UNIVERSITATEA „ALEXANDRU IOAN CUZA”
DIN IAȘI

JOURNAL OF EXPERIMENTAL AND MOLECULAR BIOLOGY

TOME XX, Number 4 **2019**

Editura Universității „ALEXANDRU IOAN CUZA” din Iași

EDITORIAL OFFICE

Universitatea „Alexandru Ioan Cuza" din Iași, Facultatea de BIOLOGIE
Laboratorul de Biochimie și Biologie Moleculară
Bulevardul Carol I, Nr. 20A, 700506, Iași, România
www.jemb.bio.uaic.ro / gbmpapers@yahoo.com

CONTENT

Academicianul PETRE JITARIU (1905-1989) - omul şi fondatorul şcolii de biomagnetism în ţara noastră[1]

Cinstirea memoriei înaintaşilor, respectul faţă de cei care au fost personalităţi de seamă ale intelectualităţii româneşti reprezintă o datorie şi o onoare pentru urmaşi, însemnând totodată cinstirea neamului şi a ţării, o dovadă a continuităţii spirituale.

Organizarea unor manifestări omagiale, ca cea de astăzi, marchează momente importante ale istoriei şi culturii româneşti. Prin aceasta dovedim că prezentul are rădăcini puternice într-un trecut cu oameni de o valoare incontestabilă, care au iniţiat şi dezvoltat noi direcţii de cercetare ştiinţifică şi au creat şcoli în care s-au format şi s-au afirmat urmaşi care au asigurat continuitatea.

Astfel, clepsidra Universităţii „Alexandru Ioan Cuza" din Iaşi şi cea a Academiei Române ne amintesc că s-au scurs aproape 115 ani de la naştere şi 30 de ani de la dispariţia unuia dintre cei mai de seamă profesori pe care i-a avut Facultatea de Biologie din Iaşi, acad. PETRE JITARIU.

Fiind născut la 11 mai 1905 şi trăind de la un început de veac şi până aproape de încheierea lui, când la 30 iunie 1989 s-a călătorit în lumea de dincolo de orizont, acad. Petre Jitariu a făcut parte din generaţia marilor spirite de cărturari români care au dominat secolul al XX-lea şi care au trăit marile drame, frământări şi prefaceri generate de cele două conflagraţii mondiale, fără a-şi pierde însă speranţa renaşterii unei societăţi mai înţelepte şi neprecupeţindu-şi efortul pentru refacerea şi reîntregirea valorilor care s-au distrus ori s-au risipit

Academician PETRE JITARIU (1905-1989) – man and founder of the Romanian school of biomagnetism

Paying homage to the memory of predecesors shows that our esteem for the great personalities of Romanian inteligentsia is both a moral duty and a dignity for their followers and a sincere homage, a sign of respect for their country and people, a mark of spiritual continuity.

Reverential manifestations, like the one of today, are dedicated to important moments of the Romanian history and culture. In this way, one shows that the present times have deep roots in a past built up by people of indisputable value, who initiated and developed new directions of scientific research and created schools through which their followers continued their work.

Today, the sand glass of the "Alexandru Ioan Cuza" University of Iaşi and of the Romanian Academy reminds us all that almost 115 years since his birth and 30 years, respectively, elapsed from the passing away of one of the most valuable professors of the Faculty of Biology of Iaşi, academician PETRE JITARIU.

Born on May 11, 1905 living therefore along almost the whole 20th century, until June 30, 1989, when he passed the line of the horizon, Petre Jitariu belonged to the generation of brilliant Romanian scholars who dominated their epoch, who participated to and suffered the great dramas, historical turmoils and transformations brought about by the two world wars, yet without losing their hope in the re-establishment of a wiser society, making all efforts for the restoration and recovery of

[1] Comunicare prezentată în cadrul „Zilelor Academice Ieşene", 17 octombrie 2019

din nesăbuinţa oamenilor.

X

X X

Prof. Petre Jitariu s-a născut în satul Giuleşti, comuna Drăgăneşti, astăzi Boroaia, judeţul Suceava. Bunicul său, Nicolae Boare, originar din zona Câmpulung Moldovenesc, neavând prea mult noroc în munca la pădure, sau ca păstor, s-a angajat ca jitar (paznic de pământ) pe moşia lui Călinderu, din ţinutul Fălticenilor. În 1866, când s-au făcut recensămintele comunale, numele lui a fost schimbat după ocupaţia sa, din Boare devenind Jitaru şi păstrat astfel şi de fiul său, iar apoi de nepotul său, dar înregistrat ca Jitariu de către notarul care a întocmit actele. Părinţii prof. Petre Jitariu au fost oameni demni şi harnici – tatăl său- Vasile Jitariu, era învăţător, iar mama – Cleopatra, era casnică – fiind nevoiţi să întreţină o familie numeroasă, cu cinci copii, dintre care ultimul născut era viitorul academician.

Prof. Petre Jitariu a urmat şcoala primară în satul natal, sub îndrumarea atentă şi exigentă a tatălui său, iar gimnaziul şi Liceul ,,Nicu Gane" în Fălticeni.

După examenul de bacalaureat, susţinut în anul 1924, s-a înscris la Secţia de Ştiinţe Naturale a Facultăţii de Ştiinţe de la Universitatea din Iaşi. La diferitele discipline de biologie a avut dascăli renumiţi precum: Ion Borcea, Paul Bujor, Alexandru Popovici, Ion C. Constantineanu, Ion Simionescu, Ioan Botez, Constantin Motaş, Nicolae Cosmovici, Elena Lupu.

După absolvirea facultăţii, în anul 1929, şi-a satisfăcut stagiul militar la Craiova, iar în 1930 este numit, de către prof. Nicolae Cosmovici, asistent suplinitor la Catedra de Fiziologie genrală şi comparată. Totodată, a activat şi ca profesor de ştiinţe naturale la Liceul ,,Cuza Vodă" din Huşi şi la Liceul Internat din Iaşi, până în anul 1936. În acest timp a realizat primele sale cercetări de fiziologie animală, iar în anul 1938 şi-a susţinut doctoratul în ştiinţe naturale, sub conducerea aceluiaşi profesor Nicolae Cosmovici. După ce obţine titlul de doctor primeşte o bursă de studii, pentru perioada 1938-1939, la Universitatea Göttingen (Germania), unde şi-a desăvârşit specializarea în domeniul fiziologiei animale, continuând mai cu seamă cercetările asupra fiziologiei ficatului.

După revenirea în ţară şi-a reluat activitatea didactică şi ştiinţifică la aceeaşi catedră, când Universitatea din Iaşi a fost nevoită să se refugieze într-un loc mai sigur.

the values destroyed by history or by people's recklessness.

X

Petre Jitariu was born in the village of Giuleşti, Drăgăneşti, today known as Boroaia, county of Suceava. His grandfather, Nicolae Boare, native of the Câmpulung Moldovenesc region, not succeeding in making his life as a forester or as a sheperd, was engaged as a watchman ("jitar") at the Călinderu estate, in the vicinity of Fălticeni county. In the year 1866, on the occasion of the usual rural census, his name was changed, to match his new job, so that, in the records of the village, Boare became Jitaru, a name to be transmitted to his son, then to his grandson. The parents of Petre Jitariu were dignified and hard working people – his father, Vasile Jitariu, was a schoolmaster, his mother – Cleopatra, a housewife watching over their numerous family: 5 children, our future academician being the youngest.

Petre Jitariu attended the first years of education in his village, under the considerate and exigent guidance of his father, being then sent to the prestigious "Nicu Gane" Lyceum of Fălticeni.

In the year 1924, following his leaving school exam, he enrolled as a student of the Faculty of Sciences of the Iaşi University – Section of Natural Sciences, where all biological disciplines were represented by reputed professors, such as: Ion Borcea, Paul Bujor, Alexandru Popovici, Ion C. Constantineanu, Ion Simionescu, Ioan Botez, Constantin Motaş, Nicolae Cosmovici, Elena Lupu.

After graduation, in the year 1929, and after the compulsory military service performed at Craiova, in 1930 professor Nicolae Cosmovici invites Petre Jitariu to become deputy assistant at the Chair of General and Comparative Physiology. In the same period, until 1936, he also worked as a teacher of natural sciences at the "Cuza Vodă" Lyceum of Huşi and at the prestigious Internate Lyceum of Iaşi. Along these years, he initiated his first researches of animal physiology, and in 1938 he publicly defended his PhD thesis, supervised by the same professor Nicolae Cosmovici. Once a PhD, between 1938-1939, he received a scholarship grant at the University of Göttingen (Germany), where he accomplished his specialization in the field of animal physiology, continuing his scientific investigations, mainly devoted to liver physiology.

After his returning to Romania, in the end of the war, Petre Jitariu reorganized the Laboratory of Animal Physiology, seriously destroyed during

La încetarea războiului şi după revenirea din refugiu la Iaşi, Petre Jitariu reorganizează Laboratorul de Fiziologie animală, răvăşit de război, dotându-l cu aparatură modernă pentru acea vreme şi cu instalaţii corespunzătoare.

În 1942 devine şef de lucrări, iar în 1947 profesor titular, predând timp de 28 de ani cursuri de o înaltă măiestrie pedagogică, cu un conţinut bogat, permanent actualizat, fiind şi unul din principalii autori ai *Manualului de fiziologia animalelor şi a omului.*

Ca student, în anul III, al prof. Petre Jitariu, în anul universitar 1955-1956, i-am audiat cursurile predate cu o claritate de cristal, având caracter novator şi dinamic, rostite cu voce caldă şi o vorbire aleasă. Faţă de studenţi, prof. Petre Jitariu era apropiat şi înţelegător, gata să-i îndrume şi să-i ajute pentru rezolvarea oricărei probleme de studiu sau de viaţă. La examene era exigent şi drept, pretenţios, dar fără exces. Cu aceeaşi dăruire şi pasiune a pregătit numeroase serii de studenţi, dintre care, unii au devenit, la rândul lor, personalităţi recunoscute ale biologiei româneşti. Aşa s-a desfăşurat întreaga sa activitate la catedră, tinp de 45 de ani, între 1930 şi 1975, când a devenit profesor consultant.

X

X X

În afară de activitatea didactică, prof. Petre Jitariu a desfăşurat şi o bogată şi apreciată **activitate organizatorică** şi de conducere a învăţământului biologic şi a cercetării ştiinţifice. Astfel, a fost şef de catedră şi decan al Facultăţii de Biologie, timp de 13 ani.

A contribuit din plin la înzestrarea laboratoarelor facultăţii cu o bază materială corespunzătoare pentru activitatea didactică şi cea ştiinţifică. Totodată, prin efortul şi sprijinul său direct s-a realizat un nou local al facultăţii, dat în folosinţă în anul 1963 şi s-a amenajat noua grădină botanică din dealul Copoului. Din dorinţa legării învăţământului cu cercetarea şi cu practica studenţilor, a reuşit să înfiinţeze, în 1956, Staţiunea de Cercetări Biologice, Geologice şi Geografice „Stejarul" de la Pângăraţi-Neamţ, devenită ulterior Staţiunea Potoci, destinată drept bază de cercetare ştiinţifică şi de practică a studenţilor, purtând numele fondatorului său. Cu acelaşi efort şi dăruire, ca decan, a coordonat şi activitatea de cercetare şi didactică a Staţiunii Biologice Marine „Prof. Ion Borcea" de la Agigea-Constanţa, precum şi a the refuge of the University, endowing it with modern installations and equipments.

In 1942 he becomes a lecturer and in 1947 full professor, a position he honoured for 28 years, delievering remarkable lectures to his students and coauthoring the prestigious volume, *Bookhand of animal and human physiology.*

I have had the privilege, as a student in my IIIrd year of study, in the university year 1955-1956, to attend his lectures, always perfectly intelligible, always offering new, updated, dynamic information; I shall not forget his warm voice and his distinguished formulations. With his students, prof. Petre Jitariu was always friendly, ready to guide and help them in any problem they would face. He was an exigent and correct examiner, yet without ever forcing any line. Along his 45 year-long career, with the same passion and abnegation, he guided numerous series of students, many of them becoming, in time, reputed personalities in Romanian biology. In the year 1975, he retired, yet continuing to activate as invited professor.

X

X X

Apart from his didactic work, prof. Petre Jitariu had a rich and highly appreciated **organizational activity** as a member of the management staff of biological education and scientific research. For 13 years, he was head of department and dean of the Faculty of Biology.

In such important positions, he contributed decisively to the acquisition, for the laboratories of the faculty, of modern endowments for didactic and scientific activities. At the same time, he actively took part to the building up of a new edifice for the faculty, inaugurated in 1963, and in the reorganization of the new Botanical Gardens of Iași. Understanding the importance of the connection between students' education and their practice and research activities, he succeeded in creating, in 1956, at Pângăraţi-Neamţ, the "Stejarul" Biological, Geological and Geographic Research Station, the actual Potoci Station, as a sound scientific theoretical and practical basis for students, which now bears the name of his founder. With the same enthusiasm, as a dean, he coordinated the research and didactic activities performed at the "Prof. Ion Borcea" Marine Biological Station of Agigea-Constanţa, as well as at the Museum of Natural History of Iași. Another realization is the foundation, in 1968, of the Center of Biological

Muzeului de Istorie Naturală din Iaşi. O altă realizare este reprezentată de înfiinţarea, în 1968, a Centrului de Cercetări Biologice, iniţial ca unitate a Filialei din Iaşi a Academiei Române, fiind director în perioada 1968-1977.

X

X X

Paralel cu activitatea didactică şi organizatorică, prof. Petre Jitariu a desfăşurat şi o susţinută şi bogată **activitate ştiinţifică**, publicând peste 130 de lucrări originale în reviste de prestigiu din ţară şi din străinătate. Cercetările sale au un caracter complex, abordând probleme majore de fiziologie animală, atât de ordin fundamental, cât şi practic. A deschis direcţii noi de cercetare şi a format un număr mare de specialişti, creând o puternică şcoală de fiziologie animală la Universitatea ieşeană, recunoscută în ţară şi peste hotare.

Pentru valoroasele sale contribuţii ştiinţifice, profesorul Petre Jitariu a fost ales, în anul 1963, membru corespondent al Academiei Române, devenind apoi membru titular al acesteia în anul 1974.

În Academia Română a desfăşurat o susţinută şi apreciată activitate, fiind secretar al Filialei din Iaşi în perioada 1963-1974 şi apoi preşedinte ale acesteia, din 1974 până în 1989, când s-a alăturat „nemuritorilor" din lumea umbrelor, lăsând urmaşilor o bogată moştenire ştiinţifică şi materială, precum şi o valoroasă tradiţie spirituală. În perioada cât a fost preşedinte al Filialei s-a dat în folosinţă clădirea în care ne aflăm şi a început seria manifestărilor ştiinţifice *Zilele Academice Ieşene*.

Pentru trăsăturile personalităţii sale spirituale a rămas în galeria marilor cărturari ieşeni de la cumpăna dintre milenii.

Activitatea de cercetare ştiinţifică a prof. Petre Jitariu a început încă de la încadrarea sa ca asistent, cu investigaţii asupra fiziologiei ficatului, în cadrul tezei de doctorat, investigaţii realizate în timpul specializării la Göttingen, rezultatele fiind semnalate la revista Naturwissenschaften Forschungen în 1942 şi incluse integral în 1959, în monografia asupra termoreglării, publicată în Journal de Physiologie din Paris.

În activitatea de cercetare a antrenat şi format numeroşi tineri, sprijinindu-i să se specializeze în străinătate, în domenii de mare actualitate ale fiziologiei animale. A reuşit astfel să formeze o puternică şcoală ieşeană de fiziologie, reluând tradiţia deschisă de înaintaşii săi, Leon Researches, first as a structure belonging to the Iaşi Branch of the Romanian Academy, whose director he was between 1968-1977.

