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# Ginsenol as a promising bio control agent: molecular docking insights against *fusarium* pathogens for sustainable agriculture

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## ABSTRACT

*The increasing challenges posed by agricultural pests and diseases highlight the need for innovative biocontrol strategies. This study investigates a novel biocontrol compound, Ginsenol, isolated from the rhizospheric fungi associated with sesame plants. Gas chromatography-mass spectrometry (GC-MS) and High performance Liquid chromatography (HPLC) analysis confirmed the presence and identified the molecular structure of ginsenol, providing a foundation for its bioactivity assessment. Following identification, we conducted molecular docking studies using two computational tools: PyRx, Discovery Studio. Molecular docking studies revealed strong interactions between Fusarium oxysporum f. sp. lycopersici (FOL) and Fusarium oxysporum f. sp. cubense (FOC). These interactions support ginsenol's mechanism of inhibiting pathogen growth and enhancing crop resilience. The outcome obtained from in silico analysis suggested that the bioactive compound namely, ginsenol bind effectively showing -7.3 kcal/mol binding energy for Fusarium oxysporum f. sp. cubense (FOC). and -6.5 kcal/mol for Fusarium oxysporum f.sp. lycopersici (FOL). These findings suggest that ginsenol may serve as a promising biocontrol agent, contributing to sustainable agricultural practices by mitigating pest and disease threats while minimizing chemical inputs.*

**Keywords:** GC-MS, HPLC, Ginsenol, Biocontrol, Molecular docking

## INTRODUCTION

Globally, tomatoes rank as the second most economically important vegetable crop (Hussain 2016). In 2017, worldwide tomato production reached over 160 million tons. Leading producers include the European Union, China, Turkey, the United States, and India. Approximately 40 million tons of the total tomato yield undergo processing (Pathak, 2018). Throughout their lifecycle, crop plants are vulnerable to diseases, which pose a significant threat to human well-being. These diseases cause yield losses of 13–22% and result in billions of dollars in economic damage (Venbrux, 2023). Phytopathogens represent the primary obstacles in tomato cultivation, leading to decreased yields. From seedling to maturity, tomato plants are susceptible to various biotic and abiotic stresses (Brahimi,2017). The *Fusarium oxysporum* species, which are extensively distributed worldwide, are particularly harmful *Fusarium* wilt, caused by *Fusarium oxysporum f. sp. lycopersici*, is a major pathological issue affecting tomato crops globally. This disease is one of the most prevalent and destructive, as the pathogen is soil-borne and can spread through water and contaminated soil (Chacón, 2021). In the absence of a susceptible host, the pathogen can survive as chlamydospores in soil and crop residues for up to six years (Asif, 2023). *F.oxysporum* can easily infect the crop at any growth stage. The pathogen damages the plant's vascular system, with visible symptoms often appearing late, typically when the plant begins to bear fruit. Due to the pathogen's highly variable nature, this disease remains a significant problem in tomato-growing regions (Chitwood-Brown, 2021).

*Fusarium oxysporum f. sp. lycopersici* (FOL) causes a severe wilt disease that affects tomatoes throughout their growth stages worldwide. As a hemi-biotrophic pathogen that colonizes the xylem, FOL can infect various hosts, leading to symptoms such as leaf chlorosis, vascular tissue browning, and plant death. In India, tomato crops grown in greenhouses experienced significant losses of up to 45 percent due to FOL infection (Vignesh, 2021; Srinivas, 2019; Yucel, 2007). The pathogen's ability to survive in soil for extended periods, coupled with its rapid germination and infection under favourable conditions, results in

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substantial yield reductions. Banana (Musa sp.) stands as a crucial food and fruit crop in numerous developing nations (Heslop Harrison and Schwarzacher, 2007). Among the most devastating diseases affecting bananas is *Fusarium* wilt, commonly known as Panama disease (Ploetz, 2000). This ailment has been documented in all banana-producing regions, including Asia, Central and South America, Africa, and Australia (Ploetz, 2006).

