

Anti-fungal and anti-mitochondrial effect of essential oils against *Guignardia citricarpa*

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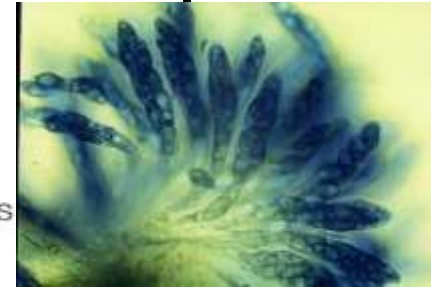
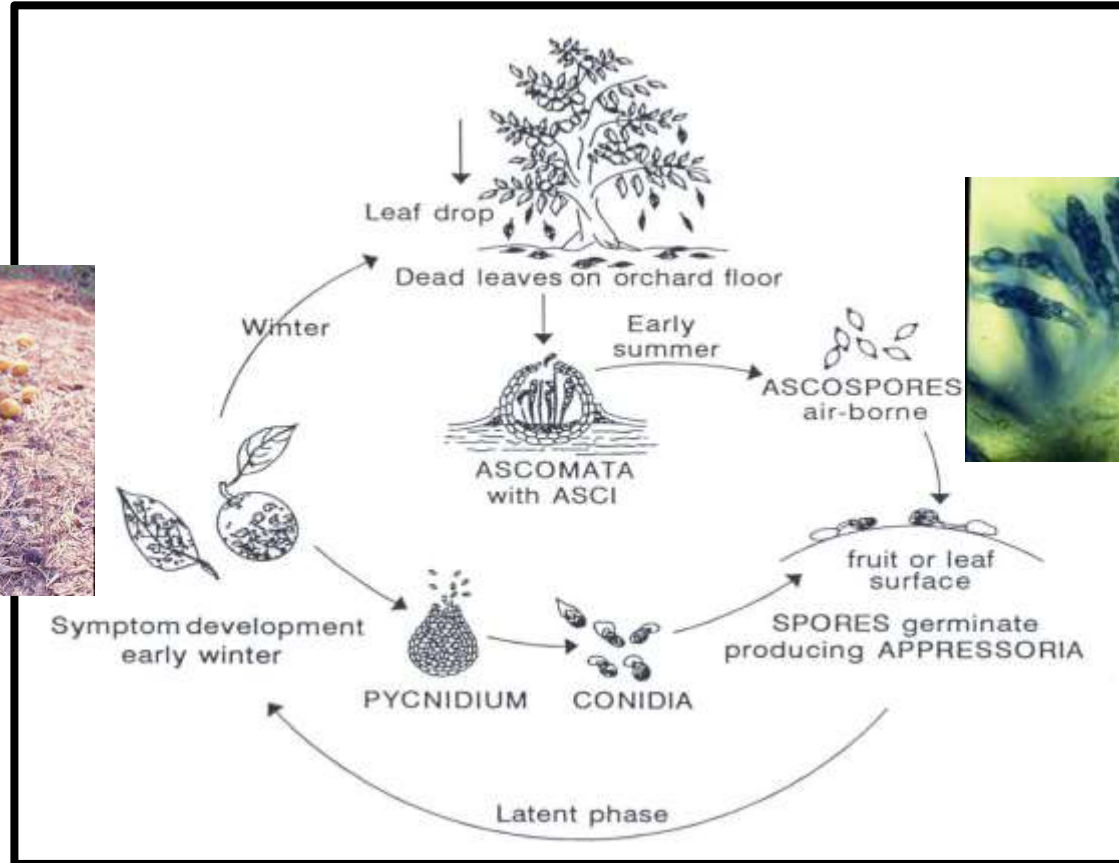
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INTRODUCTION

- One major concern currently facing Citrus growers- Citrus Black Spot (CBS) spread.
- Caused- *Guignardia citricarpa*
- Confused with *Guignardia mangiferae* (Non-pathogenic)
- Occur- warm, wet or humid climates with summer rainfall
- Incites Lesions on Citrus Fruits
- CBS infected fruits- Transferee to new areas

