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Research Article

VIRTUAL SCREENING AND IN VITRO ANALYSIS TO UNDERSTAND THE INHIBITORY EFFECT OF ALLIIN ON PYTHIUM SPLENDENS- PATHOGEN OF ROOT ROT DISEASE

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A B S T R A C T

The oomycete *Pythium splendens,* is a plant pathogen causing significant damage to broad spectrum of commercially and medicinally valuable plants such as black pepper, tomato, watermelon, bean etc. These pathogens are known to affect almost all parts of the plant by secreting effector proteins, prominent for their virulence. The most common method in practice to control these infections is usage of chemical fungicides but with severe toxicity. As an alternate, biofungicides such as crude extracts of plants is also widely used. In the present study, we aim to provide molecular evidence for the inhibitory activity of plant extracts from *Azadirachtaindica, Allium sativum* and *Curcuma longa* against *P. splendens* through molecular docking and *invitro* studies. Molecular docking analysis revealed that Alliin in *Allium sativum* showed significant molecular interactions with *P. splendens* necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins compared to commercially available chemical fungicides. The inhibitory potential of the lead compound was experimentally validated through Minimum Inhibitory Concentration (MIC) assay which was in good agreement with the *insilico* prediction. Thus, this study confirms the antifungal property of the natural lead compounds and the plant extracts to effectively combat against *Pythium* infections.

INTRODUCTION

Pythiumsplendens is an oomycete pathogen which is responsible for causing root rot disease in plants such as black pepper, tomato, watermelon, bean etc. The pathogen disrupts the plant host cell structure and its function by secreting pathogenicity related proteins that leads to infection *(*Kamoun, 2006; Lennart *et al*., 2013; Parveen and Sharma, 2015). Most of the oomycete species are reported to produce necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) that can trigger leaf necrosis and immunity-associated responses in plants (Ottmann *et al*., 2009; Birch *et al*., 2006; Kamoun, 2006).Once the pathogen succeeds in causing infection, it then leads to complete destruction of the plant. Various methods are in practice to protect the plants from pathogen attack, such as crop rotation, development of resistant cultivars, biological control and usage of chemical pesticides. But nearly all chemical pesticides or fungicides used against fungal/ oomycete pathogens are highly toxic and have shown to cause health hazards and environmental pollution (Dennis, 1993). The currently used fungicides such as Mancozeb, Metalaxyl, Mandipropamide, Pyrimorph, Fosetyl AL etc. can inhibit mycelial growth, cytospore germination and sporangium production (Yan *et al*., 2009).

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The prolonged inhalation of these fungicides by humans leads to neural and visual disturbances and lung infections (Dennis, 1993). They can also permanently silence or reprogram normal genes that last for several generations and are found to be very harmful to the environment (Komareka *et al*., 2010). As an alternative to these chemicals, practice of using bio fungicides is an effective approach to control the infections with less or no side effects (Enyiukwu *et al*., 2014; Obeidat *et al*., 2012).

Azadirachtaindica (Neem), *Nicotianatabacum* (Tobacco), *Aloe Barbadensis Miller* (Aloe vera), *Allium sativum*(Garlic), *Curcuma longa* (Turmeric*) etc.* are some of the commonly used plant extracts in order to protect crop plants from pathogen attack. *Azadirachtaindica (Neem)* is known to have numerous beneficial properties in the Indian tradition. Every part of this plant has medicinal properties and is used in human ailments and also to control household pests. The crude neem leaf oil is also active against bacterial and fungal strains (Asif, 2012; Mazid *et al*., 2011). *Curcuma longa* (Turmeric) isextensively used as a spice and also in traditional medicine due to its low side effects. Curcumin, the main chemical compound of turmeric has proven to have anti-inflammatory, antioxidant, antidiabetic, antibacterial, antifungal, hepatoprotective and anti-cancerous pharmacological activities (Zorofchian *et al*., 2014). The compound allicin found in garlic

extracts is an important antifungal agent and has antibacterial, antiviral, antitumor, anticoagulant, antihypertensive, antiparasitic and hepatoprotective effects (Joslin, 2003; Singh *et al*., 1990). Various garlic preparations have exhibited a wide spectrum of antifungal activity against species of *Candida, Cryptococcus, Trichophyton, Epidermophyton*, and *Microsporum (*Ankri *et al*., 1999; Wilson *et al*., 1997; Ark, 1959).

