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# *In silico* and Experimental Evaluation of Homoeopathic Remedies for Rice Blast Disease Management

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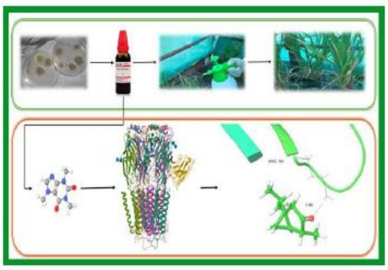
## *Abstract*

*Rice blast, caused by the fungus Pyricularia oryzae, is one of the most destructive diseases in rice cultivation, leading to significant yield losses. Current management strategies heavily rely on chemical fungicides, which pose environmental and health risks. This study explores homoeopathic medicines, Thuja and Sulphur, as eco-friendly alternatives for managing rice blast disease. The study aimed to assess the antifungal efficacy of Thuja and Sulphur in field conditions and explore the molecular interactions of Thujone, the active compound in Thuja, with key fungal proteins using in silico docking. A field experiment was conducted with rice variety IR50 treated with Sulphur (6X and 30 potencies), Thuja (6X and 30 potencies), and controls (positive and negative). Due to unfavourable conditions, molecular docking of Thujone with four target proteins (ABC1, CLP1, HRIP1, and CTI6) was performed. Protein structures were prepared using AlphaFold and optimised with Schrödinger tools. Ligand preparation and docking were carried out using Glide Docking. Field experiments were inconclusive due to climatic factors. Docking results demonstrated that Thujone interacted effectively with all four proteins, with CLP1 showing the highest binding affinity (docking score: -5.095 kcal/mol; Glide energy: -22.102 kcal/mol). Hydrogen bonding and key residue interactions were identified, highlighting Thujone's antifungal potential. Thuja, particularly through its active compound Thujone, exhibited promising antifungal activity against P. oryzae in silico. Despite field limitations, these findings support the potential of homoeopathic remedies as sustainable alternatives to chemical fungicides. Further field trials under controlled conditions are recommended to validate these results.*



***Graphical Abstract***

***Keywords:*** *Homoeopathy, Molecular Docking, Pyricularia Oryzae, Rice Blast, Thuja, Sulphur.*

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## Introduction

Rice functions as a fundamental staple crop worldwide since it supplies nutrition to over half of global community members. Artificial rice cultivation faces serious threats because of different stresses that exist alongside rice blast disease which stands out as the most harmful stress caused by the *Magnaporthe oryzae* fungal pathogen [1]. Nations with warm and humid environmental conditions suffer the most yield reduction because of this fungal disease. The ability of pathogens to adapt along with the development of resistance and high costs together with environmental worries restricts the effectiveness of traditional control measures based on chemical fungicides and resistant rice varieties. An urgent need exists for sustainable eco-friendly methods to control rice blast disease [2].

Homoeopathy which functions as an alternative medical discipline has drawn recent research interest regarding its use as a tool for plant disease management. The core concepts of homoeopathy show how dilute active plant substances potentially improve resistance mechanisms in plants to fight disease pathogens. Several homoeopathic remedies have shown promising effects against fungi while strengthening plant health mechanisms although scientists currently understand them poorly [3]. Linking in silico research methods to experimental analysis creates better comprehension of remedy-interacting molecules which guides the creation of green and innovative plant disease control solutions [4].

The emergence of *M. oryzae* as a recurrent threat to rice production necessitates innovative and sustainable solutions [5]. Synthetic fungicides together with genetic resistance breeding face limitations because they produce environmental toxicities and pathogen resistance development along with residue build-up [6]. The management of plant diseases shows promise through homoeopathy since it employs diluted natural compounds which trigger defense reactions in plants [7].

This is in contrast to the modern medical practice, which focusses on specific pharmacological interventions. This introduction lays the groundwork for understanding the rationale, methods, and outcomes of the study, with a particular emphasis on the contribution that the study makes to bridge the gap between traditional and modern approaches to healthcare for the purpose of addressing a worldwide health issue [8] The fact that rice, also known as *Oryza sativa*, is the primary source of nutrition for more than half of the world's population makes it an essential component of food security. Rice blast disease, which is caused by the fungus Pyricularia oryzae, stands out as one of the most destructive of the many biotic and abiotic pressures associated with its production. However, the production of rice faces considerable obstacles from a variety of biotic and abiotic stresses. It is possible for the disease to affect all of the plant's aerial components, which can result in yield losses of up to fifty percent in extreme outbreaks. The spread and severity of the disease are further exacerbated by several climatic conditions, including humidity, temperature, and wind speed [1, 2].

