



The 75th EAAP Annual Meeting

1/5 September 2024 - Florence, Italy

Effects of insect frass fertilizer on tomato fruits untargeted metabolomics



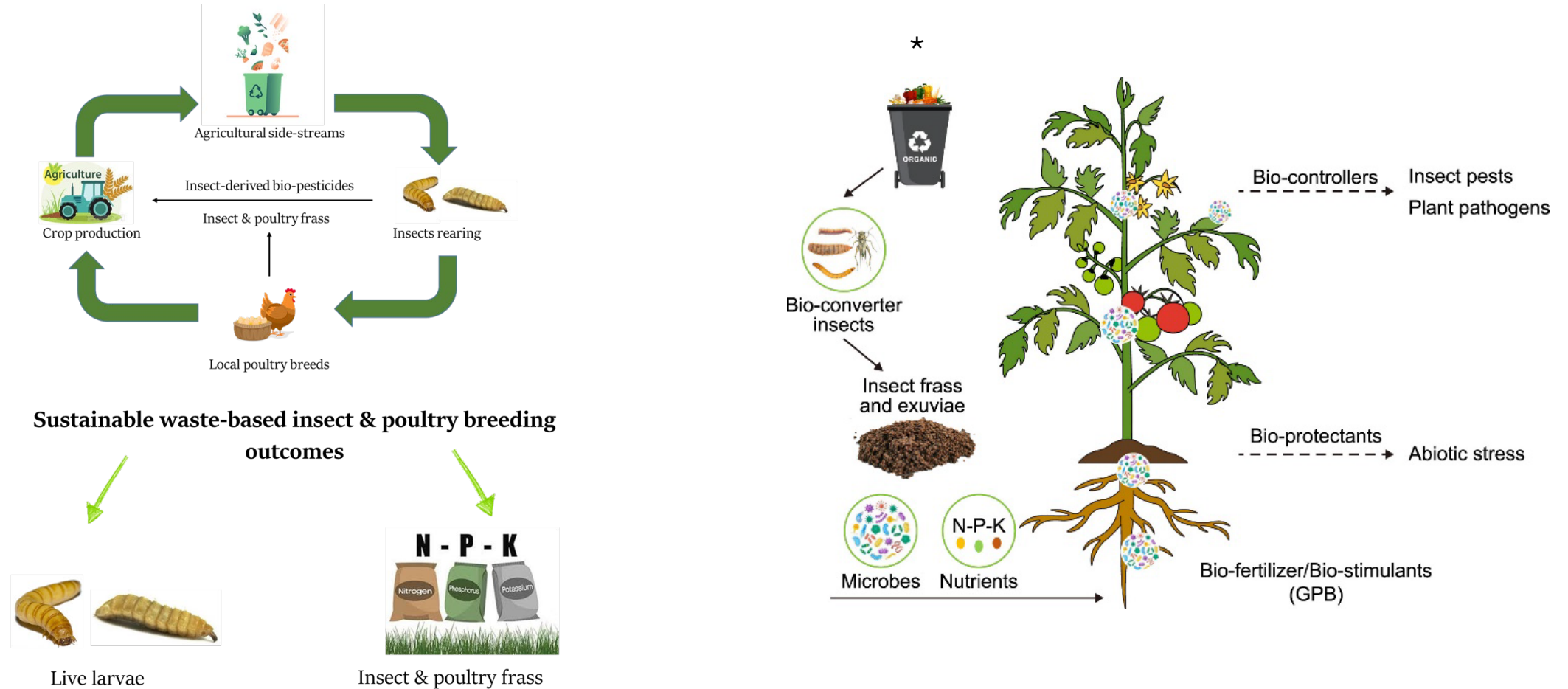
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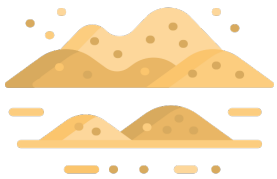


*Source: MANNAA et Al. *Environmental Science and Ecotechnology*, 17, January 2024, 100287

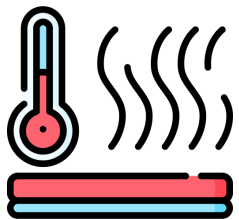
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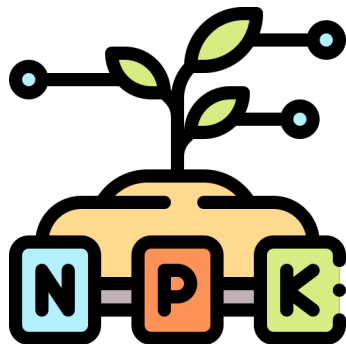
According to the **European Regulation 1925/2021**, amending certain Annexes to Regulation (EU) No 142/2011 as regards the requirements for placing on the market of certain insect products and the adaptation of a containment method, insect frass is intended:



"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."