The aim of this study is to identify the antifungal property of the selected plant extracts against *Pythium splendens* through computational methods and via *in vitro* analysis. Since the protein structures of *Pythium splendens* are not yet available in Protein Data Bank (PDB), this part of the study is performed with selected pathogenicity related proteins from other *Pythium* species (*Pythium aphanidermatum*) that are available in PDB.

MATERIALS AND METHODS

In silico studies

The three dimensional structures of the pathogenicity related proteins - 3GNZ and 5NNW in *Pythium aphanidermatum* were retrieved from Protein Data Bank (PDB: http://www.rcsb.org/pdb/). 3GNZ (Toxin fold for microbial attack and plant defense), and 5NNW (NLPPya in complex with glucosamine) are necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins (NLPs) that triggers leaf necrosis and immunity-associated responses in host plants (Ottmann *et al*., 2009; Lenarcic *et al*., 2017; Lennarcic *et al*., 2019). Using 'Prepare Protein' protocol of Discovery Studio 4.0 (Inc, A. S., 2007) various process such as clean the experimental target structure, inserting the missing atoms in incomplete residues, modeling of missing loop regions, removing the disordered conformations and water molecules, standardizing atom names, protonating titratable residues using predicted pKs and finally minimizing the energy of the protein were performed. The binding site wasdefined using 'Define and Edit Binding Site' tool of Discovery studio 4.0. Two dimensional structures of phytochemicals from *Azadirachtaindica (68)*, *Allium sativum (97)* and *Curcuma longa (127)* and the commonly used chemical fungicides such as Mancozeb, Metalaxyl, Pyrimorph, Mandipropamide and Propamocarb were retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov/) in .sdf format. Then, the ligands were optimized and neutralized using 'Prepare Ligand' tool in Discovery Studio 4.0. The 'Prepare Ligand' protocol removes duplicated structures, generates isomers and tautomers, 3D conformations etc.

Molecular docking studies were performed using LibDock tool in Discovery Studio 4.0. LibDock is a commercially available docking program developed to manage the rapid docking of the combinatorial libraries of compounds by using the physicochemical properties of the ligands with corresponding features in the protein binding sites *(*Rao *et al*., 2007). The best pose is ranked based on LibDock score which is calculated based on the hotspot map for the receptor site that contains polar and apolar groups. The hotspot map rigidly aligns the ligand conformations to form favorable interactions with the active site. A final energy minimization step was performed and the top scoring ligand poses were considered as the best result. In addition, binding energies of the docked complexes were calculated using 'Calculate Binding Energy' protocol based on the following equation: Ebind= Ecomplex-ELigand-EReceptor (Jalaie *et al*., 2006)embedded in Discovery Studio 4.0.

Plant material collection and extraction

Azadirachtaindica (leaf), *Allium sativum* (bulb) and *Curcuma longa* (Rhizome) were collected from local places in Kollam District, Kerala, India. The materials for crude and methanolic extraction were washed and shade dried. 10 gm of each sample was finely ground and extracted with methanol for 2 days at room temperature by the Continuous Hot Sauxhlate Extraction method. Methanol was concentrated to dryness using a rotary evaporator and the extract yield was stored at room temperature. The crude extract of the three samples were filtered using Whatman No.1 filter paper and kept at 4 °C.

Antifungal/Anti-oomycete studies

The antifungal activity of selected plant samples against *Pythium splendens* was determined using spread plate method (Zorofchian *et al*., 2014) and agar- well diffusion method (Singh *et al*., 1990). The *P. splendens* culture was revived using Potato Dextrose Agar (PDA). A small agar piece with the stock culture was transferred on to a fresh PDA petri plate and incubated at 27^{o}C for 48 hours and used for further studies. Spread plate method was used for preliminary analysis. A small portion from the revived plate was placed on to the center of Potato Dextrose Agar (PDA) plate and transferred 100 µl of each samples (both methanol and crude extracts). Similarly for agar well diffusion method, petri plates were prepared and wells of 6 mm in diameter were made using sterile cork borer. Then, aliquot of 100 µl of the samples were added to each wells of all the plates. Mancozeb and *P. splendens* culture were kept as control plates. Alliin and Mancozeb (1mg/ml) each was serially diluted and 100 µl aliquot of compounds having different concentrations ranging from 500 µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml, 15.62µg/ml, 7.8 µg/ml and 3.9 µg/ml were added to each well. For proper diffusion, the plates were kept undisturbed for 1 hr and then incubated at 27^{o}C for 48 hours and the Minimum Inhibitory Concentration (MIC) was determined. The MIC values were determined visually as the lowest concentration of the compound that inhibits the growth of *P. splendens*. The assay was repeated with lower concentrations (125µg/ml, 62.5µg/ml, 31.25µg/ml and 15.62µg/ml) of Alliin and Mancozeb. The diameter of zone of inhibition was measured in millimeters.