In India, the disease is pervasive across nearly all banana-growing areas, impacting most commercial cultivars except Red Banana and Nendran (Thangavelu and Mustaffa, 2010). The causal agent of banana *Fusarium* wilt has been identified as *Fusarium oxysporum formae specialis cubense* (FOC), a soil-dwelling hyphomycete (Ploetz and Pegg, 1997; Ploetz, 2006; Fourie *et al.,* 2011). This fungus enters banana plants through their roots and colonizes the vascular tissue (xylem), resulting in external symptoms such as progressive wilting, gradual leaf yellowing, eventual petiole collapse, and longitudinal splitting of the pseudostem's outer leaf sheaths (Yin *et al.,* 2011). The hallmark internal symptom of the disease is the characteristic discoloration of vascular tissues, ranging from light yellow to dark brown, initially appearing in the outermost or oldest leaf sheath before advancing up the pseudostem (Ploetz, 2006). Ultimately, the disease leads to the demise of infected banana plants.

Researchers worldwide have explored various approaches to combat the disease, including chemical control (Nel *et al.,* 2007), biological control (Saravanan *et al.,* 2003; Cao *et al.,* 2005; Lian *et al.,* 2009), and molecular techniques (Paul *et al.,* 2011; Yip *et al.,* 2011). While some of these studies demonstrated effective suppression of FOC growth in laboratory and greenhouse settings, they failed to provide efficient control of *Fusarium* wilt under field conditions. Molecular docking is a computational technique widely used to evaluate the interaction and binding affinity of bioactive compounds with target proteins. In the context of *Fusarium spp*., a significant phytopathogen affecting tomato and banana plants, molecular docking is employed to assess the antifungal potential of metabolites produced by rhizospheric fungi associated with sesame plants. The present Study was focused on the effect of various natural compound on *Fusarium* species. The docking studies were carried out in order to know the binding affinity of the ligand molecule of rhizospheric fungi produced which is associated with sesame plants root against the target protein of FOC and FOL species.

## MATERIALS AND METHODS

### Target protein identification

The molecular docking study focuses on identifying key target proteins in *Fusarium* spp. essential for its growth, pathogenicity, or survival. Common targets may include enzymes involved in cell wall synthesis (e.g., chitin synthase), membrane integrity, or signaling pathways critical for fungal virulence.

### Compound selection

Bioactive natural compounds are isolated from rhizospheric fungi associated with sesame plants. These compounds are characterized and identified using techniques like Gas chromatography-mass spectrometry (GC-MS) and High Performance Liquid Chromatography.

### Protein and ligand preparation

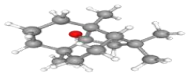
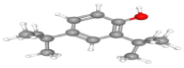
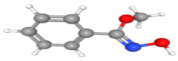
The FOL binding protein SIX6 (PDB ID: 8EBB, 1.88 Å resolution) and effector protein NADase1 (PDB ID: 8R15, 2.40Å resolution) crystal structures were obtained from the RCSB Protein Data Bank (Berman, 2002) and utilized as templates for molecular docking simulations. Discovery Studio 2019 molecular visualization software 4.5 (Sharma, 2021) were employed for protein preparation. Initially, the proteins were imported into the software to identify and eliminate extraneous elements such as default ligands, ions, and water molecules. Subsequently, the cleaned files were saved in PDB format in form of PDBqt for additional analysis. All ligands (fungal metabolites) listed in Table 1 obtained from the Pubchem database and ZINC database were based on an extensive literature review. They were used to design the three-dimensional structures by 0penbabel (O'Boyle *et al.,* 2011). All structures were saved in Mol2 format for their use in the docking process. (O'Boyle *et al.,* 2011)

### Docking of ligand against target protein

The docking simulations were conducted using PyRx0.8 The process involved several steps : (https://pyrx.sourceforge.io/).

