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VALORIZATION OF TOMATO WASTE: DEVELOPMENT OF TOMATINE-LOADED NANOPARTICELS FOR SUSTAINABLE PEST CONTROL

Marzorati S [1], Pagnoni S [2], Esposito M [2], Scapuzzi C [1], Maggioni D [3], Passera A [2], Casati P [2], Scaglia B [2]

[1] Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, Via Celoria 2, 20133, Milano, Italy.

[2] Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Via Celoria 2, 20133, Milano, Italy.

[3] Dipartimento di Chimica, Università degli Studi di Milano, Via Golgi 19, 20133, Milano, Italy.

DiSAA DIPARTIMENTO di SCIENZE AGRARIE e AMBIENTALI
ESP Dipartimento di Scienze e Politiche Ambientali
UNIVERSITÀ DEGLI STUDI DI MILANO

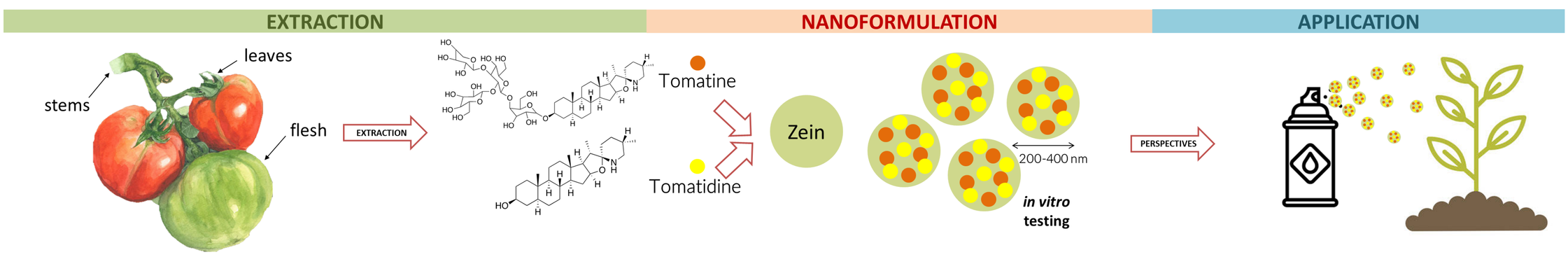
E-mail:
stefania.marzorati@unimi.it barbara.scaglia@unimi.it

SPOKE 8, WP 8.1.1

Spoke 8. Circular economy in agriculture through waste valorization and recycling. WP 8.1: Producing new products to upgrade waste value.
Task 8.1.1. Valorization of the waste by green chemistry to obtain high value molecules or new products.

ABSTRACT

Following the European directives pointing to a reduction in the use of chemical pesticides and as part of an effort to address environmental concerns to enhance the circularity of biomass residues, this project aims to reuse **tomato residues** by extracting bioactive compounds, specifically **tomatine and tomatidine**, derived from unripe tomato fruits and leaves, secondary metabolites known for their antifungal and insecticidal properties [1,2]. Tomatines were encapsulated in zein, a biodegradable and non-toxic biogenic protein sourced from maize in the form of nanoparticles acting as delivery systems, in this way overcoming the issue of tomatine water insolubility. The formulations were tested for their antifungal activity against *Botrytis cinerea*, a common fungal pathogen, in in-vitro trials to understand if the nano-scale carriers could provide increase delivery efficiency of the active principle (AP).



EXTRACTION OF TOMATINE AND NANOFORMULATIONS

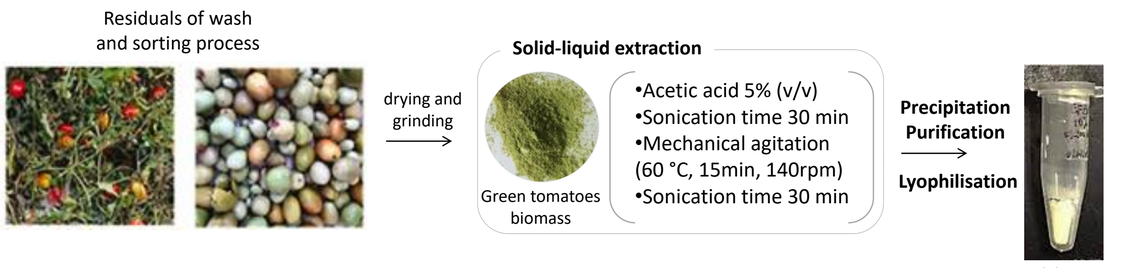


Figure 1. Schematic representation of extraction of all-tomatine followed by purification procedure [3]