RESULT AND DISCUSSION

Molecular Docking Analysis

All species of *Pythium* genus are reported to be active pathogens and *P. splendens* is regarded as one of the most important oomycete pathogen that infects both plants and animals. It is also studied that this pathogen can cause 100% crop loss due to its infection. Because of its agronomic importance, numerous studies have been carried outto understand the mode of infection and control of this pathogen. The three dimensional structures of 3GNZ and 5NNW at resolution 1.35 Å and 1.54 Å were retrieved and then analyzed in Discovery Studio Visualizer. From predicted binding sites using default parameters in 'Define and Edit Binding Site' tool, the one with highest volume (Site1) was selected for further molecular docking studies (Fig1). To obtain a lead compound, a total of 573 conformers of ligands were generated and used for further molecular docking studies.

Molecular interactions of the ligands to 3GNZ and5NNW were ranked based on various parameters such as LibDock score, covalent and non-covalent interactions such as Hydrogen bonds, pi-pi interactions, electrostatic interactions and Binding energies. Docking results showed that Alliin from *A. sativum* has obtained the high LibDock score with 5NNW and 3GNZ (72.0073, 72.5374 respectively) (Table 1).

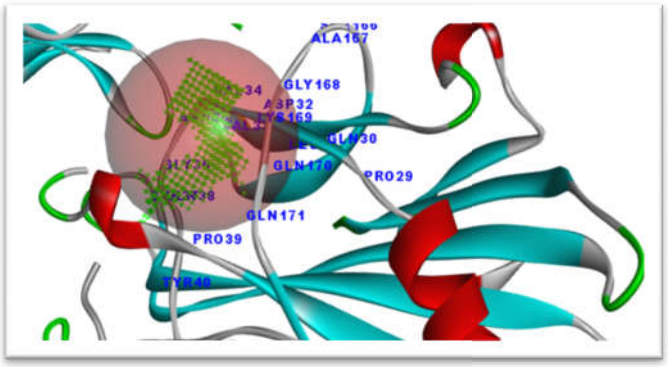

Fig 1 The largest binding site sphere of 5NNW and residues identified

Figure represents the largest binding site residues of the Nep-1 like protein 5NNW. Molecular docking of the selected phytochemicals were performed by defining this region as the active cavity of the protein.

Table 1 Molecular interactions of ligands to NLPs in *Pythium*

Protein	Ligand & Id	Libdock Score	Binding Energy (Kcal/Mol)	Molecular Interactions
5NNW	**ALLIIN** (121922)	72.0073	-146.50	ASP:32, GLN:38, LYS:169, GLN:171
3GNZ		72.5374	-132.72	GLN:38, GLN:38, GLN:171, GLN:171
5NNW	**MANCOZEB** (13307026)	41.2589	+512.95	LEU:31, GLN:38
3GNZ		55.1371	+490.15	GLN:38(3), VAL:33, LYS:169

With 5NNW, four hydrogen bonds ASP: 32, GLN: 38, LYS: 169 and GLN: 171 (Fig 2 A, B). The binding free energy estimation of Alliin was found to be -146.50 Kcal/mol. With 3GNZ, four hydrogen bonds GLN:38, GLN:38, GLN:171, GLN:171, with the binding free energy -132.72 Kcal/mol (Fig 4 A, B).

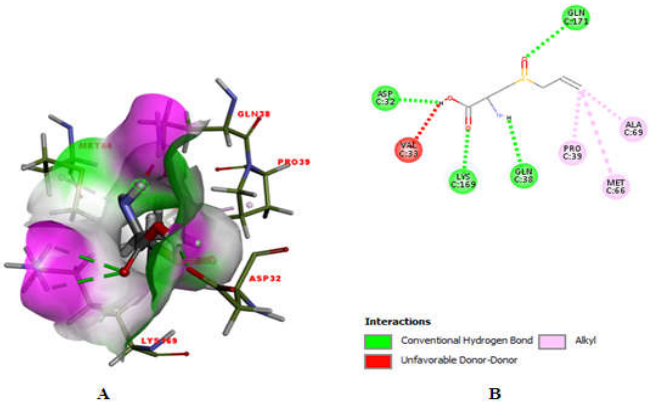

Fig 2 Molecular interaction of alliin with 5NNW

Fig 2 A: Hydrogen bond interactions of Alliin with the predicted binding residues to 5NNW are colored in green. **B:** 2D diagram showing significant interactions of Alliin with the predicted binding residues to 5NNW. The Protein –ligand interaction are colored depending on their type: Hydrogen bonds in green, electrostatic interactions in purple and unfavorable donor –donor interactions in red.

