

Herbal essential oils: Potential for development as low-risk pesticides, plant growth promoters and produce sanitizers.

ENTERPRISES/0916/0025

Start Date: 01/12/2018

End Date: 30/11/2020

Total Budget: 214.672,80 €

Requested budget: 197.231,00 €



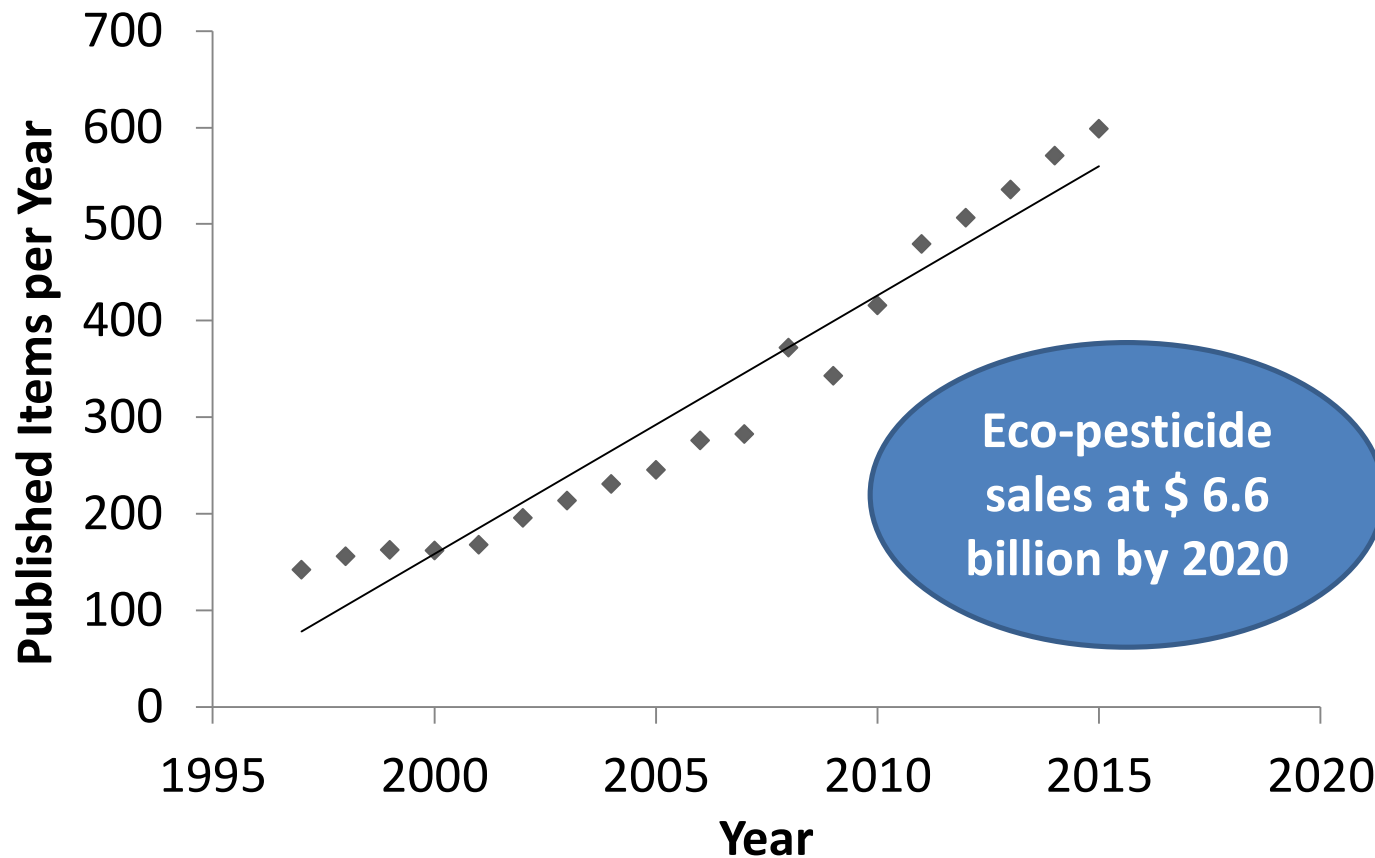
The project is co-funded by the European Regional Development Fund and the Republic of Cyprus.

Plant essential oils (EOs)

- Synthesized through secondary metabolic pathways of plants
- Communication and defense molecules – pollinator attraction, defense against pests and pathogens
- Scent and flavor of aromatic plants
- Medicinal, anti-microbial and pesticidal properties (Gorgi 2009)

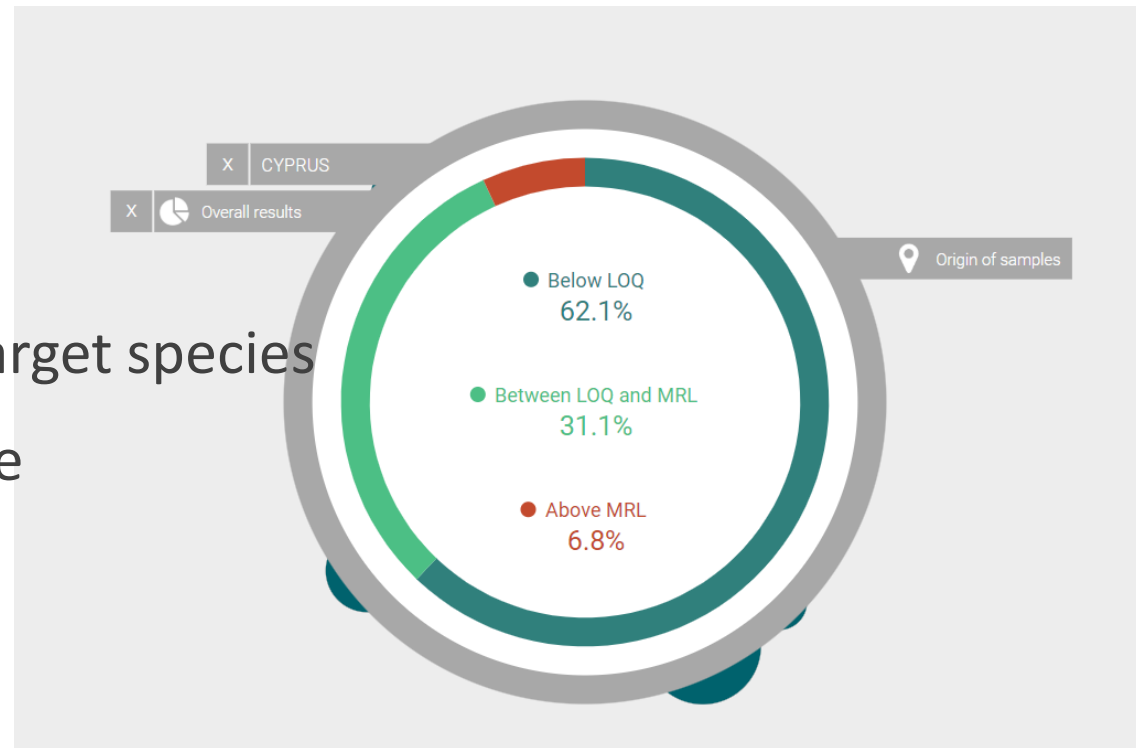


Increasing interest in the insecticidal and fungicidal activities of EOs



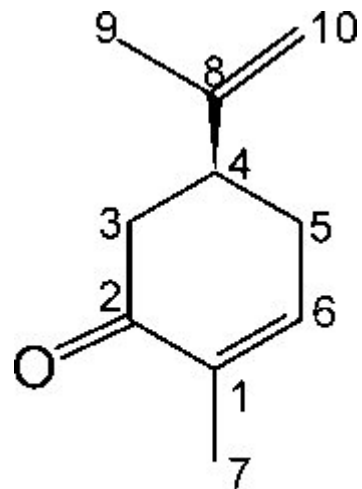
Why the increasing interest in EOs?

