STUDY ON BROWN SPOT DISEASE OF RICE AND ITS MANAGEMENT

Thesis

Submitted to the

Uttar Banga Krishi Viswavidyalaya

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in

PLANT PATHOLOGY

By

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PUNDIBARI, COOCH BEHAR

WEST BENGAL-736165, INDIA

2023


Dedicated to
my beloved
Daddy & Maa

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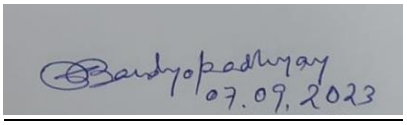
This is to certify that the work recorded in the thesis entitled **"Study On Brown Spot Disease Of Rice And Its Management"**submitted by **Ms. Diyya Rachitha** with **Registration No (A-2021-052-M)**, in partial fulfilment of the requirement for the Degree of **Master of Science (Agriculture) in Plant Pathology**, Uttar Banga Krishi Viswavidyalaya is a genuine and original one carried out under my personal supervision and guidance. The results of the investigation reported in the thesis have not so far been submitted for any other Degree or Diploma. The assistance and help received from various sources during the courses of investigation is being duly acknowledged.



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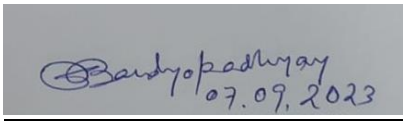
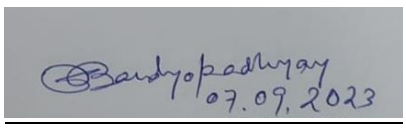
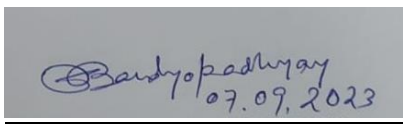
Certificated that the thesis entitled **"Study On Brown Spot Disease Of Rice And Its Management"** submitted by **Miss. Diyya Rachitha** bearing Registration number **A-2021-052-M** towards partial fulfilment of the requirement for the award of **Master Degree from the Department of Plant Pathology**, Faculty of Agriculture under Uttar Banga Krishi Viswavidyalaya has been checked against plagiarism through Urkund (Original) software on 07-09-2023 and the similarity index has been achieved as **6%** which is below the maximum tolerable range as per stipulation of this Viswavidyalaya. The thesis of **Miss. Diyya Rachitha** may be accepted for the award of the **Master Degree in M.Sc. (Agriculture)** of Uttar Banga Krishi Viswavidyalaya.







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Approval of Examiners for the Award of the Degree of Master of Science (Ag) in Plant Pathology

We, the undersigned, having been satisfied with the performance of M. Sc. Student DIYYA RACHITHA [Reg. No: A- 2021-052-M], on the final evaluation of thesis entitled **"Study on Brown Spot Disease of Rice and its Management"** conducted today, the 18/09/2023, recommended that the thesis would be accepted for the award of the degree of Master of Science (Agriculture) in Plant Pathology.

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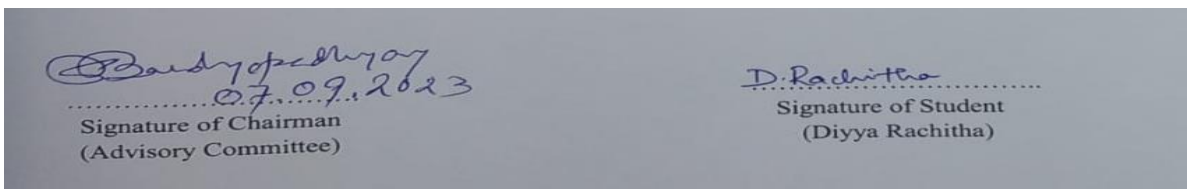
ABSTRACT

Rice (*Oryza sativa*) is the most important and predominant crop over other crops. It is the staple food for nearly half of world's population. Globally it is grown nearly in about 11% of total cultivated area.There is a huge demand for rice and this may reach 852 million tonnes by the year 2035. Rice is an integral part of Indian dietary chart, 60% people rely on this. It is one of the diverse crops grown in different Agro-climatic conditions and is the second largest produced cereal in the world. Ten isolates were collected from ten different locations of West Bengal. The locations were Pundibari, Dinhata, Kalyani, Bolpur, Tufanganj, Raiganj, Siliguri, Malda, Bankura and Bishnupur. The pathogen was named as HO 1 to HO 10, respectively. The length, breadth and number of septa of conidia of all those isolates were counted. Growth of all the isolates were studied in Potato Dextrose Agar media. Colony characteristics of the Pundibari isolate of the pathogen was studied in six different media. Growth of the pathogen in different media was seen. The pathogen was tested against different chemicals under in vitro condition using poisoned food technique. it was found that Azoxystrobin + Difenoconazole was the best treatment as it produced minimum growth of the pathogen in all the concentrations tested among all the chemicals. All the fungicides were tested for the management of brown spot disease of rice under field situation. Azoxystrobin + Difenoconazole treatment was best here too as it produced lowest PDI of 5.41, 7.96 and 10.43 at first, second and third disease scoring time, respectively. This treatment also recorded the lowest AUDPC of 79.40 and highest disease reduction over control (75.41%). The highest yield was also recorded in the same Azoxystrobin + Difenoconazole treatment with a yield of 5.44 t/ha and 39.13% increase in yield over control.Incremental benefit cost ratio over control was found out for all the treatments. It was again found that highest incremental return over control of Rs. 30,600/- per ha with incremental cost benefit ratio of 3.40 was recorded by Azoxystrobin + Difenoconazole treatment. So, this treatment can be recommended for the management of Brown spot disease of Rice in the field.



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LIST OF ABBREVIATIONS

ABBREVIATION	FULL FORM
°C	Degree Celsius
psi	Pound per Square Inch
hrs	Hours
g	Gram(s)
L	Litre(s)
kg	Kilogram(s)
ml	Millilitre(s)
%	Percentage
m	Metre(s)
@	At the rate of
Fig.	Figure
BOD	Biological Oxygen Demand
PDA	Potato Dextrose Agar
PDB	Potato Dextrose Broth
PSA	Potato Sucrose Agar
OMA	Oat Meal Agar
MEA	Malt Extract Agar
AUDPC	Area Under Disease Progress Curve
AUMGC	Area Under Mycelial Growth Curve
PDI	Percent Disease Index
DI	Disease Incidence
SE(m)	Mean Sum of Squares
CV	Coefficient of Variation
CD	Critical Difference
RH	Relative Humidity
Temp	Temperature
Max	Maximum
Min	Minimum
SC	Suspension Concentrate
WG	Water Dispersable Granule
WP	Wettable Powder
EC	Emulsifiable Concentrate

CHAPTER- 1
INTRODUCTION

1. INTRODUCTION

Rice (*Oryza sativa*) is the most important and predominant crop over other crops. It is the staple food for nearly half of world's population. Globally it is grown nearly in about 11% of total cultivated area (Rout and Tewari, 2012).

There is a huge demand for rice and this may reach 852 million tons by the year 2035. Rice is an integral part of Indian dietary chart, 60% people rely on this. It is one of the diverse crops grown in different Agro-climatic conditions and is the second largest produced cereal in the world.

At present large yield gaps are reported in several estimates especially in different states of the country in terms of productivity. The demand of today is to reduce this gap in order to meet the future estimates of the production.

Rice is used as rice flakes, puffed rice, rice wafers and canned rice. It is also used in starch and brewing industries. Most of the world's rice is grown in tropics which include South and South East Asia, West Africa, Central and South America. In Asia, where 3 billion Asians consume 60% of global calories, more than 90% of the world's rice is produced and eaten (Khush, 1997).

Next to China, India is the world's top producer of rice. According to figures given by the Indian government, rice is farmed on 43.86 million hectares in India, with an average annual output level of 104.80 million tons and a productivity of roughly 2.39 t/ha, accounting for 21.81% of the world's rice production in 2015–16. However, China produces the most rice in the world, with 205.21 million tons produced at a productivity of 6.71 t/ha and constituting 27.70% of global rice output. In Bangladesh, 75% of the land is planted with crops, while roughly 92% of farmers grow rice (Rekabder, 2004). In the European Union, rice is grown on around 4.5 lakh hectares of fertile land, with Spain accounting for nearly 25% of this total (Bacilio-Jiménez, 2001). According to Shivani *et al*., (2007), Africa (7.78%), South America (6.4%), and North America (1.4%) are the other continents where rice was farmed.

Punjab (11.82) million tons over 2.97 million hectares, Uttar Pradesh (12.5) million tons over 5.86 million, and West Bengal (15.75) million tons over 5.46 million hectares are the top states in India for producing rice (India Today Web Desk New Delhi, September 18, 2018), followed by Tamil Nadu and Haryana.

With an estimated production of 5.50 million tons and an average production of 1.57 t/ha, rice accounts for more than 80% of the total cultivated land in North East India, occupying 3.51 million hectares, or 7.8% of the country's total rice area (Das *et al*., 2014).

India's total rice production of 13.5% or 5.90 million hectares, is farmed in Uttar Pradesh. 70% of the state's overall geographical area is made up of its 11.56 million hectares of arable land. Over 13.43 million hectares are irrigated, with a 3.0 t/ha production average. It produces roughly 18–20% of the nation's rice production.

Despite having a significant share of the world's rice production and rice-growing land, India's productivity per unit of land is still poor by international standards. The low production of rice in India may be caused by a number of factors, but the prevalence of pests and diseases is a significant one. According to widely accepted estimates, diseases alone causes 20% of all crop-related damages.

Green revolution was successful in producing high yielding varieties, using adequate fertilizers and other complimentary inputs. Over the decades, rice production in Asia has increased at the rate of 2.5% per year keeping pace with population growth. Most important outcome of these efforts is securing an adequate supply of food grain which helped to prevent hunger. But now the scenario in population growth has outpaced rice production. This deficit affects the low outcome countries where people consume more rice and the population grows faster.

Rice suffers from more than 60 different diseases caused by fungi, bacteria, virus, phytoplasma, nematodes and other non-parasitic disorders. Fungi are the principal organisms associated with seeds in storage. Among the seed-borne diseases of rice, majority are caused by fungi alone. Among the biotic factors, disease is the most important factor which results in crop losses of 5 billion every year. The important seed-borne fungal diseases of rice are Brown spot (*Helminthosporium oryzae*), Blast (*Pyricularia oryzae*), Sheath rot (*Sarocladium oryzae*), Sheath blight (*Rhizoctonia solani*), leaf scald (*Microdochium oryzae*), seed rot and seedling blight, grain spot, etc. Fungi associated with discoloured rice seed resulting in poor germination and vigour and cause diseases in emerged seedling or growing plants, weight loss and loss of germination and seeding vigour (Sachin and Agarwal, 1994).