Although they are successful, chemical fungicides are the primary component of the current management measures for rice blast. These fungicides, despite their effectiveness, pose considerable environmental and health issues. The rise of fungicide-resistant fungus strains, pollution, and dangers to human health are some of the factors that are included in this category. As a consequence of this, there is an urgent requirement for alternatives that are sustainable and kind to the environment, and that continue to be effective while mitigating these issues.

Homoeopathy is an alternative medical system that provides a holistic and ecologically friendly approach. It has been extensively researched for agricultural applications due to its potential positive impact on the environment. Thuja and sulphur are two examples of remedies that have the potential to be used as innovative therapies against rice blast disease. These remedies were chosen based on the idea of symptom similarity.

The computer method known as molecular docking makes it possible to investigate the interactions that take place between ligands and proteins. This method is extremely important in the process of drug discovery as well as with the evaluation of bioactive chemicals. In this study, the active component found in Thuja, known as Thujone, was examined to determine its binding affinity to specific fungal proteins, including ABC1, CLP1, HRIP1, and CTI6. The purpose of this investigation was to discover the mechanism of action of Thujone as well as its possible therapeutic applications.

The natural monoterpene ketone compound thujone exists primarily in plants from three plant genera: Artemisia, Thuja and Salvia. Northern white cedar or arborvitae (Thuja occidentalis) serves as the primary source where people can find this compound. The stereoisomeric pair of thujone contains α-thujone as the active biological component while β-thujone is less potent.

The isolation process for thujone from Thuja occidentalis requires individuals to perform solvent extraction followed by steam distillation and chromatographic purification. Thujone contains two rings that form a bicyclic monoterpene skeleton while the ketone functional group is present within the structure. The biological compound contains ten carbon atoms ($C_{10}H_{16}O$) which forms a bicyclo[3.1.0]hexan-3-one core structure. A thujone molecule exhibits three dimensions (3D) as described below: Thujone uses a bicyclohexane layout to maintain its compact structure and rigid shape. Its reactivity is based on a C=O (carbonyl) functional group. Thujone exists in two structural isomers named α-thujone and β-thujone that show different positions of the isopropyl group. Due to its neurotoxic properties and GABAergic response thujone has become an important subject for pharmacological and toxicological research studies. The psychoactive properties of absinthe were historically associated with thujone but contemporary regulatory measures restrict this substance in commercially available items.

In order to evaluate the effectiveness of Thuja and Sulphur in the management of rice blast disease, this study combines field experiments with advanced in silico methodologies. This allows the study to bridge the gap between traditional and modern agricultural practices within the agricultural industry. Through the provision of alternatives to chemical fungicides, the findings intend to make a contribution to the implementation of sustainable farming practices. This research combines computational and laboratory-based methodologies to establish scientific evidence about homoeopathic formulation potential as eco-friendly substitutes for controlling rice blast disease. These search results will create new biocontrol methods which decrease reliance on chemical fungicides alongside promoting environmentally friendly farming systems.

## Materials and Methods

### Pathogen Collection

The fungal pathogen Pyricularia oryzae was purchased in petridish with PDA medium from Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu.

### Field Experiment Setup

A 196 $m^2$ field was divided into 16 sections and sown with rice variety IR50. Treatments included Sulphur 6X, Sulphur 30, Thuja 6X, Thuja 30 (via foliar and drenching methods), a positive control (Tricyclazole), and a negative control. High ambient temperatures (>33°C) and low humidity rendered field experiments inconclusive, necessitating an alternative approach.

## In Silico Analysis

### Protein Structure Retrieval and Preparation

The 3D structures of the target proteins (ABC1, CLP1, HRIP1, and CTI6) were retrieved from AlphaFold due to the unavailability of experimentally resolved structures. The protonation state and hydrogen-bond optimization were achieved using the ProtAssign tool, and the structures were minimized with the 'impref' tool in Schrödinger's OPLS3e force field.