- Pesticide residues in food
- Negative effects on non target species
- Development of resistance



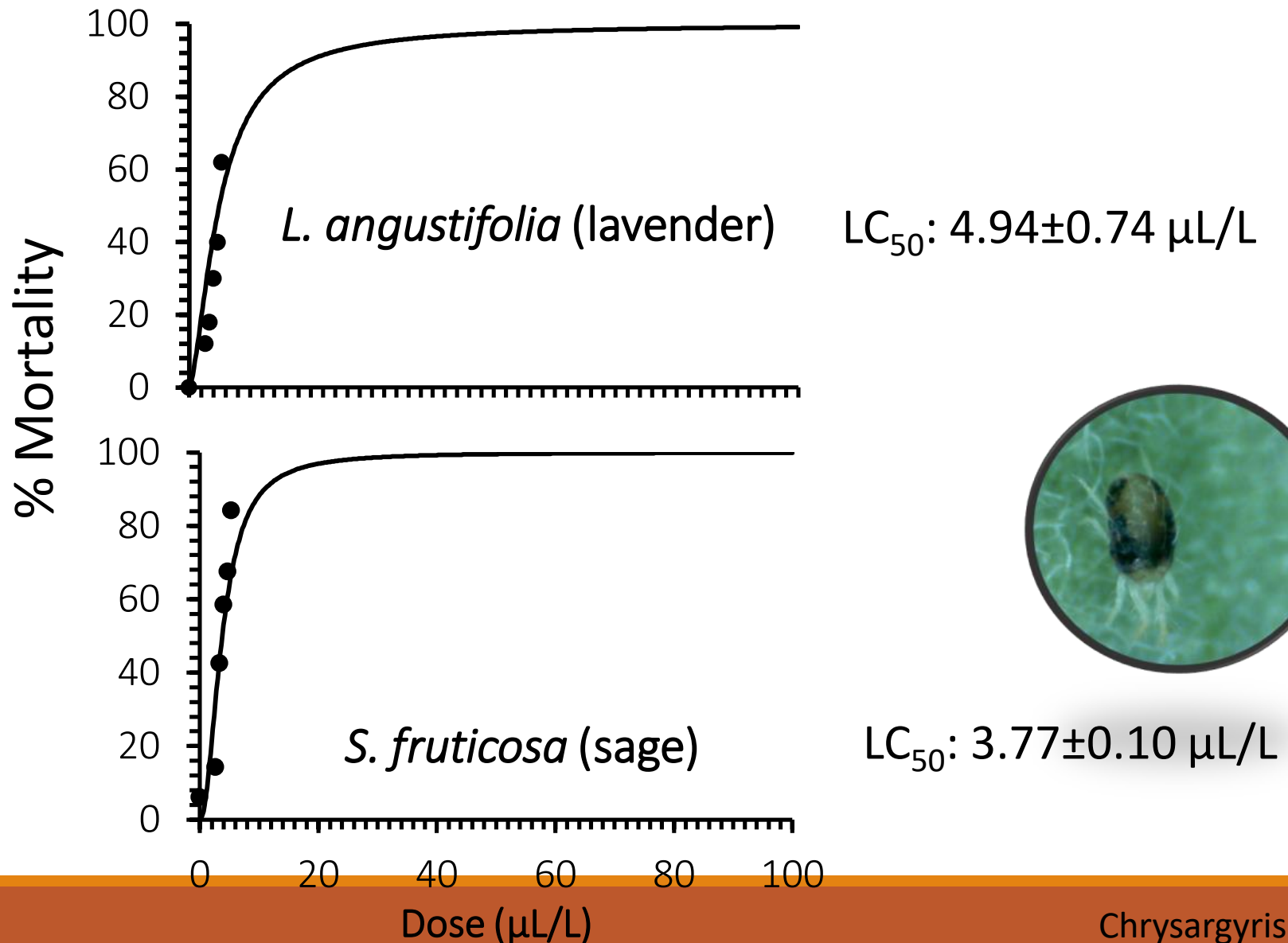
EO activity against pests

- Inhibition of P450 cytochromes (CYPs), e.g. piperamides from *Piper spp.*
- GABA receptors - thymol from *Thymus vulgaris*
- Inhibition of cholinergic system (AChE) – S-carvone from *Mentha spicata*
- Modulation of octopaminergic system -
a-terpineol from *Pinus silvestris*

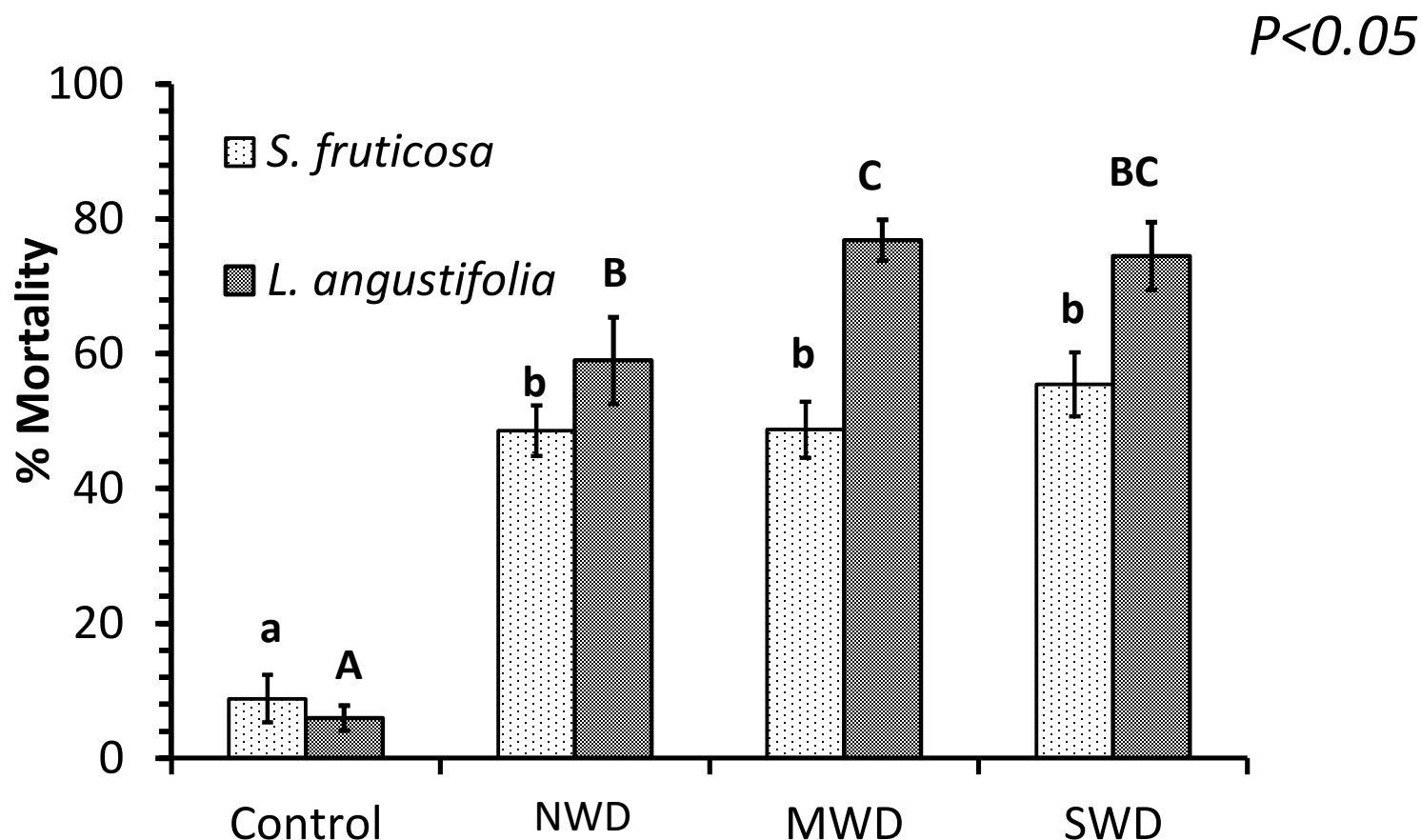


S-carvone

Toxicity of essential oils to two-spotted spider mite (*Tetranychus urticae*)



Plant-water stress increased essential oil toxicity for lavender but not sage



Advantages of EOs

- Potential effectiveness against range of pests
- Multiple mechanisms of action?
- Low toxicity against humans / other non-target species (invertebrates?)
- Simple production processes

