Journal of King Saud University – Science 34 (2022) 101747

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Terpinen-4-ol from *Trachyspermum ammi* is a potential and safer candidate molecule for fungicide development against *Alternaria solani*

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ARTICLE INFO

Article history:
Received 27 October 2021
Revised 22 November 2021
Accepted 29 November 2021
Available online 02 December 2021

Keywords:
Computer Aided Fungicide Design
Percent Disease Incidence
Minimum Inhibitory Concentration
Minimum Fungicidal Concentration
MD Simulation

ABSTRACT

Phytopathogenic fungi are serious threats to fruit and vegetable production. Use of plant essential oil (PEO), as antifungal agents, have been in practice for many years. Essential oils (EO) of five plants i.e., *Trachyspermum ammi*, *Cuminum cyminum*, *Azadirachta indica*, *Citrus sinensis* and *Eucalyptus grandis* were tested against *Alternaria solani* in tomato. The trials consisted of laboratory testing (MIC and MFC), field testing and Computer Aided Fungicide Design (CAFD) of PEOs against *A. solani*. Each PEO was used at 10 and 25 µl/ml concentrations to asses MIC, while MFC was assessed at 25, 50 and 75µl/ml concentration. *Trachyspermum ammi* EO at 25µl/ml concentration showed the largest inhibition zone (37.3 mm) followed by a not significantly different zone(20.6 mm)produced by *C. cyminum* EO. No fungal growth was observed at 50µl/ml concentration of *T. ammi* while, at 75µl/ml for *A. indica* and *C. cyminum*. In foliar application, *T. ammi* EO showed lowest percent disease incidence (2.2) which was not significantly different from that of *C. cyminum*. *Trachyspermum ammi* EO, qualified in laboratory and field trials, was selected for CAFD. Compounds of *T. ammi* PEO docked against toxin producing enzyme, solanapyrone synthase, of *A. solani*. Terpinen-4-ol was qualified as a lead compound against *A. solani*.

1. Introduction

Thevplants produce defense molecules as secondary metabolites (Hancock et al., 2015). More than 30,000 of these secondary metabolites are essential oils which have antifungal and antimicrobial properties (Beeby et al., 2020). These plant essential oils (PEOs) have been used in traditional medicines for many decades (Turek & Stintzing, 2013).

The hazardous effects of synthetic pesticides led to the concept and use of biological control methods in agroecosystems. Important agents of biological control are predators, parasitoids, microorganisms, plant semiochemicals, algae, animals (Ravensberg, 2015). Plant essential oils (PEO) are categorized as plant originated natural compounds which can be used as biological control agents. Due to their botanicals origin and antifungal and antibacterial properties PEO became an important component of IPM program (Lamichhane et al., 2016). The use of botanicals as substitute for synthetic chemicals have been highly supported in Europe by the directive 2009/128/CE. The ultimate goal of this approach is to minimize the use of chemical pesticides and encourage the application of natural products and agricultural inputs more in line with sustainable agriculture development. http://data.europa.eu/eli/dir/2009/128/oj

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2021.101747
1018-3647/© 2021 Published by Elsevier B.V. on behalf of King Saud University.

The phytopathogenic fungi are the reason for almost 30% of all agricultural crop diseases (Jain et al., 2019) and cause about 38% yield losses by affecting them in field and/or after harvest (Ons et al., 2020). Plant essential oils have shown significant activities against such plant pathogenic fungi (Tabassum & Vidyasagar, 2013).

Early blight is one of the dominant diseases of the genus *Alternaria* and causes 32–57% yield losses (Nisa et al. 2015). *Alternaria* can tolerate adverse climatic conditions and thrives a wide range of temperature and atmospheric moisture (Tomazoni et al., 2017). It causes serious economic losses in tomato production around the world.

Computer Aided Drug Design (CADD) approaches are used in pesticide development research from the last few years (Chu et al., 2012). The CADD approaches are designed to perform the drug development tasks computationally. A lead molecule can be identified by screening compound libraries containing millions of synthetic and natural compounds (Speck-Planche et al., 2011). Speedy and convenient methods of lead molecule identification by using CAPD approaches provide base line information about novel and safer pesticide molecules (Yousafi et al., 2019). With the advancement in computational methods, we might expect dramatic growth for a number of pesticides in the market with new, presumably safer, modes of action.

Keeping in view the efficacy of PEO against pathogenic fungi and non-hazardous nature they can be used for fungicide development industry.. The current study was designed to detect antimicrobial effectiveness of five medicinal PEOs against *Alternaria solani*. The findings of our study will provide a base line knowledge and data for novel and safer fungicide development and new target site identification to avoid fungicide resistance.

2. Materials and methods

2.1. Plant sample collection and essential oil extraction

The leaves (*Azadirachta indica* and *Eucalyptus grandis*) peel (*Citrus sinensis*) and seeds (*Trachyspermum ammi* and *Cuminum cyminum*) of five plants were collected from Sahiwal (Pakistan). The PEO extraction was done by following methods described by Saleem et al. (2014). Culture of *Alternaria solani* was obtained from culture bank of PCSIR, Lahore (Pakistan).