### Ligand Preparation

Thujone's structure (CID_10931629) was retrieved from the PubChem database and processed using the LigPrep tool of Schrödinger. All possible protonation and tautomeric states were generated within a pH range of 7.0 ± 2.0.

### Molecular Docking

Active sites of the target proteins were predicted using the SiteMap tool in Schrödinger. The receptor grids were generated using the receptor grid generation panel, and docking was performed using Glide Docking, treating the ligand as flexible and the protein as rigid. Docking scores, Glide energies, and interactive residues were analyzed for each target.

## Results

### Field Observations

Despite extensive preparation, the field experiment failed due to unfavorable abiotic conditions. No visible symptoms of rice blast were observed even after fungus inoculation. Thujone has promising bioactive properties, further controlled field trials, dose optimization, and environmental impact studies are needed to ensure safe and effective applications in medicine and agriculture. Due to seasonal climatic conditions, the effects on ecology, including insect repulsion and microbial interaction, were not replicated in the paddy field. The spraying through inoculation method and Lesions that failed to develop even after 10 days are shown in **Figure 1**. Field observations play a crucial role in validating the biological activity, efficacy, and potential risks associated with thujone, particularly in its natural environment and its applications in agriculture, medicine, and ecology.

### Molecular Docking

Molecular docking results revealed that Thujone (**Figure 2**) interacts effectively with all four target proteins, with docking scores ranging from -5.095 kcal/mol to -3.873 kcal/mol and Glide energies between -22.102 kcal/mol and -15.721 kcal/mol (**Table 1**). Among the targets, CLP1 exhibited the strongest interaction with Thujone, with a docking score of -5.095 kcal/mol and a Glide energy of -22.102 kcal/mol. Key residues contributing to the interaction were identified for each target.

**Figure 1.** Rice leaves before spraying, spraying through the inoculation method and Lesions failed to develop even after 10 days

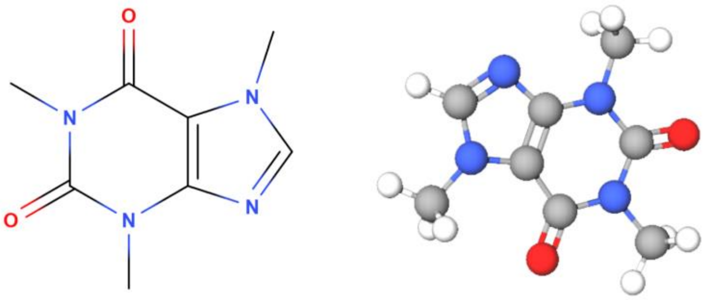


**Figure 2.** 2D and 3D Structure of Thujone

**Table 1:** Docking results of the compound Thujone with the selected targets

| Sl. No | Protein | Docking Score | Glide Energy | Number of Hydrogen bonds |
|---|---|---|---|---|
| 1 | ABC1 | -4.564 | -17.938 | 1 |
| 2 | CLP1 | -5.095 | -22.102 | 1 |
| 3 | HRIP1 | -3.873 | -15.721 | 1 |
| 4 | CTI6 | -4.878 | -16.482 | 1 |

***CTI6:*** Residues such as Phe142, Leu173, and Ser177 contributed to the active pocket, with Leu173 forming a hydrogen bond with the ketone moiety of Thujone.

***CLP1:*** Active site residues included Ala196, Gly192, Leu170, and Ser189. A hydrogen bond was formed between Leu170 and the ketone group of Thujone at a distance of 1.80 Å.

***ABC1:*** Key residues included Lys433 and Thr570, with Lys433 contributing to hydrogen bonding.

***HRIP1:*** This target showed the weakest interaction, with a docking score of -3.873 kcal/mol.

Thujone's ketone group played a significant role in stabilising interactions through hydrogen bonding, as depicted in **Figures 3–6**. CLP1's active site exhibited a favourable binding environment, highlighting its potential as a high-affinity target for Thujone.

