EVERGREEN

EU project LIFE13 ENV IT 000461 / "EVERGREEN"

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

ACTIVITIES OF CONSORTIUM INSTM
Nov. 1, 2015 – Apr. 30, 2016

Monitoring Visit and 18th month Meeting May 13, 2016

- Sergio Miele, Enrica Bargiacchi –

ACTIVITY FOR THE PROJECT Month 12-18

- **B3**-*Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale*

INSTM is preparing a document to be included in B3 delivery

concerning the legal/normative status of the polyphenols formulations under test, with reference to the Italian, and the European laws

Presently, some formulations (liquid, gel) are strictly experimental because of their concentrations and components.

The present Italian Law on Fertilizers (DLgs. 2010/75 and successive modifications) permits the use of liquid Chestnut tannin products only if they have at minimum 13% w/w of tannins, and doesn't allow the use of mixed polyphenols sources.

There is a further drawback to overcome in case of successful results: the double steps of the authorization process, both at domestic and European levels. This might require a lag of > 18-24 months to labeled field use.

ACTIVITY FOR THE PROJECT Month 12-18

- **B3**

But where shall we label EVERGREEN products?

"The purpose of Regulation (EC) No 66/2010 (hereafter referred to as 'the Ecolabel Regulation') is to provide a voluntary EU award scheme to help consumers identify products and services that have a reduced environmental impact throughout their life cycle, from the extraction of raw material through to production, use and disposal"


EUROPEAN COMMISSION

Brussels, 17.3.2016
COM(2016) 151 final

REPORT FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL

on the sustainable use of biocides pursuant to Article 18 of Regulation (EU) No 528/2012 of the European Parliament and of the Council concerning the making available on the market and use of biocidal products

(Text with EEA relevance)

*"The study however showed that **biocidal products are not perceived as suitable or eligible for the scheme**, because of their inherent properties and of their very purpose of controlling unwanted organisms"*

"Finally, the EU Ecolabel promotes available alternatives to biocidal products"

ACTIVITY FOR THE PROJECT Month 12-18

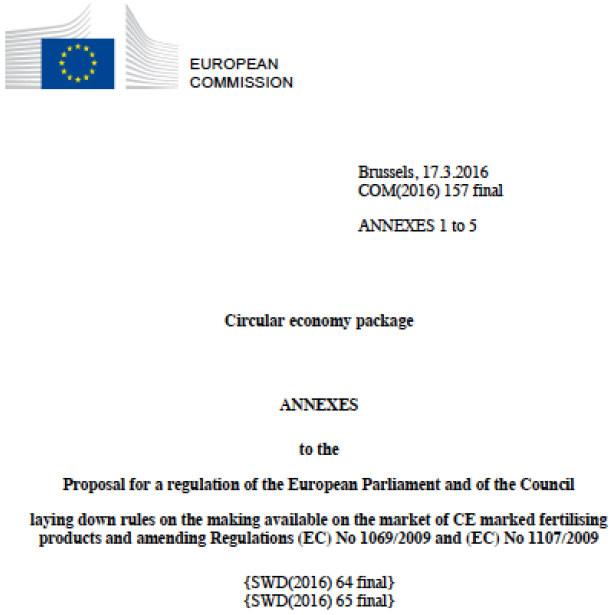
- **B3**-

What are the alternatives?

The problem of the European Law: it is still under discussion an innovative proposal for BIOSTIMULANTS in the circular economy package

(March 17, 2016)

Probably EVERGREEN formulations will find more efficient allocation **within the BIOSTIMULANT FRAMEWORK**



EUROPEAN COMMISSION

Brussels, 17.3.2016
COM(2016) 157 final

ANNEXES 1 to 5

Circular economy package

ANNEXES

to the

Proposal for a regulation of the European Parliament and of the Council

laying down rules on the making available on the market of CE marked fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009

{SWD(2016) 64 final}
{SWD(2016) 65 final}

B4 - Demonstration of the biological activity of the high quality polyphenolic extracts recovered from not edible biomass and waste, against plant pathogenic bacteria and nematode, in *planta*

- **Tobacco Virginia Bright, warm* greenhouse pot test, growing period: Oct.21st – Dec. 18th 2015**
- Treatments
  - Non-infested control
  - Infested control
  - Etoprofos 2.5 g/plant
  - Chestnut Hydrolysable Tannins (CHT) 2.5 g/plant
  - CHT+Grape Marc Meal (50%+50%) 1.25+1.25 g/plant

* Mean temperatures in the range 20-16°C, with lows 13-15°C

B4 –Tobacco Pot Experiment (Oct. 21st – Dec. 18th 2015)