X

X X

In parallels with his didactic and organizational activities, professor Petre Jitariu also developed an impressive **scientific activity**, materialized in more than 130 original papers published in prestigious journals issued in Romania and abroad. His researches have a complex character, approaching major aspects – equally theoretical and practical - of animal physiology. He opened new directions of research and trained a great number of specialists, thus creating an important school of animal physiology at the University of Iaşi, unanimously recognized both at national level and abroad.

Considering his valuable scientific contributions, in the year 1963, professor Petre Jitariu was elected corresponding member of the Romanian Academy, and full member in 1974.

Within the Romanian Academy, he developed a rich and most appreciated activity, as a secretary of the Iaşi Branch between 1963-1974, then as its president, between 1974 and 1989, when he joined the "immortals" living in the world of shadows, leaving to his followers a rich scientific and material heritage, as well as a distinguished spiritual tradition. As a president of the Iaşi Branch of the Academy, he launched the series of the scientific manifestations of the *Academic Days of Iaşi*.

The scientific activity of Petre Jitariu begun as early as his years as a university assistant, with investigations devoted to the physiology of the liver, approached in his PhD thesis, prepared during his specialization in Göttingen, the results obtained being published in the prestigious Naturwissenschaften Forschungen in 1942, then included in the monography on thermoregulation, published in Journal de Physiologie of Paris, in 1959.

In his research work devoted to most actual fields of animal physiology, he involved numerous young colleagues, helping them to be specialized abroad, thus succeeding in creating a powerful school of physiology in Iaşi, resuming the tradition installed by his predecesors, Leon Cosmovici, Nicolae Cosmovici and Elena Lupu. He invited to work together representatives of the didactic staff of the Faculty of Biology,

Cosmovici, Nicolae Cosmovici şi Elena Lupu. A unit în jurul său cadre didactice de la Facultatea de Biologie, cercetători de la Centrul de Cercetări Biologice şi de la cele două staţiuni ale Universităţii ieşene de la Agigea-Constanţa şi de la Pângăraţi-Neamţ, precum şi doctoranzi din diferite centre universitare din ţară. Prin rezultatele obţinute, această şcoală a devenit recunoscută în ţară şi în străinătate, mulţi dintre membrii ei ajungând personalităţi ştiinţifice de prestigiu, profesori universitari şi cercetători de valoare.

O deosebită atenţie a fost acordată de acad. Petre Jitariu domeniilor noi de cercetare pe plan mondial, prin sprijinirea specializării în ţară şi în străinătate a unor tineri, îndeosebi pentru cunoaşterea unor tehnici noi, de mare fineţe şi abordare a unor cercetări de fiziologie a sistemului nervos şi a sistemului endocrin, de fiziologie a membranelor, de biologie celulară şi moleculară, de biofizică.

Împreună cu colaboratorii şi doctoranzii săi, prof. Petre Jitariu a abordat diferite subdomenii ale fiziologiei animale:

- evoluţia unor funcţii în seria animală: fiziologia mediului intern, a inimii şi a aparatului circulator la amfibieni;
- raporturile ce se stabilesc între tensiunea superficială a plasmei sanguine şi a hemolimfei la mamifere;
- procesul de coagulare a hemolimfei la crustacei şi lamelibranhiate;
- ecofiziologia animalelor de apă dulce: parametri hidrochimici, hidrofizici, fiziologici şi biochimici specifici bazinelor râurilor Bistriţa şi Prut, înainte şi după realizarea barajelor şi a hidrocentralelor de la Bicaz şi Stânca-Ştefăneşti;
- comportamentul fiziologic al păstrăvului din râul Bistriţa, înainte de realizarea barajului de la Bicaz, apoi din Lacul Bicaz, după realizarea barajului; ***aceste cercetări au fost continuate de colaboratorii săi, la Staţiunea Potoci-Neamţ, având o importanţă aplicativă deosebită pentru salmonicultura dirijată.***
- nutriţia animală la unele animale domestice, păsări şi ovine, studiul vizând rolul unor alimente vegetale bogate în iod;
- fiziologia sistemului nervos: rolul său asupra epilepsiei şi schizofreniei;
- interrelaţiile hipotalamus-hipofiză;

investigators from the Center of Biological Researches and from the two scientific stations of the University: Agigea-Constanţa and Pângăraţi-Neamţ, as well as PhD students from different university centers of Romania. The results obtained made this school renowned both in the country and abroad as, along the years, many of its representatives became prestigious personalities of the field, university professors and reputed researchers.

Special attention was paid by acad. Petre Jitariu to the new domains of international research, assuring specialization stages for his young collaborators in prestigious centers of the whole world, stress being laid on the new techniques applied for better understanding and approaching the physiology of the nervous and endocrine system, membrane physiology, cell and molecular biology, biophysics, etc.

Together with his collaborators and his PhD students, professor Petre Jitariu approached various subdomains of animal physiology, such as:

- Evolution of certain functions of the animal series: physiology of the internal environment, of the heart and of the circulatory apparatus in amphibians;
- The relations established between the surface tension of sanguine plasma and of hemolympha in mammals;
- The process of hemolimpha coagulation in crustacean and lamellibranchiate organisms;
- The ecophysology of sweetwater animals: hydrochemical, hydrophysical, physiological and biochemical parameters specific to the basins of the Bistriţa and Prut rivers, both before and after the construction of dams and of the hydrocentrals of Bicaz and Stânca-Ştefăneşti;
- The physiological behaviour of the trout living in the Bistriţa river, prior to the realization of the Bicaz dam, and then in the Bicaz Lake, after the building up of the dam; ***these researches, of special practical importance for controlled salmoniculture, have been continued by his collaborators at the Potoci-Neamţ Recearch Station.***
- Animal nutrition in some domestic animals, birds and sheep, stress being laid on the role of certain vagatal iodine-rich

- fiziologia splinei: interrelaţiile funcţionale spleno-gastrice şi splenosanguine.

Unul din cele mai însemnate domenii de cercetare abordate de acad. Petre Jitariu este cel referitor la acţiunea câmpurilor electromagnetice asupra organismelor vii. Prin anvergura cercetărilor şi originalitatea rezultatelor obţinute, acesta a fost domeniul cel mai fecund al acivităţii sale, acad. Petre Jitariu fiind considerat unul dintre principalii fondatori ai şcolii româneşti de biomagnetism, urmărind acţiunea CEM la nivelul sângelui, sistemului nervos şi endocrin, efectele stimulatoare ale dinamicii formării de anticorpi la animale. Acestui important domeniu i s-a adăugat iniţierea cercetărilor în cel mai modern câmp de cercetare: fiziologia membranelor celulare.

Rezultatele a peste 50 de comunicări asupra efectelor biologice ale CEM, elaborate de acad. Petre Jitariu şi colaboratorii săi, au fost prezentate la Simpozionul Naţional de Biomagnetism din 1970, cu aprecieri unanime din partea specialiştilor. În acest domeniu au fost studiate diferite aspecte ale influenţei CM şi EM asupra organismelor animale şi vegetale, precum şi asupra microorganismelor, fiind obţinute numeroase rezultate originale, publicate în articole apărute în reviste de prestigiu din ţară şi din străinătate, sau incluse în teze de doctorat ale elevilor îndrumaţi de acad. Petre Jitariu. Sub coordonarea prof. Petre Jitariu a fost publicată, în Edit. Academiei Române, în anul 1987, **o monografie** de referinţă, cuprinzând aspecte teoretice şi rezultate experimentale obţinute de membrii şcolii ieşene de magnetobiologie.

Pe baza rezultatelor obţinute, acad. Petre Jitariu a formulat o teorie nouă privind mecanismul de acţiune a CM şi EM asupra organismelor vii. Ideea de bază a acestei teorii originale constă în existenţa unor interacţiuni specifice la nivel molecular între CEM şi structurile vii. Astfel, la nivelul macromoleculelor proteice, cu structură helicoidală, polarizate electric datorită existenţei unor atomi de carbon asimetrici, cu radicali diferiţi, au loc mişcări de electrozi care generează microcâmpuri electrice proprii.

Meritele acad. Petre Jitariu i-au fost recunoscute de contemporani, fiind investit cu diverse funcţii, ales în diferite organisme ştiinţifice, româneşti şi internaţionale şi răsplătit cu numeroase titluri onorifice, ordine şi medalii: şef de catedră, decan, director de staţiune şi de centru de cercetări biologice. Pentru meritele sale ştiinţifice deosebite a fost ales membru al Academiei Române, pe care a

aliments;
- Physiology of the nervous system: its role in epilepsy and schizophrenia;
- The hypotalamus-hypophysis inter-relations;
- Spleen physiology: functional spleen-gastric and spleenosanguine inter-relations.

One of the most important domains of research approached by acad. Petre Jitariu refers to the action of electromagnetic fields upon the living organisms, the most prolific domain of his activity, if considering the amplitude of the investigations and the originality of the results obtained, which made acad. Petre Jitariu one of the main founders of the Romanian school of biomagnetism; the main themes of scientific interest were the action of CEM at blood level, nervous and endocrine systems, the stimulating effects of the dynamics of antibodies formation in animals. To these important aspects, one should add launching of investigations in the most modern field of research, namely physiology of cell membranes.

The results communicated in more than 50 studies devoted to the biological effects of CEM, elaborated by acad. Petre Jitariu and his coworkers, were presented at the National Symposium of Biomagnetism, held in 1970, raising the interest and unanimous appreciation from the part of the specialists in the field. In this domain, different aspects of the influence of CM and EM upon animal and vegetal organisms, as well as upon microorganisms, were investigated, the original results obtained being published in prestigious Romanian and international journals, or included in PhD theses of the young students he supervised. In the year 1987, the Publishing House of the Romanian Academy issued, under the coordination of Petre Jitariu, a most valuable **monograph**, approaching both theoretical aspects and experimental results obtained by the representatives of the Iași school of magnetobiology.

On the basis of the obtained results, acad. Petre Jitariu formulated a new theory on the CM and EM mechanisms of action upon the living organisms, the main idea of this original theory involving the existence of some specific interactions, at molecular level, between CEM and the living structures. According to such an idea, at the level of proteic macromolecules with helicoidal structure, electrically polarized through the existence of some assymetrical carbon atoms with

slujit-o şi onorat-o peste 25 de ani, îndeplinind funcţia de secretar şi de preşedinte al Filialei din Iaşi.

Datorită prestigiului său ştiinţific, acad. Petre Jitariu a fost ales membru al Asociaţiei Oamenilor de Ştiinţă din New York (SUA), membru al Societăţii de anatomie comparată din Paris (Franţa), membru în comitetele de redacţie ale unor reviste româneşti.

În semn de recunoaştere şi apreciere a remarcabilei sale activităţi ştiinţifice, didactice şi obşteşti i s-au decernat distincţii, precum Ordinul Muncii (clasele 3, 2 şi 1), Ordinul „Meritul Ştiinţific", Diploma de „Profesor emeritus" ş.a.

X

X X

Eruditul dascăl a fost căsătorit cu Matilda Jitariu, fiică a compozitorului Alexandru Zirra şi nepoată a lui George Enescu, fiindu-i colegă şi colaboratoare, delicată şi harnică, în acelaşi domeniu al fiziologiei animale.

Omul Petre Jitariu a fost deschis la suflet, modest şi autentic, neatins de vanităţi şi orgolii. A fost un om de cultură aleasă, iubind cu delicateţe şi sensibilitate elevată frumuseţile şi măreţia naturii, muzica, pictura, literatura, filozofia şi istoria, fiind animat de un patriotism discret.

A fost, de asemenea, un pasionat vânător, dar nu de dragul trofeelor (căci biolog fiind preţuia şi respecta fiinţele vii), ci doar pentru că vânătoarea îi oferea prilejul de a colinda în natură, urmărindu-i frumuseţile, nestingherit de tumultul oraşului.

Avea o voce caldă şi plăcută, se antrena în discuţii şi povestiri cu cei apropiaţi, râzând cu poftă la glumele acestora şi dând replici de aceeaşi factură spirituală.

Aşa a trăit şi a muncit, iubind natura şi oamenii, cel care a dus o viaţă demnă şi bogată în realizări, de om de ştiinţă, de om preţuit pentru elevata sa distincţie intelectuală şi sufletească, până în dimineaţa zilei de 30 iunie 1989, când, împăcat cu sine şi cu oamenii, după o suferinţă neiertătoare, care i-a şubrezit sănătatea, s-a călătorit în liniştea lumii de dincolo de orizont ...

Discipolii, urmaşii, colaboratorii şi toţi cei care l-au cunoscut şi-l amintesc întotdeauna cu admiraţie şi-i dedică omagiul lor de veneraţie şi recunoştinţă, ca semn de împlinire a crezului său pe care l-a rostit la Academie, la aniversarea vârstei de 80 de ani: ***„Creaţia ştiinţifică este o înălţare a spiritului, o izbândă fericită a gândirii umane. Acesta mi-a fost crezul de o viaţă, pe care am*** different radicals, movements of electrodes, which generate their own electrical microfields, are manifested.

The scientific value of acad. Petre Jitariu has been recognized by his contemporaries, who invested him with various positions, in numerous Romanian and international institutions, which offered him prestigious honours and titles, such as: head of department, dean, director of the station and of the center of biological researches. He was also elected a member of the Romanian Academy, which he served and honoured for more than 25 years, as a secretary, than as president of its Iaşi Branch.

Due to his scientific prestige, acad. Petre Jitariu was elected member of the Association of Men-of-Science of New York (USA), of the Society of Comparative Anatomy of Paris (France), and also of various editorial boards of prestigious Romanian journals.

He also received various medals and distinctions of honour: the Romanian "Ordinul Muncii" award (classes 3, 2 and 1), the "Scientific Merit" Order, the "Emeritus Professor" Diploma, etc.

X

X X

Petre Jitariu married Matilda Jitariu, daughter of the reputed composer Alexandru Zirra and niece of George Enescu, who, along their whole active life, was his delicate and hardworking coworker in the same domain of animal physiology.

The man Petre Jitariu was an open-hearted, modest and most sincere person, never affected by vanity or self-importance. He was an authentic man of culture, a sensible and gentle lover of nature, music, painting, literature, philosophy and history, and equally a most discrete patriot.

He was also a passionate hunter, as hunting offered to him the opportunity to escape the crowded city, to relax, to wander in nature and admire its unfading beauty.

He had a warm and most pleasant voice, he was a spiritual joker, always enjoying the company of his friends, a most distinguished intellectual and moral collocutor, always animated by his love for nature and for people, living a dignified and accomplished life as a unanimously appreciated man of science... all these until the morning of June 30 iunie 1989 when, after a long suffering, reconciled and accepting the moment, he passed the line of horizon...

His disciples, his followers, coworkers

căutat să-l transmit cu încetul, pe neobservate, elevilor şi colaboratorilor mei”.

