PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 12(1): 1-13
https://doi.org/10.14719/pst.4657

HORIZON
e-Publishing Group
HePG

REVIEW ARTICLE

A comprehensive review on brown spot disease of rice: Etiology, epidemiology, management strategies and future directions

Tenzing Phurbu Bhutia[1], Yenda Damodhara Rao[2], Priyanka Kharel[1], Prayashma Rai[3], Meenakshi Rana[1] & Seweta Srivastava[1*]

[1]School of Agriculture, Lovely Professional University, Phagwara 144 411, Punjab, India

[2]Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur 208 002, Uttar Pradesh

[3]Department of Forestry, Dolphin PG Institute of Biomedical and Natural Sciences, Dehradun 248 007, Uttarakhand

OPEN ACCESS

ARTICLE HISTORY
Received: 16 August 2024
Accepted: 18 January 2025
Available online
Version 1.0 : 05 March 2025
Version 2.0 : 18 March 2025

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Bhutia PT, Rao YD, Kharel P, Rai P, Rana M , Srivastava S. A comprehensive review on brown spot disease of rice: Etiology, epidemiology, management strategies and future directions. Plant Science Today. 2025; 12(1): 1-13. https://doi.org/10.14719/pst.4657

Abstract

Rice (*Oryza sativa* L.) is the second largest staple grain among cereal crops, feeding more than half of the worlds' population. Many fungal diseases damage rice crops, leading to considerable yield loss. *Bipolaris oryzae*, the teleomorph *Cochliobolus miyabeanus*, is the cause of brown spots on rice, a global problem known to significantly reduce grain production up to 52 % quantitatively and qualitatively. Under conditions of direct seeding, drought and low input management, the brown spot disease is most significant. The disease is also historically important as it caused a disastrous outbreak in the Bengal Province that culminated in the Great Bengal Famine (1943), which left 2.1 to 3 million people starved to death. The brown spot remains terrible when considering the current scenario for rice deterioration. A broad host range, pathogenicity and molecular diversity characterize the pathogen. In this present article, we have emphasized the epidemiology, the prevention techniques that are currently in use and several quantitative and qualitative gaps regarding disease management that, if filled, would have a significant impact on crop disease control and the long-term sustainability of rice and are relevant to farmers' current circumstances.

Keywords

Bipolaris oryzae; brown spot; epidemiology; etiology; management; rice

Introduction

Oryza sativa L., a staple food for more than half the population of the world, is under threat from the brown spot of rice disease- a fungal disease that can ruin rice crops worldwide (1). Since 1900, brown spots on rice have been documented in Japan as a result of *Bipolaris oryzae* Subr. and Jain (= *Helminthosporium oryzae* Breda de Haan telemorph=*Cochliobolus miyabeanus*). It is also called "nai-yake," which includes Helminthosporiosis, sesame leaf spot and seedling blight (2). All of the countries that grow Rice, including China, Japan, Burma, Bangladesh, Sri Lanka, Africa, Iran, Russia, South America, North America, Saudi Arabia, Philippines, Australia, Thailand and Malaya, have been reported to have the disease (3, 4). Since its initial report from Madras in 1919 by Sundraraman, it has been discovered to appear in all five of Indias' rice-growing states. The disease is majorly found in dry, direct, seeded rice in Bihar, Madhya Pradesh, Chhattisgarh, Orissa, Jharkhand, Assam and West Bengal (5). Brown spot affects rain-fed and upland rice production, causing grain quality and yield losses by 6-90 % (6).

Brown leaf spot disease on rice causes rice leaves and stem necrosis, interrupting photosynthesis and weakening the plants' immune system. This may lead to substantial decreases in yield and lower grain quality, causing serious trouble for food security (7). Brown spot of rice disease is not simple, but rather complex and multi-dimensional because this disease develops due to environmental factors, malnutrition and crop residues. Brown spot of rice disease develops better under warm and humid conditions, spreading rapidly during heavy downpours (8). Nutrient deficiency or environmental stress increases their vulnerability to brown spot of rice disease infection in stressed rice plants. The residue can also host brown spots of rice disease fungus, which acts as a reservoir of inoculum for further invasion during crop season (9).

Poor farmers' disease is a term used to describe conditions that are typically associated with resource-poor farmers' fields. The disease results in a decrease in grain yield that is both quantitative and qualitative (10). It mainly occurs in environments where water supply is scarce and is often combined with imbalances in plant mineral nutrition, specifically a lack of nitrogen and a deficit of silicon (6, 11). Timely transplantation of rice (between July–August) and sufficient nitrogen levels enhance rice growth and minimize *C. miyabeanus* infection. Si is presumably not essential for plant growth and development. Applying various Si sources to Si-deficient paddy soils dramatically reduces the incidence and severity of brown spots caused by *C. miyabeanus*. Si mounts resistance to *C. miyabeanus* by preventing the fungus from hijacking the rice ethylene machinery (12).

Resistance is one of the best and most environmentally safe ways of controlling disease. To utilize resistance in the best possible way, it is essential to understand how the pathogen infects and how the plant defends itself against infection (13). Understanding the dynamics of the brown spot of rice (*Bipolaris oryzae*) at the molecular and genetic levels requires novel tools and techniques to elucidate pathogen behaviour, host-pathogen interactions and disease progression. Emerging imaging tools and high-throughput molecular technologies can significantly enhance understanding of the pathogens' life cycle, virulence factors and the plants' defence responses (14). Biosensors using CRISPR-based diagnostics to detect *B. oryzae* at early infection stages by targeting specific virulence genes in *B. oryzae* to study their roles in pathogenicity (15). Quantitative PCR (qPCR) detects and quantifies *B. oryzae* in rice tissues with high sensitivity and enables early detection of infection before visible symptoms appear (16). Loop-Mediated Isothermal Amplification (LAMP) provides a rapid and field-deployable diagnostic tool for testing pathogens and is also very useful for monitoring pathogen spread in rice fields (17).

The main goals of this review paper are to gather and summarize current information and studies on brown spot disease in rice, providing insights into its causes, spore dispersal, the various methods used to manage it and to present potential research areas and strategies that can improve the prevention, management and reduction of the disease in rice farming. Through a comprehensive analysis of existing knowledge, this article aims to provide a holistic understanding of brown spot disease while suggesting future directions for further study and more effective approaches to combatting the disease in rice cultivation.

Pathogen

The fungus was initially identified by Breda de Haan in 1900 as *Helminthosposium oryzae*, which Subramanian and Jain later changed to *Drechslera oryzae* (18). The bipolar characteristics of conidial germination led Shoemaker (1959) to propose changing *Helminthosporium oryzae* to *Bipolaris oryzae* (19, 20). Nonetheless, other researchers noticed that the fungal conidia also grew all around, leading Subramanian and Jain (18) to identify the fungus as *Drechslera oryzae*. The kind of conidial germination is influenced by the age or maturity of the conidia (21). The fungus was discovered to have a teleomorph in culture named *Ophiobolus miyabeanus* (22). However, later, the fungus moved to the genus *Cochliobolus*, which became *Cochliobolus miyabeanus*.

The graminicolous *Helminthosporium* species was split into the subgenera *Eu-Helminthosporium* and *Cylindro -Helminthosporium* (23). While species with fusiform and curved conidia germinated only from end cells were put in the subgenus *Eu-Helminthosporium* and species with straight cylindrical conidia that germinate with one or more germ tubes from any cell were placed in the old subgenus *Cylindro-Helminthosporium* (24, 25). *Drechslera* accommodated previously in subgenus *Cylindro-Helminthosporium* (26). A characteristic of *Drechslera* to produce a germ tube from any of the cells in the distoseptate conidia, as shown in Fig. 1, sets it apart from all other graminicolous helminthosporoid taxa (25, 27). *Bipolaris* and *Drechslera* may additionally be distinguished from one another by helium morphology. The lowest portion of the basal cell in *Drechslera* has a flat scar, while in *Bipolaris*, it is either undetectable or very slightly protuberant (25). While the sexual forms of *Bipolaris* were thought to belong to *Cochliobolus*, the sexual forms of

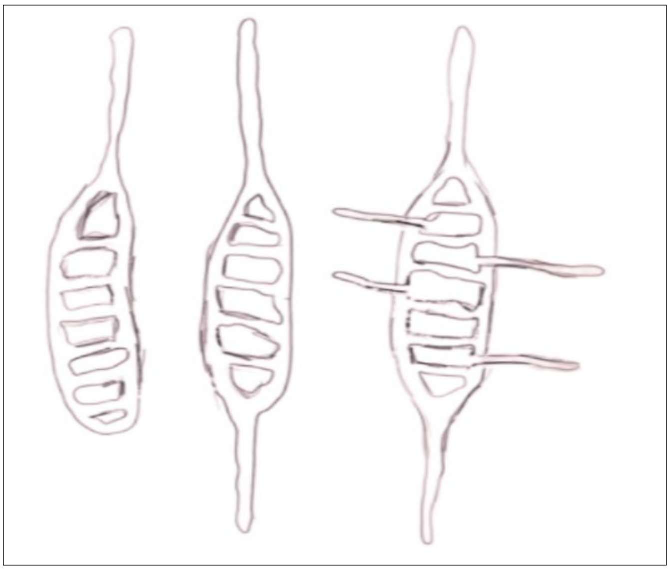

Fig. 1. Diagrammatic representation of distoseptate conidia showing germination in (A) *Helminthosporium*, (B) *Bipolaris*, (C) *Drechslera*.

Drechslera have been connected to *Pyrenophora* (14, 28).

