Natural cropping system based on Brassicas biomasses for plant management and defense

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A growing interest for non chemical systems as total or partial alternative to chemical in agriculture

✓ Chemicals in European agriculture are more and more controlled (Directive 2009/128/EC " Sustainable use of pesticides") in the framework of a wider strategy of their registration (EC Regulation REACH 1907/2006). It encourage the application of natural materials as non chemical strategies

✓ **Pesticides and fertilisers are:**

✓totally widespread in the environment

in contact with operators during and after distribution

✓ closest to food chain with all the problems of food health due to residue problem and sustainability

✓ The EU is requesting to produce more. with less chemistry (IPM techniques)

Soil fertility concept

In the last 50 years the wide application of chemistry in agriculture, linked to the climate change determined a progressive decreasing of soil fertility, moreover in coastal areas, where the soils have very low organic matter amounts

Soil fertility as the whole Physical, Chemical, and Biological characteristics of a soil is strongly linked to the organic matter amount, composition and dynamics requesting a plan for increasing soil organic matter

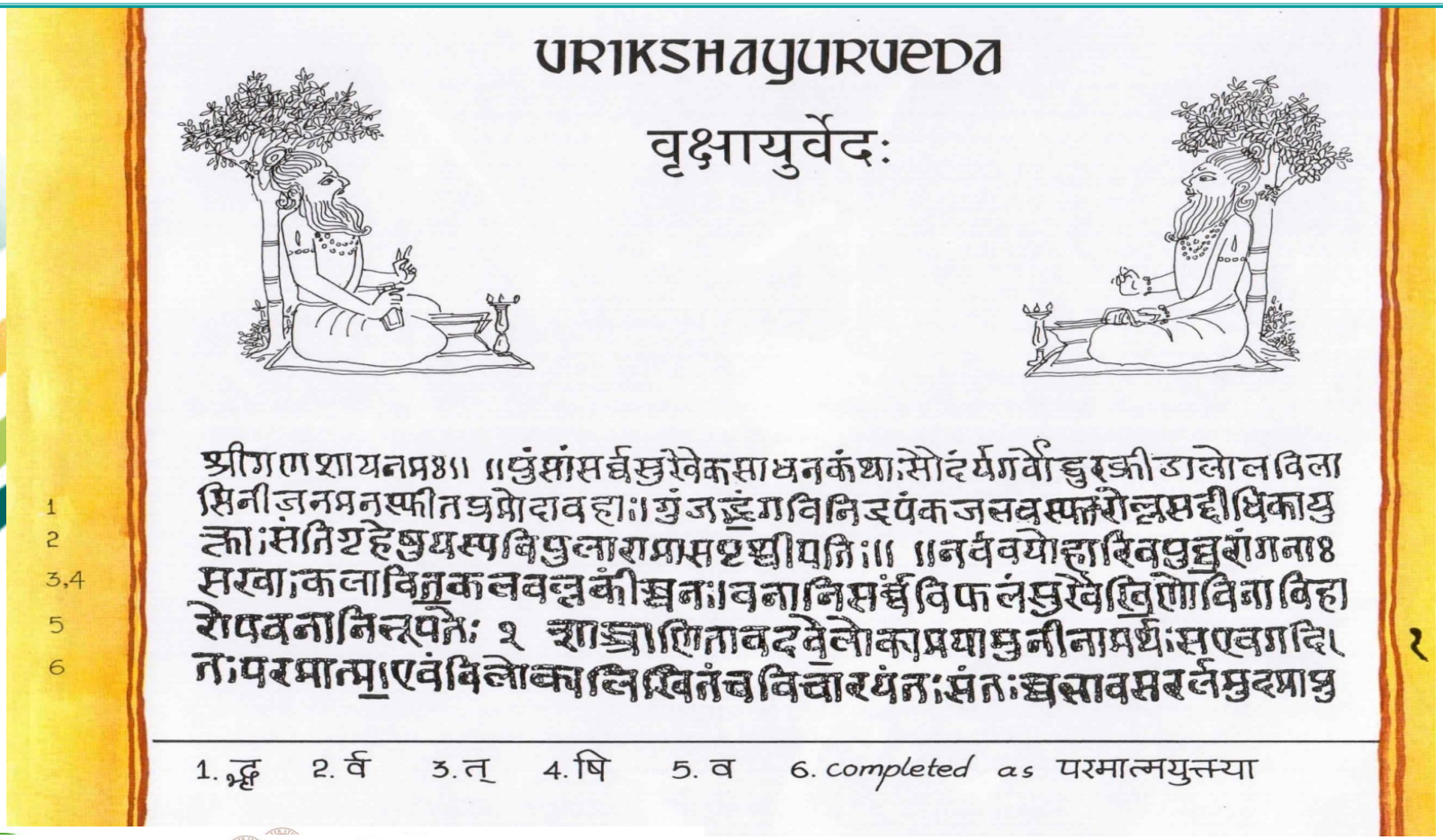
It is not possible to produce high quality agriculture on low fertility soils, without a wide application of Chemicals

The Brassicaceae Family

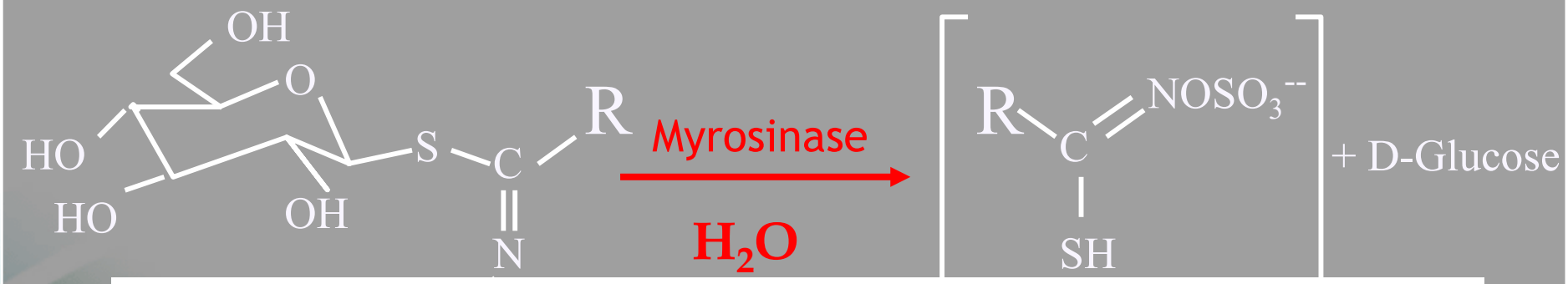
- 350 genera
- Around 3700 specie
- More than 200 glucosinolates
- Generally herbaceous
- Present in each continent