NANOFORMULATIONS

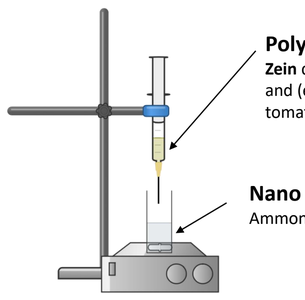


Figure 2. Set up used for zein nanoformulation

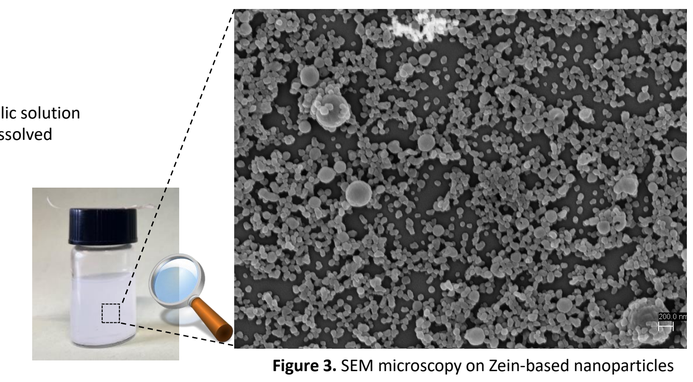


Figure 3. SEM microscopy on Zein-based nanoparticles

RESULTS: extraction and formulation

	Sample	Polymer phase	Nanoprecip. bath	Procedure	Stability by DLS after 14 days	Final concentrations
BLANK (UNLOADED) NANO PARTICLES	Zein_BLK	10 mg Zein in 3 mL EtOH/H_2O (2:1)	5 mL ammonia solution (pH 13)	Polymer phase dropped into solution while magnetically stirring the nanoprecipitation bath. Ethanol removal from suspension overnight..	Stable	[zein]=**1.33 mg/mL**
TOMATINE LOADED NANO PARTICLES	aT_Zein	10 mg Zein in 3 mL EtOH/(H_2O + 0.5% acetic acid) (2:1) + 1.2 mg alfa-tomatine	5 mL ammonia solution (pH 13)		Stable	[zein]=**1.7 mg/mL** [alfa-tomatine]=**0.2 mg/mL**
	TD_Zein	10 mg Zein in 3 mL EtOH/(H_2O + 0.5% acetic acid) (2:1) + 1.2 mg tomatidine				[zein]=**1.7 mg/mL** [tomatine]=**0.2 mg/mL**

Table 1. Nanoformulations experimental details

EXTRACTION OF ALL-TOMATINE:

- The extraction with acetic acid and the successive ammonia precipitation enabled the production of all-tomatine enriched fraction starting from the residues of the tomato cannery industry.
- The results showed that the percentage of extraction yield by green fruits and stems+leaves was 0.58 ±0.1 and 0.09 ±0.07 %, respectively. In terms of total tomatine, 989 mg g^{-1} extract was found in green fruit and 1053 mg g^{-1} extract in stems+leaves. The α-tomatine was more abundant than the tomatidine in green fruit.

NANOFORMULATIONS:

- Preliminary results on nanoparticles loaded with tomatines commercial standards are promising, with an **optimal range of size** distributions in nanometer range.
- Nanoformulation **stability** evaluations were successful: over 14 days nanoparticles exhibited an unchanged hydrodynamic diameter, as measured by DLS.

