarity index using NTSYS-pc, *Juniperus* community had the highest similarity with the *Amygdalus elaeagnifolia* Spach.- *Acer monspessulanum* L.ssp. cinerascens (Boiss.) Yaltirik. community (Figure 5).

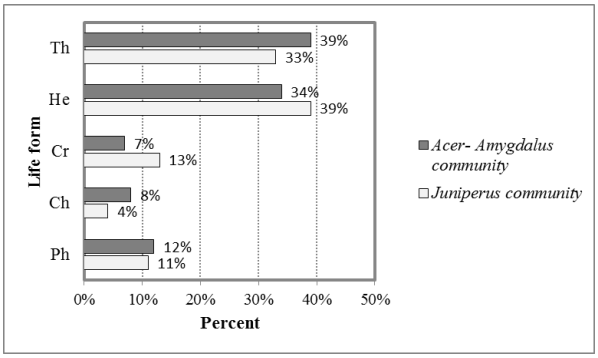

Figure 4. Life Forms of *Juniperus* and *Acer-Amygdalus* communities.

When comparing the diagrams of the frequency classes of Figure 3, the *Juniperus* type in classes I to III has a high variety of the species and in the classes with high frequency, the number of species is very low. Therefore, the plant coverage in one type is not coherent and includes plants of other groups. According to the results of life form of *Juniperus* and *Acer-Amygdalus* communities (Figure 4), the numerous quantities are related to Hemi-cryptophytes and Therophytes; that demonstrated presence of a rangeland as dominant vegetation cover of area.

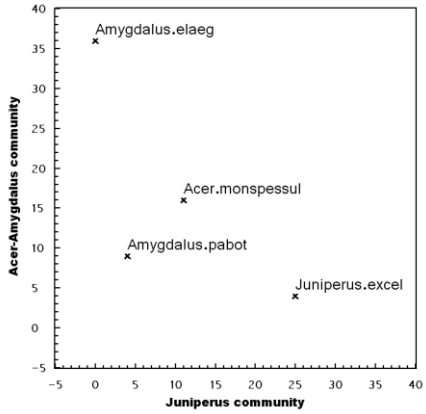

Figure 5. Ranking of similarity between *Juniperus* and *Acer-Amygdalus* communities.

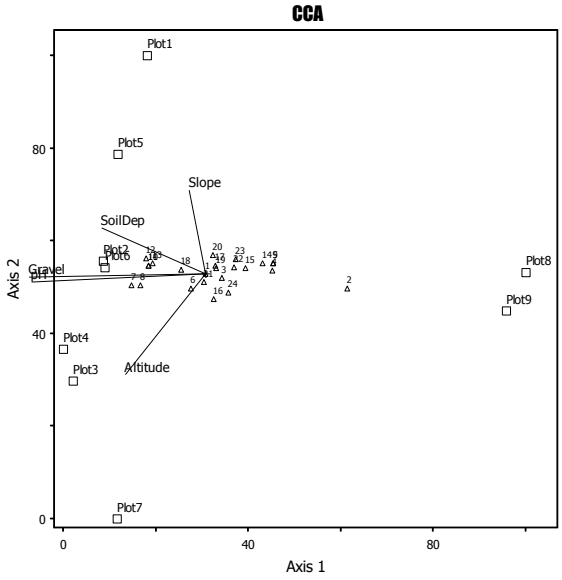

Figure 6. Ordination plot of affected ecological factors in formation of *Juniperus excelsa* communities in the studied region using PC-ORD software .

CONCLUSION

The most important ecologic factors affecting the regeneration of *Juniperus* and similar species co-growing with it are altitude, the slope degree and direction, and the soil texture and pH. It commonly grows on north and northeast slope facing and it tends to sand-clay soil in mid-dry-cold climate belongs to Irano-Turanian region. The numerous quantities of *J. excelsa* reproduction accomplish under sprout regeneration and remainder is related to seed germination. In addition to the great amount of healthy standing mass is related to sprout regenerated form. Comparing preserved and non-preserved zones, it was found out that in the preserved zone, the percentage of the eupeptic plant coverage of class I & II is high and the density of *Juniperus* and small trees with phyllode has increased and in non-preserved zones, the abundance of evading species such as *Peganum harmala* and *Cousinia grandis* is observed.

