EGYPTIAN JOURNAL OF BOTANY (EJBO)

Print ISSN: 0375-9237
Online ISSN: 2357-0350

Chairperson
PROF. DR. MOHAMED I. ALI

Editor-in-Chief
PROF. DR. SALAMA A. OUF

Fungicidal efficacy assessment of synthesized *M*onochloro *B*enalaxyl

Devarajan Chockalingam and Rajasekaran Chandrasekaran

PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Fungicidal efficacy assessment of synthesized *M*onochloro *B*enalaxyl

Devarajan Chockalingam and Rajasekaran Chandrasekaran

Department of Biotechnology, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore -632 014, Tamilnadu, India

Increase in global population demands the need for more food and farmers engaged in crop protection use pesticides to control disease, weeds, and insects to enhance agricultural productivity. However, the side effects of pesticides also increase in parallelly. That creates a need to develop pesticides with low toxicity. Discovery of a new pesticide is very difficult due to the time, cost, and regulatory challenges. Novel pesticide invention through the Intermediate Derivatization Approach (IDA) presents a productive tool that can be leveraged for new pesticide molecule discovery quickly in a sustainable way. This approach is faster, greener, and more efficient. Benalaxyl is a successful molecule that protects economically important crops like potatoes, tomatoes, onions, flowers, and grapes from *Peronosporaceae*. Monochloro Benalaxyl was prepared by Benalaxyl by controlling chlorination by simple and commercially viable procedure using hydrogen peroxide, and hydrochloric acid under mild conditions. It was purified and characterized using GCMS and ^{1}H NMR. The efficacy of Benalaxyl and Monochlro Benlaxyl is compared with herbal pesticides by molecular docking. Fungicide activity of Monochloro Benalaxyl was found to be better than Benalaxyl against *Alternaria solani* and *Phytophthora sp.* Furthermore, Monochloro Benalaxyl shows comparable Acute oral toxicity for rats (LD_{50}) when compared to Benalaxyl which was proved by Protox a promising toxicity analyzer. The present results indicate that Monochloro Benalaxyl is a promising candidate for further exploration for fungicidal activity and formulation.

Keywords: Herbal Pesticide**,** Green Chemistry, Sustainability, Chlorination, Monochloro Benalaxyl, Toxicity assessment

ARTICLE HISTORY
Submitted: October 05, 2024
Accepted: December 23, 2024

CORRESPONDENCE TO
Rajasekaran Chandrasekaran,
Department of Biotechnology, School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore -632 014, Tamilnadu, India
Email: drcrs70@gmail.com
DOI: 10.21608/ejbo.2024.324783.3029

EDITED BY: N. Khalil

©2025 Egyptian Botanical Society

INTRODUCTION

The plant pathogenic fungi affect plant growth and result in the poor production of crop yield and immune-compromised plant generations (Shuping and Eloff, 2017). The grey mold mildews of grasses, stem canker, black scurf, septoria tritici blotch, and fusarium head blight are some of the common plant diseases caused by fungi (Nikitin et al., 2018; Li et al., 2024; Zakieh et al., 2023). Most common plant pathogenic fungi include *Fusarium sp., Colletotrichum sp., Botrytis cinerea, and Cladosporium fulvum* ultimately reduces the yield of crops (Dean et al., 2012; Bi et al., 2023; Zhao et al., 2024). Fungicides/ anti-fungal agents might be either physical, chemical, or biological agents that kill pathogenic fungi support the plant growth and yield, and increase the shelf-life of the crop (Potocki et al., 2020; Brauer et al., 2019). Agricultural fungicides are mostly chemicals or synthetic agents that are being employed for the protection of crops from plant pathogenic fungi (Beckerman et al., 2023). Some widely used fungicides are azoles and triazoles followed by benzimidazoles, and strobilurins (Ribas et. al., 2016; Hof, 2001). The overexposure of these fungicides to soil and agricultural fields also the humans and animals by showing adverse effects, metabolic fluctuations, infertility especially in men, gut microbiome fluctuations, and pathogenesis such as pulmonary disorders and cancer (Lerro et al., 2015; Melgarejo et al., 2015; Selvaraju et al., 2011).

Benalaxyl is a phenyl amide-based fungicide commonly used for the protection of potato, tobacco, and tomato crops (de Albuquerque et al., 2018; Qiu et al., 2007). In general, the R-enantiomer of benalaxyl, Benalaxyl-M [methyl N-phenylacetyl-N-2,6-xylyl-DL-alaninate] is highly active than the S-enatiomer (San Jose et al., 2016; Huang et al., 2012). The combination of two fungicides Benalaxyl-M + Mancozeb (4%+65%) co-treatment was reported to control the downy mildew disease (Kundu et al., 2012). Benalaxyl specifically targets the RNA polymerase 1 of plant pathogenic fungi and disrupts/alters its nucleic acid synthesis (Lee et al., 2004). Benalaxyl exposure also leads to several toxic and adverse effects in humans and animals (Nallani et al., 2017; Qin et al., 2014).

The present study was designed to synthesize, characterize, and evaluate the chloro-substituted benalaxyl high effect and low toxicity. The Monochloro benalaxyl (modified form of benalaxyl) was synthesized, purified, and characterized by GCMS and ^{1}H NMR. In addition to that, we also compare its efficacy with the herbal pesticide. Finally, the antifungal potential of the synthesized Monochloro benalaxyl was tested against the fungal plant pathogens *Phytophthora sp.,* and *Alternaria solani* respectively.

MATERIALS AND METHODS

Glassware and chemicals

All the chemicals used were analytical grade and purchased from Sigma Aldrich. All the glassware used

in the research was borosilicate and entirely maintained in a sterile condition. We maintain a very suitable atmosphere condition during the entire research period for the clarity output.

