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Experimental and *In-Silico* Analysis of the Esterase Domain of *Alternaria alternata* Causing Wheat Black Point: Evaluating Fungicides Efficacy and Climate Change Impacts Using Explainable AI and Machine Learning

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Abstract: Wheat Black Point, caused by Alternaria alternata, is a seed-borne disease that poses significant challenges to wheat production by reducing both yield and quality. This study integrates experimental and computational methods to evaluate the efficacy of fungicides targeting the esterase domain of A. alternata. Field trials determined the effectiveness and optimal dosages of fungicides, complemented by laboratory analyses using the agar well diffusion method to assess fungal susceptibility. Computational approaches, including AlphaFold3 Protein structure predictions, molecular docking and MD simulations, explored fungicide binding mechanisms. Additionally, machine learning () and Explainable AI (XAI) techniques, specifically SHAP and XGBoost, were employed to analyze the influence of climate variables—temperature, humidity, and precipitation—on fungicide performance, supported by 1000 Monte Carlo simulations. Propiconazole (Tilt) emerged as the most effective fungicide, with Dose 4 providing superior disease suppression and yield improvement. Structural analyses identified three critical fungal proteins (AFE1, AFE2, AFE3) containing the Esterase_PHD domain, essential for fungal survival in host cells. Molecular docking and dynamics simulations highlighted Pyraclostrobin–AFAE3, Metalaxyl–AFAE2, and Cymoxanil–AFAE2 as top binders based on stability metrics. Notably, Propiconazole–AFAE1 exhibited a well-balanced interaction profile, aligning with its superior in vitro performance. SHAP analysis revealed that moisture-dependent fungicides (Propiconazole, Cymoxanil + Mancozeb) performed optimally in humid climates, while region-specific fungicides (Metribuzine, Thiophanate-methyl) were more effective in semi-arid environments. Broadly adaptable fungicides, such as Chlorothalonil + Metalaxyl-M, exhibited consistent efficacy across diverse climatic conditions. The findings underscore the importance of integrating AI tools with traditional agricultural practices to optimize fungicide application strategies, enabling climate-adapted disease management and enhancing sustainable wheat production.

Keywords: *A. alternata*; black point; Esterase_PHD; propiconazole (Tilt); *Triticum*; explainable AI (XAI); SHAP (SHapley Additive exPlanations); XGBoost

Introduction

Wheat (*Triticum* spp.) is an important crop that provides key nutrients and energy in the human diet (Shukla *et al*., 2020). Pakistan is the second most rapidly growing population at the rate of 3.6% in South Asia and the fourth largest population in general. The most significant and second largest food crop is wheat after rice. Both the biotic and abiotic factors are involved in causing black point disease which causes an estimated 22% yield losses (Figueroa *et al*., 2018). Annually about 20% of global crop yield is reduced by pathogenic fungi. The most common fungal diseases in wheat include Spot Blotch (*Cochliobolus sativus*), Black Point (*A. alternata*), and leaf Rust (*Puccinia triticina*) (Savary *et al*., 2019). In 2021 and 2022, the huge impact of fungal diseases was reported, 3.9% reduction in wheat production and 2.1% in wheat acreage resulting in approximately 15 to 20% yield losses in all parts of the country (Saeed & Hammad, 2022-23).

Black point is a fungal seed-born disease that has economic importance and negatively impacts the wheat crop globally. It is differentiated by dark brown or black discoloration at the embryo's end and surrounding areas of the wheat kernel. This disease generally reduces grain quality, germination, and crop production (Shukla *et al*., 2020). The primary pathogen of this disease is *A. alternata* which accounts for up to 24% loss of wheat crop (El-Gremi *et al*., 2017). The *A. alternata* fungus affects plants and humans and causes diseases in them, also its pathogenicity affects agricultural products. This fungus belongs to Ascomycota which is known to cause diseases in about 400 plant species and damage the postharvest outputs (Masiello *et al*., 2020). It is necessary to effectively manage diseases from the danger caused by fungal pathogens (Fisher *et al*., 2012; Strange & Scott, 2005).

To prevent fungal growth and the development of disease, fungicide application is important (Achilonu *et al*., 2023). Propiconazole is a systematic fungicide that shows a response against black point, powdery mildew, and leaf spot (Kassaw *et al*., 2021). *A. alternata* contains an esterase enzyme which is involved in the pathogenicity of this fungus so the esterase domain interaction with fungicide is important to understand (Hou *et al*., 2022). Furthermore, feruloyl esterases contribute to the pathogenicity of *A. alternata* by breaking down the cell wall of plants specifically the lignocellulose-containing part (Crepin *et al*., 2004). However, there is little information is available on how the *A. alternata's* esterases are affected by the fungicides.

Crop diseases pose a danger to food security but due to the unavailability of infrastructure, it is difficult to detect and identify them in their initial stages which is significant for the control of disease (Long *et al*., 2023; Mohanty *et al*., 2016). AI-based computer vision methods must be investigated to quickly and economically identify disease incidents on each plot of land under cropping (Kamilaris & Prenafeta-Boldú, 2018b; Abade *et al*., 2021; Arnal Barbedo, 2013; Haque *et al*., 2022). The use of AI-based techniques reduces the accidental use of chemicals, carries out regular health checks of plants, and gives information about the minimum number or amount of pesticides required to cure plant diseases (Ferentinos, 2018). Recent studies reported that deep machine models give 98% accurate results of disease identification on the dataset (Naik *et al*., 2022; Sutaji & Yıldız, 2022).

The study examined the efficacy of commercially available fungicides against *A. alternata* by integrating experimental trials and *in silico* studies. The experimental analysis includes field trials and agar well diffusion technique while *in silico* analysis includes protein structure modeling with AlphaFold3 and docking simulations to investigate the interactions between fungicides and the fungal esterase-PHD domain. Machine learning models were also used to evaluate how well the fungicides would work in different environmental conditions and the impact of climate factors such as precipitation, temperature, and humidity on fungicides' performance. This research attempts to improve fungicide application strategies and adapt them to challenges posed by climate change to ensure sustainable wheat production and efficient management of disease. It is important to know how the climate affects the fungicides' efficacy since black point seriously lowers crop yields. This study seeks to the individual and combined effects of climatic variables on pesticide efficacy to understand the interconnections and the selection of appropriate pesticides for certain

Material and Methods

This study's methodology consists of four primary components: fieldwork, laboratory work, computational analysis, and machine learning employing XGBoost (Extreme Gradient Boosting) in conjunction with Explainable AI (XAI) techniques, particularly utilizing SHAP (SHapley Additive exPlanations) to deliver interpretable and transparent insights regarding feature interactions and their influence on pesticide efficacy (Prendin *et al.*, 2023).

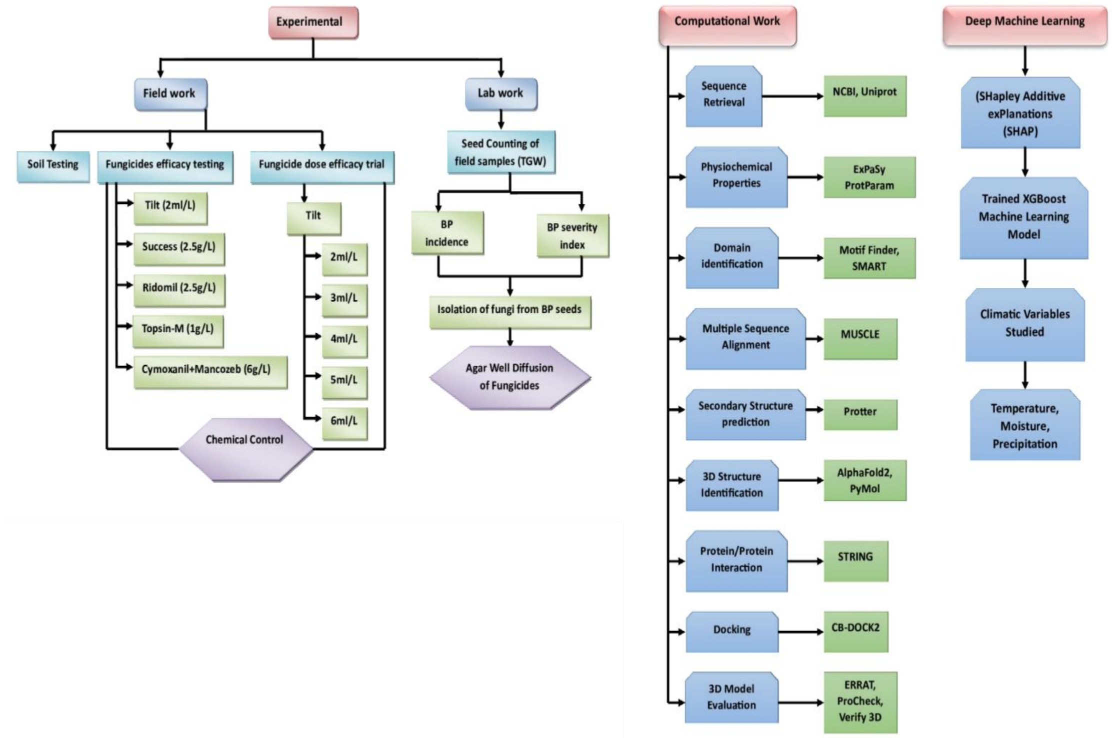

Figure 1. Depicts the systematic steps of this study such as field work, laboratory work, computational work, and deep machine learning.

Study Design

The study was designed by the Department of Microbiology, Shaheed Benazir Bhutto Women University Peshawar, in collaboration with the Plant Pathology Group at the Nuclear Institute for Food and Agriculture (NIFA) in Peshawar. The fieldwork was conducted at the NIFA farm, while the in-vitro experiments were carried out in the NIFA Plant Pathology Laboratory. The study was carried out over a year, from November 2022 to December 2023. Figure S1 shows the location of the field and experimental work.

Ethical Approval

This study was approved by the ethical committee of Shaheed Benazir Bhutto Women University Peshawar.

Field Work

During the 2022-23 cropping season, two trials were conducted at the NIFA farm to assess the efficacy of five fungicides and different doses of one fungicide against black point on the susceptible variety "Morocco." The trials were raised in a Randomized Complete Block (RCB) design with six replications. In the first trial, five fungicides (Tilt, Success, Ridomil, Topsin-M, Cymoxanil+Mancozeb) were evaluated, while in the second trial, different doses of the fungicide "Tilt" (2ml/l, 3ml/l, 4ml/l, 5ml/l, 6ml/l) were tested as shown in Table S1 to S5 (Asif *et al.*, 2021).

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Morocco was sown on 0.125 acres, with appropriate fertilizers applied (Degewione & Alamerew, 2013). Seeds were sown manually at specific densities, and fungicide applications were carried out as per company recommendations in the first trial. Harvesting took place in mid-May 2023, followed by threshing and collection of seeds for further analysis, specifically for the black point study (Nithya *et al*., 2011) (Figure S3). The physicochemical properties of the soil were also tested by the Soil and Environmental Sciences Division of NIFA, Peshawar. The soil was collected from a depth of 0-15 cm in the wheat farm (Peila *et al*., 2016).

