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Evaluation of botanical extracts and molecular docking approaches for sustainable management of rice blast disease in Mushk Budji rice

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Abstract

A study was carried out to explore and address the challenges posed by rice blast disease (*Magnaporthe oryzae* B. C. Couch.). Keeping in view the unique qualities and cultural importance of this variety, the research introduces an innovative approach by integrating plant extracts into disease management practices through in-depth analyses and field experiments. Three concentrations of ethanol-based extracts each of the twenty-one were evaluated in vitro against the control. The percentage of mycelial inhibition was recorded and based on their effectiveness; six efficient botanicals were further evaluated in the field. The field experiments were carried out on the susceptible Mushk Budji cultivar of rice for two successive years. The results demonstrated that certain botanicals, especially extracts of *Syzygium aromaticum* and *Podophyllum hexandrum*, significantly reduced disease incidence and severity, and improved grain and straw yields, performing on par with the chemical control, Tricyclazole. Additionally, molecular docking was conducted to identify active compounds responsible for antifungal effects. The ligands for docking studies quercetin, podophyllotoxin, and eugenol were chosen based on literature mining for their reported antifungal activity. These phytochemicals displayed strong binding affinities (-5.5 to -3.8 kcal/mol) to the ToxB-like effector protein of *M. oryzae*, suggesting interference with fungal pathogenicity mechanisms. Among these, quercetin and podophyllotoxin were the most promising ligands. The integration of wet-lab assays and in silico analysis strengthens the case for deploying bioactive botanicals in integrated disease management. Our findings advocate the use of eco-friendly, plant-based fungicides as a viable strategy for blast disease control, promoting

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organic farming and preservation of indigenous rice varieties. The findings highlight the importance of developing eco-friendly, plant-based alternatives to chemical fungicides, aligning with the goals of sustainable agriculture and organic farming. Future research should focus on developing stable formulations of these botanicals, evaluating their synergistic potential with biocontrol agents, and validating their effectiveness through multi-location field trials. Additionally, further investigation into the molecular mechanisms of action and the long-term ecological and economic impacts of botanical fungicides is warranted.

Keywords Botanical management, Cultural heritage, Indigenous variety, Organic management, *Magnaporthe oryzae*

Introduction

Almost one-third of the world population utilises rice (*Oryza sativa* L.) as a staple food source, making it an important cereal crop globally. Particularly in Asia, rice holds paramount economic and cultural significance, representing the cornerstone of the region's agriculture and economy. With its vital role in sustaining daily dietary requirements for nearly 3.5 billion individuals, rice plays a crucial part in global food security, a number anticipated to rise to 3.9 billion by 2025 [1–3]. In 2013, global rice production reached around 740 million tons, with projections indicating a necessary 40% increase in production by 2030 to accommodate the growing world population of 8.3 billion [4–6]. India, with an expansive 43.82 million hectares devoted to rice cultivation stands as the second-largest producer worldwide, producing 177.6 million tonnes of rice with a productivity rate of 4,057 kg per hectare, reflecting the crop's indispensable role in the nation's food security [7–9]. Notably, India has witnessed a commendable surge in cereal production from 87.4 million tons in 1961 to 324.3 million tons in 2019 [7]. Serving as a dietary staple for over 70% of the Indian population, rice in various forms remains a fundamental component of the nation's diet, while the remaining populace depends on rice alongside wheat or other grains [10]. Mushk Budji Rice, distinguished by its exquisite aroma and distinct taste, holds a crucial place in the agricultural heritage of Kashmir [11]. However, due to its susceptibility to rice blast disease caused by *Magnaporthe oryzae* B. C. Couch. (synonym: *Pyricularia oryzae*), this variety of rice faces serious economic losses [12]. Rice blast poses a severe and recurring threat to both lowland and upland rice production worldwide, leading to annual yield losses ranging from 10% to 30%, equivalent to approximately 157 million tons globally [13–15]. Under favourable conditions, this disease has the potential to decimate entire crops within a short period, causing losses as high as 100% [13]. One major difficulty with the widespread cultivation of aromatic rice is its susceptibility to blast disease. Examples of these types are Mushk Budji in the temperate Himalayas of North India, despite its export potential due to unique aroma and quality attributes [12].

India, which is ranked as the second-largest producer and exporter of aromatic Basmati rice after China, faces

blast disease challenges in rice-growing regions, notably Jammu and Kashmir, where it acts as a major constraint, significantly impacting yield [12, 16–20]. The worldwide threat brought by rice blast disease, caused by *Magnaporthe oryzae*, results in substantial yield losses, with the potential to wipe out entire crops under extreme conditions [21, 22]. With reported occurrences in over 85 countries, rice blast has been identified as the cause of around 30% of rice production declines, representing a significant threat to global food security [15, 23–25]. The pathogen owing to its variability and changing virulence patterns has been reported to cause the blast of other cereals like wheat [26] and finger millet [15].

Effective management of rice blast disease demands a careful integration of various strategies and techniques, as a single mistake can lead to crop failure [27]. The blast fungus can infect rice at any stage in the host's growth stage. Its initial symptoms comprise white to grey/brown patches or lesions on leaves, which may lead to nodal and neck blast, eventually resulting in necrosis and panicle breaking [28]. While chemical control is an option, it is economically unfeasible and environmentally hazardous at high application doses, necessitating sustainable alternatives [29]. In the realm of organic farming, emphasis is placed on eco-friendly disease control methods, including biological, cultural, and physical methods. Although systemic and contact fungicides have been utilized, only specific formulations are permitted in organic crop production [30]. Natural plant products, known for their antimicrobial properties, are explored as alternatives for blast disease management, considering their safety, biodegradability, and eco-friendly nature [31–36].

In addition to developing and identifying blast-resistant rice varieties, the application of plant extracts and biocontrol agents is pivotal in modern sustainable agriculture practices and integrated disease management. The antimicrobial characteristics of plant extracts have broadened their potential as a promising approach for controlling plant diseases [37, 38]. Plant diseases can be substantially lowered by chemical treatment; nevertheless, it may not always be desirable to utilise synthetic fungicides in the field as indicated by the extensive research conducted over the past few decades regarding the harmful effects of excessive and improper usage of chemical fungicides on the environment, animals, and

human health [39, 40]. The plant derived substances are found naturally, easily transform into common, environmentally benign organic components, and have little negative impact on plant physiological processes [41]. As bio-fungicidal compounds, plant extracts, essential oils, gums, resins, and other materials have been demonstrated to have biological activity against plant fungal infections both in vitro and in vivo [42–44]. Such products may be utilised as substitute treatments for plant diseases since it is commonly believed that they are more acceptable and less hazardous to ecosystems [45, 46]. Natural plant products are ideal for a specific target because they possess a specific mode of action and a confined target range [47]. They are also often harmless to hostile microorganisms, have a shorter shelf life, and provide no residual hazards [48]. When compared to traditional synthetic chemical pesticides, they are usually safer for people and the environment and are frequently included in integrated pest management (IPM) programmes. In accordance with Nuzhat and Vidyasagar (2013) [49], farmers in impoverished nations who have historically used plant extracts to treat human illnesses can readily embrace them. In poor nations where resources are severely restricted natural plant-based pesticides are affordable, easily accessible, and more economical for farmers than synthetic fungicides [50, 51]. Our study aims to identify the possible role of ethanol-based plant extracts in managing rice blast disease, notably in the Mushk Budji variety of rice, which is being

grown its unique aroma [52] and cooking quality [11]. By assessing the effectiveness of several plant extracts against *M. oryzae* both in vitro and in vivo, this research seeks to identify alternative disease management strategies that are safe for human consumption, environmentally friendly, and economically viable for producers.

Materials and methods

Isolation of the pathogen

PDA (potato dextrose agar) medium was used to isolate the pathogen. Infected leaves underwent a series of sterile procedures, including washing with sterile water, cutting into small fragments using a sterile scalpel blade, and subsequent sterilization with a 1% sodium hypochlorite solution for 30 s. The leaf fragments were sterilized, then placed on Whatman No. 1 filter paper and rinsed three times with sterile distilled water. These leaf fragments were then aseptically moved using sterile forceps to Petri plates that contained sterilized PDA media amended with streptomycin (50 µg/ml). Incubation was carried out in the B.O.D. Incubator at 25 °C. To create a pure culture of the fungus, a single spore was transferred to a fresh Petri plate with 15 ml of sterilised PDA media as soon as growth commencement was seen on the previous plates. This methodology corresponds with the approach used by Vanaraj et al. [53].