Microorganism

The organisms used in this study were *Alternaria solani* 5350 and *Phytophthora sp.* 7700 collected from the Indian Type Culture Collection (ITCC), Division of Plant Pathology, ICAR- Indian Agricultural Research Institute, New Delhi. These organisms were subcultured in PDB (potato dextrose agar).

Synthesis of Monochloro Benalaxyl

To one g of (3.07 mmol) Benalaxyl was added 2.7 g of acetic acid and stirred well for 10 minutes at 35^{o}C then added 0.7g of 36% HCl. To this reaction mixture, 0.45g of 27% H_2O_2 was added dropwise over 30 min and continuously mixed for 4 hours at 45-^{o}C. The reaction was monitored using thin-layer chromatography. After that added 100 mL ice cold water was followed by 0.7g of 25% sodium sulphite solution to the reaction mixture which were extracted using ethyl acetate (3 X 10 mL). The combined ethyl acetate layer was washed with 10 mL of water, dried with sodium sulphate crystals, and decanted.

Purification

The extract was further purified using the column chromatographic method. 2 feet length with 2-inch inner diameter borosilicate glass column was taken and the column was filled using 230-400 mesh silica gel as stationary phase and the mixture of 0.5% ethyl acetate in heptane as mobile phase. After the silica completely packed the extract was poured at the top of the column then the mobile phase solvent was poured continuously for 12 hours, and the samples were collected every 1 hour.

Herbal formulation

Here we formulated a special type of herbal pesticide formulation to eradicate the fungal infection in the plants for that we are mixing the most efficient antimicrobial medicinal plants in the ratio turmeric rhizome powder 25%, neem seed kernel 25%, eucalyptus leaf powder 25%, ocimum leaf powder 25% in water.

GCMS analysis

The obtained elutes were primarily analysed in GCMS to identify the molecules present in the elutes. GCMS data were obtained at 70 eV using Thermo Fisher make Trace Ultra GC and PolarisQ MS using HP-5, column 30m Length X 0.32mm Inner Diameter X 0.25µm film thickness, and the method was critically designed to identify the presence of Monochloro Benalaxyl in the elute. The method was fed into the GCMS as follows, the injection port temperature was set at 275°C with the split ratio 1:40. And the Initial oven temperature of GC was set at 80°C which is maintained for 2 min and increased with the ramp rate of 10°C/min to reach final temperature 300°C. Nitrogen gas with a flow rate of 1.0 mL/min. was used as a mobile phase. After the method was fed completely in the GCMS, the elutes were dissolved in 5mg/g of ethyl acetate and 0.2 µL of the sample as injection volume and injected into it. Then the Mass spectra were recorded at 70eV electron energy in an MS Detector with ion source temperature at 200°C.

NMR analysis

The ^{1}H NMR spectra provide evidence that Monochloro benalaxyl exists in a $CDCl_3$ solution in a pure form Here we used ^{1}H NMR spectra from Bruker Avance III NMR machine at 400 MHz, in $CDCl_3$ mixture. Expansion of one-dimensional single pulse ^{1}H NMR spectrum from 0 to 9 ppm of sample. It represents several metabolites detected using a single pulse NMR experiment. The intensities of resonances depend on their respective concentrations in the sample.

Molecular modelling

The three-dimensional structures of Multidrug Efflux Pump MepR (PDB ID: 4LLL) were retrieved from the Protein Data Bank (PDB) and the excess chains, bound molecules and ions were removed, and the protein was prepared for docking. The three-dimensional structures of Oleanolic acid (PubChem ID: 10494) were retrieved from PubChem and the energy minimization of the ligand was performed and the ligand was prepared for docking (Mekala et al., 2022).

Antifungal activity

Here we used the agar well diffusion method to study the antifungal activity of the sample and the sample was tested against 2 different fungal plant pathogens named *Alternaria solani* 5350 and *Phytophthora sp.* 7700. Muller Hinton Agar (MHA) was prepared, autoclaved and poured into the petri plates. After solidification of MHA, the non-contaminated fungal strains were inoculated by using a sterile L rod. Wells were made using a sterile well cutter and 200, 400, and 600 µg concentrations of sample to the appropriate wells as DMSO solution. Similarly, 200, 400, and 600 µg concentrations of Benalaxyl were to

the appropriate wells and used as positive control. The plates were incubated at 25℃ for 2 days and the zone of inhibition was observed and measured using a transparent ruler in millimeters. The procedure was carried out in triplicates.

Toxicity assessment

The toxicity effect was measured for Benalaxyl and Monochloro Benalaxyl using Pro-Tox II software. Probable toxicity toward target pharmacophores was predicted based on fragments and molecular fingerprints from the 38000-compound data pool.

Statistical analysis

All experiments were performed in triplets (n=3), repeated thrice and the data was represented as mean ± standard error. One-way ANOVA was conducted to analyze the significance of fungicide efficacy between Benalaxyl and Monochloro Benalaxyl at various concentration levels.

RESULTS AND DISCUSSION

A total of 600 mg of Monochloro Benlaxyl was obtained as a colorless viscous liquid from the pure fraction after column purification. This compound was subjected to GCMS analysis.

GCMS Analysis

The GCMS analysis was performed to predict the components present in the sample, and the chromatogram showed the presence of a high composition of Monochloro Benalaxyl with a specific retention time. Also, the mass spectrum of the Monochloro Benalaxyl peak was observed, and the mass spectrum data and fragmentation pattern MS (EI): m/z (%) = 359.2 (8) [$C_{20}H_{22}ClNO_3$, M+], 240 (35) [$C_{12}H_{15}ClNO_2^+$], 204 (29) [$C_{12}H_{15}NO_2^+$], 182 (100) [$C_{10}H_{12}ClN^+$], 91 (90) [$C_7H_8^+$] as shown in Figure 1.