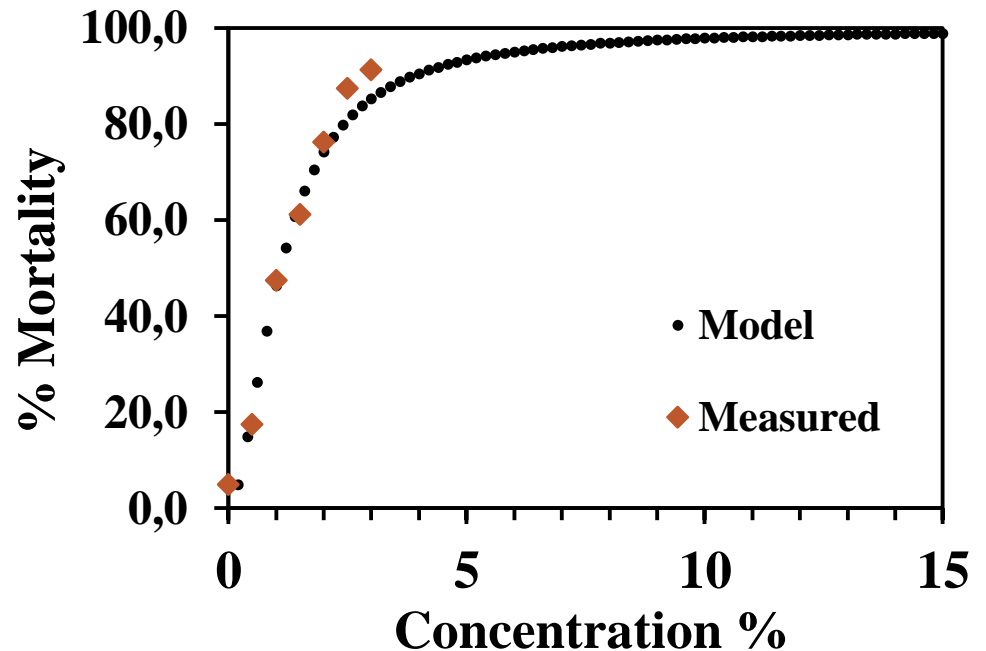


Key challenges

- Complex and costly authorization process – potential for simplification for EOs with proven history of use in the food industry / cosmetics or medicine
- Loss of efficiency in the field – need for efficient stabilization process
- Homogeneity of composition of EOs...

PlantSafe – the product

- Product: A mixture of two essential oils – eucalyptus and rosemary
- Demonstrated toxicity against *T. urticae* in laboratory trials



PlantSafe – Target Pests

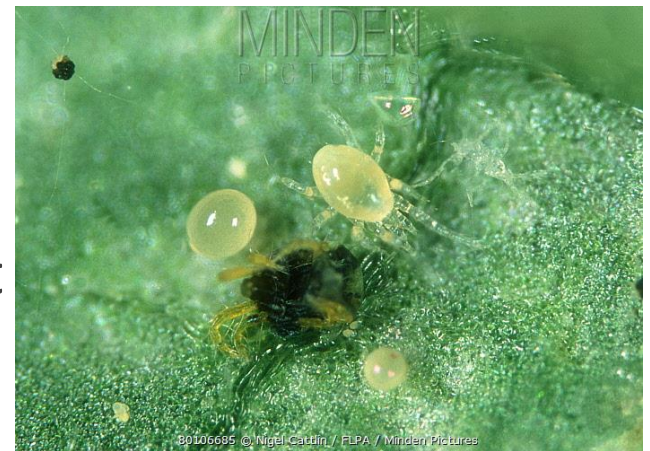
1. *Tetranychus urticae*
2. *Bemisia tabaci*
3. *Myzus persicae*

- Key pests of vegetable crops
- Highly resistant to many active ingredients
- Need for alternatives



Evaluation of effects on-target species

- Predatory mite *Typhlodromus pyri*
- Key predator in agro-ecosystems
- One of two natural enemies included in evaluations for registration of new plant protection products



Methodology

1. Laboratory trials to determine:
 - Dose-response curves and LC_{50}
 - Sublethal effects (development, fecundity, longevity)
2. Greenhouse trials to determine effectiveness



Expected results

- Baseline data on product effectiveness against the three target pests
- Baseline data on product effects on the non-target species (*T. pyri*)
- Develop a strategy for next stage of product commercialization



English EN

Search

European Commission > Food, farming, fisheries > Food Safety > Plants > Pesticides > Authorisation of Plant Protection Products >

Plants

PESTICIDES

EU Pesticides database

Sustainable use of pesticides

Approval of active substances

Procedure to apply for authorisation of a
PPP

A zonal system of authorisation operates in the EU to enable a harmonised and efficient

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Comments – Questions?

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Χρήση και επίδραση αιθέριων ελαίων στην ανάπτυξη των φυτών και στη μετασυλλεκτική διαχείριση νωπών προϊόντων

*Uses and effects of EO's on plant growth and
postharvest preservation of fresh produce*

Δρ Νικόλαος Τζωρτζάκης



The project is co-funded by the European Regional Development Fund and the Republic of Cyprus.

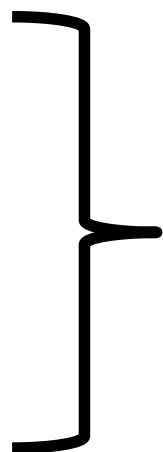


Introduction

Nowadays, there is an increasing interest both in industry and in scientific research for Medicinal and Aromatic Plants (MAP) due to their strong antioxidant and antimicrobial properties, which exceed many commonly used natural and synthetic antioxidants.

These properties are related to the presence of vitamins, carotenoids, chlorophyll, catechins, minerals, etc. and render aromatic/medicinal plants and/or their antioxidant components as food preservatives as well as necessities in cosmetics/pharmaceuticals applications.

Introduction



- Fresh biomass
- Dry biomass
- Essential oils (EO)
- Hydrosol
- Others

EO can be used for fruit and vegetables preservation either directly by decay reductions or indirectly by enhancing produce resistance to pathogens.

Introduction

Vegetable consumption is increased the last decades, with great demands in tomato, pepper and eggplant. Perishable vegetables are characterized by a high water and nutritional content and nearly neutral pH, which make them an ideal habitat for microorganisms.

Postharvest losses of fresh produce represent a critical component affecting global food losses and waste.

Mainly fungi, but also bacteria are responsible for postharvest fresh produce decay, with *Botrytis cinerea* to be one of the main postharvest pathogen.



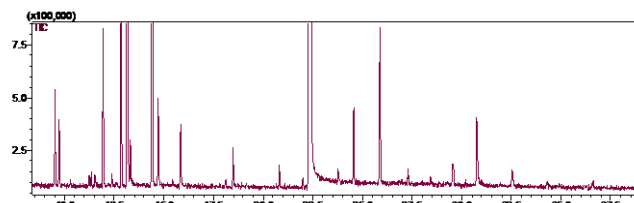
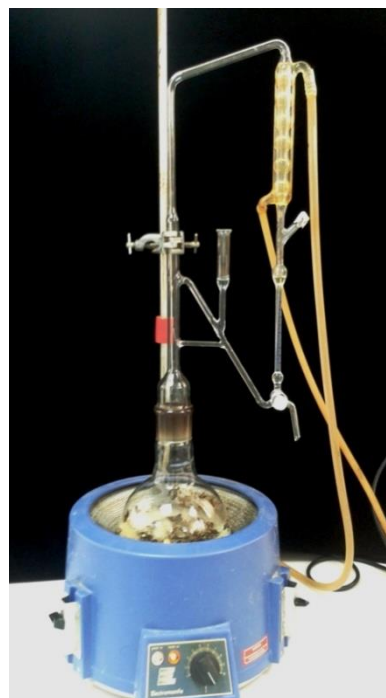
Symptoms of grey mould infection caused by *B. cinerea* as it appears externally on eggplant, cherry tomato and pepper fruits.

Introduction

There is a need for:

- Alternative way of food preservation (avoiding chemical agents)
- Ensures product quality (reduction of microbial load)
- Improves/preserves the organoleptic characteristics of fresh produce

Dittany (*Origanum dictamnus* L.)