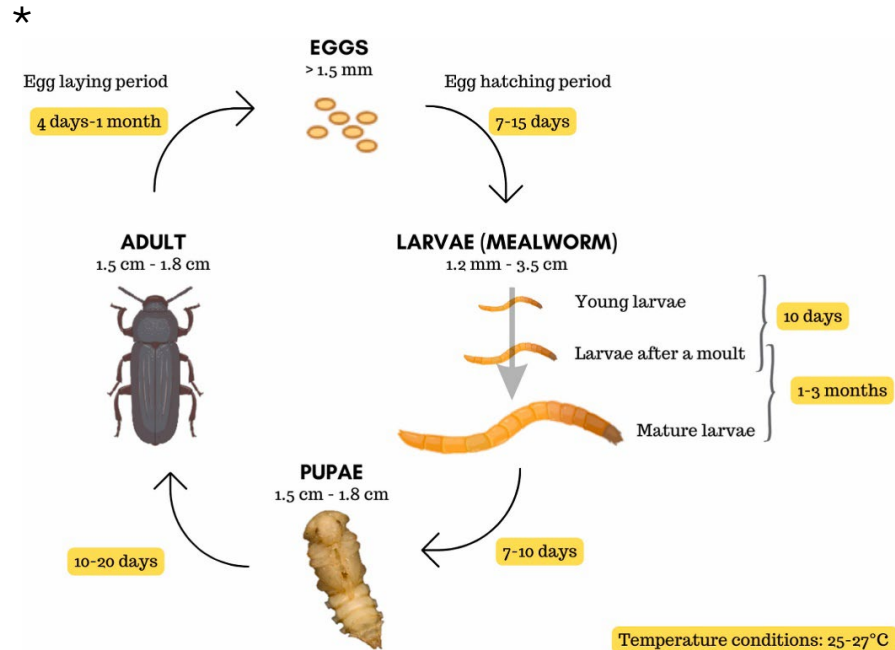


**70°C 1 h
(UE) 2021/1925**



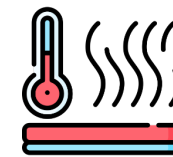
Regulation 2021/1925 setting EU standards for insect frass (i.e. heat treatment of 70 ° C for at least 60 minutes, setting of microbiological standards) entered into force on 28 November 2021.