- Characteristics of Grape Marc Meal (source: Sangiovese, Chianti area)
  - Pretreatments: oven drying at 38°C, seed separation, milling, grinding at <200mesh, homogenization
  - Analysis (% w/w @14.2% water content, except otherwise indicated)

Humidity	14.2%	Oven dry, constant weight
Ashes @550°C	3.86%	Mittem
Crude protein	0.37%	Kjeldahl
Crude fiber	27.2%	Weende
Crude fats	4.5%	Soxhlet
Carbohydrates	10.2%	Fehling
Total Phenolic Content (mg GAE/g DM)	20.9 mg/g DM	0.1% HCl / 70% acetone / 29.9% water using an ultrasonic unit

B4 –Tobacco Oct. 21st – Dec. 18th 2015 - Results

Treatm.No.	2015						
	Nematode count No/200 cm^3	TMDr (g/cm^3)	Fine roots (%)	Barker grading[a]		DM epigeal yield (g/pot)	
	40 DAT	40 DAT	40 DAT	20 DAT	40 DAT	20 DAT	40 DAT
Non infested control	NI	0.34 ab	70.6 ab	NI	NI	11.7 a	15.4 b
Infested control	916 a	0.12 d	40.8 c	3.6 a	4.1 a	4.6 c	5.7 c
Etoprofos	132 c	0.20 c	64.2 b	0.1 b	1.2 b	9.9 b	14.2 b
CHT	573 b	0.30 b	77.3 a	0.2 b	0.6 c	10.4 ab	16.7 ab
CHT+GM	428 b	0.38 a	75.6 a	0.2 b	0.8 c	10.8 ab	17.3 a

TMDr = Tissue Mass Density of roots (g fresh matter/cm^3 soil)

[a] 0=0-10%; 1=11-20%; 2=21-50%; 3=51-80%; 4=81-90%; 5=91-100% (Barker, 1985)

Means within a column followed by the same letters are not significantly different (P = 0.05).

B4 –Tobacco Oct. 21st – Dec. 18th 2015 - Results

- At the end of the experiment, tobacco growth was favorably affected by the use of CHT+GM, and CHT, with significative difference between the former treatment and the non infested control (biostimulant effect?)
- Etoprofos had a depressive effect on plant growth at the first sampling date vs. the non infested control for Tissue Mass Density of Roots and Epigeal DM yield

<u>Comments</u>

- Nematode count in the soil is only partially related to plant growth performance
- Tissue Mass Density of Roots and % of Fine roots are indicative of how plant ranks for epigeal growth, but these parameters cannot be used in conditions different from experimental pot test (time-consuming task, requiring well-trained technicians)
- Barker grading is indicative, especially when used at later stages.

B4 - Lettuce test under warm greenhouse to test extracts for their toxicity and biostimulant activity. For experiments with max. 3, 2-replicated, treatments

B7 - Demonstration of the in vivo performances of the high quality poly-phenolic bioactive preparations, recovered from vegetable not edible biomass and waste, at pilot scale level in "field" screenings

- January 2016 – May 2016 – Experiments in **hydroponics** (Float system nursery) and on soil under warm greenhouse on ***Lactuca sativa*** (Winter salad, mixed varieties), **Capsicum annuum** (Bell Pepper) and **Tobacco** transplant seedlings *in Float System nursery* using polyphenols as a complement/alternative to the ordinary protective treatments
- Formulations under test (received from UnivFI in Jan. 2016):

FORMULATION	pH	EC µS/cm
1	4,48	3229
2	4,47	3146

B7 - *Lactuca sativa* and Tobacco transplant seedling production in hydroponics (Float system nursery) & on soil under warm greenhouse

- Formulations under test (received from UnivFI in Jan. 2016):
  - **FORM. 1 (liquid)**: **TC** 2%, **O** 1% in water
  - **FORM. 2 (liquid)**: **TC** 1.5%; **O** 1%; **V** 0.3% in water
- Ingredients:
  - **TC**: Sweet Chestnut aqueous extract, liquid fraction from nanofiltration by membrane technology, 12% w/w hydrolyzable tannins
  - **O**: liquid fraction of *O. europea* leaves with a polyphenolic content of 3.22% w/v (1.13% hydroxytyrosol)
  - **V**: commercial dry grape seeds extract 82% w/w condensed tannins
- Further formulations under test (INSTM)
  - Mixture of Dry Chestnut Tannin Extract 75% w/w (dispersible) 95% + commercial dry oenologic Tannin 90% w/w gallic tannins (dispersible) 5% (courtesy Banfi)