Laboratory Work

An automatic seed counter and a Sartorius balance were utilized to generate treatment-wise/replication-wise working samples of 1,000 seeds and recorded their weight in grams (Moshatati & Gharineh, 2012) (Figure S4). Black point infection was identified by manually counting black-pointed seeds, observed through careful examination using a stereo microscope at 20X magnification. The percent disease incidence was calculated using a specific formula (Shukla *et al*., 2020). (Figure S5)

$$\%diseaseincidence = \frac{Numberofblackpointkernel \in asample}{1000} \times 100$$

The black point severity index was also calculated as described by (Draz *et al*., 2016) using the severity score from 0 to 3 (Figure S6)

The black point severity index was calculated by using the mentioned formula:

$$Blackpointindex = \Sigma \frac{Severityscore \times Noofgrains \in score \times 100}{Totalnoofgrains \times 3}$$

Pathogens associated with black-point-infected seeds were isolated using the agar plating technique on Potato Dextrose Agar (PDA) as shown in Figure S8. Surface-sterilized seeds were placed on PDA plates under a laminar flow unit and incubated for 7 days (Xu *et al*., 2018). Pure cultures of the isolated fungi were maintained on PDA and identified based on morphological features observed under a compound microscope (Manamgoda *et al*., 2015). Fresh cultures of the identified fungi were sub-cultured for further analysis. The efficacy of chemical fungicides, including Tilt, Success, Ridomil, Topsin M, and Cymoxanil+Mancozeb, against black point pathogens, was assessed using the agar well diffusion technique. Inhibition zone diameters were measured after 5 days of incubation at 25-27°C (Balouiri *et al*., 2016). (Figure S9)

The soil fungal flora of both trials was also assessed using the process of serial dilution (Alastruey *et al*., 2015). Fungal colonies were identified based on their morphological and diagnostic features using a compound microscope (Manamgoda *et al*., 2015). The soil was collected from three depths, such as 0-15 cm, 15-30 cm, and 30-45 cm, respectively as shown in Figure S10.

In-Silico Analysis

Six *A. alternata* proteins were retrieved from UniProt and NCBI databases for analysis, focusing on those without solved experimental protein structures (Griffin & Griffin, 1995; "UniProt: A Hub for Protein Information," 2015). Physicochemical properties were assessed using the ProtParam tool, including molecular weight, theoretical pI, amino acid composition, and other parameters (Gasteiger *et al*., 2005). Protein domain analysis was conducted using SMART (Schultz et al., 1998), and Motif finder (Brown *et al*., 2013). These tools were used to identify domains, motifs, and families. Sequence alignment was done using MUSCLE (Edgar & Batzoglou, 2006). Secondary structures were predicted using Protter-interactive protein feature visualization https://wlab.ethz.ch/protter/start/. Tertiary protein structures were predicted using AlphaFold 3 (Abramson et al., 2024) software and visualized with PyMol (DeLano & Bromberg, 2004). Stereochemical quality was assessed using ProCheck (Laskowski et al., 1993), ERRAT (Dym & Eisenberg, 2012), ProSA-Web (Wiederstein & Sippl, 2007), and verify3D (Eisenberg *et al*., 1997). Active sites were predicted using the Computed Atlas of Surface Topography of Proteins (CASTp) (Binkowski, 2003). To examine protein to protein interactions, STRING software was utilized (Szklarczyk *et al*., 2015). For finding reputed ligands to bind with active sites, ChEMBL (https://www.ebi.ac.uk/chembl/) database was used (Gaulton *et al*., 2012). CB-Dock2 (https://cadd.labshare.cn/cb-dock2/php/index.php) was used for blind docking purposes which is a web-based application (Liu *et al*., 2022).

Molecular Docking

CB-Dock2, a cavity-detection-based docking server, was used to blind dock the three fungal esterase homologs (AFAE1, AFAE2, and AFAE3) in order to find possible ligand-binding pockets. CB-Dock2 predicted several binding cavities for every ligand-protein set, and AutoDock Vina [1] was used to score the cavities according to their volume and binding energy. The pocket with the highest Vina score, or the top-ranked pocket, was chosen for further targeted docking.

Ligand structures were created using Open Babel's [2] 3D geometry generating feature from .mol files in PDBQT format. To ensure compatibility with rigid docking processes, protein receptors were processed using MGLTools' [3] prepare_receptor4.py and then cleaned to eliminate unnecessary torsional annotations.

AutoDock Vina (v1.2) was used for the final docking, with grid box parameters based on the chosen pocket coordinates that CB-Dock2 had supplied. A custom Python pipeline automated ligand/receptor preparation, execution of Vina docking, and parsing of results, including binding affinity values and predicted interacting residues. A structured summary table containing all of the docking data was created in order to choose ligand-protein complexes for molecular dynamics simulation.

Molecular Dynamics Simulations

GROMACS (v2021.6) [4]was used to conduct molecular dynamics (MD) simulations in order to assess the dynamic stability of the docked ligand–protein complexes. Top-ranked docking poses (based on Vina scores) were extracted, and ligand topologies were generated using ACPYPE [5] with AMBER force field parameters. Ligand coordinate and topology files were patched and standardized across all systems for consistency.

The AMBER99SB-ILDN force field and SPC/E water model were used to process protein receptors (AFAE1, AFAE2, and AFAE3) using pdb2gmx. After being solvated with explicit water and neutralised by the addition of Na+ and Cl+ ions, each ligand–protein complex was centred in a cubic box with a spacing of 0.8 nm.

Using the steepest descent algorithm, energy was minimised. Following this, 100 ps of NVT and 100 ps of NPT equilibration were performed employing position restrictions on the ligand's heavy atoms and protein. Using a Parrinello-Rahman barostat and a velocity-rescaling thermostat, final production runs were conducted for 5 ns under periodic boundary conditions.

To process all systems in a sequential manner and guarantee uniform ligand preparation, topology merging, restraint creation, and simulation execution, a completely automated Python pipeline was created. GPU acceleration was used for all simulations, and the results were post-processed to extract solvent-accessible surface area (SASA), radius of gyration (Rg), RMSD, RMSF, and hydrogen bonds.

Machine Learning and Explainable AI Analysis of Climatic Impacts on Fungicide Efficacy

Dataset Collection and Overview

In this study, the dataset used was taken from the Department of Agriculture, Peshawar which gives a detailed compilation of agricultural and environmental data collected from six key districts of wheat production in Khyber Pakhtunkhwa, Pakistan including Peshawar, Nowshera, Mardan, Dera Ismail Khan, Charsadda and Swabi. These districts constitute diverse agro-climatic zones, to ensure a thorough understanding of the agronomic and environmental factors affecting the dynamics of black point disease and wheat yield. The data collected compass 2020–2024, employing systematic field surveys and monitoring systems to capture inter-annual climate variability and cropping patterns. Rigorous validation processes were employed to ensure data accuracy, incorporating checks for consistency and completeness. The dataset consists of 2,000 rows, offering a rich and representative sample of real-world agricultural conditions.

The key climatic parameters were included in the dataset such as Humidity (%), measuring daily average relative humidity which is critical for the growth of fungus; Total Precipitation (mm), reflecting cumulative rainfall contributing to soil and atmospheric moisture; and Average Temperature (°C), capturing daily mean temperature that impacts the crop physiology and activity of pathogens. These variables provide a multidimensional view of weather conditions driving black point disease incidence. The target variable, black point disease incidence, quantifies the percentage of crops affected, serving as the outcome for prediction and analysis.

The dataset also includes detailed pesticide application records, encompassing fungicides such as Propiconazole, Metalaxyl-M + Mancozeb, Propoxycarbazone, Sulfosulfuron, Thiophanate-methyl, Metribuzine, Cymoxanil + Mancozeb, Chlorothalonil + Metalaxyl-M, and Dichlorodiphenyltrichloroethane (DDT). These records facilitated a nuanced evaluation of pesticide efficacy under varying climatic conditions.

Preprocessing

A comprehensive preprocessing pipeline was applied to the dataset to ensure data quality, enhance feature interpretability, and optimize model performance. This pipeline addressed missing values, duplicate entries, categorical encoding, feature extraction, numerical scaling, and outlier detection.

Missing Value Imputation: Addressed missing entries in climatic variables (e.g., temperature, humidity, precipitation) and pesticide efficacy data to ensure completeness.

Outlier Detection and Removal: Identified and removed anomalous values in key numeric columns, such as temperature and Black Point disease incidence, to improve the quality of the data.

Normalization: Applied Z-score scaling to normalize climatic parameters (temperature, humidity, and precipitation) for better model performance and feature comparability.

Encoding Categorical Variables: Encoded pesticide names into numeric representations (e.g., using label encoding) to prepare the data for machine learning models while maintaining interpretability.

Data Integrity Checks: Verified that the dataset was free of duplicate rows and logical inconsistencies, ensuring a clean and reliable data foundation.

SHAP Analysis to Quantify and Interpret Climate and Pesticide Interactions The Explainable AI techniques, specifically SHAP (SHapley Additive exPlanations), were utilized to quantify interaction effects and feature importance. An XGBoost model was used to facilitate SHAP analysis for its accuracy, efficiency, and ability to handle datasets with mixed numerical and categorical features, while effectively capturing interactions between predictors. The predictors included climate variables (humidity, precipitation, and temperature) and pesticide types. Black point disease incidence, expressed as the percentage of affected crops was the target variable. The preprocessed dataset, comprising normalized climatic variables and encoded pesticide types, was leveraged to train the XGBoost model, allowing it to learn complex relationships between climatic conditions, pesticide applications, and disease incidence. To ensure reliability and minimize variability, SHAP interaction values were averaged over 1,000 Monte Carlo simulations, providing robust insights into the individual and combined effects of climatic variables on fungicide performance. This comprehensive methodology enabled the identification of temperature-, precipitation-, and humidity-dependent pesticide behaviors, offering actionable data to optimize fungicide application under diverse environmental conditions (Lee *et al*., 2021; Prendin *et al*., 2023; Taylor & Cunniffe, 2023).

Data Analysis

Social Sciences software (IBM SPSS Statistics 20) was used to analyze the ANOVA, LSD, and correlation on the experimental dataset.

Results

Field Results

The soil's physicochemical properties of the wheat field were tested which include soil texture (Slit loam), pH (7.9), nitrogen (0.06%), electrical conductivity (0.50 ds/m), phosphorous (9.25ppm), potash (110 ppm) and organic matter (1%) as shown in Table S6 respectively. The field samples were evaluated in the laboratory. For the fungicides trial, tilt (Propiconazole) showed the highest efficacy, with an incidence of 5.7%, followed by Cymoxanil+Mancozeb (6.0%), Success (6.3%), Topsin-M (6.5%), and Ridomil (7.0%). The control group had the highest disease incidence i.e., 8.6%. Propiconazole also resulted in the highest TKW i.e., 35.5g, followed by Cymoxanil+Mancozeb (34g), Topsin-M (30.9g), Success (30.6g), and Ridomil (29.7g), while the control had the lowest TKW i.e., 25.9g as shown in Table S7 and Figure S11. In case of severity index, Cymoxanil+Mencozeb had the lowest severity index (2.7%), followed by Tilt (3%), Success (3.1%), and Ridomil (3.3%). The highest severity index was observed for Topsin-M (4.7%). The control group exhibited a severity index of 4.1%. as shown in Table S8 and Figure S12. The ANOVA results indicated that there were no significant differences among treatments for Severity ($F = 1.28$, $p = 0.3046$) and Incidence ($F = 1.05$, $p = 0.4109$), while TKW showed significant differences ($F = 14.92$, $p < 0.0001$). The LSD pairwise comparisons revealed significant differences among treatments for Severity and TKW, while no significant pairwise differences were observed for Incidence. Notably, TKW means varied significantly among treatments, highlighting distinct treatment effects. Correlation analysis showed a strong positive correlation between Severity and Incidence ($r = 0.7107$), moderate negative correlations between Incidence and TKW ($r = -0.2901$), and a moderate positive correlation between TKW and Treatment ($r = 0.3475$). These findings suggest significant treatment effects on TKW but not on Severity and Incidence, with notable correlations among the response variables.