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POLLINATION ECOLOGY OF *ALTERNANTHERA PARONYCHIOIDES* AND *GOMPHRENA SERRATA* (FAMILY: AMARANTHACEAE; SUB-FAMILY GOMPHRENOIDEAE)

J.S.R. ALURI*, P. R. CHAPPIDI[1]

Alternanthera paronychioides is a perennial herbaceous weed that reproduces by seed and also from perennial root system while *G. serrata* is an annual herbaceous weed and reproduces exclusively by seed. These plants appear during wet season and flower and fruit continually if soil is wet. In both the species, the flowers are hermaphroditic and display auto-selfing by themselves and also by rain drops. *A. paronychioides* is also entomophilous and *G. serrata* is psychophilous. Fruit and seed dispersal is anemo- and hydrochorous in both the species; but *G. serrata* is also myrmecochorous. *A. paronychioides* and *G. serrata* as C_4 species increase photosynthesis in warmer climates and survive well in drier and hot environments. Therefore, these species are useful in the restoration of degraded, damaged and destroyed ecosystems or habitats and support local insects by providing forage.

Keywords: *Alternanthera paronychioides*, *Gomphrena serrata*, entomophily, anemochory, hydrochory, myrmechochory.

INTRODUCTION

The sub-family Gomphrenoideae of Amaranthaceae has 14 genera of which three genera *Alternanthera, Gomphrena* and *Iresine* are represented in South India. This subfamily is further subdivided into two tribes Pseudoplantageae and Gomphreneae of which the former is monogeneric with two representatives in India. Gomphreneae has two sub-tribes, Froelichiinae and Gomphreninae. The former is represented by a single genus, *Alternanthera* in India while the latter has two genera *Gomphrena* and *Iresine* in South India. The genus *Alternanthera* has about 150 low herbaceous species, widely distributed in the American tropics and subtropics, from where many species have spread to several other countries (Tanveer *et al.,* 2013). In South India, six *Alternanthera* species: *A. brasiliana*, *A. paronychioides, A. philoxeroides, A. pungens, A. sessilis* and *A. tenella* have been reported. *A. paronychioides* is a native of tropical America but it is now naturalized in the United States of America, India, Java and other parts of the Old World

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(Anilkumar, 2006). *Alternanthera* species have been reported to be visited and pollinated by *Ceratina* and *Dialictus* bees (Kubitzki *et al.*, 1994). *Gomphrena* is a large genus of about 100 to 125 species distributed throughout the warm temperate, subtropical and tropical regions of the world, with most of its species centered in American tropics and Pacific Islands. Thirty species of this genus are endemic to Australia (Shu, 2003; Harwood & Palmer, 2011; Shih-huei & Yi-ching, 2012). In this sub-family, *Alternanthera paronychioides* A. St. Hill., and *Gomphrena serrata* L. have not been studied in any part of the world and hence, the present study was contemplated to understand how their floral morphology, floral biology, sexual systems, pollination syndromes, fruiting ecology and seed dispersal modes enable them to grow as weeds to colonize and expand their distribution range and serve as forage sources for local insects that act as pollinators.

MATERIALS AND METHODS

The seasonal annual herbs, *Alternanthera paronychioides* and *Gomphrena serrata* were selected for study during 2014-2016 in Visakhapatnam and its surroundings, Andhra Pradesh, India (17°42'N Latitude and 82°18'E Longitude). The inflorescence type and the number of flowers per inflorescence were noted. Ten inflorescences were tagged prior to commencement of their flowering and followed daily for recording the flowering duration of the inflorescence. Twenty five fresh flowers were used for each plant species to record the floral details such as flower shape, colour, odour, sex, symmetry, floral mechanism, calyx, corolla, stamens and style and stigma and ovule number. Field trips were conducted to record phenological aspects. Ten inflorescences which have not initiated flowering were tagged and followed daily to record the duration of flowering, anthesis schedule and the timing of anther dehiscence. Twenty five fresh flowers were used to record the floral morphological details. Nectar could not be measured and analyzed due to its secretion in minute quantity which was further depleted by thrips during mature bud and flower life. Twenty mature, but un-dehisced anthers were collected from different plants and examined for pollen output as per the protocol described in Dafni *et al*. (2005). The calculation of pollen output per flower and pollen-ovule ratio was done as per the formulas described in Cruden (1977). Ten flowers each from five individuals were used to test stigma receptivity. It was tested with hydrogen peroxide from mature bud stage to flower closure/drop as per Dafni *et al.* (2005). Further, the receptivity was also observed visually whether the stigma is shiny, wet or changing colours or withering. Insects foraging at the flowers were observed from morning to evening on four different days for their mode of approach, landing, probing behavior and contact with the floral sexual organs. Bees, wasps and flies were identified with the representative specimens available with the Department of Environmental Sciences, Andhra University, Visakhapatnam. Butterflies were identified by

consulting the books of Kunte (2007). The foraging visits of insects were recorded using 2 x 2 m area of flowering patch for 10 min at each hour for the entire day on four different days and the data was tabulated to record the foraging pattern and the percentage of visits made by them. The pollen/nectar collection behaviour of insects was carefully observed to assess their role in effecting pollination. Ten specimens of each insect species were captured during peak foraging period and brought to the laboratory. Each specimen was washed in ethyl alcohol, stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present. From this, the average number of pollen grains carried by each insect species was calculated to know the pollen carryover efficiency. Inflorescences with 741 buds on *D. muricata* and with 756 buds on *G. serrata* were tagged and followed to record fruit and seed set rate in open-pollinations. Fruit maturation period, the fruit and seed morphological characteristics were recorded to evaluate their adaptations for dispersal by different means. The role of wind, rain, water and ants in fruit and seed dispersal was examined in the field. The aspects of seed germination and establishment of populations were observed briefly in the field.