B7 - *Lactuca sativa* (salad) and Tobacco transplant seedling production in hydroponics (Float system nursery) & on soil (warm greenhouse) : **tests in progress since the beginning of February 2016)**

B7 - *Lactuca sativa* and Tobacco transplant seedling production in hydroponics (Float system nursery) & on soil under warm greenhouse

- **Test 1, lettuce: concentrations in the water of the Float system nursery**
  - 2 products x 3 concentrations x 3 replications
  - 0 - 100 – 150 mL/100 L water
  - NO RESULTS
- **Test 2, lettuce: concentrations in the water of the Float system nursery**
  - Form. 1 x 3 concentrations x 3 replications
  - 0 – 200 – 400 -600 mL/100 L water

Treatments	Aerial part (g FW)			Concentrations of major elements (mg/100 FW)				
	20 DAT	40 DAT	63 DAT	Na	K	Ca	Mg	Fe
Control	13.2	174.7	482.5	27	195	35	14	0.9
200 ml/100 L	15.8	168.9	467.7	28	188	33	13	0.9
400 ml/100 L	15.5	171.1	473.4	28	193	35	12	0.8
600 ml/100 L	14.2	175.5	471.7	31	192	38	15	0.8
No significative differences								

B7 - *Lactuca sativa* (salad) and Tobacco transplant seedling production in hydroponics (Float system nursery) & on soil (warm greenhouse)

Preliminary test: Foliar sprays to prevent/control Oidium

(2 products x 1 concentration x 3 replications) 50 mL / 10 Liter water, weekly

RESULTS

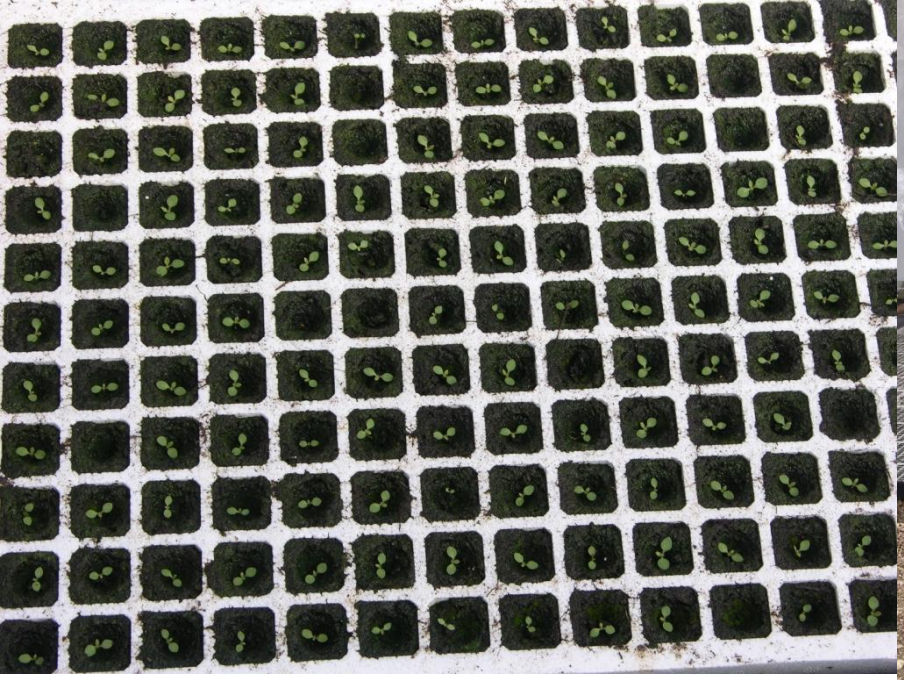
There was an apparent result soon after the treatment, but no lasting effect → **Specific tests should be repeated**

GENERAL COMMENTS:

Pre-formulated liquid products manifested problems of uneven distribution in the Float System, APPARENT INSTABILITY (change of color and density) and narrow interval between NO-EFFECT and TOXIC-EFFECT RATE.

