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"Environmentally friendly biomolecules from agricultural wastes as substitutes of pesticides for plants diseases control"

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EVERGREEN has a high Community added value, as a result of its high environmental and transnational character, thanks to the extensive use of advanced technology, which assists in overcoming the geographical and cultural barriers preventing technical progress in the agricultural sector.

The Water Framework Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishes the legal framework for an innovative and unitary approach in water management across Europe, to protect and to restore clean water and to guarantee its sustainable use in a long-time period. It also sets specific deadlines for EU countries to adopt measures to protect aquatic ecosystems, together with several economic approaches for water management and services.

In the Annexes of WFD and of Directive 2008/105/EC several priority hazardous substances whose presence in water has to be excluded or reduced have been reported, together with other pollutants such as selected existing chemicals, plant protection products, biocides, metals and other groups. In fact, concerning the control of emissions of chemicals into water, the WFD is also supported by other EU environmental legislation. In this frame environmental standards for copper and other metal contaminants in water were set out in WFD and other several EU Directives, to reduce or eliminate the risks posed by metals to the aquatic environment.

This EVERGREEN project is one of the most obvious examples of the tight and important link existing between WFD and the EU Thematic Strategy for Soil Protection, aimed to several actions concerning soil, such as to prevent its degradation, to preserve its ecological and social functions, and also to remediate polluted and degraded soils. In fact, although the framework Directive of EU Thematic Strategy for Soil Protection defines as "soil" everything that is existing between the earth surface and bedrock, it is obvious that the reductions of soil contamination by several pollutants such as copper, that can be easily runoff into water from soil, would at the end result in the protection of aquatic ecosystems, as asked by WFD.

EVERGREEN perfectly responds to the objectives of both the Water Framework Directive 2000/60/EC and of The EU Thematic Strategy for Soil Protection, offering an innovative strategy to control plant diseases caused by Gram negative phytopathogenic bacteria that also meets EU standards in terms of environmental protection of water and soil ecosystems. The use of these antifauchtive substances are a reliable and efficient alternative to the application of chemicals as bactericides against plant pathogenic bacteria, that will contribute to the reduction of chemicals contamination into agricultural soils and water.
During all the project period, Soil, plant and agricultural experts, policy makers and business people attended the two workshops. EXPO Tuscany stand, Milan, 25 June 2015

Conference Psyringae 2015, Málaga, 2
AOCS Annual Meeting, Orlando (USA), May 2
CORESTA Congress, October 12
LIFE event of ENEA and ASTRA "Dal confronto di esperienze diverse lo sviluppo di tecniche sostenibili", Faenza, "TOSCANA & AMBIENTE La sfida europea dei progetti LIFE" UNIFI LIFE event at Firenze on 24

Networking contacts at the LIFE event of ENEA and ASTRA in Faenza, on 22 January 2015
Networking contacts at the UNIFI LIFE event at Firenze on 24
Horizon 2020 “From Biodiversity to Chemodiversity: Novel Plant Produced Compounds with Agrochemical and Cosmetic interest (AGROCOS)” KBBE
Networking contacts at the UNIFI LIFE event at Firenze on 24th October 2014
Networking contacts at the LIFE event of ENEA and ASTRA in Faenza, on 22 January 2015

International Conferences and Fairs
All the project beneficiaries organized and presented EVERGREEN in the following events: “TOSCANA & AMBIENTE La sfida europea dei progetti LIFE” UNIFI LIFE event at Firenze on 24th October 2014
LIFE event of ENEA and ASTRA “Dal confronto di esperienze diverse lo sviluppo di tecniche sostenibili”, Faenza, 22/01/2015
CORESTA Congress, October 12-16/10/2014, Quebec City (Canada)
AOCs Annual Meeting, Orlando (USA), May 2-6/05/2015,
Conference Pyreinagea 2015, Málaga, 2-5 June 2015
EXPO Tuscany stand, Milan, 25 June 2015-06-26

Consequently, an increase in soil fertility will be also be obtained, as well as an important reduction in the percentage of chemicals resistant bacteria in the soil microflora, that are also a dangerous reservoir of antibiotic-resistant bacteria for humans and animals.