Acad. CONSTANTIN TOMA
Facultatea de Biologie
Universitatea „Alexandru Ioan Cuza” din Iaşi

and all those who knew him and had the chance to work with him nourish the same admiration and pay their homage of veneration and gratitude, a sign that his long-life creed, confessed by Petre Jitariu himself under the cupola of the Academy, on the occasion of his 80th anniversary: ***”Scientific creation represents a spiritual raising, a blessed victory of human thinking. This was the creed of my life, which I strived for transmitting, slowly and discretely, to my pupils and collaborators***” still bears fruits**.**

Acad. CONSTANTIN TOMA
Faculty of Biology
”Alexandru Ioan Cuza” University of Iaşi

NANOPARTICLES: AN OVERVIEW ABOUT THEIR CLASIFICATIONS, SYNTHESIS, PROPERTIES, CHARACTERIZATION AND APPLICATIONS

LĂCRĂMIOARA OPRICĂ[1*], MARIA BĂLĂȘOIU[2,3]

Received: 2nd of July 2019 / Revised: 15th of September 2019
Accepted: 23rd of October 2019 / Published: 21st of January 2020

Keywords: nanoparticles, properties, Green nanoparticles synthesis, applications

Abstract: Nanoparticles (NPs) are the main product of nanotechnologies. NPs are organic and inorganic structures, their size being less than one hundred nanometers. Due to their potential application in many fields metallic NPs are becoming increasingly important. There are numerous organisms possessing the ability to synthesise NPs and which therefore have the potential to be exploited and modified to optimise them to fulfil this purpose. Therefore, many bacteria, fungi and plants have shown the ability to synthesise metallic NPs but all have their own advantages and disadvantages. This "green" method of biological NPs production is a promising approach that allows synthesis in aqueous conditions, with low energy requirements and low-costs. The development of an environmentally friendly and inexpensive way of synthesising them is therefore crucial.

INTRODUCTION

Nanotechnology is an important field of modern research dealing with design, synthesis, and manipulation of particle structures ranging from approximately 1-100 nm. The word "nano" is derived from a Greek word meaning dwarf or extremely small. Nanobiotechnology involves research of technology in different fields of science like biotechnology, nanotechnology, physics, chemistry, and material science (Rai et al., 2008).

Nanotechnology involves intervention of novel strategies with the help of which atoms and small particles are manipulated. Progress in the field of nanotechnology has been rapid and with the development of innovative synthesis protocols and characterization techniques (Sharma et al., 2009). But most of the synthesis methods are limited to synthesis of nanoparticles (NPs) in small quantities and poor morphology (Sau and Rogach, 2010).

Chemical and physical synthesis methods often result in synthesis of a mixture of nanoparticles with poor morphology, and these methods also prove to be toxic to the environment due to the use of toxic chemicals and elevated temperatures for synthesis process (Birla et al., 2009).

The biosynthesis of NPs has been proposed as a cost-effective and as a rapid, eco-friendly alternative to chemical and physical methods. Metal nanoparticles produced using microorganisms and plant extracts are stable and can be monodispersed by controlling synthetic parameters, such as pH, temperature, incubation period, and mixing ratio. On the other hands, plant-mediated synthesis of NPs is a green chemistry approach that connects nanotechnology with plants. Among the biological alternatives, plants and plant extracts seem to be the best option because as it is known plants are nature's "chemical factories" (Parveen et al., 2016).

Recently, biological NPs were found to be more pharmacologically active than physico-chemically synthesized nanoparticles. Among the various biological NPs, those produced by medicinal plants have been found to be the most pharmacologically active, possibly due to the attachment of several pharmacologically active residues like secondary metabolites (Singh et al., 2016).

1. Classification of Nanoparticles

Nanoparticles can be classified based on the following criteria. From the point of view of origin, NPs can be natural and anthropogenic. On the other hands, they are broadly classified depending on the dimension:

● One dimensional system (thin film or manufactured surfaces) has been used for decades. Thin films (sizes 1–100 nm) or monolayer is now common places in the field of solar cells offering, different technological applications (chemical and biological sensors, optical device, fiber-optic systems);

● Two dimensions nanoparticles such as carbon nanotubes;

● Three dimensions nanoparticles such as Dendrimers, Quantum Dots, Fullerenes (Carbon 60), (QDs) (Hett, 2004).

Another classification of nanoparticles is depending on the chemical composition into the organic, inorganic and carbon based.

●*Organic nanoparticles*

Dendrimers, micelles, liposomes and ferritin, etc. are commonly knows the organic nanoparticles or polymers. These nanoparticles are biodegradable, non-toxic, and some particles such as micelles and liposomes have a hollow core (Table 1), also known as nanocapsules and are sensitive to thermal and electromagnetic radiation such as heat and light (Tiwari et al., 2008).

● *Inorganic nanoparticles*

Inorganic nanoparticles are particles that are not made up of carbon. Metal and metal oxide-based nanoparticles are generally categorised as inorganic nanoparticles.

◘ *Metal based*

These nanoparticles are synthesised from metals to nanometric sizes. Almost all the metals can be synthesised into their nanoparticles, but the commonly used metals are aluminium (Al), cadmium (Cd), cobalt (Co), copper (Cu), gold (Au), iron (Fe), lead (Pb), silver (Ag) and zinc (Zn). On the other hands, the bimetallic list includes Fe–Co, Fe–Ni, Fe–Cu, Cu–Ni and Fe–Pt nanoparticles (Figure 1). The nanoparticles have distinctive properties such sizes as low as 10 to 100nm, surface characteristics like high surface area to volume ratio, pore size, surface charge and surface charge density, crystalline and amorphous structures, shapes like spherical and cylindrical and colour, reactivity and sensitivity to environmental factors such as air, moisture, heat and sunlight etc.

◘ *Metal oxides based*

The metal oxide-based nanoparticles are synthesised to modify the properties of their respective metal-based nanoparticles. Metal oxide nanoparticles are synthesised mainly due to their increased reactivity and efficiency (Tai et al., 2007). The commonly synthesised are Aluminium oxide (Al_2O_3), Cerium oxide (CeO_2), Iron oxide (Fe_2O_3), Magnetite (Fe_3O_4), Silicon dioxide (SiO2), Titanium oxide (TiO_2), Zinc oxide (ZnO). These nanoparticles have possessed exceptional properties when compared to their metal counterparts. Some of NPs have magnetic properties like Fe_3O_4, Co–Fe_2O_4 and Mn–Fe_2O_4 (McNamara and Tofail, 2017).

●*Carbon based*

The nanoparticles made completely of carbon are knows as carbon based (Bhaviripudi et al., 2007). They can be classified into fullerenes, graphene, carbon nano

tubes (CNT), carbon nanofibers and carbon black and sometimes activated carbon in nano size (Figure 1).

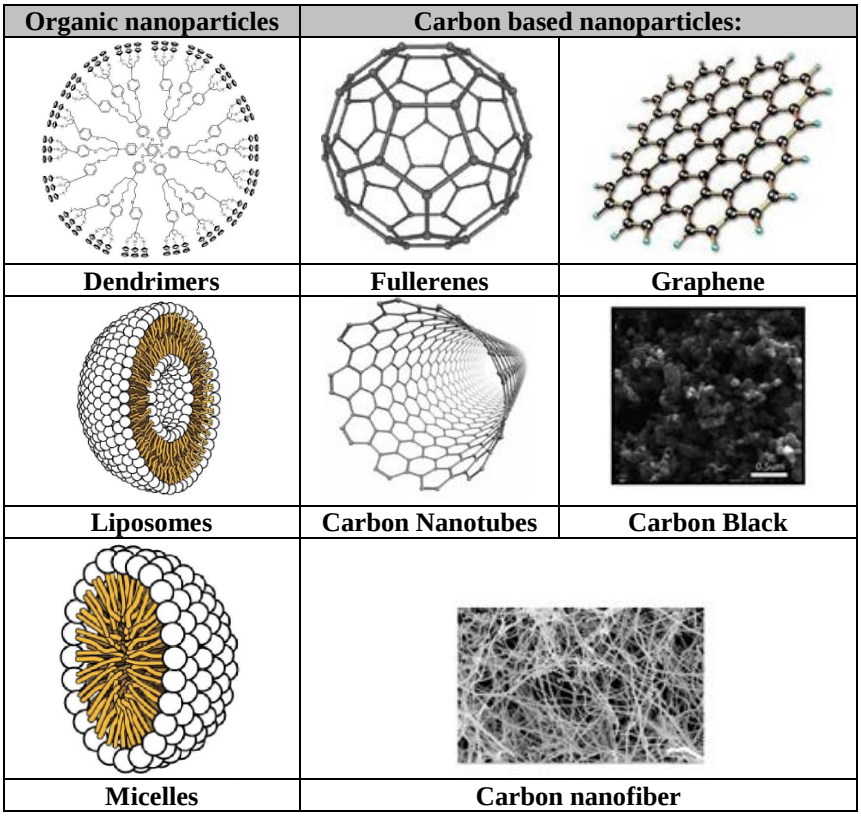

Figure 1. Organic nanoparticles (Dendrimers, Liposomes and Micelles) and Carbon-based nanoparticles (Fullerenes, Graphene, Carbon nanotubes, Carbon nanofibers and Carbon black) (Ealias and Saravanakumar, 2017)

2. Properties of Nanoparticles

The nanoparticles are of different shape, size and structure. It is spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc. or irregular and differs from 1 nm to 100 nm in size. The surface can be a uniform or irregular with surface variations. Some nanoparticles are crystalline or amorphous with single or multi crystal solids either loose or agglomerated (Machado et al., 2015). For characterization of nanoparticles there are necessary identifications of several parameters (Table 1).

The properties of nanoparticles are generally categorised into physical and chemical. The properties of few common nanoparticles are given in Table 2.

Table 1. Different parameters for characterization of nanoparticles (Wali et al., 2018)

Parameters	Instrument used
Particle size & size distribution	Zetasizer, Photon correlation spectroscopy, Mercury porosimetry, Laser diffractometry
Particle Morphology	Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Atomic force microscopy (AFM)
Charge determination	Laser droplet anemometry, Zeta potentiometer
Metallic nature	X-ray diffraction (XRD) Analysis
Identification of Functional groups	Fourier Transform Infrared (FTIR) Spectroscopy
Chemical analysis of surface	Static secondary ion mass spectrometry
Release profile	In-vitro release characteristic under physiologic & sink condition

3. Methods of Nanoparticles Synthesis

The nanoparticles can be synthesized by various protocols using the *top-down* (physical) approach which deals with methods such as thermal decomposition, diffusion, irradiation, radiation, laser ablation, arc discharge, etc., and *bottom-up* (chemical and biological) approach which involves seeded growth method, polyol synthesis method, electrochemical synthesis, chemical reduction, condensation and biological entities for fabrication of nanoparticles Figure 2, Figure 3. Different synthesis methods involve the use of different types of chemical, physical, and biological agents to yield nanoparticles of different sizes and shapes.

Table 2. Physical and chemical properties of different nanoparticles (Ealias and Saravanakumar, 2017)

Nanoparticles	Properties
Carbon based nanoparticles	
Fullerenes	Safe and inert, semiconductor, conductor and superconductor, transmits light based on intensity
Graphene	Extreme strength, thermal, electrical conductivity, light absorption
Carbon Nano Tubes (CNT)	High electrical and thermal conductivity, tensile strength, flexible and elastic
Carbon Nanofiber	High thermal, electrical, frequency shielding, and mechanical properties
Carbon Black	High strength and electrical conductivity, surface area; resistant to UV degradation
Metal based nanoparticles	
Aluminium	High reactivity, sensitive to moisture, heat, and sunlight, large surface area
Iron	Reactive and unstable, sensitive to air (oxygen) and water
Silver	Absorbs and scatters light, stable, anti-bacterial, disinfectant
Gold	Interactive with visible light, reactive
Cobalt	Unstable, magnetic, toxic, absorbs microwaves, magnetic
Cadmium	Semiconductor of electricity, insoluble
Lead	High toxicity, reactive, highly stable
Copper	Ductile, very high thermal and electrical conductivity, highly flammable solids
Zinc	Antibacterial, anti-corrosive, antifungal, UV filtering

Bottom-up method

Bottom-up or constructive method is the build-up of material from atom to clusters to nanoparticles. Sol-gel, spinning, chemical vapour deposition (CVD), pyrolysis and biosynthesis are the most commonly used bottom-up methods for nanoparticle production (Table 3).

Top-down method

Top-down or destructive method is the reduction of a bulk material to nanometric scale particles. Mechanical milling, nanolithography, laser ablation, sputtering and thermal decomposition are some of the most widely used nanoparticle synthesis methods (Table 3).

3.1. Chemical synthesis of nanoparticles, the most often used method that use the chemical reduction method, which deals with the reduction of metal particles to nanoparticles using chemical reducing agents like sodium borohydride or sodium citrate (Cao and Hu, 2009).

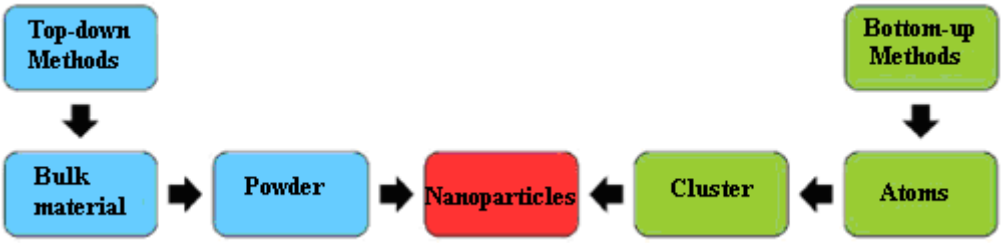

Figure 2. Nanoparticles synthesis process (Ealias and Saravanakumar, 2017)

3.2. Physical methods used for the synthesis of nanoparticles include thermal decomposition, laser irradiation, electrolysis, condensation, diffusion, etc. The thermal decomposition method is used for the synthesis of monodisperse nanoparticles. Fatty acids are dissolved in hot NaOH solution and mixed with metal salt solution which leads to formation of metal precipitate (Yang and Aoki, 2005).

3.3. Moreover, nanoparticles are synthesized through many physicochemical processes which have posed numerous pressures on the environment. Nowadays, eco-friendly attractive alternatives to chemical and physical methods are **biological synthesis** of nanoparticles synthesis using microorganisms (bacteria, yeast, fungi) and plants or plant extracts.

Thus, the *green synthesis* has been proposed as an alternative to reduce the use of hazardous compounds and harsh reaction conditions in the production of NPs. More than the biological approach is free of chemical toxins. Green synthesis of nanoparticles is a simple process, a metal salt is mixed with plant extract and the reaction completes in minutes to few hours at ordinary room temperature. Selection of solvent medium and selection of eco-friendly nontoxic reducing and stabilizing agents are the most important issues which must be considered in green synthesis of NPs. The metallic salt solutions are reduced into respective nanoparticles and for this reason have got considerable attention during the last decade because of simplicity (Wali et al., 2018).

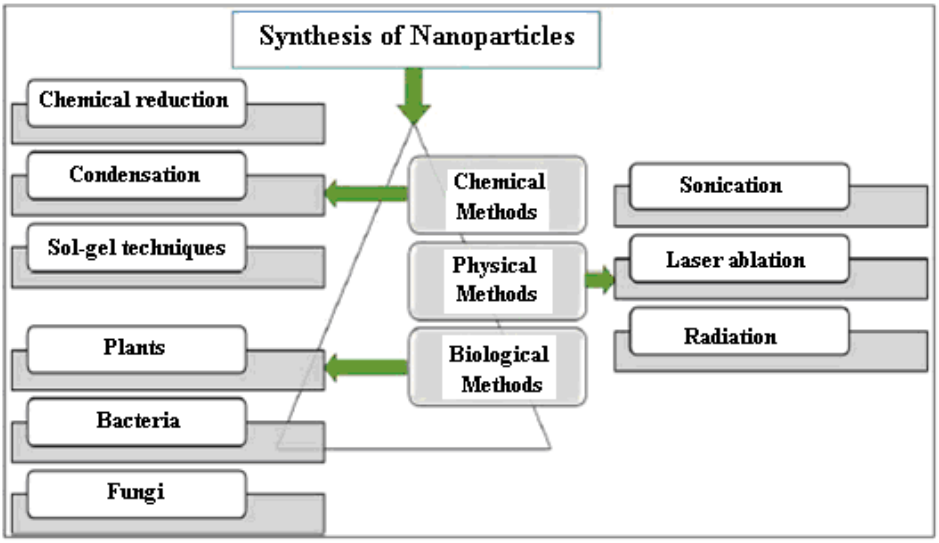

Figure 3. Various methods for making nanoparticles (Wali et al., 2018)

Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. Biogenic reduction of metal precursors to produce corresponding NPs is eco-friendly, less costly, free of chemical contaminants for medical and biological applications where purity of NPs is of major concern (Imtiyaz et al., 2016). Synthesis mediated by plant extracts is environmentally benign. The reducing agents involved include the various water-soluble plant metabolites (e.g. alkaloids, terpenoids, polyphenols, sugars, phenolic acids, and proteins) and co-enzymes (Mittal et al., 2013).