For comparison, GCMS analysis data of Benalaxyl were obtained from the “Integrated Spectral Data Base System of Organic Compounds" (Figure 2) from the National Institute of Advanced Industrial Science and Technology (Japan). Corresponding deschloro fragments 325 (21) [$C_{20}H_{23}NO_3$ M+], 206 (47) [$C_{12}H_{16}NO_2^+$], 148 (100) [$C_{10}H_{13}N^+$], 91 (59) [$C_7H_8^+$] were observed in Benalaxyl that supports structural elucidation of Monochloro Benalaxyl. In GCMS, the compound is vaporized in a Gas chromatograph and sent to a mass spectrometer, where the gaseous ions are converted into fragment ions that are measurable and indicative of the analyte. In the output GCMS spectra, the abscissa indicates the mass by charge ratio in the ions, and ordinates show the relative intensity of ions. The GCMS spectra of Monochloro Benalaxyl exhibit expected mass fragments with characteristic $^{35}Cl/^{37}Cl$ isotopes in a 3:1 ratio in the molecular ion and characteristic fragments. Corresponding GCMS spectra of Benalaxyl show expected deschloro fragments.

NMR Analysis

Peak details: ^{1}H NMR (400 Hz, $CDCl_3$) δ 7.20 (d, 1H, CH para-aniline), 7.06 (m, 3H, 2CH metaphenyl, 1CH para-phenyl), 6.93 (d, 1H, CH meta-aniline), 6.83 (m, 2H, ortho-phenyl), 4.34 (m, 1H, CH), 3.70 (s, 3H, OCH3), 3.28 (d, 1H, CH benzylic), 3.21 (d, 1H, CH benzylic), 2.3 (s, 3H, CH_3 ortho aniline), 1.79 (d, 3H, CH_3 ortho aniline), 0.90 (s, 3H, CH_3). The ^{1}H NMR was used to characterize the Monochloro Benalaxyl, and from the peak details it is observed that the chloro-substitution was present in the Benalaxyl, and thus we confirmed the presence of Monochloro Benalaxyl (Figures 3-5).

Molecular Docking

Receptor details of *Phtophthora sp.* and *Alternaria solani* are given in Tables 1 and 2 represent the Benalaxyl, Monochloro benalaxyl and Eugenol ligand details and Table 3 represents the binding energy of such ligands with receptors *G-Protein β Subunit* and *Superoxide dismutase*. Table 4 represents the binding interaction of receptor-ligand complex with residues. Figure 6 displays such binding interactions pictorially.

In the study of Molecular docking, the receptor selected were *G-Protein β Subunit* (6QWA) and *Superoxide dismutase* (1Y67) (El-Defrawy et.al., 2020; Warris et.al., 2019; Trivedi et.al., 2024). The ligands used were Benalaxyl, Monochloro Benalaxyl and Eugenol. The Herbal formulation, Eugenol has the least activity in binding interaction with the receptor on comparison with Monochloro Benalaxyl (Best docking activity) and Benalaxyl (Moderate docking activity). Thus, the herbal formulation, Eugenol cannot be used in the further study. Most fungi have G Protein and Superoxide subunits, and interaction and blocking of these targets makes a candidate effective a fungicide. The good binding interaction of Monochloro Benalaxyl with these targets makes a case for considering this to assess its fungicidal activity. Comparable binding interaction was observed with Benalaxyl, which supported the structure-activity relationship.

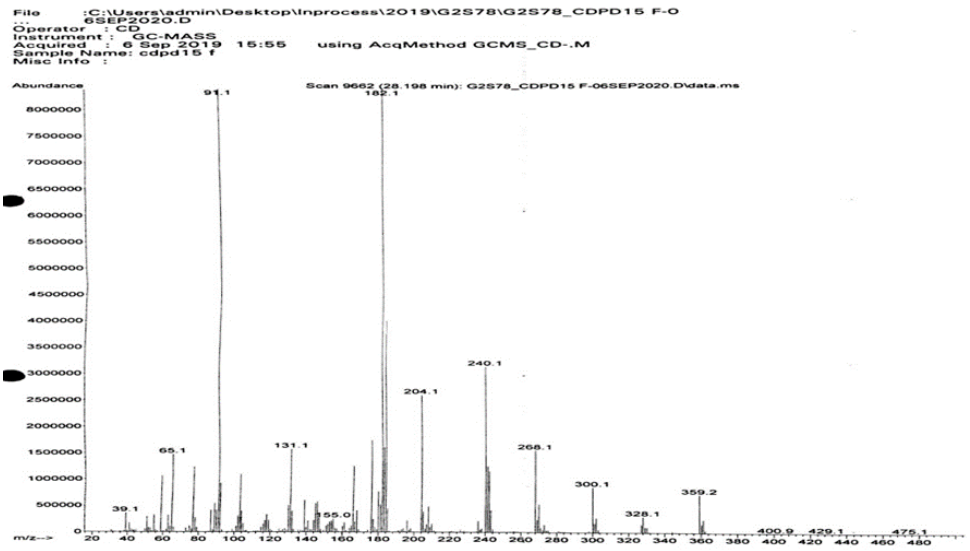


Figure 1. GCMS analysis of Monochloro Benalaxyl.

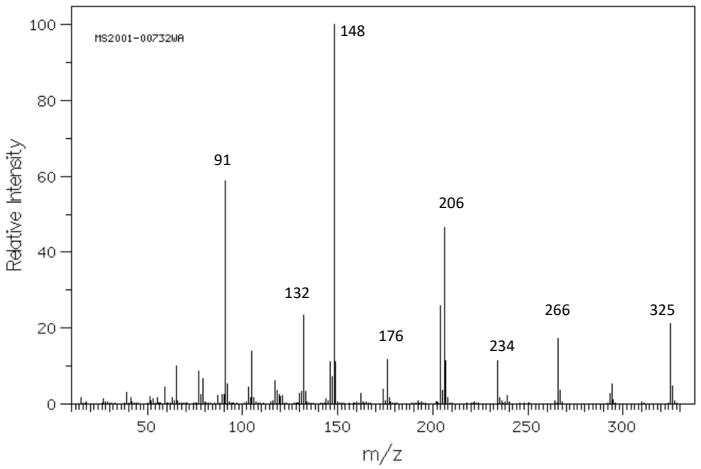


Figure 2. GCMS analysis of Benalaxyl.