Symptoms and diagnostics

Brown spot is a devastating fungal disease that can damage rice and is caused by several fungi and among them, *Bipolaris oryzae* represents one of the most significant (9). The symptoms manifest as tiny, variable-sized spots on the glumes, leaf blades, leaf sheath and coleoptile (Fig. 2). The glumes and leaf blades exhibit the most significant number of these spots (29–30). On the leaves, mature spots are small spherical and could be similar to dark brown or purplish brown dots (31). Typical spots are cylindrical or oval, brown with a grey or whitish centre, resemble mustard seeds and usually have a yellow halo around them. The damaged nursery can be immediately recognized from a distance due to the seedlings' burned look (32).

The symptoms on coleoptiles and leaf sheaths are identical to those on leaves (2). The most noticeable small brown lesions are ellipsoidal or oval, measuring around 2.8 mm by 0.5 mm and they are found on aerial parts, including glumes, leaf sheaths, coleoptiles and blades. When the leaves reached maturity, lesions with a dark-reddish brown border and a light-brown or greyish centre were observed. Severe infection is characterized by spotting of grains and browning of seeds; the infected grains may also wilt (9). Brown colour to dark brown coloured lesions (5-15 × 1-2 mm) on the stalk of the panicle at the flag leaf to stalk joint has also been linked to the disease. Typically, these lesions spread below the sheath, causing partial filling to chaffy dull grains, severe wet rotting and occasionally hanging panicles. In severe cases, greyish mycelial development can be detected between the sheath and stalk. When an infection spreads higher, dry rot symptoms appear (2, 33).

Etiology of the Causal Organism

The fungus grows inter- and intracellular mycelium on the diseased tissues, which turns into a mat that ranges from dark brown to grey. Its colour ranges from grey to olive to black in culture. Conidiophores can arise single or in clusters, be branching or simple, multiseptate, erect or flexuous, geniculate and lighter at the tip and dark at the base of the olivaceous stem. They emerge through stomata. *Bipolaris oryzae* isolates have been found to have conidiophores and conidia that range in size from 70-175 × 5.6-7 µm and 45-106 × 14-17 µm in India, respectively. Conidia are typically curved, seldom straight, navicular, fusiform, obclavate, or nearly cylindrical; while juvenile, they are hyaline, but when mature, they turn slightly brown and they have five to ten distoseptations (Fig. 3). Hilum, jutting out a little, germinates along the length of the conidia. Certain conidia exhibit secondary sporulation (2).

Cochliobolus miyabeanus has been studied by various scientists. The perithecium of the fungus was defined as globose to depressed globose with a dark yellowish brown pseudoparenchymatous outer wall (22). The asci are cylindrical to long fusiform, most commonly 4 or 8 in number, clavate or broadly fusoid. The ascospores are coiled, hyaline or pale olive-green, filamentous or long cylindrical (2).

Toxin Production

Ophiobolins (Ophs) are the sesterterpene-type (C_{25}) compounds produced by fungal species belonging to the genera *Bipolaris, Cochliobolus, Drechslera, Cephalosporium* and *Aspergillus* (34) (Table 1). Their structure is characterized by a specific tri- or tetracyclic ring system (35). Thus far, forty-nine types of natural Ophs have been reported, assigned into 23 subgroups (36). Ophiobolins are derived from isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), the basic five-carbon units in terpenoid biosynthesis. These are isoprenoid precursors that act as a building block and are involved in the biosynthesis of terpenoids (37). IPP is an intermediate in the 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase pathway and the methylerythritol

Fig. 3. Microscopic image of distoseptate conidia of *Bipolaris oryzae*, Scale bar 50 µm.

Fig. 2. Symptoms of brown spot shown in grains and leaves.

Table 1. Ophiobolin production by various fungi

S. No.	Ophiobolins	Pathogen	Perfects stage	Host	Reference
1	Ophiobolin A	*Bipolaris oryzae* *Bipolaris sorghicola* *Bipolaris maydis*	*Cochliobolus miyabeanus* *Cochliobolus carbonum* *Cochliobolus heterostrophus*	*Oryzae sativa* *Sorghum bicolor* *Zea mays*	41
2	6-epi-ophiobolin A	*B. maydis* *Bipolaris oryzae*	*Cochliobolus heterostrophus* *Cochliobolus miyabeanus*	*Zea mays* *Oryzae sativa*	42 43
3	3-anhydro ophiobolin A	*Bipolaris setariae* NY1 *Bipolaris oryzae*	*Cochliobolus setariae* *Cochliobolus miyabeanus*	*Setaria viridis* *Oryzae sativa*	44 45
4	3-anhydro-6-epi-ophiobolin A	*Bipolaris setariae* NY1	*Cochliobolus setariae*	*Setaria viridis*	46
5	3-anhydro-6-hydroxy-Ophiobolin A	*Bipolaris oryzae*	*Cochliobolus miyabeanus*	*Oryzae sativa*	47
6	Ophiobolin A lactone	*Bipolaris oryzae*	*Cochliobolus miyabeanus*	*Oryzae sativa*	48
7	Ophiobolin B	*Bipolaris maydis* *B. oryzae*	*Cochliobolus heterostrophus* *Cochliobolus miyabeanus*	*Zea mays* *Oryzae sativa*	42
8	Ophiobolin B lactone	*Bipolaris oryzae*	*Cochliobolus miyabeanus*	*Oryzae sativa*	36
9	Ophiobolin C	*Bipolaris zizaniae* *B. Maydis*	*Cochliobolus miyabeanus* *Cochliobolus heterostrophus*	*Zizania latifolia, Oryzae sativa* *Zea mays*	49
10	Ophiobolin D	-	*Cephalosporium caerulen*	*Orzya rufipogon*	50
11	Ophiobolin E	*Drechslera gigantea*	*Cochliobolus intermedius*	*Digitaria sanguinalis*	51
12	Ophiobolin F	*Bipolaris maydis*	*Cochliobolus heterostrophus*	*Zea mays*	52
13	Ophiobolin G	*Aspergillus ustus*	*Emericella* spp.	*Saccharum officinarum*	53
14	Ophiobolin H	*Aspergillus ustus*	*Emericella* spp.	*Saccharum officinarum*	53
15	Ophiobolin I	*Bipolaris oryzae* *B. maydis* *B. sorghicola*	*Cochliobolus miyabeanus* *Cochliobolus heterostrophus* *Cochliobolus carbonum*	*Oryzae sativa* *Zea mays* *Sorghum bicolor*	54
16	Ophiobolin J	*Bipolaris oryzae*	*Cochliobolus miyabeanus*	*Oryzae sativa*	55
17	Ophiobolin K	*Aspergillus ustus*	-	*Saccharum officinarum*	56
18	Ophiobolin L	*Bipolaris maydis*	*Cochliobolus heterostrophus*	*Zea mays*	57
19	Ophiobolin M	*Bipolaris maydis*	*Cochliobolus heterostrophus*	*Zea mays*	58
20 21	Ophiobolin O 6-epi-ophiobolin O	*Aspergillus* sp.	-	Marine derived fungus	59
22	Ophiobolin P-T	*Ulocladium* sp.	-	Endolichenic fungus	35
23	Ophiobolin U-W	*Aspergillus ustus*	-	Algicolous fungus	60

phosphate (MEP) pathway, while DMAPP is a product of both paths. Geranylfarnesyl pyrophosphate synthase (GFPP synthase) enzyme catalyzes the condensation of IPP and DMAPP to form geranylfarnesyl pyrophosphate (GFPP), a precursor for ophiobolin synthesis. Another enzyme, *viz.,* ophiobolin synthase, a specialized terpene synthase likely encoded by a specific gene cluster, converts GFPP into the characteristic tricyclic ophiobolin skeleton (38). After the initial formation of the tricyclic sesterterpene skeleton by ophiobolin synthase, enzymes catalyze various cyclization steps (37). Environmental conditions like temperature, pH, nutrient availability and light influence ophiobolin synthesis. Enzyme activity involved in ophiobolin biosynthesis, such as terpene synthases, is susceptible to temperature and pH. Deviations from optimal ranges can inhibit enzyme functionality and reduce yield. Among the environmental factors regulating fungal metabolism, light also plays a crucial role. The white light allowed the highest production of the metabolite. The blue and green lights showed an inhibitory effect, reducing the output to 50 %, as well as red and yellow but at a lower level (39).

Ophiobolins function as virulence factors, aiding the pathogen in suppressing plant defences and inducing cell death in host tissues. The ophiobolins are phytotoxic and cause inhibition of root and coleoptile elongation and leaf chlorosis on many plants, including hosts and non-hosts of *Bipolaris oryzae* (13). Both resistant and susceptible cultivars had fast cell death after being treated with conidial germination fluid (CGF) from *C. miyabeanus*, indicating that phytotoxins are present in CGF. Reduced levels of total carotenoids (Car), Chlorophyll (Chl) and Chl a + b/Car ratio are brought on by brown spot infection. After the beginning of the condition, a considerable decrease in biochemical components such as sodium, potassium, magnesium, calcium, iron and total soluble sugar was seen in rice plants (8). The *H. oryzae* toxin-induced electrolyte loss from rice plants' coleoptiles, leaves and roots. A rise in toxin concentration was accompanied by an increase in electrolyte discharge from rice tissue and phenol metabolism was also hampered (40). Within one hour of exposure, ophiobolin prevented tissues from absorbing exogenous $^{32}PO_4$. After 30 minutes of exposure to ophiobolin, rice root hair cells lost their capacity to plasmolyze. The overall amount of photosynthetic pigments in rice leaves decreases due to brown spot disease. Maintaining plants' net CO_2 assimilation rate depends on the leaf pigment concentration and membrane integrity. As the concentration of pigments decreases and plant tissues integrate rapidly in disease occurrence, it will drastically reduce the yield and quality of grains. In plant cells, ophiobolin A can prevent the synthesis of proteins and nucleic acids or function as an antagonist for α-1,3-glucan synthetase (8).