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MEALWORM FEED

WHEAT BRAN



NPK PROFILE

2-2-3



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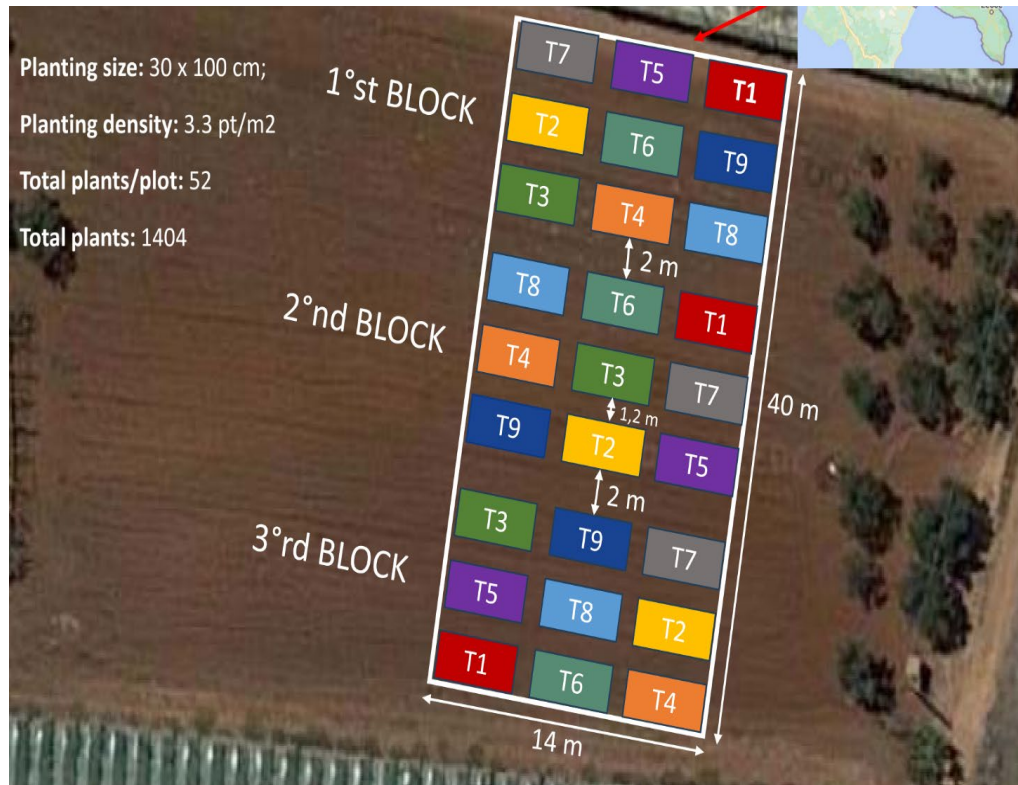
Species: tomato, local variety (cv. “Regina”) in Apulian region (southern Italy)

Growing environment: open field

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



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Treatments		
100% Chemical (CHEM) T1	100% TM frass (TMF) T2	100% organic T3
100% Poultry Manure (PM) T4	75% CHEM / 25% TMF T5	50% CHEM / 50% TMF T6
75% CHEM / 25% PM T7	50% CHEM / 50% PM T8	(Negative) Control T9

Experimental Design: randomized block with 3 replicates

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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% CHEM	100% TMF	100% organic
100% PM	75% CHEM 25% TMF	50% CHEM 50% TMF
75% CHEM 25% PM	50% CHEM 50% PM	Control

Figure 2. Burying of fertilizers before transplanting

Figure 3. Transplant

Figure 4. Growing and Fruit Harvest (August 2023)

