Consiglio Nazionale delle Ricerche
ISPA
ISTITUTO DI SCIENZE DELLE PRODUZIONI ALIMENTARI

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Session 25: Project session ADVAGROMED

Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

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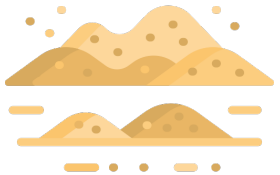
EAAP
European Federation of Animal Science

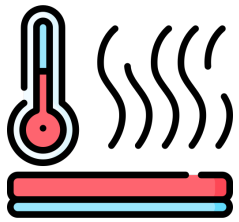
Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

European Commission

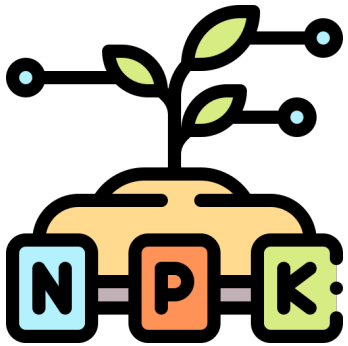
According to the **European Regulation 1925/2021**, amending certain Annexes to Regulation (EU) No 142/2011 as regards the requirements for placing on the market of insect products and the adaptation of a containment method, insect frass is intended:



”*a mixture of excrements derived from farmed insects, the feeding substrate, parts of farmed insects, dead eggs and with a content of dead farmed insects of not more than 5% in volume and not more than 3% in weight*.”



70°C 1 h
(UE) 2021/1925



Regulation 1925/2021 implementing EU standards for insect frass (i.e. heat treatment of 70 °C for at least 60 minutes, setting of microbiological standards) entered into force on 28 November 2021.

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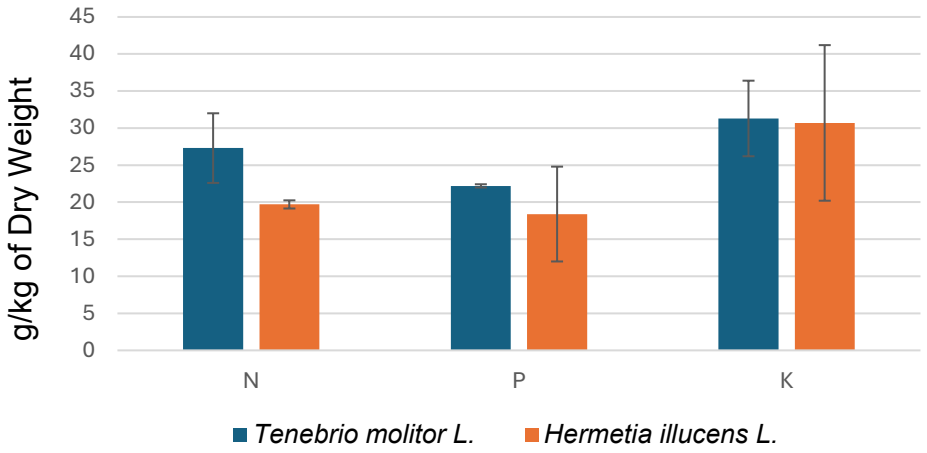
ADV agro MED

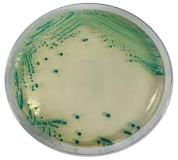
Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

Chemical and microbiological characterization of different batches of *T. molitor* and *H. illucens* insect frass

- **N,** C, ions (F, Cl, NO_2, NO_3, PO_4 and SO_4, **P**, **K,** Ca, Mg, Fe, Mn, Mo, Cd, Ni, Zn, B, Al, others..)



- After Thermal treatments (70 °C 1 h) *Salmonella* spp. and *E. coli* (absent: UNI ISO 16649-2; UNI EN ISO 6579)



Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

Aim: This study aimed to evaluate the effect of *Hermetia illucens* L. (H) and *Tenebrio molitor* L. (TM) frass on the nutritional quality of tomatoes and mizuna and rapini microgreens, in two different production contexts: open field (tomatoes) and growth chambers (microgreens).