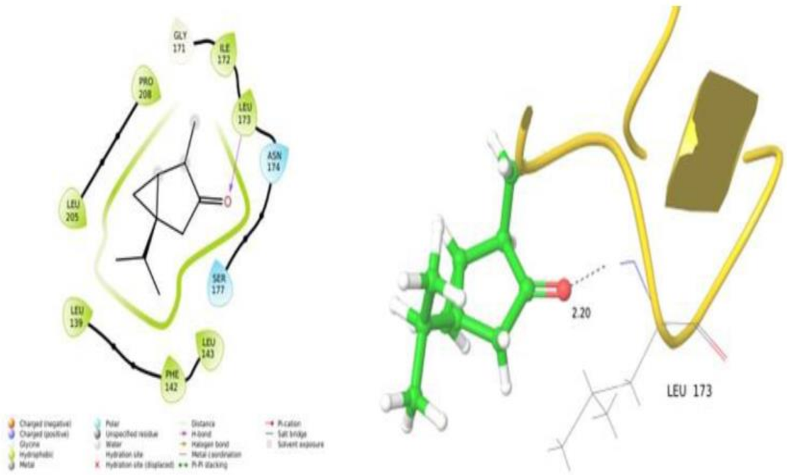
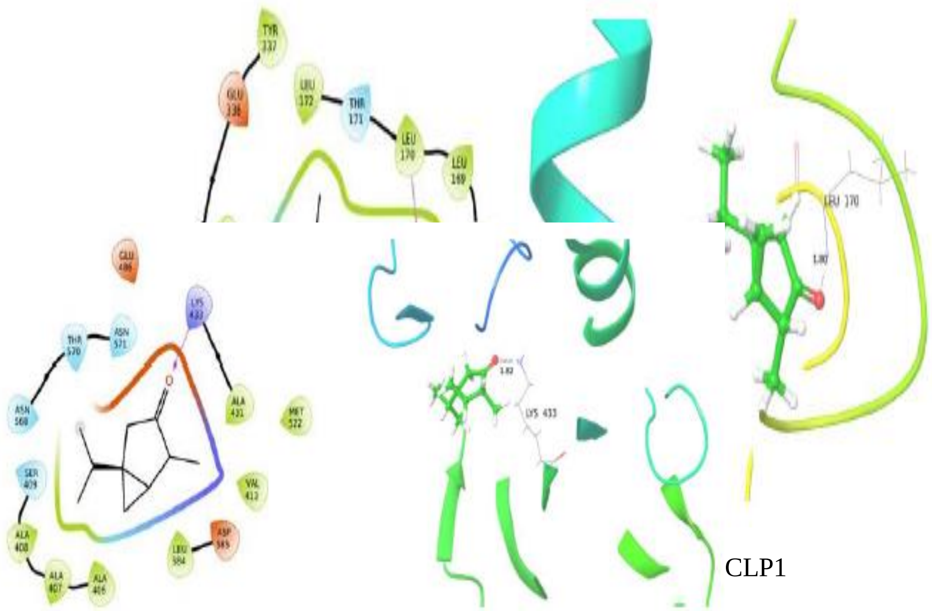



**Figure 3.** Interaction of Thujone with CTI6. A) 2D interaction showing the ligand in black and the active site residues around. B) 3D interaction showing ligand in ball and stick with green carbons.



