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In silico analysis of antifungal compounds against *Colletotrichum gloeosporioides* to control mango anthracnose disease

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Abstract
Mango fruit (*Mangifera indica*) is one of the important economic fruit crops because of its good smell, delicious taste, excellent flavor, and attractive fragrance. It also called as king of fruits. However, one of the constraints of markets is disease, especially anthracnose disease caused by fungus *Colletotrichum gloeosporioides*. It causes yield loss of about 10-50%. *In silico* analysis was done to identify the competence of the fungicides. Beta tubulin of the fungus was found to be important protein involved in causing the disease. The three dimensional structure of Beta tubulin was modeled through Homology modeling with 2XRP_A as template. The protein-Ligand interaction studies has been carried out to analyse the competence of fungicides. Docking studies reveals that ligands such as Benomyl and Azoxystrobin has good binding affinity with the receptor and express its competency towards controlling anthracnose disease.

Keywords: *Mangifera indica, Colletotrichum gloeosporioides, in silico,* beta tubulin, homology modelling

Introduction
Anthracnose, caused by *Colletotrichum gloeosporioides* is presently recognized as one of the most harmful disease bringing about postharvest decay of mango in the world. It causes a problem after harvest due to disease expression starting at the ripening stage. The pathogen causes leaf spot and blight, blossom blight, twig blight, fruit rot, and tree dieback in mangoes (Nelson *et al*., 2008) [4]. The gene TUB2 of the fungus act as vital pathogen and the protein interact with host proteins and cause modifications in the mango genome (Ru-Lin *et al*., 2007) [7]. If some compounds bind suitably to the tubulin protein, it prevents microtubule formation and consequently inhibits cell division. Hence, docking studies was performed between tubulin and about 22 ligands. This comparative analysis was carried out with an aim of identifying the interaction between fungus protein and ligands which may help to find the best fungicide to control mango anthracnose disease.

Materials and Methods
Sequence Retrieval
The Protein sequence Beta tubulin of *C.gloeosporioides* of sequence length 447 was retrieved from NCBI. The three dimensional structure of the protein was not available. Hence the Protein structure was predicted using Homology modeling.

Template selection
PDB BLAST was performed for the protein Beta tubulin of *C.gloeosporioides* and the structure with highest sequence identity with the target Protein has been selected as a template and retrieved from Protein Data Bank. The retrieved template structure has been used for Modelling the Protein structure.

Molecular modeling and validation
The three dimensional structure of Beta tubulin was modeled using Swiss model server using Homology modeling method The stereochemical quality of the model was evaluated using Procheck. (Laskowski RA *et al*., 1996) [3]. The spatial features of the residues should comply with empirically characterized constraints on torsional angles captured in Ramachandran plots (Ramachandran *et al.* 1963) [6].

Ligand Library Generation

Chemical compounds which were used effectively for controlling the fungus *Colletotrichum gloeosporioides* were selected through literature search and its structure was collected from PubChem database. The phytochemical compounds which act as an antifungal agent was retrieved from Dr. Dukes database and their structures was generated.

Protein-ligand docking

Beta tubulin protein and the ligands were subjected for docking using Glide. The Ligands was prepared using Lig prep and Protein was prepared using Protein Preparation wizard. Receptor Grid was generated for binding sites and it was calculated and stored. During the initial phase of docking calculation, the maximum poses generated from the variables were fixed to 5000 and the best variable which set the number of poses per ligand that enters the energy minimization was set to 1000. The dielectric constant of 4.0 and 1000 steps of conjugate gradient was applied in the energy minimization protocol. At the ending of each docking calculation, utmost 100 poses per ligand were generated. Using Glide scores (G-score) function, the best docked structure was chosen. (Parasuraman *et al*., 2014) [5].

Results and Discussion

Chemical compounds with antifungal activity

The chemical and phytochemical compounds having antifungal activity has been retrieved from databases and tabulated below.

Table 1: Chemical Compounds

S. No	Compound Name
1.	Carbendazim (Kongtragoul *et al*., 2011) [2]
2.	Prochloraz
3.	Benomyl
4.	Azoxystrobin
5.	Thiabendazole
6.	Benzimidazole
7.	Thiram
8.	Tolnaftate
9.	Carvone

Modelling of the Protein

The sequence of the protein was retrieved from NCBI and subjected to PDB BLAST for Template selection. The template Protein with PDB ID 2XRP_A was selected as template (Fig. 1) since it has high sequence identity of 83.45% and 95% Query coverage with the target protein. The target sequence and the Template structure was subjected for modeling using Swiss model User Template mode. The modelled structure has been shown in Fig 2. The modelled protein structure has been validated using Ramachandran plot.