RESULTS

Alternanthera paronychioides

Plant and flowering phenology

The plant is a perennial prostrate to erect profusely-branching herb that produces dense stands as a weed in open wet soils, waste lands, road sides and along the stream or canal banks of agricultural fields. Seeds germinate and produce new plants. The plants growing in perpetual wet soils produce new branches from the perennial root system. The plants appear in rainy season. The flowering occurs during August-November with a peak during October in the plants that grow in waste lands and along road sides (Fig. 1a). The plants growing along the streams or canal banks of agricultural fields show flowering and fruiting throughout the year. Inflorescence is a sessile fasciculate globose head and is borne in leaf axils only and the heads are borne on the entire length of plant (Fig. 1b). The globose head borne in each leaf axil consists of 8 ± 2 flowers (Range 3-13) and antheses within a week.

The Flower

The flowers are sessile, small, white, actinomorphic, odorless and bisexual. They are equipped with 1 papery acuminate bract and 2 papery acuminate bracteoles. The tepals are five, free, greenish white, sub-equal, outer 3 tepals oblong-lanceolate, 3-veined in proximal half with barbellate hairs; inner 2 tepals somewhat laterally compressed, 1-veined and apex acute. The stamens are five and alternating with 3-4 toothed pseudo-staminodes with half the length of stamens; the filaments are greenish-white, free but fused at the base forming a cup-like structure

enclosing the ovary. The anthers are bisponrangiate, unithecal, introrse, ellipsoid and golden yellow in color. The ovary is globose, compressed, glabrous, unilocular with one pendulous ovule (Fig. 1k). The style is very short and extends into capitate shiny wet stigma that is situated well below the height of anthers.

Floral biology

The flowers are open during 0700–1000 h but most of them open at 0800 h (Fig. 1c-g). The tepals unfold and expose the stamens and stigma following the anthesis. Anthers dehisce during anthesis by longitudinal slits (Fig. 1h). The pollen output is 501.20 ± 45.87 per anther and 2,506 per flower. The pollen- ovule ratio is 2,506:1. The pollen grains are monads, white in color, sticky, spheroidal, dodecahedral, 18.26 ± 3.49 µm in diameter, pantoporate and multiporate (Fig. 1i). The stigma is receptive from anthesis onwards, and ceases by the noon of the following day. The nectar is produced in minute volume around the base of the ovary inside the staminal tube. The tepals close back partially by the evening of the same day or by the noon of 2nd day of anthesis. The bracts, bracteoles and tepals are persistent and remain in their place until seed dispersal occurs while the stamens, style and stigma gradually wither inside as the fruit grows.

The flowers with homogamy facilitate auto-selfing by gravitational pollination (Fig. 1j). Rain drops falling on the flowers splash the dehisced anthers and in effect the pollen drops down and reaches the stigma effecting autogamy.

Figure 1. *Alternanthera paronychioides*: a. Flowering patch; b. Twing with flowering inflorescences; c. Mature bud; d. Flower; e. & f. Flower without tepals; g. Relative positions of stamens and stigma; h. Dehisced stamens; i. Pollen grain; j. Gynoecium with stigma coated with pollen; k. Ovule; l. Fruit; m. 1-seeded fruit; n. Mature and dry seeds.

Flower visitors and Pollination

Thrips were found to be using floral buds for breeding and flowers for forage. The flowers were visited by bees, wasps (Hymenoptera), flies (Diptera) and butterflies (Lepidoptera) from 0800 to 1700 h with concentrated activity during forenoon period (Fig. 5, 6). The bees except for *Xylocopa pubescens* collected both nectar and pollen in the same and/or different foraging visits while *X. pubescens*, wasps, flies and butterflies collected nectar only. The bees were *Apis dorsata* (Fig. 2a), *A. cerana*, *A. florea* (Fig. 2b), *Trigona iridipennis* (Fig. 2c) and *Xylocopa pubescens* (Fig. 2d). The wasp was not identified (Fig. 2e). The flies were *Eristalinus arvorum* (Fig. 2f) and *Helophilus* sp. (Fig. 2g). The butterflies were *Phalanta phalantha* (Fig. 2h), *Leptotes plinius* (Fig. 2i), *Zizeeria karsandra* (Fig. 2j), *Zizina otis* (Fig. 2k), *Freyeria trochylus* (Fig. 2l) and *Spindasis vulcanus* (Fig. 2m). Bees except *X. pubescens* were the regular and consistent foragers while all other insects were not consistent foragers. The forage collection behavior resulted in effecting pollination. Bees constituted 53%, wasps 5%, flies 10% and butterflies 32% of total visits made in a day (Fig. 7). All these insect species landed on the globose head inflorescence to probe flowers for the forage; while doing so, they invariably contacted the stamens and stigma with their head and ventral side effecting pollination. Reduced levels of forage, especially nectar due to feeding by thrips increased foraging visits and in effect it promoted pollination rate. The body washings of all insect visitors revealed the presence of pollen to varying extents - bees 64 to 324, the wasp 72.8, flies 11 to 93 and butterflies 16-104 (Table 1).

