

EUROPEAN COMMISSION Research Executive Agency Marie Curie Actions – International Research Staff Exchange Scheme



Project No: 316067

Project Acronym: HERBAL PROTECTION

Project Full Name: Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation

Marie Curie Actions

Mid-term Report

Period covered: from 01/01/2013 **to** 31/12/2013

Period number: 1st

Start date of project: 01/01/2013

Project coordinator name:

Prof. Stoycho Stoev

Version: 1

 $\textbf{Date of preparation:}\ 15/04/2014$

Date of submission (SESAM):

Duration: 48

Project coordinator organisation name:

TRAKIA UNIVERSITY

Mid-term Report

PROJECT MID-TERM REPORT

316067
HERBAL PROTECTION
Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation
FP7-MC-IRSES
1st
01/01/2013
31/12/2013
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1. GENERAL PROGRESS OF THE PROJECT

The project has achieved most of its objectives and technical goals for the mid-term period with relatively minor deviations:

Description of the modifications:

Most of the objectives and the tasks are achieved. There are only some amendments in regards to the time of the secondments between the partners and beneficiaries as follow

Work package 1 - without changes

Work package 2 - two of the secondment from Bulgaria to S. Africa were done, but one secondment from Hungary to South Africa was postponed for the next year and is currently realized. However, all the tasks and scientific objectives were achieved.

Work package 3 - one of the secondment from RU to Trakia University was realized, but the other from DRDO to Trakia University was postponed for the next year.

The other work packages are of planned for the next year, but part of them are already fulfilled this year as work package 4.

The reason for amendments is as follow: the transfer of money made by REA is insufficient for the first reporting period of two year, because most of the tasks and secondments were situated in the first two years. Therefore, we must postpone some of the secondments in order to adjust them to the available money supplied by REA. The second reason is that some of the researchers included in this project leaved their institutions and we should find some other researchers to take their place.

Qualitative indicators of progress and success in line with workplan and milestones (description of progress towards milestones and deliverables)

Workpackage 1 - Production of mycotoxins Fumonisin B1 and Ochratoxin A for experimental studies – partially done according to the plan – The produced mycotoxins will be used in the planned experiments on the herbal protection against mycotoxins using various kinds of animals/chicks.

- -Involved researchers: Prof. S. Denev (ER from TU), Prof. S. Stoev (ER from TU),
- -Used full-time equivalent months so far -5.81 months

Deliverables:

- D 1.1.: Ensuring of enough quantity of mycotoxin FB1 for experimental work was done
- D 1.2.: Ensuring of enough quantity of mycotoxin OTA for experimental work was done

Work package 2 - Collection of target herbs with known protective and antimicrobial effect from South Africa and India and receiving some herbal extracts – partially done

- -Involved researchers: Prof. R. Zheleva (ER from TU), Prof. V. Gadjeva (ER from TU), Prof. S. Stoev (ER from TU)
- -Used full-time equivalent months so far -4.86 months

Deliverables:

- D 2.1.: Ensuring of enough quantity of target herbs for necessary experimental work was done
- D2.2.: Establishment of biological activity of the same herbs is partially done according to the plan

Work package 3 - Selective characterisation of some South African and Indian herbs for their bioconstituents (flavanoids, etc) via EPR (Electron Paramagnetic Resonance), NIRS (Near Infrared Reflectance Spectroscopy), NMR, FTIR, UV, EA, MS, RS, MA, etc. and preparing of target herbal fractions or extracts. In vitro study on antioxidative effect of some herbal extract against oxidative stress via EPR spectroscopy - partially done

Deliverables:

- D 3.1. Establishing the bioconstituents of some target Himalayan and South African herbs partially done according to the plan
- D 3.2. Elaboration of technology for preparing some target herbal extracts or fractions and Preparing research

patents and relative publications in this regard - partially done.

