30 YEARS OF NANOSCIENCE AT BANAT UNIVERSITY OF AGRICULTURAL SCIENCES AND VETERINARY MEDICINE „King Mihai I of Romania" FROM TIMIŞOARA

1. Reflected in Different Publications

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Abstract. *In a short retrospective this paper presents the results and the important findings in the researches regarding Magnetic-Nanocomposites utilization at Banat University of Sciences (Anton, et al., 1988, Butnaru and Roosz, 1988). The paper brings attention to a pioneering research in the nanomagnetic materials field. We are briefly presenting the results of NMPs involvement with the plant's organogenesis and animal health as well as the environment protection. The researchers focused on a lot of questions and opened new domains of study but throughout the obtained results suitable application were formulated. The work resumes the results of 160 scientific works made by 291 researchers from all the Faculties of our University. It points out the interest for different topics, the improvement of producing bio-nanomaterials, their utilization and the visible results reflected in different publications.*

Keywords: 30 years of MNPs researches, publications, magnetic-nano materials, utilization.

Introduction

The staff of the Polytechnic University and the Romanian Academy, Timisoara Branch, developed a new class of materials of nano dimensions (Anton et al. 1977 and 1990). In the 1970s all over the world as well as in our country the perspective of their utilization was the industry. The idea of using them in biology was a utopia.

In our researches was introduced a new and particular topic the utilization of Romanian Nano-Materials in biology and agriculture, generally named now "agricultural nanotechnology". About 10 years before our research (1959) Feynman mentioned: ...the biological example of writing information on a small scale has inspired me to think of something that should be possible. He understood how a "smaller organism" is capable to manage his energy for a better activity to survive in improper conditions. He has believed in.... "manipulating

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and controlling things on a small scale"; ….which "would have an enormous number of technical applications"….. In "the marvellous biological system" he has seen a big hope (Feynman, 1959).

The first Magnetic Liquids (ML) prepared in Romania was obtained in Iasi at the Center of Technical Physics during 1970-1975 (Neaga and Lazar, 1987).

At the same time in Timisoara Prof. Anton I. PhD. organized the first lab to produce magnetic liquids for industry (1971). He was also the first initiator and the supporter for MNPs utilization in the bio domain. Due to his competence and his vision of the future our University was involved in the world pioneering research. Very soon the idea was taken into account by other Universities Research Centers which started to work with different types of Magnetic Liquids.

As new topic the name of the same product was changed several times. Thus at first was used the name "Magnetic Liquid" (ML), then "Ferrofluid" (FF), "Magnetic-Nanofluid" (MNF), etc. This is the reason why different research centres and authors used different names for the same product. The efficacy or "magnetization power" of the product is accounted in Gs units (cm3; Fe3O4/cm3).

The research centres involved in ML utilization in agriculture and biology were: Timisoara: Banat University of Agriculture, Faculty of Agriculture (1983) and the Faculty of Veterinary Medicine (1991); Bucharest: Research Center for Ecological Technology (1994); Craiova: Craiova University, Horticulture Faculty (1994); Arad: Vest University "Vasile Goldis" (2001); Oradea: Oradea University, Faculty of Biology (2004).

This study was structured per time periods and on interest areas.

The major goal was: to emphasize the studies with Magnetic-Nano Liquids to show the obtained results in important domains of agriculture. It was important to be established if the NMLs are useful to enhance the plant performances, would contribute to plant and animal as well as the environmental protection, or to prevent biotic and abiotic stress. Our problem was to establish the positive/negative effect of MNP on tissue, cell, and chromosome and if they are involved in the DNA structure and function.

Investigation details

Each researcher or person in charge of an experiment has chosen the biological material that was considered the most appropriate for the intended purpose. A large number of plants were used as biological material, economical important species as wheat, corn, triticale, potato, tomatoes as well as "model plants" Arabidopsis thaliana Nicotiana tabaccum and Saintpaulia ionantha. Lab and farm animals as well as company species were subjects of animal science and veterinary medicine experiments. Drosophila melanogaster was used as a model for chain of harmful insects and their entomophagous. Bacteria and fungi species were used to understand how the MNP acts in their life cycle.