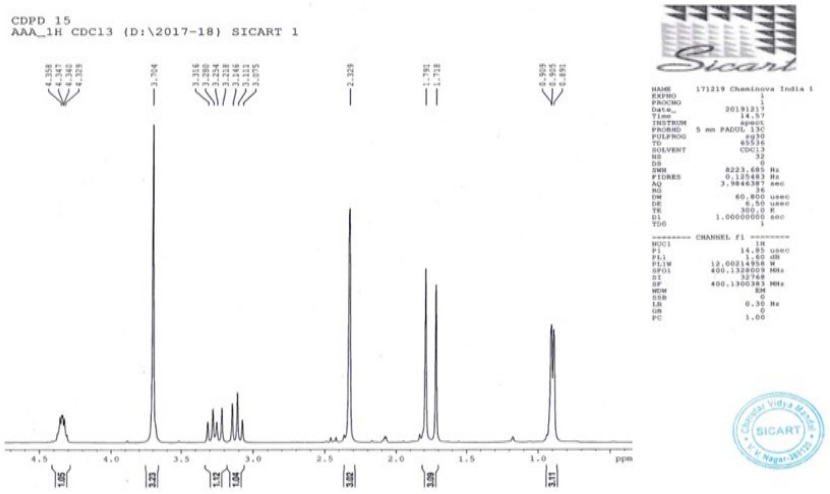


Figure 3. NMR analysis of Monochloro Benalaxyl upfield region

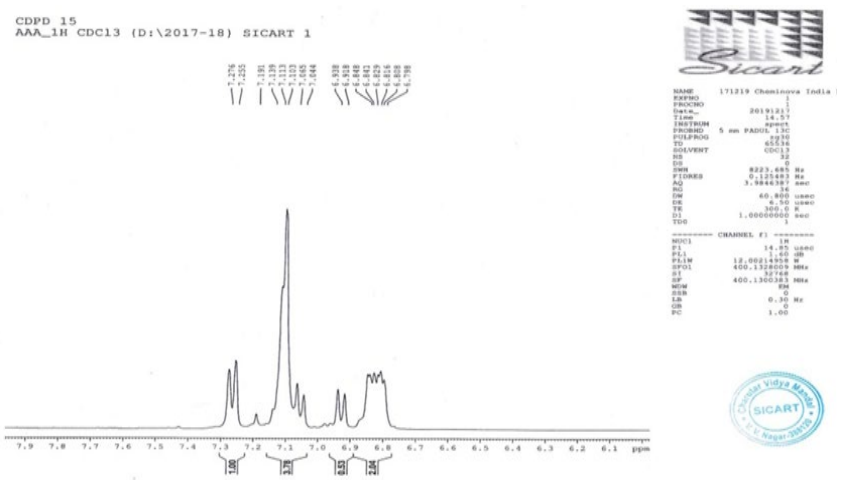


Figure 4. NMR analysis of Monochloro Benalaxyl downfield region

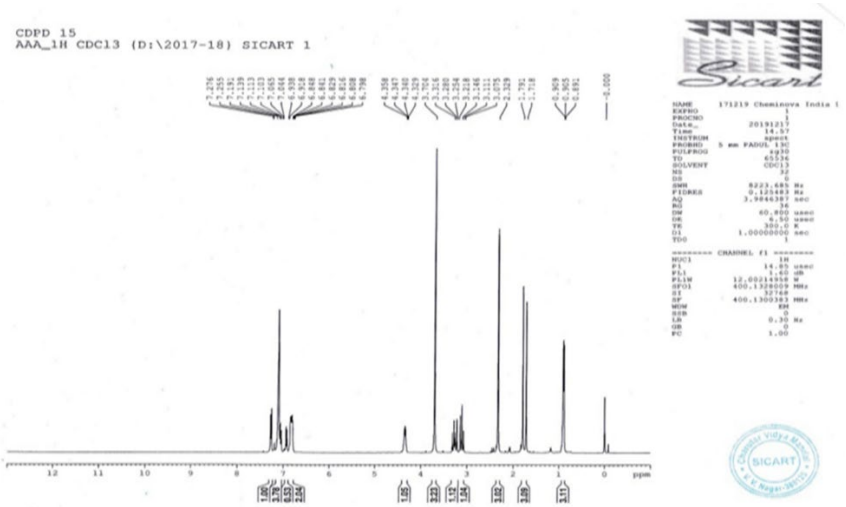


Figure 5. NMR analysis of Monochloro Benalaxyl

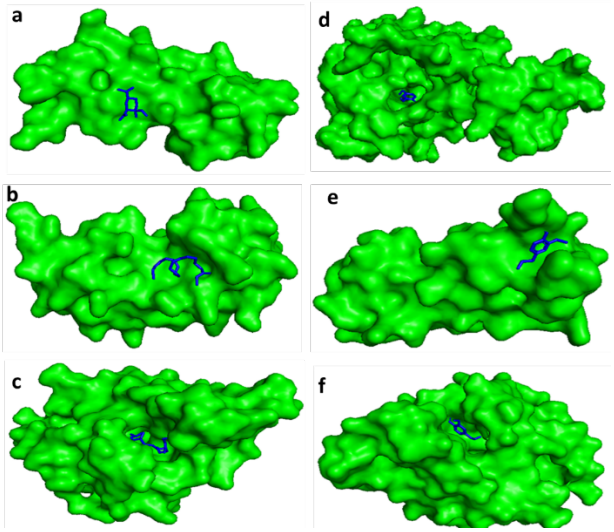


Figure 6. Binding interaction between receptor *G-Protein β Subunit* (6QWA) with Benalaxyl, Monochloro Benalaxyl and Eugenol (a, b & c). Binding interaction between receptor *Superoxide dismutase* (1Y67) with Benalaxyl, Monochloro Benalaxyl and Eugenol (d, e & f).