Fig. 1



Fig. 2



Fig. 3



Fig. 4

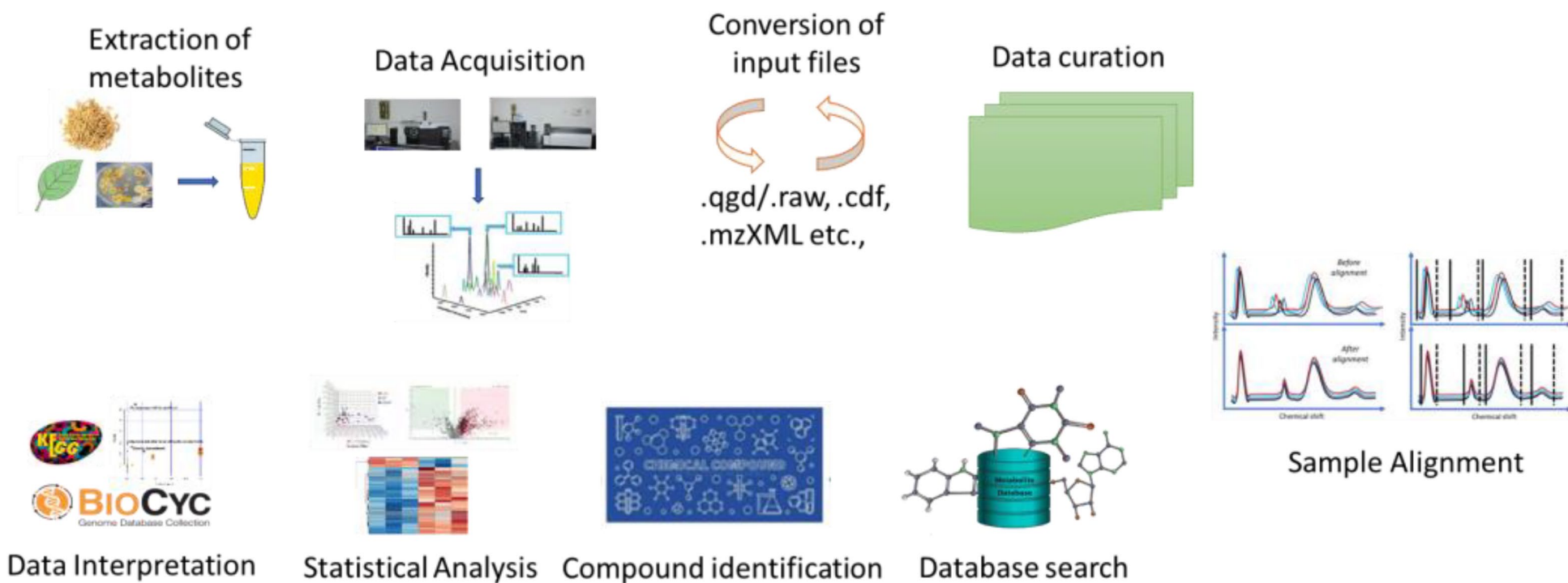


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Metabolomics

A modern approach that combines different techniques of analyses of small molecules (i.e. sugars, amino acids, organic acids, secondary metabolites) in tissues and cells that allows an understanding of the functional roles of specific metabolites in plants' physiology, development, and responses to biotic and abiotic stresses.

Workflow

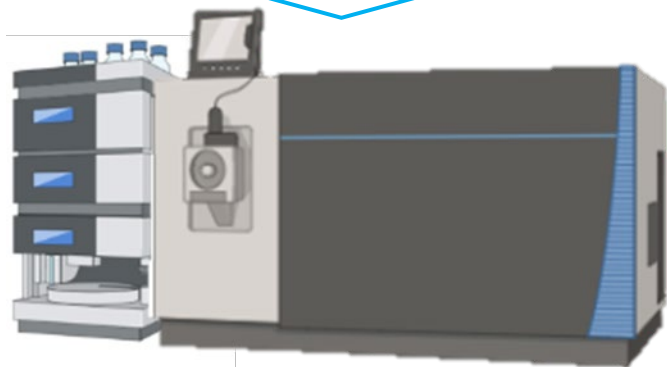


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100 mg of tomato freeze dried powder/ 5 mL Methanol

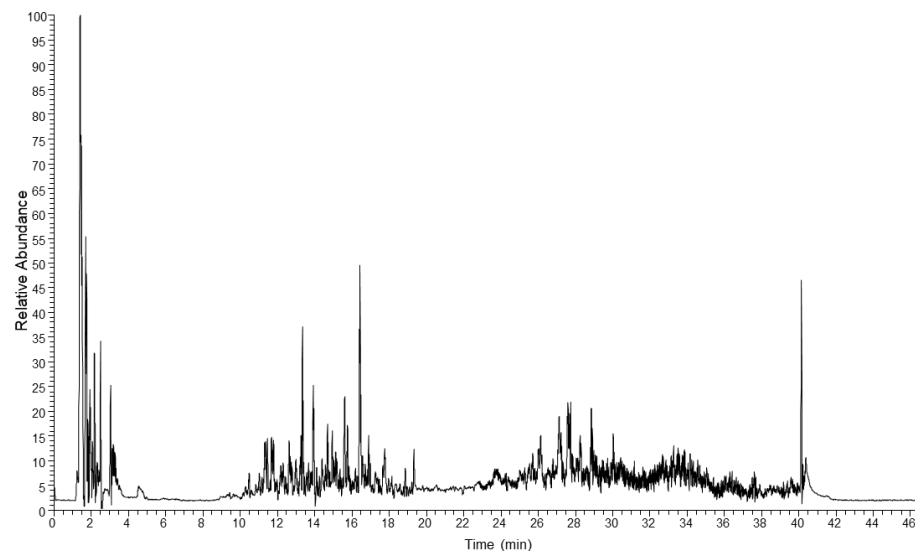


- ☐ Sonication
- ☐ Centrifugation
- ☐ Dilution (water)



Chromatography parameters:

- Dionex 3000 UHPLC
- Solvents: 0.1% formic acid in H₂O (A); ACN (B)
- Column: Luna C18 150×2 mm, 3μm
- Organic gradient 5-100% in 37 min
- Flow: 0.2 mL/min