Epidemiology

The initial appearance of brown spot symptoms and the intensity of the disease are highly impacted by epidemiological factors. The brown spot disease has been considered one of the most critical global rice diseases, being distributed in all rice-producing territories from tropical to temperate areas (61). The condition is more pronounced in Asia, especially causing a 45 % percentage of yield loss. Various factors determine the intensity of brown spot disease outbreaks, including environmental conditions and host susceptibility (61, 62).

Warm and humid conditions are favourable for brown spot disease. The fungus *Bipolaris oryzae* thrives and spreads most effectively in temperatures between 16 and 36°C and a relative humidity of over 85 %. The disease spreads rapidly when continuous rain, cloudy weather and high day temperature couples. It has been found that the optimum temperatures for conidial germination and growth are 25-30°C and 27-30°C, respectively. Conidia form at temperatures ranging from 5 to 38°C, with 25°C being ideal and for ascospore formation, it is 20-24°C. However, the sexual stage is not typical (63). Conidia germinate at pH levels ranging from 2.6 to 10.9, with a preferred range of 6.8 to 7.0. *B. oryzae* sporulation required both light and dark phases. It was, however, enhanced by near-ultraviolet light and hindered by blue light (2). Overall, temperature does not appear to influence BS epidemics (64). However, influential factors on infection efficiency include temperature and humidity, which are expressed as leaf wetness. That could be why more severe epidemics occur when daily minimum temperatures drop from 9.3 to 7.5 ºC (65). The host leaf has to remain wet for 8 to 24 hr to establish the pathogen (66). The disease is limited to areas with uneven rainfall distribution and heavy dew (67). Wind can spread the disease to new places by dispersing *Bipolaris oryzae* spores. The spores of *Bipolaris oryzae* can get trapped in fog, leading to a higher incidence of the disease among rice plants. Rice varieties with shorter leaves and thinner leaf sheaths are prone to brown spot infection (62). Brown spot disease is more likely in high-nitrogen fertilizers used in rice-growing areas. Nitrogen fertilization enhances the prevalence and severity of *Bipolaris oryzae* infection in rice fields (61).

Mode of Survival and Disease Cycle

The primary transmission mode for brown spot disease is seed-borne and the secondary inoculum is predominantly disseminated by airborne conidia, as mentioned in Fig. 4 (68, 69). The disease can live in additional weed hosts and diseased plant debris (70). According to reports, the pathogens' spores are present in the air throughout the year, with cooler seasons being when they are more likely to survive and disperse (71). The appressoria penetrates quickly the host epidermis and starts its infection (69). Some of the metabolites secreted by the host plants, like glutamic acid, aspartic acid, methionine and alanine, act as a stimulating agent for establishing the *Helminthosporium oryzae*. Glutamic acid and aspartic acid are carbon and nitrogen sources for *Bipolaris oryzae,* supporting its growth and proliferation. In contrast, alanine acts as a substrate for fungal metabolism, providing energy for infection processes because high alanine levels in the apoplast can attract fungal hyphae, enhancing pathogen colonization. *B. oryzae* may utilize methionine-derived metabolites to synthesize toxins that enhance virulence (72).

Necrotic lesions on the coleoptile and the sheath of the first leaves typically indicate an initial infection (68, 73). The secondary infection caused by airborne spores from the primary lesions is primarily responsible for the subsequent leaf lesions (69). *Bipolaris oryzae* spores were disseminated throughout the year, although they were more numerous in the cooler, more humid months (71).

Management Strategies

Various tactics for managing brown spots of rice have been tried, including using appropriate cultural practices and fungicides, introducing resistant or moderately resistant cultivars, tissue culture techniques and biocontrol measures. Late planting after mid-August during the primary Kharif season and before February in rabi and using N in high dosages should be avoided, especially when not combined with phosphorus or potassium. It has also been suggested to adopt the practice of destroying and cleaning the diseased stubble of the previous (infected) rice crop that was left in the field after harvest, especially in places where the brown spot is endemic. It has been demonstrated that using N in slow-release dosage lowers the incidence of this

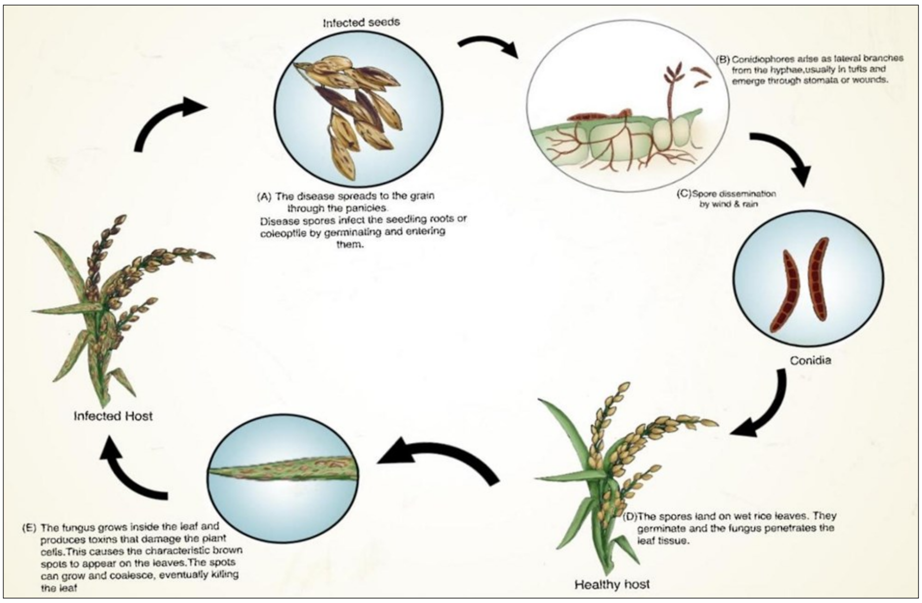

Fig. 4. Disease cycle of brown spot disease in rice.

disease (74).

Host resistance

Managing resistance is among the most effective and ecologically sound disease control methods (13, 75). Due to host plant tolerance to the disease, this is the most economical method of managing brown spots in rice. Formerly, diseases like bacterial blight and blast were the main focus of breeding research. Nevertheless, the brown spot disease of rice requires significant efforts to combat it. Inoculation techniques must be 100 % effective in identifying the origins of resistance (76). Though different resistance levels have been discovered in cultivars, no genes for total resistance to brown spots in rice have yet been found (13). Indian CH45 indica cultivar exhibits a high degree of partial resistance and has also demonstrated resistance in Japan (77, 82). The primary resistance mechanism is based on quantitative trait loci (QTLs) that provide different degrees of protection, which may change in response to shifting environmental factors (6, 78). Efforts have been made to locate and utilize quantitative trait loci (QTL) for brown spot resistance since 2008. Even though several QTLs for BS resistances have been found, most of them could not account for more than 30 % of the phenotypic variation found in the investigation (79).

The host-specific toxin of *Helminthosporium oryzae* has also been utilized to identify plants or calluses resistant to brown spots. In field experiments, several exotic and native upland rice germplasm genotypes from eastern India have demonstrated partial and complete resistance to the brown spot disease. Three rice genes-Spl7, Spl11 and Spl18-have been cloned and characterized concerning brown rice spots. These genes activate downstream defence responses, including producing pathogenesis-related (PR) proteins and secondary metabolites by enhancing pathogen recognition and subsequent immune responses. Contrary to widespread assumption, physiological stresses on rice crops, such as dryness and low soil fertility, produce brown spots, a secondary problem rather than an infectious disease. The advantages of silicon for rice yield, kernel quality and increased resistance to brown spots in paddy grains (80). Brown spot, a significant disease caused by the fungus *Bipolaris oryzae*, can be managed effectively in rice fields by applying slag-based silicon (Si) fertilizer (81).

Additionally, according to the study, a rise in Si content in the husks was promoted by the functioning Lsi1 gene. Phenylalanine Ammonia-lyase (PAL), Catalase (CAT), Polyphenol Oxidase (PPO), Peroxidase (POD) and β-1,3-Glucanase were the enzymes with the lowest quantities in the vulnerable rice genotypes, with the lowest amounts reported in the paddy variety Bas-2000 (80 % Disease Index). Based on the distinct defensive reaction between susceptible and resistant genotypes, these enzymes could be employed as biochemical indicators to identify disease-resistant genotypes (8). Larger epidermal cells and more silicate cells are two examples of rice plants' structural characteristics that have been positively correlated with resistance to brown spot disease (82). According to reports, the youngest leaf is the most resistant, while the second is the most vulnerable. Susceptibility and dry weight, total nitrogen, free amino acids and total protein inflated. Cultivars resistant to the brown spot had a more significant oxidation-reduction potential (Eh) in the leaf sap, which was altered by nutrient deficiencies or excesses, as well as the addition of H_2S or other reducing agents (2).

Biocontrol

It is well established that the continuous, improper and non -discriminatory consumption of pesticides can lead to unfavourable outcomes such as residual toxicity, the emergence of pathogen resistance to fungicides, contamination of the surroundings, risks to the well-being of humans and animals and higher costs associated with plant protection. Instead, plant pathologists have concentrated on creating long-lasting, reliable and ecologically friendly biocontrol strategies for managing plant diseases. *Trichoderma* spp. and *Pseudomonas fluorescens* were the most commonly employed fungal and bacterial biocontrol agents against various plant diseases, including brown spots of rice (Table 2). Based on research conducted over the past 20 years, biological control appears to be a more viable strategy for managing brown spot disease of rice (3).