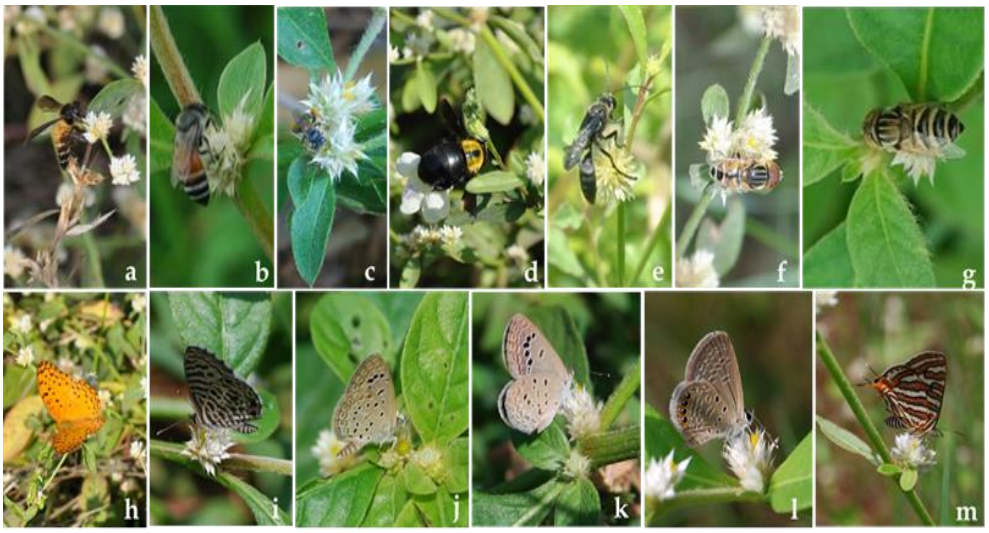

Figure 2. *Alternanthera paronychioides*: a. *Apis dorsata*; b. *Apis florea*; c. *Trigona iridipennis*; d. *Xylocopa pubescens*; e. Wasp (unidentified); f. *Eristalinus arvorum*; g. Helophilus sp.; h. *Phalanta phalantha*; i. *Leptotes plinius*; j. *Zizeeria karsandra*; k. *Zizina otis*; l. *Freyeria trochylus*; m. *Spindasis vulcanus*.

Table 1. Pollen recorded in the body washings of insect foragers on *Alternanthera paronychioides*

Insect species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Apis dorsata	10	96 - 315	208.3	71.85
Apis cerana	10	72 - 264	162.4	52.82
Apis florea	10	125 - 324	229.4	66.92
Trigona iridipennis	10	64 - 252	150.3	60.81
Xylocopa pubescens	10	74 - 208	146.1	37.31
Wasp (Unidentified)	10	46 - 102	72.8	18.06
Eristalinus arvorum	10	25 - 93	59.7	22.58
Helophilus sp.	10	11 - 82	52.2	22.39
Phalanta phalantha	10	16 - 73	45.2	17.93
Leptotes plinius	10	32 - 93	62.3	19.72
Zizeeria karsandra	10	24 - 104	64.7	27.66
Zizina otis	10	19 - 82	55.7	20.44
Freyeria trochylus	10	21 - 94	60.6	22.19
Spindasis vulcanus	10	18 - 86	53.9	21.91

Fruiting ecology and seed dispersal

Fruits mature within 3 weeks. The tepals gradually bulge and cover the growing fertilized ovary; externally the tepals are surrounded by the bract and bracteoles. Natural fruit and seed set is 93.05% . Fruit is an indehiscent membranous, obcordate utricle with a single shiny, brown, discoid somewhat lenticular and vertical seed (Fig. 1-n). The fruited globose heads fall off as single units from the mother plant. Fruits from these heads gradually separate and expose the seeds during rainy season. Globose heads are dispersed by high winds and rain water during rainy season. Therefore, seed dispersal is anemochorous and hydrochorous.