Out of different biotic stresses which influence the performance of rice crop, Brown spot of rice caused by *Cochliobolus miyabenus* is an important disease which impairs grain quality resulting in about 67% yield reduction (Kohls *et al.*, 1987 and Jones *et al.*, 1993). *Bipolaris oryzae*, the causal agent of brown spot disease of rice, belongs to

the Kingdom Fungi, phylum Ascomycota, class Loculoascomycetes, order Pleosporales and family Pleosporaceae. In case of severe infections, the spots fused together and leaves withered, spots also develop on glumes. When conditions favoured for fungal developments, a velvety growth could be seen over the seed and the fungus entered into the glumes producing blackish spots on the endosperm. Brown spot symptoms might appear on the leaf coleoptiles, leaf sheaths and panicle branches. Blackish lesions might be seen on young roots. Both upland and lowland ecosystems support brown spot development. The disease can be a serious one causing considerate yield loss worldwide, as it affects the quality as well as number of grains per panicle along with reduction in the kernel weight.

Rice brown spot disease is a major issue in many nations and has been shown to result in significant grain yield losses (up to 90%), especially when the leaf spotting phase takes on epiphytotic proportions as was seen during the Great Bengal Famine in 1942 (Ghose *et al*., 1960).

Synthetic pesticides are currently the main method for managing crop diseases. A number of fungicides *viz*., Thiophanate Methyl, Tebuconazole, Azoxystrobin, Propiconazole, Propineb, Blitox, Carbendazim, Edifenphos, Mancozeb etc., has been used extensively against various crops diseases, however its indiscriminate use has created various issues on environment, man and animal health, more over application of the kind of same chemical over time causes resistant built up and pest resurgent in many cases.

On the other hand, using antagonistic microorganisms to control plant diseases is now a successful method.

Many non-pathogenic fungi, such as *Trichoderma* spp., *Gliocladium*, etc., were also being used for management of various crop diseases. *Pseudomonas fluorescens*, a bacterial antagonist, has been successful in controlling a large number of plant diseases through different modes of action, such as competition for nutrients and space, antibiosis, production of siderophores, lytic enzymes, and induction of resistance, etc.

Given the foregoing, the research work **"Study on brown spot disease of rice and its management"** was taken up with the following objectives:

1. Isolation of Brown spot disease causing pathogen of rice from different locations of West Bengal
2. Characterization of the pathogen
3. Management of Brown spot disease of Rice both under *in vitro* and *in vivo* condition

CHAPTER- 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The relevant literature under consideration in the ongoing research endeavour, titled **"Study on Brown spot disease of Rice and its Management"** are as follows:

2.1 Symptomatology:

Mundkur and Chattopadhyay (1967) documented various symptom variations, including the presence of dark brown, ellipsoidal, or eye-shaped spots on the upper surface of rice leaves. A mature spot exhibited a central region with a greyish-brown hue, encircled by a deeper reddish-brown margin, and typically measured between 4 to 6 mm in size. These spots were also observed on leaves of more mature plants, where they tended to increase in size. In contrast, the spots on coleoptiles were smaller and brownish in appearance.

Misra (1973) documented that the spots created by *Helminthosporium oryzae* at their center displayed a dry, straw-coloured to grey appearance, encircled by a margin that ranged from brown to reddish-brown. These spots were accompanied by a longitudinally elongated green halo, spanning dimensions of 0.5 to 5 mm in width and 0.5 to 3 mm in height. On glumes, the manifestation involved brown spots. Additionally, the grains exhibited a covering of an olivaceous mat composed of conidiophores.

According to Padmanabhan (1977), characteristic water-soaked lesions with a brown colouration manifest at the node situated below the rachis or at the neck during the emergence of flowers. Additionally, he noted that brown spots develop on the grains, resulting in their shrinkage and a shrivelled appearance.

The pathogen *Helminthosporium oryzae* affects rice crops from the seedling to the milk stage. It creates tiny lesions on various parts of the plant, including the coleoptiles, leaf blade, leaf sheath, and glumes. These lesions are most noticeable on the leaf blades and glumes. On the leaves, these lesions are typically brown with a grey or whitish center. They have a cylindrical or oval shape that resembles sesame seeds and often have a yellow halo. When the lesions are young, they are small and circular, appearing as dark brown or purplish-brown dots. As the infection progresses, multiple lesions may merge, causing the affected leaf to dry out. In rice cultivars that are susceptible to the pathogen, the lesions can be much larger, growing up to 1 cm or more in length (Ramakrishnan and Subramanian, 1977).

As per Rangaswami (1979), leaf spotting represents the most prevalent and easily discernible symptom. The spots, appearing on both leaves and leaf sheaths, exhibit a brown coloration and typically adopt a round to oval shape, measuring approximately 0.5 to 2 mm in width and 2 to 5 mm in length. These spots are generally solitary, although in severe instances, they might merge to create extensive areas of withered tissue. The spots found on glumes are enveloped by an olivaceous velvety growth. In the neck region, a grey or greyish-brown appearance is noticeable.

Singh (1983) documented that the spots found on coleoptiles were brown, small, and displayed circular to oval shapes. On leaves, these spots exhibited a range in size and shapes, spanning from tiny dots to circular, eye-shaped, or oval configurations, measuring between 1 to 14 mm in width and 0.5 to 3 mm in height. The smaller spots typically displayed a dark brown or purplish-brown coloration. Conversely, the larger spots possessed dark brown edges, but towards the center, they could appear pale yellow, dirty white, brown, or grey. In some instances, these larger spots were accompanied by a yellowish halo. In cases of severe infection, the central area of the leaf might become brown or desiccate. Ganguly and Padmanabhan (1959) conducted observations around 7 to 10 days after inoculation and identified minute light brown or brownish-red spots, approximately the size of pinpoints, which could be differentiated. In certain cases, these leaf spots would grow in size and darken to a dark brown color.

Ou (1985) similarly outlined the symptoms observed on the leaves, resembling oval shapes akin to sesame seeds. Furthermore, he noted that the initial signs of brown spot involved the presence of small, circular to oval spots on the first leaves of the seedlings. As these spots aged on the leaves, a distinctive bright yellow halo developed around the lesion. The appearance of spots on the leaf sheath and hulls was consistent with those observed on the leaves. Early brown spot lesions posed challenges in differentiation from lesions caused by blast.

According to Ranganathaiah (1985), it was observed that black or dark brown spots develop on the glumes, leading to grains that become discolored and shrivelled. When conditions are favourable, these spots on the glumes exhibit the growth of dark brown conidiophores and conidia, creating a velvety texture. The fungus might also enter the glumes, leaving behind blackish spots on the endosperm. In cases of severe infection on the grains, there have been reports of inhibited germination.

As per Datnoff and Lentini (1994) findings, the initial signs of the disease manifest as small, rounded to oval-shaped brown lesions on the leaves, leaf sheath, and hull. These

lesions range in color from dark brown to reddish-brown. Larger lesions exhibit a central area that is lighter in color, appearing reddish-brown or grey, encircled by a darker boundary ranging from dark to reddish-brown. In the case of more mature leaf spots, a vibrant yellow halo can develop around the lesion.

Near the base of the culm, just above ground level, brown lesions emerge on the sheath. Initially, these lesions exhibit a greenish-grey tint, forming oval to ellipsoidal areas of discoloration (Sharma and Chahal, 1996).

In their study, Sunder *et al* (2005) documented the presence of brown to dark brown lesions, measuring 5 – 15 × 1 – 2 mm, on the panicle stalk near the connection point of the flag leaf to the stalk, which were attributed to *H. oryzae* infection. These lesions typically spread downward, often extending beneath the sheath, leading to significant moisture-induced decay. This decay results in grains that are incompletely filled, appearing dull and chaffy. Additionally, there are instances of the panicle hanging down. In more severe instances, a greyish mycelial growth becomes evident between the sheath and the stalk. When the infection progresses upward, dry rot symptoms become apparent. Furthermore, the fungus generates brown or greyish-brown spots on the neck region, in contrast to the blackened appearance associated with neck blast.

In the study by Patel *et al* (2010), it was observed that the pathogen's infection led to the blighting of coleoptiles and the development of oval-shaped, dark brown to purplish-brown spots on leaves. This resulted in significant impairment of photosynthetic activities and ultimately resulted in the death of the affected leaf.

Groth and Clayton (2013) conducted a review concerning the perfect stage of the pathogen associated with brown spot of rice, known as *Cochliobolus miyabeanus* (previously identified as *Bipolaris oryzae*). They noted that the size of brown spots on rice leaves is comparatively smaller on younger leaves when compared to older ones. The appearance of these spots can vary in terms of size and shape, ranging from tiny dark spots to larger oval or circular spots. The smaller spots exhibit a dark brown to reddish-brown coloration, whereas the larger spots are characterized by a brown margin surrounding a central area that appears light reddish-brown or grey. Notably, these larger spots also possess a distinctive gold halo. This fungus is responsible for the development of circular to oval brown spots on the coleoptiles and leaves of rice seedlings.

Sunder *et al* (2014) documented the initial manifestation of brown spot as minute lesions appearing on the coleoptiles, leaf blade, leaf sheath, and glumes of rice plants. On

leaves, the characteristic spots exhibit a brown hue, with a central area that appears grey or whitish. These spots take on a cylindrical or oval shape, resembling sesame seeds, and often possess a yellow halo. In their early stages, these spots are small, circular, and may present as dark brown or purplish-brown dots. As the infection progresses, multiple spots can merge, leading to the drying up of the affected leaf. On the glumes, the pathogen gives rise to black or dark brown spots, which subsequently cause the grains to become discoloured and shrivelled. The fungus has the ability to penetrate the glumes, leaving behind blackish spots on the endosperm.

2.2 Pathogen Life cycle:

The initial stage of the illness is frequently initiated by an infection originating from seeds. As the fungus exists in a dormant mycelium state within the husk and even on the kernel, it is plausible for it to contaminate the sprouting grain. It has been documented that the fungus can persist in the soil and within afflicted plant components like stubbles, straw, and grains for a span of 2-3 years. This extended survival acts as a primary reservoir of infective material (Ou, 1985).

The seed-borne characteristic of the pathogen was documented by Wesely *et al*, 1996. Typically, the initial infection is set in motion by the presence of the infected seed (Bernaux, 1981; Sharma and Maheshwari, 1982), leading to necrotic lesions on the coleoptile and sheath of the first leaves. Subsequent lesions on the leaves predominantly result from a secondary infection caused by air-borne spores that develop on the primary lesions (Ou, 1985). The spores of *B. oryzae* were identified to be present in the atmosphere throughout the entire year (Ghose *et al*, 1960; Kulkarni *et al*, 1982), yet their quantities were more substantial during the cooler months when humidity levels were higher (Kulkarni *et al*, 1982).

Air-borne conidia lead to secondary infection, originating either from plants initially infected or from alternate sources. Collateral hosts play a role in furnishing air-borne conidia for the commencement of secondary infection. These hosts have been identified through observations of natural field infections or via deliberate inoculation experiments.

Environmental conditions play a role in affecting the fungus's persistence within seeds and soil. The viability of the pathogen is influenced by the temperature and relative humidity during seed storage (Mia and Safeeulla, 1998; Dallagnol *et al*, 2011). The

pathogen can endure in both arid and moist soil, although its survival rate fluctuates with changes in soil moisture content (Kulkarni *et al,* 1981c).