Formulation and Storage conditions should be better tuned, otherwise it is necessary to switch to different formulations

TEST 2 – Control (left) and 600 mL/100 L treatment (right) → no differences



Activity on tobacco: aiming at reduction of Float System agrochemicals to aim at the target of ORGANIC TOBACCO

ORGANIC
TOBACCO
EXPERIMENT
2016
AT FATTORIA
AUTONOMA
TABACCHI: 3.5 ha

ACTIVITY FOR THE PROJECT Month 12-18

- **C4** – Monitoring of the short term environmental benefits from the use of the high quality standardizes polyphenolic preparations in plant disease control at pilot scale level in field screenings: **Field soil sampling for residues and nematodes (in progress)**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Action		2014				2015				2016			
Action numbe	Name of the action	I	II	III	IV	I	II	III	IV	I	II	III	IV
A. Preparatory actions (if needed)													
B. Implementation actions (obligatory)													
B.1	Demonstration of the performances of traditional pesticides for the control of bacterial and nematode diseases of plants important for the EU				■	■							
B.2	Demonstration of the qualitative and quantitative yields of extraction process for the recovery of high quality polyphenolic molecules from not edible vegetable biomass and waste at laboratory scale					■	■	■	■				
B.3	Demonstration of the biological and of the chemical stability of the crude polyphenolic extracts and of their fractions, recovered from not edible vegetable biomass and waste, at laboratory scale					■	■	■	■				
B.4	Demonstration of the biological activity of the high quality polyphenolic extracts recovered from not edible biomass and waste, against plant pathogenic bacteria and nematode, in planta						■	■	■				
B.5	Demonstration of Kilo-scale extraction of the high quality poly-phenolic bioactive molecules recovered from vegetable not edible biomass and waste							■	■	■			
B.6	Demonstration of the null toxicity profile of the high quality poly-phenolic bioactive molecules recovered from vegetable not edible biomass and waste, on model organisms andmicroorganisms.						■	■	■	■			
B.7	Demonstration of the in vivo performances of the high quality poly-phenolic bioactive preparations, recovered from vegetable not edible biomass and waste at pilot scale level in fieldscreenings							■	■	■	■	■	
C. Monitoring of the impact of the project actions (obligatory)													
C.1	Monitoring on the environmental impact of copper compounds and nematicides for the cropdefence against phytopathogenic bacteria and nematodes				■	■							
C.2	Monitoring of the absence of side effects for the high quality standardised polyphenolic preparations on common targets of any living organism at laboratory level					■	■	■	■				
C.3	Monitoring of the absence of a direct selection operated by the polyphenolic preparations towards the emergence of bacteria resistant to the polyphenolic molecules themselves, at laboratory level						■	■	■	■			
C.4	Monitoring of the short term environmental benefits from the use of the							■	■	■	■	■	

	high quality standardised polyphenolic preparations in plant disease control at pilot scale level in field screenings												
C.5	Monitoring of the economic benefits deriving from the recycling of the spent vegetable biomass after the extraction of the high quality standardised polyphenolic molecules at laboratory level						■	■	■	■	■	■	
C.6	Monitoring of the absence of a selection on the polyphenolic preparations on the selection of copper and antibiotic resistant bacteria, on plant and in soil, from laboratory to in field screenings.							■	■	■	■	■	
C.7	Monitoring of technical-socio-economic assessment of the After-Cu project										■	■	

Deliverable with 2014/2015 data

Report ready at Febr. 29, 2016

Tests in progress salad & tobacco (hydroponics & greenhouse)

Analyses in progress

Activities just begun

INSTM ACTIVITY FOR NEXT MONTHS Month 18-24

DELIVERABLE B1

"I note that you consider this Action as completed on time. However, you also mention that two further field experiments are being / will be carried out by the Associated Beneficiary Consorzio Interuniversitario Nazionale per la Scienza e Tecnologia dei Materiali, in 2015 and 2016"

B3: DISPAA (batteri) + INSTM (nematodes/formulations)

B4: DISPAA (batteri) + INSTM (nematodes/biostimulant activity on tobacco and salads) + CEBAS

B7: ASTRA + INSTM (nematodes/biostimulant activity on tobacco and salads) + CEBAS

C4: CEBAS + INSTM (residues and total microbiological activity)

C5: INSTM + Mondo Verde + Phytolab (partial contribution)

C7: INSTM + Mondo Verde + Phytolab (partial contribution)

INSTM ACTIVITY FOR NEXT MONTHS Month 18-24

Problem of re-arranging the allocation of resources

Inviato: martedì 16 febbraio 2016 11:12 **A:** Daniele Consigli; Costantino Raspi **Cc:** Smiele Agr **Oggetto:** voce nel budget Evergreen

Buongiorno,
in merito alla voce nel budget EVERGREEN " **C4, Direct treaty, Lab materials for microbiological monitoring, 30,000.00€**"
desideriamo avere i seguenti chiarimenti:
.....
- a noi servono anche analisi multiresiduali, quindi chimiche: è possibile trasformare la voce da "Lab materials for microbiological monitoring" a "**Lab materials for microbiological and chemical monitoring**"
Attendiamo una rapida risposta.
Grazie e cordiali saluti
E. Bargiacchi