MS parameters:

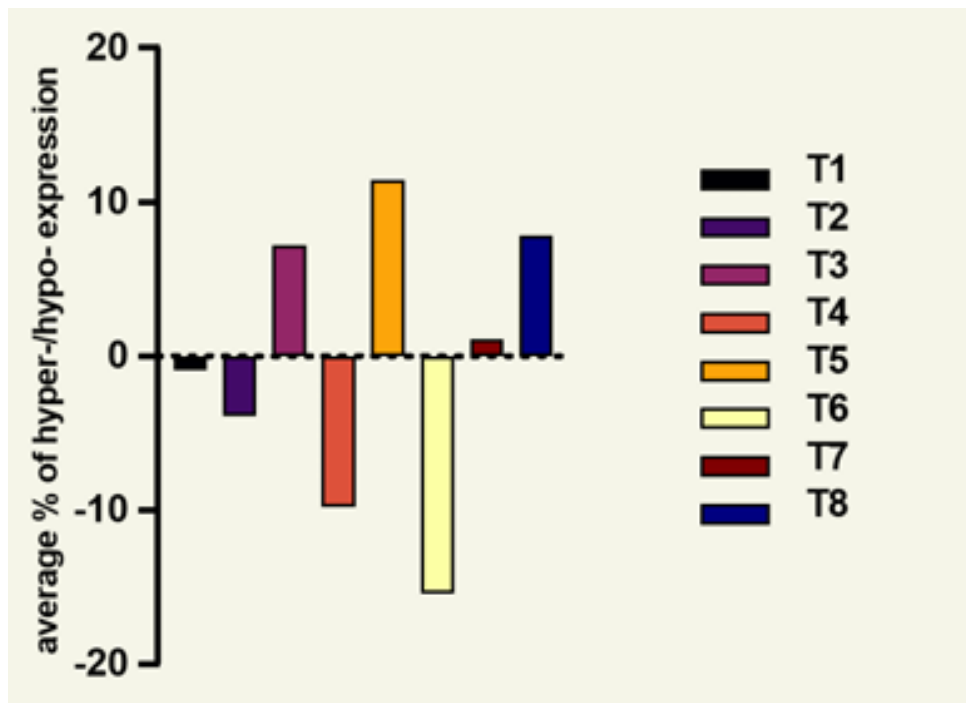
- Polarity: positive and negative ion modes
- Spray Voltage: Positive Ion = 4000 V Negative ion = 3200 V
- Acquisition range *m/z*: 100-1000
- Acquisition type: Full mass, DDA
- Resolution: 30K
- Datatype: profile