Table 1. Receptor details of *Phtophthora sp.* and *Alternaria solani*

Fungal Organism	Receptor Name	Receptor (PDB - ID)
Phtophthora sp.	*G-Protein β Subunit*	6QWA
Alternaria solani	*Superoxide dismutase*	1Y67

Table 2. Ligand details-Benalaxyl, Monochloro Benalaxyl and Eugenol

Ligand Name
Benalaxyl
Monochloro Benalaxyl
Eugenol

Table 3. Binding energies of Benalaxyl, Monochloro Benalaxyl and Eugenol with receptors of *Phtophthora sp.* and *Alternaria solani*

Receptor	Ligand	Binding energy
G-Protein β Subunit (6QWA)	Benalaxyl	-4.7
	Monochloro Benalaxyl	-3.7
	Eugenol	-7.3
Superoxide dismutase (1Y67)	Benalaxyl	-5.9
	Monochloro Benalaxyl	-4.7
	Eugenol	-6.8

Antifungal Activity

Fungicidal activity of Benalaxyl was reported against *Phytophthora sp.* and *Alternaria solani* (Davidse et.al., 1988; Guashao et.al., 2017; Schmey et.al., 2024)). Hence the antifungal potential of the newly synthesized Monochloro Benalaxyl (200-600 μg concentrations) against the *Phytophthora sp.* and *Alternaria solani* in comparison with the Benalaxyl was evaluated using a well diffusion method (Table 5). The fungal inhibition activity was observed with increasing concentrations of the compounds. The zone of inhibition indicates that the maximum inhibition was achieved at 600 μg concentrations against both the tested pathogens. Also, the Monochloro Benalaxyl potent activity than the Benalaxyl at each concentration (Figure 7). The above data were analyzed for variance between Benalaxyl and Monochloro Benalaxyl groups by ANOVA. The results indicate that there is significance ($P < 0.05$) in fungicide efficacy between the groups and Monchloro Benalaxyl is more active than Benalaxyl.

Figure 8 depicts the chemical structure of Benalaxyl and Monochloro benalaxyl. Table 6 portrays corresponding molecular study data obtained from Protox software. Tables 7 and 8 portray corresponding toxicity study data obtained from Protox software for Benalaxyl and Monochloro benalaxyl respectively Then the toxicity of the synthesized compound along with the parent compound was evaluated using ProTox II software.

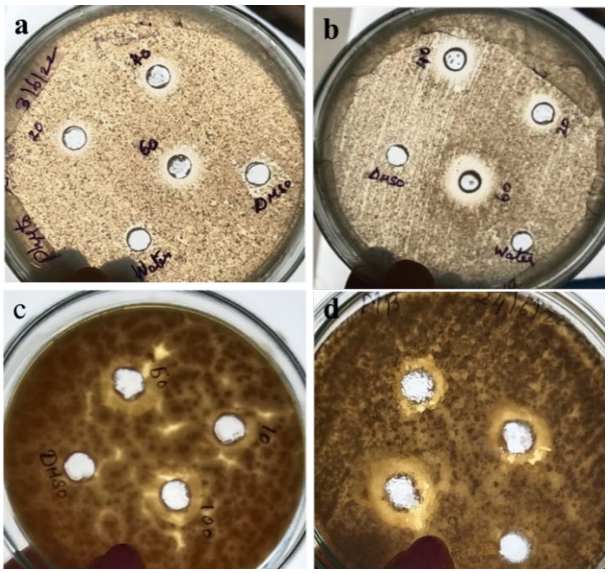


Figure 7. Zone of inhibition by (a) Benalaxyl (b) modified fungicide against the *Phytophthora sp.* (c) Benalaxyl and (d) modified fungicide showed zone of inhibition against *Alternaria solani.*

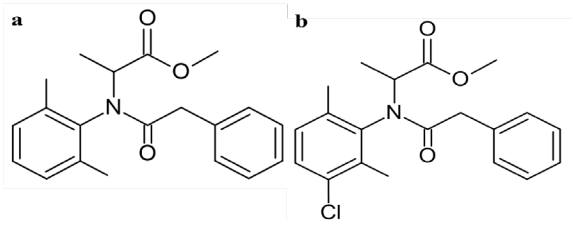


Figure 8. Chemical structure of a) Benalaxyl b) Monochloro Benalaxyl.

The Monochloro Benalaxyl shows comparable Acute oral toxicity by showing LD_{50} at 566 mg/Kg while the Benalaxyl showed LD_{50} at 680 mg/Kg for rats (LD50). Monochloro Benalaxyl shows a lower predicted toxicity target towards Amine Oxidase A, and Prostaglandin G/H Synthase 1 pharmacophores. The rationale adopted in this research construed on the hope that Monochloro Benalaxyl being structurally like Benalaxyl and having promising interaction with fungi targets could evolve as a promising fungicide candidate which is proven to be true. This enhanced fungicidal activity could be owed to the choro group introduced into the molecule.

Fungicide contamination in the environment poses a problematic threat and is complex as it depends on the toxicity of the fungicide, its persistence in the environment, and its concentration (Mitra et.al., 2024) The toxicity assessment results mentioned in this paper are estimations that need to be confirmed through laboratory-based toxicity tests or *in vivo* studies to give a more comprehensive assessment of Monochloro Benalaxyl's safety profile.