Botanicals used for *in vitro* management of *Magnaporthe oryzae*

Ethanolic extracts of twenty one botanicals viz., *Artimisia annua*, *Acorus calamus*, *Capsicum annum*, *Bergenia ciliata*, *Datura stramonium*, *Syzygium aromaticum*, *Echinacea purpurea*, *Allium sativum*, *Geranium pratense*, *Heracleum candicans*, *Inula racemose*, *Juglans regia*, *Marrubium vulgare*, *Mentha arvensis*, *Mentha Longifolia*, *Allium cepa*, *Picrorhiza kurroa*, *Podophyllum hexandrum*, *Rheum emodi*, *Saussurea costus* and *Zingiber officinale* were evaluated against *Magnaporthe oryzae* *in vitro* following poisoned food technique [54]. The 21 plant extracts were selected through a dual approach: firstly, many were chosen due to their documented antifungal activities in existing literature [35, 38]; secondly, several less-explored species native to the Kashmir Himalayas were included to investigate new sources of antifungal activity. Selection was also informed by ethnobotanical practices, as multiple extracts have traditional applications against microbial infections. The reason to select ethanol as a solvent was based on the fact that its polarity allows it to effectively extract both polar and moderately polar compounds, from plant parts [55]. Most of these plants are native to the Kashmir Himalayas, although some were sourced from local markets. The selection of forest plants was influenced by their known antimicrobial properties [35, 38, 56–59]. Table 1 provides

Table 1 List of Plants/Plant parts used for in vitro evaluation against *Pyricularia oryzae*

S.No	Botanical	Local name	Plant part used
1.	<i>Acorus calamus</i>	Wai Gander	Leaves
2.	<i>Artimisia annua</i>	Tathwan	Leaves
3.	<i>Bergenia ciliata</i>	Zakhm-e-Hayat	Leaves
4.	<i>Capsicum annum</i>	Mirchi	Fruit
5.	<i>Syzygium aromaticum</i>	Rongg	Flower buds
6.	<i>Datura stromium</i>	Datur	Leaves
7.	<i>Echinacea pupurae</i>	Echinacea	Flowers
8.	<i>Allium sativum</i>	Ruhun	Bulbs
9.	<i>Geranium pratense</i>	Ringresh	Leaves
10.	<i>Heracleum candicans</i>	Hogweed	Leaves
11.	<i>Inula racemose</i>	Poshkarmool	Roots
12.	<i>Juglans regia</i>	Doon	Leaves
13.	<i>Marrubium vulgare</i>	Trapad	Leaves
14.	<i>Mentha arvensis</i>	Mint	Leaves
15.	<i>Mentha longifolia</i>	Yenn	Leaves
16.	<i>Allium cepa</i>	Piyaz	Bulbs
17.	<i>Picrorhiza kurroa</i>	Kutki	Roots
18.	<i>Podophyllum hexandrum</i>	Wanwangun	Leaves
19.	<i>Rheum emodi</i>	Himalayan rhubarb	Leaves
20.	<i>Saussurea costus</i>	Kuth	Leaves
21.	<i>Zingiber officinale</i>	Adhrak	Rhizome

additional details regarding the common names and the specific parts of the plants utilized. These botanicals were obtained from the Regional cum Facilitation Centre for Northern Region-II (RCFC, NR-II) of the National Medicinal Plants Board (NMPB), situated at the Faculty of Agriculture, SKUAST-Kashmir in Wadura, Sopore.

Preparation of ethanolic plant extracts

Ethanolic plant extracts were prepared by first drying selected whole plants or plant parts either through shade drying or oven drying, followed by powdering using an electric blender. Subsequently, 16 g of the powdered plant material were mixed with 100 ml of sterile solvent (70% v/v Ethanol) in a conical flask to create a suspension. The tightly plugged flasks were kept in a shaker incubator at 25 °C for 4 days to facilitate optimal extraction of phytochemicals. Whatman's No. 1 filter paper was used to filter the resultant suspension, and the filtered extracts were then dried at 60 °C in a hot air oven until complete evaporation of the solvent occurred [60, 61].

Reconstitution of the extract

For reconstitution of the extract, 1 gram of the dried extract was weighed and dissolved in 5 ml of warm sterile distilled water, generating a stock concentration of 200,000 ppm [62, 63]. This stock solution was stored in plastic tubes at 4 °C in a refrigerator for subsequent studies. From this stock, three different concentrations (2000 ppm, 3000 ppm, and 4000 ppm) were prepared to assess their inhibitory effects against the *Magnaporthe oryzae* pathogen.

Determination of mycelial growth inhibition by poisoned food technique

Using the Poisoned Food Technique, the effectiveness of the plant extracts against *Magnaporthe oryzae* was assessed in vitro based on mycelial inhibition percentage [64]. Specific volumes of the plant extract from the stock solution were added to sterilized PDA medium in conical flasks with final concentrations of 2000 ppm, 3000 ppm, and 4000 ppm. Petri plates were filled with 20 ml of the PDA medium poisoned with the plant extract and allowed to solidify under aseptic conditions. Each treatment combination was inoculated with 5 mm mycelia discs of a 7-day-old *Magnaporthe oryzae* culture in the centre of the Petri plates. Control plates without plant extracts were also maintained. The experiment was set up in a completely randomized design (CRD) with three replications for each treatment. Tricyclazole (600 ppm) served as a positive control. The inoculated Petri plates were incubated at 25 ± 1 °C, and colony diameters were recorded after 7 and 14-days post-inoculation. Fungitoxicity/Inhibition Percentage of the extract, in terms of

percent of mycelial growth inhibition, was determined using the formula by Vincent (1947) [65].

$$\text{Percent Inhibition (\%)} = \frac{C - T}{C} \times 100$$

where, I= Percent inhibition;

C= Colony diameter in control plate.

T= Colony diameter in treated plate.

Evaluation of efficient medicinal plants against rice blast pathogen under field conditions

Furthermore, at the Mountain Research Centre for Field Crops, Khudwani, SKUAST-Kashmir, an assessment of the effectiveness of efficient botanicals against the rice blast pathogen under field conditions was carried out. The field experiment employed six potent ethanolic extracts identified from in vitro studies: *Podophyllum hexandrum*, *Inula racemosa*, *Syzygium aromaticum*, *Heracleum candicans*, *Allium sativum*, and *Allium cepa*. Field applications specifically targeted the 4000 ppm dose, which was found to be most effective in vitro. This study was conducted over two consecutive years, 2022 and 2023. Control treatments included the chemical fungicide Tricyclazole 75WP, marketed as Baan by Indofil Industries Ltd., applied at a concentration of 0.06%. Additionally, plain water was used as a control. The botanical extracts were administered at a concentration of 4000 ppm during three key growth stages: tillering, panicle initiation, and booting. Healthy seedlings, approximately one month old and 20 cm in height at 2–3 seedlings/hill, were transplanted in early June with a plot size of 2 m×2 m for each treatment. Square transplanting with a spacing of 15 × 15 cm was employed, and recommended agronomical practices were followed throughout the crop period. Foliar sprays of standard extract solutions were applied at tillering, panicle initiation, and booting stages, with each treatment replicated three times. Phytotoxicity was monitored during field trials by assessing plant growth, health, and yield parameters; no adverse phytotoxic effects were observed across treatments, indicating suitability for further agricultural use. One week after the final spray, data on the incidence and severity of leaf blast was collected using the SES (0–9) scale (Table 2) [66]. The calculations for disease incidence and severity followed the formulas outlined by Chiang et al. (2017) [67] and Bock et al. (2020) [68]. Also, the data on grain and straw yield (q/ha) was taken after harvest.

Disease Incidence (%)

$$= \frac{\text{No. of samples affected with disease}}{\text{No. of Samples observed}} \times 100$$

Table 2 Disease scoring scale used for determining disease severity (SES, 2013)

Score	Disease severity	Diseased leaf area (%)
0	No lesion observed	0
1	Leaf lesions are not visible upon first glance, but upon close inspection, a few lesions are seen in each row	<0.3
2	Rapid detection detects a few lesions	0.3–0.9
3	Within a plot, lesions are sporadically distributed, with a leaf affected by infection having one to four lesions altogether	1–2
4	Although the tips of the upper leaves are not necrotic (Scott et al.), they are consistently speckled with blast lesions. Several to a few brown leaves witnessed	3–7
5	There are few dead leaves visible and several to many lower leaves that are necrotic. The tips of a few top leaves become brown and start to fold	8–14
6	Several dead leaves are evident, and the lower leaves are all uniformly brown in colour. The majority of the top leaf tips are necrotic	15–24
7	The majority of upper leaf tips are curled. Lower and middle leaves are brown. A number of the tillers or plants are dead or stunted	25–39
8	The upper and middle leaves frequently exhibit extensive curling and browning. A significant number of plants are dead, and most plants are stunted	40–65
9	Most plants are brown, dead, and severely stunted. A few or more plants are severely infected and only have green leaves	>65

Disease Severity (%)

$$= \frac{\text{Sum of Score of diseased samples}}{\text{No. of Samples scored}} \times \frac{1}{\text{Highest score}} \times 100$$

Research design and statistical analysis

The in vitro test was conducted using a Completely Randomized Design (CRD), while the field experiment was performed using a Randomized Complete Block Design (RCBD) to minimize variability. The in vitro assessment of botanical extracts was performed using a Completely Randomized Design (CRD). To facilitate comparisons between the treatments in the in vitro experiment, a chord diagram was created using R-Studio software. For the field evaluation of these botanicals, a Randomized Complete Block Design (RCBD) was employed to reduce variability in the results. The data collected from the experiments were statistically analyzed using analysis of variance (ANOVA) to assess the significant effects

of treatments. The experimental design involved treatments as fixed factors and replications as random factors where applicable. Prior to conducting ANOVA, residuals were checked for normality using the Shapiro-Wilk test, to confirm that the assumptions were met. The significance of main effects was determined by the F-test at a 5% significance level ($p < 0.05$). Least significant difference (LSD) test was used for mean separation to identify which specific treatment means were significantly different. Treatments with different superscript letters in tables denote statistically significant differences based on the LSD test. All analyses were performed using R software [69].

Ligand selection and preparation

A total of six bioactive phytochemicals—Eugenol, Alliin, Germacrene D, Isoalantolactone, Podophyllotoxin, and Quercetin—were selected based on their reported antifungal and antimicrobial activities. The selection of these ligands was based on comprehensive literature mining, focusing on their documented antifungal and antimicrobial activities against plant pathogens. The 2D chemical structures of the selected compounds were retrieved from the PubChem database in SDF format [70, 71]. These structures were then converted to 3D conformations and subjected to energy minimization using the Universal Force Field (UFF) to ensure optimal geometry. The minimized structures were subsequently converted to PDBQT format using Open Babel integrated within PyRx 0.8, preparing them for molecular docking analyses [72] (Table 3).