Specific methods were applied to each group of the organism of interest to clarify the impact of MNP in their life cycle. The procedures of work have aimed to highlight the direct and indirect action of PM upon cell activity, in mitosis and in meiosis respectively and on chromosome chiasmata.

The electron microscope inquiry was used to point out the involvement of MNP with subcellular components.

The experimental regime was kept under control in the lab or growth chambers but was subject to external factors influence in field conditions. In both conditions was followed the relationship between magnetic liquids (ML) - magnetic nanoparticles (MNPs) by one hand and plants - animals - insects and microorganisms on the other hand.

In all experiments the statistical analysis was applied (Ceapoiu, 1968, Ciulcă, 2006).

The data of this work present scientific results taken into account in publications, proceedings, PhD thesis, diploma papers, and symposiums, round tables or debates. In the graphs 1 to 6 are illustrated the main domains that had been followed at different time periods.

About the Magnetic Nano Liquids used

During the years special tailored magnetic nanomaterials were prepared depending on:

- nano – core:

Fe_3O_4, γFe_2O_3 and $CoFe_2O_4$, Fe_3O_4+ $CoFe_2O_4$, and with Zn:Mn:Fe_3+

- stabilizers:

Group I produced in the 1980s was γ $Fe_3O_4 \bullet Fe_2O_3$ /H_2O or Dodecylbenzene Sulfonate (DBS);

Group II - Fe_3O_4 /C_4OH prepared between 1988 and 1990;

Group III Bio - produced during 1990-2000 - Fe_3O_4 /Lauric Acid/DBS (Fe_3O_4/LA-DBS); lauric acid/lauric acid (Fe_3O_4/LA-LA); lauric acid/citric acid (Fe_3O_4/LA-CA); myristic acid/DBS (Fe_3O_4/MA-DBS);

Group VI Bio – $CoFe_2O_4$ stabilized with oleic acid/DBS; myristic acid/DBS; lauric acid/citric acid; lauric acid /oleic acid;

Group V – Montmorillonite-clay complexes prepared in 2003 were:

montmorillonite Fe_3O_4/lauric acid (bilayer); suspension of montmorillonite Fe_3O_4 and lauric acid; suspension of montmorillonite $CoFe_2O_4$ and lauric acid. - carriers:

- Aqueous: Water - FM/W (Doina Bica Patent RO-90078/85); "vine tears" - LV; FM/LV/oleine; LV/lauric acid; (collected by us; LV have been used in traditional Romanian medicine); "depleted water" - DDW, FM/DDW/oleine; FM/DDW/lauric acid; (DDW prepared at the National Research and Development Institute for Cryogeny and Isotopic Technologies Rm. Vâlcea, Romania; DDW is used in cancer therapy);

- Oleous: Mineral oil - MO; Soya oil - FM/SO; (Doina Bica & Mirea Radu, Patent RO-93107/87); Sunflower oil - FM/SfO (Doina Bica et al, Patent RO-105049/1995);

- Lanoline FM was also prepared (Doina Bica et al, Patent RO-105048/1989).

The NMLs were prepared Center for Fundamental and Advanced Technical Research, the Romanian Academy, Timişoara Branch, by Chem Eng. Doina Bica, Ph.D. and were characterised by Phy. Ladislau Vekas, Ph.D., et al., 2001 from the National Center for Engineering of Systems with Complex Fluids, University Politehnica Timisoara.