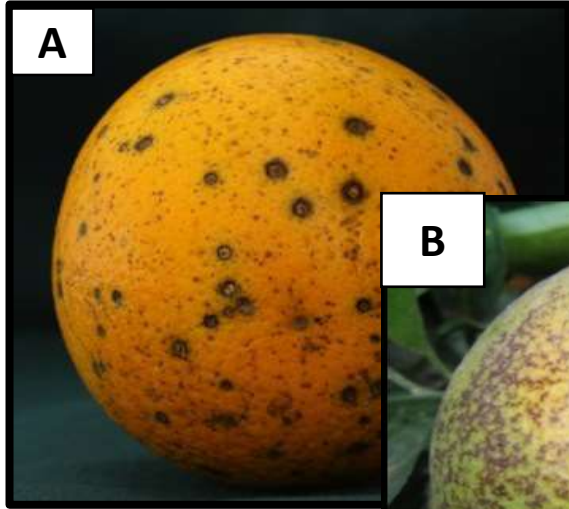
(Dharini and Silvia, 2014; Bulanon *et al.*, 2013 and Carstens *et al.*, 2012)

LIFE CYCLE



(Department of plant pathology, 2011)

SYMPTOMS



(A) hard spot, (B) False Melanose (C) Cracked spot (D) Virulent spot

(Department of Plant Pathology, 2011).

ECONOMICAL CONCERN

- Rind spots cause the economic damage
- *internal quality unaffected*
- Reduces fruit value for the fresh market
- Restricts export of fresh fruit mostly to European countries and U.S
- Causes premature fruit drop reducing yield

(Bulanon *et al.*, 2013, Halueendo, 2008 and Brent, *et al.*, 2007).

ECONOMICAL IMPORTANT

- Problem:
- CBS prevalence in South Africa (Limpopo, NW, Mp, KZN, EC & part of WC)
- EU perspective (CBS on fruit as a threat to the EU)

- Consequences:
- Loss of market
- Loss of incomes and jobs

CBS CONTROL

- Synthetic fungicides:
 - Counteract invading fungi
 - Work against most fungi
- (Elshafie *et al.*, 2015 and Ayoola *et al.*, 2008)

SYNTHETIC FUNGICIDES CONCERN

- Toxic i.e. copper sprays
- Polluting the environment
- Antimicrobial resistance i.e. Benzimidazole
- Enters nearby water source

(Dharini and Silvia, 2014 and Wightwick *at al.*, 2010)

ALTERNATIVE TO ANTIFUNGAL COMPOUNDS (EO's)

- Volatile oily liquids of the secondary metabolism
- Act as defence mechanism
- Can be applied against drug resistance microbes
- Environmental friendly
- Complex in mixture
- Chemical composition can differ according to the origin of the plants, soil composition, plant organ and climate
- Possess Anti-inflammatory properties

(Moussaoui *et al.*, 2013; Nazzaro *et al.*, 2013 and Faleiro, 2013)

PREVIOUS HYPOTHESIS

- Kock and co-worker hypothesis
- Anti-inflammatory compound target increased mitochondrial activity structures i.e. Ascospores and Conidia
- Play role in fungal life cycle and development
- Anti-mitochondrial compound limit spread of fungi (ASA)

(Kock et al., 2007, 2009; Leeuw et al., 2009; Ncango et al., 2010)

AIMS

- Assess antifungal effect of essential oils (EO's) against CBS
- Asses anti-mitochondrial of effect (EO's) against CBS

MATERIAL AND METHODS

- Organism used-*Guignardia citricarpa*
- EO's used-Thyme i and ii
- Ethanol as control (negative control)
- Acetylsalicylic acid (Positive)
- Gas Chromatography Mass Spectrometry- analyse EO's
- Bio-assay, Mitochondrion mapping ($\Delta\psi_m$) and SEM.