Gomphrena serrata

Plant and flowering phenology

It is an erect annual herbaceous weed. It produces dense stands in open wet soils, road sides and in irrigated agricultural fields (Fig. 3a). The stem produces several prostrate branches and it is densely clothed with appressed white hairs especially when young. Seeds germinate and produce new plants during rainy season. The flowering season varies with the habitat type, it is from August-November plants occurring in open areas and along the roadsides while it is from August to February in agricultural lands and in areas where soil is sufficiently wet. The plants disappear in March. The branches are borne from basal part of the stem and each branch and main stem produces a single inflorescence only. Inflorescence is a terminal, sessile, solitary globose spike with 67.27 ± 9.31 flowers which anthese acropetally over a period of 10 to 12 days (Fig. 3b). The flowers fall off sequentially in acropetal manner. The inflorescence elongates into 3.6 cm when fruiting and is subtended by 2 leafy bracts.

Figure 3. Gomphrena serrata: a. Flowering patch; b. Inflorescence; c. Mature bud; d. Flower; e. Position of stamens and stigma; f. Dehisced anthers; g. stigmas; h. Ovule; i. Mature fruit; j. & k. Ripen and dry fruits covered with thick mass of silky hairs; l. Seed.

The Flower

The flowers are snow-white with pinikish tinge, sessile, small, compressed, ovate-lanceolate, actinomorphic, odorless and bisexual. Each flower has 1 bract and 2 bracteoles; the bract is deltoid-ovate, glabrous while the bracteoles have irregularly dentate crest. The tepals are five, lanceolate, free, white; the outer 2 are fleshy, woolly on the back at base while the inner 3 are papery, woolly at base. The stamens are five and fused to form staminal tube with 5-lobes at the apex. The anthers are positioned between incisions of the lobes; they are dithecous, introrse, glabrous and yellow in color. The ovary is globose, glabrous, greenish-white and uni-locular with a pendulous ovule (Fig. 3h). The style and stigmas are together and greenish-white; the stigmas are 2, linear, equal in length, slender, glabrous and diverge slightly (Fig. 3g). The ovary, style and stigma are seated inside the staminal tube and the distance between the terminal part of the stigmas and the basal part of the anthers is 2.5 mm.

Floral biology

The flowers are open during 0700–1600 h but most of them open during 0700-1000 h (Fig. 3c,d). The stamens are positioned far above the height of the stigmas during and after anthesis (Fig. 3e). During anthesis, anthers dehisce by longitudinal slits (Fig. 3f) and stigma becomes receptive which ceases by the evening of the same day. The pollen output is 213.2 ± 17.93 per anther and 1,066 per flower. The pollen – ovule ratio is 1,066: 1. The pollen grains are monads, yellow in color, spheroidal, 18.26 ± 3.49 µm in diameter, pantoporate, multiporate, dry and fall as single grains. The nectar is produced in minute volume around the

base of the ovary within the staminal tube. The tepals close back completely by the evening of the same day. The bract, bracteoles, tepals, style and stigma are persistent and remain in their place until seed dispersal while the stamens gradually wither inside as the fruit grows.

The flowers with homogamy facilitate auto-selfing by gravitational pollination. Rain drops falling on the flowers splash the dehisced anthers and in effect the pollen drops down and reaches the stigma effecting autogamy. The test involving just bagged mature buds for three weeks indicate 70% fruit and seed set confirming auto-selfing.

Flower visitors and Pollination

Thrips were found to be using floral buds for breeding and flowers for forage. The flowers were visited exclusively by lycaenid butterflies (Lepidoptera), *Castalius rosimon* (Fig. 3a), *Leptotes plinius* (Fig. 3b), *Zizula hylax* (Fig. 3c), *Zizeeria karsandra* (Fig.3d), *Zizina otis* (Fig. 3e), *Freyeria trochylus* (Fig. 3f), *Chilades laius* (Fig. 3g), *Euchrysops cnejus* (Fig. 3h) and *Azanus jesous* (Fig. 3i) for nectar from 0900 to 1700 h with more foraging activity during 1000-1200 h (Fig. 8). All these butterflies were regular foragers. They landed on the inflorescence to probe the flowers for the forage; while doing so, they invariably contacted the stamens and stigmas with their head/proboscis effecting pollination. The body washings of all butterflies revealed the presence of pollen to varying extents for each species; the mean pollen recovered varied from 44.6 to 61.7 (Table 2) and hence proved their role in pollination.