Results

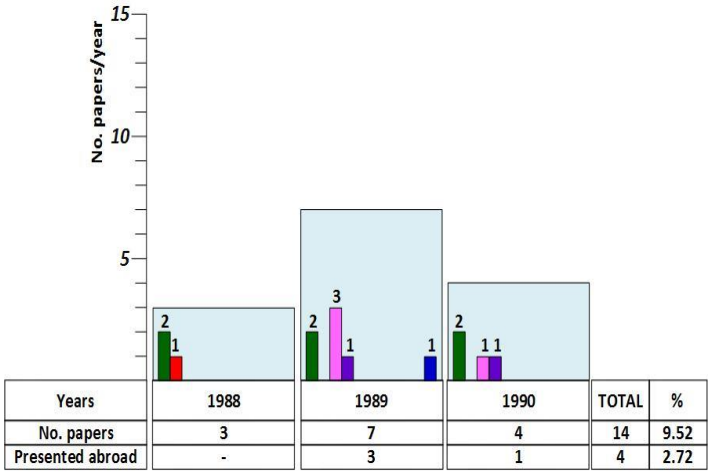
From 17 top journals of Nanoscience 52.9% are dedicated to medicine, 11.76% to biomaterials and only 35.34% for all other fields (biology, agriculture, environment, techniques of preparation, etc.). As in most cases, nanotechnology applications to the agricultural field are still relatively underdeveloped (Mastronardi et al., 2014, Achari and Kowshik, 2018).

Our first results were reported in 1988 representing 2.05% from all USAMVB reports (Anton et al., 1988, 1, 2; Butnaru and Roosz, 1988). Extensive researches started in 1982 when a large part of our university staff was involved in Magnetic Liquids (ML) experiments on plant and animal even on human voluntaries (Goian et al., 1989). Before this date in 1986 Goian and Butnaru in informal conditions presented the utilization of ML for the early sowing of thermophiles plants in soil with low temperatures.

The evolution of LM utilization during 30 years

The scientific results were grouped in 5 year periods (Figures 1 to 6). The data point out the interest to the different topics, new areas of investigation the results of ML utilization in agriculture, animal health and environment.

During 1988-1990 there were presented in 14 papers representing 9.52% from all publications (Fig. 1).



Years	1988	1989	1990	TOTAL	%
No. papers	3	7	4	14	9.52
Presented abroad	-	3	1	4	2.72

Fig. 1: The obtained results at fundamental domain and at plants presented in 1988-1990

Legend: Plants ■; Cell/Histo. ■; DNA/Chromosome ■; Animals ■; Synthesis ■

The germination, plant growth and their fertility, cell activity and chromosome *behaviour* were presented into 4.11%, 3.42% and 1.37% respectively (Borcean et al., 1989; Butnaru and Butnariu, 1989). The cell and subcellular modification in presence of NMP is still of interest (Tominaga et al., 2013, Pieuchot et al., 2015).

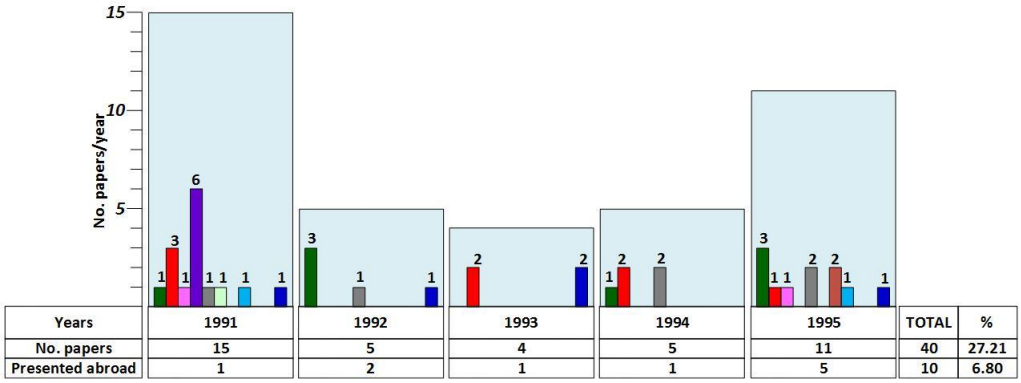
In 1989 Goian et al. in a synthesis pointed out the view of ML utilization in biotechnology and agricultural and animal husbandry technologies and in human medicine.

It was the most difficult part of research because we started from zero information and insufficient knowledge.