No	Compound	R.I. ^b	Retention time	Percentage (%) Composition
1	a-Thujene	930	9.507	0.68
2	a-Pinene ^a	939	9.746	0.56
3	Sabinene	975	11.253	0.06
4	b-Pinene ^a	979	11.353	0.10
5	1-Octen-3-ol	979	11.530	0.10
6	b-Myrcene	990	11.933	1.12
7	a-Phellandrene	1002	12.402	0.10
8	a-Terpinene	1017	12.855	1.48
9	p-cymene ^a	1024	13.163	12.65
10	Limonene ^a	1029	13.302	0.37
11	g-Terpinene ^a	1059	14.400	7.11
12	cis-Sabinene hydrate	1070	14.701	0.72
13	Linalool ^a	1096	15.841	0.54
14	Terpinen-4-ol	1177	18.479	0.29
15	Thymoquinone	1252	20.816	0.19
16	Thymol ^a	1290	22.007	0.11
17	Carvacrol ^a	1299	22.382	70.01
18	a-Cubenene	1348	23.768	0.08
19	a-Copaene	1376	24.565	0.58
20	b-Caryophyllene	1419	25.883	1.39
21	β-Bisabolene	1505	27.302	0.14
22	δ-Cadinene	1523	29.561	0.29
23	Thymohydro quinone	1555	30.761	1.00
24	Caryophyllene oxide	1583	32.548	0.20
Total (%)				99.87
Monoterpene hydrocarbons				24.33
Oxygenated monoterpenes				72.86
Sesquiterpene hydrocarbons				2.68

Dittany oil for the preservation of fresh produce

EO: Dittany (*Origanum dictamnus* L.)

Fresh Produce: eggplant, cherry tomato, pepper

Fungi: *B. cinerea*

Time: 0-24-48-144 h

Concentrations: 0-50-100-250-500 $\mu\text{L/L}$ –vapour application

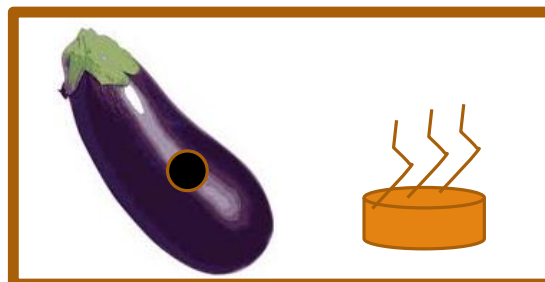
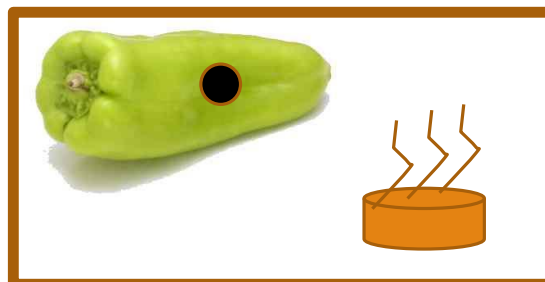
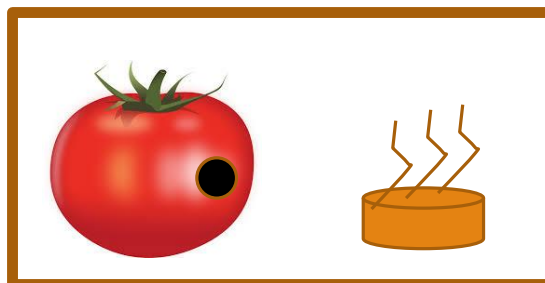
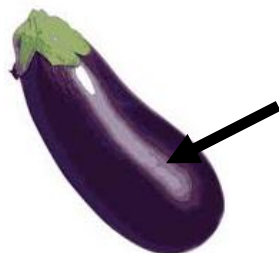
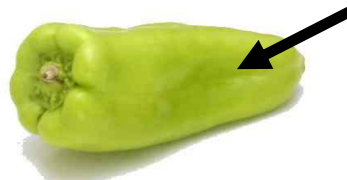
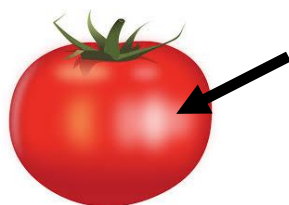


Inoculated fruits (eggplants, cherry tomatoes and peppers) placed in the containers.

Dittany oil for the preservation of fresh produce

Sustain effect-SE

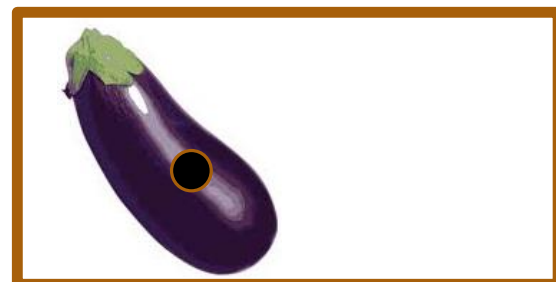
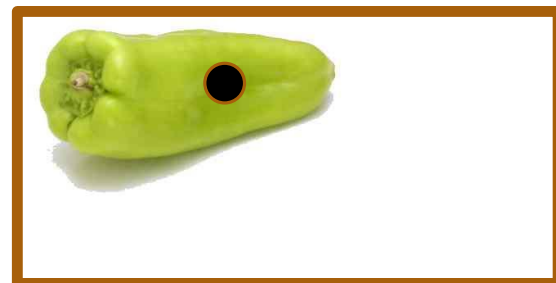
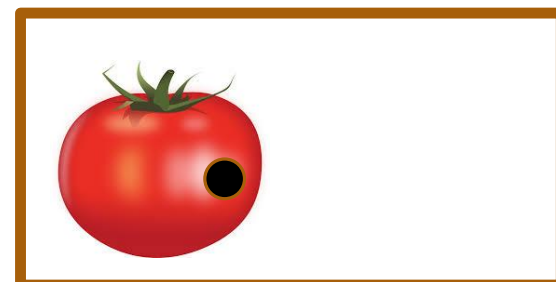
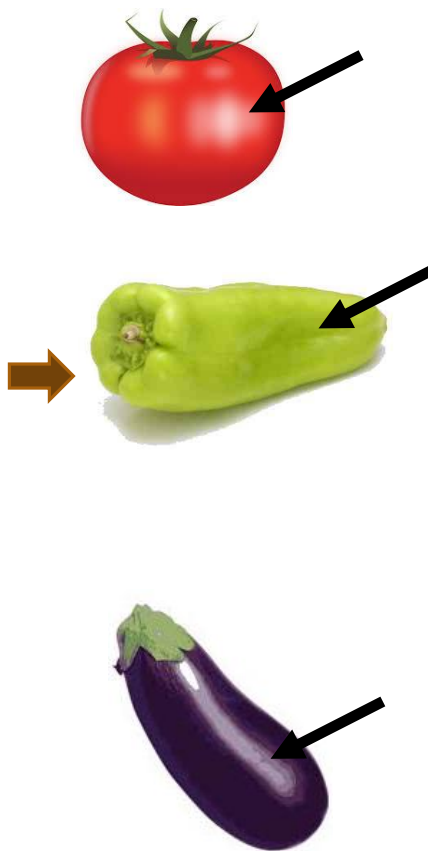
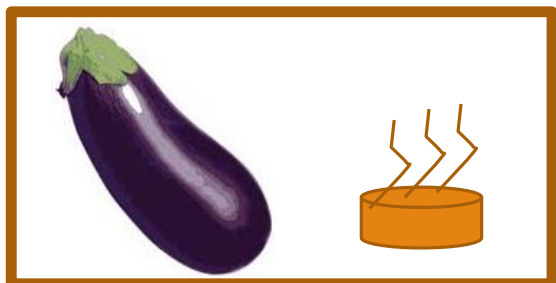
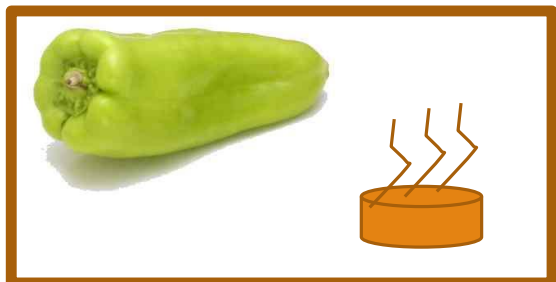
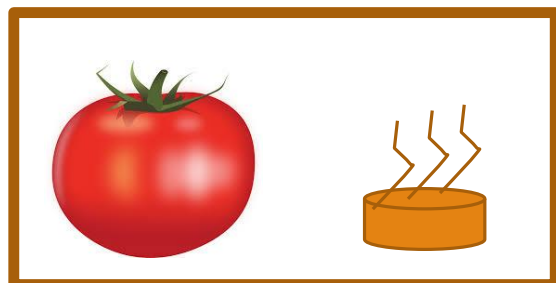
Fruits or PDA media