2.2. Laboratory evaluation of PEO

2.2.1. Minimum inhibitory concentration (MIC)

Two concentrations, 10 and 25 μl/ml of PEO were used in triplicate to determine MIC levels by agar well diffusion method. The inhibition zone was measured by a graduated scale. The lowest PEO concentration producing largest inhibition zone was considered as a MIC. Each treatment (Supplementary Table1) was replicated three times in Completely Randomized Design (CRD) experiment layout.

2.2.2. Minimum fungicidal concentration (MFC)

A measured fungal spore load of 1x10^6 cfu/ml was transferred to the tubes containing culture media broth and three concentrations, *i.e*., 25, 50 and 75μl/ml of PEOs to be tested. Broth tubes without PEOs were assigned as control. The test tubes were incubated for 48h at 25℃ to observe *A. solan*i growth. About100 microliter broth,from the tubes showing no fungal colony development, was transferred in to petriplates coated with agar. The petriplates were incubated for 48hours to observe fungal colony growth. The lowest PEO concentration,which showed no fungal colonies after incubation, was assigned as MFC.

2.3. Field trials

The PEO of *Azadirachta indica*, *Trachyspermum ammi* and *Cuminum cyminum* exhibited better antifungal activity in laboratory culture were selected for field trials. Two concentrations of PEO, 60 and 80 μl/ml, were used for field trails. Treatments (Supplementary Table2) were planned under CRD with three replications. The Plant essential oils were applied to the plant leaves with syringes. The soil was inoculated with 10^6cfu/ml fungal spore load fifteen days after transplant. Four foliar applications of PEO were done fortnightly after soil inoculation. The number of plants with symptoms were counted and percent disease incidence was recorded as under

Percent disease incidence = (Number of infected plant)/(Total number of plants assessed)X 100

2.4. Computer Aided Fungicide Designing (CAFD)

The Plant essential oil qualified in laboratory and field trials were used to identify potential lead fungicidal compounds against *A. solani*.

2.4.1. Acquisition of chemical compounds of PEO

The compounds from qualified PEO's were selected from the literature (Supplementary Table 3). The 2D and 3D structures were obtained from online databases PubChem and ChEBI. Human toxicity was checked by ToxiM, toxicity prediction tool, and only non-toxic compounds were selected for further analysis.

2.4.2. Three dimensional protein model prediction

The amino acid sequence of the toxin producing enzyme solanapyrone synthase: polyketide synthase (UniProt ID: D7UQ40) was obtained from UniProt. The protein domains were predicted by Expassy ProSiteScan. The 3D structure of domain was predicted by I-TASSER server.

2.4.3. Molecular docking

Molecular docking of FAD-binding domain (Solanapyrone synthase: *A. solani*) was accomplished with nontoxic chemical compounds/ ligands in *T. ammi* essential oil. Protein-ligand blind docking was by AutoDockVina tool. The selection of the complex model was based on the ligand-protein interaction with the lowest binding energy.

2.4.4. Molecular dynamics (MD) simulations

The Groningen Machine for Chemical Simulations (GROMACS 5.0.7) was used to evaluate the dynamic interaction profile of the selected ligand–protein complex. The trajectories of 50-ns were produced on time scale of 2-fs.

3. Results

3.1. Laboratory evaluation of PEO

3.1.1. Minimum inhibitory concentration (MIC)

The significantly largest inhibition zone (37.3 mm) was found for *T. ammi* (25μl/ml) followed by significantly smaller (25.3 mm) for *T. ammi* (10μl/ml). The inhibition zone formed by *T. ammi* (10μl/ml) was significantly larger than all other treatments, except that of *C. cyminum* (25μl/ml) (Fig. 1).

3.1.2. Minimum fungicidal concentration (MFC)

Three PEOs of *T. ammi, C. cyminum* and *A. indica* were tested for their fungicidal potential. The minimum fungicidal concentration was evaluated for three concentrations (25,50 and 75 μl/ml) of PEOs. The experiment set was replicated three times. After 72 h,

Table 1
Results of Molecular docking of *Trachyspermum ammi* essential oil compounds with FAD binding domain of solanapyrone in *Alternaria solani*.