In addition, the biological method provides a wide range of resources for the synthesis of nanoparticles. The rate of reduction of metal ions using biological agents is found to be much faster at ambient temperature and pressure conditions. For instance, in case of synthesis of nanoparticles using *Aspergillus niger* synthesis of silver nanoparticles was observed within 2 h of treatment of fungal filtrate with silver salt solution (Gade et al., 2008).

On the other hand, the biological agents secrete a large number of enzymes, which are capable of hydrolyzing metals and thus bring about enzymatic reduction of metals ions (Rai et al., 2009). In case of fungi, the enzyme nitrate reductase is found to be responsible for the synthesis of nanoparticles (Kumar et al., 2007).

Table 3. Categories of the nanoparticles synthesised from the various methods (Ealias and Saravanakumar, 2017)

Category	**Method**	**Nanoparticles**
Bottom-up	Sol-gel	Carbon, metal and metal oxide based
	Spinning	Organic polymers
	Chemical Vapour Deposition (CVD)	Carbon and metal based
	Pyrolysis	Carbon and metal oxide based
	Biosynthesis	Organic polymers and metal based
Top-down	Mechanical milling	Metal, oxide and polymer based

	Nanolithography	Metal based
	Laser ablation	Carbon based and metal oxide based
	Sputtering	Metal based
	Thermal decomposition	Carbon and metal oxide based

The biggest advantage of biological synthesis based on fungal enzymes is the possibility of developing a rational approach for the biosynthesis of nanomaterials over a range of chemical compositions, which is currently not possible by other microbe-based methods.

Mechanism of biological nanoparticles biosynthesis

Biosynthesis of nanoparticles by microorganisms is an eco-friendly technology. Diverse microorganisms, both prokaryotes and eukaryotes are used for synthesis of metallic nanoparticles (silver, gold, platinum, zirconium, palladium, iron, cadmium and metal oxides such as titanium oxide, zinc oxide, etc). The synthesis of nanoparticles may be intracellular or extracellular according to the location of nanoparticles (Table 4).

Table 4. Synthesis of metallic nanoparticles by different microorganisms

Microorganism	**Type**	**Location**	**Size (nm)**
Phoma sp.	Ag	Extracellular	71.06–74.46
Fusarium oxysporum	Au	Extracellular	20–40
Verticillium sp.	Ag	Intracellular	25 ± 12
Trichoderma asperellum	Ag	Extracellular	13–18
Phaenerochaete chrysosporium	Ag	Extracellular	50–200

Intracellular synthesis of nanoparticles by fungi: This method involves transport of ions into microbial cells to form nanoparticles in the presence of enzymes. As compared to the size of extracellular reduced nanoparticles, the nanoparticles formed inside the organism are smaller. The size limit is probably related to the particles nucleating inside the organisms.

Extracellular synthesis of nanoparticles by fungi: Extracellular synthesis of nanoparticles has more applications as compared to intracellular synthesis since it is void of unnecessary adjoining cellular components from the cell. Fungi are mostly known to produce nanoparticles extracellularly because of their enormous secretory components, which are involved in the reduction and capping of nanoparticles (Narayanan and Sakthivel, 2010). Because of their tolerance and metal bioaccumulation ability, fungi have occupied the centre stage of studies on biological generation of metallic nanoparticles.

4. Characterization of Nanoparticles

Characterization of nanoparticles is based on the size, morphology and surface charge, using such advanced microscopic techniques. Properties like surface morphology, size and overall shape are determined by electron microscopy techniques. Features like physical stability and redispersibility of the polymer dispersion, as well as, their performance *in vivo* are affected by the surface charge of the NPs (Hett, 2004).

■ *Particle size*

Characterization of NPs is primarily evaluated by the particle size distribution and morphology, using electron microscopy. The images of Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) are used for the measurement of particles and clusters whereas laser diffraction methods are used for measuring bulk samples in solid phase (Marsalek, 2014).

■ *Morphological characterizations*

The morphological features of NPs always attain great interest since morphology always influences most of the properties of the NPs. There are different characterization techniques for morphological studies, but microscopic techniques such as SEM and TEM are the most important.

■ *Surface morphology*

The NPs possess various shapes (include spherical, flat, cylindrical, tubular, conical and irregular shapes) and surface structures (like crystalline or amorphous) that play a key role in exploiting its properties. The surface is generally determined by electron microscopy imaging techniques like SEM and TEM (Hodoroaba et al., 2014).

■ *Surface Charge*

Surface charge and intensity determines the interaction of NPs with the biological environment as well as their electrostatic interaction with bioactive compounds. Generally, a zeta potentiometer is used for the measurement of surface charges and its dispersion stability in a solution. Zeta potential values can be utilized in evaluating surface hydrophobicity and the nature of material encapsulated within the nanocapsules or coated onto the surface (Pangi et al., 2003).

■ *Structural characterizations*

The structural characteristics are of the primary importance to study the composition and nature of bonding materials. It provides diverse information about the bulk properties of the subject material. The common techniques used to study structural properties of NPs are X-Ray Diffraction (XRD), energy dispersive X-ray (EDX), X-ray Photoelectron Spectroscopy (XPS), Infrared (IR) and Raman Spectroscopy.

■ *Composition*

The composition (chemical or elemental composition) measurement is usually carried out by X-ray photoelectron spectroscopy (XPS) (Sharma and Rao, 2014). Some techniques involve chemical digestion of the particles followed by wet chemical analysis such as mass spectrometry, atomic emission spectroscopy and ion chromatography. The particles in gaseous phase are collected either by filtration or electrostatically and spectrometric or wet chemical techniques are used for the analysis (Bzdek et al., 2011).

Once NPs are synthesized, it is important to fully characterize and understand their structure. Over the years, many methods and techniques have been developed for the analysis of various physicochemical properties of NPs. Different characterization techniques have been used for the analysis of various physicochemical properties of NPs.

Nanotechnology has massively grown up with the development of advanced electron microscopes and the main relevance techniques with will be presented below.

● **UV-visible absorption spectroscopy**: Absorbance spectroscopy is used to determine the optical properties of a solution. When the wavelength is varied and the

absorbance is measured at each wavelength. The absorbance can be used to measure the concentration of a solution by using Beer-Lamberts Law.

● **X-ray diffraction (XRD) analysis**: X-ray diffraction is a conventional technique for determination of crystallographic structure and morphology. There is increase or decrease in intensity with the amount of constituent. This technique is used to establish the metallic nature of particles gives information on translational symmetry size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities.

X ray diffraction analysis with various nanoparticles has been studied by various research workers to find the high crystallinity of the prepared sample.

● **Fourier Transform Infrared (FTIR)** spectroscopy measures infrared intensity vs. wavelength of light; it is used to determine the nature of associated functional groups and structural features of biological extracts with nanoparticles. The calculated spectra clearly reflect the well-known dependence of nanoparticle optical properties.

● **Photon-Correlation Spectroscopy (PCS) or Dynamic Light Scattering (DLS)** Current research demands the fastest and most popular method of determining particle size. The fastest and most popular techniques like photon-correlation spectroscopy (PCS) or dynamic light scattering (DLS), widely used to determine the size of Brownian nanoparticles in colloidal suspensions in the nano and submicron ranges. In this technique solution of spherical particles in Brownian motion causes a Doppler shift when they are exposed against shining monochromatic light (laser).

● **Scanning Electron Microscopy (SEM)**

This electron microscopy-based technique determines the size, shape and surface morphology with direct visualization of the NPs. Therefore scanning electron microscopy offer several advantages in morphological and sizing analysis.

During the process of SEM characterization, solution of nanoparticles should be initially converted into a dry powder. This dry powder is then further mounted on a sample holder followed by coating with a conductive metal (e.g. gold) using a sputter coater. Whole sample is then analyzed by scanning with a focused fine beam of electrons (Jores et al., 2004). Secondary electrons emitted from the sample surface determine the surface characteristics of the sample. This electron beam can often damage the polymer of the nanoparticles which must be able to withstand vacuum.

● **Transmission Electron Microscope (TEM)**

Experimental difficulties in studying nanostructures stem from their small size, which limits the use of traditional techniques for measuring their physical properties. TEM techniques can provide imaging, diffraction and spectroscopic information, either simultaneously or in a serial manner, of the specimen with an atomic or a sub-nanometer spatial resolution. TEM operates on different principle than SEM, yet it often brings same type of data.

The sample preparation for TEM is complex and time consuming because of its requirement to be ultrathin for the electron transmittance. High-resolution TEM imaging, when combined with nanodiffraction, atomic resolution electron energy-loss spectroscopy and nanometer resolution X-ray energy dispersive spectroscopy techniques, is critical to the fundamental studies of importance to nanoscience and nanotechnology.

5. Exemples of Metallic Nanoparticles

5.1. Silver nanoparticles (AgNPs) have unique physicochemical properties such as high electrical conductivity and thermal conductivity, chemical stability, catalytic activity, enhanced optical properties and antimicrobial efficacy against bacteria, viruses as well as other eukaryotic micro-organisms (Gong et al., 2007, McNamara and Tofail, 2017). Because of AgNPs extraordinary antimicrobial activity it is mainly used in the medical industry for textiles, wound dressings and device coatings. However, AgNPs are used in biomedical applications such as biosensors and photothermal therapy and drug delivery.

AgNPs have toxic properties that can inhibit bacterial growth, are hazardous to zebrafish and the human reproductive system, and are lethal to cell-based *in vitro* systems; they are still abundantly utilized in several commercial products such as contraceptive devices and feminine hygiene products. There are potential environmental and health alerts since the toxicity threat of AgNPs can be observed near the vicinity of consumers, particularly in the freshwater ecosystem (Syafiuddin et al., 2017). AgNPs can be synthesized by several approaches including physical, chemical, and biological (Figure 4). In general, equipment constraints, cost, and time consumption are identified as the major factors influencing the method of synthesis.

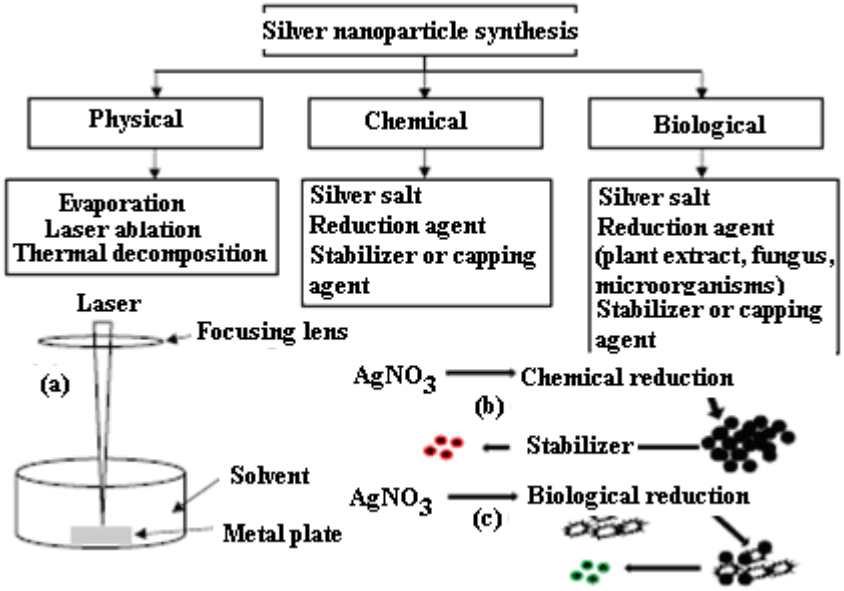

Figure 4. General procedures to synthesize silver nanoparticles by different approaches (Syafiuddin et al., 2017).

- Generally, the *physical approach* used to synthesize AgNPs employs the evaporation-condensation method. A new method was proposed by Tsuji et al., 2006 for synthesizing AgNPs by a laser ablation technique. Employing an alternative approach, AgNPs were synthesized using thermal decomposition by Lee and Kang, 2004.

●*The chemical approach* is widely used for synthesizing AgNPs using water or organic solvents. It is an easy way to synthesize AgNPs in solution (Quang Huy et al., 2013). A chemical reduction method was adopted for synthesizing AgNPs of various sizes using gallic acid (Martínez-Castañón et al., 2008). In addition, AgNPs were successfully synthesized from a silver ammonia solution (Tollens' reagent, 0.1 mol/L) where particles with sizes from 10 to 30 nm were observed on the surface of bacterial cellulose nanofibers (Wu et al., 2014). $AgNO_3$ as silver salt, aniline as reducing agent, and cetyltrimethylammonium bromide as stabilizer were also used to synthesize AgNPs (Khan et al., 2011). In case of AgNPs chemical synthesis, three main components are needed: a silver salt (usually $AgNO_3$), a reducing agent (i.e. ethylene glycol) and a stabilizer or capping agent (i.e. PVP) to control the growth of the NPs and prevent the aggregating.

● Recently, the *biological approach* for synthesizing AgNPs is being increasingly considered. This method is a green technology aimed at minimizing the negative environmental impact. The reducing agent and the stabilizer for AgNPs biological synthesis are replaced by molecules produced by living organisms such as plants, bacteria, fungi, yeast, and algae.

Thus, nowadays, a simple and eco-friendly green approach for synthesis of AgNPs by various plant extracts have drawn the attention of researchers because of its advantage over physical and chemical methods. Synthesis of NPs by green approach is emerging field because of its various advantages over the other process like nontoxic, ecofriendly and low cost (Kumar et al., 2017). Studies have already reported the successful biosynthesis of AgNPs by plants such as *Azadirachta indica, Cinnamomum camphora, Glycine max, Jatropha curcas, Cinnamomum camphora, Phyllanthus amarus, Carica papaya, Gliricidia sepium, Coriandrum sativum* (Deepak et al., 2011).

5.2. Gold nanoparticles (AuNPs)

The importance of AuNPs was recognized over 150 year back when Michael Faraday observed different properties of colloidal gold solution differing from their bulk material, gold. AuNPs are important components for biomedical applications. More precisely, AuNPs have been widely employed for diagnostics, and have seen increasing use in the area of therapeutics (Yeh et al., 2012). In recent times, biosynthesis and applications of AuNPs has been highly acknowledged.

The usual synthetic route to prepare gold nanoparticles involves the reduction of a gold salt (usually a halide, $HAuCl_4x3H_2O$) in solution by various reducing agents in the presence of a stabilizer (sodium citrate) (Andries et al., 2016, Lengke et al., 2011). The use of rapid reductants (e.g., white phosphorus, tannic acid, formamide, o-anisidine) results in bigger and generally spherical nanoparticles, while weak reducing agents (e.g., citrate, tartarate).

AuNPs are used in immunochemical studies for identification of protein interactions. They are used as lab tracer in DNA fingerprinting to detect presence of DNA in a sample. They are also used for detection of aminoglycoside antibiotics like streptomycin, gentamycin and neomycin. Gold nanorods are being used to detect cancer stem cells, beneficial for cancer diagnosis and for identification of different classes of bacteria.