Table 2 Molecular interactions of Alliin and fungicides with 5NNW

Compounds		PubChem Id	LibDock Score	Binding Energy (Kcal/mol)	Molecular interactions
Phyto chemical	**Alliin**	**121922**	**72.0073**	**-146.50**	**Asp32,Gln38, Lys169,Gln171**
Chemical Fungicides	Mancozeb	13307026	41.2589	+512.95	Leu31, Gln38
	Pyrimorph	46220487	38.5492	-14.20	Lys169, Glu117
	Metalaxyl	42586	30.0048	-38.29	Asn194
	Mandipropamide	11292824	26.8763	+ 43.0	Nil
	Propamocarb	32490	23.4327	+ 93.29	Nil

Table shows the molecular interaction o Alliin with the commonly used fungicides for *Pythium* control. Alliin has the highest libdock score and least binding energy when compared to the fungicides, which make it a stable complex. There are four hydrogen bond interactions between Alliin and 5NNW

The interaction of the chemical fungicide Mancozeb with 5NNW and 3GNZwere found to be 41.2589, 55.1371 respectively. Mancozeb has shown hydrogen bond interactions with LEU: 31 and other neighboring residue GLN: 38, but with lesser LibDock score and high binding affinity. The fungicides Mandipropamide and Propamocarb have shown no interactions with 5NNW (Table 2). Among the two NLPs selected for the study, 5NNW showed better interaction with the phytochemical Alliin. The best poses of other top ten phytochemicals were Allicin, Citral, Geraniol, Aloin, Demethoxycurcumin, DiallylTrisulfide, ar-Turmerone, Nimbosterol, Geranyl acetate and Ally Propyl disulphide. The details of the molecular interactions are listed in Table 3. From *in silico* studies, among 292 phytochemicals from *Azadirachtaindica*, *Allium sativum* and *Curcuma longa* screened against 3GNZ and 5NNW, Alliin from *Allium sativum* showed good LibDock score, molecular interactions and binding energy than compared to the commonly used chemical fungicides. Therefore, Alliin from *Allium sativum* is consideredas the lead candidate for further *in vitro* studies.

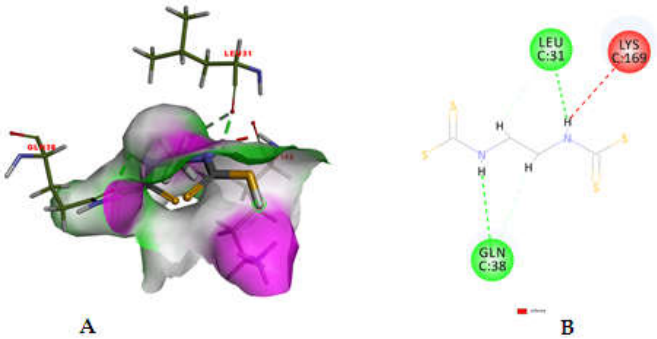

Fig 3 Molecular interaction of mancozeb with 5NNW

Fig 3 A: Hydrogen bond interactions of mancozeb with the predicted binding residues to 5NNW are colored in green. **B:** 2D diagram showing significant interactions of mancozeb with the predicted binding residues to 5NNW. The Protein –ligand interaction are colored depending on their type: Hydrogen bonds in green unfavorable donor –donor interactions in red.