Dittany oil for the preservation of fresh produce

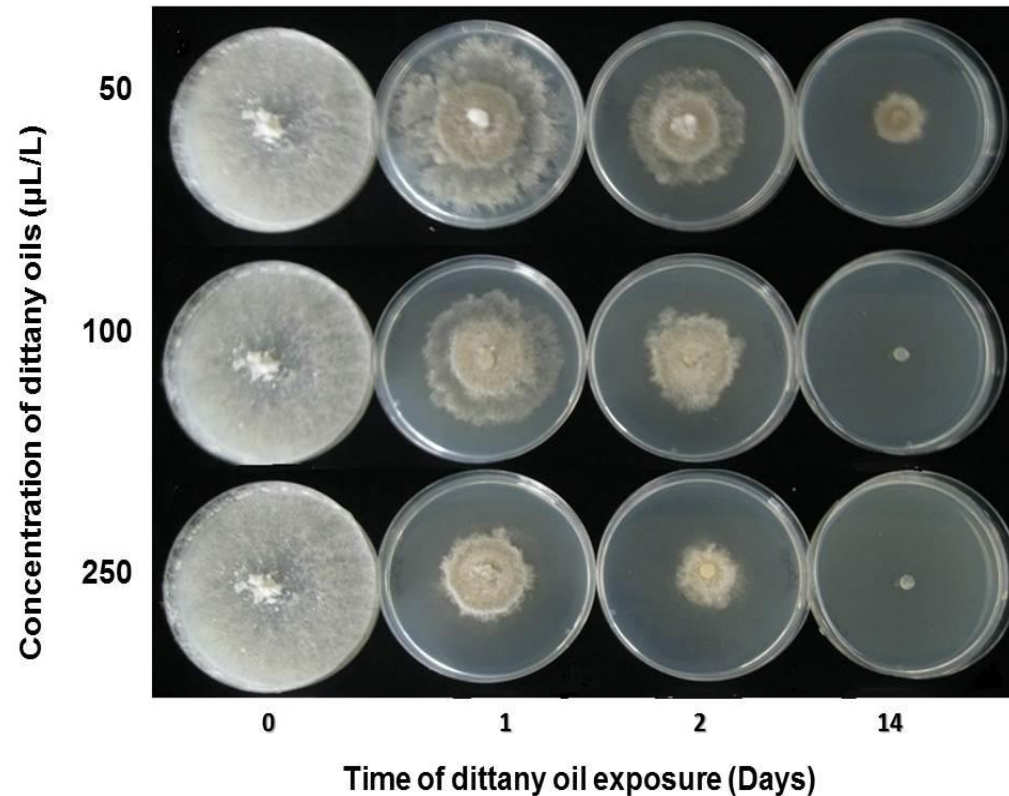
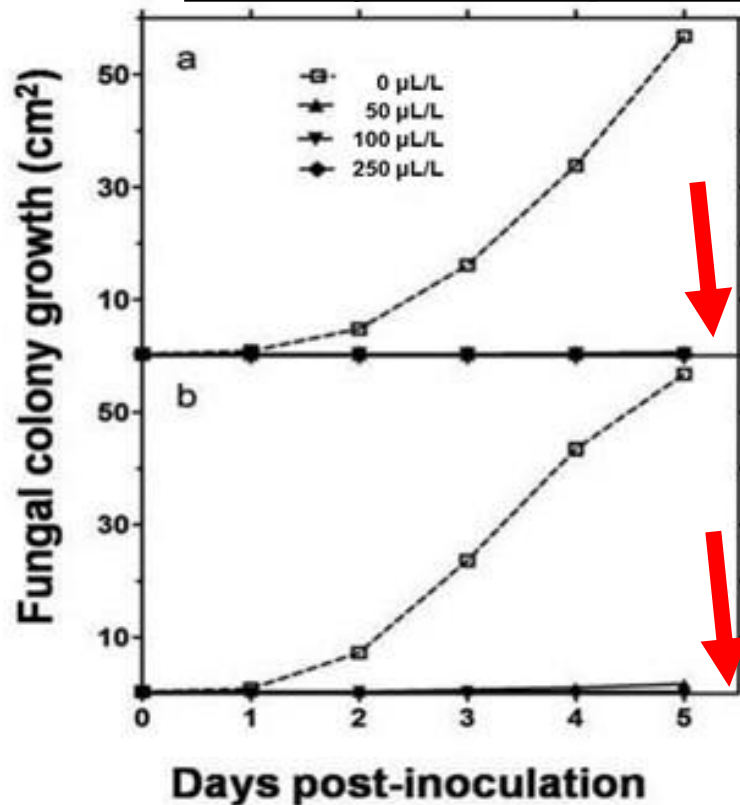
Memory effect-ME

Fruits or PDA media



In vitro: Impacts of dittany EO volatiles on *B. cinerea*

Colony development



- a) sustain effect-SE
- b) memory effect-ME in exposed PDA to volatiles

In vitro: Impacts of dittany EO volatiles on *B. cinerea*

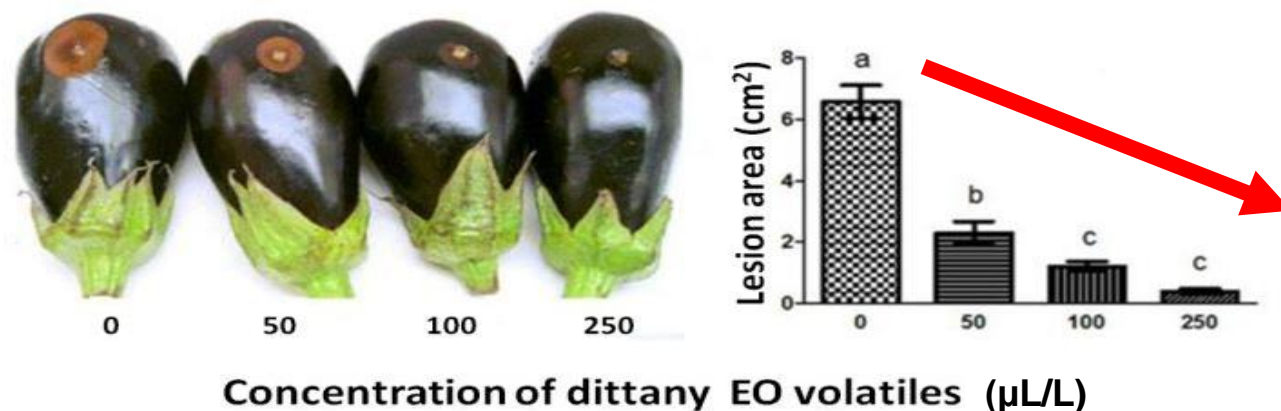
Fungal sporulation

EO ($\mu\text{L/L}$)	SE	ME
	Spore production ($10^5/\text{mL}$)	Spore production ($10^5/\text{mL}$)
0	22.70 a	33.25 a
50	21.57 a	16.31 b
100	0.00 b	0.00 c
250	0.00 b	0.00 c

a) sustain effect-SE

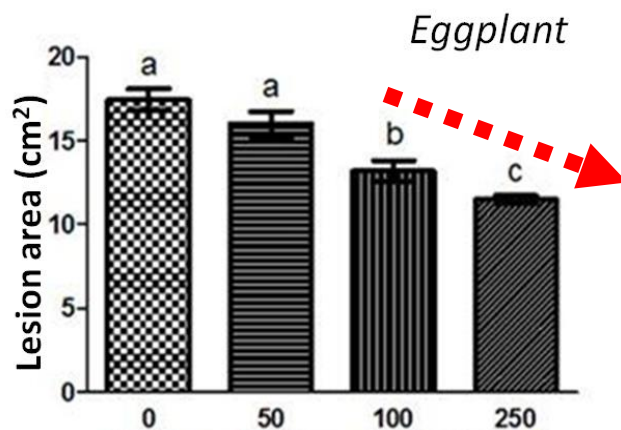
b) memory effect–ME in exposed PDA to volatiles

In vivo: Impacts of dittany EO volatiles on fruits lesion area of grey mould (*B. cinerea*) 7 days post-inoculation.