MZmine 3



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Metabolomics: Results

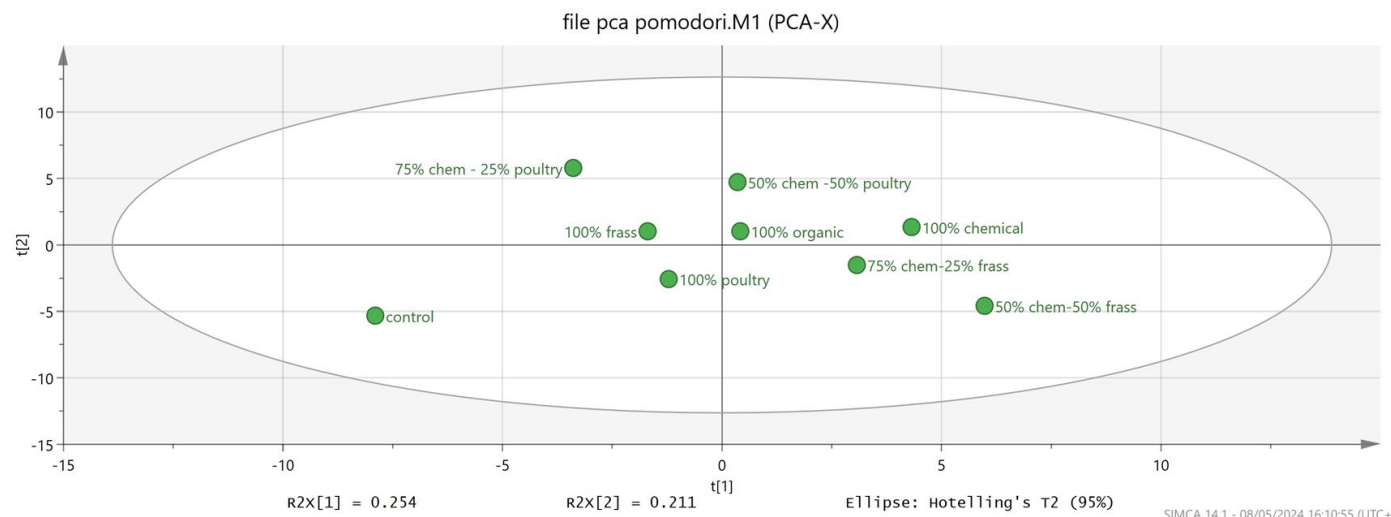


T2: 100% TMF

T6: 50% CHEM – 50% TMF

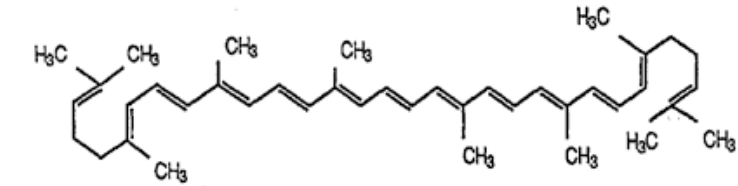
T5: 75% CHEM – 25% TMF

- In total, **72 compounds** were detected in all the samples.
- Concerning the **TM frass treatments (T2, T5, and T6)**, T2 and T6 apparently reduced, while T5 increased the expression of annotated compounds when compared to the negative control treatment (T9).

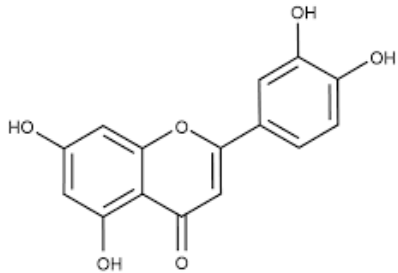


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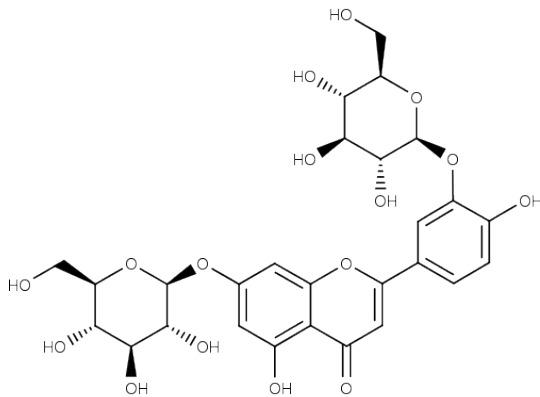
Metabolomics: Results