We had to create out a minimum of information and literature to hold them for our future results comparison. To create trust in the MNP effect it was necessary to broaden our own knowledge as to offer relevant explanations to researchers with very different views in this field. To create our own persuade the experiments were repeated many times in successive cycles till the results were close in minimum 5 of them.

The results were spectacular and were a great support to continue the efforts at other fields.

The 1991-1995 period was fruitful; the 40 scientific papers represent 27.21% from all the papers published over 30 years. At the same time the papers published abroad were considerable (6.80%; Fig. 2).



Years	1991	1992	1993	1994	1995	TOTAL	%
No. papers	15	5	4	5	11	40	27.21
Presented abroad	1	2	1	1	5	10	6.80

Fig. 2: The scientific results at different fields presented published in different magazines or proceedings in 1991-1995

Legend: Plants ■; Cell/Histo ■; DNA/Chromosome ■; Animals ■; Biotechnology ■; UV protection ■; Environment ■; Fungi/Bacteria ■; Synthesis ■;

The main results pointed out the MNP capacity to penetrate cell membrane for changing the course of mitoses (Butnaru et al., 1989, Butnaru et al., 1991, Goldstein et al., 2008) and the chromosome behaviour. In lab condition to a high concentration of MNP with 380 Gs/0.0238 g Fe_3O_4 all maize plants were short and the Sk gene was activated in the tassel. In field conditions the plants were shorter than at the control and the expression of Sk gene in the tassels was under 20%. But the conclusion was clear; in some circumstances the MNP have the power to modify the gene activity (Butnaru, 1996).

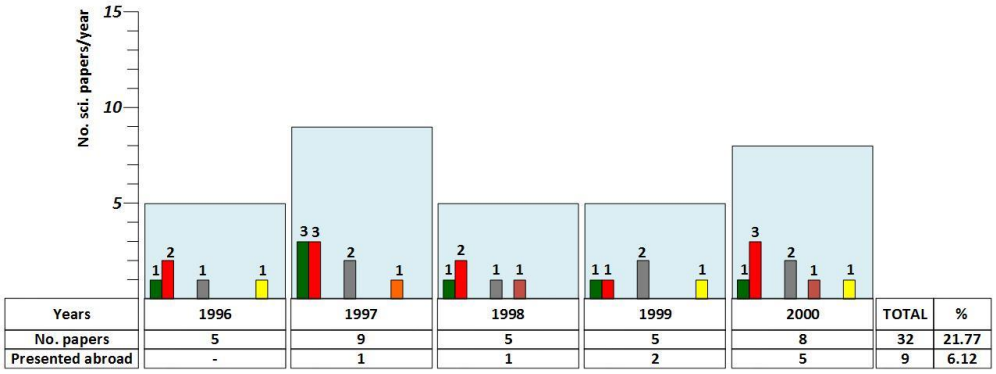
During 5 years the emulation of investigations was high and new domains of research were identified.

From all the obtained and discussed results or submitted to different reports of grants only 74% were published. In most of the rejected experiments the outcome pointed out an interesting direction of MLs utilization but the scientific argumentation and concern for environment protection was not sufficiently argued. In this period of activity 9 topics were followed from which 4 of them were new. 15% brought the results from biotechnology (Perciuleac et al., 1994) 5% from environment protection and the reaction of animal pathogens (Butnariu, et al., 1995 and Moga-Manzat et al., 1991 respectively) and 2.5% about animal defence from the UV radiation (Sincai et al., 1991).

The results pointed out that the: - Gene pool splitting was the most valuable result (Butnaru and Butnariu, 1995). The Costeni tomato local cultivar of Maramures County segregated in different plant and fruit types (with indeterminate growth/round and with determinate growth/different fruit shapes). From “Tim. 1-1137 FM” genotype with determinate growth was isolated Ileana-2 variety proposed for registration in 2018. It has been established the protection

effect of MNP at UV radiations at plants and animals. The NMLs may be used as a non-invasive material for environment protection.

In the third stage the research work results were quite rich representing 21.77% from all publications with 6.12% abroad (Fig. 3).