Initially, the prepared proteins and ligands files were loaded into the PyRx interface. Next, the docking grid was established, encompassing the active site or the anticipated binding pocket of the protein. AutoDock Vina, a docking engine integrated within PyRx, was employed to determine the ligand's binding affinity and forecast its binding position. Finally, the outcomes of the docking were evaluated based on binding affinity scores and the specific interactions observed between the ligands and proteins. Docking analysis is carried out by Discovery studio (Sharma, 2021).

Table 1: A list of ligands used in docking analysis against target proteins

| Compound (Ligands) | R.T. (Min) | Molecular Formula | Structure |
|---|---|---|---|
| Ginsenol | 53.887 | C15H26O |  |
| 2,4,Di-tert-butylphenol | 20.058 | C14H22O |  |
| Heptadecane,2,6,10,15-tetramethyl | 12.943 | C21H44 |  |
| Oxime –methoxy-Phenyl | 3.405 | C8H9NO2 |  |
| Hexadecanoic acid | 31.409 | C17H34O2 |  |
| 1-Dodecanol | 10.386 | C12H26O |  |
| Heptadecane | 20.879 | C17H36 |  |

## RESULTS

To determine the binding free energy (ΔG) between ligands and proteins docking complexes, Autodock vina engine with PyRx (https://pyrx.sourceforge.io/) was employed. The binding affinity of ligand molecules against target antimicrobial protein receptors can be assessed using binding free energy (ΔG). An analysis of the binding free energy (ΔG) for all ligands molecules revealed varying binding affinities against the antimicrobial proteins listed in Table 2. For further interaction analysis, the docking complex with the lowest binding free energy (ΔG) was selected from among different docking conformations. Discovery studio was utilized to conduct a comprehensive examination of the binding interactions and affinity of all docked complex molecules.

### Determination of the binding free energy (ΔG) between ligands and target protein using Autodock vina software

| | Binding free energy (ΔG) (Kcal/mol) | |
|---|---|---|
| Ligand | SIX6 (PDB ID:8EBB) | NADase1 (PDB ID:8R15) |
| 1 | -6.5 | -7.3 |
| 2 | -6.0 | -6.9 |
| 3 | -4.6 | -5.7 |
| 4 | -5.6 | -5.6 |
| 5 | -4.0 | -5.0 |
| 6 | -3.7 | -4.7 |
| 7 | -3.9 | -5.0 |