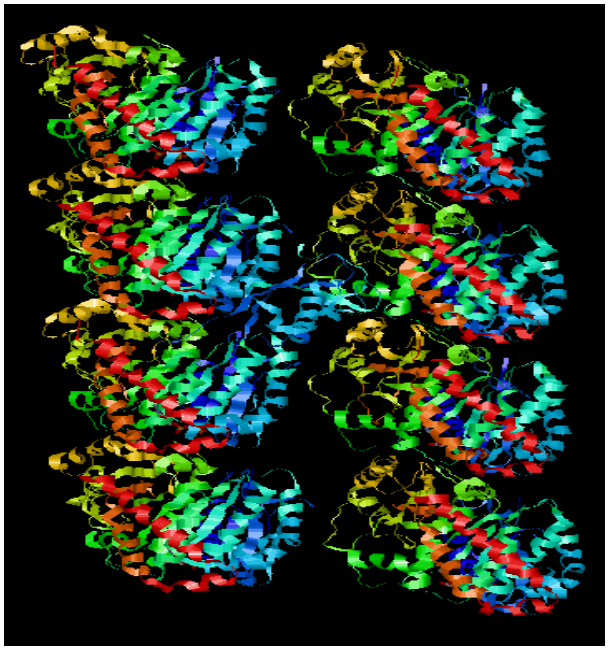

Fig 1: Template structure 2XRP

Fig 2: Modelled Protein Tubulin

Model Validation

The models were built using swiss model user template mode and it was followed by the energy minimization and loop refinement. The modelled structure was validated in Procheck (SAVS server). The stereo chemical quality of the predicted model and accuracy of the model was evaluated after the refinement using Ramachandran Plot shown in Fig 3. About 87% of residues were seen in most favorable region and only 3 residues such as His, Val and Leu were present in disallowed region. Since, residues in most favorable region was above 80%, it could be considered as valid structure used for further docking studies.

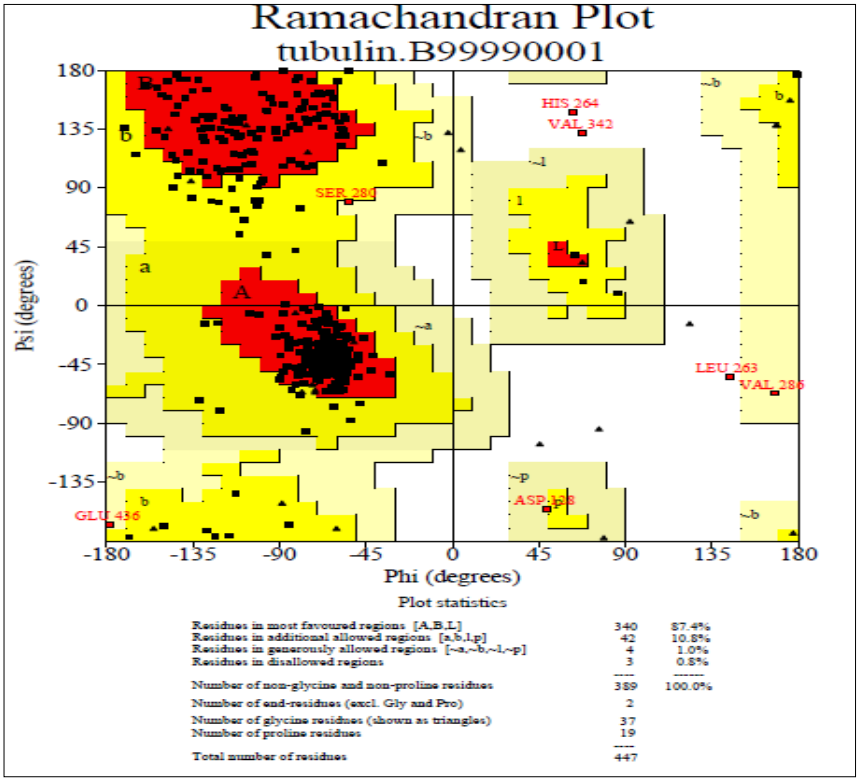

Fig 3: Model Validation using Ramachandran plot

Docking

The structures of bioactive compounds were searched and their analogues that show anti-fungal properties based on literature survey. About 9 structures has been selected from chemical category with antifungal properties and subjected for docking through Glide. The tubulin protein was optimized in protein preparation wizard and the grid was generated. The structure of the ligands were built by Ligprep in all possible conformations to dock on the target *C. gloeosporioides.* The size of the active site was determined by generating the Glide Receptor Grid. The orientations of the ligand in the binding sites are predicted. Docking was performed between the protein *C. gloeosporioides* and the selected compounds. The scoring functions of the docked compounds are obtained in the form of G-Score. G-Score indicates the binding ability of the ligand to the protein. The negative scoring indicates the better docking between the ligands and the protein. Table. 3 illustrates the scoring functions of the best docked compounds with the protein.

