Sub-theme:

Emerging innovations in phytosanitary systems

Title:

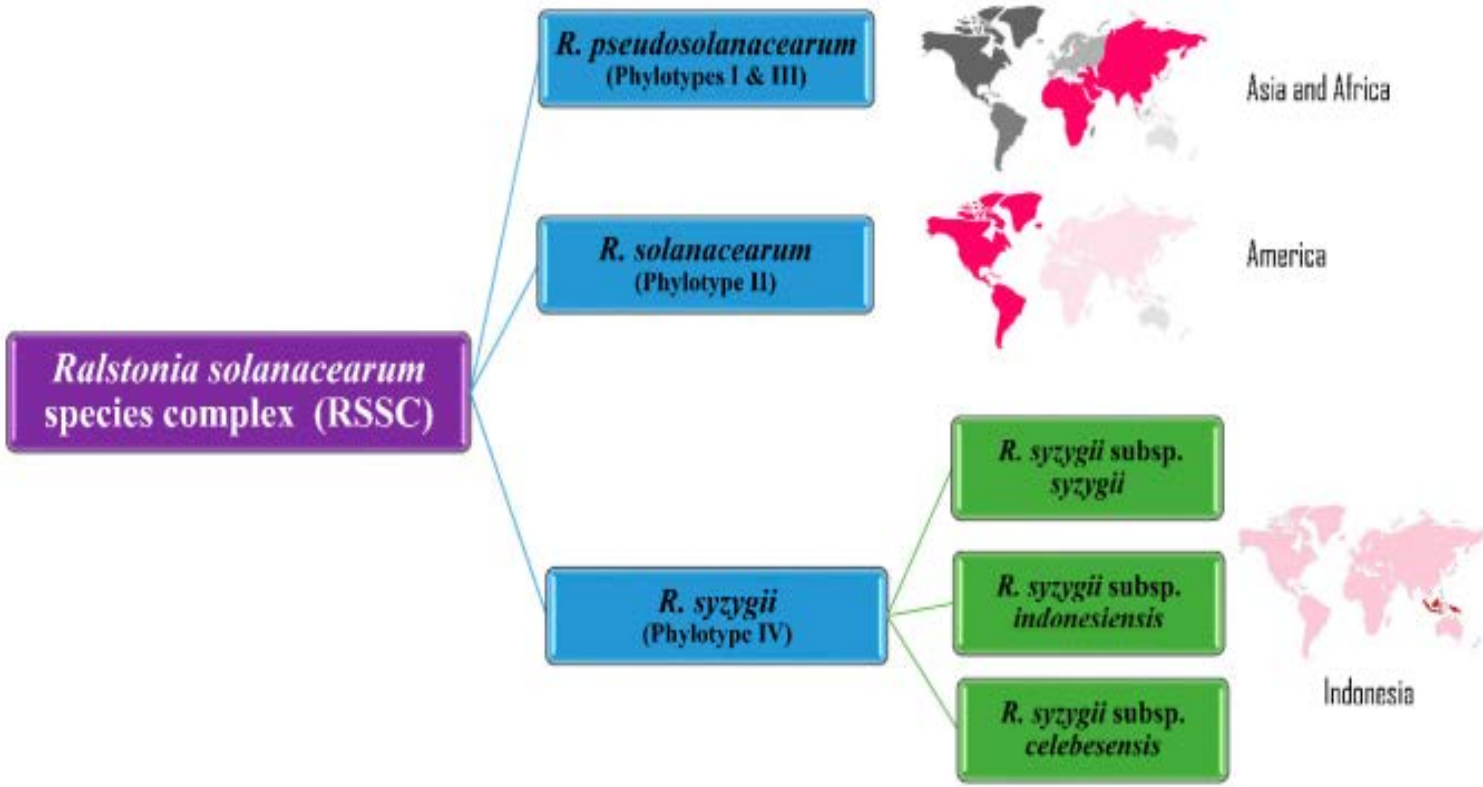
Efficacy of Nonoencapsulated Thymol and Eugenol with Chitosan Nanoparticles against Bacterial Wilt Bacterium Ralstonia solanacearum

Presented by:

George Oluoch

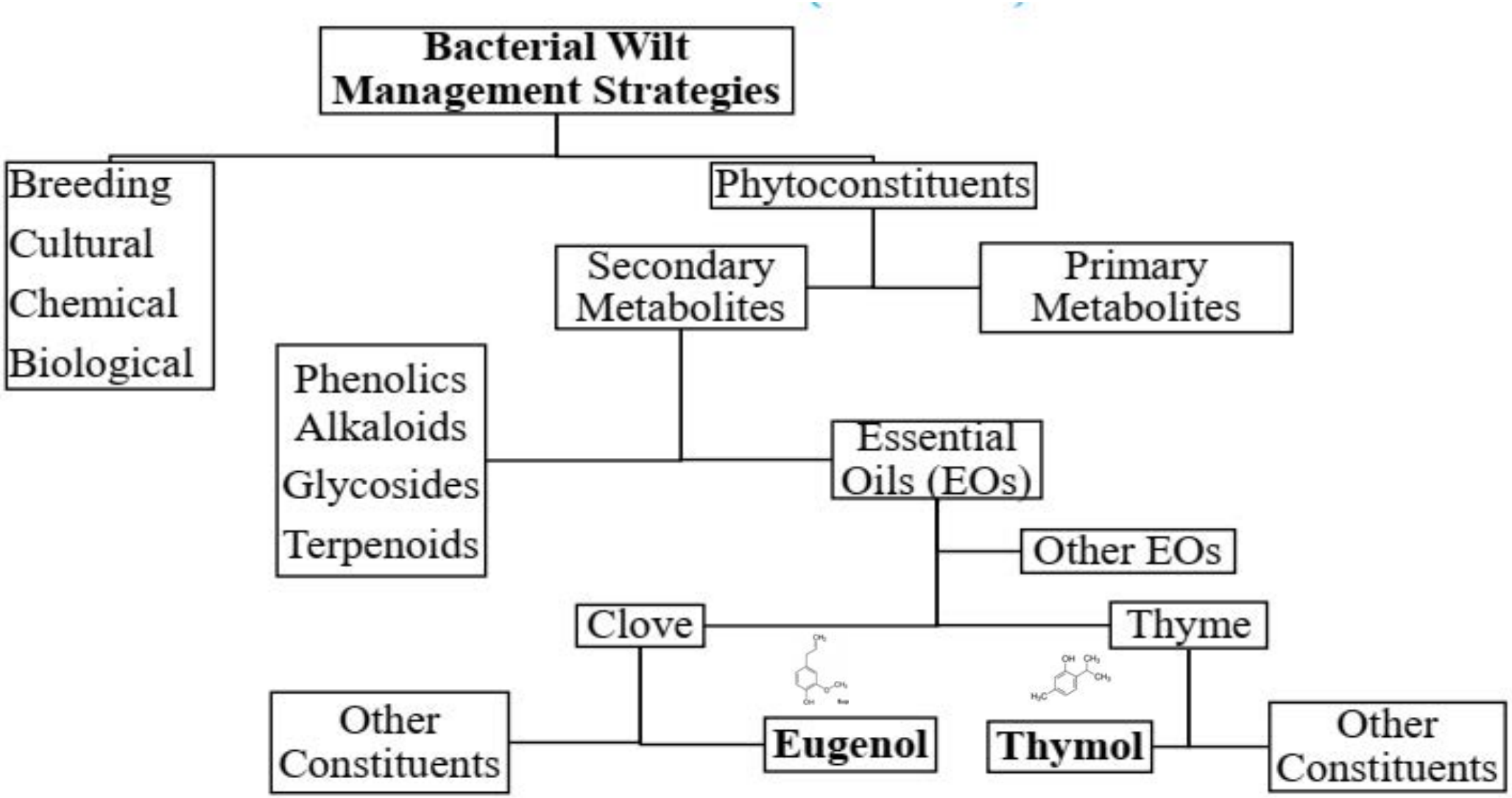
Introduction

Bacterial wilt symptoms and rapid bacterial streaming test (Mansfield et al., 2012)