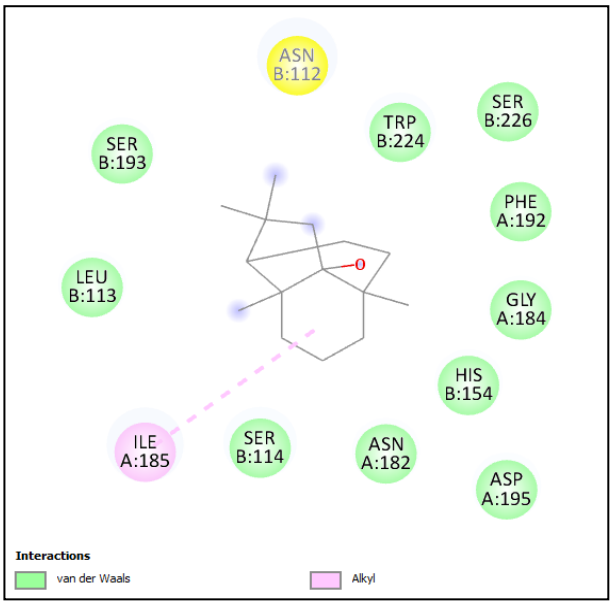



Figure 1: 2D interaction: SIX6

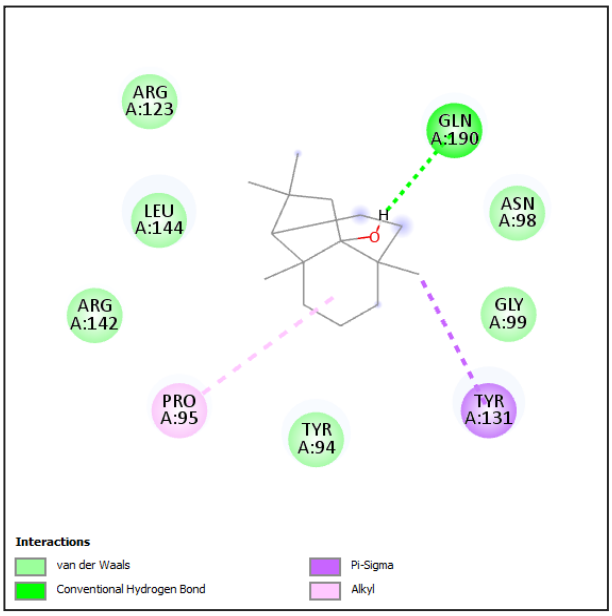



Figure 2: 2D interaction: NADase1

## Molecular docking studies against *Fusarium oxysporum* f.sp. *lycopersici* (FOL)

Analysis of the 2D interaction diagram unveiled the ligand's interactions with crucial amino acids in the SIX6 binding pocket. The ligand exhibited Van der Waals forces with SER B:114, SER B:193, and ASN B:182, which helped stabilize it within the pocket. An alkyl interaction of hydrophobic nature was detected with ILE A:185, enhancing the ligand's affinity for binding. These observed interactions align with the ligand's proposed inhibitory function, further supported by its binding energy of -6.5kcal/mol. Moreover, the ligand was found to interact with ASN B:112, a residue essential for substrate recognition, suggesting possible competitive inhibition. The calculated RMSD value was 0.0, which falls below the 2 Å threshold, suggesting that the docking process met the required standards. These observations offer valuable insights into the molecular mechanisms of ligand binding and its potential applications in biocontrol.

## Molecular docking studies against *Fusarium oxysporum f. sp. cubense* (FOC)

The molecular docking of the ligand with the target protein NADase1(PDB ID:8R15) was conducted using Autodock vina within the PyRx platform to predict the binding affinity and interaction profile. The ligand-protein interaction were visualized using Discovery Studio (Sharma, 2021), and the resulting 2D interaction diagram highlights the residues involved in the binding pocket. The analysis revealed a strong polar interaction through a hydrogen bond with the GLN190 residue. The ligand's stability within the binding pocket was enhanced by hydrophobic interactions, specifically a Pi-sigma interaction with TYR131 and an alkyl contact with PRO95. The complex's stability was further strengthened by van der Waals forces involving residues ASN98, LEU144, and ARG123. An RMSD value less than 2 Å was used to determine the best docking position between ligand and target protein. The calculated RMSD value was 0.0, which falls below the 2 Å threshold, suggesting that the docking process met the required standards. These observations, coupled with a docking score of -7.3 kcal/mol, indicate robust binding between the ligand and the protein's active site.

## CONCLUSION

Natural compounds offer an advanced and safe method for managing Fusarium wilt in tomatoes and Panama disease in bananas. This study's outcomes suggest that Ginsenol and 2, 4, Di-tert-butylphenol have potential as fungicides or lead compounds against these diseases. Notably, Ginsenol exhibited the strongest binding affinity for both target proteins, displaying numerous interactions and superior bioactivity scores. As a result, Ginsenol emerged

as the top performer among the seven compounds examined in this computational analysis. Our findings indicate that Ginsenol, a natural antifungal agent, targets proteins associated with SIX6 in FOL and NADase1 in FOC. This computational investigation marks the first detailed exploration of Ginsenol's antifungal mechanisms against wilt and Panama disease. However, further in vivo molecular and field research is essential to corroborate these in Silico results before Ginsenol can be recommended as a fungicide for FOL and FOC.

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