RESULTS: ANTIFUNGAL ACTIVITY AGAINST *B. cinerea*

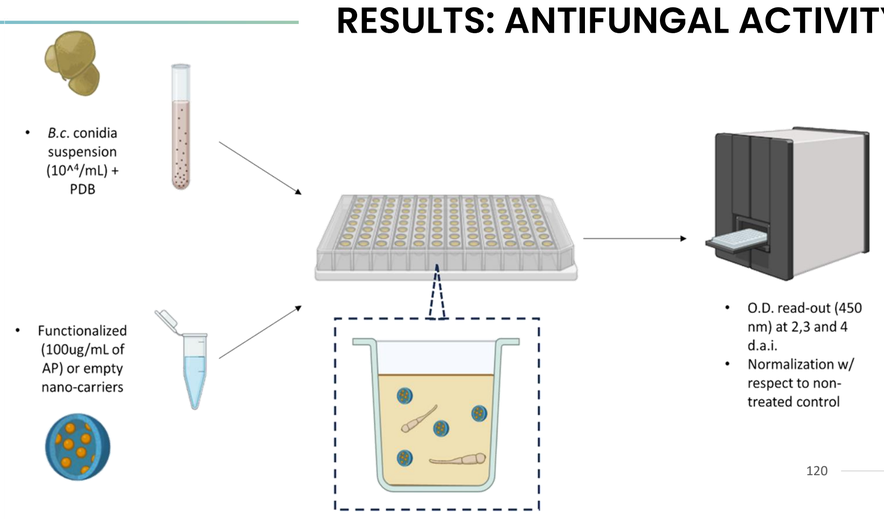


Figure 4. Set up used for O.D. measurement

Percentage of inhibition (POI)

- Estimation of POI: Optical density (O.D.) was measured at t0, 2,3 and 4 day after inoculum (d.a.i.), incubating the plate at 22° C. At each time point and for each thesis ΔO.D. was evaluated with respect to t0. POI was estimated as:

$$(1-(\Delta OD_T/\Delta OD_C))*100$$

- ΔOD_T = O.D. difference for the treated samples
- ΔOD_C = O.D. difference for untreated samples

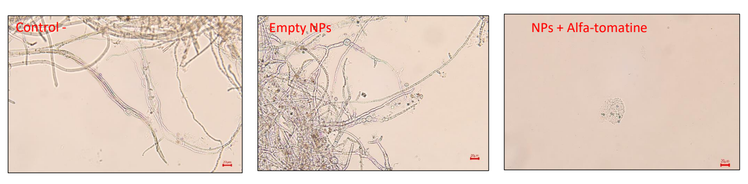
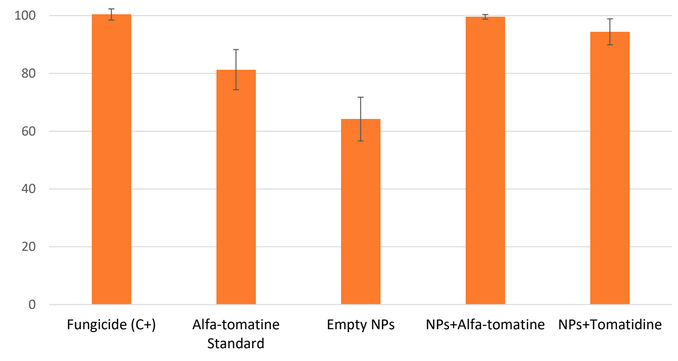


Figure 6. Wet-mounting sample observation at 4 d.a.i., 20x enlargement. From top to bottom: untreated samples, samples treated with empty NPs and samples treated with functionalized NPs.



ANTIFUNGAL ACTIVITY:

- The antifungal activity of α-tomatine or tomatidine-functionalized zein NPs is comparable to the gold standard fungicide. Moreover, the loaded active principle shows increased efficacy than the unloaded form, indicating increased bioavailability from the nano-carrier. Empty NPs exhibit a transient and partial inhibitory effect that progressively attenuates over time and ceases within 7 days (data not shown).

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