Ligands	PubChem CID	Binding Energy (Kcal/mol)	Binding Interactions	Bond Distance (A°)
(E)-Octadec-2-enoic acid	5282750	−5.1	Ser39(A)–O2:OG	2.81
(E)-Octadec-9-ene	12382046	−5.4	No	No
Palmitic acid	985	−5.9	Phe31(A)-N:01	3.02
			Tyr43(A)–OH:O2	3.04
Terpinen-4-ol	**11230**	**−6.0**	**Asn156(A)-O:O**	**3.06**
			Asn157(A)-O:O	**2.97**
Vaccenic acid	5282761	−5.2	Asn156(A)–O2:O	2.95
cis-13-Octadecenoic acid	5312441	−5.7	No	No
1,8-Cineol	2758	−5.3	No	No
α-Pinene	6654	−5.0	No	No
Myrcene	31253	−5.5	No	No
p-Cymene	7463	−5.0	No	No

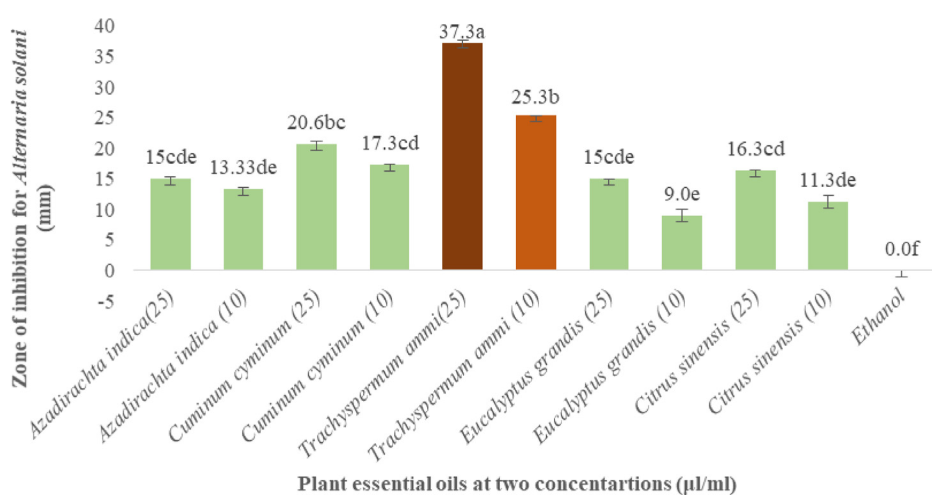

Fig. 1. Minimum inhibitory concentration of different concentrations of plant essential oils against *Alternaria solani.* ($p < 0.05$).

PEO of *T. ammi* at 50 μl/ml, *C. cyminum* at 75 μl/ml, and *A. indica* at 75 μl/ml concentration, showed no fungal colonies growth (Fig. 2).

3.2. Field trials

The percent disease incidence after first application was the lowest (2.2) for EO of *T. ammi* at 80 μl/ml concentration, which

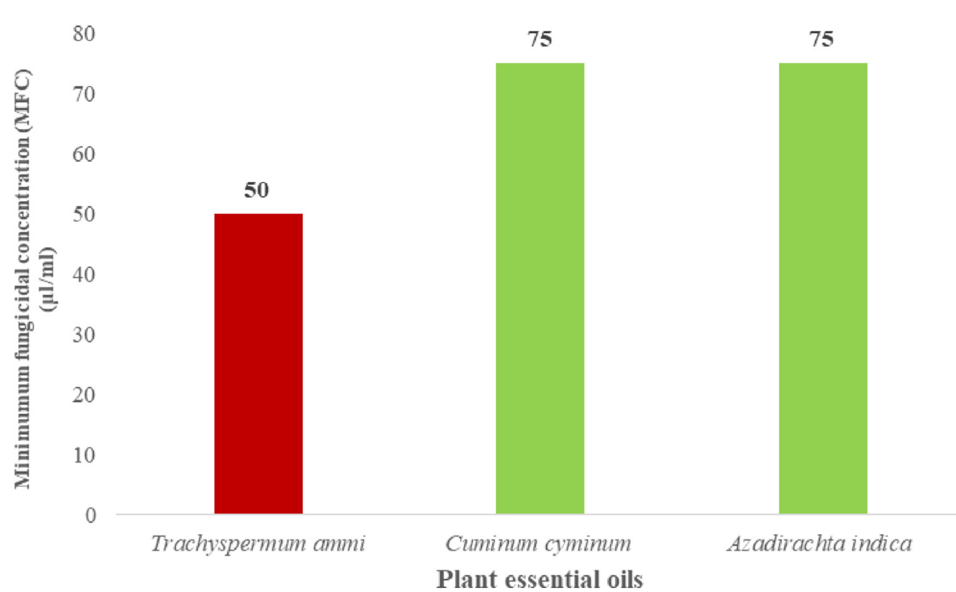

Fig. 2. Minimum fungicidal concentration activity different plant essential oils against *Alternaria solani*.

was not significantly different from that of Ridomil Gold. After four PEO foliar applications only *T. ammi* at 80 µl/ml concentration showed a significantly lower percent disease incidence than the commercial fungicide (Fig. 3). All other treatments were found not significantly different among each other for percent *A. solani* incidence. Disease incidence from different application intervals was not significantly different across the application dates.