Certain weed hosts have also been identified as reservoirs of the infectious material (Biswal and Mohanty, 1995).

2.3 Pathogen biology:

Gangopadhyay (1983) noted that the progression of brown spot disease can also extend from one year to another by utilizing alternate hosts. These hosts encompass a variety of plants such as *Cynodon dactylon, Echinochloa colona, Echinochloa frumentacea, Euchlocna mexicana, Imperata arundica, Leersia hexandra, Penicum milliare, Penicum milliaceum, Pennisetum typhoides, Saccharum officinarum, Zizania latifolia,* and several wild rice varieties including *Oryza coaracata, O. granulata, O. malampuzhensis, O. latifolia, O. ridileyi, O. perenis, O. fatua, O. jeyporensis, O. montanal,* and *Zizania aquatic.* Additionally, various species of grasses spanning 23 genera have been documented to become infected by the pathogen under both natural and artificially induced conditions of inoculation.

According to Ou (1985), the fungus demonstrates the capability to persist within the soil and in various infected components of plants, such as stubbles, straw, and grains, for a duration spanning 2 to 3 years. These components serve as the primary reservoirs of infectious agents. Consequently, the disease can re-emerge in the following season through the dissemination of the pathogen via infected seeds and plant remnants. The subsequent formation of lesions on leaves is primarily attributed to secondary infections caused by spores disseminated through the air. These spores originate from the primary lesions and lead to a recurring cycle of infection.

Damicon *et al* (2001) documented that the initial infections stem from the seeds themselves. When infected seeds germinate, the coleoptiles become affected, initiating a gradual spread to the seedling. This progression leads to the manifestation of blight symptoms on different parts of the infected seedling, including the coleoptiles and leaves. Subsequently, a substantial number of conidia are produced. These conidia, propelled by the wind, disperse and land on various segments of the same seedling or neighbouring seedlings, instigating new infections. This constitutes the secondary cycle, which iterates multiple times throughout the growing season, intensifying the advancement of the disease. As the season draws to a close, the infectious agents originating from the infected leaves

can also migrate to the inflorescence. Here, the pathogen can establish itself within the seeds as mycelium or present on the seeds as conidia.

According to the findings of Pico and Rodofil (2002), rice brown spot inflicts significant harm in regions of southern China where the weather conditions are conducive, characterized by cool summers and a lack of nitrogen availability. Disease severity is heightened under conditions of high humidity, specifically exceeding 92.5%, along with extended periods of leaf wetness and temperatures ranging from 24 to 30°C. These particular environmental factors create a favourable environment for the development of the disease.

The observations made by Datnoff and Lentini (2003) highlight that the progression of rice brown spot disease is influenced by specific environmental conditions. This includes elevated humidity levels ranging from 86% to 100%, temperatures within the range of 68-78ºF, and continuous leaf wetness for durations spanning 8 to 24 hours. The disease development is particularly favoured when temperatures fall between 25-30ºC, coupled with a humidity level of 80%. Furthermore, an excess of nitrogen also contributes to the optimal conditions for disease advancement.

Singh (2012) noted that the dispersion of spores of rice brown spot can occur through the agency of wind and rainfall, leading to their transfer to various parts of the same plant as well as to other neighbouring plants. The extent of losses incurred can be substantial, especially when weather and field conditions are conducive to the dissemination of the disease. The occurrence of rice brown spot can be observed throughout the entire growing season.

Sunder *et al* (2014) asserted that brown spot disease of rice, which is induced by *Bipolaris oryzae* (with its teleomorph known as *Cochliobolus miyabeanus*), has a global distribution and is recognized for causing significant reductions in both the quantity and quality of rice grain yield. This ailment holds particular importance in scenarios involving minimal input practices, water scarcity, and direct seeding methods. The causative pathogen displays a broad spectrum of host compatibility and demonstrates variations in both its pathogenic characteristics and genetic makeup. The complete genome sequence accessibility of *Cochliobolus miyabeanus*, along with other *Cochliobolus* species, allows for a comparative analysis of their genomes, which in turn can provide enhanced insights into the interactions between the host and pathogen, thereby contributing to improved disease management strategies. They also conducted a comprehensive analysis of historical

research. Notably, they delved into the pioneering work of Breda de Haan in 1900, who initially characterized the fungal perfect stage as *Helminthosporium oryzae*. Subsequently, in 1966, Subramanian and Jain reclassified it as *Drechlera oryzae*. However, in 1959, Shoemaker introduced the term *Bipolaris oryzae,* drawing attention to the predominant germination of conidia from two end cells. In 1927, Ito and Kuribayashi observed the teleomorph stage of the fungus in culture and labelled it as *Ophiobolus miyabeanus*. Drechsler's perspective in 1934 placed the fungus within the *Cochliobolus* genus, a classification later officially endorsed by Dastur in 1942. Presently, the name *Cochliobolus miyabeanus* (Ito and Kuribayashi, 1927) Drechler ex Dastur continues to be employed for this fungal species.

2.4 Isolates collected from different places:

In their study, Motlagh and Kaviani (2008) identified four distinct categories of brown spot pathogens affecting rice. These categories encompassed *B. oryzae, B. victoriae, B. indica,* and *B. bicolor*. It is worth noting that *B. oryzae* emerged as the predominant species within this group. Determining the precise *Bipolaris* species through morphological characteristics is a challenging and infrequent undertaking.

According to Kumar *et al* (2016), *Bipolaris oryzae*, the causative agent of brown spot disease in rice, poses a significant global threat to paddy cultivation. A comprehensive survey was undertaken during the kharif seasons of 2012, 2013, and 2014. This survey yielded 116 isolates of *B. oryzae*, extracted from affected samples gathered across diverse geographical regions of India that represent the primary rice-growing areas. The isolates underwent scrutiny for various morphological attributes, including colony traits, colony diameter, and sporulation rates. Through assessment of colony morphology and growth patterns on Potato Dextrose Agar (PDA), these isolates were classified into eight distinct categories. Furthermore, based on colony diameter, all these isolates were classified into three separate groups: slow-growing, moderate-growing, and fast-growing isolates. Among the isolates, those originating from Bihar and Jharkhand exhibited the highest levels of sporulation. In contrast, isolates collected from Gujarat displayed the lowest levels of sporulation.

2.5 Length, Breadth and septation of different isolates:

According to Kumar *et al* (2016), the majority of isolates from the Northern and Eastern regions of the country exhibited spore sizes falling within the larger range of

110-137 µm in length and 18 – 23 µm in width. However, this pattern was not universally applicable to all isolates, as certain isolates from Andhra Pradesh and Karnataka also displayed larger spore sizes. Similarly, most isolates originating from the Western and Southern regions of the country demonstrated spore sizes ranging from 90 – 109 µm in length and 15 – 17 µm in width. Nevertheless, this trend was not consistent across all isolates, with a few isolates from West Bengal and Delhi displaying larger spore sizes in both length and width. Consequently, the categorization of *B. oryzae* isolates based solely on spore size is not feasible.

Jaiganesh & Kannan (2019) had collected brown spot of Rice infected disease samples from 5 different locations and after isolating those pathogens, naming was done as Ho_1 to Ho_5. The colony morphology of all the isolates showed olivaceous, light brown to black, septate, profuse aerial/submerged and branched mycelium. They had also measured the size of conidia of all those isolates. In the current investigation, the size of conidia varied from 29.3 to 33.2 µm in length and 13.5 to 14.8 µm in width among the isolates. The conidia also exhibited varying degrees of septation, ranging between 3 and 5 septa.

Valarmathi & Ladhalakshmi (2018) had isolated 17 isolates collected from brown spot of rice infected disease samples. Conidia were usually curved, fusoid or obclavate, occasionally almost cylindrical, pale to mid golden brown, 5 to 6 septate with hilum. The conidia exhibited bipolar germination pattern and hence named the pathogen as *Bipolaris*. The size of BO conidia was measured with three microscopic fields and average was calculated. Only 3 isolates i.e. BO 12, BO 13 and BO 14 could sporulate. The length and breadth of these three isolates varied from 56.89 - 113.32 × 13.75 - 27.41 µm.

Motlagh and Kaviani (2008) isolated number of isolates from rice leaves from different places which were infected with brown spot disease. The characterization of the colonies and the morphology of different structures of the pathogen was studied under microscope. They had found 4 different groups of the pathogen. Characteristics of the first group of the pathogen were found to produce solitary conidiophores or sometimes in small clusters also, exhibiting a range from straight to slightly flexible forms. Some were even characterized by a bending or zigzag pattern i.e. having geniculation. Their coloration varied from pale to medium brown or olivaceous brown, with a paler tone toward the apex. These conidiophores displayed septation, typically measuring 430–580 × 4–7 µm (average size of conidiophore being 500 × 5 µm). The conidia, on the other hand, often showed a curved or boat-like shape, resembling a navicular, fusoid, or obclavate structure. In certain instances, they almost took on a cylindrical appearance. The colour of these conidia ranged

from pale to medium golden brown, and their surface was smooth. These conidia were noted for having 5 – 12 septa, size being 46.5–125 × 10–26 μm. The attachment point, referred to as the hilum, was tiny and could be either dark or light in colour, sometimes slightly raised like a small protrusion. Conidia germinated from polar cells and germ tube from the basal cell usually emerged immediately adjacent to the hilum and grows in the direction of the long axis. Second group of the pathogen produced conidiophores in single or in small groups, straight to flexuous, sometimes geniculate above, pale to mid brown, smooth, septate, 50–40×5–9 μm. Conidia were slightly curved, broadly fusiform or obclavate fusoid, pale or mid golden brown, smooth, 4–13 (mostly 8–10) septate, 32–143 × 9–24 μm and hilum was minute but not protruded. The third group of the isolates produced Conidiophores which were single, straight or flexuous, mid to dark olivaceous brown, smooth, septate, cylindrical, 260–350 × 7–10 μm. Conidia were straight, shortly clavate or more rarely broadly ellipsoidal, with a markedly protuberant hilum or the hilum without protuberant in some conidia, mid to dark olivaceous brown, smooth, 35–69 × 12–25 μm, 4–8 distoseptate (mostly 6). The fourth group produced conidiophores which were single or in small groups, straight to flexuous, septate, smooth, occasionally upper part geniculate, golden brown, 300–400 × 5–10 μm. Conidia were straight or rarely curved, cylindrical or rather broader in the middle, tapered towards the ends rarely obclavate, 3–8 (mostly 6) distoseptate, 31–95 × 9–17 μm central cells of mature conidia were often dark brown but end cells hyaline or very pale and frequently cut off by a very dark septum and hilum was 3–5 μm.