For the dose trail, the most effective dose was dose 4 (5.7%), followed by dose 1 (6%), dose 2 (6.3%), dose 5 (6.6%), and dose 3 (7.4%) respectively. The control group had the highest disease incidence i.e., 9.1%. The maximum TKW was recorded for dose 5 (37.0g), followed by dose 4 (36.7g), dose 3 (36.6g), dose 2 (36.3g), and dose 1 (35.8g). The control had the lowest TKW of 30.3g as shown in Table S9 and Figure S13. The severity index for kernel black point disease in the tilt fungicide trial varied across different doses of the fungicide. Dose 4 had the lowest severity index of 3.3%, followed by dose 2 (3.6%), dose 3 (3.8%), dose 5 (4.1%), and dose 1 (4.2%). The control group had the highest severity index of 4.4% as shown in Table S10 and Figure S14. The ANOVA results indicated no significant differences among treatments for Sev ($F=1.75$, $p=0.1599$), while significant differences were found for both Inc ($F=4.30$, $p=0.0059$) and TKW ($F=20.12$, $p<0.0001$). The LSD All-Pairwise Comparisons Test reveals significant differences among treatments for severity, incidence, and TKW, with specific treatments showing distinct separation from others within each variable. Correlation analysis showed a moderate positive correlation between Sev and Inc ($r=0.5170$) and Treatments and TKW ($r=0.6424$). Negative correlations were observed between Inc and TKW ($r=-0.5131$) and Sev and TKW ($r=-0.2482$), indicating potential trade-offs among these traits. These findings provide insights into the treatment effects and relationships between key agricultural variables.

Laboratory Results

Fungus detected and isolated from black-pointed seeds was identified as *Alternaria alternata* (Figure S15), exhibiting characteristic morphological features (Figure S16) (Woudenberg *et al*., 2013). The efficacy of fungicides against *A. alternata* was assessed using the agar well diffusion technique. Tilt (Propiconazole) displayed the highest efficacy with a 28 mm inhibition zone, followed by

Cymoxanil+Mancozeb with an 8 mm inhibition zone. Success, Ridomil, and Topsin M showed no significant activity against *A. alternata* under laboratory conditions. These results indicate varying effectiveness among the tested fungicides, with Tilt and Cymoxanil+Mancozeb demonstrating a positive lab-based inhibition of *A. alternata* growth as shown in Figures S17 and S18. From the serial dilution process, *Aspergillus niger, Aspergillus fumigatus*, and *A. alternata* were consistently isolated across all dilutions as shown in Figure S19. These three fungi have been identified as the causative agents of Black Point disease in wheat.

In-Silico Analysis Results

Sequence Retrieval

To identify proteins involved in the virulence of *A. alternaria*, a total of six proteins were searched and selected based on data from the Universal Protein Resource (UniProt) and the National Center for Biotechnology Information (NCBI) databases. To determine whether the structures of these proteins had been previously elucidated, a search was conducted in the Protein Data Bank (PDB). Only those proteins whose structures had not been experimentally determined were selected for further analysis, relying solely on the AlphaFold-predicted structures or not even predicted by AlphaFold. After validation, the FASTA sequences for each protein were obtained using UniProt. Among the six proteins involved in the virulence of *A. alternata*, AFAE1 (Accession no: A0A4Q4NLE1), AFAE2 (Accession no: A0A177DJG1), and AFAE3 (Accession no: A0A177DMH0) were selected for further investigation due to their functional relevance, as they feature the esterase-PHD domain. By hydrolyzing ester linkages between hemicelluloses and lignin, feruloyl esterases in particular are linked to the breakdown of plant cell walls, which is a crucial step in impairing plant defenses and promoting infection. These proteins are important targets for structural and functional research since it is thought that they contribute significantly to *A. alternata's* pathogenicity. (Table S11).

These three proteins were chosen based on a number of factors, such as their possible functional significance in *A. alternata*, their high correlation with virulence, and the presence of the esterase-PHD domain. Existing research and bioinformatic predictions that emphasized these particular proteins as important contributors to pathogenicity served as the impetus for the focus on them. A more thorough structural and functional examination is possible when the study is restricted to three proteins, guaranteeing correctness and viability. Other proteins with esterase domains may be the subject of future research, but this preliminary analysis lays the groundwork for examining the most promising possibilities first. In addition to offering important insights into the function of esterases in fungal virulence, this phased strategy guarantees that the study stays focused and manageable.

AFAE1 likely stands for *Alternaria* feruloyl esterase 1. AFAE1 can be broken down as follows: **A** stands for *Alternaria* (the genus of the fungus), **F** stands for *Feruloyl* (referring to feruloyl esterase activity), **A** stands for *Acid* (commonly linked to enzymatic function in biological nomenclature), **E** stands for *Esterase* (the enzyme class), and **1** indicates the number of protein or enzyme.

Analysis of Physiochemical Properties

Physiochemical properties of UniProt sequences were analyzed, focusing on molecular weight (kDa), amino acid composition, theoretical pI, and instability index. The instability index assesses protein stability; values <40 denote stability, while >40 suggest potential challenges in handling and expression, aiding researchers in anticipating stability issues. AFAE1 contained 324 amino acids, weighed approximately 35.20395 kDa, and had a theoretical pI of 4.67 and an instability index of 37.63, indicating potential instability. AFAE2 also comprised 324 amino acids, weighed about 35.27207 kDa, and had a theoretical pI of 4.82 and an instability index of 38.18, suggesting moderate instability. AFAE3 consisted of 330 amino acids, weighed approximately 35.59258 kDa, and had a theoretical pI of 6.70, indicating acidity. Its instability index of 43.40 suggested a higher propensity for instability under laboratory conditions. These findings provided insights for researchers to anticipate and address potential stability issues in experimental work. (Table S12)

Protein Domain Analysis

Functional domain analysis identified conserved protein domains crucial for understanding protein function and evolutionarily conserved sites. Protein domains contribute to stability and function as distinct functional or structural units within a protein. The Esterase_PHD domain, shared by AFAE1 and AFAE3, spanned residues 55–206, while for AFAE2, it spanned residues 55–209 (Figure 2). This domain in *A. alternata* facilitated the degradation of plant cell walls by targeting feruloyl-arabinose ester bonds in arabinoxylans, crucial polysaccharides found in wheat cells. The proteins' signal peptides aided in efficient trafficking within the cell, ensuring targeted deployment at specific cellular locations. A signal peptide is not considered a common domain. It is a short sequence that directs the protein to a specific cellular location, unlike domains, which are functional or structural units within a protein.

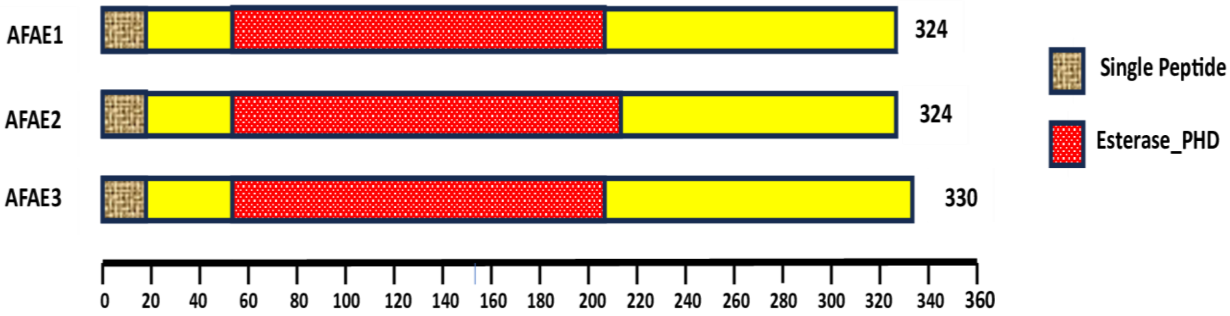

Figure 2. Diagram showing positions of Esterase PHD domain and signal peptide of three feruloyl esterase (AFAE1, AFAE2, AFAE3) proteins.

Protein Sequence Alignment

Protein sequence alignment via MUSCLE [version 3.8] was performed on Feruloyl esterase proteins to identify shared domains (Figure S21). However, the alignment results did not reveal common motifs, underscoring the diversity and complexity of Feruloyl esterase proteins. Despite this, the findings offer valuable insights for future research, particularly in understanding the role of Feruloyl esterase proteins from *A. alternata* in causing the black point of wheat. While shared domains were not observed, functional similarities or conserved regions may still exist. Further investigation using complementary approaches is warranted to explore potential functional relationships among these proteins.

Secondary Structure Prediction

The Protter was utilized to predict the secondary structures of the Esterase_PHD domains from three selected feruloyl esterase proteins. Protein secondary structure prediction (PSSP) seeks to develop a method that translates an amino acid sequence into its corresponding secondary structure, enabling the determination of the protein's secondary structure from its amino acid sequence as shown in Figure S22.

Visualization of Alphafold3 predicted Structures

The AlphaFold3 server was utilized to predict the structures of the Esterase_phd domains from three selected feruloyl esterase proteins. The predicted protein structures exhibited almost identical configurations and demonstrated generally high degrees of success. These structures were superimposed, resulting in a model with a high level of confidence and reliability. (Figure S23).

Verify 3D, Errat and ProCheck

The evaluation metrics for each protein based on its Protein ID, presented in Table S15, including the proportion of residues in the core region ("ProCheck"), the percentage of acceptable residues in the 3D structure ("Verify 3D"), and the percentage of prohibited residues ("ERRAT"). "AFAE1" achieved a quality factor of 99.3056%, with 88.0% of residues in the core region and only 0.8% disallowed residues. "AFAE2" scored 96.5753%, with 89.1% core residues and 0.8% disallowed residues. Despite generally high scores for all AlphaFold3 Esterase_phd models, "AFAE3" demonstrated lower scores of 67.11% and 90.9722%, indicating variability in model quality. This assessment offered insights into the precision and dependability of protein models across VERIFY 3D, ERRAT, and PROCHECK evaluations.

Ramachandran Plot

Ramachandran Plot analysis, as displayed in Table S16, showed the distribution of residues across different regions for each protein. For protein AFAE1, 88.0% of residues resided in the most favored region, followed by 11.2% in the additional allowed region, and 0.8% in the disallowed region. AFAE2 showed 89.1% of residues in the most preferred region, 10.2% in the additional allowed region, and 0.8% in the disallowed region. AFAE3 exhibited even higher percentages, with 88.9% in the most favored region, 9.5% in the additional allowed region, and 0.8% in both generously allowed and disallowed regions. These investigations offered standards for assessing the Ramachandran distributions of AlphaFold 3D models of *A. alternata* proteins.