3.3. Computer Aided Fungicide Design (CAFD)

3.3.1. Three dimensional protein model prediction

The Expassy ProSiteScan predicted only one 170 aa long domain in the solanapyrone synthase (UniProt ID: D7UQ40) i.e., FAD-binding domain, PCMH-type. The predicted 3D structure of domain was qualified by VERIFY 3D, 90.40% of the residues have averaged 3D-1D score >= 0.2. The quality factor by ERRAT was 83.64. More than 90% of the residues in structure were found in most favored region and 9.2% in additional allowed region of Ramachandran Plot. The refined and evaluated 3D structure was saved in pdb format for docking analysis.

3.3.2. Molecular docking

Ten non-toxic chemical compounds from *T. ammi* were selected for docking with protein FAD-binding domain. The complexes with lower binding energies and hydrogen bonds were selected (Table 1). The binding energy of the selected complexes ranged from −6.0 (Terpinen-4-ol) to −5.1 ((E)-Octadec-2-enoic acid). Two hydrogen bond interactions and lowest binding energy was observed for Terpinen-4-ol (Fig. 4).

3.3.3. Molecular dynamics (MD) simulation

RMSD was fluctuated at the start with a steep rise initially but after 25 ns a stable RMSD (1 nm) was recorded (Fig. 5a). The RMSF peaks were observed at Gly105 for 0.7 nm height with the highest

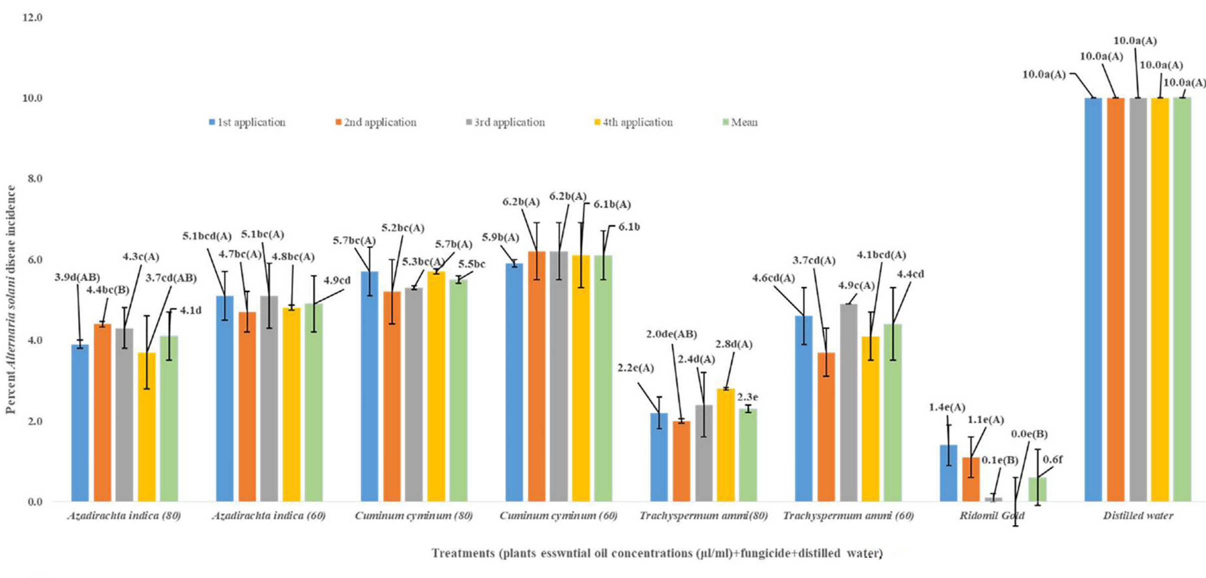

Fig. 3. Effect of foliar application of different concentrations of plant essential oils on percent disease incidence of *Alternaria solani* in tomato (($p < 0.05$).

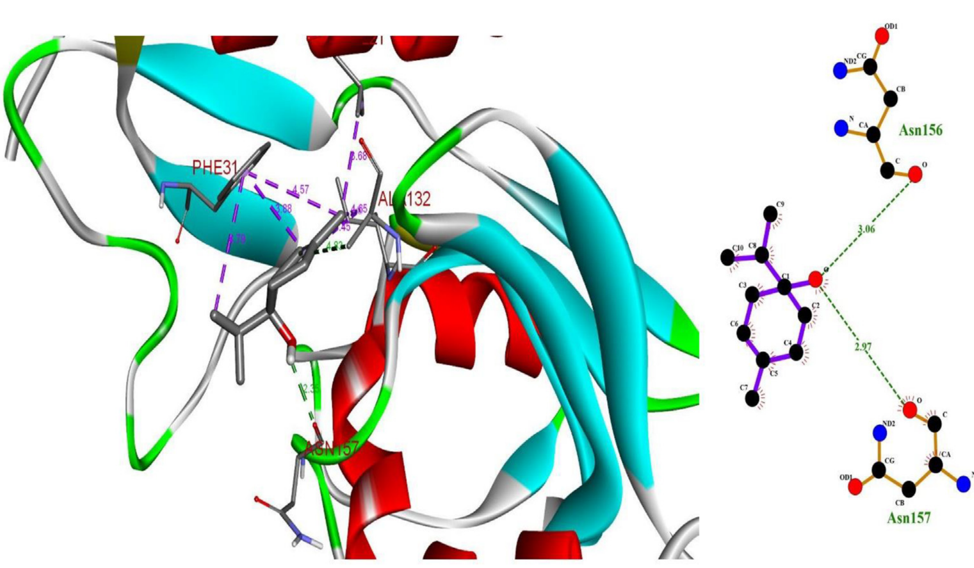

Fig. 4. Terpinen-4-ol-polyketide synthase interaction complex in *Alternaria solani*.