The conidia of this fungus display a unique structure, typically curving slightly and being widest at the midpoint. As they mature, they take on a brownish color. These conidia exhibit 5 to 10 septations, with the oldest conidium situated toward the base. Upon full maturation, the conidia exhibit a germination pattern involving two polar germ tubes, each originating from thin-walled regions. In less mature instances, subhyaline (almost transparent) spores might produce germ tubes from intermediate segments. Across various regions, there are observed differences in the size of conidiophores and conidia. In Japan, they range from 68 – 688 μm in length and 7.6 – 20 μm in width. In India, the sizes are approximately 70 – 175 × 5.6 – 7 μm and 45 – 106 × 14 – 17 μm. In China, measurements span was found to be 99 – 345 × 7.11 μm and 24 – 122 × 7 – 23 μm. In the USA, the dimensions vary between 150 – 600 × 4 – 8 μm and 35 to 170 × 11 – 17 μm (Ou, 1985).

In 1973, Misra documented that *B. oryzae* generates conidiophores of a dark olivaceous shade, occurring individually or grouped in clusters, emerging from internal

mycelium via stomata. Additionally, it was observed that the conidia displayed a curved shape and had dimensions ranging approximately from 99 – 280 µm in length and 3 – 9 µm in width, with a characteristic 2 to 5 septa.

2.6 Colony characters of different isolates of pathogen:

Valarmathi & Ladhalakshmi (2018) had collected a total of 17 isolates of *B. oryzae* from nine different rice-growing states across India. These isolates were subjected to characterization based on their colony morphology and growth patterns. As a result, they were classified into four distinct groups.

The isolates were categorized as follows:

- Group I: Characterized by black coloration and a fluffy growth pattern.
- Group II: Displaying a grey appearance with fluffy growth, accompanied by white spots.
- Group III: Exhibiting a grey coloration along with a fluffy growth pattern.
- Group IV: Showing a grey coloration and a growth pattern that appeared suppressed.

Nayak & Hiremath (2019) studied cultural and morphological characters like colony characters, colony diameter, margin, mycelia growth, spore germination and sporulation were carried out in the laboratory of ten isolates isolated from rice leaves infected with *Bipolaris oryzae* from different rice growing regions of northern Karnataka. All examined isolates displayed noteworthy variation in growth patterns and colony characteristics. The morphological traits among different isolates were evident like dark greyish black colour, light greyish black colour, greyish colonies with white cottony texture, greyish black with a white center, cottony growth. Regular margins and irregular margins were displayed.

Jaiganesh & Kannan (2019) had collected brown spot of Rice infected disease samples from 5 different locations. The colony morphology of all the isolates showed olivaceous, light brown to black, septate, profuse aerial/submerged and branched mycelium.

Kumar *et al* (2016) isolated *B. oryzae* isolated from the diseased specimens collected from different geographical locations of India representing major rice growing regions. The isolates were characterized for morphological traits like colony characters,

colony diameter, and sporulation. On the basis of colony morphology and growth pattern on Potato Dextrose Agar medium, the isolates were grouped into 8 categories viz. black with suppressed growth, black with cottony growth, black with fluffy growth, grey with suppressed growth, grey with cottony growth, grey with fluffy growth, grey and white mix with cottony growth and white with cottony growth.

In the study conducted by Motlagh & Kaviani (2008), they isolated different isolates of the pathogen infected by brown spot disease of rice. They have grouped those isolates in 4 groups and they have characterized their growth pattern in media. The characteristics are as follows:

Group I: Grey to dark grey conidial colonies grew and spread rapidly. Aerial mycelium was fluffy, cottony, grey olivaceous with brownish tinge.

Group II: Conidial colonies grew, spread, grey to dark grey. Aerial mycelium was fluffy, cottony and pale to mid yellowish.

Group III: Colonies were effuse, dark blackish brown and velvety. Aerial mycelium was fluffy, pale brown to dark brown.

Group IV: Colonies were effuse, grey to blackish brown and velvety. Aerial mycelium was fluffy, yellow, pale brown to dark brown.

2.7 Study of the pathogen in different media:

In 1963, Misra and Chatterjee conducted research on the cultural traits of two distinct isolates of *B. oryzae*, namely IS and IB. They noted that these isolates exhibited robust growth on potato dextrose agar (PDA) and glucose peptone agar compared to other types of media. In 1974, Hiremath conducted investigations into the cultural characteristics of *H. oryzae*, the causal agent behind rice's brown spot disease, using various solid media. Among these solid media options, oat meal agar and czapek's agar were identified as the most conducive for the growth of *H. oryzae*.

To achieve the sporulation of *Bipolaris oryzae*, six different media and five distinct light treatments were employed. Among these, abundant spore production was stimulated by using commercial rabbit food agar. The most effective light condition for promoting sporulation involved a 12-hour cycle of black light exposure followed by 12 hours of complete darkness. Continuous exposure to either black light or complete darkness did not result in sporulation. When the fungus was subjected to fluorescent light following the black light treatment, a lower quantity of conidia was produced (Hau and Rush, 1980).

Arshad *et al* (2013) studied five different culture media like Malt Extract Agar, Rice Polish Agar, Sucrose Proline Agar, Sash's Agar and Potato Dextrose Agar media for growth of *B. oryzae* at 24, 48, 72 and 96 hours after inoculation. It was found that Maximum growth of *B. oryzae* was observed on the Malt Extract Agar and Potato dextrose agar followed by Rice Polish Agar, Sash's Agar and Sucrose Proline agar till 96 hours of incubation.

P. Valarmathi and D. Ladhalakshmi (2018) studied the colony morphology for all the seventeen isolates in different media viz., Rice extract with potato dextrose agar, Rice extract with oat meal agar, Rice polish agar and Malt extract agar. It was found that Rice Polish Agar produced highest growth at 5 days after inoculation which is followed by Rice extract+ Potato Dextrose Agar media and Rice extract+ Oat Meal Agar media.

Nayak and Hiremath (2019) tested different isolates of *Bipolaris oryzae* in 4 different media viz. Czapeck's malt agar medium, Host extract dextrose agar medium, Potato dextrose agar medium and V8 agar medium for studying their cultural variability. Among the different media's tested, potato dextrose agar showed maximum radial growth (89.33 mm) in all the isolates followed by host extract dextrose agar (87.25 mm).

2.8 Management of disease with chemicals:

2.8.1 *In vitro* testing of fungicides:

Hunjan *et al* (2011) reported that new fungicides like trifloxystrobin + tebuconazole, tebuconazole and propiconazole showed higher level of efficacy against *D. oryzae* pathogen of rice under laboratory conditions, however, thifluzamide (Spencer 24SC) was least effective against brown spot disease of rice under the same condition.

Sandeep P (2015) conducted laboratory experiments to assess the effectiveness of fungicides in suppressing the mycelial growth of *H. oryzae*, the pathogenic agent responsible for rice leaf brown spot. Across different concentrations all fungicidal preparations exhibited noteworthy reductions in the fungus radial growth. Among the examined fungicides, Bavistin at a concentration of 1500 ppm demonstrated the most remarkable efficacy in restraining mycelial growth. Following 144 hrs of incubation, the second most effective treatment was Hinosan, which showed comparable results are same concentration.

Kumar *et al* (2016) tested four different fungicides viz (carbendazim (Bavistin) 50 WP, carboxin (Vitavax) 50 WP, propiconazole (Tilt) 25 EC and hexaconazole (Contaf) 25 EC) against brown spot pathogen of rice under *in vitro* condition. In the result, propiconazole was observed to be most effective with 96.58, 83.00, 74.00 and 63.85 % inhibition of *Drechslera oryzae* at 500, 250, 200 and 100 ppm concentrations, respectively, as compared to control.

The efficacy of ten different fungicides were tested at 50, 100, 150, 200, 250 ppm, concentrations *in vitro* against the pathogen by Channakeshava and Pankaja (2018). Among the different fungicides tested, propiconazole (25% EC) was significantly superior over the other fungicides. However. it was *on par* with hexaconazole (5% SC), where both the fungicides recorded 100% mycelial growth inhibition at 100, 150, 200 and 250 ppm concentrations.

Monisha *et al* (2019) tested the efficacy of twelve fungicides against brown spot pathogen *viz.,* Carbendazim 50% WP, Propineb 70% WP, Hexaconazole 5% EC, Tebuconazole 25% WG, Tricyclazole 75% WP, Propiconazole 25% EC, Kresoxim methyl 44.3% SC, Isoprothiolane 49% EC, Difenoconazole 25% EC, Zineb 68% + Hexaconazole 4% WP, Tebuconazole 50% + Trifloxystrobin 25% WG and Carbendazim 25% + Mancozeb 50% WP were used at different concentration of 50, 100, 250, 500, 1000, 1500 ppm by Poison Food Technique. Among the twelve fungicides, Hexaconazole 5% EC and Tebuconazole 25% + Trifloxystrobin 50% WG showed complete inhibition in all six concentrations whereas Hexaconazole 4% + Zineb 68% WP and Propiconazole 25% EC showed 100% inhibition at 250 and 500 ppm, respectively. Carbendazim 50% WP, Tricyclazole 75% WP, Tebuconazole 25% WG, Kresoxim methyl 44.3% SC, Isoprothiolane 49% EC and Difenoconazole 25% EC showed partial inhibition of 84.44, 85.56, 85.56, 72.22, 69.63 and 85.56% at 1500 ppm respectively when compared to control and Carbendazim 25% + Mancozeb 50% WP and Propineb 70% WP were less effective than all other fungicides tested. Among the twelve fungicides, Hexaconazole 5% EC and Tebuconazole 25% + Trifloxystrobin 50% WG were the most effective fungicides against brown spot pathogen of rice.

Nayak and Hiremath (2019a) worked on the efficacy of different fungicides singly and also in combinations for the control of *Bipolaris oryzae* under in vitro condition at different concentrations. It was found that maximum mean percent inhibition of 100% was recorded in propiconazole 25% EC at 0.05% concentration. Among six combi product fungicides evaluated, 100% mean mycelial inhibition was recorded in tebuconazole 50 % +

trifloxystrobin 25 % WG at 0.05% concentration which was significantly superior to all other fungicides. So, it was concluded by them that propiconazole 25% EC and tebuconazole 50 % + trifloxystrobin 25 % WG was the most effective fungicide against *B. oryzae* under *in vitro* condition.

In vitro test of the fungicidal effect of various fungicides namely Tricyclazole, Propiconazole and Hexaconazole was evaluated at 25, 50, and 100 ppm concentrations by food poison technique against *H. oryzae*. Inhibition of mycelial growth of *H. oryzae* varied significantly with different concentration of fungicides. Among them at 25ppm concentration maximum mycelial growth inhibition per cent of *H. oryzae* was recorded in hexaconazole (28.15%) after 144 hours, which is superior from all the tested fungicides followed by tricyclazole (27.04%) and then proiconazole (13.70%). Mycelial growth inhibition was recorded highest in hexaconazole (48.52%) followed by tricyclazole (42.96%) and after that propiconazole (16.67%) at 50 ppm concentration level. While at 100 ppm concentration again hexaconazole (57.04%) shows maximum growth inhibition percent followed by tricyclazole (44.81%) and after that propiconazole (23.70%) [Yadav *et al,* 2020]

Arbol *et al* (2022) tested 7 different fungicides against *Bipolaris oryzae* at different concentrations like 10, 25, 50, 100 and 250 ppm under *in vitro* condition. They noticed that azoxystrobin + difenoconazole was the most effective fungicide as it produced lowest growth of 13.15 mm at 100 ppm and at 250 ppm no growth was noticed. This treatment recorded highest growth inhibition of 85.39% over control at 100 ppm whereas propiconazole recorded second highest inhibition of growth of 83.33% over control at 100 ppm concentration. However, highest growth inhibition (100%) was recorded in azoxystrobin + difenoconazole, propiconazole + difenoconazole and tebuconazole + trifloxystrobin at 250 ppm concentration.