CLP1

**Figure 5.** Interaction of Thujone with

ABC1

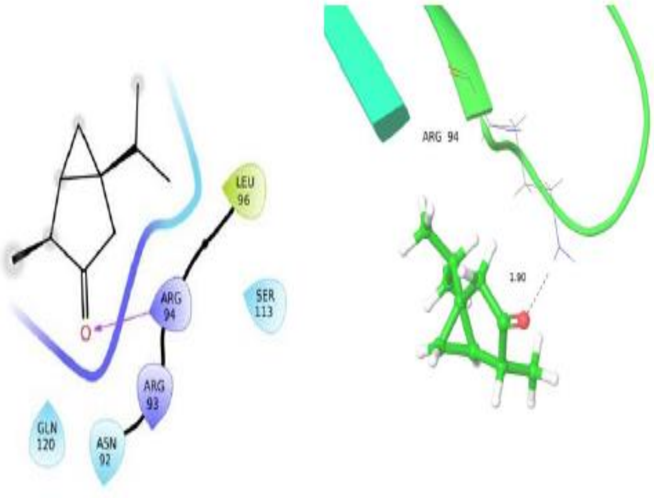



**Figure 6:** Interaction of Thujone with HRP1

## Discussion

Rice variety IR50 served as the test crop in 196 m² of field space divided into 16 sections for experimental purposes. Treatments included Sulphur (6X and 30), Thuja (6X and 30) applied via foliar spray and soil drenching, and control treatments (positive: Tricyclazole, negative: untreated). The rice blast pathogen received inoculation treatments and high-temperature/high-humidity (greater than 33°C) and low-humidity conditions in an experimental field, but failed to cause any detectable disease symptoms. Field conditions prevented fungal development, so pathogenesis research produced unclear results. The experiment results demonstrate how environmental factors control disease rates while exposing the restrictions inherent to field tests executed in unfavourable situations [10].

The results suggest that Thuja, through its active compound Thujone, exhibits promising antifungal properties against P. oryzae. The high docking scores for CLP1 highlight its potential role in disrupting fungal growth and pathogenicity. Sulphur, although not tested in silico, has established antifungal properties, as evidenced in prior studies. These findings align with the principles of symptom similarity used in homoeopathy and point to the potential of these remedies as sustainable solutions for rice blast management [11].

The field experiment's failure underscores the challenges posed by climatic variability in agricultural research. Future studies should incorporate controlled environmental setups or explore additional in silico methods to complement field data.

The ketone group present in Thujone built stable hydrogen bond interactions with all active site residues in the four proteins studied. The ketone functional group promotes protein-ligand complex stability and provides direction for improved antifungal Thujone derivatives. The binding environment of CLP1 demonstrates strong structural compatibility with Thujone that could serve as the foundation for developing new antifungal compounds [12].

## Conclusion

Molecular docking analysis data presents foundational insights that can lead to experimental laboratory validation. In silico results successfully set the groundwork for creating targeted laboratory and greenhouse experiments despite field trial constraints. Understanding the molecular connections between Thujone and pathogen proteins creates new possibilities for reasoning-based medication engineering that produces modern antifungal drugs. The research demonstrates why computational approaches need to combine with conventional experimental methods to solve weather-related and geographical research obstacles. The combination of computational approaches with experimental methods speeds chemical agent discovery for sustainable farming and food defence systems. This study demonstrates the potential of homoeopathic remedies, particularly Thuja, in managing rice blast disease [13]. Molecular docking results underscore the efficacy of Thujone against critical fungal proteins. While field experiments faced limitations, the promising in silico results warrant further validation through field trials under controlled conditions. If successful, these remedies could provide an eco-friendly alternative to chemical fungicides, benefiting farmers and the environment. The computational analysis identified CLP1 as the most promising target for Thujone, exhibiting the strongest interaction among the evaluated proteins [14]. Thujone's ability to form hydrogen bonds, particularly through its ketone group, underscores its potential as a candidate for drug development. Future experimental validation of these findings is recommended to explore Thujone's therapeutic applications further [15]. Research on thujone extraction from Thuja occidentalis generates essential knowledge about its chemical makeup as well as biological behaviour as well and useful application territory. The bicyclic monoterpene ketone thujone functions as an essential compound, so neuroscience and medicinal research centres around it because it binds to the γ-aminobutyric acid type A (GABA<sub>A</sub>) receptor for its diverse pharmacological activities [16 -19].

Laboratory researchers utilize steam distillation and solvent extraction, and chromatographic methods simultaneously to obtain pure thujone forms from Thuja occidentalis. Accurate characterisations of this bioactive compound alongside its quantitative measurements rely on advanced spectroscopic techniques that utilise NMR and FTIR, and GC-MS tools [20].

Two isomeric forms called α-thujone and β-thujone make up the structural makeup of thujone, which display varying biological effects. Studies show its ability to affect GABAergic neurotransmission, making researchers interested in potential results on the nervous system, particularly regarding absinthe-linked psychiatric effects. The toxicological assessment of excessive consumption shows that extreme usage of the substance may cause convulsant and neurotoxic outcomes, which require management in the pharmaceutical and herbal medical fields.

## Conflict of Interest

There was no conflict of interest. The authors funded this study.

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