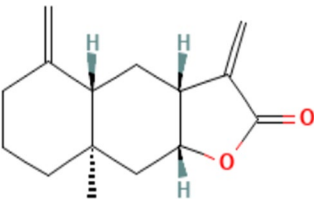
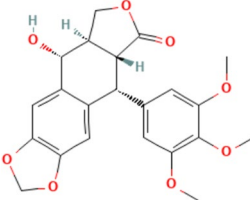
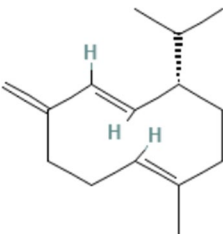
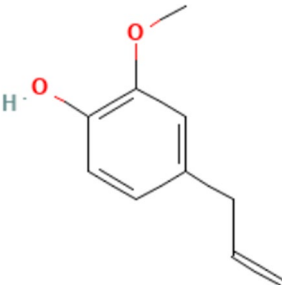
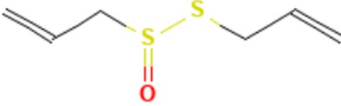
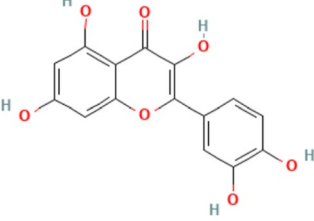
Target protein retrieval and preparation

The amino acid sequence of the ToxB-like (MAX) effector protein from *Magnaporthe oryzae* was retrieved from UniProt [73]. The ToxB-like effector protein was chosen based on its established role in modulating fungal pathogenicity and virulence, particularly in *Magnaporthe oryzae*. As no experimental 3D structure was available, the tertiary structure was predicted via SWISS-MODEL, employing homology modeling techniques [74]. The generated structure was validated through the SAVES v6.0 server, including Ramachandran plot analysis (PROCHECK), ERRAT, and VERIFY3D for structural reliability [75] (Table 4). The validated model was further refined using Swiss-PDB Viewer for energy minimization [76]. The prepared structure was saved in PDB format and later converted to PDBQT format using AutoDock Tools, with water molecules and non-essential ligands removed,

Table 3 Modelling details of target proteins obtained with the help of SWISS- MODEL

UniProt ID	Protein target & sequence length	Template protein PDB ID	Sequence identity %	Sequence similarity %	Coverage %	GMQE
G5EI17	ToxB-like (MAX) effectors-88	L7II96.1.A	100	61	100	0.86

Table 4 Structure of ligands retrieved using pubchem database

Name	Molecular formula	Molecular weight	Structure	Source
Isoalantolactone	C ₁₅ H ₂₀ O ₂	232.32 g/mol		(Liu et al., 2001) [111]
Podophyllotoxin	C ₂₂ H ₂₂ O ₈	414.4 g/mol		(Anand et al., 2022) [110]
Germacrene D	C ₁₅ H ₂₄	204.35 g/mol		(Chauhan et al., 2018) [112]
Eugenol	C ₁₀ H ₁₂ O ₂	164.20 g/mol		(Atif et al., 2024) [85]
Allicin	C ₆ H ₁₀ OS ₂	162.3 g/mol		(El-Saber Batiha et al., 2020) [118]
Quercetin	C ₁₅ H ₁₀ O ₇	302.23 g/mol		(Lanzotti et al., 2011) [109]

and polar hydrogens and Kollman charges added (Figure 4) [77].

Binding site prediction

The active binding pockets of the modeled ToxB-like protein were predicted using PrankWeb, which uses machine learning to identify ligand-accessible residues [78]. The most probable and highest scoring pocket was selected for grid box generation in docking studies.

Molecular docking

Docking simulations were performed using AutoDock Vina within PyRx [79]. The grid box was centered on the predicted binding site of the ToxB-like protein from *M. oryzae*. The ToxB-like effector protein was chosen based on its established role in modulating fungal pathogenicity and virulence, particularly in *Magnaporthe oryzae* [73, 80]. Docking studies against this protein provide molecular insight into how the selected phytocompounds may

inhibit key mechanisms of host infection. Each ligand was docked individually, and the best binding affinity (in kcal/mol) for each compound was recorded. Docked complexes were visualized using PyMOL to assess ligand-protein interactions [81].

Results

Determination of mycelium inhibition percentage

The evaluation of various plant extracts against *Magnaporthe oryzae* in vitro revealed significant findings regarding mycelial inhibition. The study indicated that increasing the concentration of the extracts resulted in a notable reduction in mycelial growth (Table 3, Figure 1). Most botanicals demonstrated a higher percentage of mycelial inhibition at 4000 ppm, both at 7 and 14 days after inoculation (DAI). Among these, the ethanolic extract of *Syzygium aromaticum* (clove) showed the highest mean percent inhibition across all concentrations, followed closely by *Inula racemosa*, *Geranium pratense*, *Heracleum candicans*, *Podophyllum hexandrum*, *Allium cepa*, *Bergenia ciliata*, and *Allium sativum*. The lowest inhibition at 7 DAI was noted for *Picrorhiza kurroa*, followed by *Mentha longifolia* and *Juglans regia* (Table 5). At 14 DAI, a similar trend was observed with *Syzygium aromaticum* again exhibiting the highest mycelial inhibition percentage, succeeded by *Inula racemosa*, *Allium cepa*, *Allium sativum*, *Capsicum annum*, and *Podophyllum hexandrum* (Table 5). While all extracts showed

maximum inhibition at 4000 ppm at 7 DAI, at 14 DAI, extracts at 3000 ppm also displayed significant effectiveness. As concentrations increased, there was a corresponding rise in the inhibition of radial growth of *M. oryzae* when using extracts like *Syzygium aromaticum*, *Inula racemosa*, and others. Based on their efficacy, these six effective plant extracts were selected for use in field conditions alongside Tricyclazole 75WP and a control treatment. Additionally, the results from the in vitro study are illustrated graphically in Figure 2 as a chord diagram, where the extracts are positioned around the circle's circumference and concentration groups are represented as arcs. The width of ribbons connecting the extracts to their respective concentration arcs corresponds to the percent inhibition of mycelial growth.

Evaluation of efficient plant extracts against rice blast disease

Disease incidence and severity

The study found that the incidence of rice blast disease was significantly higher in 2022 compared to 2023 across all treated and control plots. Detailed results for both years are summarized in Table 6 and Supplementary Figure-1. Among the six botanical treatments, *Syzygium aromaticum* extract showed the highest effectiveness, resulting in a minimum disease incidence of 40.92%. This was followed by *Podophyllum hexandrum* (48.06%), *Heracleum candicans* (54.79%), *Inula racemosa* (54.38%),



Fig. 1 Photographs of plates showing mycelial growth of *Pyricularia oryzae* on various ethanol-based plant extracts evaluated *in vitro* using the poisoned food technique at 7 days after incubation (DAI) [A] and 14 DAI [B] at three concentrations: C₁ (2000 ppm), C₂ (3000 ppm), and C₃ (4000 ppm). *P. oryzae* on: a. *Acorus calamus*, b. *Artimisia annua*, c. *Bergenia ciliate*, d. *Capsicum annum*, e. *Syzygium aromaticum*, f. *Datura stramonium*, g. *Echinacea purpurea*, h. *Allium sativum*, i. *Geranium pratense*, j. *Heracleum candicans*, k. *Inula racemose*, l. *Juglans regia*, m. *Marrubium vulgare*, n. *Mentha arvensis*, o. *Mentha Longifolia*, p. *Allium cepa*, q. *Picrorhiza kurroa*, r. *Podophyllum hexandrum*, s. *Rheum emodi*, t. *Saussurea costus*, u. *Zingiber officinale*, v. Control, w. Tricyclazole 75 WP

Table 5 Mycelial Growth Inhibition Percentage for Various Plant Extracts against Rice Blast Pathogen at 7 and 14 days after Inoculation