2.8.2 ED 50 value of fungicides

Sunder *et al* (2005) observed that among nine fungicides under *in vitro* condition, hexaconazole produced EC50 value of 0.11 ppm a.i. followed by propiconazole recording EC50 value of 0.42 ppm a.i.) and these two were most inhibitory to mycelial growth of the pathogen followed by iprobenphos and edifenphos.

Lore *et al* (2012) studied some new fungicide formulations for the control of sheath blight and brown spot of rice both *in vivo* and *in vitro* condition. Under *in vitro* condition against *Drechslera oryzae* the lowest ED 50 value of 1.2 µg/ml was found in kresoxim

methyl 40% + hexaconazole 8% treatment which is very closely followed by ED 50 value of 1.3 µg/ml in Propiconazole treatment.

Chouhan *et al* (2021) evaluated 8 different fungicides against *Bipolaris oryzae* in laboratory conditions. Propiconazole, pyraclostrobin + epoxiconazole, and tebuconazole + trifloxystrobin exhibited complete inhibition of mycelial growth at 0.75 ppm concentration of the active ingredient. Lowest EC50 value of 0.27 ppm of a.i was found in Propiconazole treatment which is followed by pyraclostrobin + epoxiconazole and tebuconazole + trifloxystrobin treatment with EC 50 values of 1.48 ppm a.i and 1.50 ppm ai., respectively.

2.8.3 *In vivo* testing of fungicides

The initial effort to address brown spot disease using fungicide was undertaken by Chattopadhyay in 1951. According to his findings, applying peronox 50 WP (containing 4 kg of Cuprous oxide per 1000 liters) through three sprays over two-week intervals, from the tillering to flowering phases of rice growth, demonstrated greater efficacy in disease control.

Mukherjee and Bagchi (1964) noted that effective control of the disease was achievable through the application of blitox, merculine, and mancozeb sprays. Additionally, a successful approach involved the application of a mixture containing fentin acetate and mancozeb at a ratio of 1:5, at a concentration of 0.2% during the heading and grain maturation stages, as demonstrated by Kulkarni *et al* (1981b).

In the research conducted by Moletti *et al* (1996), it was observed that the application of iprodione at a rate of 0.4 liters of active ingredient per hectare and propiconazole 25EC at a rate of 0.126 liters of active ingredient per hectare, done twice during the initial stages of brown spot disease development, led to a reduction and/or delay in infections caused by *H. oryzae*.

Jung and Yamunda (1972) provided recommendations for controlling *H. oryzae* from seedling to heading stages, suggesting the use of Propineb 60 WP (Antracol) and sulfamide 50 WP (Euparen) at a dosage of 2 kg per hectare.

During the Kharif seasons of 2003-2005, Hunjan *et al* (2006) conducted an evaluation of novel fungicides against rice brown spot disease across various rice varieties. The findings demonstrated that among the tested options, Armure 30 EC (consisting of propiconazole + difenoconazole) at a concentration of 0.1% exhibited the highest efficacy. This treatment notably decreased the severity of the disease to 5.18% and concurrently elevated the grain yield to 6153 kg/ha, in contrast to the untreated control group.

In a study by Sunder *et al* (2010) focused on brown spot disease in rice, the effectiveness of six fungicides was examined. Notably, propiconazole 25 EC (1 ml/l) and hexaconazole 5 SC (2ml/l) emerged as the most potent options, leading to a substantial reduction in brown spot severity. Specifically, propiconazole reduced severity from 22.34% to 5.19%, while hexaconazole decreased it from 7.98% to similarly low levels.

Dey and Hussain (2011) conducted an investigation to examine the effectiveness of azoxystrobin and propiconazole in managing brown spot disease in rice. Azoxystrobin 23 SC (1 ml/l) and propiconazole 25 EC (1 ml/l) were applied during tillering and ear initiation stages, leading a notable decrease in the severity of brown spot disease. Furthermore, these treatments resulted in a substantial increase in grain yield, with improvements of 32.17% and 26.76% respectively compared to the untreated control, particularly when administered during the tillering stage.

Gupta *et al* (2013) assessed various fungicides in their study for their efficacy in addressing brown spot disease in rice. Among these options, when applied in field conditions, propiconazole 25 EC demonstrated a noteworthy reduction in disease severity and simultaneously led to an increase in grain yield, as compared to the control plot.

In a field-based assessment by Mustafa *et al* (2013), the efficacy of seven fungicides was examined for mitigating brown spot disease in rice. Notably, difenoconazole 250 SC exhibited a substantial reduction in brown spot disease, achieving a rate of 9.33%, as opposed to the control (untreated plot) which displayed a disease incidence of 60%.

Iqbal *et al* (2015) undertook research to determine the most effective fungicide for managing brown spot disease in basmati rice variety during the Kharif seasons of 2011 and 2012. Among the range of fungicides examined, copper hydroxide 77 WP exhibited the highest level of disease control, with a recorded rate of 31.16%. Following closely, Difenoconazole 250 SC that achieved a disease control rate of 29.18% during both years.

Kumar *et al* (2017) investigated the effectiveness of four distinct fungicides in their research against *Drechslera oryzae*. Through field experiments, they found that a combination of methods yielded notable results. This approach involved seed treatment with Carbendazim 50 WP at a rate of 0.2 g active ingredient per kilogram and a foliar spray with Propiconazole 25 EC at a concentration of 1 ml per litre. This combined treatment demonstrated a significant reduction in disease severity by 37.26% and simultaneously led to a substantial grain yield increase of 55.49% in comparison to the control plot.

Monisha *et al* (2019) tested 12 fungicides against brown spot disease of rice under field condition. Among all the fungicides, lowest PDI of 14.67 was exhibited in Hexaconazole 5% EC @ 0.125ml/litre followed by PDI of 22.67 in Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.04g/litre and PDI of 28.44 in Zineb 68% + Hexaconazole 25% WG @ 0.625g/litre treatment.

Chouhan *et al* (2021) evaluated 8 fungicides against *Bipolaris oryzae* in laboratory conditions. From which five most effective fungicides were selected for field trails against rice brown spot disease in the context of direct seeding. Under field conditions, the foliar application of Propiconazole at 0.1% concentration emerged as the most effecting in disease control of 63.24% followed by Pyraclostrobin + Epoxiconazole (50.07%) and Tebuconazole + Trifloxystrobin (47%). Propiconazole, Pyraclostrobin + Epoxiconazole and Tebuconazole + Trifloxystrobin recorded low brown spot disease severity of 22.40%, 30.43% and 32.30%, respectively.

2.8.4 Disease severity (PDI) of Brown spot of Rice:

Lore *et al* (2012) worked on some fungicide formulations against sheath blight and brown spot disease of rice both *in vivo* and *in vitro* condition. Under the field condition Kresoxim methyl 40% + Hexaconazole 8% treatment recorded lowest mean brown spot disease severity of 3.1 when pooled over the results of two years of 2009 and 2010. This treatment is closely followed by Hexaconazole 75 WG treatment and Propiconazole 25 EC treatment with mean brown spot disease severity of 6.9 and 7.6, respectively.

In a study on different doses of different fungicides against brown spot of rice by Shrestha *et al* (2017) it was noted that disease severity was lowest with a reading of 9.926 in Saaf (Carbendazim+Mancozeb) treatment @ 2g/litre of water which is very closely followed by disease severity of 10.04 in Tilt (Propiconazole) treated plots with a dose of s ml/litre of water. The highest disease severity of 39.14 was found in control.

Kumar *et al* (2019) tested 3 different fungicide combinations in the farmers field which was compared with the normal farmers practice against different diseases of rice. All the three-fungicide combination significantly reduced percent disease intensity of brown spot disease, out of which Nativo 75 WG (Tebuconazole 50% + Trifloxystrobin 25%) @ 0.04% resulted into lowest disease intensity of 6.5 % followed by Monceren 250 SC (Pencycuron 22.9%SC) @ 0.10 % + Bavistin (Carbendazim) 50WP @ 0.1% and Sheathmar (Validamycin 3% L) @0.2% + Bavistin (Carbendazim) 50WP @ 0.1 % both of

which resulted into significantly lower disease intensity at par of 8.5 % in comparison of 18.4 % in the Farmers practice.

Poudel *et al* (2019) tested six different fungicides against brown spot of rice in field condition and disease severity was recorded in different dates like 85 DAT, 92 DAT, 99 DAT, 106 DAT and 113 DAT. Propiconazole treatment was found to be best with disease severity of 12.99 at the last disease recording i.e at 113 DAT. This treatment is very closely followed by Azoxystrobin + Tebuconazole treatment which produced PDI of 15.74 at 113 days after transplanting. These two treatments were statistically *at par* with each other. Control plot recorded the highest PDI of 38.39 at 113 days after transplanting.

In a study by Persaud *et al* (2022) effect of different fungicides and the combination of new fungicides were recorded against brown spot of rice. It was found that Mancozeb +Azoxystrobin produced least brown spot disease severity of 30.56 in autumn 2020 season and the highest disease severity of 44.45 was recorded in control treatment.

2.8.5 AUDPC measurement of the disease:

In 2015, Magar conducted a field experiment at Karma Research and Development Center in Jyotinagar, Chitwan. The experiment focused on fourteen different rice varieties and aimed to assess their resistance levels against the brown leaf spot disease caused by *Bipolaris oryzae*. This investigation took place during the summer of 2013. The results indicated significant variations among the rice varieties in terms of disease severity, total AUDPC value, thousand grain weight, and grain yield. Disease severity and the total AUDPC value were found to range from 21.73% to 58.07% and 614.8 to 1827, respectively.

Shrestha *et al* (2017) recorded that lowest AUDPC of Brown spot disease affected plots of 373.7 was found in Tilt (Propiconazole) @ 2 ml/litre of water which is very closely followed by AUDPC of 374.9 in Saaf (Carbendazim+Mancozeb) @ 2g/litre of water.

Poudel *et al* in 2019 studied the effect of different chemical fungicides against Rice brown leaf spot disease in the field condition. In that experiment, the Area Under disease progressive curve (AUDPC) value was calculated on the basis of the disease severity recorded in different dates i.e. 85 DAT, 92 DAT, 99 DAT, 106 DAT and 113 DAT. Significantly lowest AUDPC value was observed in the field treated with the Propiconazole (415.7) which was statistically *at par* with Azoxystrobin + Tebuconazole

with an AUDPC value of 464.3. The plot with control treatment showed the maximum AUDPC value of 723.4.

