#### Proceedings

# Innovative functional plastic films as cover of stone wool grow blocks and their effect on tomato hydroponic cultivation

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**Abstract:** Alkaloid compounds derived from tomato cultivation wastes (leaves, stems etc) are proposed as natural pesticides in this study. The tomato waste derived alkaloids (tomatidine and tomatine) were encapsulated in LDPE films through extrusion process, in order to develop polymeric films with pesticide action as covers of stone wool grow blocks used in hydroponic tomato cultivation. Reduced attraction of mealybugs and the presence of its larvae in the leaves in relation to the control culture as well as improved productivity was observed, with the total marketable production of the tested plants to be higher.

**Keywords:** Alkaloids; antimicrobial properties; natural pesticides, polymeric films, application in the field

# 1. Introduction

Pesticides protect crops from pest infestations and yield reduction. Although, pesticides pose potential risks to food safety, the environment, and all living things since they can emigrate from treated fields to air, other land, and water bodies. On 12 May 2021, the European Commission adopted the EU Action Plan: "Towards a Zero Pollution for Air, Water and Soil" a key deliverable of the European Green Deal, targeting to minimize the use and risk of chemical pesticides by 50% by 2030 [1]. Extensive use of chemical pesticides can lead to chemical pollution of the soil and the water used for irrigation [2-4] while the farmers are exposed to high chemical hazards [5]. Also, the accumulation of pesticides' residues in food products can cause serious health problems to the consumers, especially in the case of food that are mainly consumed fresh, like tomatoes [2]. Under this frame, the presented study proposed the use of alkaloid compounds (tomatidine and tomatine) derived from tomato cultivation waste (leaves, stems etc) as natural pesticides. Plants, naturally, have their own protective mechanism against pathogen microbes [6, 7]. In Solanaceae family plants, alkaloids protect them from insect attacks while offering fungal and nematode resistance. Based on previous studies, tomatine has reported fungicidal properties and is mainly located in the stems and leaves of the tomato plant [7]. The alkaloids in Solanaceae family plants protect the plant from insect attack and offer fungal and nematode resistance [8].

#### 2. Materials and Methods

2.1 Materials

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**Copyright:** © 2022 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/license s/by/4.0/). Residues of tomato cultivation (leaves, stems, green and red tomato fruit) were provided by AGRITEX Energy S.A. while low density polyethylene pellets were provided by ACHAIKA PASTICS S.A. Ammonium hydroxide (NH<sub>4</sub>OH), hydrochloric acid (HCl) were purchased from Sigma-Aldrich. Ethanol and acetic acid were of analytical grade, methanol was of HPLC grade and were purchased from Fisher Scientific.

# 2.2 Methods

## 2.2.1 Extraction of alkaloids

Ultrasound assisted extraction (UAE) (XO-SM50 Ultrasonic Microwave Reaction System, China) was used for the receipt of the tomato residues alkaloid content. Ethanol: Acetic acid (95:5 v/v) mixture was used as the extraction solvent while the solvent/ tomato residues ratio by weight was 20:1. The UAE operating conditions were: 25 kHz frequency at 450 Watt and temperature 25°C. After the extraction a centrifugation step took place at 3000 rpm for 10 min and the supernatant was collected and stored in -80 °C (Ultra-Low Temperature Freezer Panasonic MDF-U3386S, Japan). The deep frozen extracts were freeze dried in Leybold-Heraeus GT 2A (Germany) for 8 h in high vacuum (3 mbar).