Table 2. Pollen recorded in the body washings of butterflies on *Gomphrena serrata*

Species name	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Castalius rosimon	10	18 - 73	48.6	16.89
Leptotes plinius	10	27 - 86	55.7	19.97
Zizula hylax	10	21 - 65	44.6	15.27
Zizeeria karsandra	10	25 - 84	60.6	19.24
Zizina otis	10	16 - 68	44.7	17.45
Freyeria trochylus	10	22 - 76	52.1	17.84
Chilades laius	10	19 - 87	61.5	20.42
Euchrysops cnejus	10	12 - 62	47.2	16.52
Azanus jesous	10	23 - 87	61.7	17.46

Fruiting ecology and seed dispersal

Fruits mature within 3 weeks. The tepals gradually bulge, harden and cover the growing fertilized ovary; externally the tepals are surrounded by the bract and bracteoles. Natural fruit set is 95.37% while natural seed set is 71.84%. Fruit represents tepals, bract and bracteoles enclosed by abundant silky hairs; it is utricle, oblong, ovoid with a single compressed-ovoid, brown, glabrous and shiny seed (Fig. 3i-l). The fruits mature acropetally and fall off in the same way (Fig. 4j); they

fall off together with the seeds from the mother plant (Fig. 4k). Fruits after reaching the ground, gradually shed tepals and expose the mature and dry seed by the time of monsoon season. The different parts of the fruit appeared to be an adaptation to inhibit loss of soil moisture during dry season. Fruits due to their light weight are dispersed easily by wind during dry spells and rain water during rainy season. During dry spells of rainy season and dry season, *Crematogaster* ants were found to carry fruits to their nests where they consume the fleshy elaisome or feed it to their larvae without damaging the seeds (Fig. 4l). Therefore, fruit dispersal is anemochorous, hydrochorous and myrmecochorous.

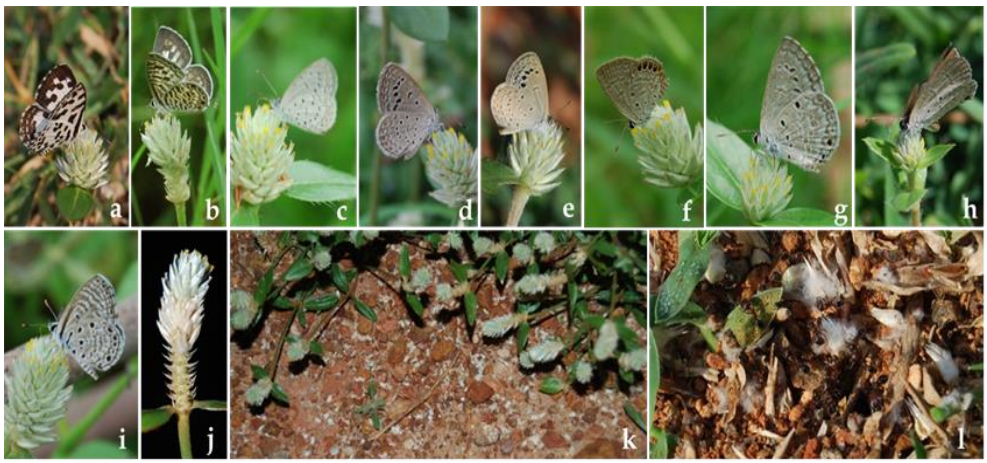

Figure 4. *Gomphrena serrata*: a. *Castalius rosimon*; b. *Leptotes plinius*; c. *Zizula hylax*; d. *Zizeeria karsandra*; e. *Zizina otis*; f. *Freyeria trochylus*; g. *Chilades laius*; h. *Euchrysops cnejus*; i. *Azanus jesous*; j. Fruited inflorescence dispersing fruits from the base to the tip; k. Fallen silky fruits with seeds inside; l. Fruit dispersal by red ants.

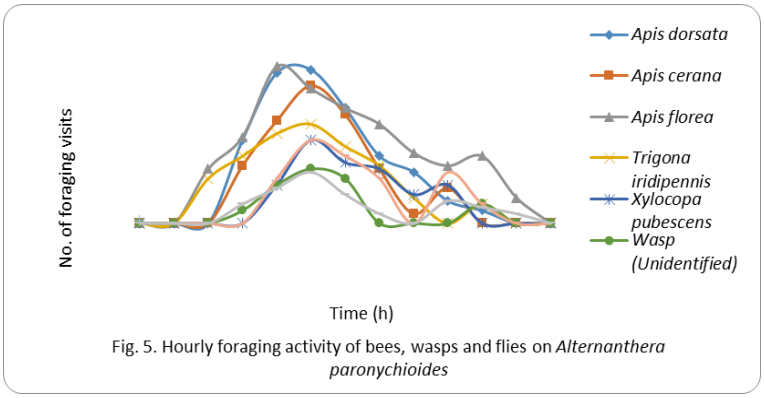

Fig. 5. Hourly foraging activity of bees, wasps and flies on *Alternanthera paronychioides*