Table 4. Binding interactions of Receptor-ligand complex

Receptor	Ligand	Binding interactions
G-Protein β Subunit (6QWA)	Benalaxyl	HIS27, HIS81, PHE84, ASN80
	Monochloro Benalaxyl	PRO52, LEU51, VAL53, ASP50, ALA49, SER82, HIS78, TYR3, ALA2, THR4, PRO6
	Eugenol	PHE595, ASP594, GLY593, HIS593, SER536, PHE383, GLY593, LEU505, LEU514, SER535, CYS535, THR529, ALA481, TRP531, VAL471, ILE463
Superoxide dismutase(1Y67)	Benalaxyl	PHE303, TRP308, PHE309, ILE310, ALA265, TYR264, ILE262, PRO263, ALA261
	Monochloro Benalaxyl	GLY91, GLN92, GLN90, GLY89, ALA14, MET88, LEU15, GLU16, HIS18, ILE19
	Eugenol	ILE463, TRP531, ALA481, CYS532, VAL471, PHE583, LEU514

Table 5. The antifungal activity of modified fungicide against the fungal plant pathogens

Concentration of Fungicide (µg)	Zone of inhibition (mm)			
	Phytophthora sp.		*Alternaria solani*	
	Benalaxyl	Monochloro Benalaxyl	Benalaxyl	Monochloro Benalaxyl
200	11 ± 0.50	12 ± 0.76	12 ± 0.55	13 ± 0.44
400	13 ± 0.44	14 ± 0.75	16 ± 0.25	18 ± 0.24
600	15 ± 0.50	16 ± 0.24	25 ± 0.88	26 ± 0.88

mm (Millimetre) – Zone of inhibition was observed in mm.

Table 6. Molecular data from protox of a) Benalaxyl b) Monochloro Benalaxyl

Protox	Benalaxyl	Monochloro Benalaxyl
Mol. Weight	325.4	359.9
Number of hydrogen bond acceptors	3	3
Number of hydrogen bond donors	0	0
Number of atoms	24	25
Number of bonds	25	26
Number of rings	2	2
Number of rotable bonds	7	7
Total charge	0	0
Molecular Polar Surface Area	46.6	46.6

Table 7. Benalaxyl toxicity study using Protox

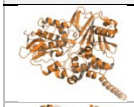
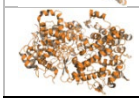
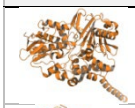
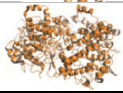
Toxicity Target		Avg. Pharmacophore Fit	Avg. Similarity Known Ligands
	Amine Oxidase A	65.8%	0%
	Prostaglandin G/ H Synthase1	61.41%	0%

Table 8. Monochloro Benalaxyl toxicity study using protox.

Toxicity Target		Avg. Pharmacophore Fit	Avg. Similarity Known Ligands
	Amine Oxidase A	44.26%	0%
	Histamine Receptor H1	48.9%	0%
	Prostaglandin G/ H Synthase1	40.07%	0%

CONCLUSION

In this study, Monochloro Benalaxyl was successfully prepared by Benalaxyl by controlling chlorination by a simple and commercially viable chlorination procedure using hydrogen peroxide and hydrochloric acid using acetic acid solvent under mild temperature condition. Monochloro Benalaxyl was purified and characterized using GCMS and ^{1}H NMR. The efficacy of Monochlro Benlaxyl is better than Benlaxyl and herbal pesticide. Further, the Fungicide activity of Monochloro Benalaxyl was found to be better than Benalaxyl against *Alternaria solani* and *Phytophthora sp*. The toxicity of Monochloro Benalaxyl was assessed using ProTox II software. Monochloro Benalaxyl shows comparable acute oral toxicity for rat (LD_{50}) and lower predicted toxicity target towards Amine Oxidase A, and Prostaglandin G/H Synthase 1 pharmacophores. Predicted lower toxicity coupled with a simple and scalable synthesis procedure makes Monochloro Benalaxyl a promising candidate for further exploration for fungicidal activity and formulation. In addition to that, this research will help other researchers to develop the number of derivatives from already available pesticides by reducing their side effects and improving their efficacy. This investigation could make a billion-dollar business around the world when it enters the market.

ACKNOWLEDGEMENT

The authors express their gratitude to the VIT management and the Dean SBST for their support and encouragement. The authors acknowledge FMC Corporation for providing an opportunity to carry out this work and TanBio R and D Solution Thanjavur for giving their research support.

REFERENCES

Shuping, D.S.S., Eloff, J.N. (2017) The use of plants to protect plants and food against fungal pathogens: A review. *African Journal of Traditional, Complementary and Alternative Medicines*, 14, 120-127.

Nikitin, M., Deych, K., Grevtseva, I., Girsova, N., Kuznetsova, M., Pridannikov, M., Dzhavakhiya, V., Statsyuk, N., Golikov, A. (2018) Preserved microarrays for simultaneous detection and identification of six fungal potato pathogens with the use of real-time PCR in matrix format. *Biosensors*, 8, 129.

Li, P., Liang, C., Jiao, J., Ruan, Z., Sun, M., Fu, X., Zhao, J., Wang, T., Zhong, X. (2024) Exogenous priming of chitosan induces resistance in Chinese prickly ash against stem canker caused by *Fusarium zanthoxyli. International Journal of Biological Macromolecules,* 259, 129119.

Zakieh, M., Alemu, A., Henriksson, T., Pareek, N., Singh, P.K., Chawade, A. (2023) Exploring GWAS and genomic prediction to improve Septoria tritici blotch resistance in wheat. *Scientific reports,* 13, 15651.

Dean, R., Van Kan, J.A., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., Foster, G.D. (2012) The Top 10 fungal pathogens in molecular plant pathology. *Molecular plant pathology*, 13, 414-430.

Bi, K., Liang, Y., Mengiste, T., Sharon, A. (2023) Killing softly: a roadmap of *Botrytis cinerea* pathogenicity. *Trends in Plant Science,* 28, 211.