Surapala: The Science of plant life

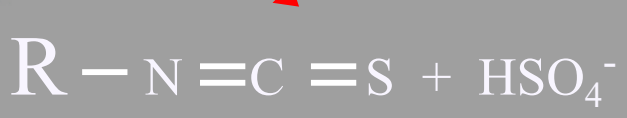
An ancient Sanskrit text dated 10 century describes the effects of organic amendments and green manures (including *crucifers*)



The glucosinolate- myrosinase system (1)



"Allyl-isothiocyanate (AITC), as component of mustard oil, is on the FDA Generally Recognized as a Safe (GRAS) Substance"



Isothiocyanate

Nitrile

Federal Register of rule and regulations: May 20, 1996, Vol. 61,No 98, page 25152No 25152--25153

EPA - 40 CFR Part 18040 180

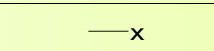
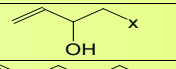
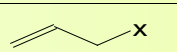
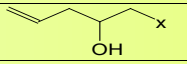
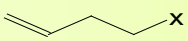
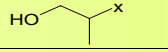
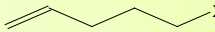
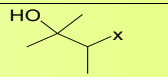
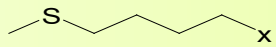
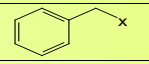
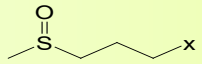
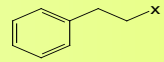
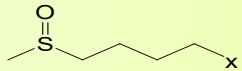
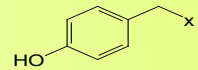
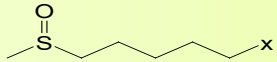
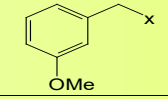
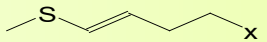
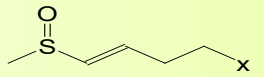
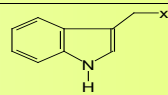
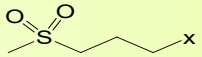
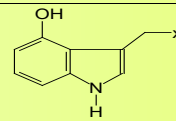
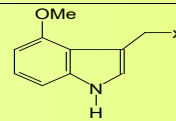
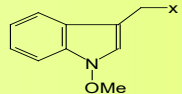
Glucosinolate Biodiversity

Different R side chain

Different Isothiocyanates

Different Physico-chemical properties

Different biological effect

Glucosinolati con catena R alchilica		Glucosinolati con catena R ossidrilica	
Nome	**Struttura**	**Nome**	**Struttura**
Glucocapparina		Progoitrina /Epiprogoitrina	
Sinigrina		Gluconapoleiferina	
Gluconapina		Glucosisimbrina	
Glucobrassicanapina		Glucoconringina	
Glucosinolati con catena R tiofunzionalizzata		**Glucosinolati con catena R aromatica**	
Glucoerucina		Glucotropeolina	
Glucoiberina		Gluconasturtina	
Glucorafanina		Sinalbina	
Glucoalissina		Glucolimnantina	
Glucorafasatina		**Glucosinolati indolici**	
Glucorafenina		Glucobrassicina	
Glucocheirolina		4-Idrossi glucobrassicina	
		4-Metossi glucobrassicina	
		Neoglucobrassicina	

The CREA CI collection of purified glucosinolates

Biofumigation definition

"Pest and desease suppression by glucosinolate containing plants arising specifically from the biocidal properties of the glucosinolate hydrolysis products, particularly the isothiocyanates, released from incorporated tissues or rotation crops, notably the *Brassicaceae*".

(Kirkegaard and Matthiessen *"Developing and refining the biofumigation concept"* Proceeding of the First International Symposium "Biofumigation: a possible alternative to methylbromide?" Firenze 29 marzo-1 aprile 2004 - Agroindustria, 2004, 3 (3),233-239)

Green manure and cover crop roles

Green manure and cover crops could offer a fundamental help to the farmer for:

(i) Improving soil fertility in nutrient (Nitrogen, soil structure etc.) and the biodiversity in the system

(ii) Playing a role in the decrease of CO_2 in the atmosphere thanks to the SINK effect of organic matter in soil