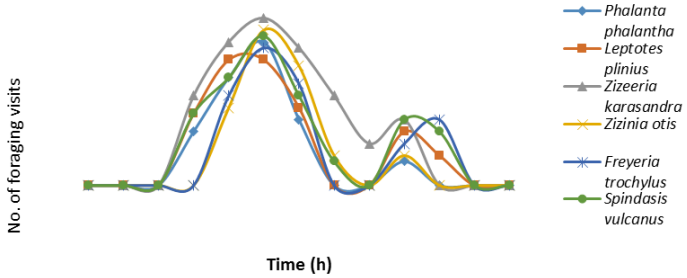

Fig. 6 Hourly foraging activity of nymphalid and lycaenid butterflies on *Alternanthera paronychioides*

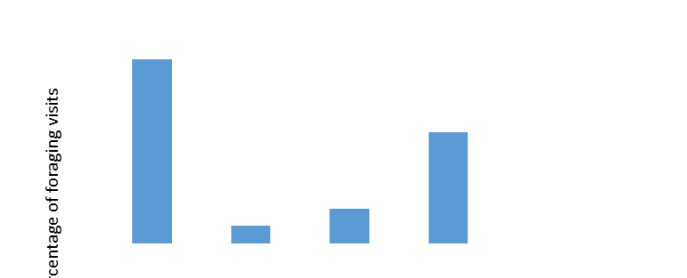

Fig. 7. Percentage of foraging visits of different categories of insects on *Alternanthera paronychioides*

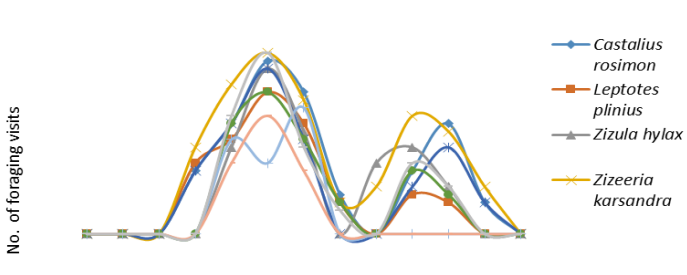

Fig. 8. Hourly foraging activity of lycaenid butterflies on *Gomphrena serrata*

DISCUSSION

Alternanthera paronychioides is a perennial herbaceous weed that reproduces by seed and also from perennial root system while *Gomphrena serrata* is an annual herbaceous weed and reproduces exclusively by seed. These plants appear during

the wet season, and flower and fruit continually if the soil is sufficiently wet. Kajale (1940) and Anilkumar (2006) stated that Amaranthaceae flowers are uni- or bisexual and display monoecious or dioecious or polygamous sexual systems. The allogamy by dichogamy is said to be the norm in this family. Further, protogyny is functional to achieve cross-pollination in species with hermaphroditic flowers and in those demonstrating monoecism. In *A. paronychioides* and *G. serrata,* hermaphroditism and homogamy are functional. Kajale (1940) and Costea *et al.* (2003) reported that self-pollination is not a rare occurrence in Amaranthaceae members. In *A. paronychioides* and *G. serrata,* autonomous selfing occurs by gravitational pollination and by rain drops falling into the flowers and its function is reflected in the highest fruit or seed set rate in bagged flowers and all seeds are filled and viable.

Kajale (1940) considered that most species of Amaranthaceae are anemophilous. Piotrowska (2008) stated that virtually all Amaranthaceae are wind-pollinated but the plants produce less pollen than other anemophilous species. Muller & Borsch (2005) stated that although anemophily is considered to be the norm in Amaranthaceae, many genera are in fact frequently visited by insects. Borsch (1998) reported that white or cream coloured flowers of many genera are insect-pollinated. Kubitzki *et al.* (1994) mentioned that entomophily appears to be functional, particularly by bees. In *A. paronychioides* and *G. serrata,* the flower characters such as nectar production and yellow anthers with white tepals are important characters that draw insects to the flowers for effecting pollination. Insect pollination occurs on the day of anthesis and also until the noon of the next day in *A. paronychoides*, but it occurs only on the day of anthesis in *G. serrata*. Bees, wasps, syrphid flies and butterflies pollinate *A. paronychioides* but only butterflies pollinate *G. serrata*; their role in pollination is realized in the pollen recovered from their body washings. Reduced levels of nectar and emptied flowers due to nectar feeding by thrips causes intense foraging activity due to which pollination rate increased manifold. Therefore, the two species are auto-pollinated and also by rain drops and insects.