Open Field

Soilless system - Vertical farm

Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

Species: *Brassica rapa* L. (mizuna and rapini)

Growing environment: Growth Chamber

Main goal: to evaluate new growing substrates based on *T. molitor* (L.) and *H. illucens* (L.) frass in mixture with peat for microgreens production.

Microgreens, the young and tender greens from vegetables, herbs, legumes, or grain seeds, represent an expanding segment of the produce market space.

Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

Use as a substitute for chemical (conventional) fertilization.


***Hermetia* frass**


***Tenebrio* frass**

Treatments:

- Peat (**+ chemical fertilization**) common agronomic practices
- Hermetia frass:peat (50:50 w/w) **without chemical fertilization**
- Tenebrio frass:peat (50:50 w/w) **without chemical fertilization**

Experimental Design: randomized block with 3 replicates

Harvesting

Ctr – 100% Peat

Hermetia frass:peat (50:50 w/w)

Tenebrio frass:peat (50:50 w/w)

Phytotoxic effect

(+ chemical fertilization)

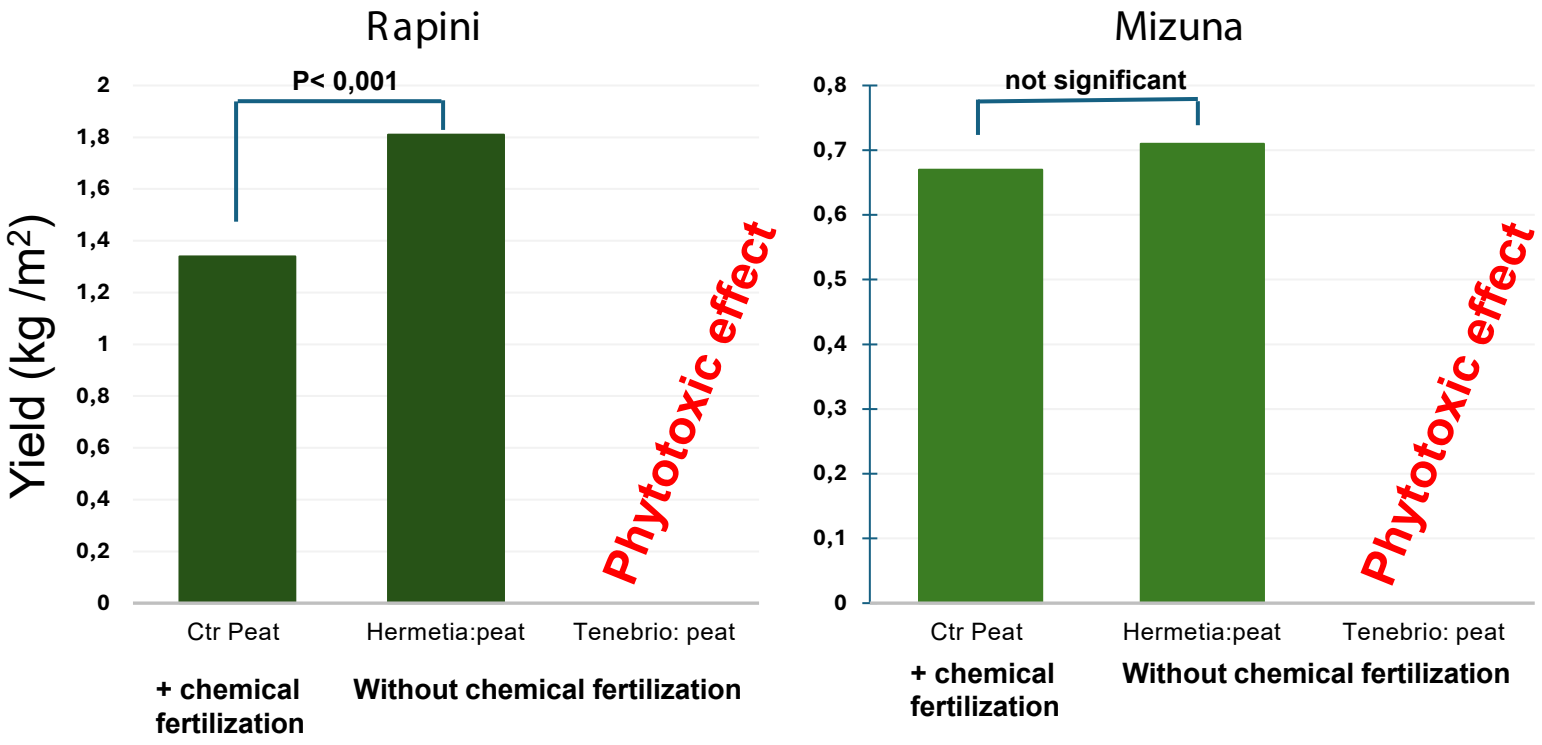
Without chemical fertilization

Mizuna microgreens (*Brassica rapa* L.)

Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

Results - Growth Chamber (1/2)

Crop performance and nutritional quality



Data are expressed as mean (n = 9).

Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

Results - Growth Chamber (2/2)

- Analysis of mineral content

Rapini microgreens

	Ca	K	Mg	P	B	Fe	Na	Zn	Al
	g/kg of Fresh weight						mg/kg of Fresh weight		
Peat	2.56	1.93	0.40	0.80	2.29	6.58	21.3	5.68	0.59
Hermetia:Peat	0.82	7.47↑	0.32	1.15	2.09	5.32	269↑	7.05	0.50
Significance	**	***	*	*	ns	ns	***	*	ns

Mizuna microgreens