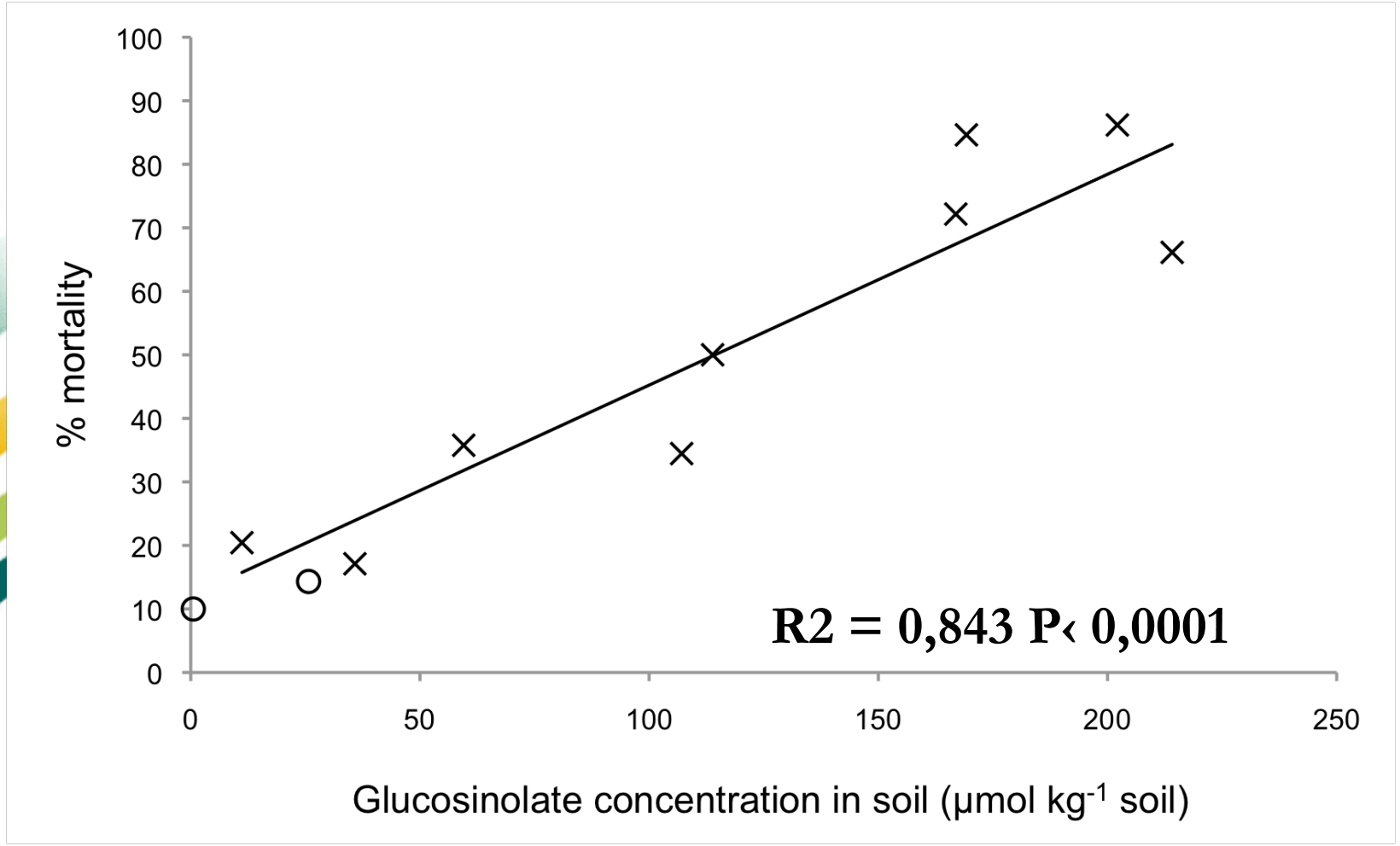
(iii) Improving the resistence of the agro-system to disease and pest for a multifunctional and more sustainable agriculture

Biofumigant green manure plants (1)

Plants selected for their capability of releasing volitile ITCs from their epigean part. (*B. juncea*. *B, rapa*, *Sinapis alba*)

They are applied in soil for containing soil borne fungi, nematodes and wireworms

Correlations of glucosinolate content in plants and mortality of *Globodera pallida* eggs



Biofumigant green manure: catch crops (2)

Plants selected for a high GL content in roots. (*Eruca sativa* sel. Nemat. *Raphanus sativum* cv. Karacter, (*Crotalaria juncea*)

Nematodes attack biofumigant plant roots, penetrates the root tissues and activates the enzymatic hydrolysis and isothiocyanates from glucoerucin or glucoraphasatin are released.

The nematode feeds a poisoned media and does not end its growing cycle into the root during the time of biocidal crop cultivation (8-10 weeks).

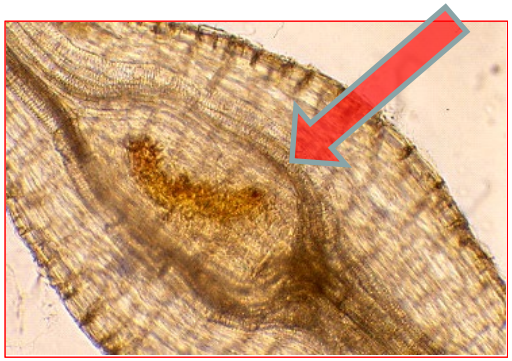
Catch crop effect of *E. sativa* green manure