## 2.2.2 Alkaloids detection

The choice of the best extracts for use as natural pesticides was made after the detection of tomatine and tomatidine with High Pressure Liquid Chromatography (HPLC) with a photodiode Array Detector (PDA) (Shimadzu LC-2030C, Japan). For this study, leaves, stem, green and red tomato freeze dried extracts were dissolved in 0.2 N HCl and the alkaloids were precipitated with 5% NH<sub>4</sub>OH until a pH = 10 was reached. Subsequently, the mixture was centrifuged at 3000 rpm for 20 min at 4 °C and the supernatant was rejected. The washing process was repeated twice. Any residual supernatant was removed by evaporation at 45 °C using a rotary evaporator (Buchi R200 rotavapor) and the collected precipitate was redissolved in methanol. Prior to HPLC, the samples were filtered through a 0.45 μm PTFE syringe filter. The column used was Phenomenex Luna® 5 μm C18 (2) 100 Å, LC Column 250 x 4.6 mm, the mobile phase was methanol (isocratic flow) with a flow rate of 1 ml/min and the injection volume was 10 µL. The absorbance was detected at 200 nm. All samples were studied in triplicates. The content of alkaloids was calculated using calibration curves of standard tomatine and tomatidine samples. The calibration curve was well linear over the concentration range from 0.02 to 1 mg·mL<sup>-1</sup>(r=0.9987); and the limit of detection was 0.01 mg·mL<sup>-1</sup>.

## 2.2.3 Polymeric film development

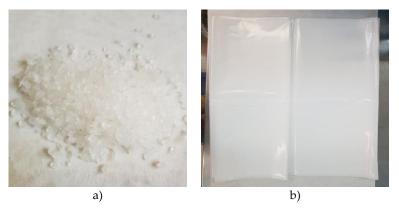
Based on the HPLC results, the extracts with the higher alkaloid content were used as bioactive agents for encapsulation in LDPE. for this purpose, two extrusion processes took place. Firstly, a twin-screw extruder (Prism EuroLab 16 mm) was used for the incorporation of the selected bioactive extracts in the LDPE. The screw speed was 50 rpm, the extrusion temperature was 180-190°C while the concentration of the bioactive agent in the extrusion product was 20% w/w. This product was pelletized with a pelletizer Brabender and was incorporated in a blown film extruder. More specifically, the pellets with the encapsulated alkaloids were mixed with pure LDPE pellets for the production of the final polymeric film. The screw speed was 50 rpm and the extrusion temperature was 170-175°C. The content of the encapsulated alkaloids in the final polymeric film was 1% w/w.

# 2.2.4 Application in the field

The experiments took place in tomato hydroponic cultivation greenhouses of AGRITEX ENERGY S.A. in Imathia, Greece. The cultivation period of reference started on July 2020 and ended on January 2021. The total area of application was 294.4 m<sup>2</sup> devided equally in two parts, the first was the control tested area where the tomato cultivation took place in commercial Grodan stone wool, whereas the second area was the experimental application of the innovative polymeric film with encapsulated alkaloids as cover of the stone wool. The stone wool dimensions in both cases were 120X20X7.5cm. The studied cultivation was Brioso tomato cultivar. The evaluation of cultivation performance was based on the following parameters: a. Total plant yield (kg/m<sup>2</sup>), b. Marketable yield of plants (kg/m<sup>2</sup>) ,c. Non-marketable yield of plants (kg/m<sup>2</sup>), d. Average catches of mealybugs in chrome-adhesive traps (5 yellow traps per operation) and e. Phytosanitary level, in all the plants of the experimental piece, visual and with a stereoscope.

# 3. Results

The measurement with HPLC showed that leaves followed by stem extract had the highest amount of  $\alpha$ -tomatine with a much lower concentration in the green tomato fruit extract, which is in agreement with literature about tomatine [7, 9]. Respectively for tomatidine, it was mainly detected in leaves and green fruit extracts with stem showing a very small content. As for the red tomato extract, it was not detected, because of its degradation during the fruit's ripening [10, 11]. Based on these results a mixture of leaves, stem and green tomato extracts was used as the bioactive agent for encapsulation with the extrusion method. The pellets produced from the twin screw extrusion (Figure 1a) were incorporated in a blown film extruder and the produced film (Figure 1b) were used as cover of stone wool grow blocks on tomato hydroponic cultivation (Figure 2).