5.3. Alloy nanoparticles exhibit structural properties that are different from their bulk samples (Ceylan et al., 2006). Since Ag has the highest electrical conductivity among metal fillers and, unlike many other metals, their oxides have relatively better conductivity, Ag flakes

are most widely used. Bimetallic alloy nanoparticles properties are influenced by both metals and show more advantages over ordinary metallic NPs.

5.4. Cooper nanoparticles is reported effective against spread by *Xanthomonas* sp. such as rise bacterial blight disease (*Xanthomonas oryzae*) and leaf spot of mung by *Xanthomonas campestris.* Esteban-Tejeda et al., (2009) reported that Cu nanoparticles have broad spectrum antimicrobial activity against Gram positive and negative bacteria and fungi at low concentration it can be used as a fungicide.

5.5. Silica nanoparticles

Silica is recognized as a vital element in plant physiological activities and growth inducer (Kanto et al., 2006) which would helpful in proliferation of stress resistance capability of diseased plants. Silica nanoparticle in combination with Ag (Ag-Si) nanoparticle showed anti-bacterial and anti-fungal activity (Park et al., 2013). Barik et al., 2008, used nano-silica as a pesticide against insect and reported that nano-silica absorbed into the cuticule lipid of insect by physio-sorption and kill insects.

5.6. Zinc nanoparticles has been used as nonfertilizer on many crops and its showed positive results in oprimal concentration, but the ZnO as fungicidal against fungal plant pathogen is less studied.

5.7. Selenium nanoparticles

Selenium (Se) is an essential micronutrient for humans, animals, and other organisms (El-Ramady et al., 2014). However, in higher plants, the role of selenium nanoparticles (SeNPs) has not been demonstrated clearly. Earlier studies have indicated that soil and/or foliar application of Selenium improved the antioxidant capacity in basil (*Ocimum basilicum* L.), (Oraghi Ardebili et al., 2015, Oprica et al., 2018) growth in tobacco (*Nicotiana tabacum* L.), (Jiang, et al., 2015) and yield in mustard (*Brassica rapa* L.) (Lyons et al., 2014), in potato (*Solanum tuberosum* L.) (Turakainen et al., 2004). Decreased lipid peroxidation and cell membrane damage through increased superoxide dismutase (SOD) and glutathione peroxidase (GPX) enzymes activity by Se application explains its antioxidative activity (Djanaguiraman et al., 2005).

Elemental Se is not soluble in water and biologically inert because of its redox state. However, nanosized elemental Se-NPs were found to possess prominent bioactivity and biosafety properties (Wang et al., 2007, Zhang, et al., 2008). Studies have shown that the biological activity and antioxidant property of SeNPs increase with their surface-to-volume ratio and decreasing particle size (Zhang et al., 2001).

Selenium nanoparticles (SeNPs) represent what it believes to be a novel prospect for nutritional supplementation because of their lower toxicity and ability to gradually release selenium after ingestion. SeNPs demonstrate anticancer and antimicrobial properties that may contribute to human health, not only as dietary supplements, but also as therapeutic agents (Skalickova et al, 2017).

6. Nanoparticles applications

Having significant applications, nanoparticles are used or being evaluated for use, in many fields like biomedical, food industry, agriculture.

6.1. Applications of nanoparticles in cosmetics and sunscreens

The conventional ultraviolet (UV) protection sunscreen lacks long-term stability during usage. Thus, the UV protection property of titanium oxide and zinc oxide nanoparticles as they are transparent to visible light as well as absorb and reflect UV rays found their way to be used in

some sunscreens. Moreover, some lipsticks use iron oxide nanoparticles as a pigment (Wiechers and Musee, 2010).

6.2. Biomedical applications of nanoparticles

Nanotechnology provides us with the opportunity of achieving smart nanostructures with complex functionalities including local heating, targeting (passive or active), improved uptake, delivery, biocompatibility, suitable biodistribution, or no immunogenicity, to name a few.

More than nanoparticle can be used in cancer treatment. There are a variety of nanoparticle systems currently investigated and explored for biomedical applications with some particular emphasis for cancer therapeutics; hence some precious metals (mainly gold and silver systems, Au, and Ag) and some magnetic oxides (in particular magnetite Fe_3O_4) received much interest including quantum dots and some of what is called natural nanoparticles (Bououdina et al., 2013).

The use of local heating by bioactive NPs can drastically reduce the side effects (cell toxicity and/or tissue radiation damage) of traditional treatments when used in combined therapies. There is also another approach to destroy tumors by NP-based heating, that is, increasing the temperature above 46^0C and, therefore, causing cell death by necrosis; in contrast to apoptosis (the other known cellular death), necrosis occurs when cells suffer an irreversible damage (Pablo del Pino and Pelaz, 2012). Hyperthermia is different from "ablative" techniques, which use heat from ultrasound waves, radio waves or lasers, to destroy cancer cells. "In those treatments, the heat itself is high enough to 'cook' the cancer". In mild temperature hyperthermia, it is use lower temperatures (1090 to 110˚ F) to allow radiation therapy or chemotherapy to work better and this often shrinks the tumour.

Nanotechnology has improved the medical field by use of nanoparticles in drug delivery. NPs have been produced to deliver drugs, proteins/peptides and genes, to be used in various biomedical areas including cancer therapy and vaccination (Ashaben et al., 2014). In fact, NPs can be used in various administration routes, such as oral, nasal, parenteral or intraocular, representing and efficient and effective improvement over current methods. The drug can be delivered to specific cells using nanoparticles (Ganesh and Archana, 2013). The total drug consumption and side effects are significantly lowered by placing the drug in the required area in required dosage. This method reduces the cost and side effects (Mudshinge et al., 2011).

6.4. Applications of nanoparticles in food

Nanofood is a term used to describe foods that use nanotechnology techniques, tools or manufactured nanomaterials that have been added during cultivation, production, processing or packaging. For example, a nanocomposite coating in a food packaging process can directly introduce the anti-microbial substances on the coated film surface (Laad and Jatti, 2016). One of the examples is the canola oil production industry includes nanodrops, an additive designed to transfer the vitamins and minerals in the food. There are several purposes for the development of nanofood like improvement of food safety, enhancement of nutrition and flavor, and cutting production and consumer costs. On the other hand, nanofood provides various benefits by which include health promoting additives, longer shelf lives and new flavour varieties.

The application of nanotechnology in food is rapidly emerging and is involving all areas of the food chain from agricultural applications to food processing and enhancing bioavailability of nutrients (Heera and Shanmugam, 2015).

6.5. Applications of nanoparticles in agriculture

6.5.1. Nanoparticles as insecticides

Synthetic agrochemicals have changed the face of agriculture, but it has also developed new challenge in form of insect pest resistance. Applications of different type of nanoparticles (silver nanoparticles, aluminium oxide, zinc oxide and titanium dioxide) in the control of rice weevil (caused by *Sitophilus oryzae*) and grasserie disease in silkworm (caused by *Bombyx mori* and baculovirus BmNPV (*B. mori* nuclear polyhedrosis virus) were studied (Goswami et al., 2010).

The entomotoxicity of silica nanoparticles against rice weevil *Sitophilus oryzae* was tested by Debnath et al. 2011, which compared the efficacy with bulk-sized silica (individual particles larger than 1.0 μm). Amorphous silica nanoparticles were found to be highly effective against this insect pest causing more than 90% mortality, indicated the effectiveness of silica nanoparticles to control insect pests. Moreover, Teodoro et al., 2010 reported that insecticidal activity of nanostructured alumina against *Sitophilus oryzae* L. and *Rhyzopertha dominica* evidenced significant mortality after 3 days of continuous exposure to nanostructured alumina-treated wheat.

6.5.2. Nanoparticles as fungicides.

Fungal diseases among crops cause major loss to the production. Nanoparticles have been experimented as antifungal agents against pathogenic fungi and their application have not causes effects to plants. Shyla et al., 2014, has been tested the antifungal activity of nanoparticles of zinc oxide (35–45 nm), silver (20–80 nm) and titanium dioxide (85–100 nm) against *Macrophomina phaseolina*. The higher antifungal effect was observed in silver nanoparticles at lower concentrations than zinc oxide and titanium dioxide nanoparticles.

6.5.3. Nanoparticles as micronutrient supply

It is known that micronutrients like manganese, copper, boron, iron, molybdenum, zinc etc. are important for the growth and development. Foliar application of micronutrients can enhance uptake by the leaves (Martens and Westermann, 1991). Nanotechnology can be used to make the availability of micronutrients to plants. Nano formulations of micronutrients can be sprayed on plants or can be supplied to soil for uptake by roots to enhance soil health and vigor (Peteu et al., 2010). Different nanoparticles have been tested to provide appropriate level of micronutrients in plants.

Foliar application of iron compounds by the nanoparticle's technology may be a solution to plants growing in iron deficiency soils with high pH and calcareous. Thus, Ghafariyan et al., 2013 showed that iron oxide nanoparticles could be used as a source of iron for soybean for reducing chlorotic symptoms of iron deficiency.

In addition, application of iron nanoparticles also improved crop black-eyed peas performance more than that by application of a regular iron salt (Delfani et al., 2014). On the other hand, manganese nanoparticles have been reported to enhance growth of mung bean (*Vigna radiata*) and photosynthesis (Pradhan et al., 2013).

6.5.4. Nanoherbicides

Nanoherbicides can play a very important role in removing weeds from crops in an eco-friendly way, without leaving any harmful residues in soil and environment (Pérez-de-Luque and Rubiales, 2009)

The resistance of weeds against herbicides appears by continuous use of same herbicide for constant period of time cause. Encapsulation of herbicide in polymeric nanoparticles also results in environmental safety (Kumar et al., 2015). These molecules enter into the roots system

of the weeds, translocate to cells and inhibit metabolic pathways such as glycolysis. This ultimately leads to death of plants (Ali et al., 2014).

CONCLUSIONS

Nanotechnology is improving our everyday lives by enhancing the performance and efficiency of everyday objects.
Having significant applications, nanoparticles are used or being evaluated for use, in many fields like biomedical, food industry, agriculture. In the biomedical field, these nanoparticles have been investigated for antimicrobial applications, biosensing, imaging, and drug delivery. In the environmental field, nanoparticles have been investigated for applications in bioremediation of diverse contaminants, water treatment, and production of clean energy. The nanotechnology has a great future due to its efficiency and environmental friendly property.

It provides a clean environment by providing safer air and water, and clean renewable energy for a sustainable future. Nanotechnology has established to be an advanced field of science where extensive research is carried out to implement the technology. It is being tested for various new applications to increase the efficiency and performance of the object or process and subsequently reduce the cost so that it is accessible for everyone. The nanotechnology has a great future due to its efficiency and environmental friendly property.

REFERENCES

1. **Ali, M.A., Rehman I., Iqbal A., Din, S., Rao, A.Q., Latif, A., Samiullah, T.R., Azam, S., Husnain, T.,** (2014): Nanotechnology: a new frontier in agriculture. Adv. Life Sci., 1,129–138.
2. **Andries, M., Pricop, D., Oprica L., Creanga, D.E., Iacomi, F.,** (2016): The effect of visible light on gold nanoparticles and some bioeffects on environmental fungi, Int. J. Pharm., 505, (1-2), 256-261.
3. **Ashaben, P., Mitesh, P., Xiaoyan Y., Ashim, K. M.,** (2014): Recent Advances in Protein and Peptide Drug Delivery: A Special Emphasis on Polymeric Nanoparticles, Protein Pept. Lett., 21(11), 1102–1120.
4. **Barik, T.K., Sahu, B., Swain, V.,** (2008): Nanosilica-from medicine to pest control. Parasitol. Res.,103, 253-258.
5. **Bhaviripudi, S., Mile E., Iii S.A.S., Zare, A.T., Dresselhaus, M.S., Belcher, A.M., Kong.J.,** (2007): *CVD* Synthesis of Single-Walled Carbon Nanotubes from Gold Nanoparticle Catalysts, J. Am. Chem. Soc., 1516–7
6. **Birla, S.S., Tiwari, V.V., Gade, A.K., Ingle, A.P., Yadav, A.P., Rai, M.K.,** (2009): Fabrication of silver nanoparticles by Phoma glomerata and its combined effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, Lett. Appl. Microbiol., 48,173–179.
7. **Bououdina, M.S., Rashdan, J.L., Bobet, Y., Ichiyanagi,** (2013): Nanomaterials for biomedical applications: synthesis, characterization, and applications. J. Nanomater., 1-4.
8. **Bzdek, B.R., Zordan, C.A., Iii, G.W.L., Murray,V., Bzdek, B.R., Zordan, C.A., Iii, G.W.L., Murray, V., Bzdek, B.R., Zordan, C.A., Iii G.W.L., Johnston M.V.,** (2011): Nanoparticle Chemical Composition During New Particle Formation, Aerosol Sci. Technol., 45, 1041-1048.
9. **Cao, J., Hu, X.,** (2009): Synthesis of gold nanoparticles using halloysites. J. Surf. Sci. Nanotechnol. 7, 813–815
10. **Ceylan, A., Jastrzembski, K., Shah, S. I.,** (2006): Enhanced solubility Ag-Cu nanoparticles and their thermal transport properties, Metall. Mater. Trans., A., 37, 2033.
11. **Debnath, N., Das, S., Seth, D., Chandra, R., Bhattacharya, S.C., Goswami, A.,** (2011): Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae (L.)*. J. Pest. Sci., 84, 99–105.
12. **Deepak, V., Kalimuthu, Kalishwaralal, Sureshbabu, Ram Kumar Pandian, and Sangiliyandi Gurunathan,** (2011): An Insight into the Bacterial Biogenesis of Silver Nanoparticles, Industrial Production and Scale-up, in Metal Nanoparticles in Microbiology (Ed. Mahendra Rai l Nelson Duran), Springer.