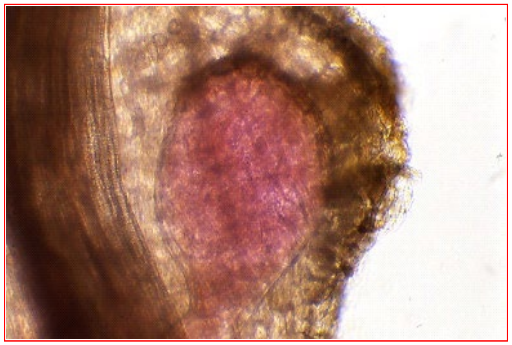
Nematode: *Meloidogyne incognita*

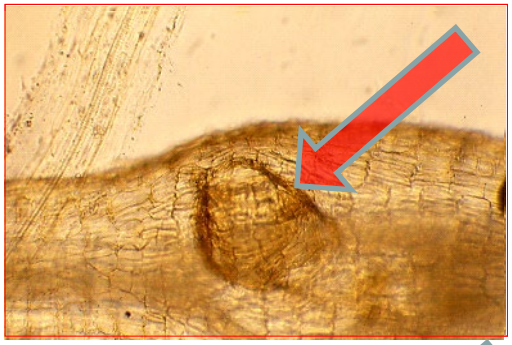
Post J2 larvae

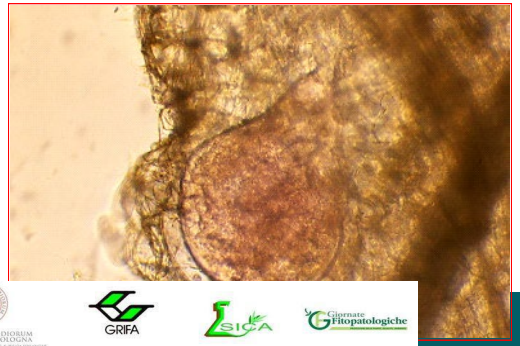
J4 young females

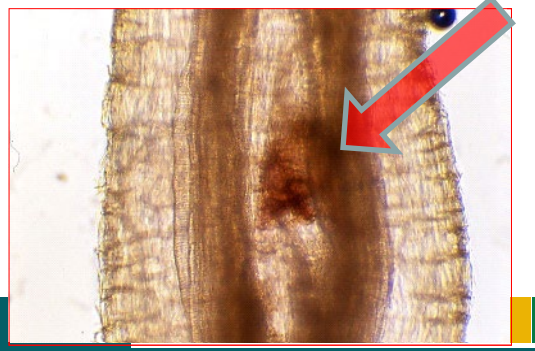
Females









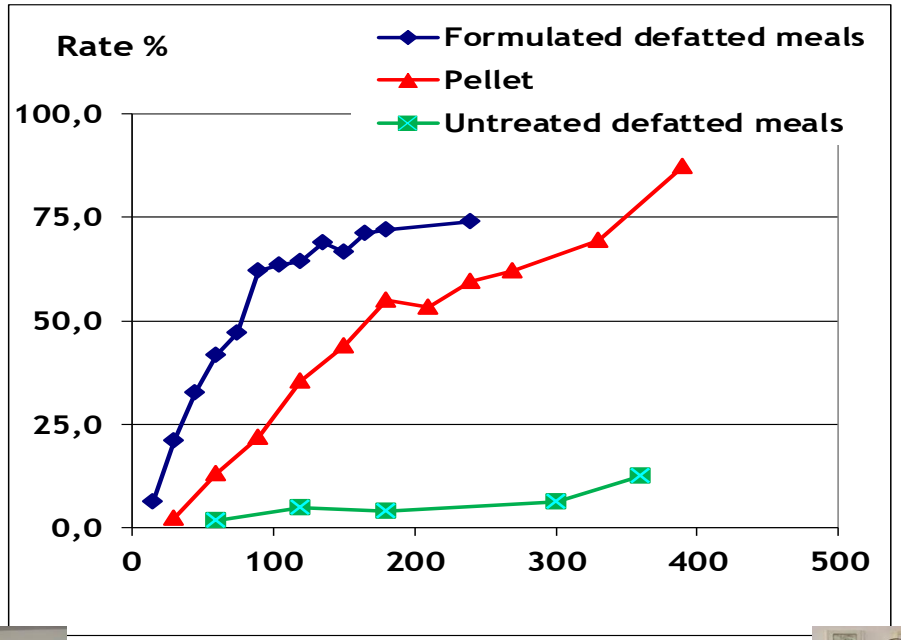


Necrosis on roots after nematode attack

Defatted seed meals

USA Patent N° US7,749,549B2 of 2010-06-06

Biofumigant pellets and meals based on *B. carinata*, *B.juncea*, *B. nigra* et al. seeds

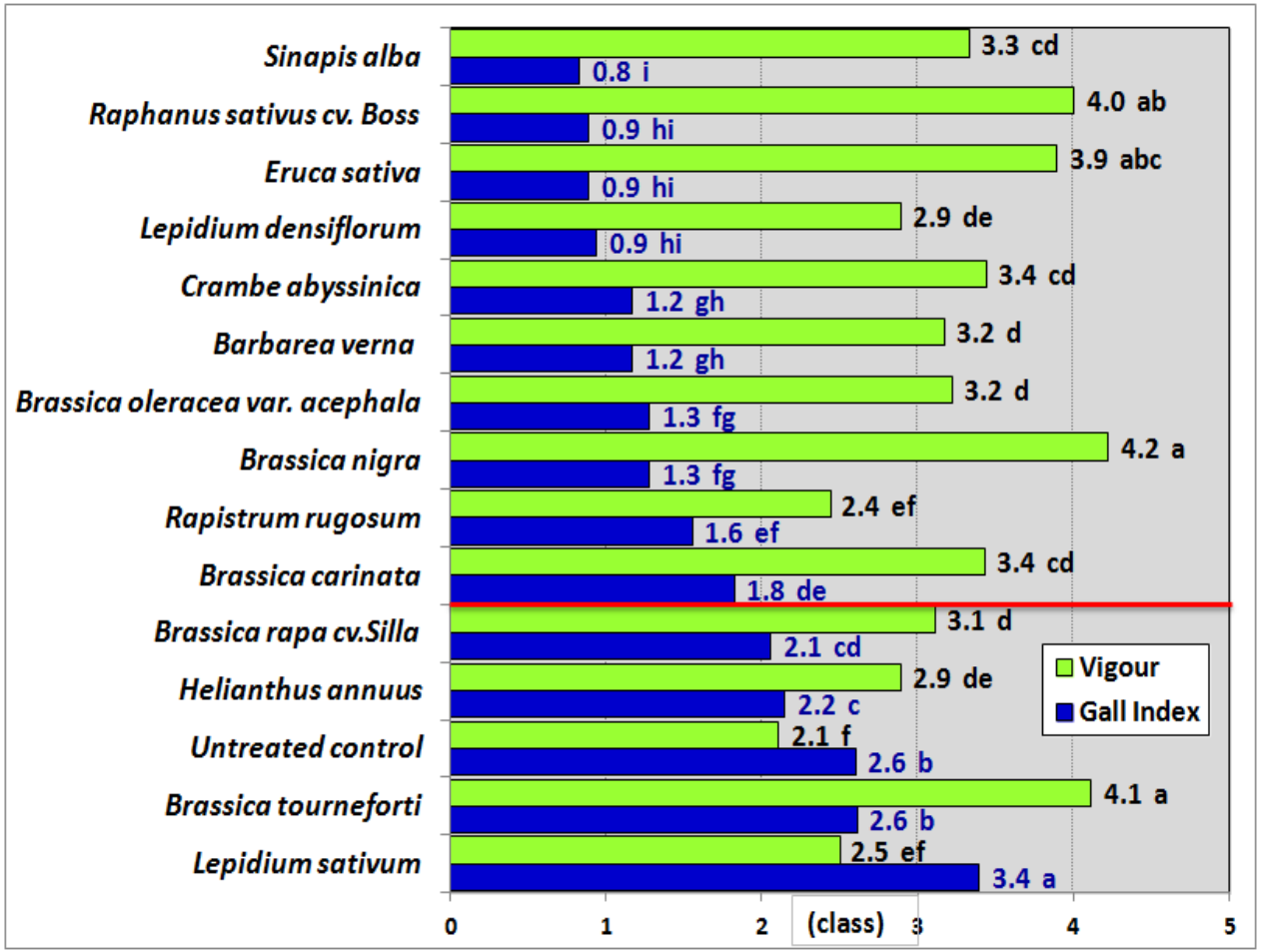


In vials

In soils

Gall index and vigour after treatment with different DSM (2)