In the EVERGREEN project we demonstrated on a pilot field scale the effectiveness and the potential benefits for the environment of the use of these anti-virulence peptides, in the respect the principles of the Directive 91/414/CEE, concerning research field trials with novel and unregistered products and in compliance with good experimental practice.

**ENVIROMENTAL PROBLEM**

The goal of the project was the demonstration of an environmentally friendly, sustainable and integrated strategy for the control of bacterial and nematode diseases of plants, in the view of an economically viable and environmental respectful reduction or replacement of the traditional pesticides used in conventional and organic agriculture.

The EVERGREEN approach perfectly matches the restrictions established within the EU Member States concerning the use and the placing on the market of plant protection products and the main aim of the landmark package very recently proposed by the European Commission to modernise, simplify and strengthen the agri-food chain in Europe, which is definitely oriented towards an holistic vision of a sustainable and more competitive agriculture.

Furthermore EVERGREEN project was also aim at demonstrating how several simple and inexpensive extraction processes can allow a cost-effective exploitation of plant biomass and non-edible portion of crops. This allows greater short and long term environmental benefits in comparison with other current approaches concerning the management of these wastes. Due to their natural origin, these extracts can be properly used without any damaging side effects for humans, animals, plants and any ecosystem. More importantly, in addition to the phenolic and polyphenolic compounds, these extracts contain other components whose presence have been analytically tested and standardised and which reinforce their biological activity against bacteria and nematodes of plants, if compared with the results obtained when using the same single purified chemical molecules.
EVERGREEN aimed at innovating the approach towards plant disease control, reducing conventional chemical pesticides with environmentally friendly and highly bioactive molecules recovered from agricultural vegetable wastes. The project had a double goal to simultaneously improve the safety profile of plant disease control products for environmental compatibility and to support the sustainability of plant production to pursue cost-effective strategies.

EVERGREEN demonstrated in vitro and in vivo efficacy and reliability of the polyphenolic-based biomolecules extracted from agricultural non-food biomasses and wastes as disease control products against phytopathogenic bacteria and nematodes, for replacing current commercial pesticides and application of copper salts in conventional and organic agriculture. Optimised field treatments were carried out on several plants and crops having a high commercial value and here used as a model (Olive, Kiwi, Potato, Tobacco).

All the project beneficiaries made sure that the EVERGREEN project gained maximum visibility at a European level. During all the project life various dissemination materials were produced and distributed in fairs, conferences, workshops and so on. A logo was designed together with brochures, gadgets and various other items which allowed for a full dissemination of the project aim and results. 29 articles were also edited on newspapers, a project website and a Facebook page were created and published online within the second month of the project.

EVERGREEN has been presented in different events, in a specific web site, in notice boards, in gadgets and in brochures. In particular the EVERGREEN dissemination has been performed during all the project life detailing the type of EVERGREEN innovative technology and product.

All the beneficiaries were involved in the development and implementation of the following main EVERGREEN dissemination activities from the start of the project (01/10/2014) until the end of the project (30/09/2016):

- 12 EVERGREEN posters
- 10,000 EVERGREEN general brochures
- 200 EVERGREEN workshop brochures
- 3,000 EVERGREEN general plastic flyers
- 1,500 EVERGREEN pendrives as project gadget (two types)
- 1,000 EVERGREEN notebooks as project gadget
- 500 EVERGREEN cups as project gadget
- 100 EVERGREEN pens as project gadget
- 3 project presentations (In English, Italian and Spanish)
- 1,000 copies of Layman’s report to general public (In English, Italian and Spanish)

AFTER-LIFE COMMUNICATION PLAN

1,000 copies of EVERGREEN video in English, Italian and Spanish sent and distributed after the project end to soil, plant and agricultural managers and technicians.
EVERGREEN perfectly meets the EU vision about sustainable agriculture, with the use of renewable resources, such as vegetable not edible biomass and waste, to obtain environmentally friendly substitutes for traditional pesticides against plant pathogenic bacteria and nematodes, avoiding environmental pollution and lowering the costs deriving from both the use of conventional pesticides and the disposal of these wastes.