Plant extracts	Mycelial growth inhibition (%) at 7DAI				Mycelial growth inhibition (%) at 14DAI			
	Concentrations				Concentrations			
	2000 ppm	3000 ppm	4000 ppm	Mean	2000 ppm	3000 ppm	4000 ppm	Mean
<i>Acorus calamus</i>	39.72 ^{ghij}	26.95 ^{mno}	29.08 ^{klmn}	31.91 ^{ef}	15.56 ^{xyzA}	31.11 ^{klm}	25.19 ^{mnpqrs}	23.95 ^{jk}
<i>Artimisia annua</i>	14.89 ^{stuvwxx}	29.08 ^{lmn}	46.81 ^{efg}	30.26 ^{efg}	24.81 ^{nopqrs}	40.74 ^{hi}	32.96 ^{ijkl}	32.84 ^g
<i>Bergenia ciliata</i>	23.90 ^{nopq}	33.33 ^{ijklm}	44.68 ^{fgghi}	33.97 ^{de}	28.52 ^{klmno}	45.19 ^{gh}	38.15 ^{ij}	37.2 ^{8f}
<i>Heracleum candicans</i>	36.88 ^{ijkl}	38.30 ^{hijk}	40.43 ^{ghij}	38.53 ^{cd}	41.11 ^{hi}	52.22 ^f	48.15 ^{fg}	47.16 ^d
<i>Syzygium aromaticum</i>	57.45 ^{cd}	80.14 ^b	89.36 ^a	75.65 ^a	67.04 ^{bc}	94.44 ^a	88.52 ^a	83.33 ^a
<i>Datura stramonium</i>	5.67 ^{zAB}	3.55 ^{AB}	31.21 ^{klmn}	13.48 ^{ij}	0.37 ^C	12.22 ^{yzAB}	2.59 ^C	5.06 ⁿ
<i>Echinacea purpurea</i>	14.18 ^{stuvwxy}	23.40 ^{nopq}	24.82 ^{nop}	20.80 ^h	18.52 ^{tuvwxx}	31.11 ^{klm}	27.41 ^{lmnop}	25.68 ^{ij}
<i>Allium sativum</i>	36.88 ^{ijkl}	40.43 ^{ghij}	39.01 ^{ghijk}	38.77 ^{cd}	45.19 ^{gh}	59.26 ^{de}	49.26 ^{fg}	51.23 ^c
<i>Geranium pratense</i>	19.78 ^{opqrst}	27.66 ^{mno}	57.45 ^{cd}	34.96 ^{de}	23.70 ^{opqrstu}	48.52 ^{fg}	41.48 ^{hi}	37.90 ^f
<i>Capsicum annuum</i>	24.11 ^{nopq}	39.72 ^{ghij}	56.03 ^{cd}	39.95 ^c	17.78 ^{uvwxyz}	51.11 ^{fg}	18.15 ^{tuvwxy}	29.01 ^{hi}
<i>Inula racemosa</i>	57.45 ^{cd}	54.61 ^{de}	63.83 ^c	58.63 ^b	61.48 ^{cd}	70.00 ^b	66.30 ^{bc}	65.93 ^b
<i>Juglans regia</i>	11.35 ^{uvwxyzA}	12.06 ^{tuvwxyz}	10.64 ^{uvwxyzA}	11.35 ^{jk}	17.04 ^{vwxyz}	26.30 ^{mnpq}	21.11 ^{qrstvwxx}	21.48 ^{kl}
<i>Marrubium vulgare</i>	26.24 ^{mno}	31.21 ^{klmn}	24.82 ^{nop}	27.42 ^{fg}	19.63 ^{stuvwxx}	24.07 ^{opqrst}	21.85 ^{pqrstuvv}	21.85 ^{kl}
<i>Mentha arvensis</i>	22.90 ^{nopqr}	17.02 ^{pqrstuv}	9.22 ^{vwxyzA}	16.38 ^{hi}	29.63 ^{klmno}	22.22 ^{pqrstuv}	21.11 ^{qrstvwxx}	24.32 ^{ik}
<i>Mentha Longifolia</i>	8.39 ^{wxyzA}	7.80 ^{xyzA}	14.18 ^{stuvwxy}	10.13 ^{jk}	12.22 ^{yzAB}	25.56 ^{mnpqr}	19.26 ^{stuvwxx}	19.01 ^{lm}
<i>Allium cepa</i>	38.30 ^{hijk}	42.55 ^{ghi}	46.10 ^{fgh}	42.32 ^c	61.48 ^{cd}	65.93 ^{bc}	64.81 ^{bcd}	64.07 ^b
<i>Picrorhiza kurroa</i>	8.51 ^{wxyzA}	6.38 ^{yzAB}	5.67 ^{zAB}	6.86 ^k	10.37 ^{AB}	30.33 ^{klmn}	11.85 ^{zAB}	17.53 ^m
<i>Podophyllum hexandrum</i>	14.89 ^{stuvwxx}	37.59 ^{ijk}	52.48 ^{def}	34.99 ^{de}	33.70 ^{jk}	54.07 ^{ef}	41.48 ^{hi}	43.09 ^e
<i>Rheum emodi</i>	1.42 ^B	27.66 ^{mno}	29.08 ^{lmn}	18.44 ^h	8.89 ^B	41.85 ^{hi}	38.52 ^{ij}	29.75 ^{gh}
<i>Saussurea costus</i>	17.73 ^{pqrstu}	27.66 ^{mno}	16.31 ^{qrstuvv}	20.57 ^h	20.74 ^{qrstuvwxx}	15.93 ^{wxyzA}	30.74 ^{klmn}	22.47 ^{ijkl}
<i>Zingiber officinale</i>	33.33 ^{klm}	23.40 ^{nopq}	20.57 ^{opqrs}	25.77 ^g	18.15 ^{tuvwxy}	34.07 ^{jk}	25.56 ^{mnpqqr}	25.93 ^{ij}
Mean	24.34 ^A	30.02 ^B	35.80 ^C		27.43 ^A	41.73 ^B	34.97 ^C	
CD (p<0.05)	Extracts(E) = 4.82; Concentration (C) = 1.82 Extracts*Concentration (E*C) = 8.35				Extracts(E) = 3.47; Concentration (C) = 1.31 Extracts*Concentration (E*C) = 6.01			

Values with different letters in a column are significantly different at P<0.05

The superscripts in capital letters indicate significance with respect to various

The superscripts in small letters indicate significance with respect to various Concentrations

The superscripts in italic letters indicate the significance with respect to E*C interaction

* has been used as a symbol of interaction between two components

Bold letters indicate mean values)

Allium sativum (75.77%), and *Allium cepa* (83.03%) during the year 2022. The synthetic fungicide Tricyclazole 75WP served as a positive control, exhibiting the lowest disease incidence at 33.36%. In 2023, *Syzygium aromaticum* again demonstrated the lowest incidence (34.12%), followed by *Heracleum candicans* (41.25%) and others, while untreated control plots had an incidence of 81.63%. The pooled results indicated that treatments with *Syzygium aromaticum* (37.52%) and Tricyclazole 75 WP (31.75%) were statistically similar in terms of disease incidence, with a critical difference (CD) of 0.58 at $p < 0.05$, suggesting their effectiveness in reducing disease incidence. Conversely, *Allium cepa* was ineffective, showing an incidence rate of 82.07%, comparable to the untreated control at 84.07%. *Allium sativum* also exhibited a significant incidence rate of 73.13%, lower than that of *Allium cepa*. Both *Podophyllum hexandrum* (44.55%) and *Heracleum candicans* (48.02%) showed moderate effects, with

the latter being statistically similar to *Inula racemosa* (51.90%) (Table 6 and Supplementary Figure-1). Analysis of variance (ANOVA) indicated a highly significant effect of treatments on disease incidence across the two years. Mean separation was conducted using the Least Significant Difference (LSD) test at the 5% significance level. Treatments with different superscript letters are statistically different.

Disease severity

In terms of disease severity, Tricyclazole 75WP consistently outperformed other treatments, recording a severity of 20.60%. In 2022, both *Syzygium aromaticum* (24.12%) and *Podophyllum hexandrum* (23.43%) showed comparable efficacy to Tricyclazole 75WP (22.14%) (Table 6 and Suppl. Figure 1). The range of disease symptoms observed in field are presented in Figure 3. Other botanical treatments had comparatively higher severity

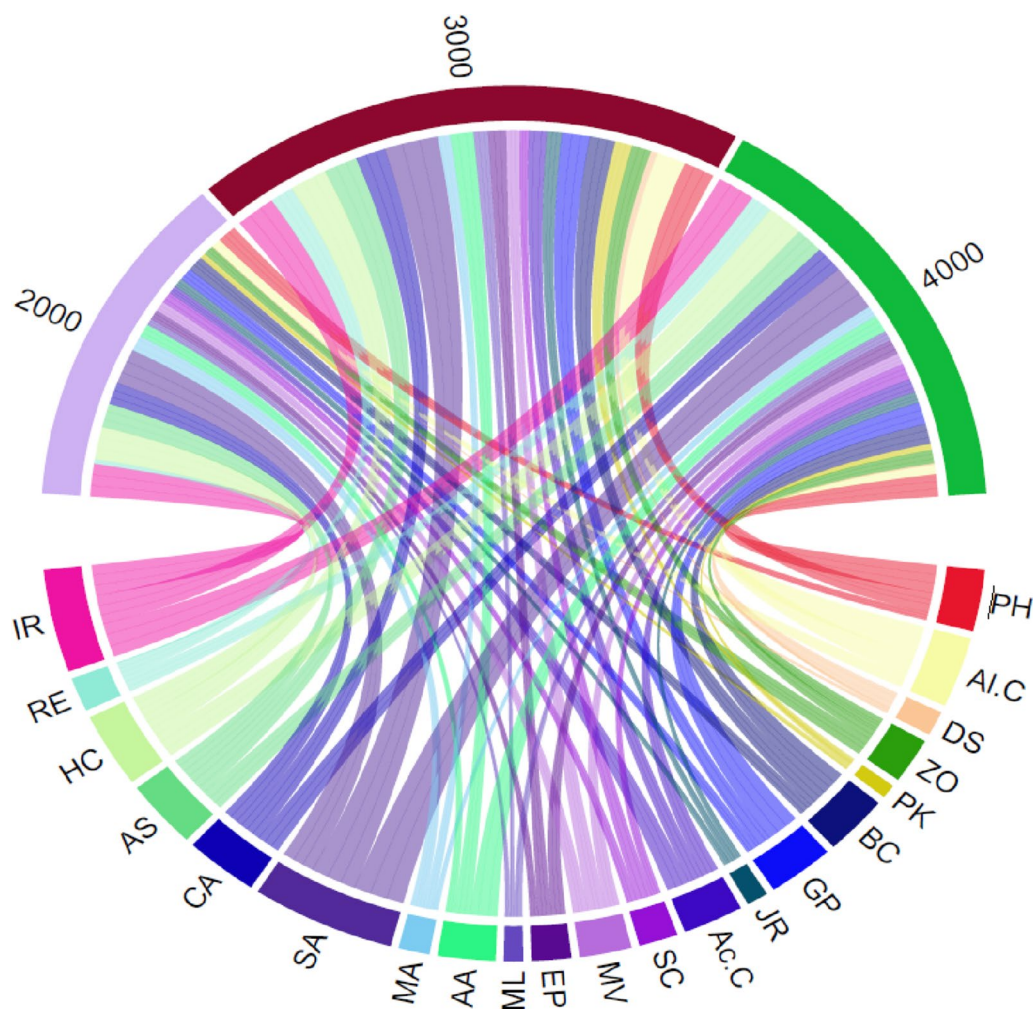


Fig. 2 Chord diagram representing the in vitro inhibition (%) of mycelial growth of the rice blast fungus *Pyricularia oryzae* when treated with different botanical extracts at concentrations of 2000, 3000, and 4000 ppm. The extracts are arranged along the circumference of the circle and the three concentration groups are shown as arcs. The widths of the ribbons connecting the extracts to the concentration arcs are proportional to the percent inhibition of mycelial growth. Each of the extract is represented by short forms viz., *Inula racemosa* (IR), *Rheum emodi* (RE), *Heracleum candicans*, *Allium cepa* (AI. C), *Allium sativum* (AS), *Capsicum annum* (CA), *Syzygium aromaticum* (SA), *Mentha arvensis* (MA), *Artimisia annua* (AA), *Mentha longifolia* (ML), *Echinacea purpurea* (EP), *Marrubium vulgare*, *Saussurea costus* (SC), *Acorus calamus* (Ac.C), *Juglans regia* (JR), *Geranium pratense* (GP), *Bergenia ciliate* (BC), *Picrorhiza kurroa* (PK), *Zingiber officinale* (ZO), *Datura stramonium* (DS), *Allium cepa* (AI. C), *Podophyllum hexandrum* (PH).