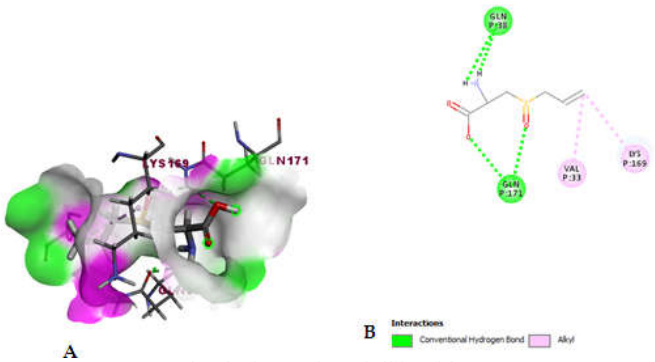

Fig 4 Molecular interaction of alliin with 3GNZ

Fig 4 A: Hydrogen bond interactions of alliin with the predicted binding residues to 3GNZ are colored in green. **B:** 2D diagram showing significant interactions of alliin with the predicted binding residues to 3GNZ. The Protein –ligand interaction are colored depending on their type: Hydrogen bonds in green and electrostatic interactions in purple.

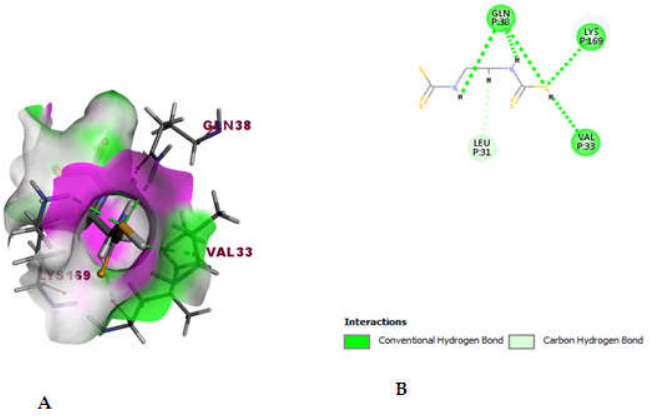

Fig 5 Molecular interaction of mancozeb with 3GNZ

Fig 5 A: Hydrogen bond interactions of mancozeb with the predicted binding residues to 3GNZ are colored in green. **B:** 2D diagram showing significant interactions of mancozeb with the predicted binding residues to 3GNZ. Green color indicates the hydrogen bond interactions of ligand with protein.

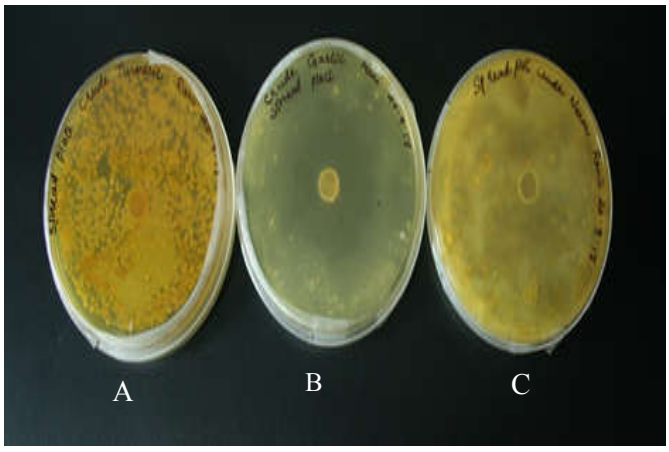

Fig 6 Spread plate assay on *Pythiumsplendens*

Antifungal studies on *P. splendens* against natural fungicides using spread plate method, from left crude extracts of (A) *Curcuma longa* (B) *Allium sativum* (C) *Azadirachtaindica*

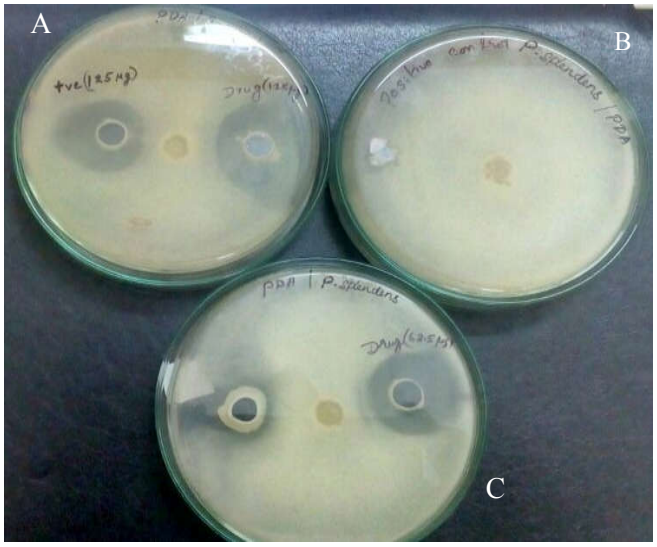

Fig 7 Minimum inhibitory concentration assay

Minimum inhibitory concentration studies at 125 µg/ml and 62.5 µg/ml. (A) Alliin and Mancozeb (125 µg/ml) (B) *P. splendens* control (C) Alliin and Mancozeb (62.5µg/ml).