- -Involved researchers: D. T. Ndinteh (ESR from RU)
- -Used full-time equivalent months so far -1.77 months

Work package 4 - Elaboration of mixtures of target herbal extracts for preparing of some sprays/unguents designed for stimulation of wound granulation - partially done in advance

- -Involved researchers: M. L. Dlamini (ESR from UJ), T. Fonkui (ESR from UJ)
- -Used full-time equivalent months so far -2,78 months
- -Description of work finished:
- -Antifungal screening of plant extracts against 5 mycotoxin producing fungal species via "MIC of highest activity measurement" and "preparing of TLC-Bioautogram of antifungal activity via TLC-Bioautography" showed that most activity recorded for P. africanum, and less activity for M. longifolio and L. leonorus
- -Chromatographic separation of active components was done via TLC and column chromatography

2. PROJECT ACHIEVEMENTS

Scientific highlights and research achievements:

-Description of work finished in work package 1:

FB1 is produced using the strain Fusarium verticillioides (isolate MRC 826 - this strain was used under material transfer agreement with Medical Research Council of PROMEC Unit, Tygerberg, South Africa). The strain was grown on moistened ground corn kernels (50 g ground maize kernels in 1000 ml conical flasks moistened by addition of 70 ml sterile water and then autoclaved 30 min at 121°C and 120 kPa). The moistened ground corn kernels were inoculated with 1 ml of a spore suspension (lyophilized conidia of F. verticillioides MRC 826 in 100 ml of sterile distilled water) and incubated on a rotary shaker at 25°C in the dark for 2 weeks. The medium was then freeze-dried. The FB1-rich moulded ground maize kernels will be then homogenised into chick/pig/rat ration to give the required concentration of FB1 in diet.

Aspergillus ochraceus (isolate D2306, as used by Stoev et al., 2000b, 2002b,c) supplied by our UK collaborator from Imperial College (London) was used for production of OTA as described in our previous studies (Stoev et al, 2000b, 2002b,c). The sterilised shredded wheat (40 g) in 500 ml conical flasks, moistened by a 40% (v/w) addition of sterile water and incubated on a rotary shaker at 27 °C for 2 weeks was used for growing of the same strain of A. ochraceus. The final product was sterilised at 80 °C for 1 h (yield 2 kg) and stored at –20 °C. The ochratoxin-rich moulded shredded wheat will be then homogenised into chick/pig/rat ration (diluted more than 2000-fold) to give the required concentration of OTA in diet.

-Description of work finished in work package 2:

Some indigenous herbs from India with possible activity to reduce the deleterious effects of mycotoxins were collected from different regions with the help of Dr. Rajesh Arora and his team in order to be studied for possible effects on wound granulation or for protective effects on kidneys and liver via the respective studies. Similar collection of herbs was done in S. Africa under the guidance of Prof. Krause (RU), Dr. P. Njobeh and Dr D. Ndinteh (UJ). Some of the herbs currently supplied via the SA firm Parceval (Ulrich Feiter) include:

-Centella asiatica – suggested as spindle poison for skin operation (as it stops cell division). It was suggested to have antibacterial, anti-fungal, anti-inflammatory, wound-healing, nephroprotective and antidiabetic activity, contains flavonoids, ameliorate tubulointerstitial fibrosis by reducing tubular injury. It was known by traditional healers in Kwazulu-Natal to heal wounds, cuts, grazes and burns. It contains triterpenoid saponoids that help to promote cell replication. It increases the reproduction of peripheral blood vessels and connective tissue, improves circulation and helps to retain/restore elasticity of the skin. It speeds up collagen formation and increases antioxidant levels within the wound in the early stages of tissue repair.

-Withania somnifera (leaves used for wound healing) -anti-inflammatory, immunostimulating and rejuvenating properties, improving thinking ability, anti-oxidant, known to increase sexual desire, described as "Indian ginseng, positive influence on the endocrine, cardiopulmonary, and central nervous systems, healing hypertension, hepatoprotective effect, flavonoid content, anti-tumour effect -Silybum marianum – it was suggested to have anti-oxidative effect and anti-cancerogenic, immunostimulating or nephroprotective effects, improve wound granulation and has flavonoid content.

- -Description of work finished in work package 3:
- -Antioxidant screening of active components was done. Ten different extracts and fractions from medical plants and trees have been studied for their free radical scavenging capacity against the stable free radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) by EPR (electron para-magnetic resonance) spectroscopy and for superoxide dismutase enzyme (SOD) activity by spectrophotometry (in vitro before and after 2 hrs UV irradiation).
- -Among all studied South African samples, the methanol extracts isolated from stem bark of the trees Piptadenastrium africanum and Millettia Laurentii showed the highest DPPH scavenging capacity and highest SOD like activity.
- -Further these both extracts were investigated by EPR spectroscopy for their in vivo effects on liver and kidney oxidative status of healthy mice treated by them and were compared to that effect of the leaves methanol extract isolated from Bulgarian medicinal plant Haberlea Rodopensis. Moreover, by EPR methods in vivo protective effects of the above extracts against oxidative stress induced by xenobiotics (antitumor drugs) were evaluated and compared in mice pretreated by them
- -Structure characterization /elucidation of active components/ fractions was done via UV-VIS spectrum, FT-IR spectrum, NMR spectrum, GCXGC spectrum, but the structure elucidation confirmation is still pending
- -Cytotoxicity screening of active components and effects in reducing cytotoxicity against OTA and FB1 is also currently studied it was found that the tested compounds are not toxic for human lymphocytes