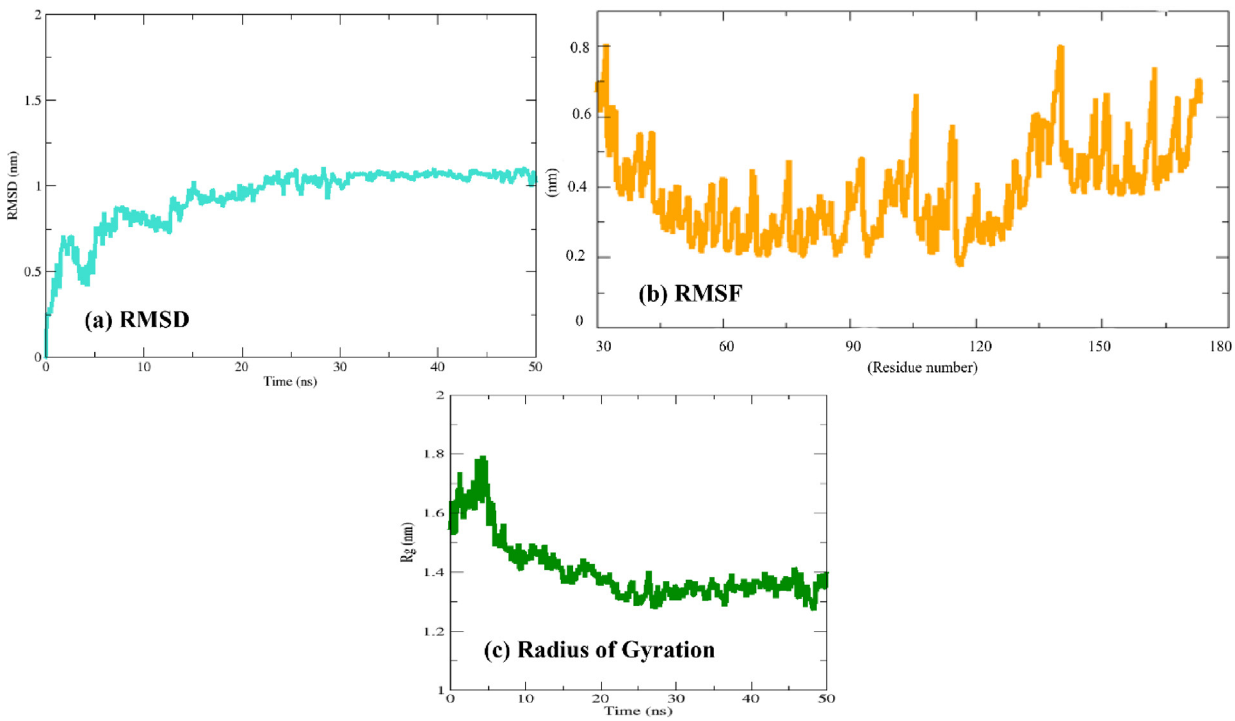

Fig. 5. Results for MD simulation of Terpinen-4-ol-polyketide synthase complex (a: RMSD, b:RMSF, c: Radius of Gyration)

peak (0.8 nm) observed at Gly35 and Ala 137 (Fig. 5b). Radius of gyration was 1.6 nm at the start with a peak of 1.8 after 5 ns, then started dropping and ended up at 1.4 nm at 50 ns. A very little drop in radius of gyration was observed (Fig. 5c).

4. Discussion

Phytopathogenic fungi are a serious threat to agriculture s worldwide (Fisher et al. 2012). Indiscriminate use of fungicides, especially on vegetables and fruits is very deleterious for human health (Hakala et al., 2011). Moreover, it causes environmental pollution and pesticide resistance (Boxall et al., 2009). These issues are less addressed for fungicides than insecticides (Zubrod et al., 2019). The fungicidal properties of PEO have been reported in different research findings (Jiménez-Reyes et al., 2019). The basic advantage of using plant essential oils in fungicide development is that they are biodegradable, so are least toxic to mammals and cause no environmental pollution (Raja, 2014).