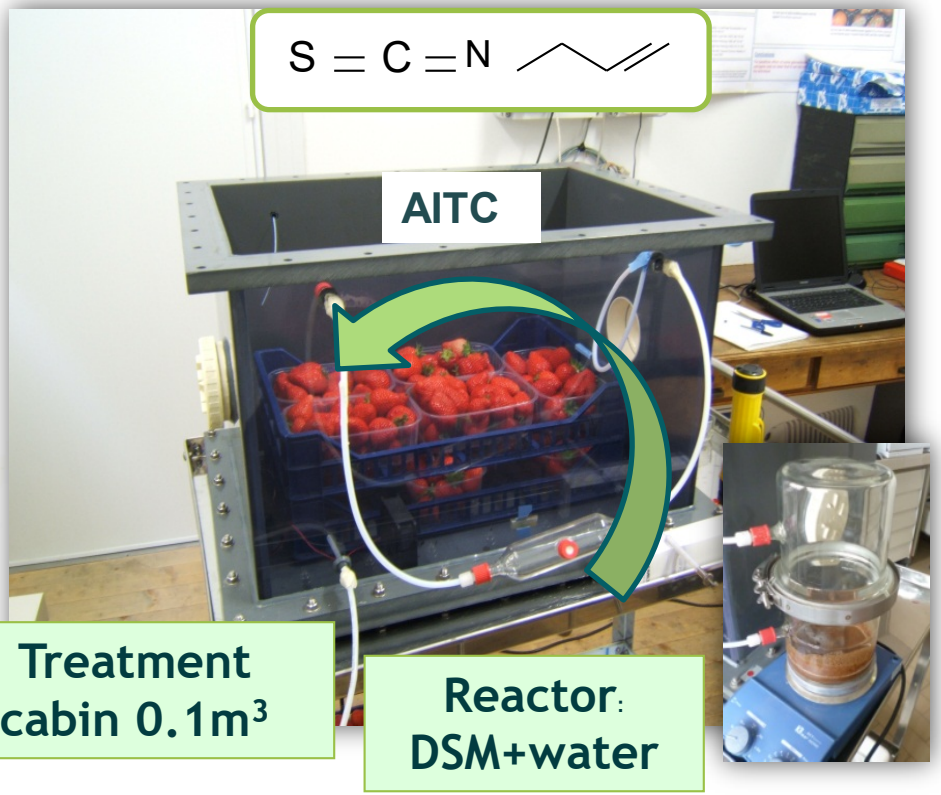
On soils with 30 J2 larvae in 100 cm^{-3} inoculum of *Meloidogyne incognita*



Biofumigant pellets composition

	Oil	N	P	K	C	C/N	OM	GI
% on DM	9-12	5-6	0.7-1	1-1.5	40-45	7-8	80/85	4.1/4.7

Pilot plant for post harvest biofumigation



Quality Maintaining,
Extension of shelf-life

improvement of nutraceutical properties

Liquid biofumigant formulations

Liquid formulation are essentially vegetable oil/water emulsions where DSM are mixed

The emulsion activates the hydrolysis reaction of DSM, releasing isothiocyanates (ITCs) that naturally are solubilised in the oil phase

Oil in the emulsion plays a suffocating effect on pests or pathogens and protecting plants from ITCs phytotoxicity

DSM in the emulsion play the well known «biofumigant effect»

Different final preparation of the liquid formulations

Foliar formulations

Duolif

Duofruit

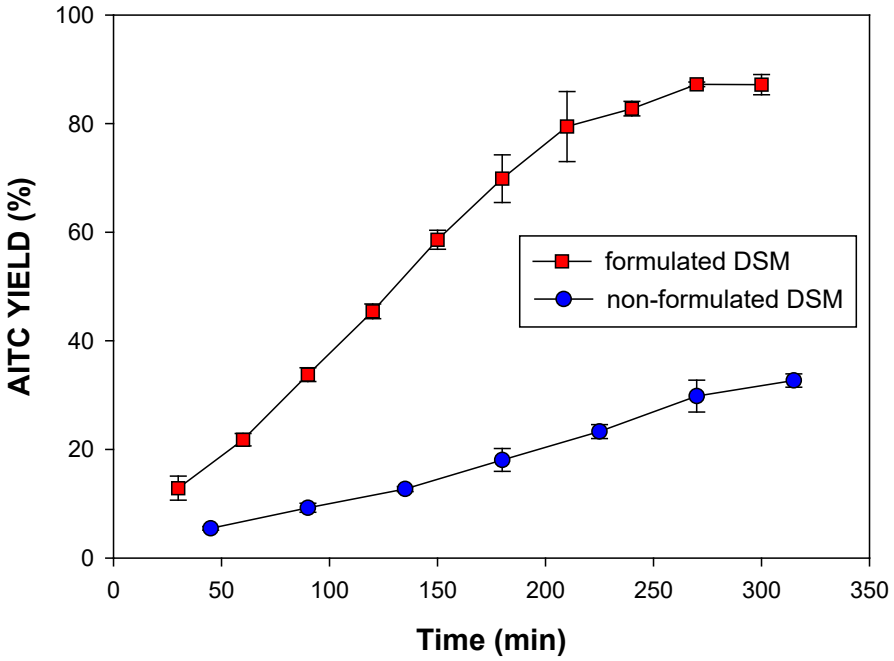
Root formulation

Biofence FL

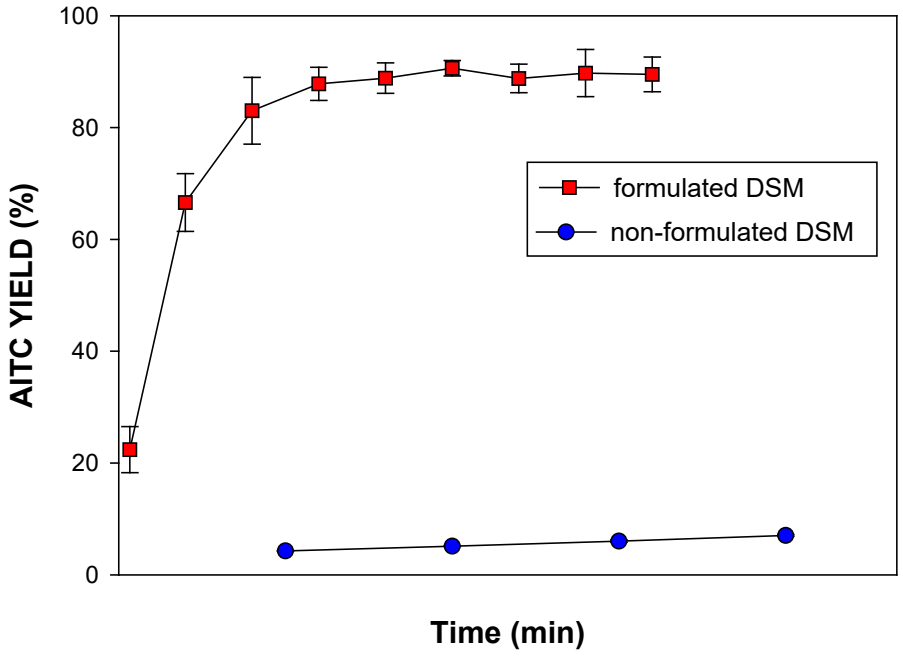
The suspension is taken at the requested concentration in water.
DSM are micronized at a size lower than 150 micron, a size that permits the distribution of the emulsion still containing DSM by mechanical pumps.