Commercially accessible bioagents, *i.e.*, *P. fluorescens* and *Trichoderma* species, can reduce disease either directly on the pathogen by competition for nutrients and iron, mycoparasitism and antibiosis or indirectly through enhanced plant immunity via induced resistance (67). Isolates of fluorescent *Pseudomonas* from soil inhibited fungal growth and the occurrence of brown spots. Spraying talc-based *P. fluorescens* has been shown to reduce brown spot severity (83-84). *Bacillus megaterium* suppressed the

Table 2. Efficacy and mode of action of different biocontrol agents against brown spot pathogen

Biocontrol agent	% of Inhibition	Cost (Rs/ha)	Mode of action	References
Bacillus megaterium	90-100 %	15000-22500	Antibiosis, siderophore production	88z
Bacillus subtilis	60-70 %	2000-3000	Antibiosis, competition for resources	88
Beauveria bassiana	70-80 %	4000-6000	Infection	83
Chaetomium globosum	70-80 %	3000-5000	Antibiosis	95
Flavobacterium sp.	80-90 %	4000-6000	Antibiosis, competition for resources	96
Gliocladium virens	80-90 %	5000-7500	Parasitism	89
Paenibacillus sp.	70-80 %	3000-5000	Antibiosis, induced systemic resistance	97
Pseudomonas fluorescens	70-80 %	3000-5000	Antibiosis, competition for resources, induced systemic resistance	86
Serratia marcescens	80-90 %	2000-3000	Antibiosis, competition for resources	98
Streptomyces avermitilis	80-90 %	4000-6000	Antibiosis	99
Streptomyces griseoviridis	80-90 %	5000-7500	Antibiosis	100
Trichoderma harzianum	70-80 %	7500-15000	Antibiosis, competition for resources, mycoparasitism	83
Verticillium lecanii	60-70 %	2000-3000	Infection	101

development of *B. oryzae* at 1 × 10^4 bacterial cells/ml with an ED50 value of 1 × 10^3 cells/ml in a field experiment (85). It has been demonstrated that *Trichoderma* spp. enhance nutrient uptake and mobilization, as well as nitrogen utilization efficacy. *Trichoderma* also increases plant biomass and root growth and strengthens resistance against soil salinity and drought (86, 87). *Cladosporium* spp. was the most efficient phylloplane microorganism in reducing *B. oryzae* mycelial development and spore germination, followed by *Penicillium* spp. and *Aspergillus flavus* (88). It has additionally been shown that treating rice leaves with *Trichoderma harzianum* foliar reduces the severity of this disease and significantly increases grain yield, total grain proteins and carbohydrates and total photosynthetic pigment (89). *Bacillus amyloliquefaciens* can effectively control rice brown spot disease strain BS5 (90). *Bacillus amyloliquefaciens* employs several strategies as a biocontrol agent to combat plant infections. These mechanisms encompass plants' capacity to manufacture various antibiotics, the pathogens' direct suppression, the encouragement of plant development and the activation of defensive enzymes (91). Brown spot severity was 90 % reduced by *Bacillus cereus, Priestia megaterium, Stenotrophomonas nitritireducens, Serratia marcescens and Serratia nematodiphila* and the radial colony formation of *Bipolaris oryzae* was slowed down suggests that these isolates may be helpful in the biocontrol of brown spot in rice (4).

Research on biological control using microorganisms is experiencing and gaining remarkable momentum, although applications in the field are still limited (92). The factors determining the use of BCAs in the field include the inconsistent efficiency in protecting plants under field conditions, decreased availability in the market and farmers' wide unacceptance of BCAs. To ensure effective biocontrol, it is critical to choose effective agents in various situations, including soil texture, wetness, temperature extremes and competition (93). In addition to the factors mentioned earlier, the need for scientists to publish harmful data and their ability to do so appears to be critical to the success of biological control. The researchers will be able to determine biopesticide weaknesses such as lack of efficacy, uneven field performance and/or unfavourable economic considerations to tackle them in the future (94).

Effect of botanicals

The adoption of alternative disease management techniques is rapidly gaining popularity to reduce the harmful effects of agrochemicals and promote sustainable food production (93). These techniques focus on using organic plant oils and extracts, rich sources of antimicrobial active chemicals that can be applied to integrated disease treatment (102). Plant extracts are an outstanding source of novel agrochemicals with broad antibacterial spectrum qualities for managing plant diseases (103). Plants provide plentiful substances containing novel, physiologically active compounds with antibacterial qualities with thousands of elements (104). Certain plants can produce aromatic secondary metabolites, such as phenols, phenolic acids, quinones, flavones, flavonoids, tannins, coumarins and so on (105). Carvacrol, eugenol and thymol are compounds with phenolic structures that are very effective against pathogens (104). Because plant extracts have different roles in managing diseases, ethnobotanical research on plants is essential for advancing medicine. Plant extracts are inexpensive and practical, lessen risks associated with chemicals and are environmentally beneficial, making them readily available to farmers. It is evident from reading numerous articles that various plant extracts can be employed for managing plant diseases (106).

Some essential oils and phytochemicals in the planting materials containing organosulphur, which was sourced from garlic (*Allium sativum* L.) showed very high efficacy in controlling brown spot disease of rice caused by *Bipolaris oryzae*. This bulb possesses several sulfur compounds, such as allicin, ajoene and diallyl sulfides, all of which have massive antifungal properties. These compounds affect the structural composition of fungal cells, preventing the growth of these fungi and their potential harm. For example, allicin, the principal bioactive compound of garlic, has been investigated for its broad-spectrum antimicrobial potentials, with the studies showing effectiveness against many plant pathogens (107). Several studies have established that garlic extract could influence the funguss' target cell wall and membrane, showing that it is a potential fungicide for agricultural use (108). Some other plants used for the extracts against brown spots of rice are *Azadirachta indica, Nerium oleander, Pithecellobium dulce, Curcuma longa, Solanum indicum, Calotropis gigantean, Mimordica charantia, Vitis quadrangularis, Vinca rosea, Astercantha longifolia, Solanum nigrum, Cymbopogon citratus, Thymus vulgaris, Ocimum gratissimum, Chromolaena odorata, Callistemon citrinus, Embilica officinalis* and *Pachira glabra* enumerate in Table 3.

Plant extracts are very eco-friendly and straightforward to use. Still, there are a few drawbacks when managing plant diseases, such as relatively quick deterioration, the fact that formulations are not readily available commercially and the fact that they work less well than chemicals (109). Variations in the chemical composition of botanicals due to different plant sources, harvesting conditions and extraction methods reduce their reliability. Standardization of extraction techniques and active compound quantification is crucial. Botanicals often degrade quickly under environmental conditions like rain, UV light and temperature fluctuations. Developing stable formulations through encapsulation technologies, such as nanoemulsions or micro-encapsulation, can improve their shelf life and field efficacy (110). Adding adjuvants that improve spread, adhesion and penetration can make botanicals more comparable to synthetic agrochemicals. Advanced delivery systems, such as bio-based carriers or slow-release formulations, can enhance the bioavailability and persistence of botanicals on crop surfaces (111).

Disease management by fungicides

Though applying these chemical fungicides in the field might not always be ideal, chemical treatment may significantly and successfully lessen the impact of brown spot disease on young plants. The environment, animal and

Table 3. Different botanical extracts used to manage brown spots in rice

Botanical	Common name	Part of plant	Application	% of inhibition	References
Abutilon indicum	India abutilon, monkey bush	Seeds, roots and leaves	Aqueous extract	55.5 %	112
Aloe vera Mill.	Ghrito kumari	Leaf pulp and leaf gel	Aqueous extract	37.6 %	112
Azadirachta indica	Neem	Bark extracts, fruits, seeds, leaves	Oil cake extracts	81.13 %	112
Callistemon citrinus	Crimson bottlebrush	Flower, leaves and stem	Essential oil application	77-100 %	113
Calotropis gigantean	Crown flower	Roots and leaves	Aqueous extract	60-70 %	112
Chromolaena odorata	Bitter bush	Leaves	Aqueous extract	57 %	113
Curcuma longa L.	Turmeric	Rhizome (root)	Aqueous extract	68.8 %	112
Cyanodon dactylon L.	Bermuda grass	Leaves, roots and rhizome	Aqueous extract	52.6 %	112
Cymbopogon citratus	Fever Grass, Lemon grass	Leaves	essential oils application	57 %	113
Lawsonia inermis,	Henna	Stem bark, roots, flowers and seeds)	Aqueous extract	76.4 %	114
Madhuca longifolia	Mahua	Flowers, fruits, roots, bark, seeds and leaves	Oil cake extracts	71.41 %	112
Mimordica charantia	Bitter melon	Fruit	Aqueous extract	60-70 %	112
Nerium oleander	Kaner, oleander, rosebay, rose bay, rosebay, rose laurel	Seeds and leaves	Aqueous extract	75 %	112
Ocimum gratissimum	Basil, basil-clove, or alfavaca.	Leaves	Essential oil application	57 %	113
Phyllanthus niruri L.	Gale of the wind	Leaves and roots	Aqueous extract	71 %	112
Pithecellobium dulce	Madras thorn	Pulp and seed	Aqueous extract	76.4 %	114
Ricinus communis	Castor	Seed	Oil cake extracts	64.82 %	112
Schistosoma indicum	Sesame and til	Roots	Aqueous extract	60-70 %	112
Sesamum indicum	Gingelly	Leaves, stem and roots	Oil cake extracts	47.37 %	112
Solanum indicum L.	African eggplant	Roots and seeds	Aqueous extract	78.5 %	112
Thymus vulgaris	Common thyme	Flowers, leaves and oil	Essential oil application	57 %	113
Vettiveria zyzanoides L.	Vetiver grass	Roots, stem and leaves	Aqueous extract	61.3 %	112
Vinca rosea	Madagascar periwinkle	Leaves	Aqueous extract	60-70 %	112
Vitex negundo L.	Chinese chaste tree	Leaves, flowers, twigs, roots and seeds	Aqueous extract	69.9 %	112
Vitis quadrangularis	Veldt grape	Roots and stems	Aqueous extract	60-70 %	112