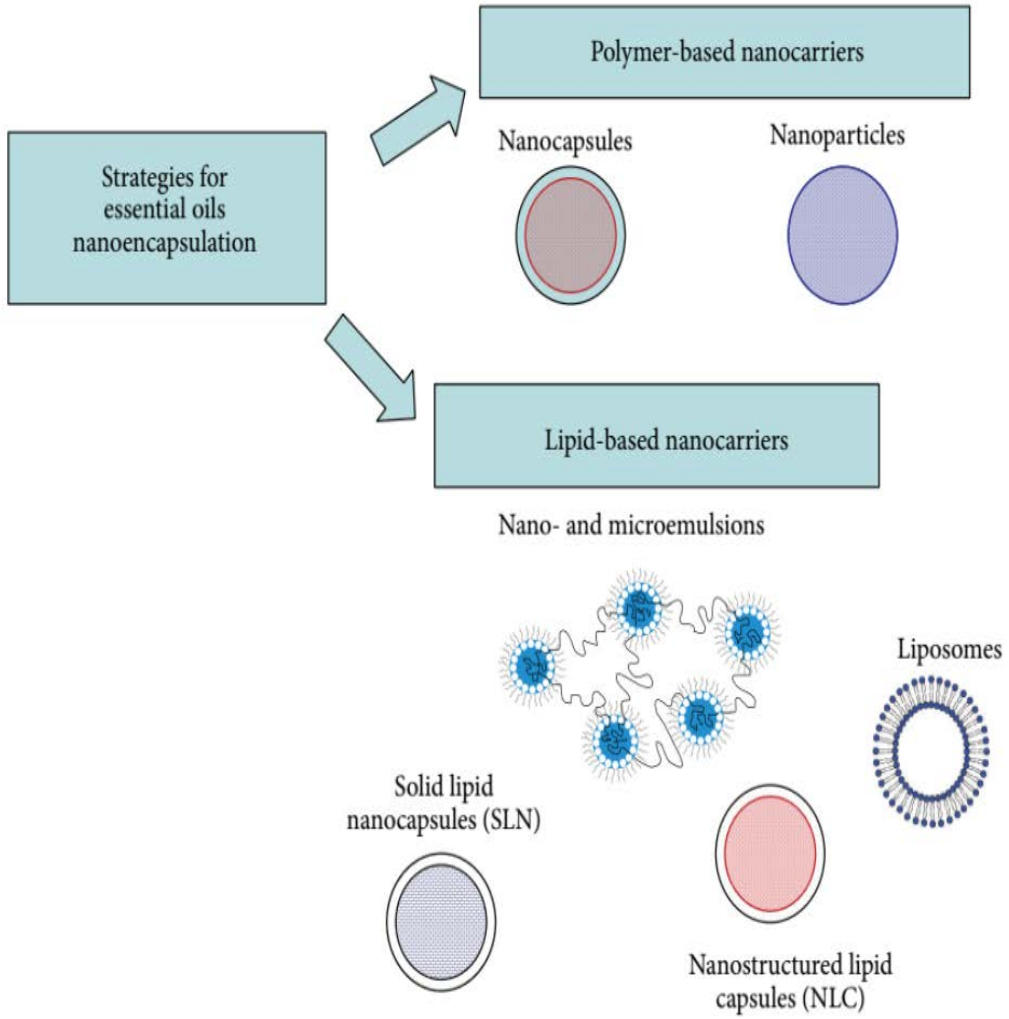
Taxonomic revision of *Ralstonia solanacearum* species complex phylotypes into three distinct species (Safni *et al.*, 2014; Paudel *et al.*, 2020).

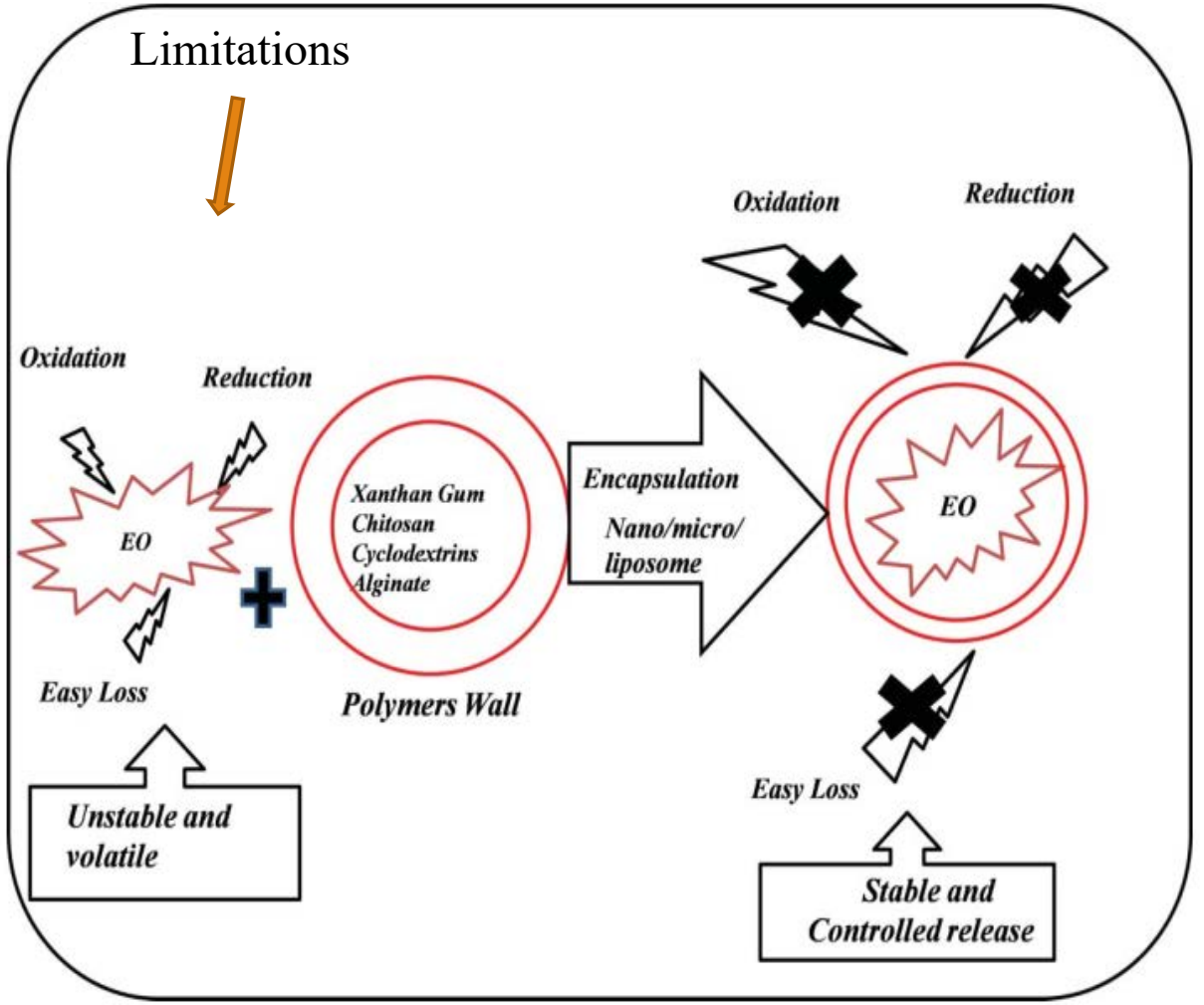
Introduction cont’



Introduction cont'

Encapsulation of EOs in Chitosan nanoparticles





Problem Statement

- To date, there is no known commercialized chemical management method for controlling *R. solanacearum*.
- Currently, bacterial wilt management is mainly through cultural control methods which have limited efficacy and bacterial wilt continues to be an economically important problem for farmers.
- The potential of essential oils as an antimicrobial agent against *R. solanacearum* pathogen is limited by their volatility, hydrophobicity, rapid degradation as well as solubility in water. Their stability and biological activity is also reduced when they are exposed to environmental factors such as heat, light, pH, oxygen and moisture hence the need for an appropriate carrier material.