Years	1996	1997	1998	1999	2000	TOTAL	%
No. papers	5	9	5	5	8	32	21.77
Presented abroad	-	1	1	2	5	9	6.12

Fig. 3: The scientific results at different fields presented published in different magazines or proceedings in 1996-2000

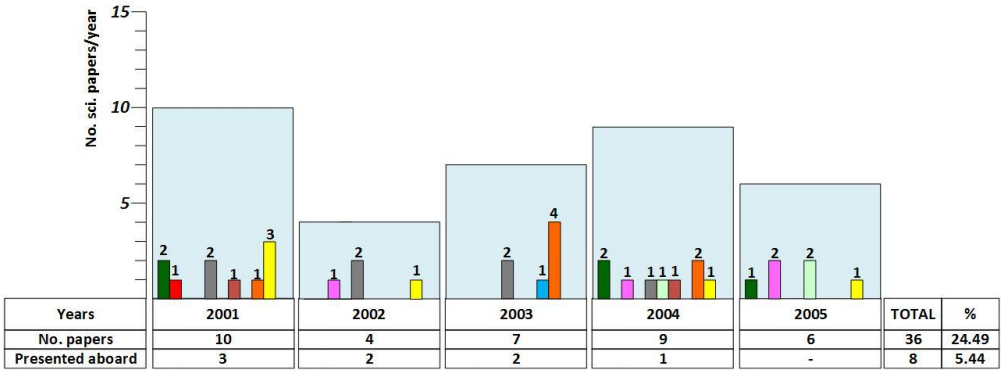
Legend: Plants ■; Cell/Histo. ■; Biotechnology ■; Environment ■; Insects ■; Vet. Med/Tumours ■

Other two new fields of research have been initiated: the tumour treatment and insect's behaviour cognition at MNP presence (Sincai et al., 19991, 2, Butnaru, 1997). To understand the mechanism of MNP involvement in the insect's life cycles the Drosophila melanogaster was used as model. Apart sensitivity was pointed out of drosophila genotypes. The mutant w+w+ reacted extremely negatively. The wild genotype resisted up to θ=3.70×10-3g/cm3.

If the "petite" adults were transferred in standard culture the dimensions were increased but never reached the normal size.

It was also noticed the enhancement of nodulation at alfalfa if the Rhizobium inoculums was prepared into solution with a low concentration of MNPs (θ=0.037×10-3g/cm3 (Butnaru et al., 1998).

In the 2001-2005 period 24.49% of the papers were published (Fig. 4).



Years	2001	2002	2003	2004	2005	TOTAL	%
No. papers	10	4	7	9	6	36	24.49
Presented aboard	3	2	2	1	-	8	5.44

Fig. 4: The scientific results at different fields presented published in different journals or proceedings in 2001-2005

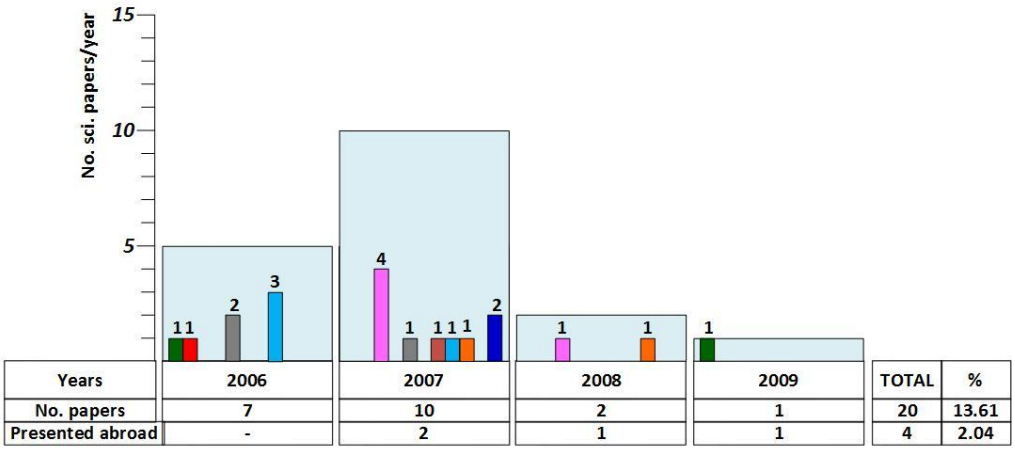
Legend: Plants ■; Cell/Histo ■; DNA/Chromosome ■; Biotechnology ■; UV protection ■; Environment ■; Fungi/Bacteria ■; Insects ■; Vet. Med/Tumours ■