human health are all at risk when these fungicides are used excessively and improperly. The cost of many of these chemicals is likewise prohibitive for the resources of underprivileged farmers (115). Several researches on the application of artificial fungicides to manage the disease known as brown leaf spot have been published. The four different fungicides are carbendazim 50WP, carboxin 50 WP, propiconazole 25 EC and hexaconazole 25 EC @ 500ppm concentrations (116). Propiconazole proved to be the best-performing fungicide, preventing 96.58 % of fungal growth in laboratory conditions. Research indicates experimental work on the antifungal activity of several synthetic fungicides under *in vitro* conditions (117). Propiconazole proved to be the most efficacious chemical, with notable outcomes also demonstrated at 0.75 ppm concentration by tebuconazole + trifloxystrobin and pyraclostrobin + epoxyconazole. The spread of the fungal mycelium of *Bipolaris oryzae* was shown to be significantly inhibited by difenoconazole 25 % EC at a 200 ppm concentration (118). Numerous chemical fungicides have already been investigated against brown spots of Rice under lab and field conditions. Mycelial development is effectively inhibited by dithane M-45, kitazin, hinosan thiram, shield, foltaf, ridomil, bitoxazol and PP 296; other potent inhibitors include tridemorph, edifenphos and triadimenol (119). Hexaconazole (EC 50, 0.11 ppm concentration) and propiconazole (EC 50, 0.42 ppm concentration) were the most repressive to the growth of mycelium (33). In addition, strobilurins, including azoxystrobin, trifloxystrobin and kresoxim methyl, are effective inhibitors of pathogen mycelial growth (120). Iprodione is another such strobilurin. Numerous techniques for applying fungicides have been established (121). It has been demonstrated that foliar applications are more successful in suppressing brown spots than seed treatments or root dips (122). It has been shown that edifenphos, aureofungin and dithane M-45 as foliar sprays can help prevent airborne infections and the spread of secondary infections. After treating seeds with tricyclazole (0.4 %), 0.25 % mancozeb + 0.08 % tricyclazole was sprayed on top. The brown spot was effectively controlled by treating seeds with thiram (2 g/kg) and three sprays of ridomyl MZ (123). The combination of propiconazole+thiophanate methyl was effective in managing brown spots of rice when applied under greenhouse conditions during pre-inoculation, post-inoculation and after symptoms appearance, respectively, compared to control (122). Grain output was increased and leaf spot and stalk rot stages were substantially reduced by spray application with hexaconazole and propiconazole at initial booting and 50 % panicle emergence of panicle stage (33). Brestan (fentin acetate) + dithane M-45 (mancozeb) in 1:5 proportions as a 0.2 % spray at heading and during grain maturation provided reasonable disease control. Furthermore, it was discovered that antibiotics such as versicolin and mycobacillin work well as foliar and seed protectants against *C. miyabeanus*. The drug that reduced the severity of the condition the most was Armure 30 EC (0.1 %), followed by Tilt 25 EC (124). Three safe phenolic antioxidants- salicylic acid, benzoic acid and hydroquinone-were investigated against rice brown spot as a potential substitute for chemical fungicides (115). At low doses, compounds such as propiconazole and azoxystrobin showed growth inhibition of *Bipolaris*, indicating their potential for treatment of the corresponding disease (125).

With the high cost and potential harm associated with chemical fungicides, it is crucial to prioritize sustainable alternatives for disease management. To sustainably control brown spot disease in rice, smallholder farmers can adopt an integrated management approach combining cultural, biological and chemical strategies. Cultural practices include using resistant varieties, seed treatment with biological agents like *Trichoderma*, balanced nutrient management (avoiding excessive nitrogen while enhancing potassium and silicon), proper plant spacing and water management to reduce plant stress. Field sanitation, crop rotation and residue removal help minimize pathogen reservoirs. Biological control options, such as applying antagonistic microorganisms like *Pseudomonas fluorescens* or beneficial endophytes, further suppress the disease while promoting plant health. Limited and judicious use of environmentally friendly fungicides can complement these measures when necessary. Training farmers on disease monitoring, group-based pest management and leveraging ICT tools for early warnings ensure community-wide adoption of sustainable practices while reducing health and environmental risks.

Conclusion

Brown spot disease is a complex challenge that threatens the rice supply. It is caused by a fungus that can infect all the parts of the rice plant, including leaves and seeds. This disease has consequences leading to reduced crop yields and lower grain quality. The impact extends beyond food security; it affects the livelihoods of farmers and communities worldwide who depend on rice. Despite the obstacles presented by spot disease, there are promising avenues for finding resilient solutions. In this review paper, we explore the diseases' origins, symptoms, impact and management strategies. This can be achieved through breeding techniques or advanced methods like marker-assisted selection. Another crucial approach involves implementing pest management (IPM) strategies. IPM combines methods like practices, biological control measures and limited use of pesticides to minimize disease risk.

Furthermore, it is vital to customize interventions based on contexts and communities. Climate conditions, agricultural practices and cultural norms should be considered when designing intervention plans. To tackle this challenge, stakeholders, researchers and policymakers must come and collaborate. By joining forces, we can create a foundation for rice production. Guarantee food security for billions of individuals across the globe.

Acknowledgements

I want to express my sincere gratitude to the Lovely Professional University for the invaluable digital assistance during the research and writing of this review paper. The library's extensive online resources, databases and support services significantly enhanced the quality and depth of the information presented in this work.

Authors' contributions

All authors made equal contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflicts of interest: The authors report no financial or other conflicts of interest in this work.

Ethical issues: This study does not involve experiments on animals or human subjects.

References

1. Das D, Chowdhury N, Sharma M, Suma R, Saikia B, Velmurugan N, Chikkaputtaiah C. Screening for brown–spot disease and drought stress response and identification of dual–stress-responsive genes in rice cultivars of Northeast India. Physiol Mol Biol Plants. 2024;30:647–63. https://doi.org/10.1007/s12298-024-01447-4
2. Sunder S, Singh RAM, Agarwal R. Brown spot of rice: an overview. Ind Phytopatho. 2014;67(3):201–15.
3. Khalili E, Sadravi M, Naeimi S, Khosravi V. Biological control of rice brown spot with native isolates of three *Trichoderma* species. Braz J Microbiol. 2012;43(1):297–305. https://doi.org/10.1590/S1517-83822012000100035
4. Ajulo AA, Bezerra AG, de Oliveira RS, Gonçalves AR, Moura MFGG, Cardoso MAA, de Filippi MCC. Efficiency of bacterial isolates on suppressing brown spot in rice plants. J Phytopathol. 2024;172(2):e13294. https://doi.org/10.1111/jph.13294
5. Dhruw S, Sahu S. Assessment of brown spot disease incidence and severity in rice cultivation across different region of Chhattisgarh. The Pharma Innov J. 2023;12(11):1663–7.
6. Barnwal MK, Kotasthane A, Magculia N, Mukherjee PK, Savary S, Sharma AK, et al. A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps. Eur J Plant Pathol. 2013;136:443–57. https://doi.org/10.1007/s10658-013-0195-6
7. Surendhar M, Anbuselvam Y, Johnny ISJ. Status of rice brown spot (*Helminthosporium oryzae*) management in India: A review. Agric Rev. 2022;43(2):217–22. https://doi.org/10.18805/ag.R-2111
8. Chavan SS, Sawant K, Aspak, Kumar S, Shukla M, Rana M, Srivastava S. Brown spot: An embryonic disease of rice. Agrica. 2023;11:172–81. https://doi.org/10.5958/2394-448X.2022.00023.2
9. Shamshad A, Rashid M, Hameed A, Arshad IHM. Identification of biochemical indices for brown spot (*Bipolaris oryzae*) disease resistance in rice mutants and hybrids. PLoS One. 2024;19(4):e0300760. https://doi.org/10.1371/journal.pone.0300760
10. Ashfaq B, Arshad HMI, Atiq M, Yousaf S, Saleem K, Arshad A. Biochemical profiling of resistant phenotypes against *Bipolaris oryzae* causing brown spot disease in rice. Front Agron. 2021;3:675895. https://doi.org/10.3389/fagro.2021.675895
11. Cao FY, Zeng Y, Lee AR, Kim B, Lee D, Kim ST, Kwon SW. OsFBN6 enhances brown spot disease resistance in rice. Plants. 2024;13:3302. https:// doi.org/10.3390/plants13233302

12. Van Bockhaven J, Spíchal L, Novák O, Strnad M, Asano T, Kikuchi S, et al. Silicon induces resistance to the brown spot fungus *Cochliobolus miyabeanus* by preventing the pathogen from hijacking the rice ethylene pathway. New Phytol. 2015;206:761–73. https://doi.org/10.1111/nph.13270

13. Thuy TTT, Lübeck M, Smedegaard–Petersen V, de Neergaard E, Jørgensen HJL. Infection biology of *Bipolaris oryzae* in rice and defence responses in compatible and less compatible interactions. Agron. 2023;13:231. https://doi.org/10.3390/agronomy13010231

14. Nizamani MM, Zhang Q, Muhae–Ud–Din G, Wang Y. High–throughput sequencing in plant disease management: a comprehensive review of benefits, challenges and future perspectives. Phytopathol Res. 2023;5:44. https://doi.org/10.1186/s42483–023–00199–5