Protein to Protein Interactions

Significant connections involving the esterase domain of *A. alternata's* Feruloyl esterase proteins were discovered through the identification of protein interaction partners using the STRING webserver. The significance of protein-protein interactions in cellular processes was highlighted by the Esterase_PHD domain's notable strong connections with both AA0117_g1633 and with itself. This implied a functional link with AA0117_g1633 and possible oligomerization or dimerization events that are essential for function (Figure S24 and S25). Deciphering intricate biological processes and disease mechanisms required an understanding of protein-protein interactions. These discoveries clarify the functions of proteins in networks and provide information for the creation of treatments that target these relationships.

Ligand Binding

The ChEMBL database, which provides bioactive compounds with drug-like qualities, was used to identify a potential molecule for binding to certain proteins. The Yang Cao Laboratory's web-based program CB-Dock2, which offers a sophisticated tool for protein-ligand blind docking, was utilized to help with ligand binding. Various fungicides were tested as ligands for docking with the Esterase_PHD of *A. alternata*. Propiconazole (Tilt) was found to disrupt ergosterol biosynthesis, potentially affecting ester bond hydrolysis. Chlorothalonil (Cymoxanil) interfered with multiple fungal processes, potentially hindering esterase function. Additionally, Cymoxanil inhibited enzyme activity by targeting cytochrome C oxidase. These interactions were visually represented, offering insights into their mechanisms within the Esterase_phd domain (Table S17).

Docking and MD Simulations

Molecular Docking Identifies Metalaxyl and Pyraclostrobin as Top Candidates

Pyraclostrobin–AFAE3 (–6.6 kcal/mol) , thiophanate-methyl_AFAE2 (–5.7 kcal/mol) and propiconazole_ AFAE1 (-5.6 kcal/mol) exhibited the most favorable binding characteristics among the simulated complexes, with AutoDock Vina-based docking scores ranging from –6.6 to –4.4

kcal/mol (Table. 1). These values suggest strong potential for stable, energetically favorable binding across the conserved catalytic sites.

Molecular Dynamics Confirms Binding Stability for Metalaxyl and Cymoxanil Complexes

For every chosen ligand–protein complex, 5 ns all-atom MD simulations were run to confirm the stability of docked postures in a dynamic environment. The findings showed significant variations in complicated behaviour after docking.

RMSD (Root Mean Square Deviation)

The best positional stability of the ligand at the binding site over time was indicated by the lowest RMSD values for Metalaxyl–AFAE2 (0.13 nm), Pyraclostrobin–AFAE3 (0.14 nm), and Cymoxanil–AFAE2 (0.14 nm) (shown in Figure 6A). Although not one of the absolute lowest, propiconazole–AFAE1 maintained an acceptable RMSD (~0.17 nm), indicating moderate positional stability.

Radius of Gyration (Rg)

The Rg values of all complexes remained between 1.56 and 1.60 nm, suggesting that the protein was generally stable and not unfolding. Comparable Rg to other ligands was displayed by propiconazole-containing complexes, indicating the receptor's structural integrity throughout simulation (Figure 6C).

RMSF (Root Mean Square Fluctuation)

All systems displayed minimal residue flexibility (average RMSF < 0.1 nm). Interestingly, the binding site residues in Metalaxyl–AFAE2 and Cymoxanil–AFAE2 were particularly rigid, indicating that the ligands had stabilising effects. In catalytic regions, propiconazole–AFAE2 also showed minimal RMSF, which is consistent with its capacity to sustain close connections (Figure 6B)).

SASA (Solvent Accessible Surface Area)

Among the SASA values, which varied from 81.62 to 93.23 nmÂ² , cymoxanil–AFAE3 had the lowest value, signifying profound burial. The moderate SASA of propiconazole–AFAE2 (88.16 nmÂ²) indicates partial but consistent pocket accommodation (Figure 6E).

Hydrogen Bonding

The only complex that consistently formed hydrogen bonds during simulation was cycloxanil–AFAE2 (mean = 0.12). Despite having a high in vitro efficacy, propiconazole–AFAE2 did not show substantial hydrogen bonding, suggesting that hydrophobic or shape-complementary binding rather than polar interactions may be the primary governing factors in their interaction (Figure 6D).

While Pyraclostrobin and Metalaxyl depend primarily on nonpolar and steric interactions for stable complex formation, Cymoxanil seems to use particular polar connections. Notably, propiconazole has a hybrid mode that is consistent with its potent experimental antifungal action, producing some hydrogen bonds while preserving favourable burial and RMSD.

Table 1. Docking Affinities, Structural Stability (RMSD, Rg), Hydrogen Bonding, and Solvent Accessibility (SASA) of Selected Ligand–AFAE1–3 Complexes Based on 5ns MD Simulations.

Ligand_Protein	Docking_Affinity (kcal/mol)	RMSD_Mean (nm)	Rg_Mean (nm)	Hbond_Avg	SASA_Avg (nmÂ²)
pyraclostrobin_e3	-6.6	0.147	1.566	0	83.58
pyraclostrobin_e1	-6.4	0.209	1.603	0.01	93.23
thiophanate-methyl_e2	-5.7	0.165	1.584	0.39	92.4

propiconazole_e1	-5.6	0.178	1.572	0.26	88.16
metalaxyl_e2	-5.4	0.135	1.564	0	90.54
cymoxanil_e2	-5.1	0.148	1.576	0.12	90.9
metalaxyl_e3	-5	0.157	1.583	0	85.78
cymoxanil_e1	-4.6	0.151	1.581	0	88.95
cymoxanil_e3	-4.5	0.122	1.569	1.05	81.62
chlorothalonil_e1	-4.4	0.17	1.569	0	90.54

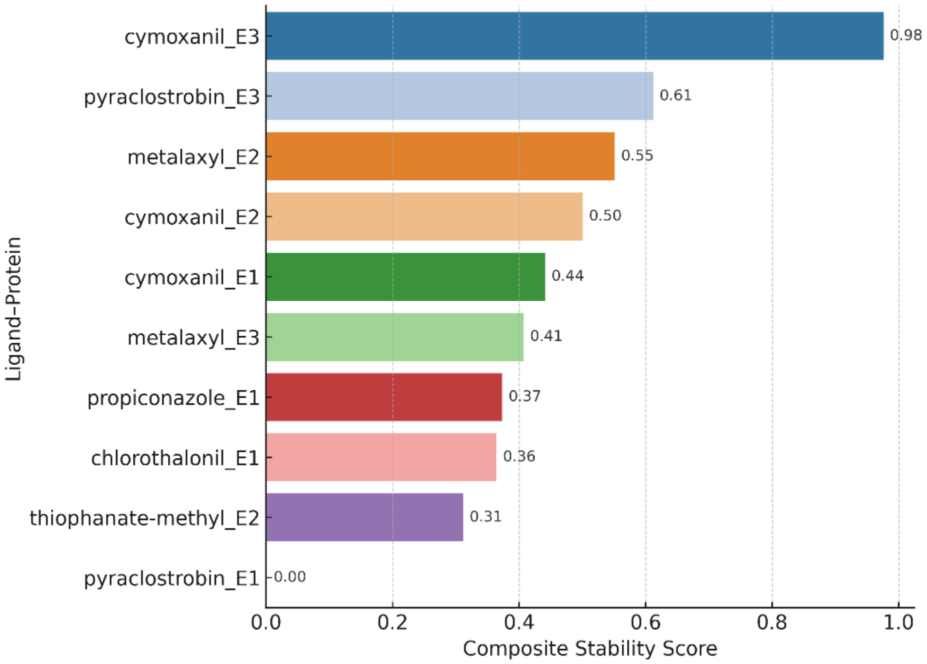

Figure 3. Composite Stability Ranking of Ligand–AFAE Complexes.

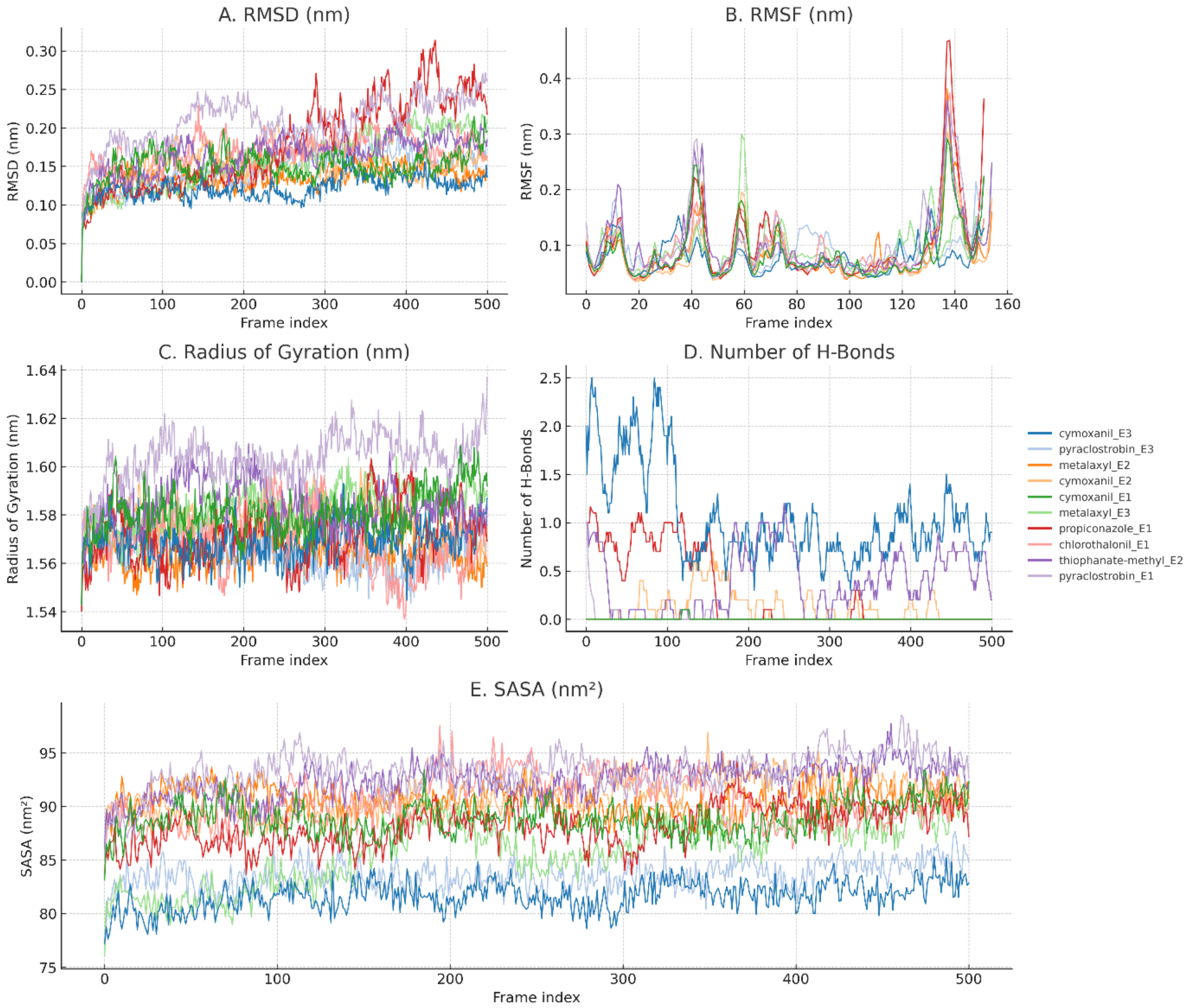

Figure 6. Molecular Dynamics Simulation Metrics for Ligand–AFAE Complexes. **(A)** Root Mean Square Deviation (RMSD) trajectories of ligand–protein complexes over 5 ns show overall positional stability, particularly for *metalaxyl_E2*, *cymoxanil_E2*, and *pyraclostrobin_E3*. **(B)** Root Mean Square Fluctuation (RMSF) profiles demonstrate local flexibility of protein residues, with most complexes showing limited fluctuations (<0.15 nm). **(C)** Radius of Gyration (Rg) indicates compactness of the protein-ligand complex, with consistent values (~1.56–1.60 nm) across all systems. **(D)** Number of Hydrogen Bonds between ligand and protein throughout the trajectory. Notably, *cymoxanil_E3* and *thiophanate-methyl_E2* formed more stable hydrogen bonds compared to other ligands. **(E)** Solvent Accessible Surface Area (SASA) shows burial trends of ligands in the binding pocket; lower SASA values suggest deeper binding and reduced solvent exposure.