Zhao, X., Liu, Y., Huang, Z., Li, G., Zhang, Z., He, X., Du, H., Wang, M., Li, Z. (2024) Early diagnosis of *Cladosporium fulvum* in greenhouse tomato plants based on visible/ near-infrared (VIS/NIR) and near-infrared (NIR) data fusion. *Scientific reports,* 14, 20176.

Potocki, L., Baran, A., Oklejewicz, B., Szpyrka, E., Podbielska, M., Schwarzbacherová, V. (2020) Synthetic Pesticides Used in Agricultural Production Promote Genetic Instability and Metabolic Variability in Candida spp. *Genes*, 11, 848.

Brauer, V.S., Rezende, C.P., Pessoni, A.M., De Paula, R.G., Rangappa, K.S., Nayaka, S.C., Gupta, V.K., Almeida, F. (2019) Antifungal agents in agriculture: friends and foes of public health. *Biomolecules*, 9, 521.

Beckerman, J., Palmer, C., Tedford, E., Ypema, H. (2023) Fifty Years of Fungicide Development, Deployment, and Future Use. *Phytopathology,* 113, 694.

Ribas e Ribas, A.D., Spolti, P., Del Ponte, E.M., Donato, K.Z., Schrekker, H., Fuentefria, A.M. (2016) Is the emergence of fungal resistance to medical triazoles related to their use in the agroecosystems? A mini review. *brazilian journal of microbiology*, 47, 793-799.

Hof, H. (2001) Critical annotations to the use of azole antifungals for plant protection. *Antimicrobial agents and chemotherapy*, 45, 2987-2990.

Lerro, C.C., Koutros, S., Andreotti, G., Friesen, M.C., Alavanja, M.C., Blair, A., Hoppin, J.A., Sandler, D.P., Lubin, J.H., Ma, X., Zhang, Y. (2015) Organophosphate insecticide use and cancer incidence among spouses of pesticide applicators in the Agricultural Health Study. *Occupational and environmental medicine*, 72, 736-744.

Melgarejo, M., Mendiola, J., Koch, H.M., Moñino-García, M., Noguera-Velasco, J.A., Torres-Cantero, A.M. (2015) Associations between urinary organophosphate pesticide metabolite levels and reproductive parameters in men from an infertility clinic. *Environmental research*, 137, 292-298.

Selvaraju, S., Nandi, S., Gupta, P.S.P., Ravindra, J.P. (2011) Effects of heavy metals and pesticides on buffalo (Bubalus bubalis) spermatozoa functions in vitro. *Reproduction in domestic animals*, 46, 807-813.

de Albuquerque, N.C.P., Carrão, D.B., Habenschus, M.D., de Oliveira, A.R.M. (2018) Metabolism studies of chiral

pesticides: A critical review. *Journal of Pharmaceutical and Biomedical Analysis*, 147, 89-109.

Qiu, J., Wang, Q., Zhu, W., Jia, G., Wang, X., Zhou, Z. (2007) Stereoselective determination of benalaxyl in plasma by chiral high-performance liquid chromatography with diode array detector and application to pharmacokinetic study in rabbits. *Chirality: The Pharmacological, Biological, and Chemical Consequences of Molecular Asymmetry*, 19, 51-55.

San Jose, G., Jackson, E.R., Haymond, A., Johny, C., Edwards, R.L., Wang, X., Brothers, R.C., Edelstein, E.K., Odom, A.R., Boshoff, H.I., Couch, R.D. (2016) Structure–activity relationships of the MEPicides: N-acyl and O-linked analogs of FR900098 as inhibitors of Dxr from Mycobacterium tuberculosis and Yersinia pestis. *ACS infectious diseases*, 2, 923-935.

Huang, L., Lu, D., Diao, J., Zhou, Z. (2012) Enantioselective toxic effects and biodegradation of benalaxyl in Scenedesmus obliquus. *Chemosphere*, 87, 7-11.

Kundu, C., Goon, A., Bhattacharyya, A. (2012) Persistence behaviour of fungicide mixture (benalaxyl-M 4%+ mancozeb 65%) WP in grapes. *Bulletin of environmental contamination and toxicology*, 89, 1253-1257.

Lee, J.K., Park, S.H., Lee, E.Y., Kim, Y.J., Kyung, K.S. (2004) Development of an enzyme-linked immunosorbent assay for the detection of the fungicide fenarimol. *Journal of agricultural and food chemistry*, 52, 7206-7213.

Nallani, G.C., ElNaggar, S.F., Shen, L., Chandrasekaran, A. (2017) In vitro metabolism of [14C]-benalaxyl in hepatocytes of rats, dogs and humans. *Regulatory Toxicology and Pharmacology*, 84, 26-34.

Qin, F., Gao, Y.X., Guo, B.Y., Xu, P., Li, J.Z., Wang, H.L. (2014) Environmental behavior of benalaxyl and furalaxyl enantiomers in agricultural soils. *Journal of Environmental Science and Health, Part B*, 49, 738-746.

Fischer, R.A., Connor, D.J. (2018) Issues for cropping and agricultural science in the next 20 years. *Field Crops Research*, 222, 121-142.

Liu, C., Guan, A., Yang, J., Chai, B., Li, M., Li, H., Yang, J., Xie, Y. (2016) Efficient approach to discover novel agrochemical candidates: intermediate derivatization method. *Journal of agricultural and food chemistry*, 64, 45-51.

Sparks, T.C. (2013) Insecticide discovery: an evaluation and analysis. *Pesticide biochemistry and physiology*, 107, 8-17.

Guan, A., Liu, C., Yang, X., Dekeyser, M. (2014) Application of the intermediate derivatization approach in agrochemical discovery. *Chemical reviews*, 114, 7079-7107.

Guan, A., Liu, C., Chen, W., Yang, F., Xie, Y., Zhang, J., Li, Z., Wang, M. (2017) Design, synthesis, and structure–activity relationship of new pyrimidinamine derivatives containing an aryloxy pyridine moiety. *Journal of agricultural and food chemistry*, 65, 1272-1280.