Mau *et al* (2020) tested different cultivars of rice to assess the brown spot disease severity to find out resistant or susceptible cultivars. Mean AUDPC of brown spot was also significantly different among rice genotypes, indicating variability in accumulation of the disease during the observation period. Rice genotypes exhibiting the lowest and the highest total AUDPC were, respectively, PJ-01 (398.42) and SBD-04 (1081.30) at 70 days after planting.

In 2022, Kamei *et al* tested 5 different fungicides and one bio control agent against brown spot disease of rice under field condition during two cropping seasons. They have calculated AUDPC value also. During the first cropping season (2014-15) studies found that disease severity was lowest in Propiconazole application AUDPC (143.35) followed by Propineb (150.80), Myclobutanil (191.65) and bio-agent *P. fluorescens* (209.70). Among the treatment of chemicals maximum disease severity index was observed in Thiophanate (AUDPC 312.00) and Carbendazim (AUDPC 222.60). Similarly, severity of brown spot disease incidence in the following cropping season (2015-16) found minimum per cent disease incidence in Propiconazole and Propineb treatment with AUDPC of 140.70 and AUDPC of 136.20, respectively followed by Myclobutanil AUDPC (171.10), Bioagent (*P. fluorescens*) AUDPC (194.50) whereas maximum disease severity was observed in Thiophanate AUDPC (279.30) and Carbendazim AUPDC (185.10). The pooled mean data analysis of disease severity also revealed that disease severity was significantly highest in Thiophanate AUDPC (295.65) and Carbendazim AUDPC (203.85) and lowest in Propiconazole AUDPC (142.02), Propineb AUDPC (143.50) and bio-agent (*P. fluorescens*) AUDPC (202.10). From the above analysis it was evidence that Propiconazole and Propineb are the most effective chemicals in reducing the brown spot diseases severity of rice.

2.9 Yield of Rice:

Hossain *et al* (2011) studied the effect of different fungicides on the brown spot disease severity and also its yield. They found that application of Azoxystrobin @ 1 ml/litre of water at tillering stage recorded highest rice yield of 6.08 t/ha and this treatment is followed by Propiconazole @ 1 ml/litre water application at tillering stage producing

5.83 t/ha yield. The application of Azoxystrobin and Propiconazole at tillering stage increased yield by 32.17% and 26.74%, respectively over control

Shrestha *et al* (2017) found that highest economic yield of rice of 5.220 t/ha was found in Saaf (Carbendazim+Mancozeb) @ 2g/litre of water treated plots which is very closely followed by a yield of 5.210 t/ha in Tilt (Propiconazole) @ 2 ml/litre of water treatment.

Kumar *et al* (2019) when tested different fungicide combinations on rice against different diseases found that Nativo 75 WG (Tebuconazole 50%+Trifloxystrobin 25%) @ 0.05% resulted highest mean grain yield of 5.23 t/ha. The lowest yield of 3.72 t/ha was found in control plot.

Poudel *et al* in 2019 found that maximum test weight (12.68 g) and grain yield of rice (4.277 t/ha) was obtained by Propiconazole treatment followed by Azoxystrobin + Tebuconazole spraying with test weight of 12.62 g and grain yield of 4.12 t/ha. Control plot recorded the lowest yield of 2.793 t/ha.

2.10 Benefit Cost Ratio:

Manzoor *et al* (2017) studied different nitrogen and potash fertilizer level on the incidence of bacterial leaf blight of rice. It was found that minimum disease incidence of BLB (15.76%) in rice was observed when 75 kg ha^{-1} N was applied with no application of K and these results were followed by the application of 75 kg ha^{-1} N with 100 kg ha^{-1} K where disease incidence was 21.22%. However, application of 75 kg ha^{-1} N with 0 kg ha^{-1} K was statistically at par with the application of 75 kg ha^{-1} N with 100 kg ha^{-1}. Application of 75 kg N ha^{-1} with 100 kg K ha^{-1} gave maximum net returns amounting Rs. 37159 which is followed by net return of Rs. 32449 with 100 kg N ha^{-1} and 0 kg K ha^{-1}. The highest cost benefit ratio of 1.32 was obtained by 75 kg N ha^{-1} with 100 kg K ha^{-1} and 100 kg N ha^{-1} and 0 kg K ha^{-1}.

The benefit-cost ratio (B:C) analysis for various fungicides indicated that Pencycuron 22.9% SC (with a B:C of 5.06) and Azoxystrobin 18.2% + Difenoconazole 11.4% SC (with a B:C of 4.65), both applied at single or recommended doses of 1 ml/l, proved to be highly cost-effective in effectively managing sheath blight disease in rice. The application of a double dose of Pencycuron 22.9% SC further increased the B:C ratio to 7.24, while using a double dose of Azoxystrobin 18.2% + Difenoconazole 11.4% SC was found to be less economically viable (with a B:C of 2.84) when compared to their recommended doses Kumar *et al* (2018).

The study for the control of brown leaf spot disease of rice crop by using different combinations of fungicide (Difenoconazole) with various concentrations of sprayable formulations of macronutrients (NPK) was conducted by Asghar *et al* in 2019. In that study economic analysis computed on the basis of grain yield revealed that rice crop sprayed with Difenoconazole @ 315 $mlha^{-1}$ + NPK (20:20:20) @ 500gha^{-1} gave the highest additional income of Rs. 26305 ha^{-1} followed by Difenoconazole @ 315 ml ha^{-1} + NPK (8:8:6) @ 500 g ha^{-1} (Rs. 22869 ha^{-1}) and Difenoconazole @ 315 ml ha^{-1} (Rs.18323 ha^{-1}).

Highest net profit of Rs 28,900 / ha with B:C Ratio as 2.24 was recorded with the application Trifloxystrobin + Tebuconazole treatment when 3 different technologies with 3 different fungicide combinations were tested in the farmers field. Lowest net return of Rs.15700 and B:C ratio of 1.73 was found in farmers field (Kumar *et al* , 2019).

Kamei and Singh (2021a) have analysed the benefit cost ratio for the application of five different botanicals against brown spot disease of rice. It was found that highest benefit cost ratio of 1.45:1 was found in the better treatments with less disease which is followed by 1.36: 1, 1.33:1 and 1.30:1 in other effective treatments.

When subject to BCR (Benefit-Cost Ratio) analysis for five selected fungicides and the bioagent *Pseudomonas fluorescens*, the highest ratio was recorded with Propiconazole (1.72:1), followed by Propineb (1.47:1), *Pseudomonas fluorescens* (1.46:1), Myclobutanil (1.45:1), Carbendazim (1.34:1), Thiophanate (1.33:1), and the Control (1.13:1). This implies that in the Propiconazole treatment, an investment of Re. 1.00 would yield a gross income of Rs. 1.72, resulting in a net return of Re. 0.72. Similar calculations for other treatments indicate net returns of Re. 0.47 for Propineb, Re. 0.46 for *Pseudomonas fluorescens*, Re. 0.45 for Myclobutanil, Re. 0.34 for Carbendazim, Re. 0.33 for Thiophanate, and Re. 0.13 for the control (Kamei and Singh, 2021b).

CHAPTER- 3
MATERIALS & METHODS

3. MATERIALS AND METHODS

3.1 Collection of disease samples:

The infected leaves with brown spot symptom were collected from different places of West Bengal

Sl. No.	Location
1.	Pundibari
2.	Dinhata
3.	Kalyani
4.	Bolpur
5.	Tufanganj
6.	Raiganj
7.	Siliguri
8.	Maldah
9.	Bankura
10.	Bishnupur

3.2 Examination of diseased samples in Laboratory:

After collecting the sample, it was examined for causal organism under compound microscope. Morphological features of pathogen associated with the disease was examined. First the infected tissue was teased nicely on a slide with water mount and cover slip was placed on it with the help of dissecting needle. Then excess fluid was removed with the help of blotting paper. In this way the slide was prepared and was ready for examining under compound microscope.

3.3 Glassware cleaning and sterilization:

The experimental equipments like petri plates, conical flasks, test tubes, measuring beakers are washed with liquid detergent and then placed in chromic acid for 24 hours.

After 24 hours with gloves in hands the glasswares were carefully taken out from chromic acid and then washed them and let them dry. Petri plates were dried and were cleaned with cotton dipped in ethanol. After the plates are dried, they were wrapped in newspaper and sterilized. Blotting paper, corkborer, inoculation needle, forceps, scissors was also sterilized before use. Then the prepared media and distilled water was sterilized in the autoclave at 121°C at 15 lbs / inch^2 pressure for 20 minutes.

3.4 Disease Symptomatology:

The appearance of tiny spots on the coleoptile, leaf blade, leaf sheath, and glume were examined as brown spot symptoms. On leaves, the usual spots have an oval or cylindrical shape, are brown in colour, and have a grey or whitish centre, usually yellow-halo sesame seeds (Ou, 1985).

3.5 Isolation of the pathogen:

Rice leaves showing Brown spot symptom were collected from the field of different places of West Bengal. The isolation of the pathogen was followed by standard isolation technique. The infected leaf portions were surface sterilized in 1: 1000 sodium hypochlorite solutions for 30 seconds. To remove the remaining amounts of sodium hypochlorite, leaf bits are dipped in sterilized distilled water twice for 45 seconds each time. The leaf bits were again transferred on a sterilized blotting paper to remove excess water. Two leaf segments were placed on each plate with sterilized PDA media and then petri plates were kept in BOD Incubator set at 26±1ºC. The cultures developed from the plates were transferred and then incubated to get pure cultures.

3.6 Pathogenicity Test:

Koch's postulates of the Pundibari isolate was established using a pure culture of the isolated pathogen following standard protocol.

3.6.1 Host Establishment:

Disease free viable seeds of MTU 7029 were selected. They were sown in soil taken in plastic pots.

3.6.2 Inoculum Preparation:

Multiplication of pure culture of pathogen was done in PDB (Potato Dextrose Broth) media. On the PDA plate, the chosen *Helminthosporium oryzae* isolate was grown. Cork borer was used to pick a 5 mm actively growing mycelial bit, which was then placed aseptically into a 500 ml conical flask containing 250 ml of PDB medium. After 20 days from inoculation, mycelia was harvested and was grinded in mixture grinder.

3.6.3 Artificial Inoculation:

Rice seedlings were sprayed with spore suspension by atomise hand sprayer during evening hours. Seedlings were then covered with polythene cover to facilitate infection. The cover was taken off the very next morning and kept clean with water devoid of nitrogen to encourage effective symptom expression.

3.6.4 Re-isolation:

Re-isolation was carried out in the lab to confirm *Helminthosporium oryzae* as the causal organism of rice brown spot. The diseased spots were cut into small pieces of 1 mm, surface sterilised in sodium hypochlorite for 45 seconds, rinsed with sterile water for 2times, and transferred aseptically the segments into petri plates containing sterilized PDA media with supplemented chloramphenicol and incubated inverted at 26°C in BOD incubator for 72 hours and observed for colony development. The culture of *Helminthosporium oryzae* was purified and maintained by periodic culturing on PDA petri plates at regular intervals.