Machine Learning & xAI: Performance of Pesticides Under Various Climate Conditions

Effect of Individual Weather Parameters on Pesticide Performance

This study highlighted the effects of individual parameters and their combined impact on pesticide performance. The analysis examined the interactions between individual weather parameters (temperature, humidity, and precipitation) and various pesticides in controlling Black Point disease. Using averaged SHAP interaction values from 1,000 Monte Carlo simulations, the examination offered solid insights into the separate effects of each climate variable on pesticide efficacy. The various impacts of meteorological conditions on pesticide performance are reported in Table S19.

Temperature and Fungicides Combined Efficacy

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The findings shed important light on how temperature affects the effectiveness of pesticides. Propiconazole, Metribuzine, and Propoxycarbazone are examples of extremely temperature-dependent pesticides that were found to significantly improve their potency under warm settings, making them ideal for usage in hotter climates or throughout the summer. Thiophanate-methyl, on the other hand, worked best in colder temperatures, indicating that it is appropriate for early-season use or temperate regions. Pesticides that are widely adjustable, such as Metalaxyl-M + Mancozeb, Sulfosulfuron, and Chlorothalonil + Metalaxyl-M, showed steady performance throughout a wide range of temperatures, giving them flexible choices for areas with erratic or fluctuating climates. Indicating broad performance without particular optimization for extreme temperature circumstances, moderate performers like Dichlorodiphenyltrichloroethane and Cymoxanil + Mancozeb showed consistent yet moderate efficacy over the temperature spectrum (Figure S26). Black Point disease is more effectively managed by coordinating pesticide use with climate, improving crop rotation, reduce resource waste. To maintain the sustainability of agriculture in the face of shifting environmental conditions, these results highlighted that climate-smart pest management techniques are crucial.

Humidity and Fungicides Combined Efficacy

Because high moisture levels foster the growth of fungal pathogens, targeted treatments of fungicides are essential in high-humidity environments, which boosts their efficacy. On the other hand, fungicide efficacy decreases under low humidity, which is indicative of decreased disease pressure and decreased pathogen activity.

Based on SHAP interaction values averaged over 1,000 Monte Carlo simulations, Figure S27 shows how different pesticides and humidity conditions interact to suppress black point disease. Propiconazole and Cymoxanil + Mancozeb demonstrated robust positive responses under high humidity circumstances, according to the analysis, which makes them very useful in tropical areas or during rainy seasons. Metribuzine, Propoxycarbazone, and Thiophanate-methyl, on the other hand, worked best at moderate humidity levels, which makes them appropriate for temperate climates with consistent moisture levels. Broadly versatile pesticides, such as Chlorothalonil + Metalaxyl-M, Metalaxyl-M + Mancozeb, and Sulfosulfuron, showed consistent efficacy at all humidity levels, making them dependable choices for areas with erratic or changing moisture conditions. Dichlorodiphenyltrichloroethane performed steadily and moderately in a variety of settings; it was not significantly optimized for extremely dry or wet climates. Overall, the results highlighted how crucial it is to adjust pesticide applications to local humidity levels in order to maximize crop yields, minimize resource waste, and control pests. The need of climate-smart pest management techniques for sustainable agricultural production was emphasized by this study.

Precipitation and Fungicide Efficacy

Higher fungicide utility is correlated with increased precipitation. In order to reduce the severity of the disease, timely fungicide applications are necessary because wet circumstances create an ideal setting for fungal growth. However, the disease's decreased frequency in arid environments reduces the requirement for fungicides.

Based on SHAP interaction values averaged over 1,000 Monte Carlo simulations, Figure S28 shows how different pesticides and precipitation levels interact to affect the incidence of Black Point Disease. Propiconazole and Cymoxanil + Mancozeb showed the most effectiveness under high rainfall, according to the analysis, which makes them perfect for tropical or subtropical areas with a lot of precipitation. Propoxycarbazone, Metribuzine, and Thiophanate-methyl, on the other hand, worked well in areas with moderate rainfall, which makes them ideal for temperate or semi-arid climates with consistent precipitation. Pesticides that are widely adjustable, such as Metalaxyl-M + Mancozeb, Sulfosulfuron, and Chlorothalonil + Metalaxyl-M, showed dependable performance at all precipitation levels, guaranteeing dependability in regions with erratic or changeable rainfall. Despite its lack of optimization for harsh conditions, Dichlorodiphenyltrichloroethane demonstrated stable but moderate performance throughout a range of precipitation levels. Climate-smart pest control tactics can be implemented by coordinating pesticide treatments with regional rainfall patterns, which can enhance crop yields, improve disease management, and encourage sustainable farming practices.

Pairwise Interactions Between Climate Variables and Pesticides

In controlling black point disease, important information about the effectiveness of pesticides was uncovered by examining the pairwise interactions between humidity, temperature, and precipitation. With their great dependence on high humidity and precipitation, Propiconazole, Metalaxyl-M + Mancozeb, and Cymoxanil + Mancozeb were the most effective in wet and humid conditions, making them perfect for tropical or subtropical regions. Sulfosulfuron and Chlorothalonil + Metalaxyl-M showed steady performance across a range of climates, demonstrating their flexibility in response to changing weather patterns. Propoxycarbazone and Metribuzine showed their most effectiveness in balanced conditions with moderate temperature, humidity, and precipitation, whereas Thiophanate-methyl and Sulfosulfuron functioned consistently in semi-arid areas with moderate precipitation and reduced humidity. These results underlined how crucial it is to match pesticide application methods to local climate circumstances to maximize crop protection, minimize waste, and improve sustainability. It was advised to prioritize moisture-dependent pesticides like Propiconazole or Cymoxanil + Mancozeb in wet conditions, while Thiophanate-methyl or Sulfosulfuron produced more dependable outcomes in dry areas. To increase agricultural productivity, this study laid the groundwork for climate-smart pest management techniques (Figure 4).

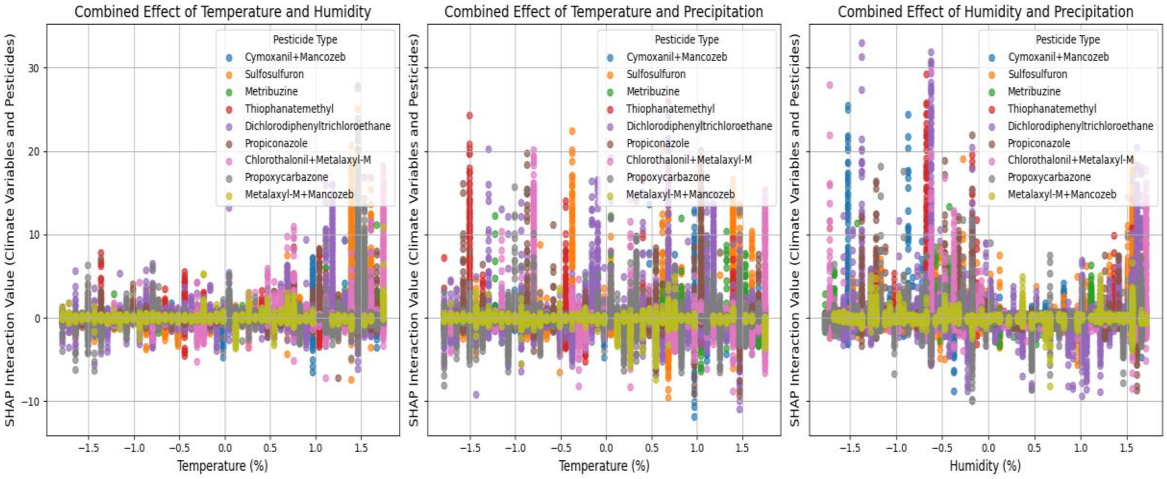

Figure 4. The pairwise interaction of Temperature, humidity, and precipitation with pesticides.

Triple Interaction Analysis of Climate Variables on Pesticides Efficacy

Different patterns in the effectiveness of pesticides in treating black point disease were found by analyzing the triple interaction of temperature, humidity, and precipitation. Metalaxyl-M + Mancozeb is perfect for rainy areas because it showed maximum effectiveness in conditions of moderate temperature, high humidity, and regular precipitation. Propoxycarbazone was less effective in harsh environments but did well in areas with steady temperatures and moderate rainfall. Sulfosulfuron and Chlorothalonil + Metalaxyl-M showed extensive adaptability, delivering constant performance in a range of conditions, making them dependable for erratic weather patterns. Propiconazole and Cymoxanil + Mancozeb flourished in wet and humid settings but were less effective in dry ones, whereas metribuzine worked best in semi-arid areas with mild temperatures and balanced moisture levels. In semi-arid regions with moderate precipitation and reduced humidity, thiophanate-methyl proved to be dependable. Dichlorodiphenyltrichloroethane, on the other hand, had an uneven performance in extreme weather conditions and performed better in stable, moderate temperatures, necessitating cautious administration. These results highlight how crucial it is to match pesticide use to local climate conditions in order to improve sustainability and efficacy.