In the current study we tested the efficacy of five plant essential oils (PEOs) viz. *Azadirachta indica, Eucalyptus grandis, Citrus sinensis, Cuminum cyminum,* and *Trachyspermum ammi,* against *Alternaria solani* in tomato. The testing of essential oils was done in three trials, i.e., *in vitro*, in field and computational analysis. In the laboratory, five PEOs were tested by investigating their minimum fungicidal concentration (MFC) and minimum inhibitory concentration (MIC). In our study *T. ammi* EO had the lowest MIC and MFC against *A. solani*. Our results confirmed the finding of Khan and Jameel (2018), who found antifungal potential of *T. ammi* seed oil, by using disk diffusion method, against different fungal species. Current findings also confirmed the results of Sharifzadeh et al. (2015), who reported 50% MFC of *T. ammi* essential oil against *Candida albicans.*

Three qualified PEOs, *i.e.*, of *T. ammi, C. cyminum* and *A. indica*, from the laboratory experiment were selected to be tested against *A. solani* in tomato under field conditions. The lowest percent disease incidence and severity were observed for *T. ammi* PEO. This is a good parameter for testing the efficacy of a chemical against the target (Naziya et al., 2020). *Trachyspermum ammi* belongs to the family Apiaceae, commonly known as ajwain. The seeds and leaves extracts of *T. ammi* have antimicrobial properties and has been effectively used as medicines (Dwivedi et al., 2012).

The best PEO (*T. ammi*) was selected for Computer Aided Fungicide Design (CAFD). Computer aided drug designing (CADD) has become a significant approach for novel pharmaceutical drug designing (Khan et al., 2019). But this approach is lacking in agricultural pesticide development. However, the pharmacodynamics and tools used in CADD can be used for computer aided pesticide designing (CAPD) except pharmacokinetic considerations (Tice, 2001). The number of known experimental findings and infrastructure of targets sites and new drug molecules identification is very least studied in the field of pesticide chemistry (Bordas et al., 2003). Little work has been done for CAPD for phytopathogenic fungi (Jiménez-Reyes et al., 2019). If we adopt these approaches for pesticide development we can develop safer and effective pesticides speedily in a least expensive manner.

In this study, we selected the active compounds in *T. ammi* PEO from the literature. The target enzyme of *A. solani,* bifunctional solanapyrone synthase, was selected to be inhibited. It is a polyketide synthase responsible for biosynthesis of crucial phytotoxin, solanapyrone, which causes early blight infection in potato and tomato (Kasahara et al., 2010). A protein domain is a region in a protein's polypeptide chain which controls its function independently and a conserved region which is self-stabilizing . To be more precise in inhibiting toxin production we chose the toxin producing domain to be blocked by an efficient and non-toxic inhibitor molecule from *T. ammi*. FAD-binding domain, PCMH-type, a toxin producing domain, (Fraaije & Mattevi, 2000) of solanapyrone synthase was selected to be blocked.

The top compound, Terpinen-4-ol, with lowest binding affinity (−6.0 kcal/mol) and 2 hydrogen bonds with target, was selected for further confirmation of its binding and stability with the target site. Molecular Dynamics (MD) simulations technique was used for this purpose. This analysis of MD simulation is used to evacuate the movements of the highly complex macromolecular systems (Phillips et al., 2005). The estimation of structural fluctuations of the macromolecule is the most crucial feature of this analysis (Karplus et al., 2002), which reflects the stability and flexibility in the ligand receptor complex. For the present study, Root-Mean-Square-Fluctuations (RMSF) and Root-Mean-Square-Deviation (RMSD) are most important parameters to be analyzed (Humphrey et al., 1996). The value of RMSD reflects the stability interaction profile. In this study, the average ligand-receptor RMSD was 1 nm, indicating that the system was stable (Faraj et al., 2016). The Root Mean Square Fluctuation (RMSF) is an estimation of the displacement of a specific atom, or group of atoms, relative to the reference structure, averaged over the number of atoms (Martínez, 2015). RMSF values in our study reflect stable complex conformation.

Results of the current study indicated Terpinen-4-ol from *T. ammi* EO had antifungal activities as reported by different studies (Morcia et al., 2012). Terpinen-4-ol from *Thymus villosus* essential oil was found effective for fungicide resistance management in *Candida*, *Cryptococcus* and *Aspergillus* species (Pinto et al., 2013). The compounds nontoxic to humans were selected for docking against toxin producing domain in *A. solanim,* for its inhibition. The predicted lead molecule, Terpinen-4-ol, in the current study is not only non-toxic to humans but has been aiso reported to have therapeutic properties (Mondello et al., 2006) especially against cancer (Shapira et al., 2016). Therefore, this compound can be used in fungicide formulation as an effective and safe active ingredient. Terpinen-4-ol molecule can be used alone or in combination with other suitable molecules for better and safer management of *A. solani*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors extend their appreciation to the deanship of Scientific Research at King Khalid University, Abha KSA for supporting this work under grant number (R.G.P.2/61/42).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2021.101747.