Table 3: Glide score

Name	GScore	Hbond	Electro	LowMW
Benomyl	-6.6	-2.9	-2	-0.5
Azoxystrobin	-6	-1	-3.1	-0.1
Benomyl-2	-5.7	-1.6	-1.7	-0.5
Benomyl-3	-5.7	-2	-1.9	-0.5
Benomyl-4	-5.4	-1.9	-1.7	-0.5
Benomyl-5	-5.2	-1.9	-1.7	-0.5
Azoxystrobin-2	-6.4	-0.9	-3.1	-0.1

Benomyl and Azoxystrobin has been docked perfectly with modeled tubulin protein with G Score -6.6 and -6.0, Hydrogen Bond -2.9 and -1.0 shown in Fig.4, 5, 6 and 7.

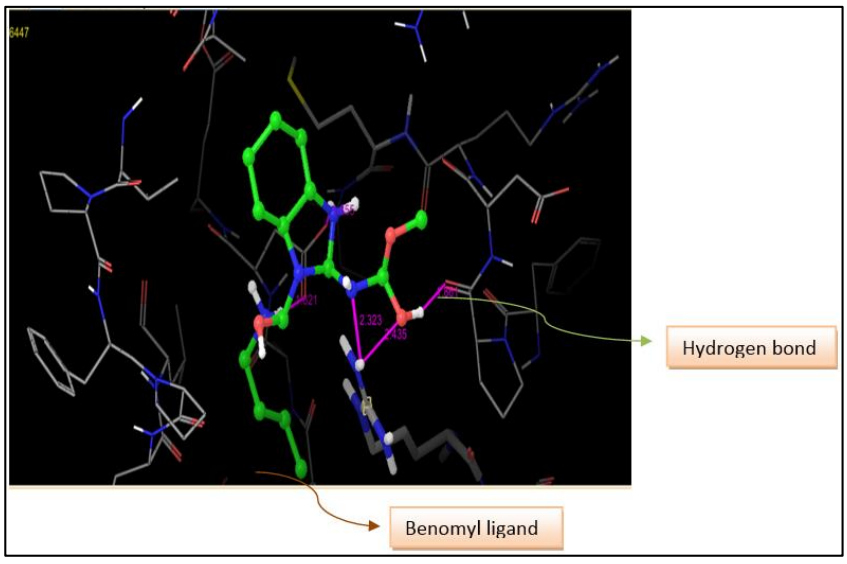

Fig 4: Benomyl ligand with Tubulin protein

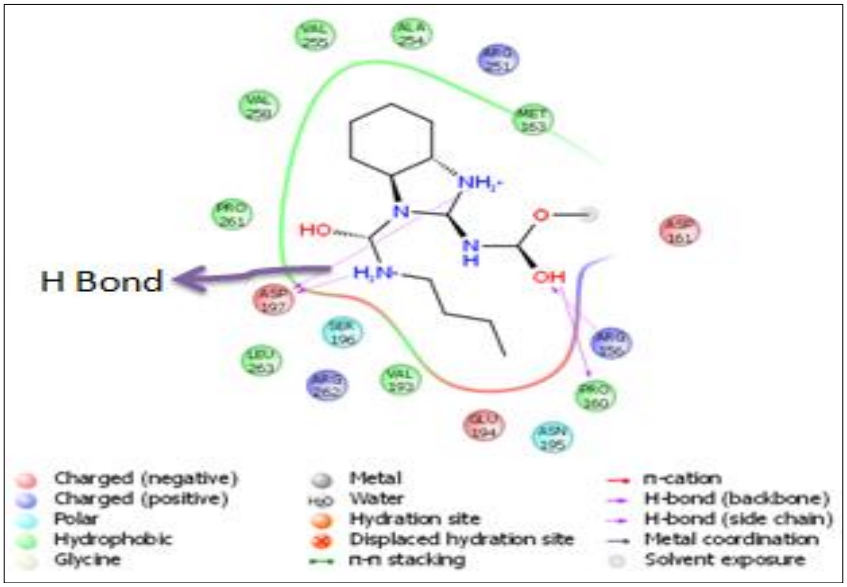

Fig 5: Residues forming Hydrogen bond with the compound Benomyl

Here the aminoacid residues Asp 197, Arg 156 and Pro 160 of tubulin protein has formed H-bond with Benomyl ligand.

