# ABSTRACT

*Capsicum annuum* L. is a significant horticulture crop known for its pungent varieties and use as a spice. The pungent character in the plant, known as capsaicinoid, has been discovered to have various health benefits. However, its production has been affected due to various biotic factors, including fungal diseases such as anthracnose, vascular wilt, and damping-off caused by *Colletotrichum capsici*, *Pythium aphanidermatum*, and *Fusarium oxysporum*, respectively. In contrast to chemical fungicides, which often come with drawbacks such as environmental concerns and the potential for resistance development, various plant extracts and essential oils are gaining significant recognition as plant protectants due to their inherent antifungal effectiveness and environmentally friendly attributes. Contemplating these findings, the present research entitled "*Development of botanical formulations for managing fungal phytopathogens in Capsicum annuum L.*" primarily focuses on developing multi-targeted fungicidal nanoformulations employing plant extracts and essential oils. This comprehensive study encompasses various stages, starting with the assessment of numerous plant extracts and essential oils against the targeted phytopathogens. Subsequently, it involves the characterization of the bioactive components within the selected botanicals, an *in-silico* evaluation of these identified compounds against the phytopathogens, exploration of synergistic antifungal interactions among these botanicals, formulation development, and culmination in a thorough *in-planta* assessment of the developed formulation.

The study commenced by evaluating the *in-vitro* antifungal activity of several plant extracts and essential oils against the targeted phytopathogens. Within this investigation, licorice (*Glycyrrhiza glabra*) cold water extract (LAE), and thyme (*Thymus vulgaris*) essential oil (TO) emerged as remarkably potent botanicals against the *C. annuum* pathogens. LAE at 200 mg $ml^{-1}$ demonstrated the maximum antifungal activity of 89.9% against *P. aphanidermatum*, whereas TO at 0.25 mg $ml^{-1}$ showed 100% inhibition of *C. capsici*. Enhanced cellular

component leakage observed in response to TO and LAE treatments provided clear evidence of damage to the fungal cell wall and plasma membrane. This phenomenon can be attributed to the TO lipophilicity and triterpenoid saponins of LAE. Furthermore, TO and LAE treatments also led to a decrease in ergosterol biosynthesis.

Metabolite profiling using gas chromatography-mass spectrometry and high resolution-liquid chromatography-mass spectrophotometry analysis showed the presence of several bioactive compounds. The co-application of TO and LAE, at notably lower concentrations of 100 mg $ml^{-1}$ LAE and 0.125 mg $ml^{-1}$ TO, demonstrated a remarkable synergistic effect in controlling fungal pathogens. The combined application also resulted in more significant cellular component leakage and a more substantial reduction in ergosterol biosynthesis compared to TO or LAE applied alone. Additionally, the research extended its focus to the identification of the key bioactive molecules within the thyme oil and licorice water extract that act to inhibit the Cox1 enzymes of the phytopathogens, employing the *in-silico* approach. From a wide range of bioactive molecules screened, the molecular docking indicated trans-carveol, carvacrol, kaempferol 3-rhamnoside 7-xyloside, kaempferitrin, and astragalin 7-rhamnoside as the potential inhibitors for Cox1 of *C. capsici*, β-Caryophyllene, Caryophyllene acetate, hispaglabridin A, kaempferol 3-rhamnoside 7-xyloside and licorice glycoside A for Cox1 of *F. oxysporum* and (+)-Longifolen, Caryophyllene acetate, Hispaglabridin A, Neoliquiritin 2''-apioside and Licorice-saponin A3 for Cox1 of *P. aphanidermatum*. Molecular dynamic simulations confirmed the stability of docked complexes when evaluated through multiple descriptors. Additionally, MM/PBSA analysis supported the findings, indicating the spontaneous binding of the enzymes to the screened ligands.

Furthermore, the study involves developing oil-in-water nanoemulsions using thyme oil (TO) alone and in combination with licorice aqueous extract (LAE) and comparing their efficacies against the targeted phytopathogens. The optimized nanoemulsions developed with TO alone

(TFO4) and with LAE (TFO4-10LAE) with droplet size (Z-average diameter) of 24.33 ± 0.86 nm and 217.0 ± 5.9 nm, respectively, exhibited promising results pertaining to their stability and shelf-life. This stability was substantiated through rigorous two-year shelf-life testing.. The life cycle assessment showed that the production of TFO4-10LAE nanoemulsion has a lesser negative impact on the environment than that of chemical pesticides. TFO4-10LAE, characterized by the preservation of the bioactive molecules present in LAE and TO (as confirmed through GC-MS and FTIR analyses), demonstrated a synergistic and superior antifungal activity when compared to TFO4, which contains only TO. During the *in-planta* assay against chilli anthracnose, nanoemulsion was applied as seed, foliar and seed-foliar treatment. TFO4-10LAE applied as a seed-foliar application significantly reduced the disease severity index (DSI) to 20.37% over control (62.96%), along with the improved activity of induced resistance enzymes and plant growth parameters. Nanoemulsion was applied as a seed, seedling, and seed-seedling treatment during the *in-planta* experiment against Fusarium wilt in chilli. TFO4-10LAE applied as a seed-seedling application against Fusarium wilt significantly reduced the disease severity index (DSI) to 25.33% over control (68.92%), with enhanced induced resistance enzyme activity and plant growth metrics. TFO4-10LAE applied as a seed application against the pythium damping-off exhibited a high reduction in disease incidences, showing pre- and post-emergence damping-off incidence of 11.9 and 5.9%, respectively, as opposed to 43.7 and 12.6% in control.

Drawing from the achieved results, this work will pave the path for future research and development of fungicide alternatives that are not only safer and more sustainable but also surpass the effectiveness of traditional chemical options.