Table 6 Per Cent Disease Incidence and Disease Severity recorded using efficient botanicals under field conditions during the years 2022 and 2023

Treatments	Disease incidence (%)			Disease severity (%)		
	Year 2022	Year 2023	Pooled	Year 2022	Year 2023	Pooled
Syzygium aromaticum	40.92 ^f	34.12 ^e	37.52 ^g	24.12 ^e	17.44 ^g	20.78 ^e
Allium sativum	75.77 ^c	70.50 ^b	73.13 ^c	44.00 ^c	39.73 ^d	41.87 ^c
Heracleum candicans	54.79 ^d	41.25 ^d	48.02 ^e	47.63 ^b	42.11 ^c	44.87 ^b
Inula racemosa	54.38 ^d	49.43 ^c	51.90 ^d	35.50 ^d	30.72 ^e	33.11 ^d
Allium cepa	83.03 ^b	81.11 ^a	82.07 ^b	47.37 ^b	43.72 ^b	45.54 ^b
Podophyllum hexandrum	48.06 ^e	41.07 ^d	44.55 ^f	23.43 ^e	18.82 ^f	21.13 ^e
Tricyclazole 75 WP	33.35 ^g	30.15 ^f	31.75 ^h	20.60 ^f	17.21 ^g	18.90 ^f
Control	86.51 ^a	81.63 ^a	84.07 ^a	53.13 ^a	49.04 ^a	51.10 ^a
CD (p<0.05)	0.95	0.76	0.58	1.29	0.90	0.75

Values with different letters in a column are significantly different at P<0.05
The superscripts indicate significance with respect to various

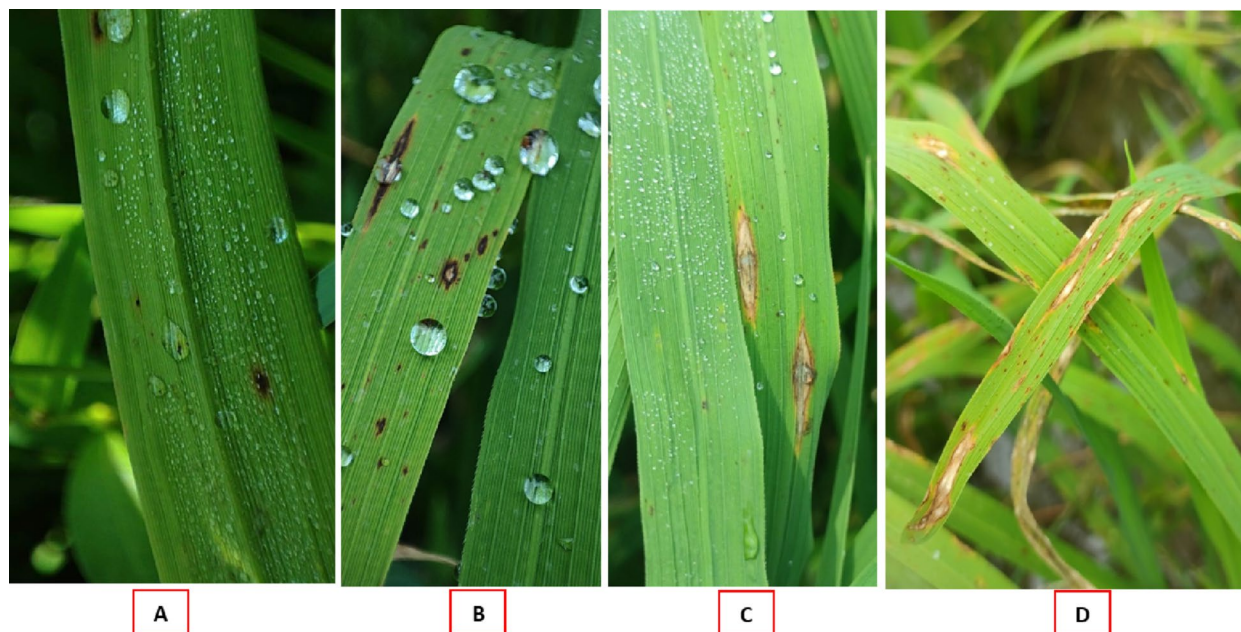


Fig. 3 Photographs with different stages of infection of rice blast pathogen on leaves, (A) Initial infection; (B) Enlargement of lesions; (C) Typical diamond shaped lesions; (D) Coalescing of lesion

levels in the following order: *Inula racemosa* (35.50%), *Allium sativum* (44.00%), *Heracleum candicans* (47.63%), and *Allium cepa* (47.37%). Untreated plots exhibited a severity of 53.13%. In the year 2023, Tricyclazole 75WP again showed the lowest disease severity at 17.21%, followed by *Syzygium aromaticum* (17.44%) and *Podophyllum hexandrum* (18.82%). The pooled analysis revealed that Tricyclazole 75 WP (18.90%), *Syzygium aromaticum* (20.78%), and *Podophyllum hexandrum* (21.13%) had statistically similar severity levels with a CD of 0.75 at $p < 0.05$, indicating their effectiveness in managing disease severity. Overall, while Tricyclazole 75 WP, *Syzygium aromaticum*, and *Podophyllum hexandrum* were identified as the most effective treatments against rice blast disease, *Heracleum candicans* and *Allium cepa* were found to be less effective, with varying degrees of effectiveness observed for other treatments like *Inula racemosa* and *Allium sativum* against the disease (Table 6 and Supplementary Figure-1).

ANOVA results demonstrated significant differences among treatments in disease severity ($F = 19.44$, $p < 0.001$) with no violation of normality (Shapiro-Wilk $W = 0.968$, $p = 0.623$). The LSD test was applied for mean separation, identifying significantly different treatment means at the 5% level. Tricyclazole 75WP, *Syzygium aromaticum*, and *Podophyllum hexandrum* had the lowest severity values, statistically similar, indicating effective disease reduction. Mean values with different letters indicate significant separation.

Grain yield

In terms of grain yield, *Syzygium aromaticum* treatment showed a notable increase from 41.91 qha^{-1} in 2022 to 42.13 qha^{-1} in 2023, highlighting its potential to enhance crop productivity. In 2022, the yields were as follows: *Syzygium aromaticum* (41.91 qha^{-1}), *Podophyllum hexandrum* (41.14 qha^{-1}), *Inula racemosa* (36.69 qha^{-1}), *Allium sativum* (31.75 qha^{-1}), *Allium cepa* (30.40 qha^{-1}), and *Heracleum candicans* (29.82 qha^{-1}). The control treatment yielded significantly lower at 26.02 qha^{-1} . In 2023, the grain yield varied among treatments, with Tricyclazole 75WP achieving the highest yield of 44.94 qha^{-1} . The yields for other treatments included *Syzygium aromaticum* (42.13 qha^{-1}) and *Podophyllum hexandrum* (41.78 qha^{-1}), both statistically similar to Tricyclazole with a critical difference of 4.86 at $p < 0.05$ (Table 7 and Supplementary Figure-2). Other yields were recorded as follows: *Inula racemosa* (38.48 qha^{-1}), *Allium sativum* (36.04 qha^{-1}), *Heracleum candicans* (34.63 qha^{-1}), and *Allium cepa* (32.10 qha^{-1}). The untreated control plots yielded the lowest at 30.16 qha^{-1} . Statistical analysis of pooled data showed that Tricyclazole 75WP consistently outperformed other treatments with a yield of 43.64 qha^{-1} , followed by *Syzygium aromaticum* (42.02 qha^{-1}) and *Podophyllum hexandrum* (41.46 qha^{-1}). The untreated control plots had the lowest grain yield at 28.09 qha^{-1} (Table 7 and Supplementary Figure-2). Treatment effects on grain yield were statistically significant based on ANOVA ($F = 6.70$, $p = 0.0004$). The LSD test was used for mean comparison, revealing that *Syzygium aromaticum*, *Podophyllum hexandrum*, and Tricyclazole 75WP

Table 7 Grain and Straw Yield recorded using efficient botanicals under field conditions during the years 2022 and 2023

Treatments	Grain Yield (q/ha)			Straw Yield (q/ha)		
	Year 2022	Year 2023	Pooled	Year 2022	Year 2023	Pooled
<i>Syzygium aromaticum</i>	41.91 ^a	42.13 ^a	42.02 ^a	66.31 ^{ab}	66.42 ^{abc}	66.37 ^b
<i>Allium sativum</i>	31.75 ^c	36.04 ^b	33.89 ^c	59.94 ^c	60.30 ^{bc}	60.12 ^c
<i>Heracleum candicans</i>	29.82 ^{de}	34.63 ^c	32.22 ^{cd}	65.09 ^b	58.89 ^{cd}	61.99 ^c
<i>Inula racemosa</i>	36.69 ^b	38.48 ^b	37.58 ^b	61.86 ^{bc}	61.63 ^{bc}	61.74 ^c
<i>Allium cepa</i>	30.40 ^{cd}	32.10 ^c	31.25 ^{cd}	64.44 ^{bc}	63.80 ^{abc}	64.12 ^{bc}
<i>Podophyllum hexandrum</i>	41.14 ^a	41.78 ^a	41.46 ^a	66.54 ^{ab}	66.55 ^{ab}	66.54 ^{ab}
Tricyclazole 75WP	42.35 ^a	44.94 ^a	43.64 ^a	71.01 ^a	70.52 ^a	70.77 ^a
Control	26.02 ^e	30.16 ^c	28.09 ^e	52.57 ^d	52.48 ^d	52.53 ^d
CD (p<0.05)	5.45	4.86	2.02	4.71	7.57	4.26

Values with different letters in a column are significantly different at $P < 0.05$

The superscripts indicate significance with respect to various Extracts

produced significantly higher grain yields compared to control and less effective treatments. Yields of these treatments were statistically similar, as indicated by shared superscripts. The untreated control had the lowest yield. The LSD critical difference was 2.02 at $p < 0.05$.