(Cadena *et al*., 2018; Oboo *et al*., 2014b; Kalagatur *et al*., 2018)

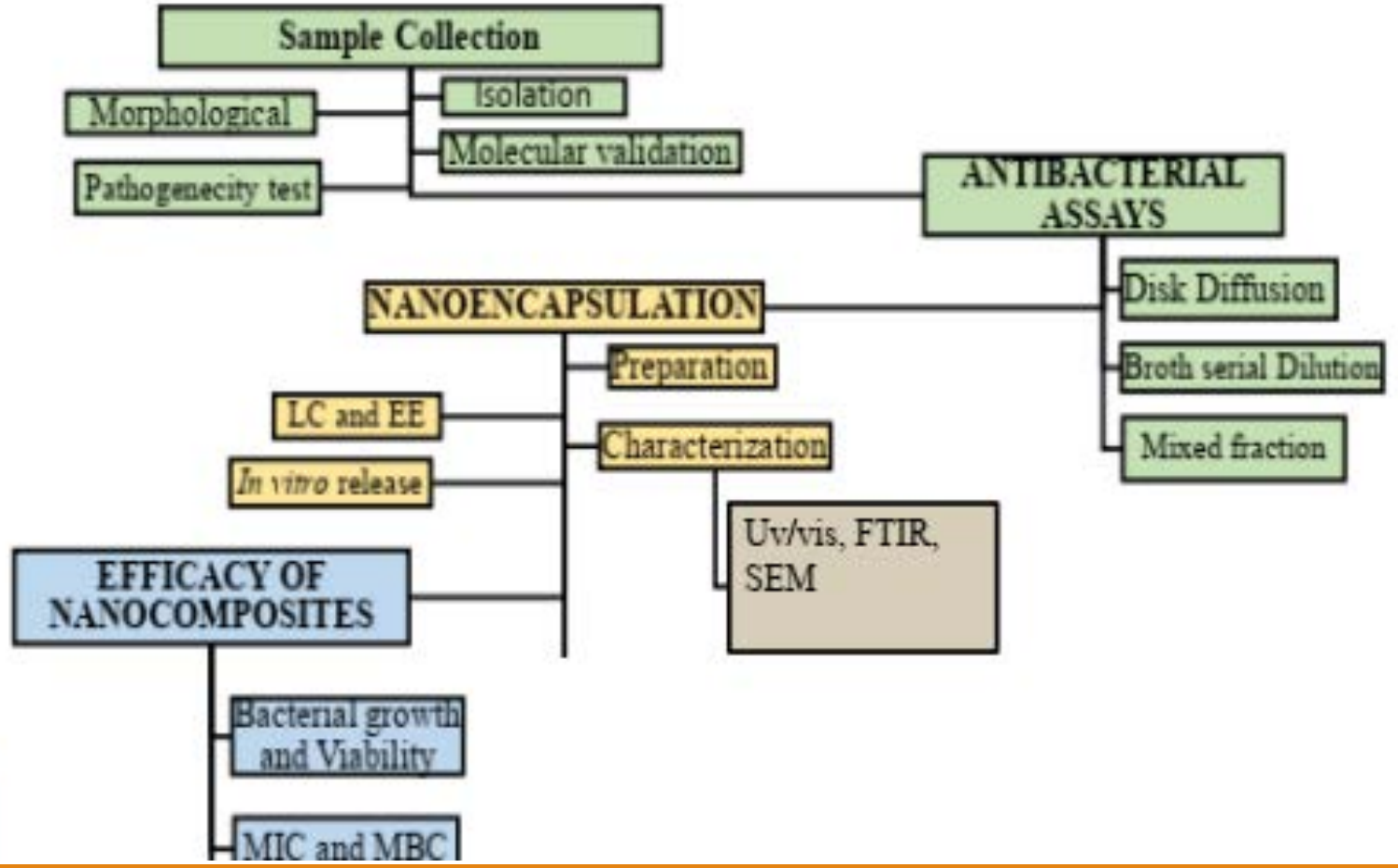
Justification

- Worldwide, it has been reported that *R. solanacearum* infestation leads to yield losses of between 33 to 90% in potato yield and up to $1 billion yearly in lost revenue (Elphinstone, 2005). While in Kenya, it has affected over 70% of potato farms and causes yield losses of between 50% and 100% (Muthoni *et al*., 2014).
- Nanoencapsulation can be used to overcome the challenges that come with the use of essential oils as antibacterial against phytopathogens.

Objective

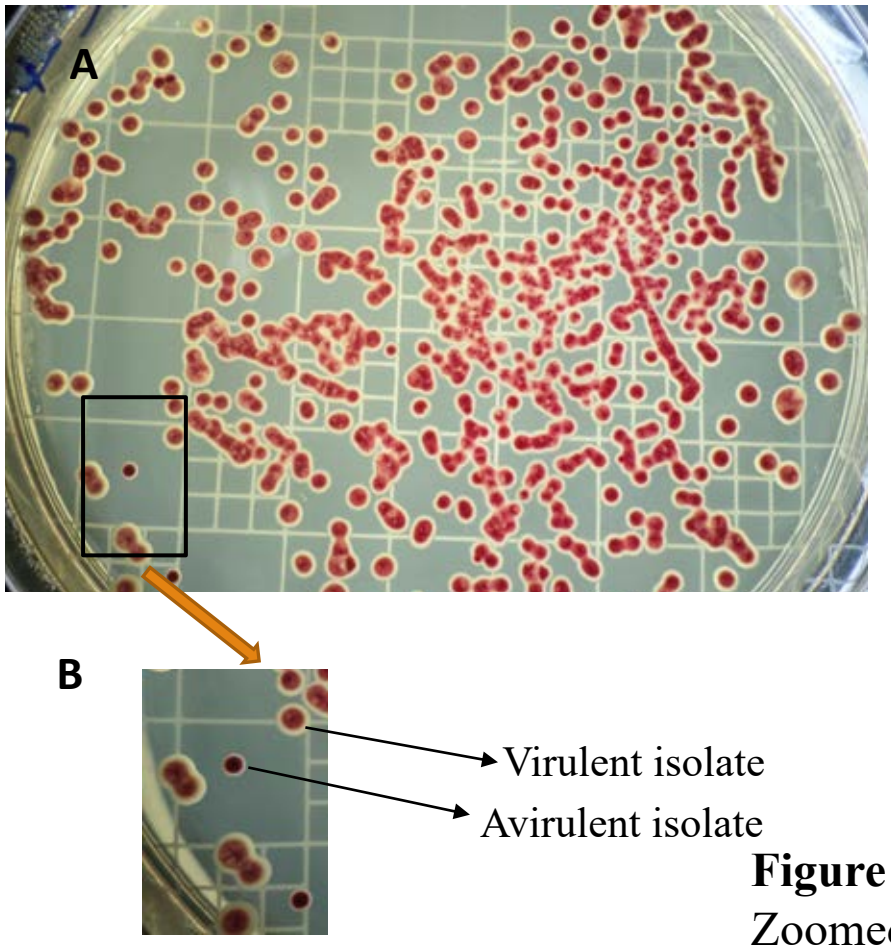
- This study aimed at evaluating the efficacy of the antibacterial potential of thymol and eugenol loaded chitosan nanoparticles (TCNPs and ECNPs) against *Ralstonia solanacearum*

Methodology



Results

Isolation and Morphological identification of *R. solanacearum*



- The pathogen was isolated as described previously (EPPO, 2018).
- Typical virulent *R. solanacearum* colonies were identified by their distinctive fluidal and pinkish red centers and whitish periphery
- Avirulent colonies were identified by their small, round, non-fluidal butyrous colonies which are entirely deep red.

Figure 1: Profile of *R. solanacearum* isolation on TZC medium **(A)**; Zoomed section showing virulent and avirulent colonies **(B)**.