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The use of the EVERGREEN antiinfective substances will be a reliable and efficient alternative to the application of chemicals as bactericides against plant pathogenic bacteria, that will contribute to the reduction of chemicals contamination into agricultural soils and water. Consequently, an increase in soil fertility will be also be obtained, as well as an important reduction in the percentage of chemicals resistant bacteria in the soil microflora, that are also a dangerous reservoir of antibiotic-resistant bacteria for humans and animals.

**LONG TERM BENEFITS**

**TECHNICAL SOLUTION**

EVERGREEN consisted on actions aiming to:

- demonstrate the negative environmental impact of the use of conventional chemical pesticides for the control of bacterial and nematode diseases of plants
- demonstrate the performances as plant disease control products of the polyphenolic-based molecules extracted from agricultural vegetable wastes, at laboratory, pilot and field scale
- demonstrate the efficiency of tailored formulations of these polyphenolic-based molecules as plant disease control products, to achieve the highest activity on the different plant pathogens
- demonstrate the reliability and the compliance with REACH document of EU legislation of the extraction processes for these polyphenolic-based molecules, from the lab-scale to the kilo-lab scale
- demonstrate the project technical validity for optimizing the up-scaling of treatments with these polyphenolic-based molecules recovered from agricultural vegetable wastes, from laboratory to pilot scale and to semi-industrial scale
- demonstrate the more efficient and ecotoxicologically compatible profile of these bioactive molecules than conventional pesticides, at laboratory, pilot and field scale
- demonstrate the absence of any side unexpected effect of the bioactive polyphenolic-based molecules on common molecular targets of living organisms, at laboratory, pilot and semi-industrial scale
- demonstrate the absence of any direct or cross-acting selection operated by the bioactive polyphenolic-based molecules towards the emergence of unwanted resistance phenomena in their respect or for conventional pesticides, copper or even antibiotics, at laboratory, pilot and semi-industrial scale
- demonstrate the short term environmental benefits and the economical advantages from the use of polyphenolic-based molecules recovered from agricultural vegetable wastes in the control of biotic plant diseases
The project had the following technical results:

- **Long term reduction (80-100%)** of pollution in agricultural soils given by conventional pesticides used against plant pathogenic bacteria and nematodes, following their replacement with the project polyphenolic-based bioactive molecules recovered from agricultural non-food vegetable biomasses and wastes;

- Increased performances in the control of the bacterial and nematode diseases of plants (55%) for the wider temporal application of the project polyphenolic-based bioactive molecules in comparison with conventional pesticides, even in period of plant life cycle which are critical for infections but during which traditional chemical plant protection treatments are not allowed;

- **Reduction (80-100%)** of point-source and diffuse pollution from the disposal of agricultural vegetable wastes in the environment;

- **Reduction (45%)** of costs for disposal of agricultural non-food vegetable biomasses and wastes;

- **Reduction (20%)** of energy consumption used for remediation processes of pesticides-contaminated soils;

- **Improved soil fertility (70%)** following the reduction/replacing of conventional agrochemicals products for plant diseases control with the project polyphenolic-based bioactive molecules;

- **Increased soil microbial diversity (65%)** with positive impact on soil biology and on the transformation and dynamic of nutrients;

- **Increased (45%)** natural competence in agrosystems for suppression against plant pathogenic bacteria and fungi;

- **Long term reduction (90%)** of pesticides pollution in watercourses;

- **Reduction (80%)** of toxicological impact of pesticides pollution on terrestrial, aerial and aquatic fauna;

- **Short term reduction (85%)** of pesticide- and drug-resistant bacteria and nematodes in agricultural sites;

- **Long term reduction (60%)** of the reservoir of environmental antibiotic-resistant bacteria and of their spread with reduction of the risks for the health of humans and animals;

- **Reduction (40%)** of pesticides residues on fruit and vegetable for human and animal consumption;

- **Increased quality (40%)** of food and feed from vegetable origin with benefits on human and animal health.