Straw yield

For straw yield, Tricyclazole 75WP again emerged as the superior treatment, maintaining high biomass production at 71.01 qha⁻¹ in 2022 and 70.52 qha⁻¹ in 2023 (Table 7 and Supplementary Figure-2). In comparison, the control treatment yielded significantly lower straw biomass at 52.57 qha⁻¹ and 52.48 qha⁻¹ for the respective years. Statistical analysis indicated substantial differences among treatments during both years, with *Syzygium aromaticum* yielding 66.31 and 66.42 qha⁻¹, *Podophyllum hexandrum* yielding 66.54 and 66.55 qha⁻¹, and *Inula racemosa* yielding 61.86 and 61.63 qha⁻¹, all demonstrating comparable efficacy. The pooled analysis revealed that Tricyclazole 75WP led with a straw yield of 70.77 qha⁻¹, followed closely by *Podophyllum hexandrum* (66.54 qha⁻¹) and *Syzygium aromaticum* (66.37 qha⁻¹). Other treatments like *Allium cepa* (64.12 qha⁻¹), *Inula racemosa* (61.74 qha⁻¹), *Allium sativum* (60.30 qha⁻¹), and *Heracleum candicans* (61.99 qha⁻¹) showed moderate straw yields, while the untreated control had the lowest straw yield at 52.53 qha⁻¹ (Table 7 and Supplementary Figure-2). ANOVA showed significant treatment effects on straw yield ($F = 6.70$, $p = 0.001$). The mean separation by LSD at 5% significance level showed Tricyclazole 75WP outperformed other treatments, followed closely by *Podophyllum hexandrum* and *Syzygium aromaticum*, which were statistically similar. Treatments such as *Allium cepa*, *Inula racemosa*, and *Allium sativum* produced moderate yields. The untreated control had the lowest straw yield. Treatment means sharing the same letters are not significantly different. The LSD value was 4.26 at $p < 0.05$.

Model validation and docking analysis

The Ramachandran plot analysis showed 86.5% of residues in the most favored regions and 13.5% in additionally allowed regions, with none in disallowed regions, indicating good stereochemical quality (Figure 5). A total of 74 residues were analyzed, including glycine and proline residues. MolProbity analysis further confirmed the structural validity with no steric clashes or outliers (Figs. 4 and 5; Table 8). Further binding sites predicted with PrankWeb are mentioned in Fig. 6. The docking results indicated that all six phytochemicals are bound to the ToxB-like effector of *Magnaporthe oryzae* with varying binding affinities. Quercetin demonstrated the strongest binding with a binding energy of -5.5 kcal/mol, followed closely by Podophyllotoxin (-5.3 kcal/mol). The weakest binding was observed for Allicin (-2.8 kcal/mol) (Fig. 7).

Discussion

The present study investigates the antifungal properties of various plant extracts sourced from both widely utilized and lesser-known herbal species native to the Himalayan region of Kashmir, India, against *Magnaporthe oryzae*. The in vitro assessments revealed that clove (*Syzygium aromaticum*) buds exhibited the most pronounced antifungal activity at concentrations of 2000, 3000, and 4000 ppm, significantly inhibiting mycelial growth. Other botanicals, including *Inula racemosa*, *Allium cepa*, *Allium sativum*, *Heracleum candicans*, and *Podophyllum hexandrum*, also demonstrated varying degrees of efficacy. Amongst the plant extracts, clove buds showed an enormous promise in reducing the mycelial growth of fungus, possible due to the presence of an active phenolic compound eugenol, that has been widely reported to inhibit the growth of various plant pathogenic fungi by disrupting their cell membrane, leading to leakage of cellular contents and eventual cell death [82–85]. The concentration-dependent effects observed indicate that higher concentrations generally yield greater inhibition

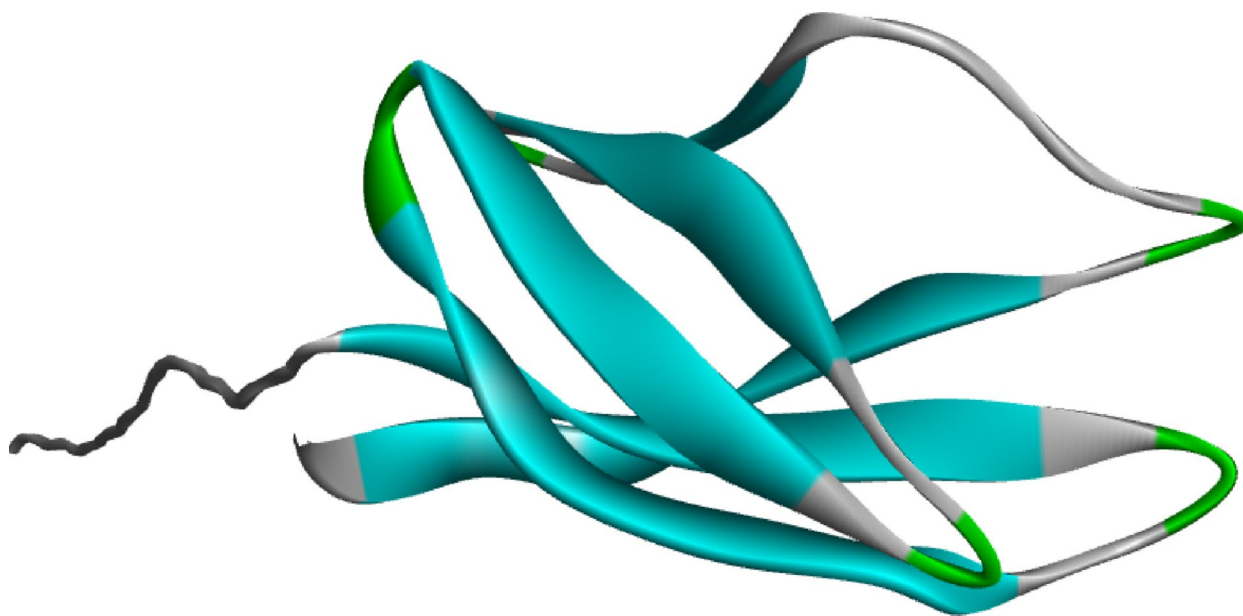


Fig. 4 SWISS-modelled structure of the ToxB-like (MAX) effector *Pyricularia oryzae*

rates at 7 days after inoculation (DAI), while at 14 DAI, similar trends were noted at 3000 ppm. This suggests that both the concentration of the active compounds and their inherent antifungal activities play crucial roles in their effectiveness [86, 87]. When active compounds are present in significant amounts, they maintain potent antifungal effects even at lower concentrations. Conversely, if these compounds are less abundant but highly effective, their antifungal activity becomes more pronounced as concentration increases [86, 87].

The antifungal efficacy of extracts from lesser-known Himalayan species such as *Inula racemosa*, *Heracleum candicans*, and *Podophyllum hexandrum* represents a novel contribution to the field. While these species have been recognized for their medicinal properties against human ailments, including cancer [88–90], their potential in managing plant diseases has not been extensively studied. This research is among the first to demonstrate their effectiveness against *Magnaporthe oryzae*, suggesting that bioactive compounds within these plants may disrupt essential cellular processes in fungal pathogens due to the presence of terpenoids, flavonoids, and other secondary metabolites known for their antimicrobial properties [89, 90]. Moderate antifungal activity was observed in extracts from *Allium cepa* and *Allium sativum*, attributed to specific organic sulfur compounds such as diallyl disulfide and allicin [91–93]. These sulfur-containing compounds are known to compromise fungal cell membrane integrity and inhibit enzymes involved in cell wall biosynthesis while inducing oxidative stress through reactive oxygen species generation [93–95].

The antifungal activity exhibited by extracts from the unexplored Himalayan plant species *Inula racemosa*,

Heracleum candicans, and *Podophyllum hexandrum* against the rice blast pathogen represents a novel finding. While the therapeutic potential of these species has been documented in traditional medicine systems, against various human diseases, including cancer [88–90], their specific roles in plant disease management remain largely unexplored. The present study is one of the first to demonstrate their efficacy against the phytopathogenic fungus, *Magnaporthe oryzae*. Several factors could contribute to the observed antifungal effects. The bioactive compounds present in these plant species may disrupt crucial cellular processes or structural components in the fungal pathogen, due to the presence of terpenoids, flavonoids, and other secondary metabolites in these plants, some of which are known to possess antimicrobial properties as revealed by the previous phytochemical analyses [89, 90]. However, the precise mechanisms by which these extracts impede fungal growth and pathogenesis remain to be clarified.