Results cont'

Molecular validation of the identities of the virulent isolates

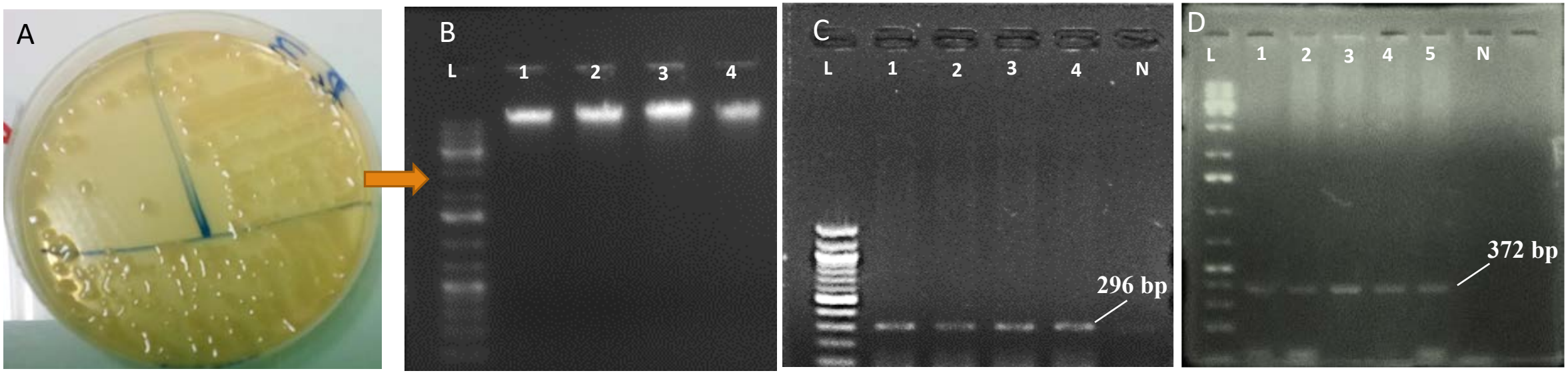
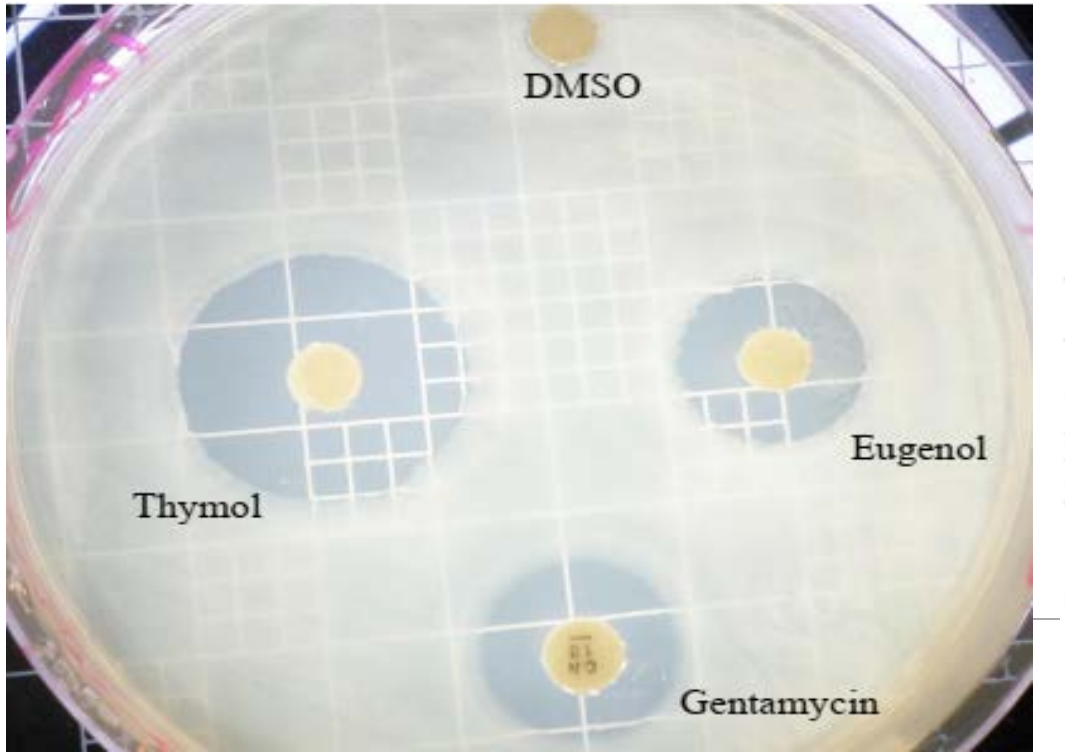


Figure 2: Virulent *R. solanacearum* on CPG medium **(A)**, DNA isolated from the presumptive *R. solanacearum* isolates 1-4; L-1kb plus DNA ladder **(B)**, Validation of *R. solanacearum* species complex using RsSC-F and RsSC-R primers; L-100 bp DNA ladder, 1-4 bacterial isolates, N-negative control (**C**), *R. solanacearum* species validation for isolates 1-5 with Nmult21:2F/Nmult22:RR primer pair; L-1kb plus DNA ladder; N-Negative control (**D**)

Results cont'

c) Disc diffusion assay



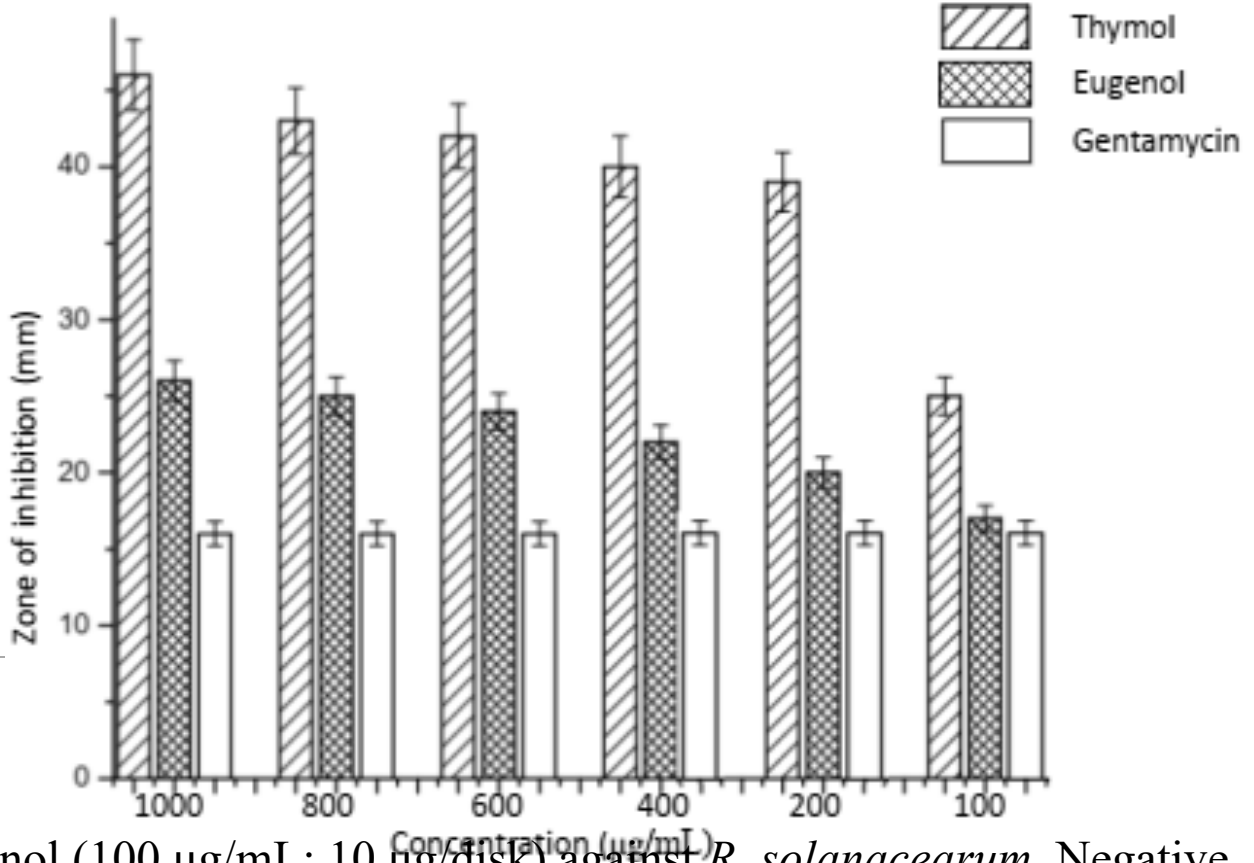


Figure 3: Antibacterial activities of thymol and eugenol (100 µg/mL; 10 µg/disk) against *R. solanacearum*. Negative control (disc impregnated with DMSO), positive control (gentamycin disc (10 µg/disk). Zone of inhibition exhibited by thymol and eugenol (100 µg/mL; 10 µL/disk) against *R. solanacearum*. Error bars indicate standard errors of mean(n = 3).