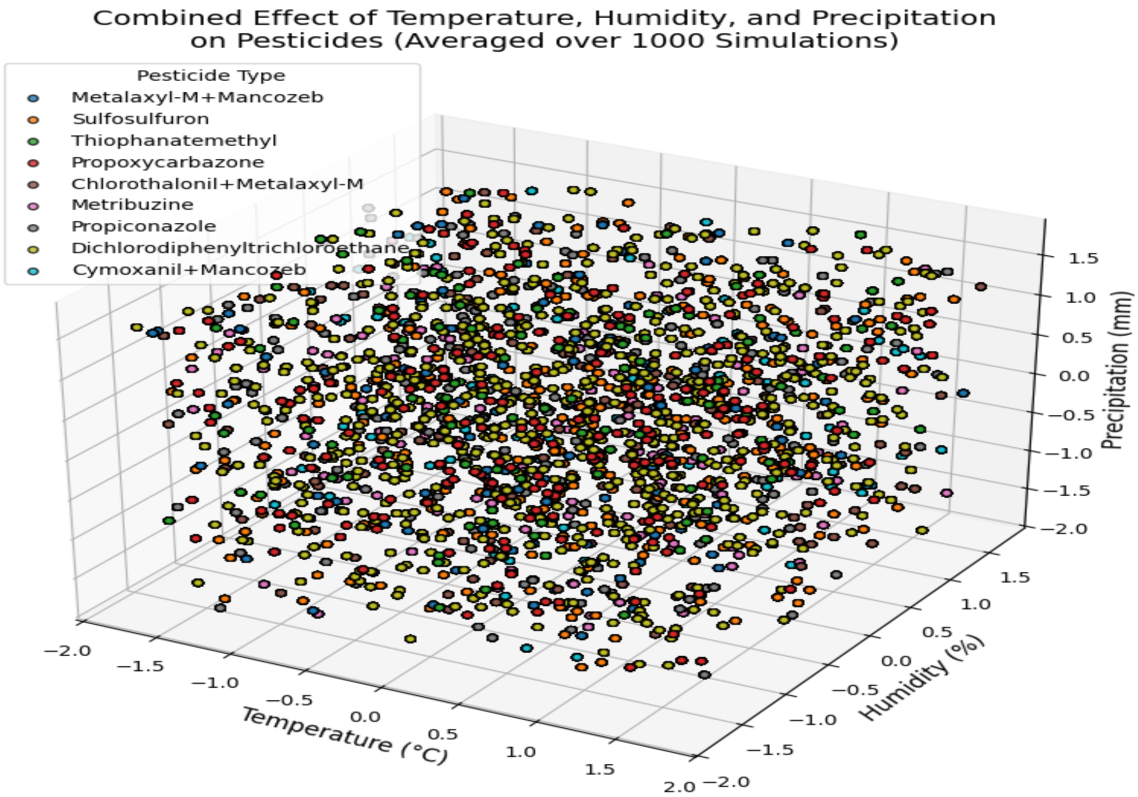

Figure 5. Pesticides combined impact on precipitation, temperature, and humidity.

Discussion

Black point is an economically significant disease of wheat prevalent in wheat-growing regions worldwide. It causes both qualitative and quantitative losses. In sensitive cultivars, disease-related grain losses range from 24 to 27% (El-Gremi *et al*., 2017). This study tested the efficacy of various systemic fungicides in two trials (a fungicides trial and a Tilt fungicide dose trial) conducted at NIFA, Peshawar, using the common variety "Morocco." Parameters such as disease incidence, disease severity index, thousand kernel weight (TKW), and the pathogens responsible for causing the disease were examined. The efficacy of field-used chemical fungicides, including Tilt (Propiconazole, foliar), Success, Ridomil, Topsin M, and Cymoxanil+Mancozeb, against *A. alternata* was assessed using the agar well diffusion technique. Additionally, the study aimed to identify and model the virulent proteins of *A. alternata*, for which the 3D structures have not yet been solved or predicted. The study also analyzed the individual and combined influence of temperature, humidity, and precipitation on pesticide efficacy against black point disease using SHAP interaction values and Monte Carlo simulations.

In this study, the efficacy of five selected fungicides was evaluated against black point disease. Tilt (5.7%) was the most effective fungicide, resulting in the lowest disease incidence compared to the control (8.6%), followed by Cymoxanil+Mancozeb (6.0%), Success (6.3%), Topsin-M (6.5%), and Ridomil (7.0%). Different doses of Tilt fungicide also reduced disease incidence compared to the control. In the dose trial, the most effective dose was dose 4 (5.7%), followed by dose 1 (6%), dose 2 (6.3%), dose 5 (6.6%), and dose 3 (7.4%). At 9.1%, the illness incidence was highest in the control group. Tilt successfully manages black point infection, according to other researchers. According to

Malaker *et al*., (2009), black point incidents were successfully reduced by foliar spraying Tilt-250EC (Propiconazole) on different schedules (Malaker *et al*., 2009). According to Iqbal *et al*., (2014), Tilt (propiconazole @ 500 ml/ha) was the most effective against black point disease and yielded an outstanding economic return of Rs. 13200/ha (Iqbal *et al*., 2014). Other authors also examined the effectiveness of six fungicides in 3 concentrations (100, 300, and 500 ppm) against *A. alternata* on PDA in vitro. The mycelial growth of *A. alternata* was reduced with propiconazole (94, 100, and 100%), followed by trifloxystrobin+tebuconazole (75, 90.11, and 95.88%). The mycelial growth was less affected by fungicides such as hexaconazole (70, 76.33, and 85.44%), carbendazim + mancozeb (64.25, 69.15, and 80.10%), azoxystrobin (57.00, 62.33, and 70.00%), and mancozeb (55.70, 59, and 65.66%). Then the most effective fungicides such as propiconazole and trifloxystrobin + tebuconazole in vitro were also studied as foliar sprays against *A. alternata* in microplots. Both fungicides considerably outperformed controls in terms of lowering disease intensity at 60 and 90 days of transplantation (DAT). At 60 and 90 DAT, trifloxystrobin + tebuconazole (7.80 and 27.90%) and propiconazole (5.00 and 23.00%) exhibited the lowest disease intensity (Yadav *et al*., 2020). Propiconazole fungicide is essential for resistance control measures. Farmers can mix or rotate fungicides with different modes of action to prevent the development of resistance. Because of its unique mode of action, propiconazole is essential for integrated pest management (IPM) programs. Environmentally, Propiconazole has a favorable profile when used correctly. Adhering to recommended rates and intervals minimizes environmental impact and health risks. However, efficacy may vary based on timing, conditions, and targeted pathogens. Following local regulations and label instructions is imperative for all agricultural chemicals (Box, 2015).

In this study, the severity index for both trials was also evaluated. Cymoxanil+Mancozeb had the lowest severity index (2.7%), followed by Tilt (3%), Success (3.1%), and Ridomil (3.3%). The highest severity index was observed for Topsin-M (4.7%). The control group exhibited a severity index of 4.1%. The severity index for kernel black point disease in the Tilt fungicide trial varied across different doses of the fungicide. Dose 4 had the lowest severity index (3.3%), followed by dose 2 (3.6%), dose 3 (3.8%), dose 5 (4.1%), and dose 1 (4.2%). The control group had the highest severity index of 4.4%. Cromey and Mulholland (1988) described the black point severity index, noting significant variation in black point infection levels in grain samples (Cromey and Mulholland, 1988). In another study, Draz *et al*., (2016) examined the black point disease severity index over two consecutive years, finding that the illness index ranged from 0.29 percent to 19.48 percent on average across both seasons (Draz *et al*., 2016). Black point disease severity varied widely between seed samples. Wheat cultivars with the lowest black point disease incidence and severity index may be valuable as sources of black point resistance germplasm. According to Srivastava *et al*., (2015), relatively higher temperatures, high relative humidity, and intermittent rains during anthesis promote the manifestation of black point symptoms. It was also noted that delayed watering (irrigation) combined with high wind velocity increased the severity of black point illness (Srivastava *et al*., 2015). The infection of winter wheat seeds by black point varies depending on the year of research and the variety.

For both fungicide experiments in this investigation, the weight of a thousand kernels was measured. TKW affects germination, seed vigor, seedling establishment, and yield, making it a crucial indication of seed quality. At 35.5 g, tilt (Propiconazole) had the greatest TKW, followed by Ridomil (29.7 g), Cymoxanil + Mancozeb (34 g), Topsin-M (30.9 g), and Succes (30.6 g). Compared to the control group, which had the lowest TKW (25.9 g), samples with the highest TKW showed a decreased incidence of black point illness. According to Shahwani *et al*., (2014), the most promising agronomic performance was shown by wheat with big seeds (Shahwani *et al*., 2014). Muhsin *et al*., (2021) found that bold seeds yielded the highest TKW (36.97 g) and the most grains per spike (45.17), whereas medium seeds produced the lowest TKW (32.03 g) and the fewest grains per spike (34). Bold seeds produce the greatest growth and increase yield as compared to other seed size classes. Therefore, wheat yield can be increased even under late-sown conditions because of their bold seed size, which enhances yield-related features and encourages vigorous germination (Muhsin *et al*., 2021). Another study found no significant difference between TKW and germination percent, germination rate, or mean germination time using a standard germination test. Nonetheless, germination percent and germination rate grew in conjunction with TGW, with the highest TGW being linked to the highest germination rate and percent. According to Moshatati and Gharineh (2012), the treatments with the highest and lowest TKW had the largest and lowest mean germination times, respectively (Moshatati & Gharineh, 2012). In conclusion, bold seeds significantly enhanced growth and yield as compared to other seed size classes. In late-planted situations, bold seed size can increase wheat production because of its strong and quick germination and improved yield-related characteristics. Before offering suggestions to the farming community, more research is necessary on different soil types and agroecosystems.

From the black-pointed diseased seeds, the fungus was isolated in the study. The frequently isolated fungus from all the disease seeds was *A. alternata*. Draz *et al*., (2016) and El-Gremi *et al*., (2017) conducted a study in which *A. alternata*, *Cochliobolus sativus* (*Helminthosporium sativum*), and *Fusarium graminearum* were commonly isolated from diseased seeds (El-Gremi *et al*., 2017; Draz *et al*., 2016). Cotuna *et al*., (2020) described that *A. alternata* is a common causal agent of wheat kernels infected by black point disease (Cotuna *et al*., 2020). In a study conducted by Draz *et al*., (2016), *A. alternata* isolated from black-pointed wheat grains was responsible for the occurrence of leaf spots, reduced grain germination, limited seedling growth, and finally losses in yield quantity and quality (Draz *et al*., 2016). Numerous bacteria are linked with the discoloration of wheat grains which destroys the appearance of the final product (Fernandez & Conner, 2011). However, the prevalence and incidence of these pathogens differ. The disease's prevalence also affects how much rain falls during seed development or early leaf senescence, which encourages black point. The pathogen's capacity to infect wheat types and raise the incidence and prevalence of black point disease is facilitated by increased precipitation and the frequency of wet days. The growth of black point or kernel smudge is highly weather-dependent. There is limited control over this issue, particularly in regions that receive significant rainfall (Ijaz *et al*., 2018). SESİZ (2023) also stated that current temperature, precipitation, and humidity, particularly during grain-filling periods, create an ideal environment for the development of black point components, which could be caused by fungal pathogens or oxidation reactions (SESİZ, 2023).