	Ca	K	Mg	P	B	Fe	Na	Zn	Al
	g/kg of Fresh weight						mg/kg of Fresh weight		
Peat	3.31	5.50	0.35	1.16	4.02	13.9	30.5	3.46	6.73
Hermetia:Peat	1.77	3.16	0.47	0.81	2.62	31.8↑	326↑	6.06↑	34.0↑
Significance	*	*	*	*	**	***	***	**	***

Data are expressed as mean of the treatment (n = 3). Ns. not significant; *P < 0.05; ** P < 0.001; *** P < 0.0001.
Cd, Co, V, Mo, Sr < limit of detection

- Safety evaluation: *E. coli* e *Salmonella* spp. were not detected

Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

Open field trial

Species: ***Solanum lycopersicum*** L., local variety (cv. "Regina") in Apulian region (southern Italy)

Main goal: evaluation of insect frass (*Tenebrio molitor* L.) as organic fertilizers in the sustainable agricultural process in the framework of the local agro-biodiversity enhancement, as partial or total substitutes of chemical fertilizers on the bio-morphology, quality and yield of local vegetable crops

Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

Application in open field

Figure 1. Distribution of fertilizers along the rows.

Quantity:

Rate of Nitrogen (**N**) fertilizer as reference: **130 kg/ha** according to Apulia Region Official Bulletin n. 38 31/03/2022

Treatments		
100% chemical	100% frass	100% organic
100% poultry manure	75% chem 25% frass	50% chem 50% frass
75% chem 25% poultry	50% chem 50% poultry	Control

Figure 2. Burying of fertilizers before transplanting.

Figure 3. Transplant.

Figure 4. Growing and Harvest

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Experimental Design: randomized block with 3 replicates

Measurements and analysis: crop performance, yield, nutritional quality, mineral composition of leaves, stems and fruits