Results cont'

Minimum inhibitory concentration

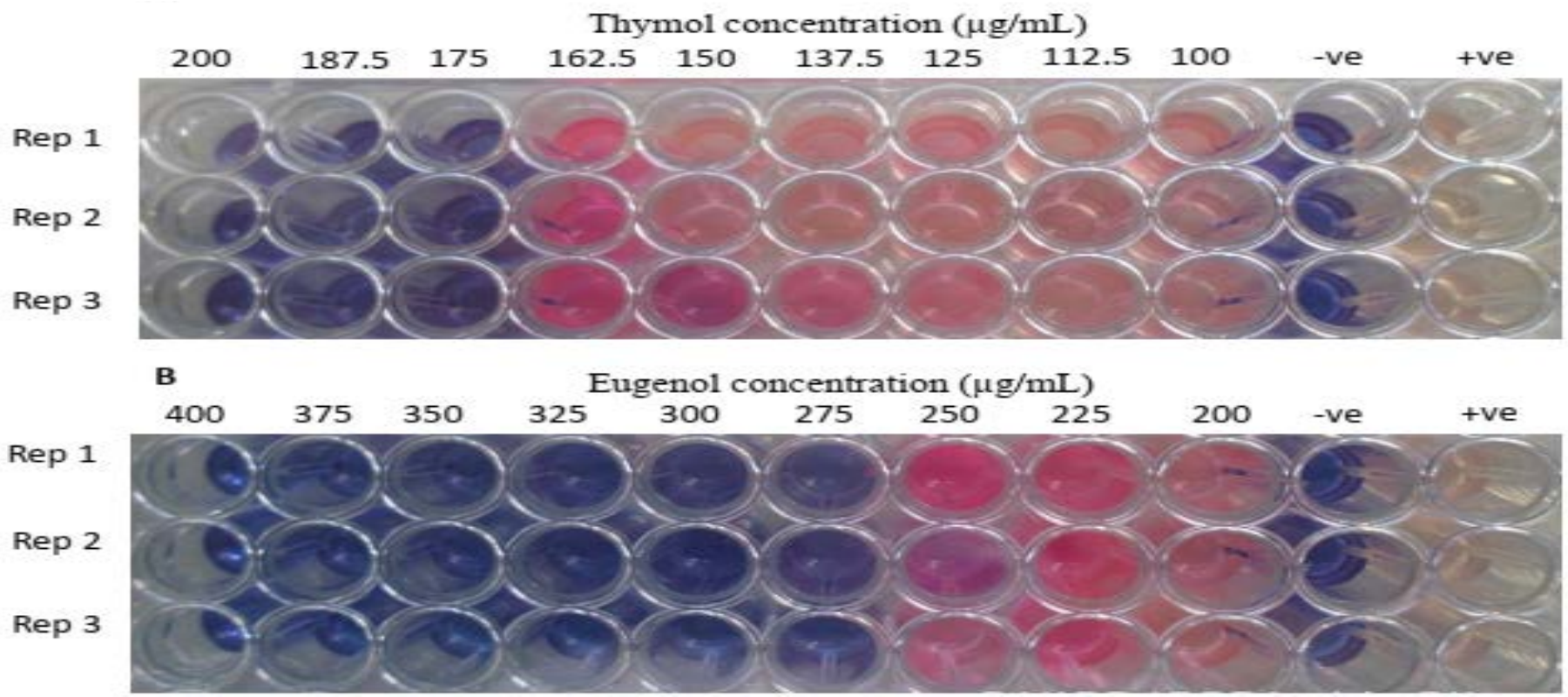


Figure 4: Minimum inhibitory concentration of thymol (**A**) and eugenol (**B**) against *R. solanacearum* using resazurin aided microdilution; negative control (broth only); positive control (broth + *R. solanacearum*).

Results cont'

b) Preparation of Chitosan-Eugenol/Thymol Nanocomposites

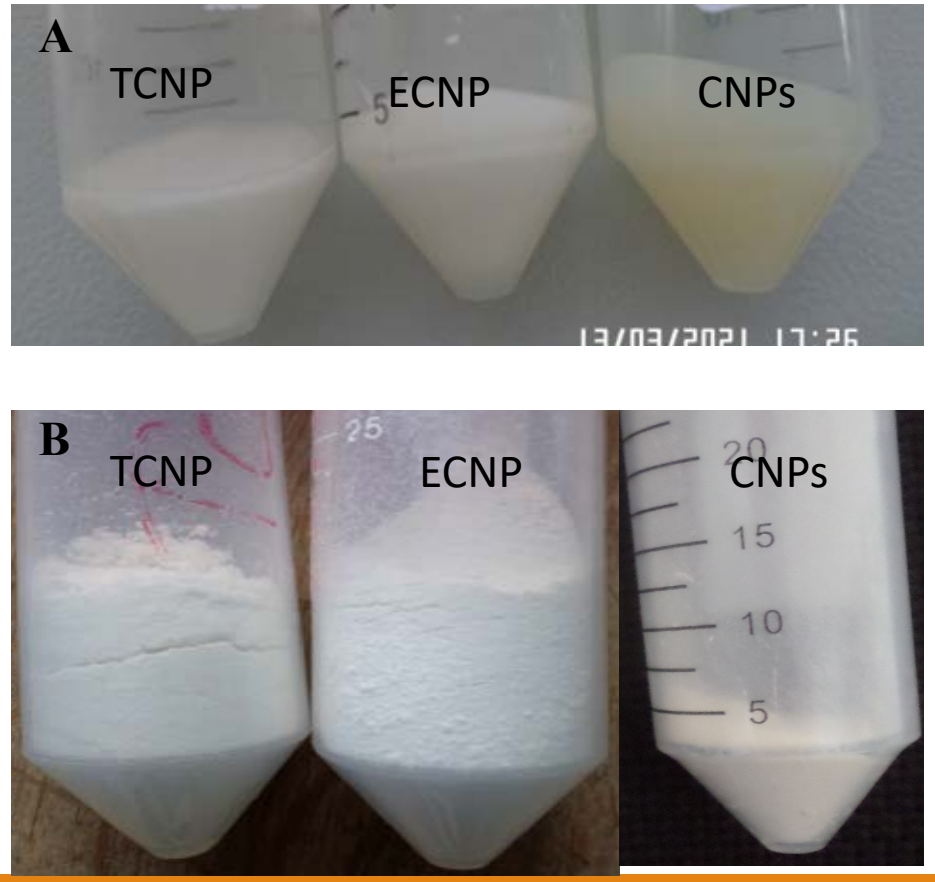


Figure 5: Minimum inhibitory concentration of thymol (**A**) and eugenol (**B**) against *R. solanacearum* using resazurin aided microdilution; negative control (broth only); positive control (broth + *R. solanacearum*).

Results cont’

Characterization of nanoparticles

a) Scanning Electron Microscope (SEM)

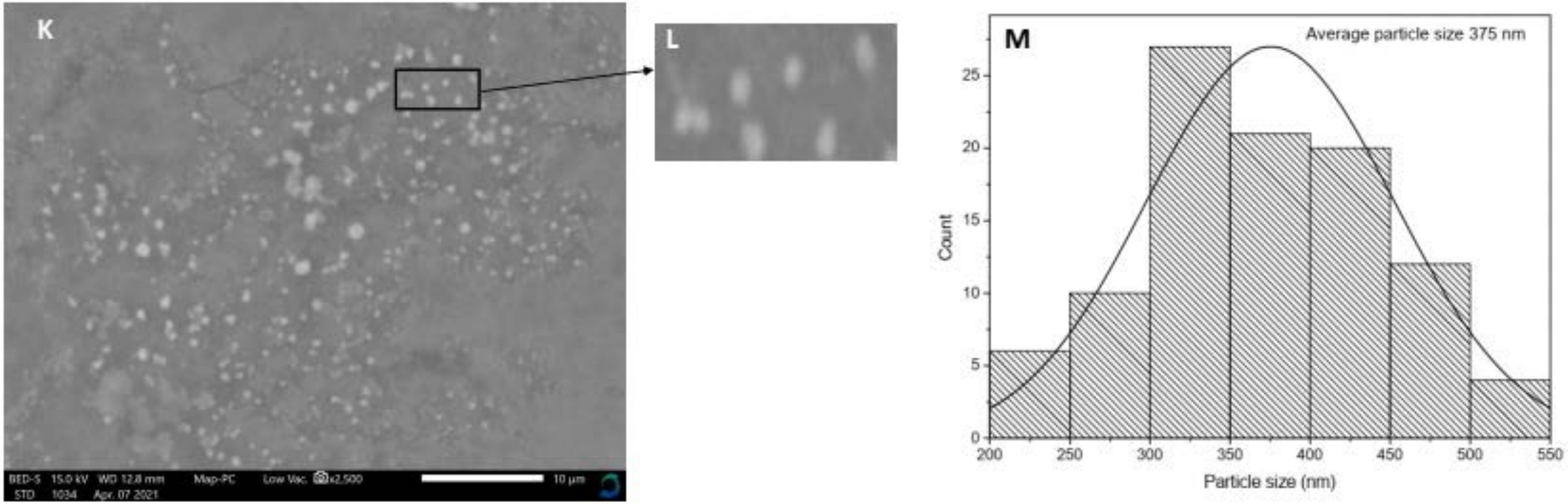


Figure 6: Scanning electron Microscope (SEM) image of CNP (**K**), its zoomed image (**L**) and particle size distribution(**M**)