Fig 8 Minimum inhibitory concentration assay using alliin and mancozeb

Minimum inhibitory concentration studies at 15.62 µg/ml. (A) *P. splendens* control (B) zone of inhibition shown by alliin and mancozeb.

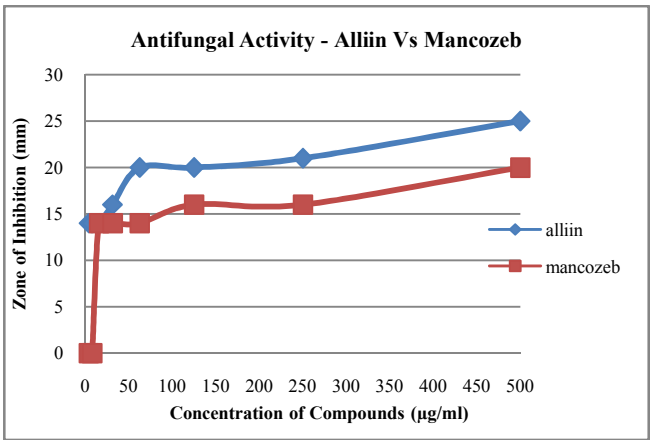

Fig 9 Graphical representation of antifungal activity of AlliinVsMancozeb

Antifungal activity of alliin Vsmancozeb with concentration of compounds (µg/ml) on X axis and zone of inhibition (mm) on Y axis showed that lower concentrations of alliin (blue line)

has better antifungal activity than the commonly used fungicide mancozeb (red line)

Table 3 Molecular interactions of top ten phytochemicals to 5NNW

Sl.No.	Compounds	PubChem Id	LibDock Score	Binding Energy (Kcal/mol)	Molecular interactions
1.	Allicin	65036	53.5592	-36.4724	ASP 119,Tyr 118, Gly 116
2.	Citral	638011	42.4566	-30.3054	Arg115, Leu110
3.	Geraniol	637566	40.4572	-30.3054	Thr114, Ser112, Lys 111
4.	Aloin	9866696	42.9044	-95.8338	Leu 110,Arg115
5.	Demethoxycurcumin	5469424	40.6272	-42.9458	Asp119
6.	DiallylTrisulfide	16315	49.0343	-38.2924	Ser 112
7.	ar-Turmerone	160512	21.2902	+62.5412	Tyr 118
8.	Nimbosterol	222284	30.3944	+143.6664	Gly 116
9.	Geranyl acetate	1549026	20.5153	+124.8348	Tyr118, Gly 116
10.	Allyl propyl disulphide	9793905	39.8103	+298.8029	Leu 110, Ser 112, Thr 114

Table shows the interaction of top ten phytochemicals with 5NNW based on the libdock score, binding energy and molecular interactions. Allicin in *Allium sativum*, shows better interaction with a libdock score 53.5592, binding energy -36.4724 and three hydrogen bonds with ASP119, Tyr118, and Gly116.

In vitro antifungal activity assay

Minimum Inhibitory Concentration (MIC) determination against *P. splendens* with the plant extracts and the standard was performed. Spread plate method showed that crude extract of *Allium sativum* possesses better inhibitory action when compared to other crude extracts (Fig 6). The plates with the crude extract of *Curcuma longa* and *Azadirachtaindica* showed little or no zone of inhibition. Based on these results, different concentrations of lead compound and standard were performed and analyzed. At a concentration of 125 µg/ml, Alliin showed a growth inhibition zone of 20 mm diameter and that of Mancozeb was 16 mm diameter. At 62.5 µg/ml concentration Alliin showed a zone with 20 mm diameter. Mancozeb showed very less inhibition at this concentration. At 15.62 µg/ml concentration, the zone of inhibition shown by Alliin was 14 mm and for Mancozeb, the zone of inhibition was 14 mm (Table 4). The assay was repeated with the lowest concentrations of the compounds and confirmed the inhibitory effect of Alliin (Table 5).