At the same time 5.44% of them were presented and published abroad. No new domain was initiated. The accumulated information allowed us to apply MNP in biotechnology and to design new techniques of utilization (Baciu et al., 2004, Butnaru et al., 2004).

At the Saintpaulia plants risen from seeds germinated into NMPs mixtures (θ=0.37×10-3g/cm3) the descendants had very different phenotypic expression. Olympia and Ni-Na completely apart varieties were patented (CR. 2264/03 and CR. 2263/03, Butnaru, 2000 and Butnaru and Sarac 2003 respectively). In a proper MLs type and amount the static criteria of quality (Y1) increased by 119% to Trichogramma spp. entomophagous used in the ecological agriculture. To Fe_3O_4/LA/DBS or at Fe_3O_4/LA/DBS/DDW and at θ=0.231x10-3 g/cm3 concentration the viability and prolificacy was higher with 29.33% and 9.49% respectively (Firu, 2004).

In this period of research the important results were deeply examined and practical extension was offered (Fig. 5).

The analysis of chromosomes and DNA was the important subject of our investigation because it offers relevant response to many unclearly solved questions. The results were presented in 3.40% of the total papers. It was concluded that the MNP induced changing in molecular profile of DNA at Nicotiana tabacum L. and at triticale at storage proteins profile (Butnaru et al., 2007).



Years	2006	2007	2008	2009	TOTAL	%
No. papers	7	10	2	1	20	13.61
Presented abroad	-	2	1	1	4	2.04

Fig. 5: The scientific results at different fields presented published in different magazines or proceedings in 2006-2009

Legend: Plants ■;Cell/Histo ■; DNA/Chromosome ■; Biotechnology ■; Environment ■; Fungi/Bacteria ■; Insects ■; Synthesis ■

Due to their useful peculiarities for ecological agriculture the Rhizobium bacteria was studied in hydroponic experiments and in field and also molecular analysis were made (Blidar et al., 2006). To have enough information regarding MNP involvement with nitrogen fixing bacteria were studied 2 different Romanian strains of Rhizobium spp.: the R. lupini (LP-83) and R. phaseolus (FL-F8).

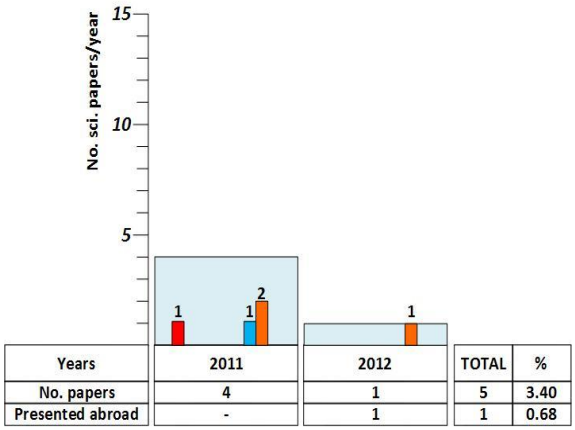
In YMA growth medium and in the presence of small concentrations of MNP to (0.037×10-3 g/cm3) after 38 days of growth the CFU number (colony-forming unit) was significantly enhanced with an average of 19% and 15.41% at LP-83 and at FL-F8 respectively (Paun, 2007). As usual, at a high concentration of MNP (3.7×10-3 g/cm3) the bacteria growth was repressed. Similar results were obtained by Jurca et al (2003) in the experiments with Bacillus sp.