Stavropoulou et al., 2014

In vivo: Impacts of dittany EO volatiles on lesion area of grey mould (*B. cinerea*), in pre-exposed fruits to volatiles, 7 days post-inoculation



Stavropoulou et al., 2014

***In vivo*:** Impacts of dittany EO volatiles on spore production of grey mould (*B. cinerea*), in pre-exposed fruits to volatiles, 7 days post-inoculation

DIT	Vapour-induced “memory” effect	
	Spore production (10 ⁵ /mL)	Spore germination (%)
<i>In vitro</i>		
0	33.25 ^a	98.66 ^a
50	16.31 ^b	97.83 ^a
100	0.00 ^c	—
250	0.00 ^c	—
<i>In vivo</i>		
0	51.67 ^a	99.08 ^a
50	16.67 ^b	97.41 ^a
100	10.77 ^c	97.00 ^a
250	5.49 ^d	97.41 ^a

Stavropoulou et al., 2014

In vivo: Impacts of dittany EO volatiles on quality related attributes on eggplant fruit exposed to DIT volatiles

DIT	Weight loss (%)	Firmness (kg cm ⁻²)	TSS (°Brix)	TA (% citric acid)	pH
0	1.06 ^a	13.52 ^a	4.01 ^a	0.77 ^a	5.63 ^a
50	1.07 ^a	13.34 ^a	3.88 ^a	0.76 ^a	5.50 ^a
100	0.97 ^{ab}	14.61 ^a	3.95 ^a	0.77 ^a	5.54 ^a
250	0.88 ^b	15.59 ^a	3.70 ^a	0.75 ^a	5.56 ^a

(b)

DIT	Colour						Pulp <i>L</i>
	<i>L</i>	Calyx <i>C</i>	<i>h</i>	<i>L</i>	Skin <i>C</i>	<i>h</i>	
0	54.88 ^a	29.87 ^a	113.08 ^a	23.47 ^b	3.73 ^a	355.68 ^{bc}	82.74 ^c
50	54.72 ^a	30.03 ^a	114.56 ^a	24.22 ^a	4.63 ^a	357.27 ^a	84.44 ^{bc}
100	54.52 ^a	29.72 ^a	114.25 ^a	24.68 ^a	4.21 ^a	355.39 ^c	85.10 ^{ab}
250	54.07 ^a	31.21 ^a	114.60 ^a	24.08 ^{ab}	4.48 ^a	357.00 ^{ab}	86.60 ^a

Stavropoulou et al., 2014

Application of essential oils and extracts on fresh produce

Effect of mint EO, mint hydrosol and pomegranate juice against *E. coli* and *L. monocytogenes* (Shredded carrot)



Shredded carrot
(*Daucus carota* L.)

Water

Mint EO
1:1000

Mint Hydrosol
1:10

Pomogranate
Juice 1:10

Aqueous solution application

Solution removal and storage at 4 °C

Analysis (Day 0, 3 and 6)

- *E. coli* ATCC 11775
- *L. monocytogenes* NCTC 7973

Mint
(*Mentha piperita* L.)

Pomegranate
(*Punica granatum* L.)



(Xylia et al., 2017a)

Effect of spearmint and lavender EO against food borne pathogens (Endive)



Endive leaves
(*Cichorium endivia* L.)

- *E. coli* ATCC 11775
- *L. monocytogenes* NCTC 7973
- *S. aureus* ATCC 6538
- *S. Enteritidis* NCTC 5188

Water

Chlorine
(300 ppm)

0.001%

0.01%

0.1%

Aqueous solution application

Solution removal and storage at 4 °C

Analysis (Day 0, 2 and 4)

Spearmint
(*Mentha spicata*)

Mixture
(50:50)

Lavender
(*Lanadula angustifolia*)

(Xylia et al., 2017b)

Take-home message :

- EO strong biocidal (antimicrobial, antioxidant etc) activities can be explored further, with promising applications.
- Coating fruits with plant volatiles and/or modified atmosphere packaging on a wider range of fresh produce must also be studied.
- Dose responses (time * concentration) needed to be consider as well.

Effects of EOs on plant growth. Any stimulatory effect:

- Can we apply EOs during plant growth?



- Any stimulatory effects/induced resistance?

 is working to that direction



Thank you for your attention

Hydro-Aromatic Plants



<https://web.cut.ac.cy/hydro-aromatic-plants/>

Εκτίμηση Επικινδυνότητας Αιθέριων Ελαίων σε Θηλαστικά

(Evaluation of possible side-effects of the product on mammals)

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Use of pesticides

The use of pesticides often relies on the fact that levels used to kill insects are generally low enough not to affect humans

Impact of use:

- On the environment: Over 98% of sprayed pesticides reach a destination other than their target species, including non-target species, air, water and soil.
- On humans: Due to their wide variety in common use, they affect a broad cross section of people differently

THE VICIOUS CYCLE OF PESTICIDES

Pesticides are manufactured by companies and sold for profits

PESTICIDES FIND THEIR WAY TO THE ENVIRONMENT AND TO OUR BODIES

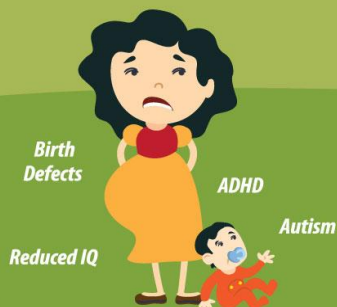
PESTICIDES CONTAMINATE SOIL

PESTICIDES CONTAMINATE THE AIR, WATER AND FOOD

PESTICIDE RESIDUE RUN-OFF INTO RIVER

School children are continuously being exposed to the pesticides from adjacent or neighboring farms

Impacts of Pesticides on children in utero



Impacts of Pesticides on Farmers and Agricultural Workers



Impact on Aquatic Organisms – fish death, loss of biodiversity



Poor Mental Development

Cancer

Leukemia

Respiratory Diseases

What are Highly Hazardous Pesticides (HHPs)

- Highly hazardous pesticides are those that have high potential to cause illness, injury or death to humans and animals or damage to the environment.
- Pesticides are widely used in agriculture and food production, homes, schools, gardens and public places.
- Pesticides can also pollute our water, air and living environment.
- Pesticides can travel far and wide and are found almost anywhere in the world.
- Some pesticides can stay in our bodies and in the environment for a very long time.

Water Contamination & Pesticides Issue in Cameron Highlands, Malaysia

- Widely banned highly hazardous pesticides have been found contaminated tap water and rivers water in Cameron Highlands.
- Endosulfan, endrine Ketone, aldrin and DDE were found in five sampling sites conducted by Universiti Kebangsaan Malaysia (UKM) from August – December 2014 at the Bertam and Terla rivers, as well as in the tap water in Brinchang town.

Schools are no longer safe learning environments

- Many school children in Asia (both rural and urban areas) are exposed to pesticides while they are in supposedly safe learning environments.
- Due to pesticide drift, school children are continuously being exposed to pesticides from adjacent or neighboring farms. School children and their teachers are unknowingly being exposed to pesticides without their consent.
- Pesticide drift poses serious environmental and health risks, especially to children.

Protect Our Children from Toxic Pesticides

- Call for a 2km buffer zone to protect children from toxic pesticides. This would be a comprehensive zone where pesticides cannot be applied.
- Assist farmers, particularly in these zones, to transition to agroecology so that they can replace pesticides with non-chemical methods of management.
- Ban and phase out of Highly Hazardous Pesticides especially the "Terrible Twenty".