15. Chen K, Shen Z, Wang G, Gu W, Zhao S, Lin Z, et al. Research progress of CRISPR–based biosensors and bioassays for molecular diagnosis. Front Bioeng Biotechnol. 2022;10:986233. https://doi.org/10.3389/fbioe.2022.986233

16. Hariharan G, Prasannath K. Recent advances in molecular diagnostics of fungal plant pathogens: A mini-review. Front Cell Infect Microbiol. 2021;10:600234. https:// doi.org/10.3389/fcimb.2020.600234

17. Prasannakumar MK, Parivallal PB, Pramesh D, Mahesh HB, Raj E. LAMP–based foldable microdevice platform for the rapid detection of *Magnaporthe oryzae* and *Sarocladium oryzae* in rice seed. Sci Rep. 2021;11(1):178. https:// doi.org/10.1038/s41598–020–80644–z

18. Subramanian CV, Jain BL. A revision of some graminicolous *Helminthosporia*. Curr Sci. 1966;35:352–55.

19. Shoemaker RA. Nomenclature of *Drechslera* and *Bipolaris*, grass parasites segregated from '*Helminthosporium*'. Can J Bot. 1959;37:879–87. https://doi.org/10.1139/b59-073

20. Srivastava S. Plant pathology–compare and contrast. New Delhi: Kalyani Publishers; 2019

21. Drechsler C. Phytopathological and taxonomic aspects of *Ophiobolus*, *Pyrenophora*, *Helminthosporium* and a new genus, *Cochliobolus*. Phytopathol. 1934;24:953–85.

22. Ito S, Kuribayashi K. Production of the ascigerous stage in culture of *Helminthosporium oryzae*. Annals Phytopathol Soc Japan. 1927;2:1–8. https://doi.org/10.3186/jjphytopath.2.1

23. Nisikado Y. Studies on *Helminthosporium* diseases of Graminae in Japan (in Japanese). Reports of the Ohara Institute of Agricultural Research. 1928;4:1–384.

24. Nisikado Y. Studies on the *Helminthosporium* diseases of Gramineae in Japan. Berichte Des Ohara Institute Landwirtschaftliche Forschungen. 1929;4:111–26.

25. Alcorn JL. The taxonomy of *Helminthosporium* species. Ann Rev Phytopatho. 1988;26:37–56. https://doi.org/10.1146/annurev.py.26.090188.000345

26. Ito S. On some new ascigerous stages of the species of *Helminthosporium* parasitic on cereals. Supplement of Proceedings of the Imperial Academy of Japan. 1930;6:352–55. https://doi.org/10.2183/pjab1912.6.352

27. Sivanesan A. Graminicolous species of *Bipolaris*, *Curvularia*, *Drechslera*, *Exserohilum* and their teleomorphs. Mycol Pap. 1987;158:1–261.

28. Alcorn JL. On the genera *Cochliobolus* and *Pseudocochliobolus*. Mycotaxon. 1983;16:353–79.

29. Keerthana U, Phalguni M, Prabhukarthikeyan SR, Naveenkumar R, Yadav MK, Parameswaran C, et al. Elucidation of the population structure and genetic diversity of *Bipolaris oryzae* associated with rice brown spot disease using SSR markers. 3 Biotech. 2022;12(10):281. https://doi.org/10.1007/s13205-022-03347-4

30. Maurya AK, John V, Pant H, Srivastava DK. Rice brown spot disease (*Helminthosporium oryzae*): Ecology, Epidemiology and Identification Measures. In: Srivastava D, Shamim M, Mobeen MA, Upadhyay RS, editors. Fungal diseases of rice and their management, New York: Apple Academic Press; 2024. p. 223–34 https://doi.org/10.1201/9781003332169-11

31. Terensan S, Fernando HNS, Silva JN, Perera SCN, Kottearachchi NS, Weerasena OJ. Morphological and molecular analysis of fungal species associated with blast and brown spot diseases of *Oryza sativa*. Plant Dis. 2022;106:12–16. https://doi.org/10.1094/PDIS-04-21-0864-RE

32. Livitha R, Christopher DJ. Morphological and molecular characterization of *Bipolaris oryzae* causing brown leaf spot of rice in Cauvery delta region of Tamil Nadu. The Pharma Innov J. 2023;12(10):1011–6.

33. Sunder S, Singh R, Dodan DS, Mehla DS. Effect of different nitrogen levels on brown spot (*Drechslera oryzae*) of rice and its management through host resistance and fungicides. Plant Dis Res. 2005;20(2):111–4.

34. Bencsik O, Papp T, Berta M, Zana A, Forgó P, Dombi G, et al. Ophiobolin A from *Bipolaris oryzae* perturbs motility and membrane integrities of porcine sperm and induces cell death on mammalian somatic cell lines. Toxins (Basel). 2014;6(9):2857–71. https://doi.org/10.3390/toxins6092857

35. Wang QX, Bao L, Yang XL, Liu DL, Guo H, Dai HQ, et al. Ophiobolins P–T, five new cytotoxic and antibacterial sesterterpenes from the endolichenic fungus *Ulocladium* sp. Fitoterapia. 2013;90:220–7. https://doi.org/10.1016/j.fitote.2013.08.002

36. Tian W, Deng Z, Hong K. The biological activities of sesterterpenoid–type ophiobolins. Mar Drugs. 2017;15(7):229. https://doi.org/10.3390/md15070229

37. Chai H, Yin R, Liu Y, Meng, H, Zhou, X, Zhou, G, et al. Sesterterpene ophiobolin biosynthesis involving multiple gene clusters in *Aspergillus ustus*. Sci Rep. 2016;6:27181. https:// doi.org/10.1038/srep27181

38. Yuan W, Lv S, Chen L, Zhao Y, Deng Z, Hong K. Production of sesterterpene ophiobolin by a bifunctional terpene synthase in *Escherichia coli*. Appl Microbiol Biotechnol. 2019;103(21–22):8785–97. https:// doi.org/10.1007/s00253–019–10103–x

39. Fanelli F, Reveglia P, Masi M, Mulè G, Zonno MC, Cimmino A, et al. Influence of light on the biosynthesis of ophiobolin A by *Bipolaris maydis*. Nat Prod Res. 2017;31(8):909–17. https://doi.org/10.1080/14786419.2016.1253084

40. Srivastava S, Chandrapati A, Gupta A, Rana M, Karnwal A, Katyayani KKS, et al. Toxins in plant pathogenesis: Understanding the role of toxins in host–pathogen interaction. J Appl Biol Biotechnol. 2024;12(4):47–61. https://doi.org/10.7324/JABB.2024.156511

41. Canales MW, Gray GR. 6–Epiophiobolin A and 3–anhydro–6–epiophiobolin A–host specific phytotoxins of *Drechslera maydis* (race T). Phytochem. 1988;27(6):1653–63. https://doi.org/10.1016/0031-9422(88)80421-7

42. Shen X, Krasnoff SB, Lu SW, Dunbar CD, O'Neal J, Turgeon BG, et al. Characterization of 6–epi–3–anhydroophiobolin B from *Cochliobolus heterostrophus*. J Nat Prod. 1999;62(6):895–97. https://doi.org/10.1021/np980462e

43. Phuwapraisirisan P, Sawang K, Siripong P, Tip–pyang S. Anhydrocochlioquinone A, a new antitumor compound from *Bipolaris oryzae*. Tetrahedron Lett. 2007;38:5193–95. https://doi.org/10.1016/j.tetlet.2007.05.151

44. Zhao XL, Niu YC, Deng H, Luo DQ. Secondary metabolites from *Bipolaris setariae* NY1 strain. Mycosystema. 2013;32:286–91.

45. Xiao JZ, Tsuda M, Doke N, Nishimura S. Phytotoxins produced by germinating spores of *Bipolaris oryzae*. Phytopathol. 1991;81:58–64. https://doi.org/10.1094/Phyto-81-58

46. Zhao XL, Niu YC, Deng H, Luo DQ. Characterization and phytotoxicity of ophiobolins produced by *Bipolaris setariae*. Mycosci. 2021;62(1):64–70. https://doi.org/10.47371/mycosci.2020.11.002

47. Wang QX, Yang JL, Qi QY, Bao L, Yang XL, Liu MM, et al. 3–Anhydro–6–hydroxy–ophiobolin A, a new sesterterpene inhibiting the growth of methicillin–resistant *Staphylococcus aureus* and inducing the cell death by apoptosis on K562, from the phytopathogenic fungus *Bipolaris oryzae*. Bioorg Med Chem Lett. 2013a;23:3547–50. https://doi.org/10.1016/j.bmcl.2013.04.034

48. Rossi C, Tuttobello L. A new natural ophiobolin. Tetrahedron Lett. 1978;19(3):307–08. https://doi.org/10.1016/S0040-4039(01)85110-8

49. Nozoe S, Hirai K, Tsuda K. The structure of zizannin–A and–B, C25–terpenoids isolated from *Helminthosporium zizaniae*. Tetrahedron Lett. 1966;7(20):2211–16. https://doi.org/10.1016/S0040-4039(00)72403-8

50. Nozoe S, Itai A, Tsuda K, Okuda S. The chemical transformation of cephalonic acid. Tetrahedron Lett. 1967;8(42):4113–17. https://doi.org/10.1016/S0040-4039(01)89702-1

51. Evidente A, Andolfi A, Cimmino A, Vurro M, Fracchiolla M, Charudattan R, Motta A. Ophiobolin E and 8–epi–ophiobolin J produced by *Drechslera gigantea*, a potential mycoherbicide of weedy grasses. Phytochem. 2006;67(20):2281–87. https://doi.org/10.1016/j.phytochem.2006.07.016