The extracts from onion and garlic depicted relatively moderate effectiveness against *M. oryzae* owing to the presence of some specific organic sulfur compounds. Previous studies have identified diallyl disulfide [91], allicin [92], and diallyl trisulfide [96] as active principle components contributing to the antifungal properties of these *Allium* species. These sulfur-containing compounds have been implicated in disrupting crucial cellular processes in various fungal pathogens like, disruption of cell membrane integrity, inhibition of enzymes involved in cell wall biosynthesis, and induction of oxidative stress through the generation of reactive oxygen species [94–96]. Of all the plant extracts evaluated in vitro, there were some instances where a few plant extracts showed

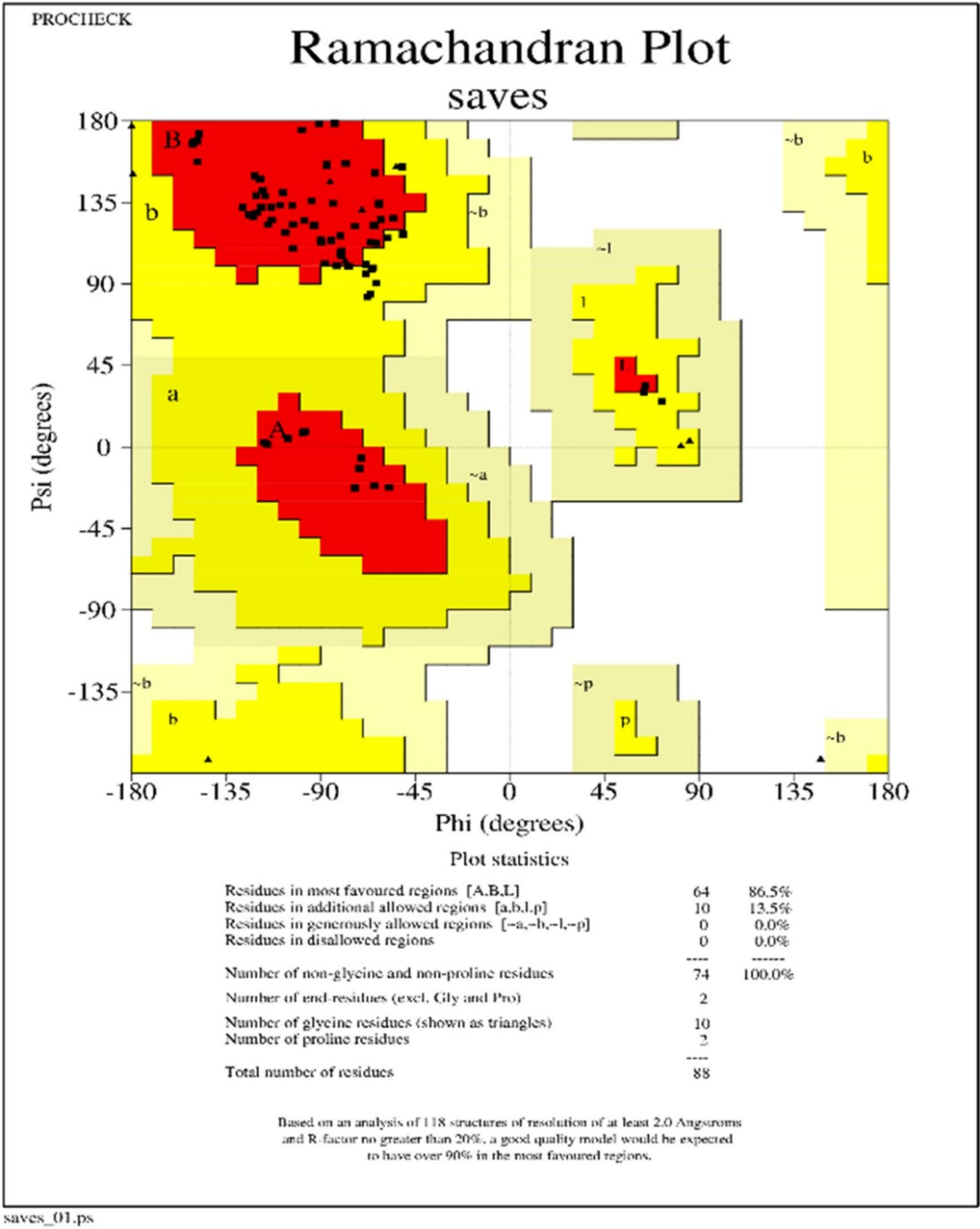


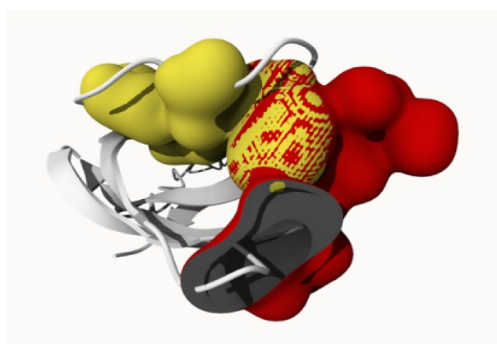
Fig. 5 Ramachandran plot of ToxB-like (MAX) effector *Pyricularia oryzae*

Table 8 MolProbity analysis of ToxB-like (MAX) effector

Geometry	Poor rotamers	1	1.43%	Goal: <0.3%
	Favored rotamers	67	95.71%	Goal: >98%
	Ramachandran outliers	1	1.16%	Goal: <0.05%
	Ramachandran favored	80	95.70%	Goal: >98%
	Rama distribution Z-score	−1.45 ± 0.95		Goal: abs(Z score) < 2
	Cβ deviations >0.25Å	1	1.28%	Goal: 0
	Bad bonds:	0/637	0.00%	Goal: 0%
	Bad angles:	3/867	0.35%	Goal: <0.1%
Peptide Omega	Cis Prolines:	0/2	0.00%	Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	0	0.0%	Goal: <1.0%
	CA Geometry outliers	0	0.00%	Goal: <0.5%
Additional validations	Chiral volume outliers	0/105		

reduced mycelial inhibition, at higher dosages, possibly due to the reason that those plant extracts may contain compounds that interfere with nutrient uptake or availability at higher concentrations, therefore, the fungus may be able to circumvent this limitation or utilize alternative nutrient sources, leading to reduced growth inhibition [97].

The field evaluation of the selected plant-derived ethanolic preparations revealed their differential efficacies against the economically important rice leaf blast disease caused by *M. oryzae*. Our findings indicate that the extract from clove buds (*Syzygium aromaticum*) exhibited superior potential in reducing disease severity, followed by extracts from *Podophyllum hexandrum*, *Hericium candicans*, onion, garlic, and *Inula racemosa*, in descending order of efficacy. These extracts significantly reduced disease intensity for the year 2022 and 2023, with clove and *Podophyllum* exhibiting comparable efficacy to the commercial fungicide Tricyclazole.



A_31 A_32 A_33 A_37 A_53 A_54 A_78

Fig. 6 Binding sites predicted with PrankWeb server

The superior performance of clove bud extracts aligns with previous reports highlighting the antifungal properties of eugenol and other phenolic constituents present in *S. aromaticum* [98, 99]. While the antifungal properties of clove extracts have been demonstrated against various fungal pathogens, however, the underlying modes of action responsible for this antifungal activity against the rice blast pathogen are yet to be determined. Previous studies on other fungal species have suggested that compounds like eugenol, a major constituent of clove, can induce structural alterations in fungal cell membranes, disrupt organelle integrity, and interfere with essential enzymatic processes [100]. However, the extent to which these mechanisms contribute to the antifungal effects observed in the rice blast pathosystem remains unclear. Similarly, the antifungal activity of *P. hexandrum* and *H. candicans* extracts could be attributed to the presence of bioactive components such as podophyllotoxins [101] and furanocoumarins [102, 103], respectively, which have been implicated in inhibiting fungal growth and development.

The reason for reduction of disease severity observed in our study may be linked to the fact that the extracts from these plants possess the potential to disrupt the life cycle of *M. oryzae*, either by directly inhibiting the growth of the fungal mycelium or by interfering with the spore germination [104] or disrupt the cellular redox balance [105] and induce oxidative stress [105] and penetration processes. While many studies have demonstrated the antifungal and antibacterial properties of plant extracts like garlic and onion [106, 107], there is still a lack of detailed research explaining the specific mechanisms by which these botanicals inhibit fungal and bacterial growth. Further, the underperformance or reduced efficacy of some plant extracts in managing rice blast in the field conditions could potentially be attributed to issues related to the stability of the active compounds present in these botanical preparations [108]. The superior performance of clove and garlic extracts was reflected in higher grain and straw yields, respectively, indicating effective mitigation of the pathogen's detrimental effects.

In this study, we investigated the interaction of six naturally occurring phytochemicals—Eugenol, Allicin, Germacrene D, Isoalantolactone, Podophyllotoxin, and Quercetin—with the ToxB-like effector (MAX family) protein of *M. oryzae* using molecular docking. These compounds were selected as major bioactive constituents of plant extracts that have previously shown promising in vitro antifungal activity against *M. oryzae* in our wet-lab experiments. Among the tested ligands, Quercetin and Podophyllotoxin exhibited the highest binding affinities (− 5.5 kcal/mol and − 5.3 kcal/mol, respectively), indicating strong interaction potential with the fungal effector protein. Quercetin is a flavonoid widely distributed