3.7 Characterization of the isolates:

3.7.1 Morphology of Conidia:

All the isolates were studied for the morphology of the conidia. Length, breadth and septation of conidia (10 for each isolate) of all the isolates was found out with the help of Magnus trinocular microscope fitted with Magnus MAGCAM camera. The photographs of all the isolates were also taken.

3.7.2 Colony characteristics study:

All the isolates were grown on Potato Dextrose Agar media for studying their colony characteristics. Colony characters like growth rate, colony colour, margin of colony, colony growth type, sporulation was observed. Radial growth of the colony of each isolate was taken at 2, 4 and 6 days after inoculation. Area Under Mycelial Growth Curve (AUMGC) was calculated based on the same formula of AUDPC given by **Das *et al* (1992)**

$$AUDPC = \sum_{i=1}^{n-1} [(y_i + y_{i+1})/2] (t_{i+1} - t_i)$$

3.7.3 Study of the pathogen in different media:

The Pundibari isolate was grown on the following media for characterization. The colony morphology was studied and radial growth was measured.

Sl. No.	Name of the media
1.	Potato Dextrose Agar
2.	Potato Sucrose Agar
3.	Malt Extract Agar
4.	Czapek Dox Agar
5.	Host Extract Agar
6.	Corn Meal Agar
7.	Oat Meal Agar

Colony characters like growth rate, colony colour, margin of colony, colony growth type, sporulation was observed. Radial growth of the colony was taken at 2, 4 and 6 days after inoculation. Area Under Mycelial Growth Curve (AUMGC) was calculated based on the same formula of AUDPC given by Das *et al* (1992).

$$AUDPC = \sum_{i=1}^{n-1} [(y_i + y_{i+1})/2] (t_{i+1} - t_i)$$

3.7.4 Media Preparation:

3.7.4. (i) Potato Dextrose Agar (PDA) Media:

Peeled Potato	:	200.00 g
Dextrose	:	20.00 g
Agar-agar	:	20.00 g
Distilled water	:	1000.00 ml
pH	:	6.4

Two hundred grams of cleaned, washed and peeled potato tubers were chopped into pieces. Later these pieces were boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose and agar agar 20 g of each were dissolved in the potato extract and the volume was made up to 1000 ml by adding sterile distilled water.

3.7.4. (ii) Potato Dextrose Broth (PDB) Media:

Peeled Potato : 200.00 g

Dextrose : 20.00 g

Distilled water : 1000.00 ml

pH : 6.4

Two hundred grams of cleaned, washed and peeled potato tubers were chopped into pieces. Later these pieces were boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose of 20 g was dissolved in the potato extract and the volume was made up to 1000 ml by adding sterile distilled water.

3.7.4. (iii) Host Extract Agar (HEA) Media:

Host extract : 200.00 g

Dextrose : 20.00 g

Agar-agar : 20.00 g

Distilled water : 1000.00 ml

pH : 6.4

Two hundred grams of cleaned, washed paddy leaves were chopped into pieces. Later these pieces were boiled in distilled water and the extract was collected by filtering through muslin cloth. Dextrose and agar agar 20 g of each were dissolved in the host extract and the volume was made up to 1000 ml by adding sterile distilled water.

3.7.4. (iv) Czapeck's Dox Agar (CDA) Media:

Sucrose : 30.00 g

Sodium nitrate : 03.00 g

Potassium dihydrogen phosphate : 1.10 g

Magnesium sulphate : 0.050 g

Potassium chloride	:	0.50 g
Ferrous sulphate	:	0.01 g
Agar-agar	:	20.00 g
Distilled water	:	1000.00 ml

Agar-agar was melted in 500 ml distilled water. Two solutions were mixed thoroughly and the volume was made up to 1000 ml and was sterilized.

3.7.4. (v) Oat meal Agar (OMA) Media:

Rolled oats	:	40 g
Agar	:	20 g
Distilled Water	:	1000 ml

Rolled oat was boiled in 500 ml distilled water for 15minutes, filtered through muslin cloth, agar was melted in 500 ml distilled water. Both the solution were mixed and the volume was made to 1000 ml and then sterilized as above.

3.7.4. (vi) Malt Extract Agar (MEA) Media:

Malt extract	:	25 g
Agar	:	20 g
Distilled water	:	1000 ml

Malt extract was mixed thoroughly in 500 ml of distilled water and agar was melted separately in 500 ml of distilled water. Both the solutions were mixed thoroughly and the volume was made up to 1000 ml by adding distilled water and autoclaved as mentioned above.

3.7.4. (vii) Potato Sucrose Agar (PSA) Media:

Peeled Potato	:	200.00 g
Sucrose	:	20.00 g
Agar-agar	:	20.00 g

Distilled water : 1000.00 ml

pH : 6.4

Two hundred grams of cleaned, washed and peeled potato tubers were chopped into pieces. Later these pieces were boiled in distilled water and the extract was collected by filtering through muslin cloth. Sucrose and agar agar 20 g of each were dissolved in the potato extract and the volume was made up to 1000 ml by adding sterile distilled water.

3.8 Efficacy of chemicals on growth inhibition of pathogen (*in vitro*):

3.8.1 Poisoned Food Technique:

In order to determine the efficacy of different chemicals for inhibiting the mycelial growth of the pathogen causing brown spot disease of rice during the period of investigation, poisoned food technique suggested by Dhingra and Sinclair (1995) was followed. The efficacy of chemicals on the growth of the test fungus was studied at different doses as per the selected chemicals.

Sl. No.	Treatments
1.	Carbendazim 50% WP @ 2g / litre
2.	Mancozeb 75%WP @ 3g / litre
3.	Carbendazim 12% + Mancozeb 63% WP @ 2 g / litre
4.	Difenoconazole 25% EC @ 1ml / litre
5.	Azoxystrobin 23% SC @ 1ml / litre
6.	Mancozeb 66.7%+Azoxystrobin 8.3% WG @ 1.5 g / litre
7.	Azoxystrobin 18.2% + Difenoconazole 11.4% SC @ 1ml / litre
8.	Control

- 5000 ppm stock solution was prepared for each fungicide.
- By using $V_1S_1=V_2S_2$, different concentrations of 50 ppm, 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm were prepared
- Control plate was without any fungicide
- After one week radial growth was measured

The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth, which was calculated by using the formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where, I = per cent inhibition, C = growth in control, T = growth in treatment

- ED 50 was also calculated by Probit analysis

During the study, required quantity of individual fungicide was added separately into molten and sparingly cooled Potato Dextrose Agar so as to get the desired concentration of fungicides in the medium. Later 20 ml of the poisoned medium was poured into sterile Petri dishes under aseptic conditions inside an inoculation chamber. Medium without any fungicide served as control. Each Petri plate was inoculated at the center with a mycelia disc of 5mm diameter taken from the periphery of 5-day old colony of the test fungus with the help of sterilized cork borer and replicated four times. The Petri plates were incubated at 27±1°C for 7 days. The colony diameter was measured in each concentration at 7^{th} day when the control plate was fully covered by the pathogen growth.

3.9 Effect of different chemicals against brown spot of rice in field condition (*in vivo*):

3.9.1 Details of the Experimental Field:

3.9.1.1 Experimental site:

In the year 2022, the field tests were carried out in the Uttar Banga Krishi Viswavidyalaya's instructional farm in Pundibari, Cooch Behar, West Bengal, India. During the proper study years, the laboratory research was conducted at the Department of Plant Pathology, UBKV, Pundibari, Cooch Behar. This chapter discusses the research techniques employed and the materials utilized in the studies.

The experimental area is physically a part of West Bengal's sub-himalayan terai region, and it is situated between 26° 19′ 86″N latitude and 89° 23′ 53″E longitude. The location is elevated since it is 43.0 m above mean sea level. The entire Coochbehar district, the Siliguri subdistrict of Darjeeling district, the Islampur subdivision of North Dinajpur district, and the entire Jalpaiguri district are all included in the terai zone. This region is surrounded by Bangladesh, Bhutan, the Kalimpong and Kurseong hills in the north, the Assam border in the east, Bihar in the west, and the Assam border in the south. The overall geographic area of this zone is 12,025 sq km, of which 8,567 sq km are cultivated. The percentage of residents who reside in rural areas is higher than the state's average at over 90%.

3.9.1.2 Experimental Soil:

The soil in the experimental area has a sandy loam texture and a dark brown top layer that is 1-3 feet deep. The soil is porous, has a medium to strong acidic nature, is abundant in raw humus, and has a low capacity to retain water. K concentration is low to medium, secondary micronutrient proportion is low, and total nitrogen content is moderate to high.

3.9.1.3 Climate:

The terai region of West Bengal is known for its high relative humidity (average maximum 95% and average minimum 65%) and significant rainfall (annual average more than 3000 mm), which distinguish it from other warm and humid regions. During the rainy kharif season, which lasts from June to September, almost 80% of the total rainfall occurs. The amount of bright sunshine each day is roughly constant from January through early June, or shortly before the onset of monsoon season. After that, the amount of daylight hours each day starts to instantly decline and continues to do so until early October. Brilliant sunny hours that last an average of more than 8 hours are the norm from November to March, with the exception of the monsoon season. Winter season starts from November and continue up to February here.

3.9.1.4 Experimental details:

Crop	Rice
Season	*Kharif*, 2022
Experimental site	Instructional Farm, UBKV
Experimental design	Randomized Block Design (RBD)
Number of treatments	8
Number of replications	3
Size of each plot	4m × 4 m
Variety	MTU 7029
Date of Transplanting	25-07-2022
Spacing	20 cm × 15 cm
Date of Harvesting	02-11-2022

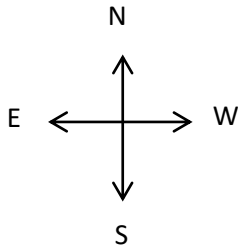
All the standard package of practices are followed for rice cultivation. Artificial inoculation with the pathogen of Pundibari isolate was done @ 10^6 cfu/ml of the pathogen population at 45 Days After Transplanting (DAT) following standard artificial inoculation technique. Spraying of the fungicides according to the below mentioned doses were done 3 times at 55 DAT (just little after disease appearance stage), 65 DAT and 75 DAT stage.

Treatments:

T_1 - Carbendazim 50% WP @ 2g / litre
T_2 - Mancozeb 75%WP @ 3g / litre
T_3 - Carbendazim 12% + Mancozeb 63% WP @ 2g / litre
T_4 - Difenoconazole 25% EC @ 1ml / litre
T_5 - Azoxystrobin 23% SC @ 1ml / litre
T_6 - Mancozeb 66.7%+Azoxystrobin 8.3% WG @ 1.5g / litre
T_7 - Azoxystrobin 18.2% + Difenoconazole 11.4% SC @ 1ml / litre
T_8 - Control

LAYOUT OF THE EXPERIMENTAL FIELD

T_1R_1	T_3R_2	T_8R_3
T_6R_1	T_1R_2	T_2R_3
T_2R_1	T_4R_2	T_6R_3
T_7R_1	T_2R_2	T_5R_3
T_3R_1	T_5R_2	T_7R_3
T_8R_1	T_6R_2	T_1R_3
T_4R_1	T_8R_2	T_3R_3
T_5R_1	T_7R_2	T_4R_3



Disease severity was recorded by following the standard evaluation scale of 0 – 9 for brown spot disease of rice (IRRI, 2002).