The suspension is taken at the requested concentration of water. DSM are applied at a size of 1000/2000 micron to make a simple remval the suspension by a simple filtration. In this way, no DSM remains in the emulsion to avoid problem to drip irrigation system

Isothiocyanate release during liquid formulation preparation



Foliar treatment



Root treatment

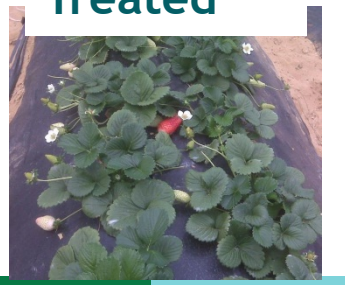
New open field application for foliar liquid

The main target of foliar liquids is in epigeal apparatus defense, not only in horticulture, but even in floriculture, fruit, grape and several other crops

1) Pests and disease control
2) 2) Biostimulant effect
3) Earliness of ripening
4) Leaf washing
5) leaves functionality

Untreated

Treated



Pest and disease control (1)

The main target for foliar liquid formulation are pests and diseases, limited to organisms of small dimension to permit a sufficient persistence of oil microfilm enriched by ITCs

Scientific studies on their effect on :

- **Aphids.** Cotton aphid (*Aphis gossypii* G.), (*Aphis fabae* S.), (*Myzus persicae* S.)
- **Mites.** Twospotted spider mites (*Tetranychus urticae* K.), Citrus red
- mite *(Panonycus citri* McG.).
- **Scales.** (*Aonidiella aurantii*, A.), Pyriform scale (*Protopulvinaria pyriformis* C.), Japanese citrus scale (*Unaspis yanonensis* K.), Cottonycushion scale (*Icerya purchasi* M.).
- **Whitefly.** Woolly whitefly (*Aleurothrixus floccosus* M.).
- **Oidium** (*Podosphaera xanthii B.*), *(Golovinomyces cichoracearum DC)*, sugar beet oidium *(Erysiphe betae V)*, cucurbitacee oidium *(Erysiphe cichoracearum DC)*.

Effect of the liquid foliar treatments on spiders on eggplants and Oidium on melon

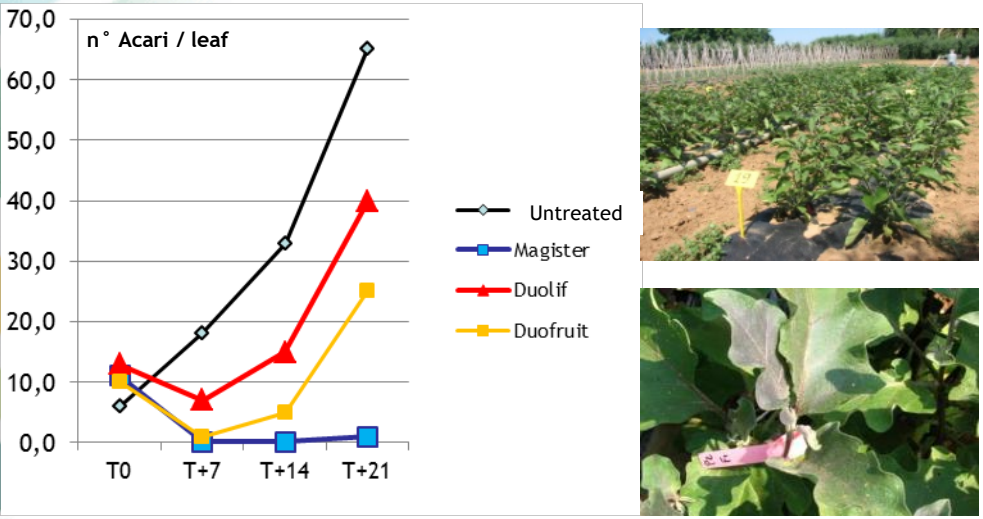
UNITA' DI RICERCA
PER L'ORTICOLTURA
Monsampolo del
Tronto (AP) Italy

Evaluation of Duofruit and Duolif

Dr. V. Ferrari
Dr. E. Piccinini

EGGPLANT

Compared to the chemical fenazaquin (*Magister*) in the control of Red spider (*Tetranycus urticae*)



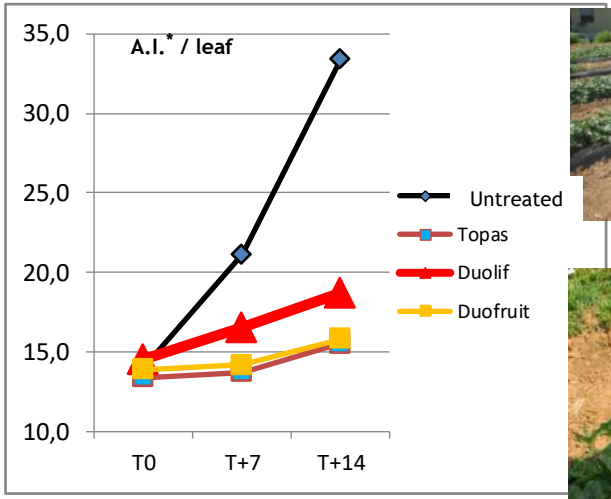
Results

Duofruit showed a higher containment effect in comparison to Duolif with a persistence of around 14 days

Defense strategy : a treatment every 15 days

MELON

Compared to the chemical product *Penconazol (Topas)* in the control of Oidium (*Podosphaera xanthii, Golovinomyces cichoracearum*)