Results cont'

Characterization of nanoparticles

a) Scanning Electron Microscope (SEM)

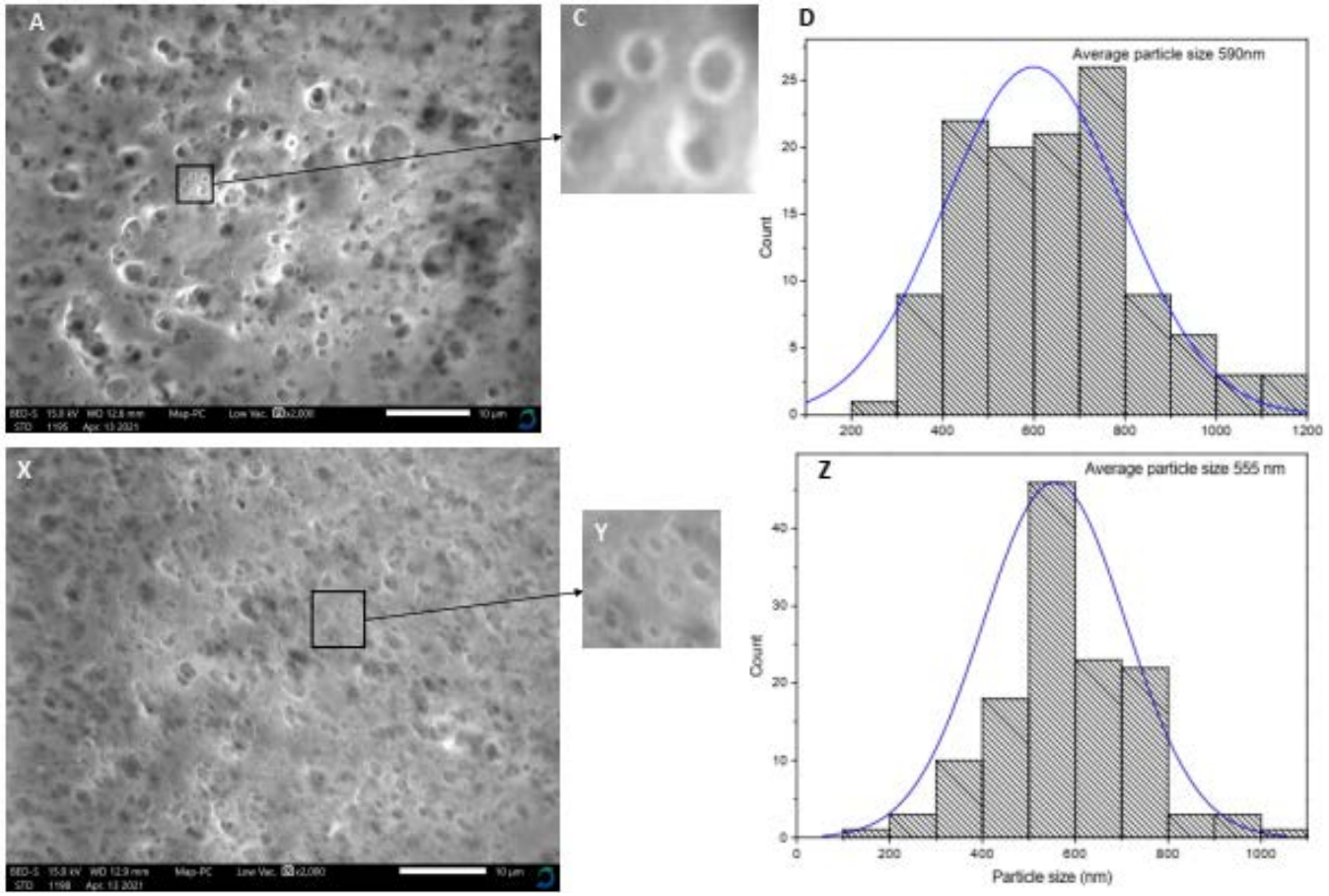
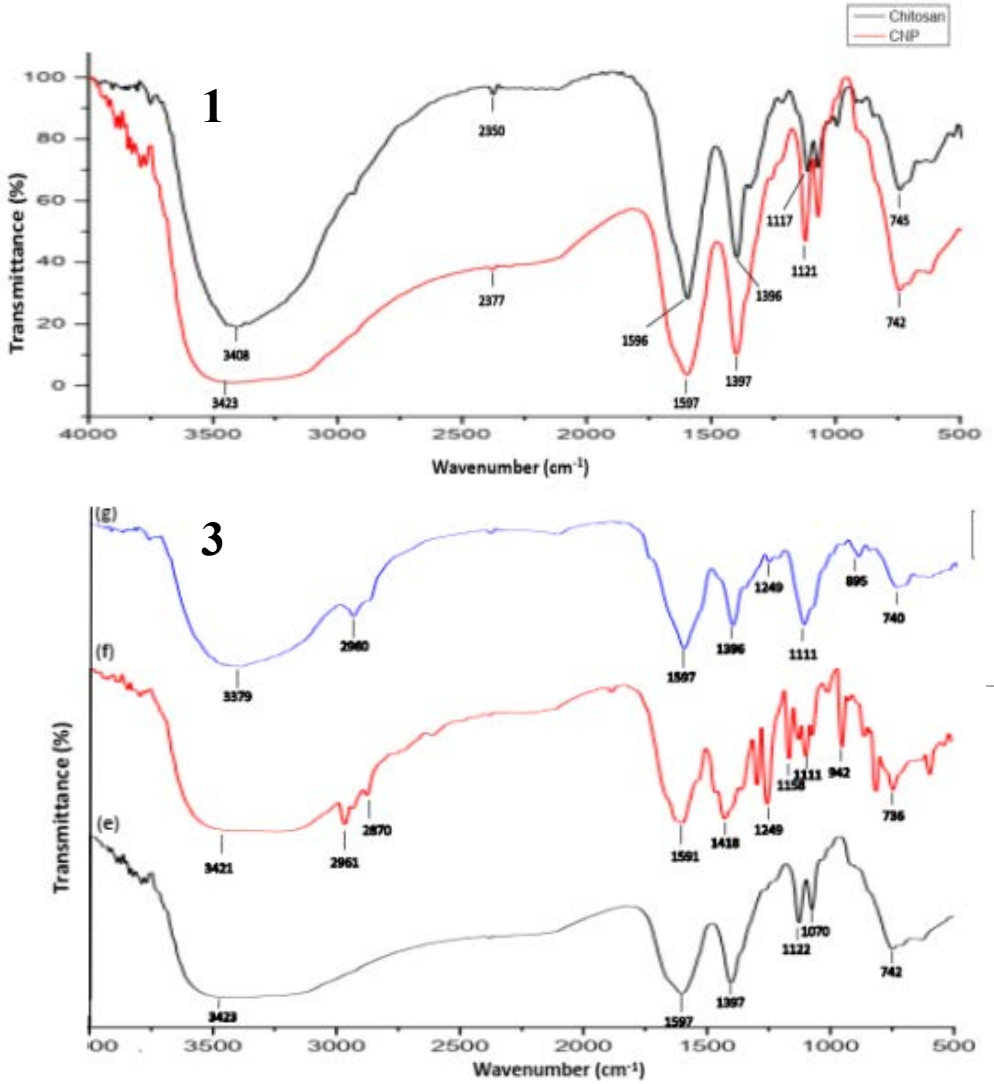


Figure 7: SEM image of TCNP (**A**), its zoomed image (C) and particle size distribution(**D**); SEM image of ECNP (**X**), its zoomed image (Y) and particle size distribution (**Z**).