Transfer of knowledge and Training activities (workshops):

Staff secondments and transfer of knowledge in work package 1:

-ER2 moved from Trakia University (Bulgaria) to University of Johannesburg (South Africa) for 3 months in order to perform the following objectives: Production and supply with mycotoxin FB1 for experimental studies in Bulgaria and Hungary, and exchange of knowledge on this subject via participation in target experimental work with target FB1-producers and via presentations

Staff secondments and transfer of knowledge in work package 2:

- -ER2 moved from Trakia University (Bulgaria) to DRDO (India) in order to perform the following objectives: Collection of some target Himalayan herbs with known protective effects on human/animal health or known to have a potent immunostimulating and/or antibacterial effects via visits of some tribal areas in higher ranges of mountains and exchange of knowledge in regard to collecting and preserving all necessary herbs (leaves, barks, seeds, fruits or roots) in different stages of their growth, etc
- -ER1 moved from Trakia University (Bulgaria) to University of Johannesburg (South Africa) in order to perform the following objectives: Collection of some target South African herbs with known protective effects on human/animal health or known to have a potent immunostimulating and/or antibacterial effects via visits of some tribal areas and exchange of knowledge in regard to collecting and preserving all necessary herbs (leaves, barks, seeds, fruits or roots) in different stages of their growth, etc.
- -ER1 from Kaposvar University (Hungary) will be moved to University of Johannesburg (South Africa) next year

Staff secondments and transfer of knowledge i work package 3:

- -ER1 will be moved from DRDO (India) to Trakia University (Bulgaria) next year
- -ESR1 from RU (South Africa) moved to Trakia University (Bulgaria) in order to perform the following objectives:
- A) Receiving some knowledge in regard to selective characterization of some SA herbs for their bioconstituents (as the levels of flavonoids, carotenoids, etc) using EPR (Electron Paramagnetic Resonance) and NIRS (Near Infrared Reflectance Spectroscopy)
- B) Exchange of knowledge between Bulgarian and South African ways of selective characterization of herbs and the respective ways of preparing various herbal extracts or fractions via mutual presentations & workshops

Work of master students involved in our IRSES project:

- 1) Masters student project by Fonkui Youmbi T, "Mitigating the occurrence of mycotoxins and their effects thereof using nano-enabled binders", Supervisor Dr Patrick Njobeh and Co-supervisor Prof R. Krause (all of them participated in our IRSES project)
- 2) Masters student in the new MSC (Nanoscience Programme), Kulani J, "Chitosan nano-particles functionalized with protective plant extracts for the inhibition of aflatoxin B1 and Ochratoxin A activity", Supervisor Dr Patrick Njobeh and Co-supervisor Prof. A. Mishra (all involved in our IRSES project).
- 3) Masters student project by Dlamini ML, "The application of some target formulations of active herbal plant components in reducing animal exposure to mycotoxins and their possible effects", Supervisor Dr Patrick Njobeh and Co-supervisor Prof R. Krause (all of them participated in our IRSES project).

All these projects are currently active and there will be additional publications in due course. All mentioned above students and supervisors travelled to Bulgaria for some training.

Projects of students from Rhodes University partly connected with our IRSES project

- 1) Synthesis of iron nanoparticles coated with plant exudates for mycotoxin concentration and extraction
- 2) Coated nanoparticles for mycotoxin destruction in food and feed
- 3) Coated Nanoparticles for pre-concentration of secondary metabolites from marine bacteria and fungi

These new projects are still in their infancy, two are developing at the postdoc level and the other as an Honours project (4th-year BSc)

Dissemination of results (conferences, publications...):

THE PRINCIPAL PUBLICATIONS IN AUTHORITATIVE PEER-REVIEWED INTERNATIONAL JOURNALS:

- 1) Stoev, S. D., S. A. Denev, Porcine/Chicken or Human Nephropathy as the Result of Joint Mycotoxins Interaction, Special issue "Recent Advances in Ochratoxins Research", Toxins, 2013, 5 (9), 1503-1530, doi:10.3390/toxins5091503 (http://www.mdpi.com/2072-6651/5/9/1503). IF=2,12
- 2) Pósa, R., T. Magyar, S. D. Stoev, R. Glávits, T. Donkó, I. Repa, and M. Kovács, Use of Computed Tomography and Histopathologic Review for Lung Lesions Produced by the Interaction Between Mycoplasma hyopneumoniae and Fumonisin Mycotoxins in Pigs, Veterinary Pathology, 2013, doi:10.1177/0300985813480510. IF=1.95
- 3) Stoev, S. D. Food safety and increasing hazard of mycotoxin occurrence in foods and feeds, Critical Reviews in Food Science and Nutrition, 2013, 53 (9), 887-901 (DOI:10.1080/10408398.2011.571800)

- (http://www.tandfonline.com/doi/abs/10.1080/10408398.2011.571800). IF=5.78
- 4) Abia WA, Warth B, Sulyok M, Krska R, Tchana AN, Njobeh PB, Dutton MF, Moundipa, PF. Determination of multi-mycotoxin occurrence in cereals, nuts and their products in Cameroon by liquid chromatography tandem mass spectrometry (LC-MS/MS) Food Control, 2013, 41, 438-453. IF=3.01
- 5) Horvatovich K, Hafner D, Bodnár Z, Berta G, Hancz C, Dutton MF, Kovács M. Dose-related genotoxic effect of T-2 toxin measured by comet assay using healthy pigs' peripheral blood mononuclear cells. Acta Veterinaria Hungarica, 2013, 61, 175-186. IF=0.67
- 6) Ndluvo C, Dutton MF. Survey of South African Maize Silage for Fungi and Mycotoxins. African Journal of Agriculture, 2013, 8(32), 4299-4397.
- 7) Abia WA, Warth B, Sulyok M, Krska R, Tchana AN, Njobeh P, Turner P, Kouanfack C, Eyongetah, M, Dutton M.F., Moundipa, PF. Bio-monitoring of mycotoxin exposure in Cameroon using a urinary multi-biomarker approach. Food and Chemical Toxicology, 2013 62, 231-237. IF=3.01
- 8) Egbuta, MA, Chilaka, CA,, Phoku, J.Z., Mwanza M, Dutton, M.F. Co-contamination of Nigerian Cocoa and Cocoa-Based Powder Beverages Destined for Human Consumption by Mycotoxins. Journal of Ethno-Medicine, 2014, 7, 187-194.
- 9) Dutton, MF, Anderson, G., Reiter, E.V., Razzazi-Fazeli, E., Mwanza, M. The analysis of a feed component imported into South Africa for aflatoxin in relation to fungal and mycotoxin contamination under the auspices of the EU Frame work 6, Biotracer programme. Agricultural Science. 2014 (in press)
- 10) Grigorov B., Y. Karamalakova, G. Nikolova, B. Popov, D.T. Ndinteh, V. Gadjeva and A. Zheleva, Comparative study on extracts isolated from Piptadenastrium africanum and Haberlea rhodopensis by Electron Paramagnetic Resonance spectroscopy (in press)

Monographs, chapters in books

1) Agarwal, P., R, Arora, R. Chawla, D. Gupta, A. Zheleva, V. Gadjeva, S. Stoev, Mycotoxins: Novel Approaches for Biological Threat Mitigation, In: Toxigological Problems, Chapter 60, Christophor Dishovsky, Julia Radenkova (Eds), Military Publishing House Ltd, Bulgarian Toxicological Society, Sofia, Bulgaria, ISBN 978-954-509-509-2, 2014, pp. 433-444

Presentations of our IRSES project and participation in Workshops and Seminars:

- 1) 03 April 2013 (Wednesday) Rajesh Arora, Presentation of the Marie Curie IRSES (International Research Staff Exchange Scheme) project 316067 "HERBAL PROTECTION" and experience with the IRSES scheme at the EURAXESS Share Workshop being organized in Delhi, India 2) 09 May 2013 (Thursday) Scientific seminar "Community of science and success" took place in hotel Meridian Palace of Stara Zagora, Bulgaria Stoycho Stoev, Presentation of the Marie Curie IRSES (International Research Staff Exchange Scheme) project 316067 "HERBAL PROTECTION": Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation (2013-2016)
- 3) Stoev, S. D., Mycotoxic nephropathy in Bulgarian and South African pigs: complex etiology and similarity with Balkan Endemic Nephropathy, Mycotoxins Workshop 2013 "Mycotoxins research in food: challenges and perspectives", 7-8 October 2013, Department of Animal Health, North West University, Mafikeng Campus, Mafikeng, South Africa
- 4) Stoev, S. D., The specific multi-mycotoxic nature of some foodborne mycotoxicoses and the hazard for animals or humans, Mycotoxins Workshop 2013 "Mycotoxins research in food: challenges and perspectives", 7-8 October 2013, Department of Animal Health, North West University, Mafikeng Campus, Mafikeng, South Africa
- 5) Dutton, M.F., Mycotoxins research in Africa: challenges mycotoxin analysis: sampling, modern

rapid test methods, data validations, Mycotoxins Workshop 2013 "Mycotoxins research in food: challenges and perspectives", 7-8 October 2013, Department of Animal Health, North West University, Mafikeng Campus, Mafikeng, South Africa

6) Dutton, M.F., Mycotoxins in Africa: perspectives, publications in peer reviewed mycotoxins journals, Mycotoxins Workshop 2013 "Mycotoxins research in food: challenges and perspectives", 7-8 October 2013, Department of Animal Health, North West University, Mafikeng Campus, Mafikeng, South Africa

3. PROJECT MANAGEMENT

Overview of the activities carried out by the partnership; Identification of problems encountered and corrective action taken:

- 1) 03 April 2013 (Wednesday) A presentation on our project and experience with the IRES scheme at the EURAXESS Share Workshop being organized in Delhi, India.
- 2) 09 May 2013 (Thursday) Scientific seminar "Community of science and success" took place in hotel Meridian Palace of Stara Zagora, Bulgaria Presentation of the Marie Curie IRSES (International Research Staff Exchange Scheme) project 316067 "HERBAL PROTECTION": Studies on some herbal additives giving partial protection against toxic or immunosuppressive effects of some mycotoxins and improving wound granulation (2013-2016)
- 3) 19.07.2013 Meeting with the Dean of Faculty of Science in University of Johannesburg Prof. Bhekie Mamba at Auckland Park Kingsway Campus on the advance of our Marie Curie IRSES project.
- 4) 26.07.2013 Visiting a Zulu Open Herbs Market in Johannesburg for collecting more information from the source about the healing or protective effects of some local herbs used by traditional healers.
- 5) 29.07.2013 Meeting with the Vice Chancellor and Principal of UJ and Chairperson of Higher Education South Africa Prof. Ihron Rensburg as well as with the Dean of Faculty of Science in UJ Prof. Bhekie Mamba, the Dean of Faculty of Health Science in UJ Prof. Andre Swart and the Executive Director Internationalisation at Auckland Park Kingsway Campus on the advance of our Marie Curie IRSES project.
- 6) 30.07.2013 Meeting with the Registrar of UJ at Auckland Park Kingsway Campus on the needs of our Marie Curie IRSES project.
- 7) 04.08.2013-03.09.2013 Visit to Rhodes University and Meeting with the Head of Department of Chemistry of RU in Grahamstown on the possibility to visit some rural areas in order to collect some information about the medicinal plants and the way of their use by traditional healer in the tribes in the rural areas.
- 8) 08.08.2013 Meeting with the Dean of Faculty of Science of Rhodes University Professor Ric Bernard on the advance of our Marie Curie IRSES project.
- 9) 14.08.2013 Meeting with the Deputy Vice Chancellor on Research and Development of Rhodes University Dr Peter Clayton on the advance of our Marie Curie IRSES project and the measure to be taken to facilitate the successful performance of the project tasks.
- 10) 15.08.2013 Introducing and specialization in Nuclear Magnetic Resonance (NMR) technics using the help and guidance of Dr Xavier
- 11) 23.08.2013 Visit to Nelson Mandela Metropolitan University (NMMU) in Port Elizabeth and meeting with the Head of Chemistry and the Head of Biochemistry Departments. Meeting with the Chief of CSIR (Council of Science and Industrial Research) Biosciences in Pretoria Dr Rajesh Lalloo and the Chief Researchers of CSIR Materials Science in Port Elizabeth Rajesh Alandjiwala on the possible ways to collect some herbs and to introduce herbs products in the market in South Africa.
- 12) 11.09.2013 Meeting with the Chief of Scientist-Bioprocess of CSIR (Council of Science and Industrial Research) Biosciences in Pretoria Dr Rajesh Lalloo and Chief Researcher and Research Group Leader of CSIR Materials Science and Manifacturing Dr Rajesh Anandjiwala and the Commercialisation Manager of CSIR Biosciences Dr Stephanus Francois Maraisthe and the Technology Manager of CSIR Biosciences Dr Vinesh Maharaj in regard to the measure to be taken to facilitate the successful performance of the project tasks and the possibility for introducing new medicinal plant extracts in the trade market.
- 13) 7-9 October 2013 Mycotoxin Workshop in Department of Animal Health in Mafikeng Campus