Acute toxicity hazard categories of pesticides

WHO Class		LD ₅₀ for the rat (mg/kg body weight)	
		Oral	Dermal
Ia	Extremely hazardous	< 5	< 50
Ib	Highly hazardous	5–50	50–200
II	Moderately hazardous	50–2000	200–2000
III	Slightly hazardous	Over 2000	Over 2000
U	Unlikely to present acute hazard	5000 or higher	

The Lethal Dose 50% (LD₅₀) value is a statistical estimate of the number of mg of toxicant per kg of bodyweight required to kill 50% of a large population of test animals

Biopesticides

- **Biopesticides** (biological pesticides): derived from natural sources / materials such as animals, plants, bacteria and certain minerals
- Biopesticides have attracted attention in pest management in recent decades, and have long been promoted as prospective alternatives to conventional synthetic/chemical pesticides.

Considered as reduced risk pesticides

Essential oil-based biopesticides

Among eco-pesticides, botanical ones are experiencing a revival due to their eco-toxicological properties:

- Essential oils are among the best-known substances tested against insects
- Broad activity due to their multiple modes and site of action: act as fumigants, contact insecticides, repellents and may affect some biological parameters such as growth rate, life span and reproduction
- Effective in small quantities, resulting in lower operator exposure
- Decompose quickly
- Less likely to have resistance issues
- Often less toxic to mammals. The LD₅₀ for rats were 800-3000 mg/kg for pure compounds and ≥ 5000 mg/kg for formulating insecticides

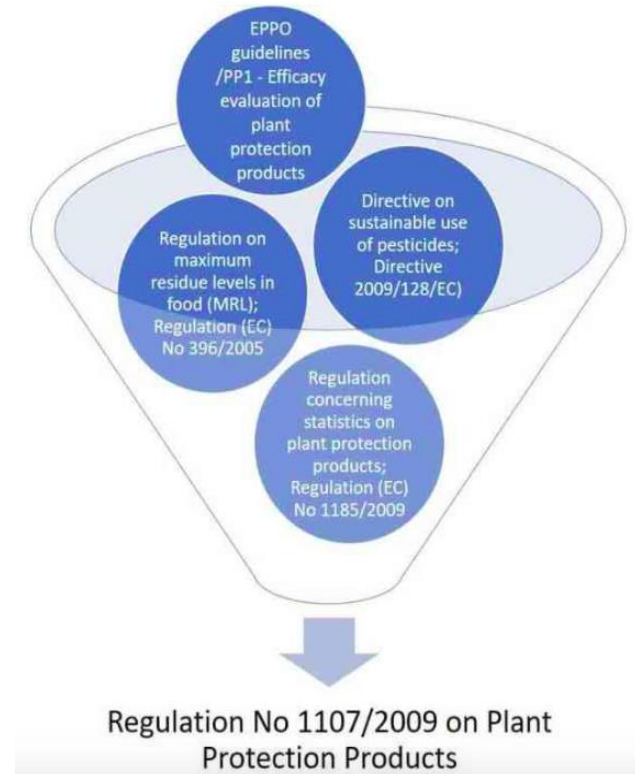
Toxicity of essential oil compounds to experimental animals

Compound	Animal	Route	LD ₅₀ mg/kg. b.wt.	WHO category
Category II (Moderately hazardous), 50-2000 mg/kg				
Thujone	Mice	Subcutaneous	87.5	II
Pulegone	Mice	Intraperitoneal	150	II
3-Isothujone	Mice	Subcutaneous	442.2	II
Apiol	Dogs	Intravenous	500	II
2-Acetonaphthone	Mice	Oral	599	II
2-Methoxyphenol	Rats	Oral	725	II
Thymol	Rats	Oral	980	II
Linalool	Rats	Oral	>1000	II
Cinnamaldehyde	Guinea pigs	Oral	1160	II
Methyl eugenol	Rats	Oral	1179	II
Dillapiol	Rats	Oral	1000-1500	II
Anisaldehyde	Rats	Oral	1510	II
(+) Carvone	Rats	Oral	1640	II
γ -terpinene	Rats	Oral	1680	II
Thymol	Mice	Oral	1800	II
Methyl chavicol	Rats	Oral	1820	II
Category III (Slightly hazardous), over 2000 mg/kg				
trans-Anethole	Rats	Oral	2090	III
Cinnamaldehyde	Rats	Oral	2220	III
Maltol	Rats	Oral	2330	III
1,8-Cineole	Rats	Oral	2480	III
Eugenol	Rats	Oral	2680	III
Menthol	Rats	Oral	3180	III
Terpinen-4-ol	Rats	Oral	4300	III
d-Limonene	Rats	Oral	4600	III
Citral	Rats	Oral	4960	III
Category U (unlikely to present acute hazard), 5000 mg/kg				
Myrcene	Rats	Oral	5000	U

major compound found in rosemary and eucalyptus

Biopesticides and the EU regulatory process

- Differences exist between conventional and low risk plant protection products after the low risk status of an active ingredient is confirmed.
- The regulatory approach in Europe, according to EU plant protection Regulation 1107/2009, does not recognize biopesticides as a regulatory category of plant protection.
- In the lack of an unequivocal regulation, in many cases decisions are made on a case-to-case basis
- The evaluation is mainly based on already existing evaluations for a substance, regarding possible effects on human or animal health or the environment.
- Low risk substances have to fulfill certain criteria such as being not carcinogenic, mutagenic or toxic to reproduction.



Biomechanics and Living Systems Analysis (BioLISYS) lab

Work Package 5: Evaluation of possible side-effects of the product on mammals



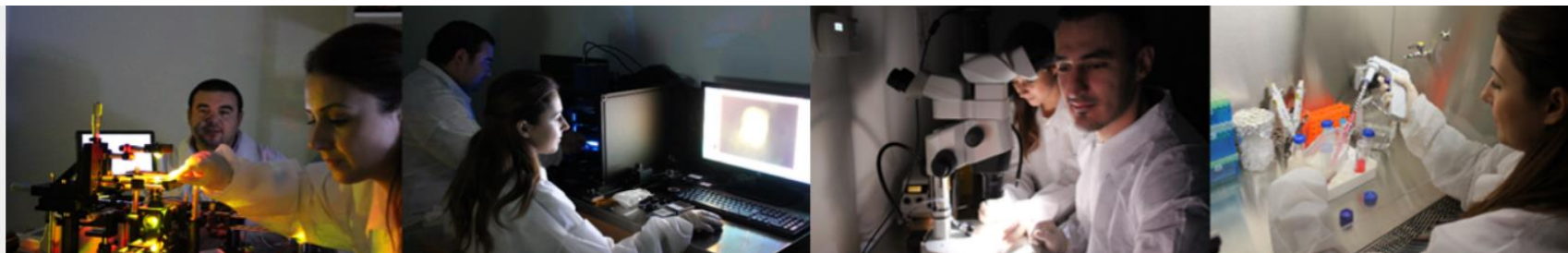
Task 5.1:

Side effects of the product
on mammalian cell lines



Task 5.2:

Side effects of the product
on mammals



ΥΠΟΥΡΓΕΙΟ ΓΕΩΡΓΙΑΣ, ΑΓΡΟΤΙΚΗΣ ΑΝΑΠΤΥΞΗΣ ΚΑΙ ΠΕΡΙΒΑΛΛΟΝΤΟΣ
ΚΤΗΝΙΑΤΡΙΚΕΣ ΥΠΗΡΕΣΙΕΣΟΙ ΠΕΡΙ ΠΡΟΣΤΑΣΙΑΣ ΚΑΙ ΕΥΗΜΕΡΙΑΣ ΤΩΝ ΖΩΩΝ (ΠΡΟΣΤΑΣΙΑ ΤΩΝ ΖΩΩΝ ΠΟΥ
ΧΡΗΣΙΜΟΠΟΙΟΥΝΤΑΙ ΓΙΑ ΕΠΙΣΤΗΜΟΝΙΚΟΥΣ ΣΚΟΠΟΥΣ)
ΚΑΝΟΝΙΣΜΟΙ ΤΟΥ 2013 και 2017ΑΔΕΙΑ ΕΡΓΟΥ

ΜΑΤΑ

Αρ. άδειας: CY/EXP/PR.L2/2019

Ασκώντας τις εξουσίες που του παρέχονται, δυνάμει του περί Προστασίας και Ευημερίας των Ζώων Νόμου του 1994 έως 2013 και των περί Προστασίας και Ευημερίας των Ζώων (Προστασία των Ζώων που Χρησιμοποιούνται για Επιστημονικούς Σκοπούς) Κανονισμών του 2013 και 2017, ο Διευθυντής των Κτηνιατρικών Υπηρεσιών χορηγεί την με Αριθμό CY/EXP/PR.L2/2019 άδεια διεξαγωγής του έργου με τίτλο «Herbal essential oils: Potential for development as low risk pesticides, plant growth promoters and produce sanitizers» το οποίο θα διενεργείται σε ποντίκια στα με αριθμό εγγραφής CY.EXP.108 υποστατικά του Τεχνολογικού Πανεπιστημίου Κύπρου, σύμφωνα με το πρόγραμμα εργασίας και στην βάση της σχετικής, με ημερομηνία 02/10/2018, αίτησης που υποβλήθηκε από τον Δρ. Ανδρέα Αναγιωτό.