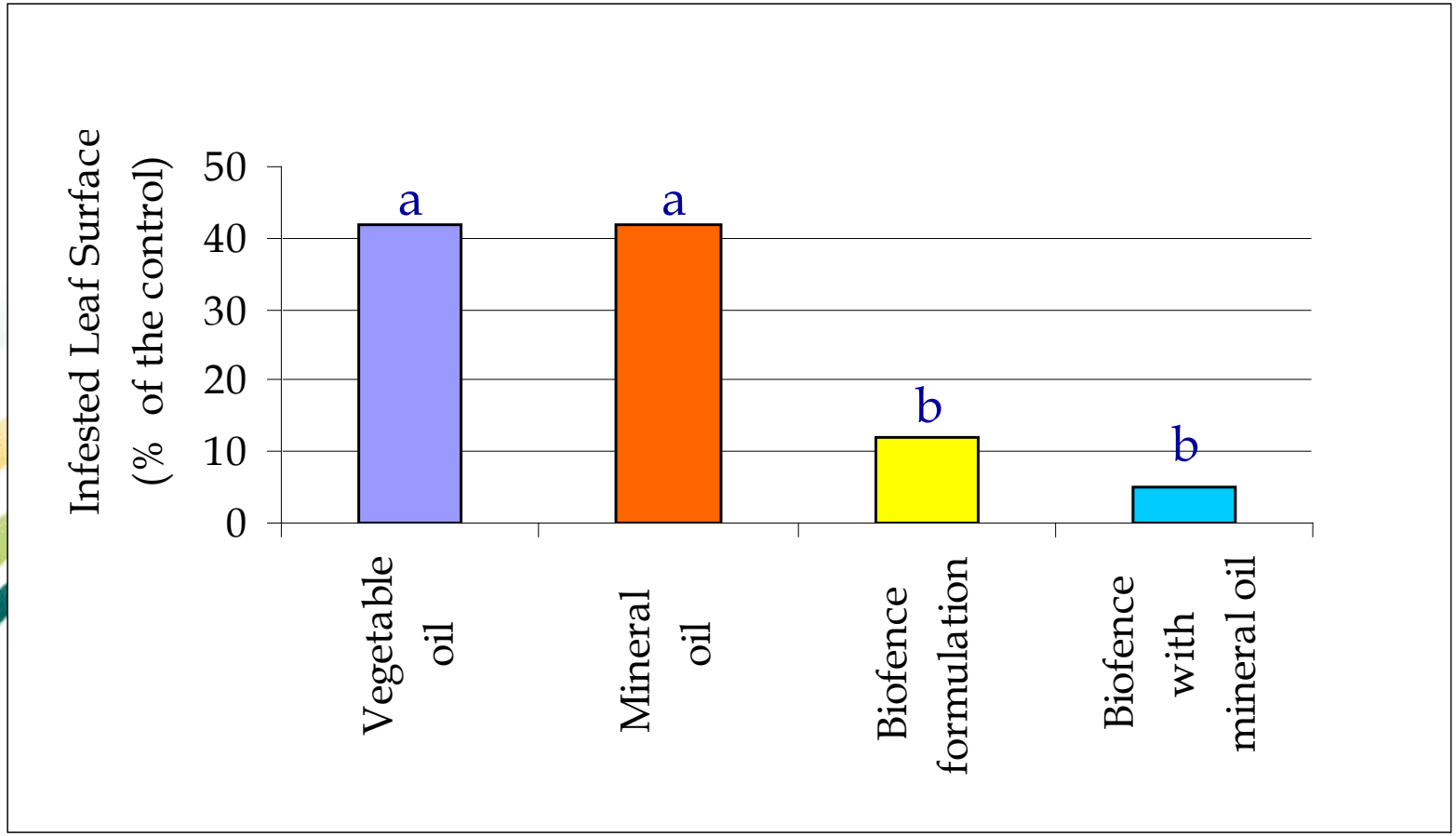
$$I.A.^{*} = \frac{\sum \%\ \text{superficie fogliare coperta da macchie}}{\text{Log}_{10}\ \%\ \text{foglie malate}}$$

Results

Duofruit showed a similar containment effect in comparison to Topas

Defense strategy : a treatment every 20 days

Biofence liquid formulation efficacy on citrus in the control of Aonidiella (*Erysiphe betae*