of North West University

- -7 October 9:20-10:20AM Prof. M. F. Dutton presentation: Mycotoxins research in Africa: challenges (Mycotoxin analysis: sampling, modern rapid test methods, data validations
- -8 October 09:00-10:30 AM Session 1: Chair: Professor D. Stoev
- -9:00-9:50AM Prof. M. F. Dutton presentation: Mycotoxins in Africa: perspectives, publications in peer reviewed mycotoxins Journals
- -11:00-11:50AM- Prof. Stoev presentation: The specific multi-mycotoxic nature of some foodborne mycotoxicoses and the hazard for animals or humans.
- 14) 9 October 2013 Meeting with Vice rector in North-West University Prof Mashudu Davhana-Maselesele on the current progress of our project.
- 15) 16.10.2013 Meeting with Prof. Ben-Eric van Wyk (Chairman of the Aloe Council in SA, Chairman of the Indigenous Plant Use Forum in SA, Member of the Association for African Medicinal Plant Standards AAMPS, Member of the Presidential Task Team on African Traditional Medicine) from Dept of Botany, Fac of Science UJ about the top 10 herbs, which would be useful for our study and experiments
- 16) 12.12.2013 Meeting of Prof Stoycho Stoev of Trakia University and Dr Patrick Njobeh of University of Johannesburg with Prof. J. van Staden, Director of Research Centre for Plant Growth and Development, School of Life Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa about the top 10 herbs, which would be useful for study and experiments under Marie Curie International Research Staff Exchange Project of European Union PIRSES-GA-2012-316067.

 17) 13.12.2013 Meeting of Prof Stoycho Stoev of Trakia University and Dr Patrick Njobeh of University of Johannesburg with Prof. JN (Kobus) Eloff, Leader Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, South Africa about the top 10 herbs, which would be useful for study and experiments under Marie Curie International Research Staff Exchange Project of European Union PIRSES-GA-2012-316067.

Drawbacks and Problems we Faced:

We faced some problems with Bulgarian Embassy giving to some SA researchers only 1 month visa instead 2 or 3 months visa without giving us any reasonable explanation for that – I already informed REA and the Head of Marie Curie unit Alessandra Luchetti about this problem.

The both beneficiaries and the three partners of the project don't have enough money available to cover all planned visits in the work-packages (researchers/months), because only 60% of the planned money was sent to us by REA

Most of my colleagues, participated in our IRSES project have possibilities to realize only 1 month or shorter visits to our partners or beneficiaries – unfortunately IRSES project is not suitable for short time visits and the money available for 1 month can only cover the ticket price. Therefore, my proposal is to suggest some amendments in the financial rules of IRSES projects – such as some additional money for ticket to be ensured for short-time visits.

Another problem we faced is the customs taxes and duties which we had to pay for the herbs received from South Africa for our experiments in Bulgaria and Hungary (more than 400 Euro were paid by our Hungarian colleagues for customs duties and taxes, etc).

We also have a PROBLEM with the Regional Service of Phytosanitary Control and the Customs Service in Sofia, where our imported herbs from S. Africa via DHL were retained and – consignment 6078281145 – and we were asked to make a registration and to pay some taxes as importer of herbs,

etc.

NOW ALL MENTIONED ABOVE PROBLEMS WERE SUCCESSFULLY RESOLVED. WE ONLY ARE GOING TO POSTPONE SOME OF THE SECONDMENTS FOR THE NEXT REPORTING PERIOD IN ORDER TO ADJUST THEM TO THE AVAILABLE MONEY SENT BY REA AS WAS MENTIONED ABOVE (only 60% of the necessary money were sent to us)

4. ADDITIONAL INFORMATION

Additional information, which may be considered useful to assess the work done during the reporting period:

Attachments

Date:

Person in charge of the project for the beneficiary(ies):