52. Nozoe S, Morisaki M, Fukushima K, Okuda S. The isolation of an acyclic C25–isoprenoid alcohol, geranylnerolidol and a new ophiobolin. Tetrahedron Lett. 1968;9(42):4457–58. https://doi.org/10.1016/S0040-4039(01)99159-2

53. Cutler HG, Crumley FG, Cox RH, Springer JP, Arrendale RF, Cole RJ, Cole PD. Ophiobolins G and H: New fungal metabolites from a novel source, *Aspergillus ustus*. J Agri and Food Chem. 1984;32(4):778–82. https://doi.org/10.1021/jf00124a019

54. Liu M, Sun W, Shen L, Hao X, Al Anbari WH, Lin S, et al. Bipolaricins A–I, ophiobolin–type tetracyclic sesterterpenes from a phytopathogenic *Bipolaris* sp. fungus. J Nat Prod. 2019;82(10):2897–906. https://doi.org/10.1021/acs.jnatprod.9b00744

55. Sugawara F, Takahashi N, Strobel G, Yun CH, Gray G, Fu Y, Clardy J. Some new phytotoxic ophiobolins produced by *Drechslera oryzae*. J Org Chem. 1988;53(10):2170–72. https://doi.org/10.1021/jo00245a008

56. Singh SB, Smith JL, Sabnis GS, Dombrowski AW, Schaeffer JM, Goetz MA, Bills GF. Structure and conformation of ophiobolin K and 6–epiophiobolin K from *Aspergillus ustus* as a nematocidal agent. Tetrahedron. 1991;47(34):6931–38. https://doi.org/10.1016/S0040-4020(01)96148-4

57. Li E, Clark AM, Rotella DP, Hufford CD, Rotella DP. Microbial metabolites of ophiobolin a and antimicrobial evaluation of ophiobolins. J Nat Prod. 1995;58(1):74–81. https://doi.org/10.1021/np50115a009

58. Tsipouras A, Adefarati AA, Tkacz JS, Frazier EG, Rohrer SP, Birzin E, et al. Ophiobolin M and analogues, noncompetitive inhibitors of ivermectin binding with nematicidal activity. Bioorg Med Chem. 1996;4(4):531–36. https://doi.org/10.1016/0968-0896(96)00036-3

59. Zhang D, Fukuzawa S, Satake M, Li X, Kuranaga T, Niitsu A, et al. Ophiobolin O and 6–epi–ophiobolin O, two new cytotoxic sesterterpenes from the marine derived fungus *Aspergillus* sp. Nat Prod Commun. 2012;7:1411–14. https://doi.org/10.1177/1934578X1200701102

60. Liu XH, Miao FP, Qiao MF, Cichewicz RH, Ji NY. Terretonin, ophiobolin and drimane terpenes with absolute configurations from an algicolous *Aspergillus ustus*. RSC Adv. 2013;3:588–95. https://doi.org/10.1039/C2RA22701K

61. Savary S, Pathak MD, Khush GS. Global distribution and prevalence of brown spot disease of rice. Plant Dis. 2022;106(11):3444–52.

62. Chaudhry MF, Akhtar MS. Influence of climate change and nitrogen fertilizer use on brown spot disease of rice. Int J Agron Pl Prod. 2023;14(4):243–51.

63. Kulkarni S, Ramakrishnan K, Hegde RK. Genetic analysis of genes involved in melanin biosynthesis of *Cochliobolus miyabeanus*. Int Rice Res Newslett. 1980;5:13–4.

64. Dasgupta MK, Chattopadhyay SB. Effect of environmental factors on the development of brown spot of rice caused by *Helminthosporium oryzae*. J Soc Exp Agric. 1977;2:24–7.

65. Minnatullah MD, Sattar A. Brown spot development in Boro rice as influenced by weather condition. J App Biol. 2002;12:71–3.

66. Luo WH. Simulation and measurement of leaf wetness formation in paddy rice crops. PhD [Thesis]. Wageningen: Wageningen Agricultural University; 1996

67. Singh RK, Singh CV, Shukla VD. Phosphorus nutrition reduces brown spot incidence in rainfed upland rice. Int Rice Res Notes. 2005;30(2):31–2.

68. Damicone J, Moore B, Fox J, Sciumbato G. Rice diseases in Mississippi: A guide to identification. Mississippi State University Extension Service; 2001

69. Ou SH. Rice disease. 2nd edn. Kew: Commonwealth Mycological Institute; 1985

70. Biswas SK, Ratan V, Srivasta S, Singh R. Influence of seed treatment with biocides and foliar spray with fungicides for management of brown leaf spot and sheath blight of paddy. Ind Phytopathol. 2008;61(1):55–9.

71. Kulkarni S, Ramakrishnan K, Hegde RK. Epidemiology and control of brown leaf spot of rice in Karnatka. IX. Atmospheric variation in spore load of *Drechslera oryzae*. Ind Phytopathol. 1982;35:80–2.

72. Purkayastha RP, Mukhopadhyay R. Factors affecting colonization of rice leaves by *Helminthosporium oryzae*. Trans British Mycol Soc. 1974;62:402–6. https://doi.org/10.1016/S0007-1536(74)80049-5

73. Sharma SK, Maheshwari SK. Efficacy of fungicides for the control of brown spot. Intern Rice Res Newsl. 1982;7:17–8.

74. Carvalho MP, Rodrigues FA, Silveira PR, Andrade LCCL, Baroni JCP, Paye HS, Junior LJE. Rice resistance to brown spot mediated by nitrogen and potassium. J Phytopathol. 2010;158(3):160–6. https://doi.org/10.1111/j.1439-0434.2009.01593.x

75. Khan MFA, Riaz A, Ahmad I, Ullah S, Raja MU, Shaheen FA, et al. A review on current status of brown leaf spot in rice growing areas of Punjab, Pakistan. Pak J Phytopathol. 2022;34(2):255–61. https://doi.org/10.33866/phytopathol.034.02.0813

76. Vu–Van B, Sangchote S. Seed borne and transmission of *Bipolaris oryzae*, the causal pathogen of brown spot of rice. Kasetsart J. 2006;40:353–60.

77. Bag MK, Raghu S, Banerjee A, Prabhukarthikeyan SR, Baite MS, Yadav M. Durable resistance of rice to major and emerging diseases: current status. The Open Agric J. 2023;17:1–14. https://doi.org/10.2174/18743315-v17-e20230109-2022-HT14-3623-6

78. Dariush S, Darvishnia M, Ebadi AA, Padasht–Dehkaei F, Bazgir E. Population structure, genetic diversity and trait association analysis in rice (*Oryza sativa* L.) genotypes for brown spot disease resistance. Trop Plant Pathol. 2021;46:265–81. https://doi.org/10.1007/s40858-020-00405-1

79. Ota Y, Matsumoto K, Nakayama Y, Yamakawa T, Ohno T, Mizobuchi R, Sato H. QTL analysis for brown spot resistance in American rice cultivar 'Dawn'. Breed Sci. 2021;71(4):491–5. https://doi.org/10.1270/jsbbs.21015

80. Wang M, Gao L, Dong S, Sun Y, Shen Q, Guo S. Role of silicon on plant–pathogen interactions. Front Plant Sci. 2017;8:701. https://doi.org/10.3389/fpls.2017.00701

81. Jamaloddin M, Mahender A, Gokulan CG, Balachiranjeevi C, Maliha A, Patel HK, Ali J. Molecular approaches for disease resistance in rice. In: Ali J, Wani SH, editors. Rice improvement. Cham: Springer; 2021. p. 315–78 https://doi.org/10.1007/978-3-030-66530-2_10

82. Tripathi R, Tewari R, Singh KP, Keswani C, Minkina T, Srivastava AK, et al. Plant mineral nutrition and disease resistance: A significant linkage for sustainable crop protection. Front Plant Sci. 2022;13:883970. https://doi.org/10.3389/fpls.2022.883970

83. Joshi N, Brar KS, Pannu PPS, Singh P. Field efficacy of fungal and bacterial antagonists against brown spot of rice. J Biol Control. 2007;21:159–62.

84. Rabiço F, Borelli TC, Alnoch RC, Polizeli MLTM, da Silva RR, Silva–Rocha R, Guazzaroni ME. Novel *Pseudomonas* species prevent the growth of the phytopathogenic fungus *Aspergillus flavus*. BioTech (Basel). 2024;13(2):8. https://doi.org/10.3390/biotech13020008

85. Stancu MM. Biosurfactant production by a *Bacillus megaterium* strain. Open Life Sci. 2020;15(1):629–37. https://doi.org/10.1515/biol-2020-0068

86. Shoresh M, Harman GE, Mastouri F. Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol. 2010;48:21–43. https://doi.org/10.1146/annurev-phyto-073009-114450

87. Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk–Ściseł J. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Int J Mol Sci. 2022;23(4):2329. https://doi.org/10.3390/ijms23042329

88. Harish S, Saravanakumar D, Kamalakannan A, Vivekananthan R, Ebenezar EG, Seetharaman K. Phylloplane microorganisms as a potential biocontrol agent against *Helminthosporium oryzae* Breda de Hann, the incitant of rice brown spot. Arch Phytopathol Plant Prot. 2007;40(2):148–57. https://doi.org/10.1080/03235400500383651

89. Abdel–Fattah GM, Shabana YM, Ismail AE, Rashad YM. *Trichoderma harzianum*: a biocontrol agent against *Bipolaris oryzae*. Mycopathologia. 2007;164:81–9. https://doi.org/10.1007/s11046-007-9032-9

90. Prabhukarthikeyan SR, Yadav MK, Anandan A, Aravindan S, Keerthana U, Raghu S, et al. Bio–protection of brown spot disease of rice and insight into the molecular basis of interaction between *Oryza sativa*, *Bipolaris oryzae* and *Bacillus amyloliquefaciens*. Biol Cont. 2019;137:104018. https://doi.org/10.1016/j.biocontrol.2019.104018

91. Elanchezhiyan K, Keerthana U, Nagendran K, Prabhukarthikeyan SR, Prabakar K, Raguchander T, Karthikeyan G. Multifaceted benefits of *Bacillus amyloliquefaciens* strain FBZ24 in the management of wilt disease in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Physiol Molecular Pl Patho. 2018;103:92–101. https://doi.org/10.1016/j.pmpp.2018.05.008

92. Lahlali R, Ezrari S, Radouane N, Kenfaoui J, Esmaeel Q, El Hamss H, et al. Biological Control of plant pathogens: a global perspective. Microorganisms. 2022;10(3):596. https://doi.org/10.3390/microorganisms10030596

93. He DC, He MH, Amalin DM, Liu W, Alvindia DG, Zhan J. Biological control of plant diseases: an evolutionary and eco–economic consideration. Pathogens. 2021;10:1311. https:// doi.org/10.3390/pathogens10101311.