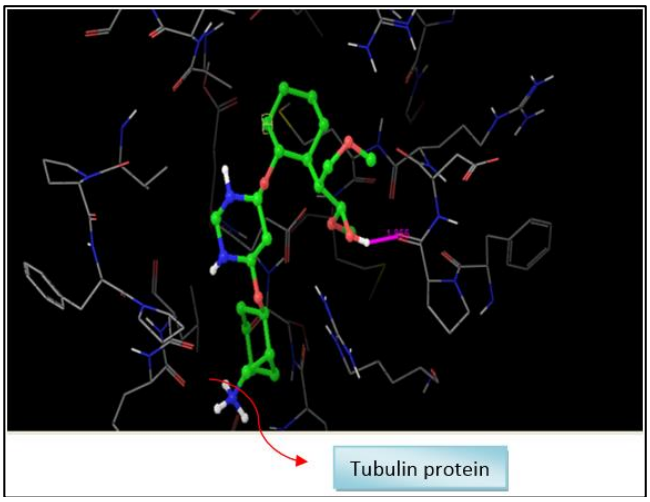

Fig 6: Azoxystrobin ligand with Tubulin protein

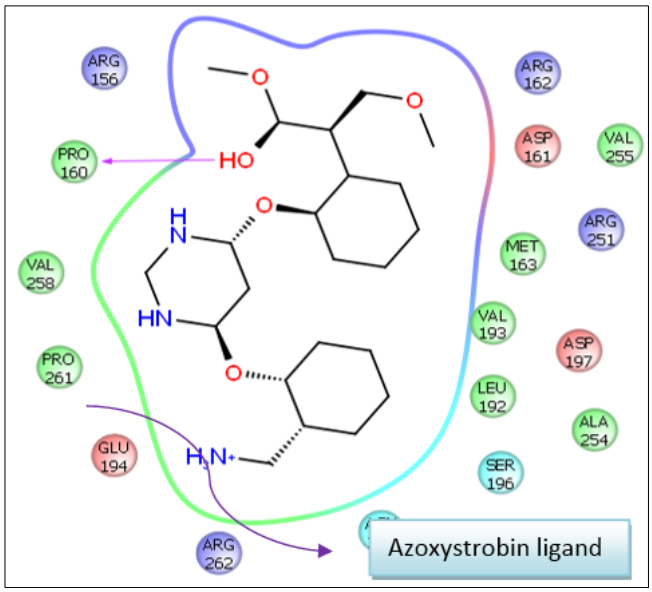

Fig 7: Residues forming Hydrogen bond with the compound Azoxystrobin

Here, Pro 160 has formed H-bond with Azoxystrobin. If it was applied in mango field, it inhibits microtubule formation and cell division of tubulin protein.

Conclusion

In this study, *In silico* analysis has been used for modeling the target protein and to find the interaction between the selected antifungal compounds and the target. A comparative analysis has been carried out for analyzing the competence of the fungicides and it has been concluded that use of Benomyl and Azoxystrobin will control the fungus *Colletotrichum gloeosporioides* better than other fungicides. This has been concluded based on the binding affinity of the compounds towards the receptor protein Beta tubulin.

References

1. Flicker C, Smith ML, Akpagana K, Gbeassor M, Zhang J, Durst T *et al*. Bioassay-guided isolation and identification of antifungal compounds from ginger. Phytotherapy Research. 2003; 17(8):897-902.
2. Kongtragoul P, Nalumpang S, Miyamoto Y, Izumi Yu, Akimitsu K. Mutation at codon 198 of tub2 gene for carbendazim resistance in *Colletotrichum gloeosporioides* causing mango anthracnose in Thailand. Journal of Plant Protection Research. 2011; 51(4):378.
3. Laskowski RA, Rullmannn JA, MacArthur MW, Kaptein R, Thornton JM. AQUA and PROCHECK-NMR: Programs for checking the quality of protein structures solved by NMR. J Biomol NMR. 1996; 8:477-86.
4. Nelson. Scot Charles. *Mango anthracnose* (*Colletotrichum gloeosporiodes*). University of Hawai at Manoa, College of Tropical Agriculture and Human Resources, Cooperative Extension Service, 2008.
5. Parasuraman P, Suresh R, Premnath D. Balancing anti-amyloid and anti- cholinesterase capacity in a single chemical entity: *In silico* drug design. Int J Pharm Pharm Sci. 2014; 6(2):571.
6. Ramachandran GN, Ramakrishnan C, Sasisekharan V. Stereochemistry of polypeptide chain configurations. Journal of Molecular Biology. 1963; 7:95-99.
7. Ru-Lin Z, Jun-Sheng H. Cloning of a carbendazim-resistant gene from *Colletotrichum gloeosporioides* of mango in South China. Afr. J Biotechnol. 2007; 6(2):143-147.

8. Sharma Abhishek, Verma KS. *In vitro* cross pathogenicity and management of Collectotrichum gloeosporioides causing anthraenose of mango. Ann. Pl. Protec. Sci. 2007; 15:186-188.
9. Yenjit P, Issarakraisila M, Intana W, Chantrapromma K. Fungicidal activity of compounds extracted from the pericarp of Areca catechu against *Colletotrichum gloeosporioides in vitro* and in mango fruit. Postharvest Biology and Technology. 2010; 55(2):129-132.