Table 4 Different concentrations of Alliin and Mancozeb with their zone of inhibition

Concentration (µg/ml)	Zone of inhibition (diameter in mm)	
	Mancozeb	Alliin
500	20	25
250	16	21
125	16	20
62.5	14	20
31.25	14	16
15.62	14	14
7.8	0	14
3.9	0	14

Table shows the zone of inhibitions at different concentrations of mancozeb and alliin on *Pythiumsplendens*. At both higher and lower concentrations, alliin showed greater zone of clearance compared to mancozeb.

Table 5 Results of antifungal activity assay repeated with lower concentration of the compounds

Concentration (µg/ml)	Zone of inhibition (diameter in mm)	
	Mancozeb	Alliin
125	34	42
62.5	14	35
31.25	14	16
15.62	23	32

Table shows the results of the repeated assay. The zone of inhibition in mm for alliin and mancozeb with four different concentrations

At a concentration of 125µg/ml, Alliin showed a growth inhibition zone of 42 mm diameter and Mancozeb was 34 mm diameter. At 62.5 µg/ml concentration Alliin showed a zone with 35 mm diameter and Mancozeb showed a zone of inhibition of 14 mm diameter (Fig 7). At 31.25 µg/ml concentration, the zone of inhibition shown by Alliin was 16 mm diameter and that of Mancozeb was 14 mm diameter. At 15.62 µg/ml concentration, the zone of inhibition shown by Alliin was 32 mm diameter and the inhibition zone for Mancozeb was 23 mm diameter (Fig 8). The repeated assays also indicated that Alliin has better inhibitory effect than Mancozeb. The crude extracts *of Azadirachtaindica, Allium sativum* and *Curcuma longa* were more potent than Mancozeb with their minimal inhibitory concentration values in the range of 3.9-500 µg/mL. The zone of inhibition studied using well diffusion method and spread plate method indicates that the phytochemicalAlliin in *Allium sativum* is a good antifungal agent which can be used as potential candidate for the control of *P. splendens* infection in plants. A graphical representation characterizing the antifungal activity repeated at lower concentrations of Alliin and Mancozeb is shown in Fig 9. The inhibition activity of Alliin is prominent even at lower concentrations unlike Mancozeb. In higher concentration region also Alliin gives comparably better inhibition rate.

When oomycetes infect their hosts, they employ a large arsenal of effector proteins in order to establish a successful infection. Some effector proteins secreted are translocated and they function inside host cells. 3GNZ are 5NNW are necrosis and ethylene-inducing peptide 1 (Nep1)-like proteins whose x -ray crystallographic structures have been well studied and deposited in PDB. Virulence proteins are studied to cross the defense barriers by the plant cells where they can interact with the resistance proteins (R proteins) secreted by the plants as part of its defense mechanism. The infection caused by these proteins will lead to a huge yield loss and also adversely affects the survival of the plant. Therefore, proper management and control measures to eradicate the pathogen attack are very much essential. A large number of fungicides are being used by the farmers to prevent the plants from infection. Application of high doses of chemical fungicides for the pathogen control can be very toxic to the plants as well as to other living beings. The use of phytochemicals for the control of pathogen infections would definitely serve as an effective alternative to synthetic fungicides. In the present study, the ability of bio fungicides such as *Azadirachtaindica, Allium sativum* and *Curcuma longa* inhibiting the plant pathogen *P. splendens* were scientifically validated through computational and *in vitro* methods. So far no other intensive studies were reported on the interactions considering virulence proteins in *P. splendens* as a drug target and the natural compounds of the

selected plants as ligands. The findings of the present study clearly shows that Alliin in *Allium sativun* has better antifungal activity against *P. splendens* than chemical fungicide Mancozeb even at lower concentrations. The proposed work provides experimental validation of the potent lead compound through Minimum Inhibitory Concentration (MIC) assay. As a preliminary study, we provide the molecular evidence for the inhibitory activity of plant extracts from *Azadirachtaindica*, *Allium sativum* and *Curcuma longa* which is known to report for their strong antifungal activities. Further work can be extended to study the proper combinations of different plant extracts and their synergistic effects which would be useful for promoting the use of natural fungicides against *P. splendens* infections.

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References

Ankri, S., &Mirelman, D. (1999). Antimicrobial properties of allicin from garlic. Microbes and infection, 1(2), 125-129.

Ark, P. A., & Thompson, J. P. (1959). Control of certain diseases of plants with antibiotics from garlic (Allium sativum L.). Plant Disease Reporter, 43, 276-282.

Asif M. (2012). Antimicrobial potential of Azadirachtaindica against pathogenic bacteria and fungi. *Journal of Pharmacognosy and phytochemistry* 1(4).