b) Fourier-transform Infrared Spectroscopy analysis (FTIR)

Results cont'



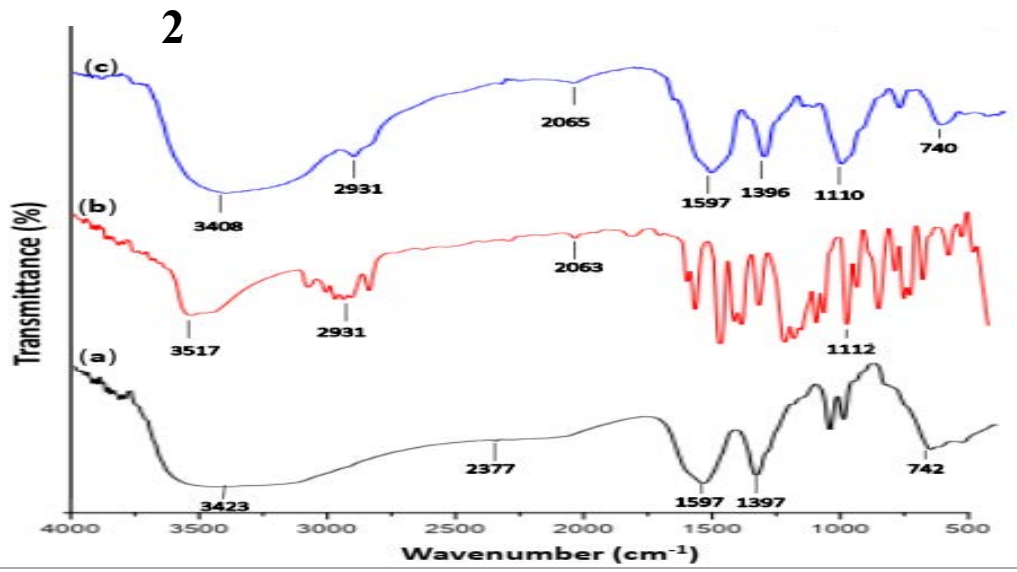


Figure 8: **1,** FTIR spectra of chitosan powder and CNPs; **2**, **(a)** chitosan nanoparticles (CNP), **(b)** eugenol, and **(c)** eugenol-loaded chitosan nanoparticles (ECNP); **3**, **(e)** chitosan nanoparticles (CNP), **(f)** Thymol, and **(g)** chitosan encapsulated thymol essential oil nanoparticles (TCNP).

Results cont’

a) Investigation of the antibacterial activities of the nanoparticles

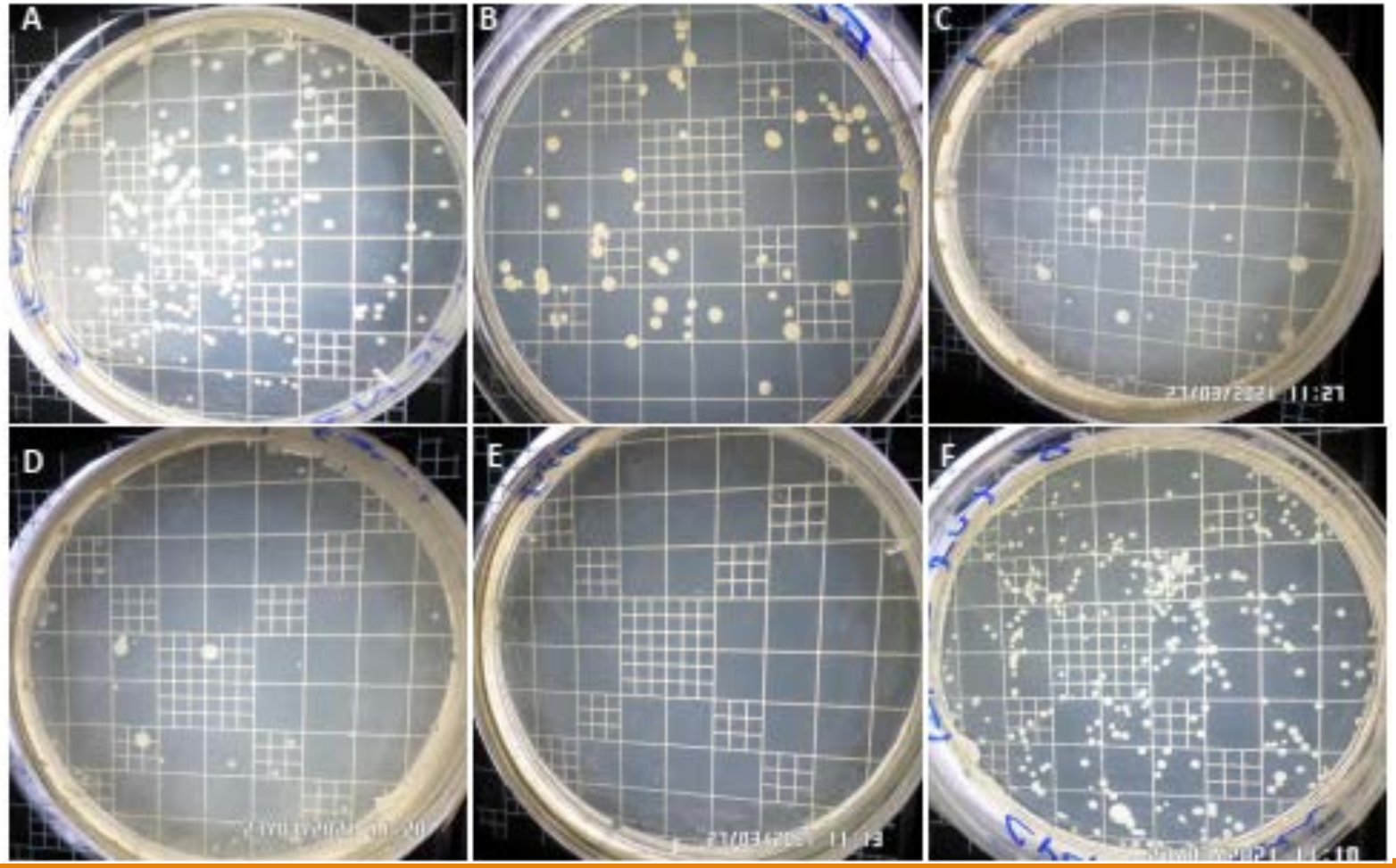
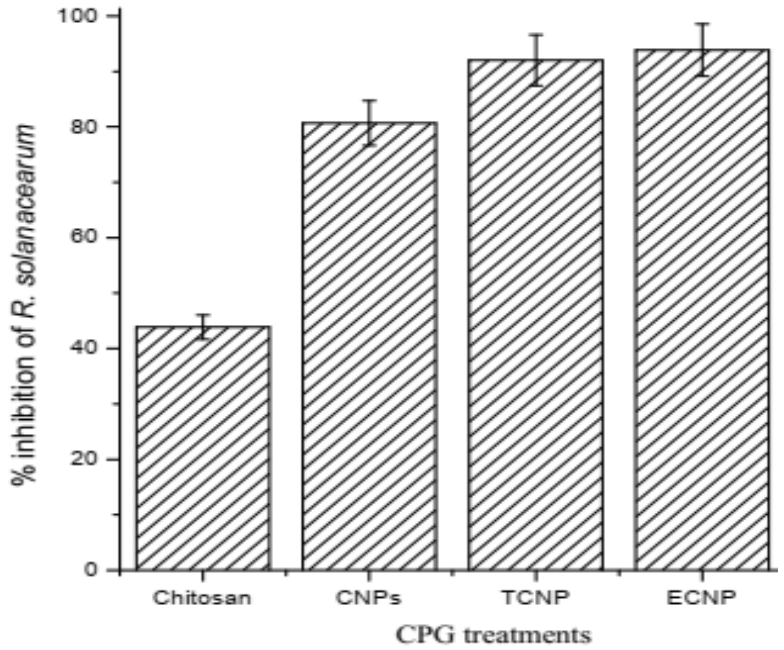


Figure 9: Representative photographs of the bactericidal activity towards *R. solanacearum* by chitosan **(A)**, CNPs **(B)**, TCNP **(C)** and ECNP **(D)**. Negative control **(E)** and positive control **(F)**.