The five fungicides—Tilt (Propiconazole), Success, Ridomil, Topsin M, and Cymoxanil+Mancozeb—used in the field were also utilized in the laboratory to assess their efficacy against *A. alternata* using the agar well diffusion technique. Among these fungicides, tilt (Propiconazole) demonstrated the highest efficacy in inhibiting the growth of *A. alternata*, displaying a zone of inhibition measuring 28 mm. Cymoxanil+Mancozeb showed an 8 mm inhibitory zone after tilt. However, in lab settings, Success, Ridomil, and Topsin M—the other fungicides—did not show any discernible efficacy against *A. alternata*. This means that the fungicides had different degrees of efficacy, with propiconazole showing the highest reduction in the growth of *A. alternata* in the laboratory. Achilonu *et al*., (2023), studied the effect of six fungicides on the mycelial development of isolates of *A. alternata* derived from Alternaria Black Spot symptoms in vitro. Three different concentrations (0.2, 1, and 5 μg mL−1) of fungicides were tested, including Boscalid + Pyraclostrobin, Fentin Hydroxide, Azoxystrobin, and Propiconazole. Notably, Tilt and Bumper 250 EC, which has propiconazole as an active ingredient and acts as a demethylation inhibitor, showed the best performance, completely stopping mycelial growth for as long as six days after incubation (Achilonu *et al*., 2023). Dasharathbhai Ajayabhai *et al*., (2018) assessed nine fungicides and five bioagents against *A. alternata* (Fr.) Keissler causing leaf blight disease, at three concentrations (250, 500, and 1000 ppm). These included Carbendazim 50 WP, Hexaconazole 5 EC, , Chlorothalonil 75 WP, Propiconazole 25 EC, Benomil 50 WP, Difenconazole 20 + Azoxystrobulin 12.5 EC, Mancozeb 75 WP, Tebuconazole 25.9 EC, and Carbendazim 12% + Mancozeb 63% 75 WP. The fungal growth was completely inhibited by Propiconazole and Hexaconazole (250 ppm) at all three concentrations (Dasharathbhai Ajayabhai *et al*., 2018). Based on these investigations, *A. alternata* was best controlled by propiconazole as compared to other fungicides. Propiconazole had a high capacity to prevent the growth of *A. alternata*.

Propiconazole is more efficient in the treatment of *A. alternata* infections which emphasizes its applicability in agricultural settings.

Ipyrobin (Iprodione + Pyraclostrobin), is a combination fungicide, which is used to manage a variety of fungal diseases in crops in agriculture. In lab work studies, this fungicide was used as a positive control to compare its effectiveness with field-applied fungicides. It showed the ability to limit the *A. alternata's* growth. However, we suggested propiconazole because it is less harmful to human health. Further *in silico* analysis was performed for Propiconazole, due to its lower toxicity to humans. Propiconazole often shows a reduced toxicity when compared to ipyrobin. Propiconazole is categorized as moderately dangerous when taken orally and slightly toxic when used topically but ipyrobin may have higher toxicity levels depending on the precise formulation and concentration (Wang *et al*., 2018). Propiconazole generally reduces environmental persistence by controlling the risk of environmental buildup and exposure to fungal disease which may result in a quicker breakdown in soil and water.

This study combines Explainable AI (XAI) techniques, notably SHAP (SHapley Additive exPlanations), with an XGBoost (Extreme Gradient Boosting) machine learning model to provide interpretable insights into the effect of climatic variables on the efficacy of fungicides. The lab-based inhibitory zone data and simulated climate conditions were used and the model evaluated the individual and combined effects of precipitation, temperature, and humidity on fungicide performance. The impact of these factors and their combinations on pesticide effectiveness was brought to light by the SHAP analysis (Prendin *et al*., 2023). The results of this study showed that different fungicides performed differently in a range of meteorological circumstances, such as temperature, humidity, and precipitation. Propiconazole and Cymoxanil + Mancozeb, two moisture-dependent fungicides, showed their best results in areas with high precipitation and humidity. While Thiophanate-methyl proved more effective in semi-arid settings with moderate rainfall and lower humidity, region-specific fungicides such as Metribuzine worked best at slightly warmer temperatures and moderate humidity. Adaptable fungicides, such as Sulfosulfuron and Chlorothalonil + Metalaxyl-M, on the other hand, demonstrated steady performance over a broad range of temperatures, humidity levels, and precipitation circumstances, making them appropriate for regions with erratic weather patterns. These results emphasize that while designing fungicide treatment techniques for efficient black point disease control, particular meteorological conditions, such as temperature, humidity, and precipitation, must be taken into account. Extreme weather patterns such as increasing temperatures, fluctuating precipitation, and changes in humidity provide serious obstacles to sustainable agriculture as climate variability increases (Kharin *et al*., 2013). According to research, a 1°C rise in temperature can result in a 10–20% drop in agricultural yield worldwide (Barlow *et al*., 2015). These temperature changes have a major effect on yield, grain weight, and grain size, especially during crucial phases of wheat growth including blooming, anthesis, and the milking stage (Talukder *et al*., 2014). For example, it has been demonstrated that dry climates in northern Europe can interfere with flowering timing, resulting in significant output losses (Bodner *et al*., 2015). The necessity for dynamic, climate-responsive solutions to sustain fungicide efficacy and agricultural output is highlighted by the rising frequency of extreme climate events. Globally more robust and sustainable wheat production systems can be ensured by incorporating climate data into disease management frameworks to help reduce yield losses brought on by climate-induced variability.

Conclusions

It is concluded from this study that propiconazole was the most effective fungicide in both field trials and lab work and showed the highest efficacy in the field resulting in lower disease incidence and increased yield. It also proved effective in the laboratory against *A. alternata*, showing significant growth inhibition. Numerous *A. alternata* proteins implicated in virulence against the host were identified by computational research. To better understand the methods used by *A. alternata* to tolerate even advanced-generation fungicides, it is still necessary to conduct experiments to clarify the structure of these proteins. Additionally, the study found that three targeted proteins' Esterase_PHD domain is essential to *A. alternata's* ability to survive inside the host cell. By changing the esterase_PHD domain via active ligand binding, *A. alternata* infections may be possibly controlled. Propiconazole resulted in the highest vina scores by interacting with specific amino acid residues of targeted proteins at the C1 pocket.

Climate variables like as temperature, humidity, and precipitation all have a substantial impact on fungicide effectiveness in the management of Black Point Disease. Moisture-dependent fungicides, such as Propiconazole and Cymoxanil + Mancozeb, performed best in areas with high humidity and frequent rainfall. Region-specific fungicides, such as Metribuzine, worked best in moderate temperature and humidity environments, but Thiophanate-methyl proved more effective in semi-arid regions with moderate rainfall and lower humidity. Adaptable fungicides like Chlorothalonil + Metalaxyl-M and Sulfosulfuron have shown consistent effectiveness over a wide range of climatic circumstances, making them ideal for places with changing weather. These findings emphasize the need of taking into account local meteorological parameters when selecting fungicides to maximize disease control and agricultural operations.

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References

1. Abade, A., Ferreira, P. A., & de Barros Vidal, F. (2021). Plant diseases recognition on images using convolutional neural networks: A systematic review. *Computers and Electronics in Agriculture*, *185*, 106125.
2. Achilonu, C. C., Gryzenhout, M., Ghosh, S., & Marais, G. J. (2023). In Vitro Evaluation of Azoxystrobin, Boscalid, Fentin-Hydroxide, Propiconazole, Pyraclostrobin Fungicides against Alternaria alternata Pathogen Isolated from Carya illinoinensis in South Africa. *Microorganisms*, 11(7).
3. Alastruey-Izquierdo, A., Melhem, M. S., Bonfietti, L. X., & Rodriguez-Tudela, J. L. (2015). Susceptibility test for fungi: clinical and laboratorial correlations in medical mycology. *Revista do Instituto de Medicina Tropical de São Paulo*, 57, 57-64.
4. Ali, Y., Abbas, T., Aatif, H. M., Ahmad, S., Khan, A. A., & Hanif, C. M. S. (2022). Impact of Foliar Applications of Different Fungicides on Wheat Stripe Rust Epidemics and Grain Yield. *Pakistan Journal of Phytopathology*, 34(1), 135–141.
5. Arnal Barbedo, J. G. (2013). Digital image processing techniques for detecting, quantifying and classifying plant diseases. *SpringerPlus*, 2(1), 660.
6. Asif, M., Strydhorst, S., Strelkov, S. E., Terry, A., Harding, M. W., Feng, J., & Yang, R. C. (2021). Evaluation of disease, yield and economics associated with fungicide timing in Canadian Western Red Spring wheat. *Canadian Journal of Plant Science*, 101(5), 680–697.
7. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, 6(2), 71–79.
8. Binkowski, T. A. (2003). CASTp: Computed Atlas of Surface Topography of proteins. *Nucleic Acids Research*, 31(13), 3352–3355.
9. Barlow, K. M., Christy, B. P., O'Leary, G. J., Riffkin, P. A., & Nuttall, J. G. (2015). Simulating the impact of extreme heat and frost events on wheat crop production: A review. *Field crops research*, *171*, 109-119.
10. Box, P. O. (2015). States Environmental Protection Agency. Toxics Release Inventory (TRI) National Analysis, 703.

11. Brown, P., Baxter, L., Hickman, R., Beynon, J., Moore, J. D., & Ott, S. (2013). MEME-LaB: motif analysis in clusters. *Bioinformatics*, 29(13), 1696–1697.
12. Chi, D. H., Giap, V. D., Anh, L. P. H., & Nghi, D. H. (2017). Feruloyl esterase from Alternaria tenuissima that hydrolyses lignocellulosic material to release hydroxycinnamic acids. *Applied Biochemistry and Microbiology*, 53(6), 654–660.
13. COTUNA, O., PARASCHIVU, M., SĂRĂȚEANU, V., DURĂU, C., & RECHIȚEAN, I. (2020). Research Regarding the Identification of the Fungus"Black Point" in Several Wheat Varieties Cultivated in Western Romania (Case Study). *Life Science and Sustainable Development*, 1(2), 25–31.
14. Crepin, V. F., Faulds, C. B., & Connerton, I. F. (2004). Functional classification of the microbial feruloyl esterases. *Applied Microbiology and Biotechnology*, 63(6), 647–652.
15. Cromey, M. G., & Mulholland, R. I. (1988). Blackpoint of wheat: Fungal associations, cultivar susceptibility, and effects on grain weight and germination. *New Zealand Journal of Agricultural Research*, 31(1), 51–56.
16. Dasharathbhai Ajayabhai, C., Kedar Nath, Bekriwala, T., & Mabhu Bala. (2018). Management of Alternaria leaf blight of groundnut caused by Alternaria alternata. *Indian Phytopathology*, 71(4), 543–548.
17. Degewione, A., & Alamerew, S. (2013). Genetic Diversity in Bread Wheat (Triticum aestivum L.) Genotypes. *Pakistan Journal of Biological Sciences*, 16(21), 1330–1335.
18. DeLano, W., & Bromberg, S. (2004). PyMOL User's Guide (Original). *DeLano Scientific LLC*, 1–66.
19. Draz, I. S., El-Gremi, S. M., & Wassief Abd-Elsamad Youssef. (2016). Pathogens associated with wheat black-point disease and responsibility in pathogenesis. *Journal of Environmental and Agricultural Sciences*, 8(September 2016), 71–78.
20. Draz, I. S., El-Gremi, S. M., & Youssef, W. A. (2016). Response of Egyptian wheat cultivars to kernel black point disease alongside grain yield. *Pakistan Journal of Phytopathology*, 28(1 PG-15–17), 15–17.
21. Edgar, R. C., & Batzoglou, S. (2006). Multiple sequence alignment. *Current Opinion in Structural Biology*, 16(3), 368–373.
22. Eisenberg, D., Lüthy, R., & Bowie, J. U. (1997). [20] VERIFY3D: Assessment of protein models with three-dimensional profiles (pp. 396–404).
23. El-Gremi, S. M., Draz, I. S., & Youssef, W. A.-E. (2017). Biological control of pathogens associated with kernel black point disease of wheat. *Crop Protection*, 91, 13–19.
24. Ferentinos, K. P. (2018). Deep learning models for plant disease detection and diagnosis. *Computers and Electronics in Agriculture*, *145*, 311–318.
25. Fernandes, C., Casadevall, A., & Gonçalves, T. (2023). Mechanisms of Alternaria pathogenesis in animals and plants. *FEMS Microbiology Reviews*, 47(6).
26. Fernandez, M. R., & Conner, R. L. (2011). Black Point and Smudge in Wheat Kernel Discolouration in Common and Durum Wheat. 4, 158–164.
27. Figueroa, M., Hammond-Kosack, K. E., & Solomon, P. S. (2018). A review of wheat diseases-a field perspective. *Molecular Plant Pathology*, 19(6), 1523–1536.
28. 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. h
29. Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein Identification and Analysis Tools on the ExPASy Server. In The Proteomics Protocols Handbook (pp. 571–607). Humana Press.
30. Gaulton, A., Bellis, L. J., Bento, A. P., Chambers, J., Davies, M., Hersey, A., Light, Y., McGlinchey, S., Michalovich, D., Al-Lazikani, B., & Overington, J. P. (2012). ChEMBL: a large-scale bioactivity database for drug discovery. *Nucleic Acids Research*, 40(D1), D1100–D1107.
31. Griffin, H. G., & Griffin, A. M. (1995). National centre for biotechnology information (NCBI): Services provided for the molecular biotechnologist. *Molecular Biotechnology*, 4(2), 206–206.
32. Guillermo Fuentes-Dávila, Ivón Alejandra Rosas-Jáuregui, Carlos Antonio Ayón-Ibarra, José Luis Félix-Fuentes, Pedro Félix-Valencia, & María Monserrat Torres-Cruz. (2023). Incidence of black point (Alternaria sp.) in elite advanced bread wheat (Triticum aestivum) lines. *GSC Biological and Pharmaceutical Sciences*, 25(2), 222–229.