Rhizobium lupini (LP-83) showed a high adaptability its growth almost equalized the control revealing an average of 99.06%. Based on this property it was proposed to be used for the preservation of the bacteria strains into a mixture of media with MNP. New researches point out the NMP potential to be used in agriculture (Liu and Lal, 2015).

Outside from a financial regulate research the experiments were reduced to minimum and only for practical domains. The interest was to capitalize unused experimental results to formulate new possibility of MNP utilization. The preoccupation was to discover some niches were the ML can be used for practical interest.

It was a normal decrease of results and publications (Fig. 6) but the interest in MNPs utilization is still in our attention.

The biotechnology through in vitro culture pointed out the morphogenesis intensification, root and plantlets rapid growth, a lengthy time preservation and re-juvenilization of senescent caulli. In this field, there are good results, but to establish an appreciated technology more tests are necessary. The negative response to hypogravity was reduced if the plants were watered with diluted solutions of MNP. Those results are useful to plant cultivation for fresh food for cosmonauts.



Years	2011	2012	TOTAL	%
No. papers	4	1	5	3.40
Presented abroad	-	1	1	0.68

Fig. 6: The scientific results at different fields presented published in different magazines or proceedings in 2011-2012

Legend: Cell/Histo ■; Fungi/Bacteria ■; Insects ■;

Quantifiable results point out in best mode our work. It was finished 4 PhD theses and 11 student's diploma work. 2 National symposiums, 14 mini-symposiums and round tables were organized. From the 7 patents 2 were patented abroad. 160 scientific papers were published and 22.97% of them were presented and published at International Conferences. "The Frontier: Plant - Magnetic Fluids" published in 1996 by Ed. Mirton Timisoara was the first book in the world dedicated involvement of NMP with plants. It outlined the important results in this domain out of print in a week (Butnaru, 1996). 9 research grants were accepted in which 291 researchers were involved and in this way it was possible to collect a huge amount of data that were used only partially.

We made 12 offers to private companies for the practical utilization of the obtained results (accepted 41.67%).

In our work with Saintpaulia ionantha of in vitro culture an unexpected phenomenon was observed. On the injured explants and on vitrified leaves new

plantlets arise from which healthy plants are formed. The same process was observed on potato calli. In MNP presence minituberculs were made in a larger number than usual (30% upper than control). If similar changes can be made in vivo, a new method to increase potato yield could be performed. But in the next decades you won't see FM-plants in the fields.

In vitro conservation of germplasm is promising nanotechnology utilization. The most useful application is for the species and landraces with vegetative proliferation (potatoes, carrots, and Jerusalem artichokes). It is important for the preservation to maintain the biological material in the state of cell viability and its ability to be active when the growth conditions are suitable.

The explants (neoplantlets and calli) of the mentioned species were preserved for 5 years at the room temperature on the same medium MS-0 supplemented with MNPs (θ=37×10-3g/cm3). The phenotype of regenerated plants was normal. No chromosomal or molecular analyses were made. For practical reasons it is essential to establish the proper technology for each species and maybe for each genotype.

Even if the MNP utilization has much useful application in agriculture, biotechnology and animal health their utilization implied some risks. The risks are due to unknown aspects. Up to now it is unknown how to detect the MNP traceability in nature and in soil. The capacity of MNP to induce modifications in the DNA, chromosome structure and cellular activity it is a benefit but also a risk.

It is not clear how NPM recognizes cancerous cells and how they interact with normal cells. A few researches are focused on patients' health in general and on their safety on long therm.

All risks have to be evaluated and prevented. To protect the environment were performed methods to produce potato tubers free of viruses (Patent: MD. 554/1996) and a technology to enhance the biological parameters on Trichogramma spp. (Patent: MD. 1974/2002).

Conclusions

Considering the climatic change the great challenge of the future the application of nanotechnology in agriculture can contribute to its sustainability. Magnetic Nanoparticles' ability consists in acting efficiently upon the genetic stock on the cellular activity and in the life cycle of plants and other organisms.

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