Insect Frass Fertilizer: A Green Solution for Tomato and Brassica Microgreens

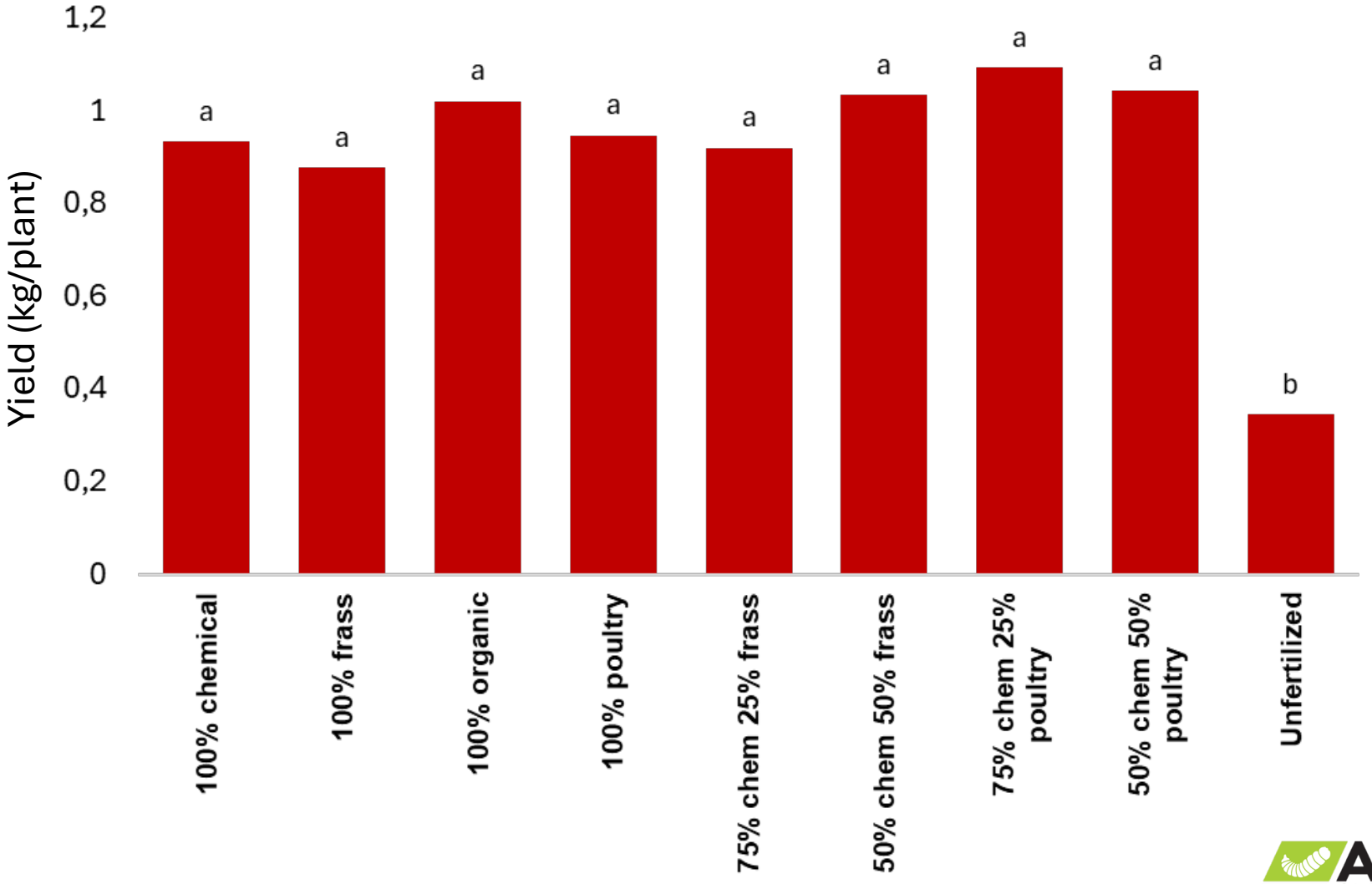
Results - Open Field (1/2)

Crop performance and nutritional quality

Yield (kg of tomato fresh fruit/plant)

The use of TM frass did not change the following tomato nutritional parameters:

- Color
- °Brix
- Titratable acidity.



Data are expressed as mean of the treatments. Different letters indicate values statistically different, according to LSD test (α = 0.05).

Results - Open Field (2/2)