94. Wilson MJ, Jackson TA. Progress in the commercialization of bionematicides. BioControl. 2013;58:715–22. https://doi.org/10.1007/s10526–013–9511–5.

95. Kumar A, Kumar V, Singh R. Biocontrol potential of *Chaetomium globosum* against brown spot disease of rice caused by *Bipolaris oryzae*. J App Microbio. 2018;125(4):1073–84.

96. Kumari M, Yadav R, Verma P. *Flavobacterium* sp. as a potential biocontrol agent against brown spot disease of rice caused by *Bipolaris oryzae*. Plant Patho J. 2022;41(6):516–25.

97. Yadav R, Kumari M, Verma P. Biocontrol potential of *Paenibacillus* sp. against brown spot disease of rice caused by *Bipolaris oryzae*. J Gen Plant Pathol. 2023;89(1):69–77.

98. Verma P, Yadav R, Kumari M. Antagonistic potential of *Serratia marcescens* against brown spot disease of rice caused by *Bipolaris oryzae*. Arch Phytopathol Plant Prot. 2023;56(1):127–37.

99. Singh P, Singh M, Singh A. Biocontrol potential of *Streptomyces avermitilis* against brown spot disease of rice caused by *Bipolaris oryzae*. Biological Control. 2020;149:104306.

100. Sivakumar D, Suresh S, Jeyarajan R. Evaluation of *Streptomyces griseoviridis* for the biocontrol of brown spot disease of rice caused by *Bipolaris oryzae*. J Plant Dis Prot. 2019;126(6):547–6.

101. Singh A, Singh B, Singh D. Biocontrol efficacy of *Verticillium lecanii* against brown spot disease of rice caused by *Bipolaris oryzae*. J Plant Dis Prot. 2021;128(3):365–74.

102. Dorneles KR, Pazdiora PC, Hofmann JF, Chaves FC, Monte LG, Rodrigues FA, Dallagnol LJ. Wheat leaf resistance to *Pyrenophora tritici–repentis* induced by silicon activation of phenylpropanoid metabolism. Plant Patho. 2018;67:1713–24. https://doi.org/10.1111/ppa.12876

103. Shuping DSS, Eloff JN. The use of plants to protect plants and food against fungal pathogens: A review. Afr J Tradit Complement Altern Med. 2017;14(4):120–7. https://doi.org/10.21010/ajtcam.v14i4.14

104. Khameneh B, Iranshahy M, Soheili V, Bazzaz BSF. Review on plant antimicrobials: A mechanistic viewpoint. Antimicro Resis Inf Cont. 2019;8:1–28. https://doi.org/10.1186/s13756-019-0559-6

105. Kisiriko M, Anastasiadi M, Terry LA, Yasri A, Beale MH, Ward JL. Phenolics from medicinal and aromatic plants: characterisation and potential as biostimulants and bioprotectants. Molecules. 2021;26(21):6343. https://doi.org/10.3390/molecules26216343

106. Choudhury D, Dobhal P, Srivastava S, Saha S, Kundu S. Role of botanical plant extracts to control plant pathogens. Ind J Agric Res. 2018;52(4):341–6. https://doi.org/10.18805/IJARe.A-5005

107. Sarfraz M, Nasim MJ, Jacob C, Gruhlke MCH. Efficacy of allicin against plant pathogenic fungi and unveiling the underlying mode of action employing yeast based chemogenetic profiling approach. Appl Sci. 2020;10(7):2563. https://doi.org/10.3390/app10072563

108. Teixeira A, Sánchez–Hernández E, Noversa J, Cunha A, Cortez I, Marques G, et al. Antifungal activity of plant waste extracts against phytopathogenic fungi: *Allium sativum* peels extract as a promising product targeting the fungal plasma membrane and cell wall. Horticulturae. 2023;9(2):136. https://doi.org/10.3390/horticulturae9020136

109. Parajuli M, Khadka GB, Chaurasia J. A review on comparative effect of chemicals and botanicals in management of brown spot diseases of rice (*Oryza sativa* L.). Arch Agric Environ Sci. 2022;7(1):127–31. https://doi.org/10.26832/24566632.2022.0701018

110. Munteanu BS, Vasile C. Encapsulation of natural bioactive compounds by electrospinning—Applications in food storage and safety. Polymers. 2021;13:3771. https:// doi.org/10.3390/polym13213771

111. Kyriakoudi A, Spanidi E, Mourtzinos I, Gardikis K. Innovative delivery systems loaded with plant bioactive ingredients: formulation approaches and applications. Plants (Basel). 2021;10(6):1238. https:// doi.org/10.3390/plants10061238

112. Harish S, Saravanakumar D, Radjacommare R, Ebenezar EG, Seetharaman K. Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. BioControl. 2008;53(3):555–67. https://doi.org/10.1007/s10526-007-9098-9

113. Nguefack J, Wulff GE, Dongmo LJB, Fouelefack FR, Fotio D, Mbo J,

Torp J. Effect of plant extracts and an essential oil on the control of brown spot disease, tillering, number of panicles and yield increase in rice. Euro J Pl Pathol. 2013;137(4):871–82. https://doi.org/10.1007/s10658-013-0298-0

114. Akila R, Mini ML. Solvent extraction and antifungal assay of *Lawsonia inermis* Linn. Against the brown spot fungus *Bipolaris oryzae*. J Pharma Phytochem. 2020;9(1):5–8.

115. Shabana YM, Abdel–Fattah GM, Ismail AE, Rashad YM. Control of brown spot pathogen of rice (*Bipolaris oryzae*) using some phenolic antioxidants. Braz J Microbiol. 2008;39(3):438–4. https://doi.org/10.1590/S1517-83822008000300006

116. Kumar V, Chaudhary VP, Kumar D, Kumar A, Sagar S, Chaudhary S. Efficacy of botanicals and fungicides against *Rhizoctonia solani* inciting sheath blight disease on rice (*Oryza sativa* L.). J Appl Nat Sci. 2017;9(4):1916–20. https://doi.org/10.31018/jans.v9i4.1463

117. Chouhan V, Kumar A, Singh R. Evaluation of fungicides for management of brown leaf spot disease of rice. Mid East J App Sci Technol. 2021;4(4):75–9. https://doi.org/10.46431/MEJAST.2021.4407

118. Nargave S, Gehlot J, Burria R, Jakhar M, Damor JS, Jain S. Biological and chemical management strategy to control brown spot disease in rice caused by *Bipolaris oryzae*. Int J Plant Soil Sci. 2023;35(23):531–7. https://doi.org/10.9734/ijpss/2023/v35i234270

119. Arshad HMI, Hussain N, Ali S, Khan JA, Saleem K, Babar MM. Behaviour of *Bipolaris oryzae* at different temperatures, culture media, fungicides and rice germplasm for resistance. Pak J Phytopathol. 2013;25:84–90.

120. Pereira LAA, Coutinho WM, Machado JC, Magalhaes FHL, Pena RCM. Fungitoxicity in vitro of iprodione on mycelial growth of fungi associated with rice seeds. Revista Brasileira de Sementes. 2002;24:67–70.

121. Thind TS, Mohan C, Prem Raj, Arora JK. Activity spectrum of strobilurins, a new generation of ecofungicides against some fungal pathogens. Indian Phytopathol. 2004;57:104–6.

122. Shaheen HMU, Rajput NA, Atiq M, Amrao L, Arshad WR, Kachelo GA, et al. Synthetic fungicides for controlling brown leaf spot of rice caused by *Bipolaris oryzae*. Int J Biol Chem. 2023;16(2):33–42. https://doi.org/10.26577/IJBCh2023v16i2a4

123. Ratan V, Biswas SK, Kumar K, Yadav MD. Evaluation of fungicides for the management of brown leaf spot (*Drechslera oryzae*) and blast (*Pyricularia oryzae*) of paddy. Farm Sci J. 2005;14:38–9.

124. Hunjan MS, Lore JS, Goel RK. Emergence of brown leaf spot of rice in Punjab and efficacy of some chemicals against it. J Res Punjab Agril Univ. 2007;44:113–7.

125. Chavan SS, Kumar R, Gaurav, Manda RR, Rana M, Srivastava S. Management of southern corn leaf blight (SCLB) with antagonists, botanicals and fungicides. Pestic Res J. 2024;36(1):74–9. https://doi.org/10.5958/2249-524X.2024.00013.6