13. **Delfani, M., Firouzabadi, M.B., Farrokhi, N., Makarian, H.,** (2014): Some physiological responses of black-eyed pea to iron and magnesium nanofertilizers. Commun. Soil Sci. Plant Anal., 45, 530–540.
14. **Djanaguiraman, M., Devi, D.D., Shanker, A.K., Sheeba, J.A., Bangarusamy, U.,** (2005): Selenium-an antioxidative protectant in soybean during senescence. Plant Soil., 272, 77– 86.
15. **Ealias, A.M, Saravanakumar, M.P.,** (2017): A review on the classification, characterisation, synthesis of nanoparticles and their application, IOP Conf. Series: Materials Science and Engineering, IOP Publishing, 263.
16. **El-Ramady, H.R., Domokos-Szabolcsy, E., Abdalla, N. A., Alshaal,T.A., Shalaby, T.A., Sztrik, A., Prokisch,J., Fari, M**, (2014): Selenium and nano-selenium in agroecosystems. Environ. Chem. Lett., 12, 495– 510.
17. **Esteban-Tejeda, L., Malpartida, F., Esteban-Cubillo, A., Pecharromán, C., Moya, J.S.,** (2009): Antibacterial and antifungal activity of a soda-lime glass containing copper nanoparticles. Nanotechnology, 20 (50)
18. **Gade, A.K., Bonde, P., Ingle, A.P., Marcato, PD, Duran, N, Rai, M.K.** (2008): Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. J. Biobased. Mater Bioenergy., 2, 243–247
19. **Ganesh, K., Archana, D.,** (2013): Review Article on Targeted Polymeric Nanoparticles: An Overview, Am. J. Adv. Drug Deliv. 3(3), 196-215
20. **Ghafariyan, M.H., Malakouti, M.J., Dadpour, M.R., Stroeve, P., Mahmoudi, M.,** (2013): Effects of magnetite nanoparticles on soybean chlorophyll. Environ. Sci. Technol., 47,10645–10652.
21. **Gong, P., Li, H., He, X., Wang, K., Hu, J., Tan, W., Tan, S., Zhang X.Y.,** (2007): Preparation and antibacterial activity of Fe_3O_4 Ag nanoparticles, Nanotechnology, 18, 604–611.
22. **Goswami, A., Roy, I., Sengupta, S., Debnath, N.,** (2010): Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. Thin Solid Films, 519,1252–1257.
23. **Heera, P., Shanmugam, S.,** (2015): Review Article Nanoparticle Characterization and Application: An Overview Int. J. Curr. Microbiol. App. Sci. 4(8), 379-386.
24. **Hett, A.,** (2004): Nanotechnology: small matters, many unknown. Zurich, Switzerland: Swiss Re.
25. **Hodoroaba, V., Rades, S., Unger W.E.S.,** (2014): Inspection of morphology and elemental imaging of single nanoparticles by high- resolution SEM / EDX in transmission mode, Surf. Interface Anal., 1-4.
26. **Imtiyaz, H., Singh N. B., Email, A. Singh, Singh H., Singh S.C.,** (2016): Green synthesis of nanoparticles and its potential application, Biotechnol. Lett., 38 (4), 545-560.
27. **Jiang, C., Zu, C., Shen, J., Shao, F., Li, T.,** (2015): Effects of selenium on the growth and photosynthetic characteristics of flue-cured tobacco *(Nicotiana tabacum L.),* Acta Soc. Bot. Pol., 84, 71– 77.
28. **Jores, K., Mehnert, W., Drecusler, M., Bunyes, H., Johan, C., MAder, K.**, (2004): Investigation on the stricter of solid lipid nanoparticles and oil-loaded solid nanoparticles by photon correlation spectroscopy, field flow fractionasition and transmission electron microscopy. J Control Release.,17, 217–27.
29. **Kanto, T., Miyoshi, A., Ogawa, T., Maekawa, K., Aino M.,** (2006): Suppressive effect of liquid potassium silicate on powdery mildew of strawberry in soil. J. Gen. Plant. Pathol., 72,137–142.
30. **Khan, Z., S.A. Al-Thabaiti, A.Y. Obaid, A.O. Al-Youbi,** (2011): Preparation and characterization of silver nanoparticles by chemical reduction method, Colloids Surf. B: Biointerfaces, 82, 513.
31. **Khan, I., Khalid, S., Khan, I.,** 2017, Nanoparticles: Properties, applications and toxicities, Arab.J. Chem., 1-24.
32. **Kumar, A.S., Ansary, A.A., Ahmad, A., Khan, M.I.**, (2007): Extracellular biosynthesis of CdSe quantum dots by the fungus, *Fusarium oxysporum.* J. Biomed. Nanotechnol., 3,190–194.
33. **Kumar, R., Ghoshal, G., Jain, A., Goyal, M.,** (2017): Rapid Green Synthesis of Silver Nanoparticles (AgNPs) Using (*Prunus persica*) Plants extract: Exploring its Antimicrobial and Catalytic Activities. J. Nanomed. Nanotechnol., 8, 4, 1-8.
34. **Kumar, S., Bhanjana, G., Sharma, A., Sarita, Sidhu M.C., Dilbaghi N.,** (2015): Herbicide loaded carboxymethyl cellulose nanocapsules as potential carrier in agrinanotechnology. Sci. Adv. Mater., 7,1143–1148.
35. **Laad, M., Jatti, V.K.S** (2016): Titanium oxide nanoparticles as additives in engine oil J. KING SAUD Univ. - Eng. Sci. 0–6.
36. **Lee, D. K., Young, S.K.**, (2004): Synthesis of Silver Nanocrystallites by a New Thermal Decomposition Method and Their Characterization, Etri.J., 26(3), 252-256
37. **Lengke, M.F., Sanpawanitchakit, C., Southam, G.**, (2011): Biosynthesis of Gold Nanoparticles: A Review, in Metal Nanoparticles in Microbiology (Ed. Mahendra Rai l Nelson Duran), Springer.
38. **Lyons, G.H., Genc, Y., Soole, K., Stangoulis, J., Liu, F., Graham, R.,** (2009): Selenium increases seed production in Brassica. Plant Soil 318, 73– 80.

39. **Machado, S., Pacheco, J.G., Nouws, H.P.A., Albergaria, J.T., Delerue-Matos, C.,** (2015): Characterization of green zero-valent iron nanoparticles produced with tree leaf extracts. Sci.Total Environ. 533, 76–81.
40. **Marsalek, R.,** (2014): Particle Size and Zeta Potential of ZnO. APCBEE Procedia, 9, 13–7.
41. **Martens, D.C., Westermann, D.T.,** (1991): Fertilizer applications for correcting micronutrient deficiencies. In: Alloway B.J., editor. Micronutrient Deficiencies in Global Crop Production. Springer, 549–553.
42. **Martínez-Castañón, G.A., N. Niño-Martínez, F.Martínez-Gutierrez, J.R. Martínez-Mendoza, F.Ruiz,** (2008): Synthesis and antibacterial activity of silver nanoparticles with different sizes, J. Nanopart. Res., 10 (8), 1343–1348.
43. **McNamara K., Tofail S.A.M.,** (2017): Nanoparticles in biomedical applications, Advances in Physics:X, 2(1), 54-88
44. **Mittal A.K., Yusuf C., Uttam C.B.,** (2013): Synthesis of metallic nanoparticles using plant extracts, Biotechnol. Adv., 31 (2), 346-356.
45. **Mudshinge S.R, Deore A.B., Patil S., Bhalgat C.M.,** (2011): Nanoparticles: Emerging carriers for drug delivery. Saudi Pharm. J., 19, 129-41.
46. **Narayanan K.B., Sakthivel N.,** (2010): Biological Synthesis of metal nanoparticles by microbes, Adv. Colloid Interface Sci., 156, 1- 13.
47. **Oprica L., Molchan O., Grigore M.N.,** (2018): Salinity and selenium nanoparticles effect on antioxidant system and malondialdehyde content in *Ocimum basilicum L.* seedlings, J. Exp. Molec. Biol., 19(4), 99-106.
48. **Oraghi, Ardebili Z.,** Oraghi Ardebili, N., Jalili, S., Safiallah, S., (2015): The modified qualities of basil plants by selenium and/or ascorbic acid, Turk. J. Bot., 39, 401– 407.
49. **Pablo, del Pino, Pelaz, B.,** (2012): Hyperthermia Using Inorganic Nanoparticles, Cap. 13, Frontiers of Nanoscience (Edited by Jesus M. de la Fuente, V. Grazu), Elsevier Ltd.
50. **Pangi, Z., Beletsi, A., Evangelatos, K.,** (2003): PEG-ylated nanoparticles for biological and pharmaceutical application. Adv. Drug Del. Rev., 24, 403–19.
51. **Park, Y.H., Bae, H.C., Jang, Y., Jeong, S.H., Lee, H.N., Ryu, W.I., Yoo, M.G., Kim, Y.R., Kim, M.K., Lee, J.K., Jeong, J., Son, S.W.** (2013): Effect of the size and surface charge of silica nanoparticles on cutaneous toxicity. Mol. Cell. Toxicol., 9(1), 67–74.
52. **Parveen, K., Banse V., Ledwani L.,** (2016): Green synthesis of nanoparticles: Their advantages and disadvantages, AIP Conference Proceedings, 1724, (1).
53. **Pérez-de-Luque A., Rubiales, D.**, (2009): Nanotechnology for parasitic plant control. Pest. Manag. Sci., 65, 540–545.
54. **Peteu, S.F., Oancea, F., Sicuia, O.A., Constantinescu, F., Dinu, S**., (2010): Responsive polymers for crop protection. Polymers, 2, 229–251.
55. **Pradhan, S., Patra, P., Das, S., Chandra, S., Mitra, S., Dey, K.K**., (2013): Photochemical modulation of biosafe manganese nanoparticles on *Vigna radiata*: a detailed molecular biochemical, and biophysical study. Environ. Sci. Technol., 47, 13122–13131.
56. **Quang Huy, T., N. Van Quy, L. Anh-Tuan,** (2013): Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives, Adv. Nat. Sci. Nanosci. Nanotechnol., 4.
57. **Rai, M., Yadav, A., Bridge, P., Gade, A.,** (2009): Myconanotechnology: a new and emerging science. In: Rai MK, Bridge PD (eds) Applied mycology, vol 14. CAB International, New York, 258–267
58. **Rai, M., Yadav, A., Gade A.,** (2008): Current trends in phytosynthesis of metal nanoparticles. Crit. Rev. Biotechnol. 28(4), 277–284.
59. **Sau T.K., Rogach A.L.,** (2010): Nonspherical noble metal nanoparticles: colloid-chemical synthesis and morphology control. Adv. Mater 22(16), 1781–1804.
60. **Singh P., Kim Y-J, Zhang D., Yang D.-C.,** (2016): Synthesis of nanoparticles from Plants and Microorganisms, Trends Biotechnol., 34 (7), 588-599.
61. **Sharma V., Rao L.J.M.,** (2014): An overview on chemical composition, bioactivity and processing of leaves of *Cinnamomum tamala*. Crit. Rev. Food Sci. Nutr. 54, 433–48
62. **Sharma, V.K., Yngard, R.A., Lin, Y.,** (2009): Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid Interface Sci.,145, 83–96.
63. **Shyla, K.K., Natarajan, N., Nakkeeran, S.,** (2014): Antifungal activity of zinc oxide, silver and titanium dioxide nanoparticles against *Macrophomina phaseolina*. J. Mycol. Plant Pathol., 44, 268–273.
64. **Skalickova, S., Vedran M., Cihalova K., Horky P, Richtera L., Vojtech A.,** (2017): Selenium nanoparticles as a nutritional supplement. Nutrition, 33, 83-90.
65. **Syafiuddin A., Salmiati, Mohd Razman Salim, Ahmad Beng Hong Kueh, Tony Hadibarata,Hadi Nur,** 2017, A Review of Silver Nanoparticles: Research Trends, Global Consumption, Synthesis, Properties, and Future Challenges, J. Chin. Chem. Soc., 64 (7), 732-756.

66. **Tai, C. Y., Tai, C., Chang, M., Liu H.,** (2007): Synthesis of Magnesium Hydroxide and Oxide Nanoparticles Using a Spinning Disk Reactor Ind. Eng. Chem. Res., 5536–41
67. **Teodoro, S., Micaela, B., David, K.W.,** (2010): Novel use of nano-structured alumina as an insecticide. Pest. Manag. Sci., 66, 577–579.
68. **Tiwari, D.K., Behari J., Sen P.,** (2008): Application of Nanoparticles in Waste-Water Treatment. World Appl. Sci. J., 3, 417–33
69. **Tsuji, M., Kisei M., Masatoshi K., Nobuhiro M.,Takeshi T.,** (2006): Effects of chain length of polyvinylpyrrolidone for the synthesis of silver nanostructures by a microwave-polyol method. Mater. Lett., 60(6), 834-838
70. **Turakainen M, Hartikainen H, Seppänen MM.,** 2004, Effects of selenium treatments on potato (*Solanum tuberosum* L.) growth and concentrations of soluble sugars and starch, J Agric Food Chem., 2004, 52(17), 5378-82.
71. **Wali, M., Safia, H., Warda, K.,** (2018): Phytosynthesized nanoparticles as an alluring step for antimicrobial drug delivery: A brief Introduction. Int. J. Chem., 6(1): 2092-2097.
72. **Wang, H., Zhang, J., Yu, H.,** (2007): Elemental selenium at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: Comparison with selenomethionine in mice. Free Radical Biol. Med., 42, 1524– 1533.
73. **Wiechers, J.W., Musee, N.,** (2010): Engineered Inorganic Naoparticles and Cosmetics: Facts, Issues, Knowledge Gaps and Challenge. J Biomed Nanotechnol., 6, 408-431.
74. **Wu, J., Y. Zheng, W. Song, J. Luan, X. Wen, Z. Wu, X. Chen, Q. Wang, S. Guo,** (2014): In situ synthesis of silver-nanoparticles/bacterial cellulose composites for slow-released antimicrobial wound dressing. Carbohydr. Polym., 102, 762-771.
75. **Yang, N., Aoki, K.,** (2005): Voltammetry of the silver alkylcarboxylate nanoparticles in suspension. Electrochim. Acta, 50:4868–4872
76. **Zhang, J., Wang, X.F., Xu, T.W.,** (2008): Elemental selenium at nanosize (Nano-Se) as a potential chemo preventive agent with reduced risk of selenium toxicity: comparison with Se-methylselenocysteine in mice. Toxicol. Sci., 101, 22– 31.
77. **Zhang, J.S., Gao, X.Y., Zhang, L.D., Bao, Y.P.**, (2001): Biological effects of a nano red elemental selenium. Biofactors, 15, 27– 38.

Acknowledgements

The authors acknowledge the JINR-Romania Project No 397/27.05.2019 Item 7, as well as, „This project is funded by the Ministry of Research and Innovation within Program 1 – Development of the national RD system, Subprogram 1.2 – Institutional Performance – RDI excellence funding projects, Contract no.34PFE/19.10.2018”

[1]“Alexandru Ioan Cuza “University, Faculty of Biology, 20A Carol I Bd., 700506 Iasi, Romania
[2] Frank Laboratory of Neutron Physics-Condensed Matter department, JINR, Dubna, Russian Federation
[3] Department of Nuclear Physic, IFIN HH, Bucharest-Magurele, Romania

***Corresponding author: lacramioara.oprica@uaic.ro**

SIDE COMPARATION OF TWO METHODS FOR QUANTIFYING MALONDIALDEHYDE LEVELS IN ANIMAL TISSUE EXTRACTS

VAIDEȘ – NEGUSTOR ROXANA – NICOLETA[1], MIHĂȘAN MARIUS[1*]

Received: 3rd of September 2019 / Revised: 27th of September 2019
Accepted: 22nd of November 2019 / Published: 21st of January 2020

Keywords: MDA, stress marker, HPLC, spectrophotometry;
Abstract: Malondialdehyde (MDA) as an important marker used for the assessment of the oxidative level in a tissue of biological fluid. The standard assay method uses tiobarbituric acid for spectrophotometric detection but suffers from the lack of specificity. Here we show that a HPLC based method for quantitating the MDA has the advantages of increase sensitivity as well as a better specificity, allowing the detection of lower MDA levels in tissue extracts with an increased accuracy.

INTRODUCTION

Malondialdehyde (MDA) is an organic compound considered to be one of the most important indicators of lipid peroxidation of polyunsaturated fatty acids (Davey et al. 2005). MDA is the main product of the arachidonic acid conversion to prostaglandin PGH2 by cyclooxygenase 1 or cyclooxygenase 2. Prostaglandin PGH2 is further metabolized by thromboxane synthase to thromboxane A2, 12-hydroxyheptadecatrienoic acid, and MDA. Alternatively, prostaglandin PGH2 can also suffer a non-enzymatic rearrangement into a mixture of 8-cis and 8-trans isomers of 12-hydroxyeicosaheptaenoic acid and MDA (Pryor and Stanley 1975).

Reactive oxygen species degrade the polyunsaturated lipids form MDA as well. As a reactive electrophile species, MDA forms covalent adducts with important molecules such as proteins and DNA. The protein adducts are referred to as **a**dvanced **l**ipo-oxidation **e**nd-products or ALE, while the DNA adducts are mutagenic (Marnett 1999). Thereby, measuring MDA levels are used as an important marker for the oxidative level in a given tissue (Del Rio, Stewart, and Pellegrini 2005).