33. Haque, M. A., Marwaha, S., Deb, C. K., Nigam, S., Arora, A., Hooda, K. S., ... & Agrawal, R. C. (2022). Deep learning-based approach for identification of diseases of maize crop. Scientific reports, 12(1), 6334.
34. Hou, Y.-H., Yang, Z.-H., Wang, J.-Z., & Yang, Q.-Z. (2022). Characterization of a thermostable alkaline feruloyl esterase from Alternaria alternata and its synergism in dissolving pulp production. *Biochemical Engineering Journal*, 187, 108657.
35. Ijaz, M., Afzal, A., Ashraf, M. M., Ali, S. R., & ul Haq, M. I. (2018). Integrated Disease Management of Black point in Wheat in Punjab (Pakistan). *Plant Protection*, 2(2), 63-68.
36. Iqbal, M. F., Hussain, M., Ali, M. A., Nawaz, R., & Iqbal, Z. (2014). Efficacy of fungicides used for controlling black point disease in wheat crop. *Int. J. Adv. Res. Bio. Sci*, 1(6), 59-64.
37. Kassaw, A., Mihretie, A., & Ayalew, A. (2021). Rate and Spraying Frequency Determination of Propiconazole Fungicide for the Management of Garlic Rust at Woreilu District, Northeastern Ethiopia. *Advances in Agriculture*, 2021, 1–9.
38. Kamilaris, A., & Prenafeta-Boldú, F. X. (2018b). Deep learning in agriculture: A survey. *Computers and Electronics in Agriculture*, *147*, 70–90.
39. Kharin, V. V., Zwiers, F. W., Zhang, X., & Wehner, M. (2013). Changes in temperature and precipitation extremes in the CMIP5 ensemble. *Climatic change*, *119*, 345-357.
40. Kustrzeba-Wójcicka, I., Siwak, E., Terlecki, G., Wolańczyk-Mędrala, A., & Mędrala, W. (2014). Alternaria alternata and Its Allergens: a Comprehensive Review. *Clinical Reviews in Allergy & Immunology*, 47(3), 354–365.
41. Laskowski, R. A., MacArthur, M. W., Moss, D. S., & Thornton, J. M. (1993). PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography*, 26(2), 283–291.
42. Lee, G. Y., Alzamil, L., Doskenov, B., & Termehchy, A. (2021). A survey on data cleaning methods for improved machine learning model performance. *arXiv preprint arXiv*:2109.07127.
43. Liu, Y., Yang, X., Gan, J., Chen, S., Xiao, Z.-X., & Cao, Y. (2022). CB-Dock2: improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. *Nucleic Acids Research*, 50(W1), W159–W164.
44. Long, M., Hartley, M., Morris, R. J., & Brown, J. K. M. (2023). Classification of wheat diseases using deep learning networks with field and glasshouse images. *Plant Pathology*, *72*(3), 536–547.
45. Lore, J. S., & Thind, T. S. (2012). Performance of different fungicides against multiple diseases of rice. *Indian Phytopathology*.
46. Malaker, P. K., & Mian, I. H. (2009). Effect of seed treatment and foliar spray with fungicides in controlling black point disease of wheat. *Bangladesh Journal of Agricultural Research*, 34(3), 425-434.
47. Manamgoda, D. S., Rossman, A. Y., Castlebury, L. A., Chukeatirote, E., & Hyde, K. D. (2015). A taxonomic and phylogenetic re-appraisal of the genus Curvularia (Pleosporaceae): Human and plant pathogens. *Phytotaxa*, 212(3), 175–198.
48. Masiello, M., Somma, S., Susca, A., Ghionna, V., Logrieco, A. F., Franzoni, M., Ravaglia, S., Meca, G., & Moretti, A. (2020). Molecular identification and mycotoxin production by alternaria species occurring on durum wheat, showing black point symptoms. Toxins, 12(4).
49. Merck. (2006). Potato Dextrose Agar. *Merck Microbiology Manual*, 12, 1–2.
50. Mohanty, S. P., Hughes, D. P., & Salathé, M. (2016). Using Deep Learning for Image-Based Plant Disease Detection. *Frontiers in Plant Science*, *7*.
51. Moshatati, A., & Gharineh, M. H. (2012). Effect of grain weight on germination and seed vigor of wheat. *International Journal of Agriculture and Crop Sciences*, 4(8), 458–460.
52. Muhsin, M., Nawaz, M., Khan, I., Chattha, M. B., Khan, S., Aslam, M. T., Iqbal, M. M., Amin, M. Z., Ayub, M. A., Anwar, U., Hassan, M. U., & Chattha, M. U. (2021). Efficacy of Seed Size to Improve Field Performance of Wheat under Late Sowing Conditions. *Pakistan Journal of Agricultural Research*, 34(1).
53. Naik, B. N., Malmathanraj, R., & Palanisamy, P. (2022). Detection and classification of chilli leaf disease using a squeeze-and-excitation-based CNN model. *Ecological Informatics*, *69*, 101663.
54. Nithya, U., Chelladurai, V., Jayas, D. S., & White, N. D. G. (2011). Safe storage guidelines for durum wheat. *Journal of Stored Products Research*, 47(4), 328–333.

55. Peila, D., Picchio, A., Martinelli, D., & Negro, E. D. (2016). Laboratory tests on soil conditioning of clayey soil. *Acta Geotechnica*, 11(5), 1061–1074.
56. Rani, P., & Singh, A. (2018). Effect of Black Point Infection on Germination of different varieties of Wheat Seed. I.
57. Prendin, F., Pavan, J., Cappon, G., Del Favero, S., Sparacino, G., & Facchinetti, A. (2023). The importance of interpreting machine learning models for blood glucose prediction in diabetes: an analysis using SHAP. *Scientific reports*, *13*(1), 16865.
58. Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 3(3), 430–439.
59. Schultz, J., Milpetz, F., Bork, P., & Ponting, C. P. (1998). SMART, a simple modular architecture research tool: Identification of signaling domains. *Proceedings of the National Academy of Sciences*, 95(11), 5857–5864.
60. SESİZ, U. (2023). The Screening of Black Point in Commercial Bread Wheat Cultivars Grown in Turkey, and The Effect of Black Point on Thousand Grain Weight. *Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 28(1), 230–238.
61. Shahwani, A. R., Sana, U. B., Shahbaz, K. B., Baber, M., Waseem, B., Hafeez, N. B., Rameez, A. B., Sial, A. H., Sabiel, S. A. I., Kamran, R., Ayaz, A. S., & Ashraf, M. (2014). INFLUENCE OF SEED SIZE ON GERMINABILITY AND GRAIN YIELD OF WHEAT (Triticum Aestivum L .) VARIETIES. *Journal of Natural Sciences Research*, 4(23), 1–11.
62. Shukla, D. N., Shrivastava, J. P., & Yadav, M. K. (2020). Occurrence of Black Point Disease Complex of Wheat in Eastern Uttar Pradesh. Www. Groupexcelindia. Com, November.
63. Srivastava, J. P., Kushwaha, G. D., & Shukla, D. N. (2015). Black point disease of wheat and its implications on seed quality. *Crop Research*, 47(1to3), 21-23.
64. Abramson, J., Evans, R., Bryant, P., Ingale, A., Johansson-Åkhe, I., Pan, J. et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature 629, 289–298 (2024).
65. Strange, R. N., & Scott, P. R. (2005). Plant Disease: A Threat to Global Food Security. *Annual Review of Phytopathology*, 43(1), 83–116.
66. Sutaji, D., & Yıldız, O. (2022). LEMOXINET: Lite ensemble MobileNetV2 and Xception models to predict plant disease. *Ecological Informatics*, *70*, 101698.
67. Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K. P., Kuhn, M., Bork, P., Jensen, L. J., & von Mering, C. (2015). STRING v10: protein–protein interaction networks, integrated over the tree of life. *Nucleic Acids Research*, 43(D1), D447–D452.
68. Talukder, A. S. M. H. M., McDonald, G. K., & Gill, G. S. (2014). Effect of short-term heat stress prior to flowering and early grain set on the grain yield of wheat. *Field Crops Research*, *160*, 54-63.
69. Taylor, N. P., & Cunniffe, N. J. (2023). Coupling machine learning and epidemiological modelling to characterise optimal fungicide doses when fungicide resistance is partial or quantitative. *Journal of the Royal Society Interface*, *20*(201), 20220685.
70. UniProt: a hub for protein information. (2015). *Nucleic Acids Research*, 43(D1), D204–D212.
71. Wang, Y., Wu, S., Chen, J., Zhang, C., Xu, Z., Li, G., Cai, L., Shen, W., & Wang, Q. (2018). Single and joint toxicity assessment of four currently used pesticides to zebrafish (Danio rerio) using traditional and molecular endpoints. *Chemosphere*, 192, 14–23.
72. Wiederstein, M., & Sippl, M. J. (2007). ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. *Nucleic Acids Research*, 35(Web Server), W407–W410.
73. Xu, K. G., Jiang, Y. M., Li, Y. K., Xu, Q. Q., Niu, J. S., Zhu, X. X., & Li, Q. Y. (2018). Identification and Pathogenicity of Fungal Pathogens Causing Black Point in Wheat on the North China Plain. *Indian Journal of Microbiology*, 58(2), 159–164.
74. Yadav, R. K., Ghasolia, R. P., & Yadav, R. K. (2020). Management of Alternaria alternata of Tomato (Lycopersicon esculentum Mill.) through Plant Extract and Fungicides in vitro and Natural Condition. *International Journal of Current Microbiology and Applied Sciences*, 9(5), 514–523.
75. 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? *Environmental Science & Technology*, 53(7), 3347.