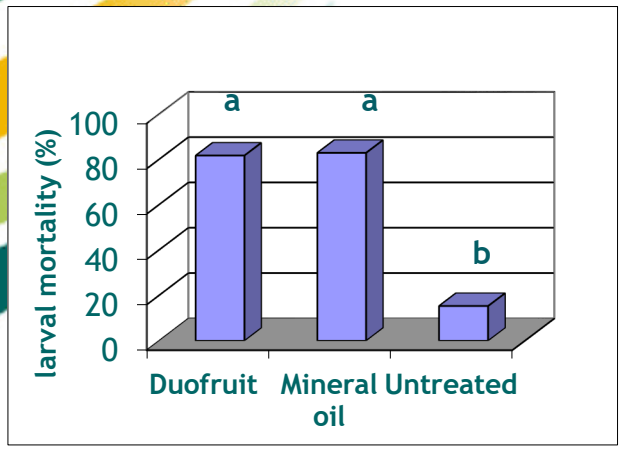


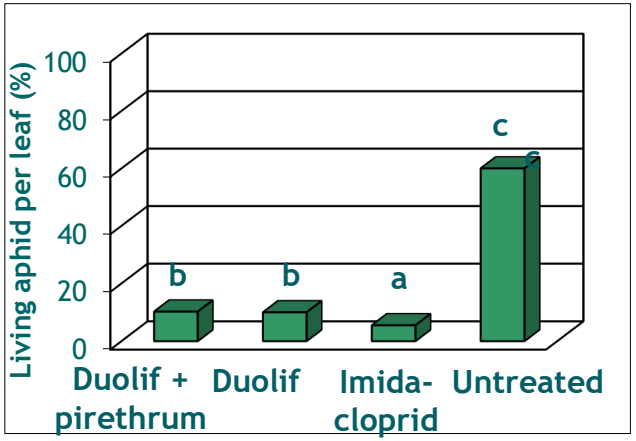
Some trials on orange cultivation

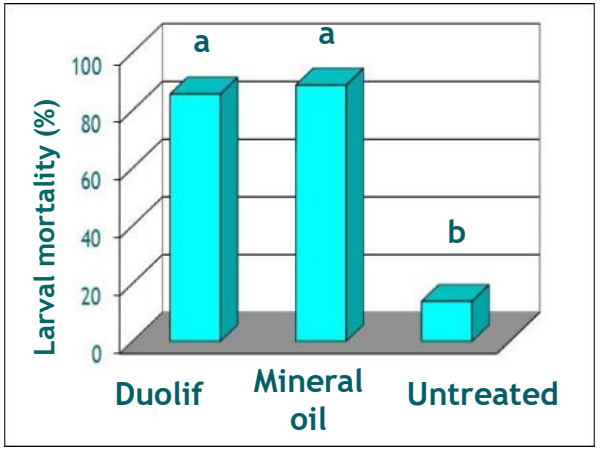
Unaspis yanonensis

Aphid gossypi

Protopulvinaria pyriformis







New application fields for root liquid formulates (1)

The main target for root liquid formulation are soil borne pests, even as rescue treatment after pre-plant with biofumigant green manure and defatted seed meals.

Positive scientific results on:

- **Nematodes:** Southern root-knot nematode (*Meloydogine incognita* Chitw. Kofoid *et* White).
- **Wireworms:** (*Agriotes sordidus*, *Agriotes litigiosus*)
- **Soilborn Fungi:** (*Armillaria mellea V.*)

Unsatisfactory effects of foliar liquid formulations

At moment unsatisfactory effect on:

Diseases: *Plasmopara viticola* B., *Aspergillus* and fungi producing mycotoxins

Pests: *Ceratitis capitata* W., Red weevil (*Rhynchophorus ferrugineus* O.), Spotted Wing Drosophila (*Drosophila suzukii* M. and all the insect of "bigger" size (including bees) and with rapid movement as Thrips (*Frankliniella occidentalis*)

New field of application for root liquid formulations

This root liquid was studied as a support of plant during cultivation, but it permitted to open new application fields not only in horticulture for nematode control, but even on fruit replant (apple, peaches, grape) and other crops

1) Containment of root infestation
2) Improvement of soil biological and chemical fertility
3) Biostimulation of root system
4) Lengthening of harvesting period

Plants and materials for biofumigation

Green manure catch crop
Eruca sativa sel Nemat

Biofumigant green manure
B. juncea sel ISCI20 - ISCI99

Biofumigant pellets
EU Patent N° 03 792 616.9 - 1219

Liquid foliar treatment
PCT W 2006/136933 A2

Liquid root treatment
PCT EP 2009/050143

Brassica cropping system

The biofumigant purposal, even considering all the potential sinergies with other non chemical alternatives (viz. Solarization, antagonists etc.) has to be considered as a real cropping system that has to be applied with a year after year strategy for a total or partial alternative to chemicals.
The production line can be considered as a biorefinery approach in the ambit of circular economy

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The production line follows a biorefinery approach in the ambit of circular economy

Environmental balance

It becomes crucial to evaluate not only the economic balance (€/kg), but even the environmental one (kg CO_2eq/kg)

Inputs

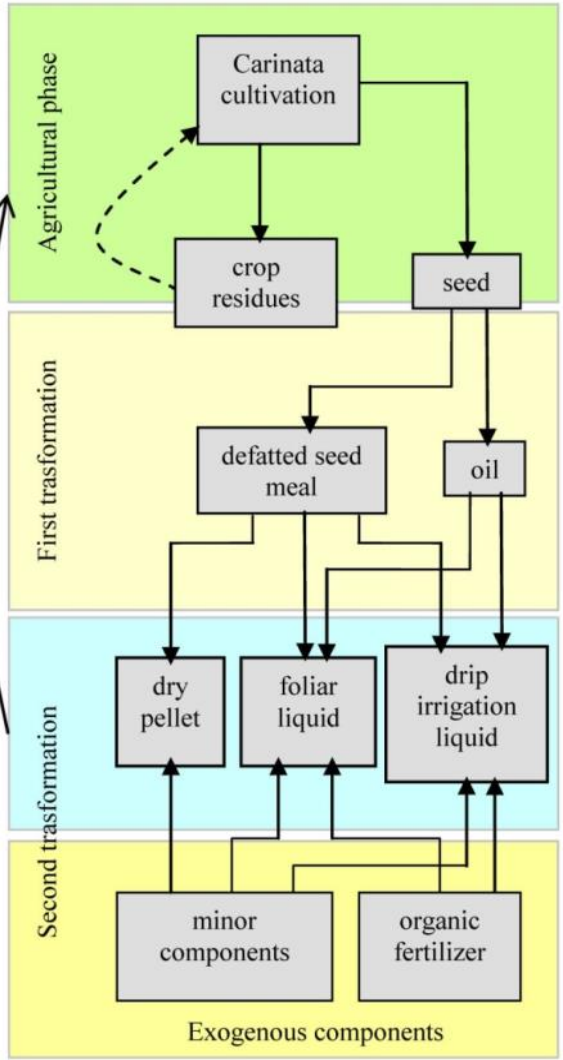




Outputs


Seeds **Residues**

Biofumigation material evaluation by the Biorefinery approach of carbon foot print



GHG emission by Biofumigation	Kg CO_2e ha^{-1}
e_c from cultivation phase	1523±707
e_p from processing	159
e_f for pellet production	41
e_f for epigeal formulation	166
e_f for hypogeal formulation	449
E_b Emissions of biorefinery = $e_c + e_p + \Sigma (e_f + e_{pk} + e_{td})$	2338
GHG emission by Conventional	
e_{ai} from Metham sodium production	2127
e_{ai} from paraffinic oil production	943
e_{ai} from Oxamyl production	746
E_c Emission of conventional proposal = $\Sigma (e_{ai} + e_{pk} + e_{td})$	3816

Biofumigant plants and materials…

- Have not to be considered a simple product innovation but a system one, that considers soil fertility management and plant health as a fundamental starting point for a real sustainable production.

The GL-MYR system management plays a fundamental role of bioactive

- products from a biorefinery chain linked to the territory with positive environmental balances

- Can be surely improved and diversified for new materials and applications. The topic request more and more research for defining the system limits.

- Could change the farmer role from user of chemicals to producer of its own bio-based materials for plant management and defense in the framework of a new multifunctional agriculture