Ο Δρ. Ανδρέας Αναγιωτός θα είναι ο υπεύθυνος για τη συνολική υλοποίηση του έργου και τη συμμόρφωση προς την αδειοδότηση του.

Δεν απαιτείται αναδρομική αξιολόγηση του έργου.

Η παρούσα άδεια δύναται να ανασταλεί ή να ανακληθεί ανά πάσα στιγμή από το Διευθυντή, εφόσον κριθεί πως δεν τηρούνται οι διατάξεις των Κανονισμών στη βάση των οποίων και εκδόθηκε.

Σφραγίδα

Ημερομηνία: 08/03/2019
Ισχύει Μέχρι: 07/03/2021

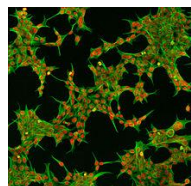
FIS No. F473236

Υπογραφή:

Ιωάννης Ιωάννου
Για Αναπληρωτή Διευθυντή

Task 5.1: Side effects of the product on mammalian cell lines

Objective



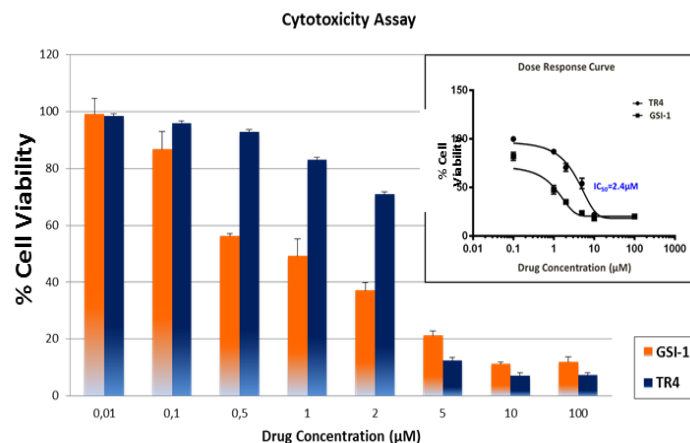
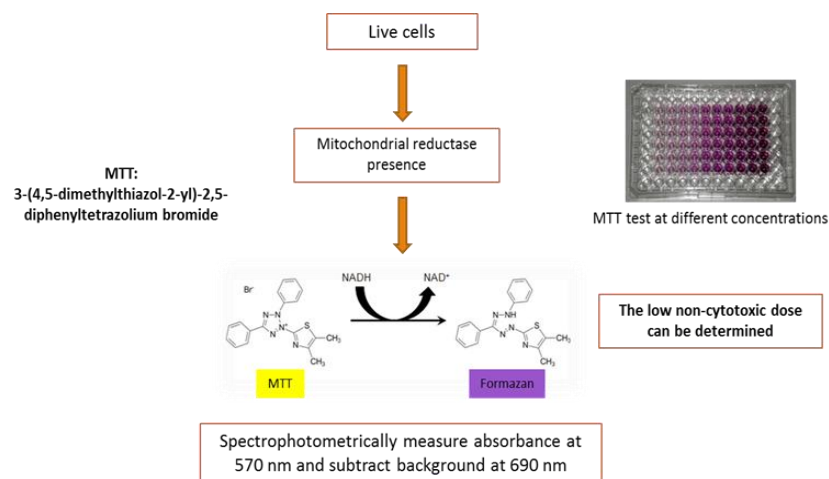
Methods

Determine the viability of cultured cells following administration of the formula

- HEK 293 (human embryonic kidney) cells
- Cell viability assessed using the MTT assay
- Escalating concentrations of the formula (3-5%)
- Four time points (day 0, 2, 4, 7)

Cell Viability – MTT assay

- Widely adopted method, suitable for high throughput screening
- Viable cells with active metabolism convert MTT into a **purple** colored formazan product (tetrazolium reduction assay)
- The color change (with an absorbance maximum near 570 nm) can be read on a microplate reader equipped with a spectrophotometer
- The generated signal is proportional to the number of viable cells present
- The amount of viable cells is represented as a fraction based on the ratio of color generation in the sample wells to the color found in positive control wells



Task 5.2: Side effects of the product on mammals

Objective

Nonclinical safety evaluation to detect toxic or adverse events that may possibly occur following exposure to the formula

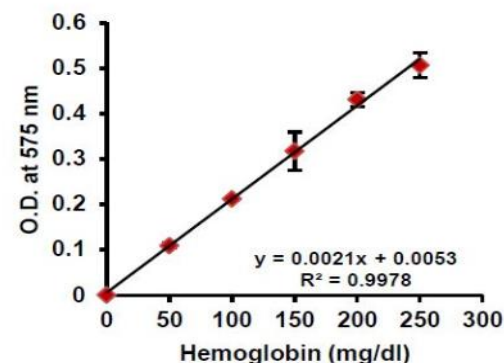
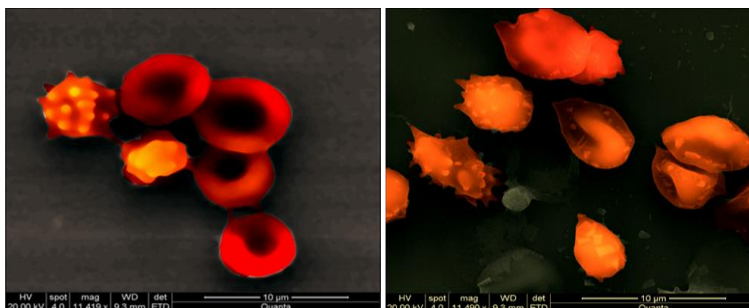


Methods

- Systemic tolerance following oral administration in specific pathogen-free CD1 mice
- 3 different concentrations of the formula in the drinking water supply (n=8 mice for each concentration, 4 for each sex)
- Small volumes of blood samples (~10µl) will be obtained from each mouse (once weekly for 5 weeks) via tail vein nick
- Hemolysis tests will be performed using blood samples from the 3 groups of mice as well as from an additional control group

Hemoglobin Colorimetric Assay Kit

- Quick, reliable method for determining total hemoglobin concentration in a variety of biological samples, including blood
- The *in vitro* hemolysis assay evaluates hemoglobin release in the plasma (as an indicator of red blood cell lysis) following test agent exposure
- Drug-induced hemolysis is a toxicity liability. Serum free hemoglobin causes damage to various vital organs such as the liver, kidney and heart
- Analysis method: spectrophotometry at 575 nm



Expected Key Results

In vitro assays will provide a dose-dependent measure of the cytotoxic potency of the formulated developing compound

In vivo tests will identify clinical signs of toxicity and discomfort/pain

- **Important for obtaining baseline data in order to support the registration of the products as an eco-pesticide.**
- **No data currently exist for potential effects of essential oils of rosemary and eucalyptus in mammalian cells → the research conducted in WP5 will reveal such effects and will yield novel scientific results.**

THANK YOU

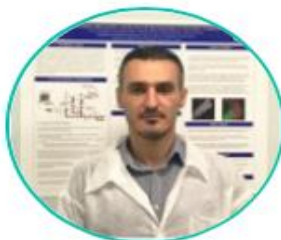
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