As a thiobarbituric reactive substance or TBARS, MDA reacts with two equivalents of tiobarbituric acid (TBA) and form a fluorescent red compound (MDA-TBA2) that can be easily quantified spectrophotometrically (Nair, O'Neil, and Wang 2008). Although alternative dyes exist such as 1-Methyl-2-phenylindole, the spectrophotometric method based on TBA is the most widely used assay method for MDA.

The main drawback of the TBA spectrophotometric method for MDA assay is its specificity. MDA is not the only compound that reacts with TBA found in a given tissue. Some other compounds, un-related with oxidative stress such as aliphatic aldehydes, metals or glyoxylic acid and sugars also react with TBA. Moreover, end-product of the MDA-TBA assay is almost identical to the end-product of the pyridine-barbiturate cyanide assay. Thereby, although the MDA-TBA spectrophotometric assay is very convenient due to its simplicity and robustness (Artenie, Ungureanu, and Negură 2008), the results must be evaluated with caution and further validated by other indicators. When an increase in specificity is needed, HPLC is the methods of choice. It is not a surprise thereby that several authors managed to put together a more specific HPLC-based assay method to measure MDA levels (Domijan et al. 2015; Moselhy et al. 2013; Lykkesfeldt 2001; Karatas, Karatepe, and Baysar 2002; Grotto et al. 2007; Khoschsorur et al. 2000). The current works focuses on adapting one of the available HPLC methods for assaying the MDA levels to the existing infrastructure at the Biology Department, UAIC Iasi and comparing it to the well-established and used spectrophotometric method in terms of specificity and ease of use.

MATERIAL AND METHODS

Chemicals. All chemicals were purchased from well-known suppliers and were of greatest purity available. As standard, 1,1,3,3-tetraethoxypropane (TEP, Sigma Aldrich) was used. Biological samples used for testing the real-life applicability of the method were clarified rat brain extracts prepared as described before. (Hritcu et al. 2013). All mobile phases were filtered through a 22 microM filter (Milipore) and degassed by applying low pressure under constant steering for 20 minutes.

MDA-TBA assay. Samples or standards up to 50 microL were mixed with 12,5 microL 3M NaOH and incubated for 30 minutes at 60^0C with constant shaking (300 rpm). 0.5 ml of H_2SO_4 98% were added, the tubes were mixed and then 0.25 ml TCA 20% was added to precipitate the proteins and DNA. The tubes were centrifuged for 10 min at 3000 rpm/min. 0.5

ml of the supernatant was further mixed with 0.25 ml 0.35% TBA and incubated at 90^0C for 40 min with constant shaking (300 rpm). Before quantification by spectrophotometry and HPLC, the samples were centrifuged for 30 min at 13000 rpm.

Spectrophotometric assay. Each sample was measured at 532 nm against a blank with water instead of the sample using a DU 730 UV/VIS Spectrophotometer (Beckman Coulter).

HPLC assay. 20 microL of the supernatant prepared as depicted above were injected on a Zorbax Eclipse XDB - C18, 250 mm length, 3 microm particle size coupled to a Shimadzu Prominence HPLC system (2x LC20AD pumps, SIL20AC autosampler, CT20AC oven, SPD M20A DAD detector). As mobile phase, methanol (Carlo Erba Reagents): 30 mM KH_2PO_4, pH 6,7 35:65 was used at a flow rate of 1 ml/min for a total of 20 minutes. The MDA-TBA adduct eluted at 9,520 ± 0,2 minutes, example chromatograms being presented in figure 1.

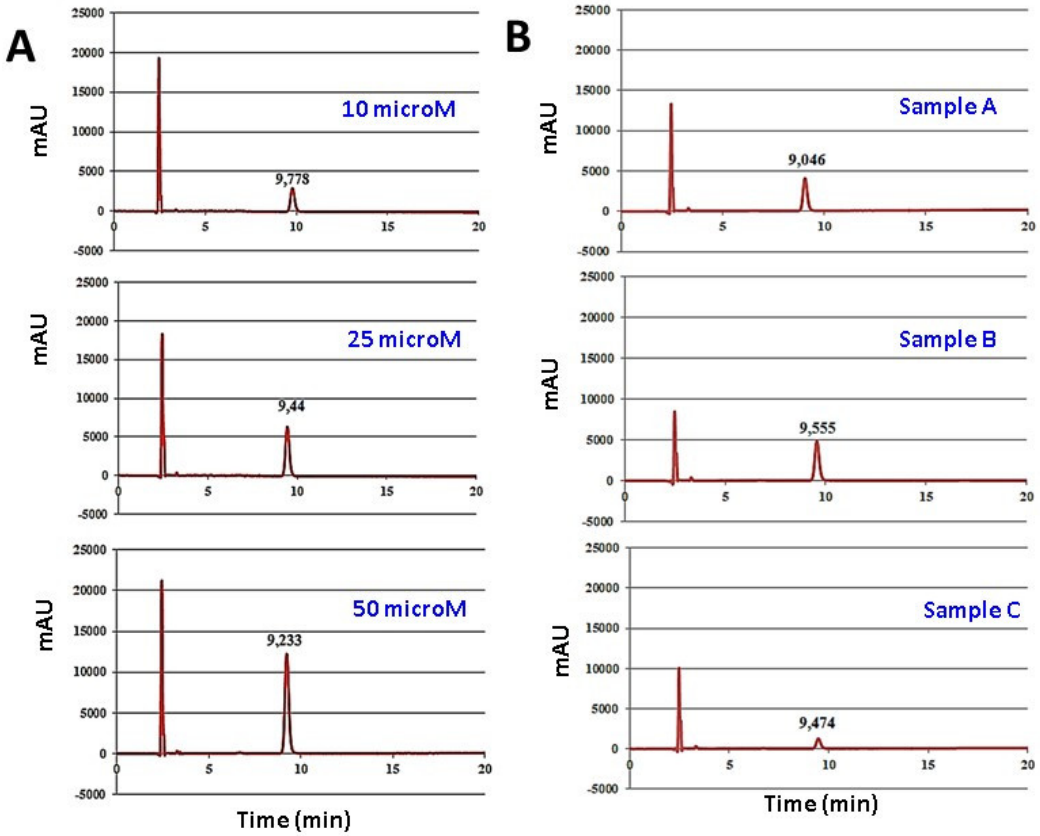

Figure 1. Typical chromatograms for (A) TEP-TBA adduct at various concentrations and (B) MDA-TBA adducts in various biological samples.

The chromatographic data was acquired using the Shimadzu LC solution Software and manually interpreted. Peak areas and peak height were measured and used. All calibration curves were built in Microsoft Execel using 3 technical replicates/point.

RESULTS AND DISCUSSIONS

Linearity and sensitivity. In order to assess the linearity of the two methods, a calibration curve using TEP was built on a very wide concentration interval 10-500 microM, while in order to evaluate the sensitivity a narrower interval was chosen, but with significantly lower

concentrations – 10-50 microM. The data obtained is depicted in Figure 2, where A are the spectrophotometric measurements, B is the HPLC data based on peak height and C is the HPLC data based on peak area.

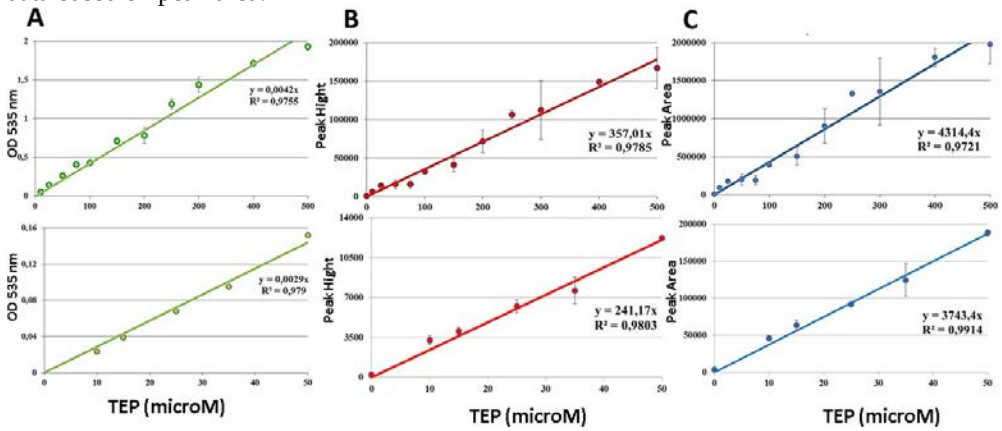

Figure 2. Linearity and sensitivity of the two MDA quantitation methods. **A**. spectrophotometric **B**. HPLC, using peak height for quantitation **C**. HPLC, using peak area for quantitation.

A quick evaluation of the regression coefficient R^2 for all the calibration curves depicted in figure 2 indicates that both methods have the same linearity, both methods providing good, but not perfect calibration curves on the 10-500 microM TEP interval with an R^2 around 0.97. In the lower concentration interval, the HPLC quantitation method based on the peak area outperforms the spectrophotometric method with a near perfect R^2 of 0.99. There is no clear difference between the performance of the spectrophotometric method and the HPLC method based on peak height. Overall, although similar in linearity, the HPLC method for quantitation of MDA based on peak height is apparently much sensible. Indeed, most of the methods we could find in the literature are used to detect low concentrations of MDA as fallows: 0.28 - 6.6 microM (Karatas, Karatepe, and Baysar 2002), 0 – 24,3 microM (Moselhy et al. 2013) and 0,15 – 3,0 microM (Domijan et al. 2015).

Real-life biological samples. In order to assess the real-life application of the method, cleared, cell-free rat brain lysates from an ongoing experiment were used to compare the MDA levels reported by the two methods. Each of the 4 samples labeled A, B, C and D were processed as stated in the Materials and methods section and the supernatant from the same tube was consecutively measured using both methods. The values were then converted into MDA concentrations using the calibration curves from figure 2. As one can see from the data plotted in figure 3, the spectrophotometric method always reported higher values of MDA comparing with the HPLC methods, independent of the parameter used for quantitation (area or height). A close inspection of the typical chromatograms from figure 1 indicates the presence several peaks at 532 nm close to the void-volume peak in the case of the biological samples. These peaks are compounds from the samples that reacted with TBA, compounds that are quantified as MDA by the spectrophotometric method and not by the HPLC method.

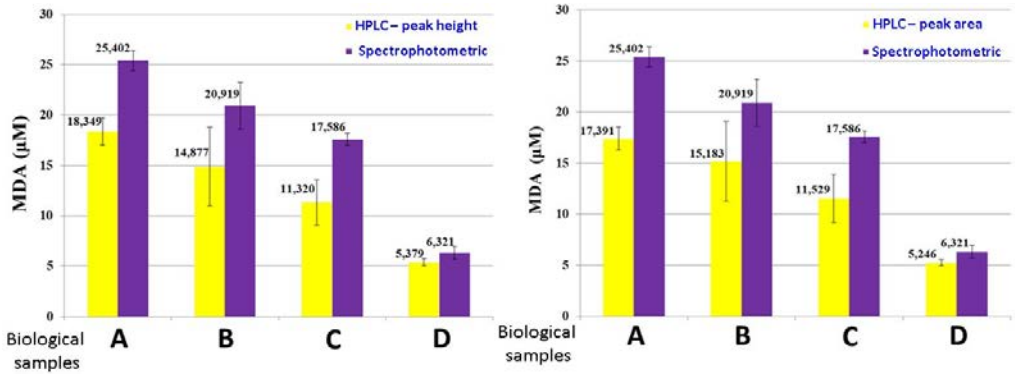

Figure 3. Data reported by analyzing real-life biological samples using the two methods described here.

CONCLUSIONS

A HPLC based method to assay for the MDA levels in biological extracts was established in the lab. Although requiring more skilled manpower, the methodology offers better sensibility for measuring lower amounts of MDA and increased specificity.

REFERENCES

Artenie, Vlad, Eugen Ungureanu, and Anca Mihaela Negură. 2008. *Metode de Investigare a Metabolismului Glucidic și Lipidic - Manual de Lucrări Practice*. Iași: Editura Pim, p. 149-152.

Davey, M. W., E. Stals, B. Panis, J. Keulemans, and R. L. Swennen. 2005. "High-Throughput Determination of Malondialdehyde in Plant Tissues." *Analytical Biochemistry* 347 (2): 201–7. https://doi.org/10.1016/j.ab.2005.09.041.

Domijan, Ana-Marija, Jovica Ralić, Sandra Radić Brkanac, Lada Rumora, and Tihana Žanić-Grubišić. 2015. "Quantification of Malondialdehyde by HPLC-FL - Application to Various Biological Samples." *Biomedical Chromatography : BMC* 29 (1): 41–46. https://doi.org/10.1002/bmc.3361.

Grotto, D, L D Santa Maria, S Boeira, J Valentini, M F Charão, A M Moro, P C Nascimento, V J Pomblum, and S C Garcia. 2007. "Rapid Quantification of Malondialdehyde in Plasma by High Performance Liquid Chromatography-Visible Detection." *Journal of Pharmaceutical and Biomedical Analysis* 43 (2): 619–24. https://doi.org/10.1016/j.jpba.2006.07.030.

Hritcu, Lucian, Marius Stefan, Roderich Brandsch, and Marius Mihasan. 2013. "6-Hydroxy-L-Nicotine from Arthrobacter Nicotinovorans Sustain Spatial Memory Formation by Decreasing Brain Oxidative Stress in Rats." *Journal of Physiology and Biochemistry* 69 (1): 25–34. https://doi.org/10.1007/s13105-012-0184-9.

Karatas, F., M. Karatepe, and A. Baysar. 2002. "Determination of Free Malondialdehyde in Human Serum by High-Performance Liquid Chroma Tography." *Analytical Biochemistry* 311 (1): 76–79. https://doi.org/10.1016/S0003-2697(02)00387-1.

Khoschsorur, G. A., B. M. Winklhofer-Roob, H. Rabl, Th Auer, Z. Peng, and R. J. Schaur. 2000. "Evaluation of a Sensitive HPLC Method for the Determination of Malondialdehyde, and Application of the Method to Different Biological Materials." *Chromatographia* 52 (3–4): 181–84. https://doi.org/10.1007/BF02490453.

Lykkesfeldt, J. 2001. "Determination of Malondialdehyde as Dithiobarbituric Acid Adduct in Biological Samples by HPLC with Fluorescence Detection: Comparison with Ultraviolet-Visible Spectrophotometry." *Clinical Chemistry* 47 (9): 1725–27. http://www.ncbi.nlm.nih.gov/pubmed/11514418.

Marnett, Lawrence J. 1999. "Lipid Peroxidation - DNA Damage by Malondialdehyde." *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis* 424 (1–2): 83–95. https://doi.org/10.1016/S0027-5107(99)00010-X.

Moselhy, Hamdy F, Raymond G Reid, Saeed Yousef, and Susanne P Boyle. 2013. "A Specific, Accurate, and Sensitive Measure of Total Plasma Malondialdehyde by HPLC." *Journal of Lipid Research* 54 (3): 852–58. https://doi.org/10.1194/jlr.D032698.