References

Beeby, E., Magalhães, M., Poças, J., Collins, T., Lemos, M.F.L., Barros, L., Ferreira, I.C.F. R., Cabral, C., Pires, I.M., 2020. Secondary metabolites (essential oils) from sand-dune plants induce cytotoxic effects in cancer cells. J. Ethnopharmacol. 258, 112803. https://doi.org/10.1016/j.jep.2020.112803.
Bordas, B., Komíve, T., Lopata, A., 2003. Ligand-based computer-aided pesticide design. A review of applications of the CoMFA and CoMSIA methodologies. Pest Manag. Sci. 59, 393–400.
Boxall, A.B.A., Hardy, A., Beulke, S., Boucard, T., Burgin, L., Falloon, P.D., Haygarth, P. M., Hutchinson, T., Kovats, R.S., Leonardi, G., Levy, L.S., Nichols, G., Parsons, S.A., Potts, L., Stone, D., Topp, E., Turley, D.B., Walsh, K., Wellington, E.M.H., Williams, R.J., 2009. Impacts of climate change on indirect human exposure to pathogens and chemicals from agriculture. Environ. Health Perspect. 117 (4), 508–514.
Chu, H., Wang, J., Shen, H., Yang, Y., Zhu, W., Li, G., 2012. Investigation of family 18 Chitinases and inhibitors by computer-aided approaches. Curr. Drug Targets 13, 502–511.
Dwivedi, S.N., Mishra, R.P., Alava, S., 2012. Phytochemistry, pharmacological studies and traditional benefits of *Trachyspermum ammi* (Linn.) sprague. Internat. J. Pharm. Life Sci. 3, 1–5.
Faraj, S.E., González-Lebrero, R.M., Roman, E.A., Santos, J., 2016. Human frataxin folds via an intermediate state. Role of the C-terminal region. Sci. Rep. 6, 20782.
Fisher, M.C., Henk, D.A., Briggs, C.J., Brownstein, J.S., Madoff, L.C., McCraw, S.L., Gurr, S.J., 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484 (7393), 186–194.
Fraaije, M.W., Mattevi, A., 2000. Flavoenzymes: diverse catalysts with recurrent features. Trends Biochem. Sci. 25 (3), 126–132.
Hakala, K., Hannukkala, A.O., Huusela-Veistola, E., Jalli, M., Peltonen-Sainio, P., 2011. Pests and diseases in a changing climate: a major challenge for Finnish crop production. Agric. Food Sci. 20, 3–14.
Hancock, R.D., Hogenhout, S., Foyer, C.H., 2015. Mechanisms of plant-insect interaction. J. Exp. Bot. 66 (2), 421–424.
Humphrey, W., Dalke, A., Schulten, K., 1996. VMD: Visual molecular dynamics. J. Mol. Graph. Model. 14 (1), 33–38.
Jiménez-Reyes, M.F., Carrasco, H., Olea, A.F., Silva-Moreno, E., 2019. Natural compounds: A sustainable alternative to the phytopathogens control. J. Chil. Chem. Soc. 64 (2), 4459–4465.
Kasahara, K., Miyamoto, T., Fujimoto, T., Oguri, H., Tokiwano, T., Oikawa, H., Fuji, I., 2010. Solanapyrone synthase, a possible Diels–Alderase and iterative type I polyketide synthase encoded in a biosynthetic gene cluster from *Alternaria solani*. ChemBioChem 11, 1245–1252.
Khan, M.S., Mehmood, B., Yousafi, Q., Bibi, S., Fazal, S., Saleem, S., Kamal, M.A., 2019. Molecular Docking studies reveals Rhein from rhubarb (Rheum rhabarbarum) as a putative inhibitor of ATP-binding Cassette Super Family G member 2. Med. Chem. 17, 273–288.
Khan, N.T., Jameel, N., 2018. A Neutraceutical Assessment of *Trachyspermum ammi*. J. Appl. Pharm. Sci. 10, 1–2.
Lamichhane, J.R., Dachbrodt-Saaydeh, S., Kudsk, P., Messéan, A., 2016. Toward a reduced reliance on conventional pesticides in European agriculture. Plant Dis. 100 (1), 10–24.
Martínez, L., Kleinjung, J., 2015. Automatic identification of mobile and rigid substructures in molecular dynamics simulations and fractional structural fluctuation analysis. PloSOne 10 (3), e0119264.
Mondello, F., De Bernardis, F., Girolamo, A., Cassone, A., Salvatore, G., 2006. *In vivo* activity of terpinen-4-ol, the main bioactive component of Melaleuca alternifolia Cheel (tea tree) oil against azole-susceptible and-resistant human pathogenic Candida species. BMC Infect. Dis. 6, 1–8.
Morcia, C., Malnati, M., Terzi, V., 2012. *In vitro* antifungal activity of terpinen-4-ol, eugenol, carvone, 1, 8-cineole (eucalyptol) and thymol against mycotoxigenic plant pathogens. Food Addit. Contam. 29, 415–422.
Naziya, B., Murali, M., Amruthesh, K.N., 2020. Plant growth-promoting fungi (PGPF) instigate plant growth and induce disease resistance in *Capsicum annuum* L. upon infection with Colletotrichum capsici (Syd.) Butler & Bisby. Biomolecules 10, 41.
Nisa, T., Gul, A., Khan, S., Jabeen, Z., Anwar, H.M., Khan, A., 2015. In vivo assesment of efficacy of different fungicides for control early blight of tomato. Life Sci. Internat. J., 3257–3263
Ons, L., Bylemans, D., Thevissen, K., Cammue, B., 2020. Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. Microorganisms 8 (12), 1930.
Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R.D., Kalé, L., Schulten, K., 2005. Scalable molecular dynamics with NAMD. J. Comput. Chem. 26 (16), 1781–1802.
Pinto, E., Gonçalve, M.J., Hrimpeng, K., Pinto, J., Vaz, S., Vale-Silva, L.A., Cavaleiro, C., Salgueiro, L., 2013. Antifungal activity of the essential oil of *Thymus villosus* subsp. lusitanicus against *Candida, Cryptococcus*, *Aspergillus* and dermatophyte species. Ind. Crops Prod. 51, 93–99.
Raja, N., 2014. Botanicals: sources for eco-friendly biopesticides. J. Biofert. Biopest. 5, 1.
Ravensberg, W., 2015. Crop protection in 2030: Towards a natural, efficient, safe and sustainable approach. Proceedings of the IBMA International Symposium. Swansea University, Swansea, Wales.
Saleem, S., Hasan, M.U., Sagheer, M., Sahi, S.T., 2014. Insecticidal activity of essential oils of four medicinal plants against different stored grain insect pests. Pakist. J. Zool. 46, 1407–1414.
Shapira, S., Pleban, S., Kazanov, D., Tirosh, P., Arber, N., Goel, A., 2016. Terpinen-4-ol: A novel and promising therapeutic agent for human gastrointestinal cancers. PLoS ONE 11 (6), e0156540.
Sharifzadeh, A., Khosravi, A.R., Shokri, H., Sharafi, G., 2015. Antifungal effect of *Trachyspermum ammi* against susceptible and fluconazole-resistant strains of *Candida albicans*. J. Mycol. Med. 25 (2), 143–150.
Speck-Planche, A., Cordeiro, M.N., Guilarte-Montero, L., Yera-Bueno, R., 2011. Current computational approaches towards the rational design of new insecticidal agents. Curr. Comput. Aided Drug Design 7, 304–314.
Tabassum, N., Vidyasagar, G.M., 2013. Antifungal investigations on plant essential oils. A review. Internat. J. Pharm. Pharm. Sci. 5, 10.
Tice, C.M., 2001. Selecting the right compounds for screening: does Lipinski's Rule of 5 for pharmaceuticals apply to agrochemicals? Pest Manag. Sci. 57 (1), 3–16.
Tomazoni, E.Z., Pauletti, G.F., da Silva Ribeiro, R.T., Moura, S., Schwambach, J., 2017. *In vitro* and *in vivo* activity of essential oils extracted from Eucalyptus staigeriana, *Eucalyptus globulus* and *Cinnamomum camphora* against *Alternaria solani* Sorauer causing early blight in tomato. Sci. Hortic. 223, 72–77.
Turek, C., Stintzing, F.C., 2013. Stability of essential oils: A review. Compr. Rev. Food Sci. Food Saf. 12 (1), 40–53.