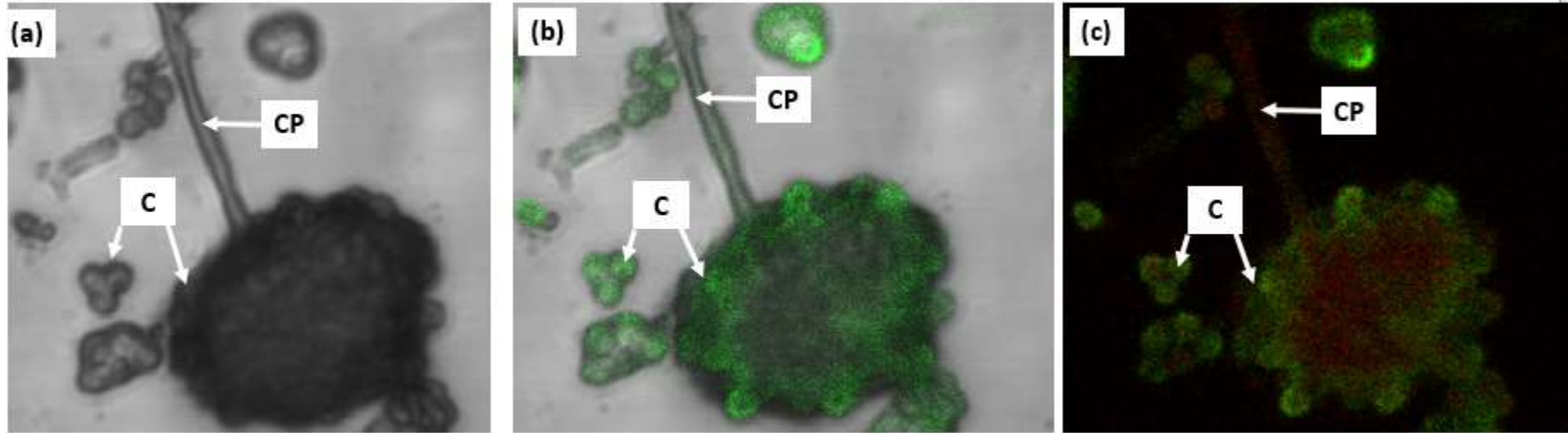
MATERIALS AND METHODS



GC/MS RESULTS

COMPOSITION	THYME I	THYME II
Caryophyllene	1.2	9.0
Thymol	32.1	-
ρ -Cymene	20.4	-
Phenol-2methyl-5-(1-methylethyl)	-	7.6

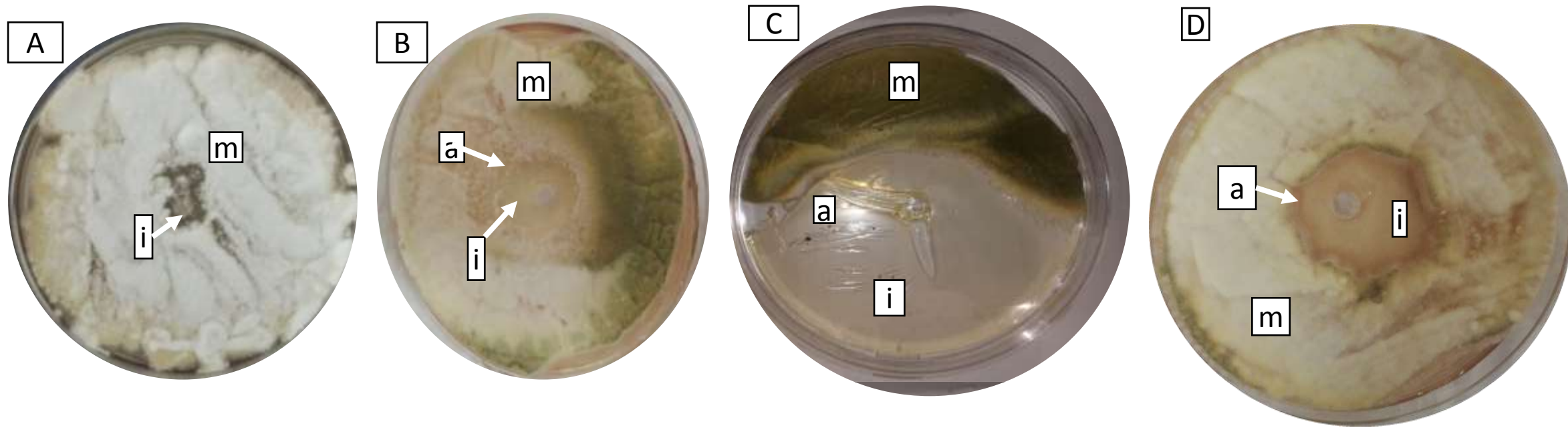
Results- Mitochondrion mapping $\Delta\psi_m$



Confocal laser Scanning Micrographs of *Guignardia citricarpa* stained with Rhodamine (Rh) 123. (a) Light Micrographs showing cluster conidia. (b) Immunofluorescence superimposed on corresponding light micrograph of cluster conidia. (c) Only immunofluorescence micrograph of cluster conidia. C, conidia; CP, conidiophores.