Nair, Vasu, Crystal L. O'Neil, and Peng George Wang. 2008. "Malondialdehyde." In *Encyclopedia of Reagents for Organic Synthesis*. Chichester, UK: John Wiley & Sons, Ltd. https://doi.org/10.1002/047084289X.rm013.pub2.
Pryor, W A, and J P Stanley. 1975. "Letter: A Suggested Mechanism for the Production of Malonaldehyde during the Autoxidation of Polyunsaturated Fatty Acids. Nonenzymatic Production of Prostaglandin Endoperoxides during Autoxidation." *The Journal of Organic Chemistry* 40 (24): 3615–17. https://doi.org/10.1021/jo00912a038.
Rio, Daniele Del, Amanda J. Stewart, and Nicoletta Pellegrini. 2005. "A Review of Recent Studies on Malondialdehyde as Toxic Molecule and Biological Marker of Oxidative Stress." *Nutrition, Metabolism and Cardiovascular Diseases*. Elsevier. https://doi.org/10.1016/j.numecd.2005.05.003.

1. Al I Cuza University of Iasi, Romania
* marius.mihasan@uaic.ro

ACKNOWLEDGEMENTS
This work was supported by the project „Developing the innovation capacity and increasing the impact of advanced research at UAIC" financed by the Romanian Ministry of Research and Innovation Program 1 – Development of the National system of RD, Subprogram 1.2 – Institutional performance – Projects for funding excellence in RD, Contract no. 34PFE/19.10.2018

INDIRECT ORGANOGENESIS OF *SYMPHYTUM OFFICINALE* L.

SMARANDA VÂNTU[1]

Received: 3rd of August 2019 / Revised: 27th of September 2019
Accepted: 30th of October 2019 / Published: 21st of January 2020

Keywords:, *Symphytum officinale*, callus, micropropagation

Abstract: *Symphytum officinale* L. (Boraginaceae) is a well-known medicinal plant and a source of natural compounds with high antioxidant activity. The initiation of "*in vitro*" cultures of *Symphytum officinale* L aimed not only to assess the dedifferentiation capacity depending on explant origin and growth regulators, but also to develop a multiplication protocol based on indirect regeneration through shoots, followed by roots development induction. The proliferative capacity was tested on leaf and shoots explants, cultivated on Murashige-Skoog basal medium (MS), testing two auxins:: naphtalenacetic acid (NAA) and indolylacetic acid (IAA) and two cytokinines: kinetine (K) and benzylaminopurine (BAP).
The MS medium with 1.0 mg/l IAA and 0,1 mg/l BAP proved to be the best for callus induction from leaf explants. Shoot regeneration was achieved after subculturing the calli on MS medium supplemented with 1 mg/l BAP and 0,1 mg/l IAA. It was found to be the best for multiple shoot regeneration from callus through organogenesis
Multiple shoot proliferation was noticed at 3th subculture in medium and shoot proliferation was decreased with the increased number of subculture. Root system development was achieved on MS medium without growth regulators. Rooted shoots (plantlets) were gradually acclimatized.

INTRODUCTION

Symphytum officinale L. is a perennial plant belonging to the Boraginaceae family. The presence of polyphenols, triterpenoids and tannins in this species represents a promising source of natural compounds with high antioxidant activity (Noorwala et al., 1994).
Many members belonging to the Boraginaceae family, including *S. officinale*, are found to contain the chemical constituent allantoin. Comfrey contains about 0.8% in the root and 0.4% in the leaf .(Winship, 1991).
Like other members the Boraginaceae, the roots of *Symphytum officinale* L contain pyrrolizidine alkaloids, which are hepatotoxic and carcinogenic agents (Winship, 1991). the highest alkaloid levels were found in bulk comfrey roots and leaves (Betz et al. 1994, Frolich et al, 2007, Dreger et al, 2009).
The root extracts of *S. officinale* were tested for their antimitotic and mutagenic activity (Furmanowa et al., 1983, Mei et al, 2005).
"*In vitro*" cultivation of *Symphytum officinale* L was initiated in order to evaluate the cell dedifferentiation and redifferentiation, as an unconvential alternative for plant biomass multiplication, the main source of bioactive compounds with pharmaceutical value (Harris et al, 1989, Huizing et al., 1983, Tacke et al., 1993, Shimon-Kerner et al., 2000).
The objective of the present investigation was to establish in vitro culture and plant regeneration methods from leaf and stem explants of *Symphytum officinale* L.

MATERIAL AND METHODS

The cultures of *Symphytum officinale* L. were based on explants taken from mature individuals, harvested from spontaneous flora.
Foliar and stem explants taken from *Symphytum officinale* L, produced in aseptic conditions were tested " *in vitro*" for proliferative capacity (Haaß et al, 1991).
Callus induction was performed on leaf and shoot explants, cultivated on different variants of MS medium (Table).
The preparation of explants for inoculation was the chemical sterilization, using a solution of 3% Na hypochlorite. Treatment duration was 12 minutes, followed by washing repeatedly with sterile distilled water.
The diversification of MS induction media was based on two types of auxins: naphtalenacetic acid (NAA) and indolylacetic acid (IAA) and two cytokinines: kinetine (K) and benzylaminopurine (BAP).
The biomass accumulation was measured by regular weighting on analytical balance. For callus induction, it were tested some variants of MS medium, using indolylacetic acid (IAA) in combination with benzylaminopurine (BAP).in same concentration (variants 1) or with indolylacetic acid (IAA) in excess (variants 2). The second tested auxin was

naphtalenacetic acid (NAA) used in combination with kinetine (K), in same concentration (variants 4) or in excess (variants 5).
Indirect micropropagation consisted in shoots development from callus cultures., cultivated on different variants of MS medium, supplemented with benzylaminopurine 1 mg/l with 0,1 mg/l indolylacetic acid (IAA) (Table)) or 1 mg/l kinetine with 0,1 mg/l naphtalenacetic acid (NAA) .
All the cultures were maintained at 25^0 C under 12 hr photoperiods.
The cultures growing on various levels of growth regulators were scored for the number of shoots per culture and rooting after every 2 weeks.
The morphogenic response of cultures was followed every 4 weeks by monitoring the fresh weight, number and height of regenerants , as well as root formation.

Table - Variants of MS medium

Variants	Growth regulators			
	BAP	**IAA**	**NAA**	**K**
1	1 mg/l	1 mg/l	-	-
2	0,1 mg/l	1 mg/l	-	-
3	1 mg/l	0,1 mg/l		
4	-	-	1 mg/l	1 mg/l
5	-	-	1 mg/l	0,1 mg/l
6			0,1 mg/l	1 mg/l
7	-	-	-	-

IAA - β indolylacetic acid
NAA - α-naphtalenacetic acid
BAP - benzylaminopurine
K – kinetin

RESULTS AND DISCUSSIONS

The initiation of "*in vitro*" cultures aimed to evaluate the capacity of cell dedifferentiation and redifferentiation at *Symphytum officinale* l., depending on the origin of the explant and hormone stimuli in the culture medium.
The diversification of the composition Murashige – Skoog (Murashige et Skoog, 1962) was based on the use of auxins and cytokinins in various combinations and concentrations.
Among the auxins tested were used: IAA - β-indolylacetic acid and NAA - α-naphthalenlacetic acid and the cytokinins : BAP- benzylaminopurine and K - kinetine.
Testing the proliferative capacity of explants on different types of induction media (Table) aimed in the first instance, to obtain primary callus cultures.
Leaf and stem explants taken from *Symphytum officinale* L, obtained aseptically were tested "*in vitro*" for proliferative capacity.
Callus cultures were periodically transferred every 3 weeks on fresh medium. The next step is to multiply the callus over a period of 6 rounds of subculturing.
The samples were kept at 12 hours light and 12 hours of darkness.
Callus was achieved using surface cultures on agar medium and callus proliferation during several stages of subculturing was assessed by periodic weighting of fresh biomass.
Initiation of "*in vitro*" cultures from *Symphytum officinale* L on the basal MS medium variants (table) generated different reactions, depending on the origin of explant, especially auxins type and hormone balance.
The dedifferentiation has been difficult, in the case of stem explants, while leaf segments were generated primary callus, within 4 weeks after cultures initiation (Photo 1, 2, 3).

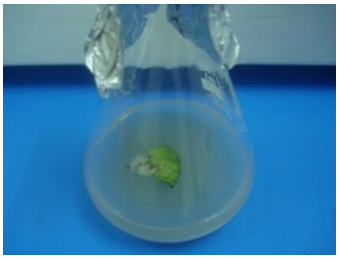

Photo 1- The initiation of callus cultures from foliar explant at *Symphytum officinale* L

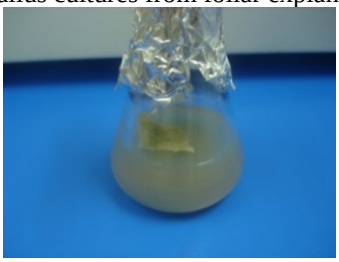

Photo 2- Early stages of cell dedifferentiation at *Symphytum officinale* L.

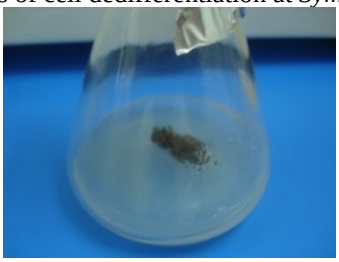

Photo 3- Primary callus culture from foliar explant

Some variation in the frequency of callus formation and in callus growth rate was noted among explants of different origins.
The response of stem explants to IAA or NAA was slower and was initiated only after 6 weeks culture.
Both β-indolylacetic acid (IAA) and α-naphtalencetic acid (NAA), have caused similar effects on leaf explants, completed to the development of primary callus. Excess of auxin in both variants generated callus.
Undifferentiated tissue developed 7-14 days after placement on the medium, at the edges of the cut borders of the foliar explant.
Two weeks later, when calli reached 0,5-1,0 cm diameter, they were cut from the explant and subcultured on medium. Subculturing on medium was done by transferring 6-10 mm diameter pieces of callus onto fresh medium.
Callus of foliar origin was tested for evidence the regenerative capacity.
The growth regulators balance tested in this case is based on the use of cytokinine in higher concentration than auxin. It were tested two cytokinine: BAP – benzylaminopurine and K-kinetine.

The caulogenesis induction was performed on MS medium, supplemented with 1 mg/l BAP – benzylaminopurine in combination with 0,1 mg/l IAA – β-indolylacetic acid (Photo 4, 5, 6).

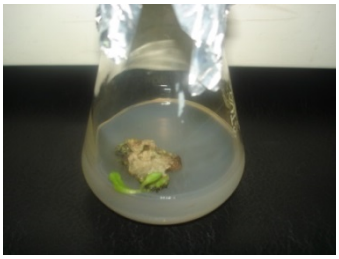

Photo 4 -Early stage of caulogenesis

Photo 5 - First shoot forming on callus culture

Photo 6- Early stages of cell redifferentiation at *Symphytum officinale* L.

Calli with morphogenetic capacity were compact and yellowish to green in colour. Shoot regeneration was always preceded by an early stage of callus growth (Photo 7).

Photo 7- Advanced stages of caulogenesis

Foliar origin calli in the presence of BAP showed the development of multiple shoots, within 4-5 weeks culture. New shoots were continuously formed during the observation period (Photo 8).

Photo 8- Multiple shoot development

Single shoots were excised from 5 week old regenerating cultures and transferred on MS medium for root induction (MS- without growth regulators) (Photo 9).

Photo 9- Regenerated shoot of *Symphytum officinale* L

The time of root initiation and rooting percentage (percentage of shoots forming roots) was determined. A shoot having one or more macroscopically visible roots (0,5 cm long) was considered rooted (Photo 10).

Photo 10- Roots development

Morphogenetic potential expressed in primary callus cultures decreased during the 6 stages of subcultivation. Whole plantlets were transferred to pots containing agricultural substrate and sand (1:1).

CONCLUSIONS

The dedifferentiation has been difficult, in the case of stem explants, while leaf segments were generated primary callus**.**

Both β-indolylacetic acid (IAA) and α-naphtalencetic acid (NAA), have caused similar effects on leaf explants, completed to the development of primary callus.
Foliar origin calli in the presence of BAP showed the development of multiple shoots.
The caulogenesis induction was performed on MS medium, supplemented with 1 mg/l BAP – benzylaminopurine in combination with 0,1 mg/l IAA – β-indolylacetic acid.

REFERENCES

Abou-Mandour, A. A., Czygan, F.C., Haaß, D., Franz, G. (1987): Fructan Synthesis in Tissue Cultures of *Symphytum officinale;* L. Initiation, Differentiation, and Metabolic Activity, Planta Med 53(5): 482-487
Betz, J. M., Eppley, R. M., Taylor, W. C. , Andrzejewski, D. (1994): Determination of pyrrolizidine alkaloids in commercial comfrey products (*Symphytum* sp.). *J Pharm Sci* 83 (5):649-53
Dreger, M., Stanisławska, M., Krajewska-Patan, A., Mielcarek, S., Mikołajczak, P. Ł., Buchwald, W. (2009): Pyrrolizidine alkaloids–chemistry, biosynthesis, pathway, toxicity, safety and perspectives of medicinal usage. *Herba Pol*, *55*(4), 127-47.
Ehmke, A., Von Borstel, K., Hartmann, T. (1988): Alkaloid N-oxides as transport and vacuolar storage compounds of pyrrolizidine alkaloids in Senecio vulgaris L. *Planta*, 176.1: 83-90.
Frolich, C., Ober, D., Hartmann, T. (2007): Tissue distribution, core biosynthesis and diversification of pyrrolizidine alkaloids of the lycopsamine type in three Boraginaceae species. *Phytochemistry*, 68.7: 1026-1037.
Furmanowa, M, Guzewska, J., Beldowska, B.(1983**)** : Mutagenic effects of aqueous extracts of Symphytum officinale L. and of its alkaloidal fractions. *J Appl Toxicol* 3 (3):127-30
Haaß, D., Abou-Mandour, W., Blaschek, G., Franz, F., Czigan, C. (1991): The influence of phytohormones on growth, organ differentiation and fructan production in callus of *Symphytum officinale* L., 10 (8): 421-424
Harris, P. J. C., Grove, C. G., Havard, A. J. (1989): In vitro propagation of *Symphytum* species. *Scientia horticulturae*, *40*(4):275-281.
Huizing, H. J, Pfauth, E. C., Malingré, Th. M., Sietsma, J. H. (1983) : Regeneration of plants from tissue- and cell suspension cultures of Symphytum officinale L. and effect of in vitro culture on pyrrolizidine alkaloid production. *Plant Cell, Tissue and Organ Culture* Vol: 2(3):227-238
Mei, N., Guo, L., Fu, P. P., Heflich, R. H., Chen, T., (2005): Mutagenicity of comfrey (Symphytum officinale) in rat liver. British Journal of Cancer 92(5): 873–875.
Murashige T., Skoog, F.(1962**)**: A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.
Noorwala M, Mohammad, F.V, Ahmad V., Sener, B. (1994**)**: A bidesmosidic triterpene glycoside from the roots of *Symphytum officinale, Phytochemistry* 36 (2):439-43
Shimon-Kerner, N., Mills, D., Merchuk, J. C. (2000.): Sugar utilization and invertase activity in hairy-root and cell-suspension cultures of *Symphytum officinale*. *Plant cell, tissue and organ culture*, 62.2: 89-94.
Tacke, R., Wagner, S. A., Brakmann, S., Wuttke, F., Eilert, U., Fischer, L., Syldatk, C. (1993*):* Synthesis of acetyldimethyl(phenyl) silane and its enantioselective conversion into (*R*)-(1-hydroxyethyl) dimethyl(phenyl) silane by plant cell suspension cultures of *Symphytum officinale* L. and *Ruta graveolens* L. *Journal of Organometallic Chemistry*, *Volume 458, Issues1–2,13-17*
Winship, K. A. (1991): Toxicity of comfrey. Adverse Drug React. *Toxicol. Rev*. 10 (1):47-59

[1]"Al. I. Cuza " University, Faculty of Biology, Iasi , B-dul Carol I, 20 A