Crop performance and nutritional quality

Mineral content in tomato fruits

Treatments	P	Ca	K	Mg	Fe	Mn	Zn	B	Al	Cu
	g/kg of dry weight				mg/kg of dry weight					
100% chemical	4.9	1.4	57.3^{a}	2.7^{a}	91.1	20.6	19.9	13.2^{b}	121	14.8^{a}
100% frass	4.1	1.3	44.2^{b}	1.8^{b}	83.1	14.5	13.8	14.4^{b}	137	9.1^{b}
100% organic	4.7	1.0	47.3^{b}	1.9^{b}	85.8	18.3	17.6	12.0^{b}	151	9.8^{b}
100% poultry	4.3	1.5	43.1^{b}	1.7^{b}	80.5	15.6	13.2	14.2^{b}	147	9.0^{b}
75% chem 25% frass	4.2	0.9	42.8^{b}	1.7^{b}	80.3	16.2	16.6	11.3^{b}	183	9.3^{b}
50% chem 50% frass	4.7	1.0	46.6^{b}	1.8^{b}	85.8	17.4	17.1	12.2^{b}	140	10.6^{b}
75% chem 25% poultry	4.8	1.0	46.6^{b}	2,0^{b}	80.1	17.8	20.3	13.4^{b}	170	11.1^{b}
50% chem 50% poultry	4.2	0.9	47.0^{b}	1.9^{b}	79.8	15.4	17.8	13.9^{b}	139	10.7^{b}
Unfertilized	5.3	1.4	48.3^{b}	2,0^{b}	119.2	16.7	20.0	22.5^{a}	140	10.6^{b}

Data are expressed as mean (n=3) of the treatments. Different letters indicate values statistically different, according to LSD test (α = 0.05).

V, Mo, Sr < limit of detection

Conclusion – Open Field and Growth Chamber

- TM insect frass can be used as an organic fertilizer in open fields, while H insect frass is suitable for soilless systems without affecting crop performance, nutritional quality, or visual quality.

The insect frass:

- Can have a key role into circular economy and organic fertilization of agroecosystems

- Can be safe to use, due to the absence of human pathogens

ACKNOWLEDGEMENTS

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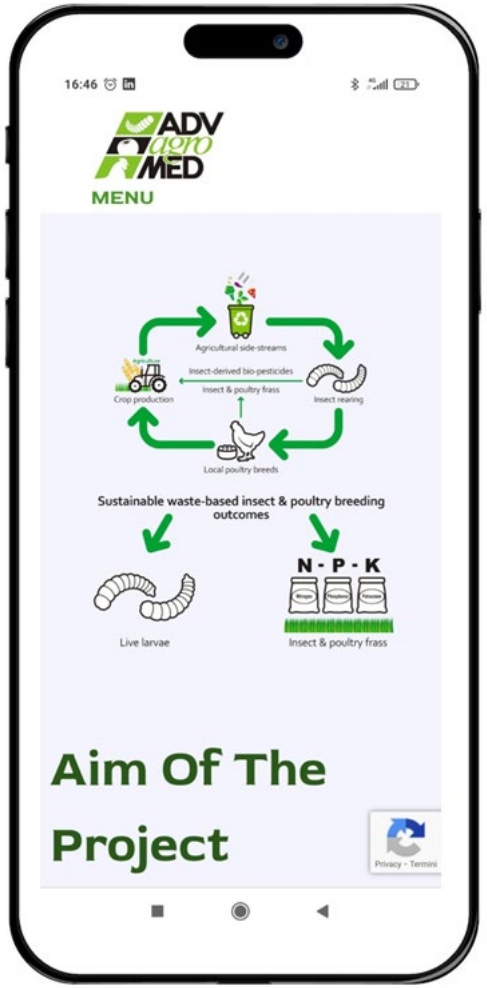