Kapralov *et al.* (2012) reported that many genera in Amaranthaceae produce 1-seeded fruits with a firm apex bearing the style and very thin, membranous walls which appear to be an adaptation to the xerophytic condition in which most species with fruits of this nature grow, when rains come, the ripe seed swells, bursts the capsule and falls to the ground. *A. paronychioides* and *G. serrata* produce 1-seeded fruits due to production of a single ovule per flower. The fruits do not have a firm apex bearing the style and stigma and very thin, membranous pericarp. Borsch (1998) reported that a high proportion of Amaranthaceae have dry capsular fruits which open by irregular rupturing of thin walls. But, *A. paronychioides* and *G. serrata* produce seed inside the fruit formed of bulged tepals enclosed by the bract and bracteoles. The fruited globose heads fall to the ground as individual units in *A. paronychoiodes* whereas the individual fruits

consisting of seed enclosed by bulged tepals, the bract and bracteoles fall off to the ground in *G. serrata*. The fruits gradually free the seed from the enclosed parts by the time of rainy season. The well developed fruit components suggest a role to inhibit loss of moisture from the seed as in case of several new world genera including *Gomphrena* (Kapralov *et al.*, 2012). Further, the very light weight fruit components accompanied by thick hairy growth in case of *A. paronychioides* and dense silky hair in case of *G. serrata* enable the fruits to disperse easily by wind and also to float in water during rains. It is pertinent to mention the finding of Kapralov *et al*. (2012) that fruits of some *Alternanthera* species produce corky cells that enable them to float in the permanent or seasonal water in which they grow. Since *A. paronychioides* also grows in watered agricultural lands, such corky cells if present in the fruit layers would definitely benefit this plant to float during rains or in well irrigated crop lands.

Costea *et al.* (2003) reported that the seed dispersal in Amaranthaceae is performed by wind, water, animals and humans. *A. paronychioides* exhibits anemochory and hydrochory while *G. serrata* shows anemochory, hydrochory and myrmecochory. In the latter species, aril form of elaisome at the terminal portion of the seed attracts *Crematogaster* ants which elicit the transport of the entire fruit to the nest by them. These ants separate the seed from the fruit components and then consume the elaisome or feed it to their baby ants after which they deposit in garbage piles either in the nest or outside the nest. Edwards *et al.* (2006) stated that elaiosomes function as rewards for ants. Gorb & Gorb (2003) reported that myrmecochory provides the seed with protection from seed predators, a safe place for seed survival during unfavorable periods such as fires and/or a microsite rich in nutrients. Goldblatt (1997) stated that the physiological and energetic costs of developing elaiosomes are likely to be much smaller than developing fleshy fruits, so they are cheap to make. Milewski (1983) mentioned that ants need to be abundant at levels that guarantee that seeds will be picked up and the seed traits need to directly influence the subsequent fate of seeds. Myrmecochory may be favoured by selection in more open, drier or less predictable habitats due to the higher availability of ants or to the lower costs of developing a reward for dispersal. It is true in case of *G. serrata* which grows in open and less predictable habitats. All the three seed dispersal syndromes (anemochory, hydrochory and myrmecochory) functional in this species enable it to colonize new environments or habitats. These two plant species propagate seasonally during rainy season, *A. paronychioides* from seed and perennial root stock and *G. serrata* exclusively from seed.

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Engineering of Microorganisms for the Production of Chemicals and Biofuels from Renewable Resources, 2017, Ed. Guillermo Gosset, Springer International Publishing, ISBN 978-3-319-51728-5, 200p.

This interesting book focuses on both prokaryotes (thermophilic bacteria- with special emphasis on species such as *Clostridium*, *Caldanaerobium*, *Thermoanaerobacterium* etc., – *Escherichia coli* and *Zymomonas mobilis*) and eukaryote – *Saccharomyces cerevisiae* which are very important in nature and in biotechnologies for the degradation of complex substances to simple ones, some of them being useful chemicals for mankind. The main task is to present updated information concerning targeted genetic modification of the cells in order to achieve a given metabolic trait enabling it to convert cheap, renewable or waste materials to useful compounds. The scientific trip deep inside cell metabolism is really interesting and, the examples of specific inactivation of a given gene in order to divert the flux of matter and energy toward the increased production of a desired chemical, really useful. The examples are very clearly described, the reader entering in the magnificent (and versatile!) world of (modified) metabolic pathways. The substrates to be converted are biomass with special emphasis on lignocellulose and glycerol whereas useful chemicals refer to ethanol, value added products, lactic acid, fuels (apart of ethanol), molecular hydrogen, etc. When it comes to the methods, this book presents both general and strain-specific genetic engineering strategies and methodologies aiming at increasing the yield of transformation of row materials to useful chemicals. Furthermore, two chapters are totally focused on one of the main drawbacks, namely lignocellulosic hydrolysate toxicity, following the physical and chemical treatments of the biomass in order to release the mixture of sugar. The editor and the contributors are very well known scientists, with very good knowledge and important contributions in their fields. The style is clear and the illustrations, besides not very rich, are very comprehensive and useful for the reader. In my opinion, this book can, indeed, become a reference for researchers and students working in this field.

I.I. Ardelean