Results cont'

% Inhibition of *R. solanacearum*



- Inhibition of *R. solanacearum* growth

$$(\%) = \frac{C-A}{C} \times 100$$ (Costerton *et al.*, 1999)

Where C and A are the bacterial colonies of positive control and treated plates respectively.

- Percentage inhibition of *R. solanacearum* was 92 and 94% for TCNP and ECNP respectively.

Figure 11: Inhibitory effect of chitosan, CNPs, TCNPs and ECNPs against *R. solanacearum*. Error bars indicate standard errors of mean (n = 3).

Results cont'

Minimum inhibitory concentration of TCNP and ECNP

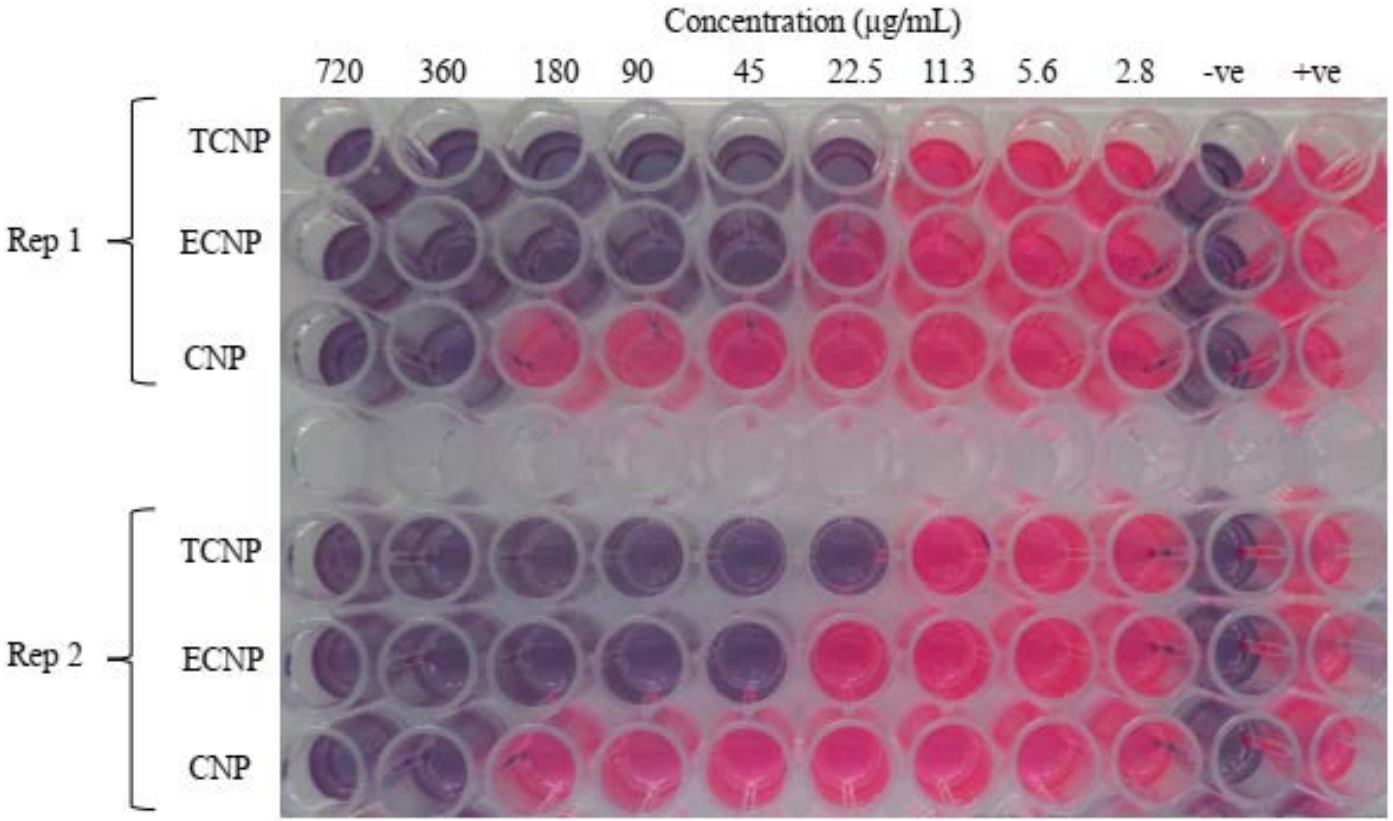


Figure 12: Minimum inhibitory concentration of TCNP, ECNP and CNP against *R. solanacearum* using resazurin aided microdilution; negative control (broth only); positive control (broth + *R. solanacearum*).

Results cont'

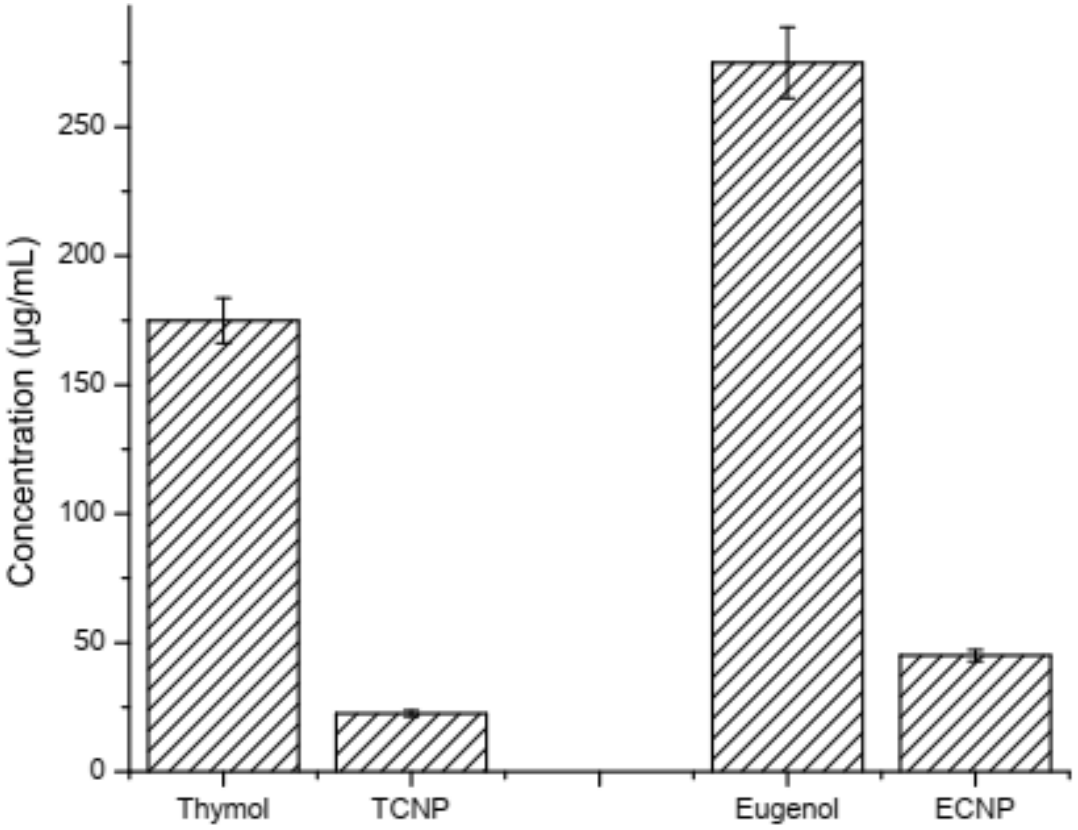


Figure 13: Minimum inhibition concentration (MIC) of thymol, eugenol, TCNP and ECNP against *R. solanacearum.*

Conclusion

- Thymol showed a higher antibacterial activity against *R. solanacearum* in comparison to Eugenol.
- Both compounds had bactericidal effect against the pathogen.
- A combination between eugenol and thymol had indifferent effect against *R. solanacearum*.
- Thymol and eugenol were successfully encapsulated in CNP through ionic gelation method with an average particle size of 590 and 555 nm respectively.
- TCNP and ECNP inhibited the growth of *R. solanacearum* with up to 92 and 94% respectively.
- The MIC of thymol reduced from 175 to 22.5 µg/mL after encapsulation with CNPs while that of eugenol reduced from 275 to 45 µg/mL.

Recommendations

- The need to move from *in vitro* to *in vivo* (Currently being done)
- Field trials

Acknowledgements

Theme: *"Enhancing Phytosanitary Systems for Healthy Plants, Safe & Sustainable Trade"*
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