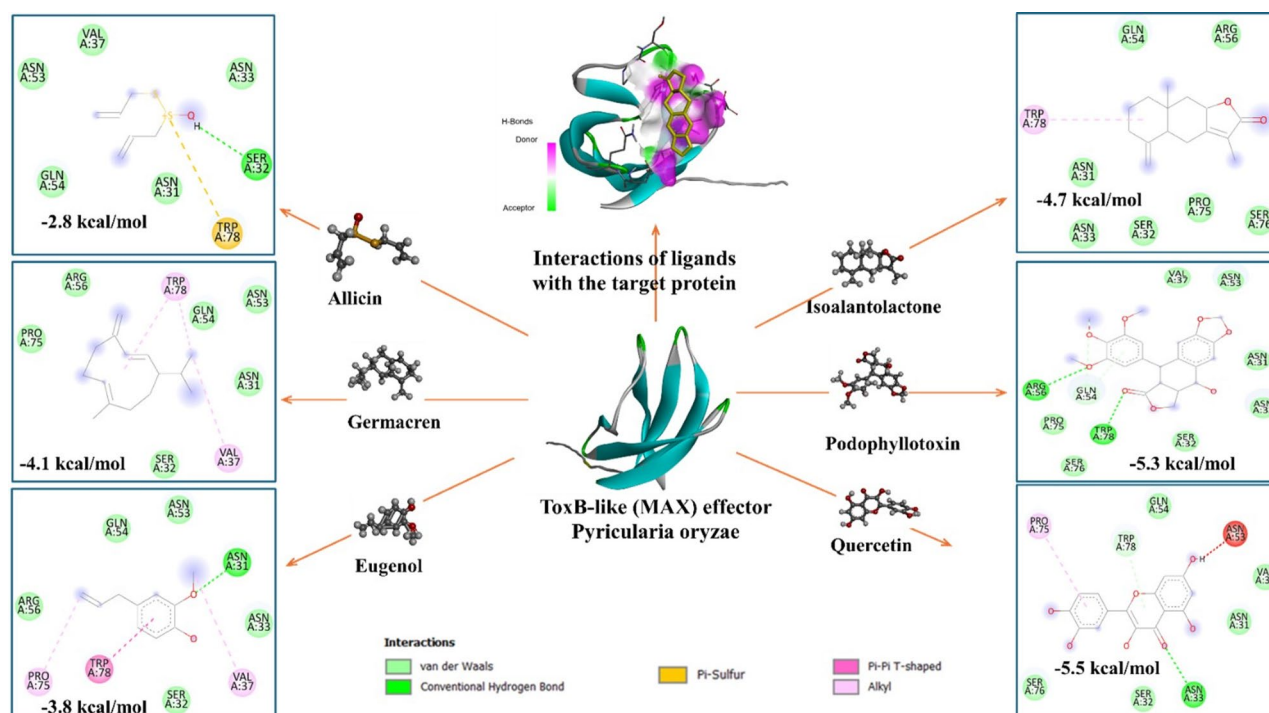


Fig. 7 Molecular docking analysis of selected ligands with the target protein. The central panel shows the 3D ribbon structure of the target protein with highlighted ligand binding regions. Surrounding the central panel are binding poses of different ligands (represented in ball-and-stick models) docked to the active site. For each ligand, the 2D interaction diagram displays key binding interactions with surrounding amino acid residues, along with the corresponding binding affinity (in kcal/mol). Types of interactions include van der Waals forces (green), conventional hydrogen bonds (light green), pi-sulfur interactions (yellow), pi-pi T-shaped interactions (pink), and alkyl interactions (lavender). Binding affinities range from -2.8 to -5.5 kcal/mol, indicating varied binding strengths of the ligands to the protein active site

in plants such as *Allium cepa*, *Capsicum annuum*, and *Camellia sinensis*, and is well-known for its antimicrobial and antioxidant properties. Its strong interaction with the ToxB-like effector, as revealed by docking, suggests that it may interfere with the fungal virulence mechanism, potentially inhibiting host invasion and colonization. Its well-documented antifungal activity further supports this hypothesis [109]. Podophyllotoxin, a lignan derived primarily from *Podophyllum hexandrum*, is known for its cytotoxic and antimicrobial properties. In our docking study, it showed one of the highest binding affinities to the fungal effector protein. Previous work by Anand et al. (2022) highlights its potent bioactivity and potential as a therapeutic agent [110]. The strong binding observed in silico reinforces the idea that Podophyllotoxin may be a key compound in the antifungal activity observed from *P. hexandrum* extracts in our wet-lab assays. Isoalantolactone, a sesquiterpene lactone isolated from *Inula racemosa*, demonstrated a binding affinity of -4.7 kcal/mol. It has been shown to possess significant antimicrobial and antifungal properties [111]. The moderate binding observed in this study aligns with its bioactivity profile and supports its potential role in suppressing fungal effectors involved in virulence. Germacrene D, a sesquiterpene hydrocarbon found in *Heracleum candicans* and

other aromatic plants, showed moderate docking affinity (-4.1 kcal/mol). Chauhan et al. (2018) reported the abundance of Germacrene D in essential oils with antimicrobial properties [112]. Though its interaction energy is lower than Quercetin or Podophyllotoxin, it may contribute synergistically when used as part of a complex plant extract, as reflected in our crude extract results [113, 114]. Eugenol, the main constituent of clove oil (*Syzygium aromaticum*), has well-known antifungal properties. It showed a docking score of -3.8 kcal/mol with the ToxB-like protein. According to Atif et al. (2024), Eugenol demonstrates significant inhibition of *Fusarium oxysporum*, suggesting a broad-spectrum antifungal potential [85]. Despite its relatively lower binding score, its role in disrupting fungal membranes and signaling pathways makes it a plausible candidate for biocontrol [115–117]. Allacin, a sulfur-containing compound from garlic (*Allium sativum*), showed the lowest binding affinity (-2.8 kcal/mol) in our study. Nevertheless, its antifungal potential, as reported by El-Saber Batiha et al. (2020), includes the ability to oxidize thiol groups in microbial enzymes, contributing to cell death [118, 119]. Though the direct binding to ToxB may be weak, Allacin likely acts through complementary mechanisms in a natural context [120, 121]. Therefore, the use of plant extracts derived from

natural sources could potentially moderate the negative influences associated with the indiscriminate use of synthetic fungicides, such as environmental pollution, development of resistance in pathogens, and potential harm to non-target organisms. Our study does not report any negative impact of the botanical treatments on beneficial soil microbes or plant-associated microorganisms. While the primary assessments focused on disease control and crop performance, the botanical extracts selected are considered environmentally safe based on published reports and traditional use. It is important to note that the observed antifungal activity was obtained using crude extracts, which implies that the purification and isolation of the active compounds could potentially enhance their efficacy. The findings have broader potential applications. Several botanicals and phytochemicals tested are known to possess activity against diverse phytopathogenic fungi. Since the mechanism of action involves inhibition of fungal growth and pathogenesis, these extracts could be adapted for use in other crops after tailored efficacy testing. There is a need for more studies addressing the stability, formulation, and potential synergistic effects with other bio-control agents or integrated pest management strategies that would be beneficial in translating these findings into practical applications.

Mushk Budji rice, distinguished by its exquisite aroma and distinct taste, holds a crucial place in the agricultural heritage of Kashmir. The results highlight the diverse impacts of different treatments on disease incidence, disease severity, grain and straw yield, providing valued understandings for optimizing agricultural practices. The variability observed between years emphasizes the importance of considering temporal dynamics in agricultural research. The botanical extracts investigated as alternatives to chemical fungicides like Tricyclazole, have demonstrated comparable efficacy. In specific instances, such as in the case of Clove and *Podophyllum* during the year 2022, their disease severity percentages were found to be very close to that of Tricyclazole. Additionally, in the subsequent year, in addition to Clove, and *Podophyllum*, *Heracleum* and other botanicals showed disease severity percentages in a closer range to Tricyclazole, suggesting that these botanicals can be considered as effective alternatives to chemical fungicides. The results from in-depth analyses and field experiments demonstrate the potential efficacy of botanical interventions in mitigating disease impact while adhering to the principles of organic farming. In our prior wet-lab assays, crude extracts from these six plant sources exhibited strong antifungal effects against *M. oryzae*. Considering that the tested phytochemicals are major constituents of these extracts, we hypothesize that these molecules play a central role in the observed suppression of fungal growth. The docking interactions with the ToxB-like effector protein suggest

that these compounds may disrupt effector-mediated host-pathogen interactions, a key step in disease development. This computational validation complements our experimental results and provides molecular-level insight into the possible mode of action of these biomolecules. Future directions should include molecular dynamics simulations, expression analysis of effector genes, and in planta trials to establish their efficacy and mechanism more definitively. Thus, there is a need for more studies addressing the stability, formulation, and potential synergistic effects with other bio-control agents or integrated pest management strategies that would be beneficial in translating these findings into practical applications. The use of bio-control agents derived from natural sources could potentially moderate the negative influences associated with the indiscriminate use of synthetic fungicides, such as environmental pollution, development of resistance in pathogens, and potential harm to non-target organisms. This highlights the importance of considering alternative treatments to optimize straw yield in agricultural practices. Therefore, in the near future, more focus and effort will need to be put into producing of more commercially available botanical fungicides if we are to move towards the production of safer agricultural products. The botanicals evaluated are especially suited for smallholder farmers due to their regional availability, affordability, and eco-friendly nature. Most extracts can be formulated locally, reducing reliance on expensive commercial fungicides. While scaling up for large-scale farms may require development of stable, commercial-grade formulations and more rigorous safety assessments, smallholders can readily adopt these botanicals as part of integrated, organic management systems.

Supplementary Information

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Supplementary Material 1

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Author contributions

Zakir Amin and Fayaz A. Mohiddin designed the study and led the manuscript writing. Suhail Ashraf contributed to molecular docking experiments and performed data analysis along with Fehim Jeelani Wani, Tauseef A. Bhat, Shugufta Parveen, and Aasiya Qadir. Zakir Amin, Fayaz A. Mohiddin, Shugufta Parveen, Tauseef A. Bhat, Arif Bashir, Rajapandiyar Krishnamoorthy and Tariq A. Raja conducted botanical extract evaluations and field experiments. Sajad Un Nabi, Fazil Fayaz Wani, Heena Altaf, Arif Bashir and Rajapandiyar Krishnamoorthy assisted with sample collection and laboratory assays. Fayaz (A) Mohiddin, Asif (B) Shikari, Tauseef A. Bhat, Fehim Jeelani Wani, Tariq A. Raja,

and Vishal Gupta provided supervision throughout the study. Heena Altaf and Fazil Fayaz Wani, Arif Bashir contributed to literature review and manuscript editing. Vishal Gupta, Arif Bashir and Rajapandiyan Krishnamoorthy helped revise the manuscript.

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Data availability

The data generated during the study is available with the manuscript. No additional data was generated.

Declarations

Competing interests

The authors declaring no conflict of interest.

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