Yousafi, Q., Anwar, H., Rashid, H., Ali, Q., Khan, M.S., Mehmood, A., Saleem, S., Wasim, M.W., Irfan, R., Masroor, A., 2019. Insecticide resistance management strategy for *Aedes aegypti* L. and *Anopheles gambiae* G. through prediction of potential chorion peroxidase inhibitors using computer aided drug designing approach (CAAD). Pakistan J. Agric. Sci. 56, 675–686.

Zubrod, J.P., Bundschuh, M., Arts, G., Brühl, C.A., Imfeld, G., Knäbel, A., Payraudeau, S., Rasmussen, J.J., Rohr, J., Scharmüller, A., Smalling, K., Stehle, S., Schulz, R., Schäfer, R.B., 2019. Fungicides: an overlooked pesticide class? Environ. Sci. Technol. 53, 3347–3365.

Further Reading

Jain, A, Sarsaiya, S, Wu, Q, Lu, Y, Shi, J, 2019. A review of plant leaf fungal diseases and its environment speciation. Bioengineered 10, 409–424.

Karplus, M, McCammon, JA, 2002. Molecular dynamics simulations of biomolecules. Nature Structural & Molecular Biology 9, 646–652.

Wheelan, SJ, Marchler-Bauer, A, Bryant, SH, 2000. Domain size distributions can predict domain boundaries. Bioinformatics 16, 518–613.