. a high $\Delta\psi_m$ is signified by a yellow-green fluorescence (collected at 450 nm), while a low $\Delta\psi_m$ is signified by a red fluorescence collected at 625 nm.

Bio-Assay Results



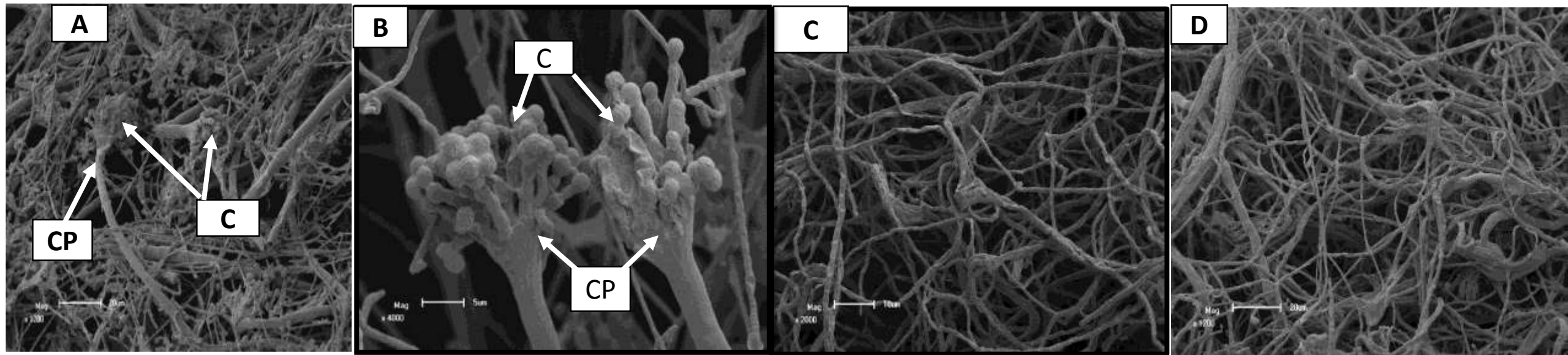
(A) Bio-assays results of ethanol (negative control) treated *Guignardia Citricarpa*, (B) *Guignardia citricarpa* cells treated with Acetylsalicylic acid (ASA) (positive control) (C) *Guignardia Citricarpa* treated with Thyme oil I (D) *Guignardia Citricarpa* treated with Thyme oil ii. (i), Growth inhibition-zone, (a), asexual zone, (m) maximum growth zone .

BIO-ASSAY RESULTS

EO' s and anti-mitochondrial tested	Inhibition zone diameter (mm)
Thyme oil (i)	55
Thyme oil (ii)	15
Acetylsalicylic acid	12
Ethanol	0.5

Bio-assays results of *Guignardia citricarpa* showing effects of different essential oils were the inhibition zone was measured in mm. Thyme oil (i), thyme oil (ii), Acetylsalicylic acid and ethanol

SEM RESULTS



Scanning Electron Micrographs. (a) Ethanol Treated cell of Guignardia citricarpa. (b) ethanol Treated cell of Guignardia citricarpa. (c) thyme oil treated cells of Guignardia, (D)thyme oil treated cells of Guignardia



Conclusion

- Rh 123 selectively stain conidia, indicating increased mitochondrial activity in conidia when compared to other structures
- Bio-assay indicated that all the zone of inhibition formed by ethanol used as the control was smaller than those of EO's dissolved in ethanol
- Indication of antifungal effect of EO's
- Therefore ethanol does not inhibit the growth of *G.citricarpa*
- All EO's used affected the fungal growth by targeting conidia with increased mitochondrial activity observe by SEM
- Indication of the anti-mitochondrial effect of these EO's

Conclusion

- The results of the current study imply that EO's can be used as alternative anti-fungal against *G.citricarpa*
- However in-vivo studies need to be carried out in future to determine the effect of EO's on the ascospores

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QUESTION?