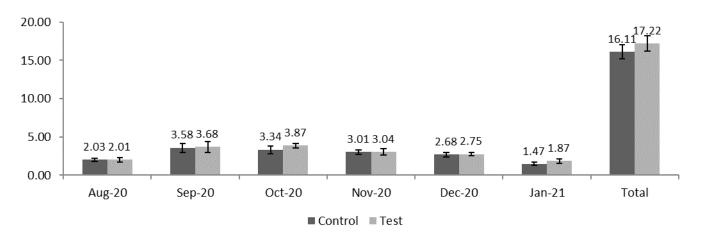


**Figure 1.** Presentation of a) the pellets of the encapsulated tomato leaves-stem extract in LDPE and b) the final polymeric film with 1% bioactive compounds



**Figure 2.** Application of the polymeric films with the encapsulated bioactive agents on tomato hydroponic crops cultivation.

In Figures 3-6, the results from the application of the innovative films in the tomato hydroponic crops are presented in comparison with the same crops covered with commercial stone wool grow blocks.



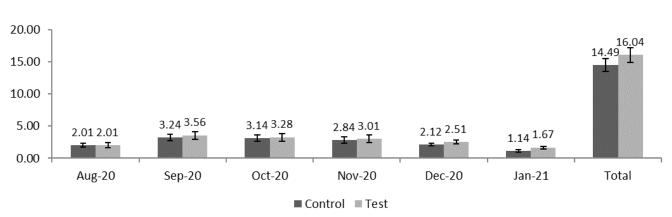


Figure 3. Total yield (kg / m<sup>2</sup>) per experimental piece per month.

Figure 4. Marketable yield (kg / m<sup>2</sup>) per experimental piece per month.

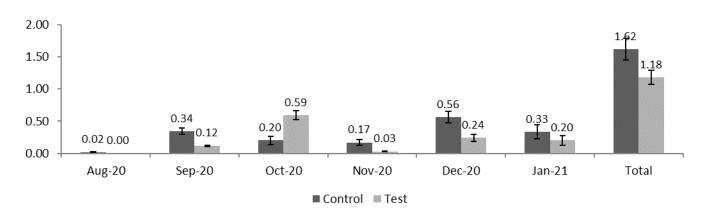


Figure 5. Non marketable yield (kg / m²) per experimental piece per month

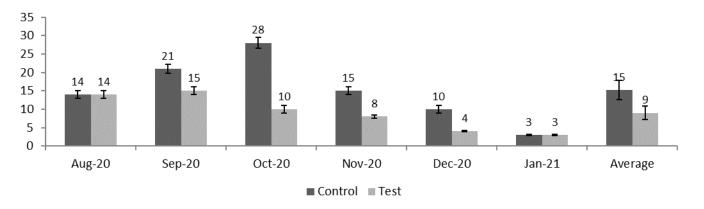


Figure 6. Average catches of mealybugs in chrome-adhesive traps per experimental piece per month.

Table 1. Phytosanitary	v level pe	er experimental	piece sample	per month.
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Sample/ month	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20	Jan-21
Control	Good*	Presence of mealybug larvae (average: 8±2 / leaf)	Presence of mealybug larvae (average: 5±2/ leaf)	Presence of mealybug larvae (average: 2±1/ leaf)	2 plants infected by botrytis	Good
Test	Good	Presence of mealybug larvae (average: 6±2 / leaf)	Presence of mealybug larvae (average: 3±1/ leaf)	Good	3 plants infected by botrytis	Good

\*Good phytosanitary level: No presence of mealybug larvae, absence of infected plants

# 4. Discussion

As it is observed in Figures 3-6, test samples gave better results than control. More specifically, test plants proved to be more productive compared to the control ones with higher total marketable yields. Moreover, reduced attraction of mealybugs and the presence of larvae on the leaves was observed in the control plants, although: 14 prosperous / 5 traps).

b) The plant protection operations that followed (biological and chemical) in both the control and the test were common. c) climatic conditions and hydro-lubrication were common.

## 5. Conclusions

Based on the results of the presented study, it is observed that the innovative polymeric films with encapsulated alkaloids derived from tomato cultivation by-products as cover of the stone wool show significant activity towards the pesticide control of the Brioso tomato cultivation. This study served as a model for the practical application of the circular economy and zero pollution crop protection.

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Data Availability Statement: Not applicable

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Conflicts of Interest: The authors declare no conflict of interest.

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