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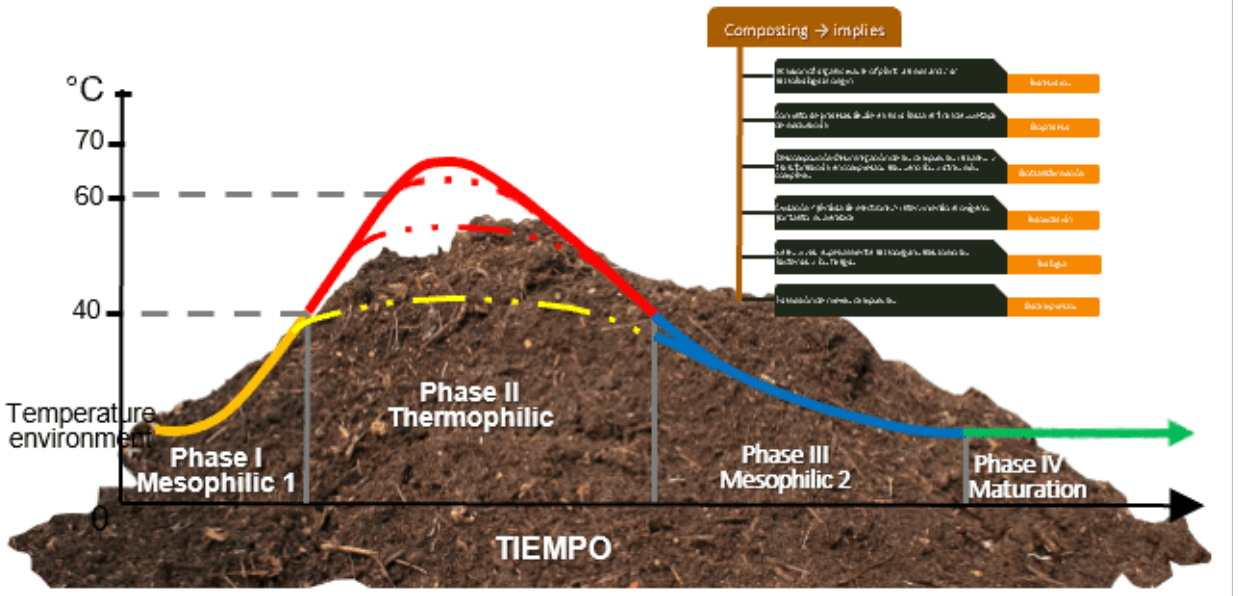
Limit and possible solution

agritech
National Center for Technology in Agriculture

Alternative to thermal treatment in an oven

Considering the increasing cost of energy, we are actively working on the development of innovative methods for the sanitation of insect frass through composting.



Limit and possible solution

Improving distribution

The transformation into pellets makes distribution easier and more precise compared to a powdery product.

Powder

Pellet

Prepare suspensions inoculated with nitrifying bacteria and apply them through fertigation.

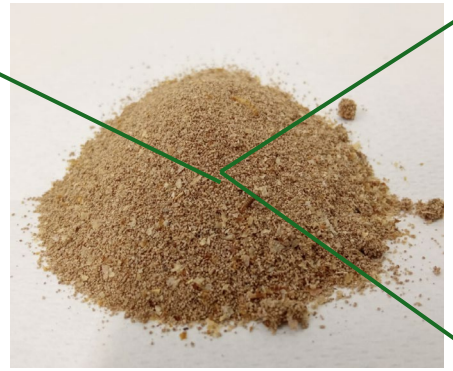
Powder

Fertigation

Chemical, microbiological and molecular characterization

Chemical

pH
EC
F, Cl, NO_2, NO_3, SO and PO_4 (IC)
K, P, Ca, Mg, B, Fe, Zn, Al, Ni , Cd, Cr, Sr, Ba, Mo, Mn, Cu, others (ICP-OES)
C and N content (Dumas method)



Microbiological

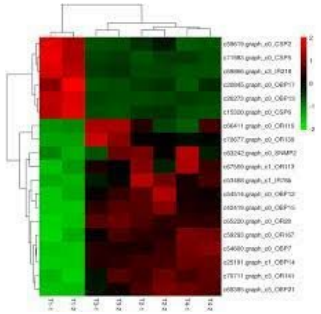
microbiological
E. coli
Salmonella spp.
Enterobacteriaceae

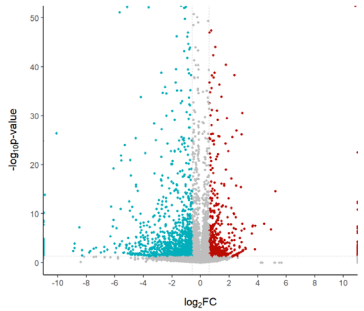
Before and
after thermal treatment
70 °C 1 h

Metagenomic analysis

Transcriptomic study

The aim of the experiment is to assess fertilization impact on the gene expression of tomato cultivar "Regina" and Brassica (Rapini) through a transcriptomic study





Plant growth

Treatments

- Chemical fertilizer
- Insect frass

→ Sampling →

Analysis

RNAseq

(currently ongoing)