Birch, P. R., Rehmany, A. P., Pritchard, L., Kamoun, S., &Beynon, J. L. (2006). Trafficking arms: oomycete effectors enter host plant cells. Trends in microbiology, 14 (1), 8-11.

Dennis D. Weisenburger, MD. (1993). Human health effects of agrichemical use. Human Pathology 24: 571–576.

Enyiukwu, D. N., Awurum, A. N., Ononuju, C. C., &Nwaneri, J. A. (2014). Significance of characterization of secondary metabolites from extracts of higher plants in plant disease management. *International Journal of Advance Agricultural Research*, 2, 8-28.

Inc, A. S. (2007). Discovery studio modeling environment, release 4.0.

Jalaie, M., & Shanmugasundaram, V. (2006). Virtual screening: are we there yet? Mini reviews in medicinal chemistry, 6 (10), 1159-1167.

Josling, P. (2003). Allicin The heart of garlic.

Kamoun S. (2006). Effectors-Role in Host-Pathogen Interaction. *Journal of Agriculture and Environmental Sciences*, June 2014, Vol. 3, No. 2, pp. 265-285.

Kamoun, S. (2006). A catalogue of the effector secretome of plant pathogenic oomycetes. Annual review of phytopathology, 44.

Komárek, M., Čadková, E., Chrastný, V., Bordas, F., & Bollinger, J. C. (2010). Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environment international, 36(1), 138-151.

Lenarčič, T., Albert, I., Böhm, H., Hodnik, V., Pirc, K., Zavec, A. B., &Greimel, P. (2017). Eudicot plant-specific sphingolipids determine host selectivity of microbial NLP cytolysins. Science, 358(6369), 1431-1434.

Lenarčič, T., Pirc, K., Hodnik, V., Albert, I., Borišek, J., Magistrato, A., &Anderluh, G. (2019). Molecular basis for functional diversity among microbial Nep1-like proteins. PLoS pathogens, 15(9), e1007951.

LennartWirthmueller, Abbas Maqbool& Mark J. Banfield, (2013) on the front line: structural insights into plant–pathogen interactions, Nature Reviews Microbiology 11, 761–776.

Mazid M, Khan TA, Mohammad F. (2011). Role of secondary metabolites in defence mechanisms of plants. Biology and Medicine, 3 (2) Special Issue: 232-249. doi: 10.4172/0974-8369.10000128.

Obeidat, M., Shatnawi, M., Al-alawi, M., Al-Zu'bi, E., Al-Dmoor, H., Al-Qudah, M., &Otri, I. (2012). Antimicrobial activity of crude extracts of some plant leaves. *Research Journal of Microbiology*, 7(1), 59.

Ottmann, C., Luberacki, B., Küfner, I., Koch, W., Brunner, F., Weyand, M. &Nürnberger, T. (2009). A common toxin fold mediates microbial attack and plant defense. Proceedings of the National Academy of Sciences, 106(25), 10359-10364.

Ottmann, C., Luberacki, B., Küfner, I., Koch, W., Brunner, F., Weyand, M. &Nürnberger, T. (2009). A common toxin fold mediates microbial attack and plant defense. Proceedings of the National Academy of Sciences, 106(25), 10359-10364.

Parveen, T., & Sharma, K. (2015). Pythium diseases, control and management strategies: a review. *Int. J. of Plant, Animal and Environ. Sci*, 5(1), 244-257.

Singh, U. P., Pandey, V. N., Wagner, K. G., & Singh, K. P. (1990). Antifungal activity of ajoene, a constituent of garlic (Allium sativum). *Canadian journal of botany*, 68(6), 1354-1356.

Wilson, C. L., Solar, J. M., El Ghaouth, A., & Wisniewski, M. E. (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against Botrytis cinerea. Plant disease, 81(2), 204-210.

Yan, X., Qin, W., Sun, L., Qi, S., Yang, D., Qin, Z., & Yuan, H. (2009). Study of inhibitory effects and action mechanism of the novel fungicide pyrimorph against Phytophthoracapsici. *Journal of agricultural and food chemistry*, 58(5), 2720-2725.

ZorofchianMoghadamtousi, S., Abdul Kadir, H., Hassandarvish, P., Tajik, H., Abubakar, S. &Zandi, K. (2014). A review on antibacterial, antiviral, and antifungal activity of curcumin. BioMed research international.

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