Wang, X., Wang, D., Zhou, Z., Zhu, W. (2018) Subacute oral toxicity assessment of benalaxyl in mice based on metabolomics methods. *Chemosphere*, 191, 373-380.

Park, O.J., Lee, S.H., Park, T.Y., Chung, W.G., Lee, S.W. (2006) Development of a scalable process for a key intermediate of (R)-metalaxyl by enzymatic kinetic resolution. *Organic process research & development*, 10, 588-591.

Liu, Y., Li, Y., Chen, N., Lv, K., Zhou, C., Xiong, X., Li, F. (2014) Synthesis and fungicidal activity of novel chloro-containing 1-aryl-3-oxypyrazoles with an oximino ester or oximino amide moiety. *Molecules*, 19, 8140-8150.

Nikishin, G.I., Kapustina, N.I., Lyubov'L, S., Bityukov, O.V., Terent'ev, A.O. (2020) H2O2/HCl system: Oxidation-chlorination of secondary alcohols to α, α'-dichloro ketones. *Tetrahedron Letters*, 61, 152154.

Srivastava, A.K., Kesavachandran, C. (2019) "Health effects of pesticides". CRC Press, Taylor & Francis Group, London.

Yin, J., Zhu, F., Hao, W., Xu, Q., Chang, J., Wang, H., Guo, B. (2017) Acylamino acid chiral fungicides on toxiciepigenetics in lambda DNA methylation. *Food and Chemical Toxicology*, 109, 735-745.

van Boxtel, A.L., Kamstra, J.H., Fluitsma, D.M., Legler, J. (2010) Dithiocarbamates are teratogenic to developing zebrafish through inhibition of lysyl oxidase activity. *Toxicology and applied pharmacology*, 244, 156-161.

Hernández, A.F., Gómez, M.A., Pérez, V., García-Lario, J.V., Pena, G., Gil, F., López, O., Rodrigo, L., Pino, G., Pla, A. (2006) Influence of exposure to pesticides on serum components and enzyme activities of cytotoxicity among intensive agriculture farmers. *Environmental research*, 102, 70-76.

Atkinson, P.W., Binnington, K.C., Roulston, W.J. (1974) High monoamine oxidase activity in the tick Boophzlus microplus, and inhibition by chlordimeform and related pesticides. *Australian Journal of Entomology*, 13, 207-210.

Martin, P.G., Dupouy, V., Leghait, J., Pineau, T., Polizzi, A., Lasserre, F., Roques, B.B., Viguié, C. (2020) Transcriptomic modifications of the thyroid gland upon exposure to phytosanitary-grade fipronil: Evidence for the activation of compensatory pathways. *Toxicology and Applied Pharmacology*, 389, 114873.

Purushothaman, B., PrasannaSrinivasan, R., Suganthi, P., Ranganathan, B., Gimbun, J., Shanmugam, K. (2018) A comprehensive review on *Ocimum basilicum*. *Journal of Natural Remedies*, 71-85.

Purushothaman, B., Suganthi, N., Shanmugam, K. (2020) Qualitative and quantitative determination of various extracts of *Ocimum basilicum* L. leaves. *Journal of natural remedies*, 53-60.

Ramalingam, P.S., Sagayaraj, M., Ravichandiran, P., Balakrishnanan, P., Nagarasan, S., Shanmugam, K. (2017) Lipid peroxidation and anti-obesity activity of

Nigella sativa seeds. *World Journal of Pharmaceutical Research*, 6, 882–92.

Sethuraman, J., Nehru, H., Shanmugam, K., Balakrishnan, P. (2017) Evaluation of potent phytochemicals and antidiabetic activity of *Ficus racemosa* Linn. *World Journal of Pharmaceutical Research*, 6, 909-920.

Mekala, J.R., Kurappalli, R.K., Ramalingam, P., Moparthi, N.R. (2021) N-acetyl l-aspartate and Triacetin modulate tumor suppressor MicroRNA and class I and II HDAC gene expression induce apoptosis in Glioblastoma cancer cells in vitro. *Life sciences*, 286, 120024.

El-Defrawy, M.M.H., Hesham, A.E.L. (2020) G-protein-coupled Receptors in Fungi. *Fungal Biotechnology and Bioengineering*, 37.

Warris, A., Ballou, E.R. (2019) Oxidative responses and fungal infection biology. *Seminars in Cell & Developmental Biology,* 89, 34.

Trivedi, H.D., Patel, B.Y., Hadiyal, S.D., Italiya, G., Ramalingam, P.S. (2024) A green one-pot synthetic protocol of hexahydropyrimido[4,5-d]pyrimidin-4(1H)-one derivatives: molecular docking, ADMET, anticancer and antimicrobial studies. *Molecular Diversity,* 28, 183.

Davidse, L.C., Gerritsma, O.C.M., Ideler, J., Pie, K., Velthuis, G.C.M. (1988) Antifungal modes of action of metalaxyl, cyprofuram, benalaxyl and oxadixyl in phenylamide-sensitive and phenylamide-resistant strains of *Phytophthora megasperma f. sp. medicaginis* and *Phytophthora infestans. Crop Protection, 7-6, 347.*

Guashao, S., Linga, W., Shuhui, J., Yanhong, D., Huizhe, L., Jianjun, Z. (2017) Synthesis and Fungicidal Activity of Diamide Compounds Based on the Metabolite of Benalaxyl. *Chinese Journal of Organic Chemistry,* 37, 157.

Schmey, T., Tominello-Ramirez, C.S., Brune, C., Stam, R. (2024) *Alternaria* diseases on potato and tomato. *Molecular Plant Pathology,* 25, 13435.

Mitra, S., Saran, R.K., Srivatsava, S., Rensing, C. (2024). Pesticides in the environment: Degradation routes, pesticide transformation products and ecotoxicological considerations. *Science of The Total Environment,* 935, 173026.