Lycopene

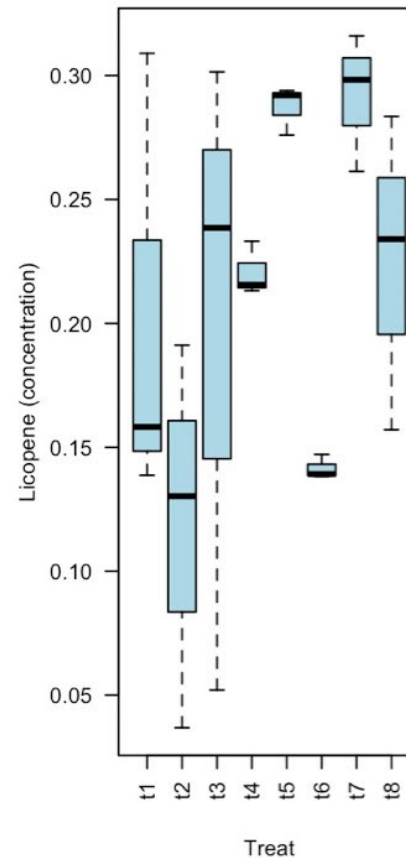


Luteolin

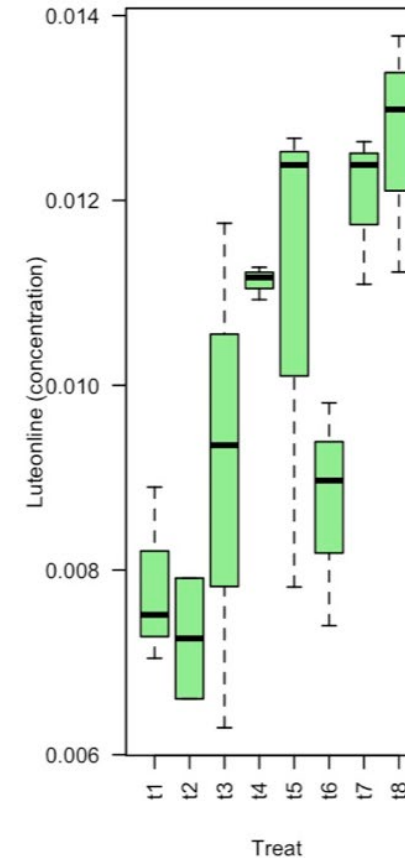


Luteolin 3,7
Diglucoside

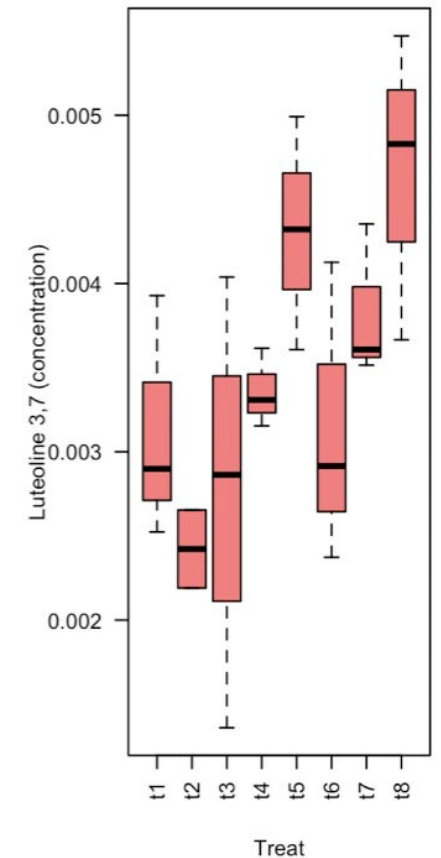
*Lycopene



Luteolin



Luteolin 3,7 DiGluc.



*[LYCOPENE, mg/g] : 25% TMF > 50% TMF > 100% TMF

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Conclusions

The insect TM frass :

- Can have a key role into circular economy and organic fertilization of agroecosystems;
- Can be used as partial or total substitute of chemical fertilizers in plant fertilization;
- at lower % (25-50) replacement level did NOT negatively impact on the nutritional (carotenoids) content of the tomato fruits



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ACKNOWLEDGEMENTS



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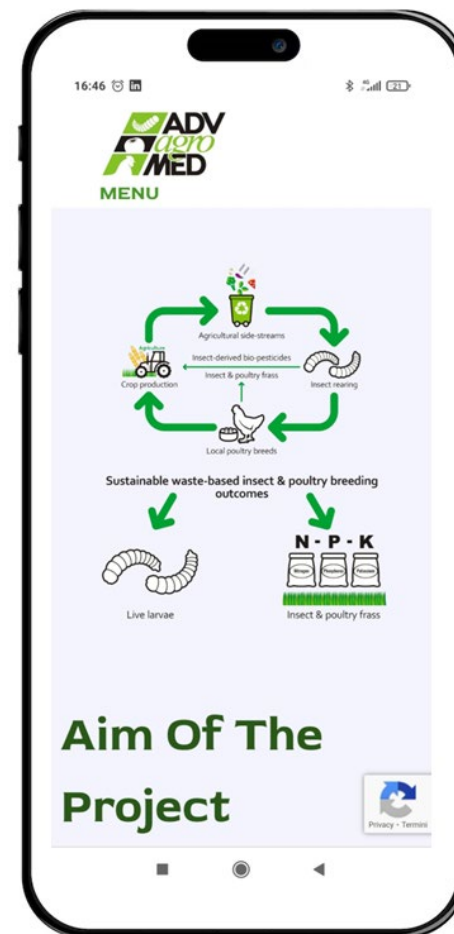


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