Brown leaf spot disease rating scale:

Score	Infection rate (%)
0	No infection
1	<1
2	1-3
3	4-5
4	6-10
5	11-15
6	16-25
7	26-50
8	51-75
9	76-100

The formula given by Wheeler (1969) was used to calculate the percent disease index (PDI) as follows:

3.9.1.5 Percent Disease Index (PDI):

$$PDI = \frac{\text{Sum of all individual disease ratings}}{\text{Total number of leaves observed} \times \text{Maximum disease grade}} \times 100$$

Disease severity (PDI) was taken 3 times starting from the disease appearance (55 DAT) at 10 days interval (65 DAT and 75 DAT). PDI was calculated on the basis of above-mentioned disease scoring scale.

3.9.1.6 Area Under Disease Progressive Curve (AUDPC):

Multiple observations of disease progression are commonly combined into a single number converting multiple PDIs into the area under the disease progress curve (AUDPC). The formula used for calculation of AUDPC was followed which was given by Das *et al* (1992) and it is given as under:

$$AUDPC = \sum_{i=1}^{n-1} [(x_i + x_{i+1})/2] (t_{i+1} - t_i)$$

where, x_i = disease severity on the *i*th date, t_i = *i*th day, n = number of dates on which disease was recorded

AUDPC of each treatment was calculated according to the above-mentioned formula. Percent reduction in disease over control was found out on the basis of AUDPC results.

3.9.1.7 Yield:

Yield of the crop was taken from each replication and each treatment. Yield of 1 m^2 area was taken from each plot and that was converted to per hectare yield which was measured in ton per hectare. Percent increase in yield over control for all the treatments was also found out.

3.9.1.8 Incremental benefit cost ratio:

Here incremental benefit cost ratio was found out. Now-a-days, this is the good method for finding out the benefit from any experiment. Here, extra money that is required for applying a particular treatment is divided by the extra return received for applying that treatment and in this way incremental benefit cost ratio is found out. The common expenditure for all the treatments is not taken into account here. That means the increment amount for applying each treatment over control was found out, the increased yield of all the treatments for applying that treatment was found out and increment in return for that extra yield was found out. Now, that increment in return was divided by extra cost that was required for application for a particular treatment and in this way incremental benefit cost ratio for each treatment was calculated.

3.10 Statistical Analysis:

The statistical analysis of all the applicable data was carried out with the OPSTAT software developed by CCSHAU, Haryana. The percentage values were transformed (if required) by angular transformation using the same OPSTAT software.

CHAPTER-4
RESULTS & DISCUSSION

4. RESULTS AND DISCUSSION

Number of leaf samples of Rice infected with Brown spot disease were collected from ten different locations of West Bengal. Those locations were Pundibari, Dinhata, Kalyani, Bolpur, Tufanganj, Raiganj, Siliguri, Malda, Bankura and Bishnupur. Those isolates were named by some name for identifying them.

Table 1: Different Isolates of Brown spot pathogen of Rice collected from different locations of West Bengal

Name of the isolates	Location	Soil type	Latitude (^{0}N)	Longitude (^{0}E)	Other disease
HO-1	Pundibari	Sandy loam	26.40	89.39	Blast
HO-2	Dinhata	Sandy loam	26.15	89.46	Sheath blight
HO-3	Kalyani	Sandy loam to clay	22.99	88.44	Blast
HO-4	Bolpur	Sandy loam	23.67	87.69	Sheath blight
HO-5	Tufanganj	Sandy loam to Clay	26.33	89.67	Sheath blight
HO-6	Raiganj	Sandy loam	25.62	88.12	Blast
HO-7	Siliguri	Clay loam	26.72	88.40	Blast
HO-8	Malda	Clay loam	22.64	88.53	Sheath blight
HO-9	Bankura	Sandy loam to Clay	23.23	87.09	Sheath blight
HO-10	Bishnupur	Sandy loam	23.07	87.33	Blast

Brown spot of rice affected disease samples were collected from ten different locations of West Bengal. The pathogen was isolated from those samples and they were named as HO 1 to HO 10. This type of naming was also given by Jaiganesh and Kannan (2019) who had collected brown leaf spot of rice sample from 5 different locations and after isolation the pathogen was named as Ho_1 to Ho_5. The soil type of those locations, latitude, longitude is mentioned in the Table No. 1. Other diseases like blast, sheath blight was also noticed in those areas on rice crop (Table 1).

KOCH POSTULATE

Koch Postulate of the Pundibari isolate was carried out. The pathogen *Helminthosporium oryzae* was isolated from the diseased leaf. It was grown *in vitro* condition in the laboratory in Potato Dextrose Agar Media, growth characteristics were observed and the pathogen was identified under a compound microscope. The pathogen was then grown in Potato Dextrose Broth medium and this pathogen was inoculated in the leaf of a susceptible rice variety. The similar and typical symptom of brown spot disease of rice was noticed having small sesame seed like brown spots 7 days after inoculation. From

that spot the pathogen was re- isolated in Potato Dextrose Agar media and similar growth characteristics of the pathogen was noticed. In this way Koch postulate of the pathogen is proved and Pathogenicity test is completed (Plate 1).

Pathogen cultured on PDB Media is sprayed on rice plants

Later brown spot symptom is noticed

Plate 1: Koch Postulate establishment

Table 2: Length, breadth and septation of conidia of different isolates of the pathogen

Isolates	Length (µm)	Breadth (µm)	No. of septa
Pundibari	110.26	16.71	9.10
Dinhata	53.94	16.54	7.00
Kalyani	116.49	15.68	7.50
Bolpur	62.63	12.88	7.50
Tufanganj	103.47	16.40	8.60
Raiganj	173.42	17.41	9.10
Siliguri	144.17	18.02	8.20
Malda	50.37	15.29	4.40
Bankura	71.27	19.07	4.80
Bishnupur	99.41	19.19	8.90
SE(m)±	0.677	0.592	0.235
CD (5%)	1.906	1.667	0.661
CV	2.174	11.203	9.895

The pathogen *Helminthosporium oryzae* was isolated from different locations of West Bengal. Their length, breadth and number of septa of the conidia was also found out. From Table 2, it was noticed that conidia of Raiganj isolate was highest in length (173.42 µm) and Siliguri isolate conidia produced the second highest length of 144.17 µm. The length of conidia of all the isolates showed statistically significant difference among each other. The lowest length of conidia was found in Malda isolate with 50.37 µm length. Highest breadth of conidia was found in Bishnupur isolate with 19.19 µm which is closely followed by Bankura isolate with 19.09 µm breadth. These two isolates are statistically *at par* with each other in respect of breadth. The lowest breadth was found in Bolpur isolate with 12.88 µm breadth (Plate 2). Highest number of septation was found in both Pundibari and Raiganj isolate with an average of 9.10 septa which is very closely followed by Bishnupur isolate with and average number of septa of 8.90 (Fig 1). These three isolates are statistically *at par* with each other. This result is in accordance with Ou (1985) who found that size of conidia of Indian isolate are approximately 70 – 175 × 5.6 – 7 µm and 45 – 106 × 14 – 17 µm. He found that these conidia produced 5 – 10 septations. This result is also in conformity with Kumar *et al* (2016) who reported that conidia of *Bipolaris oryzae* of Northern and Eastern regions of the country are 110-137 µm in length and 18 – 23 µm in width. The lowest average number of septa of 4.40 was found in Malda isolate.

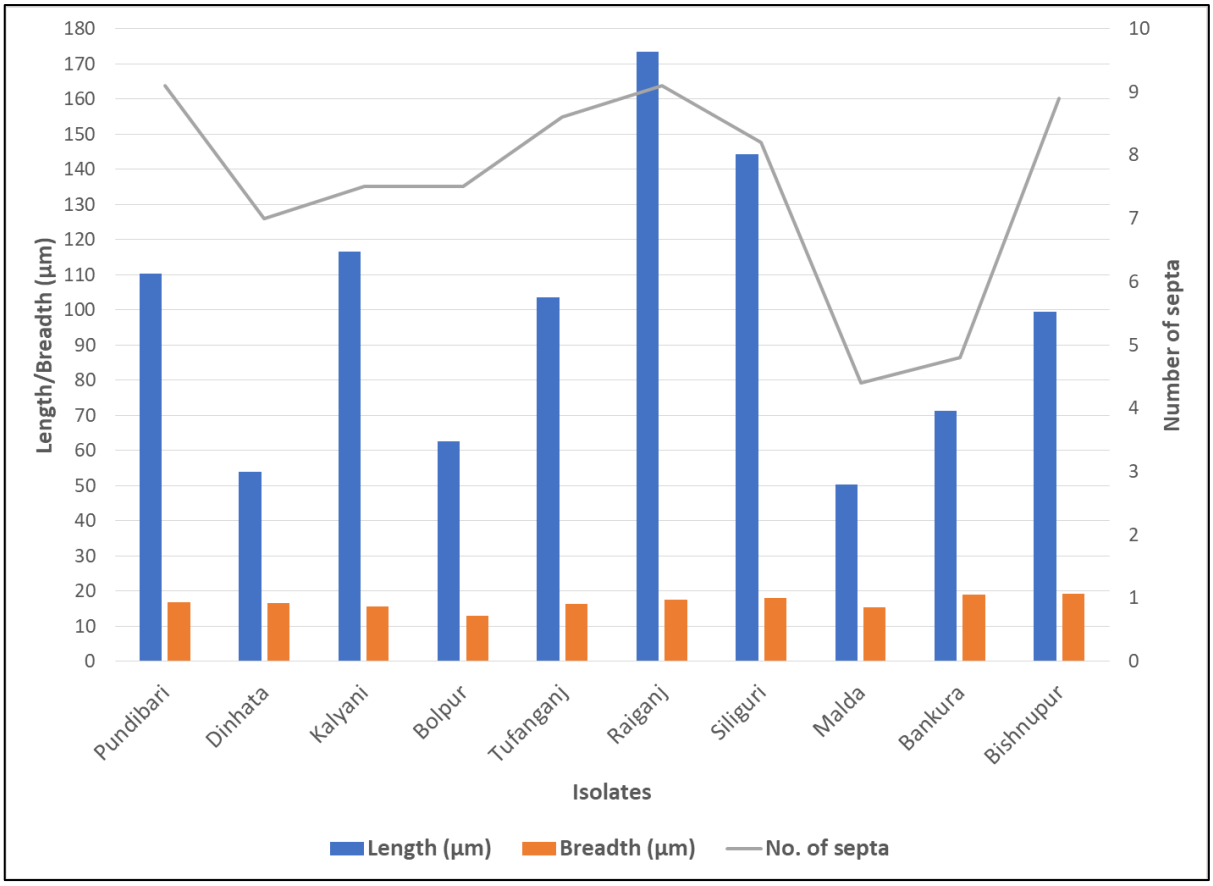
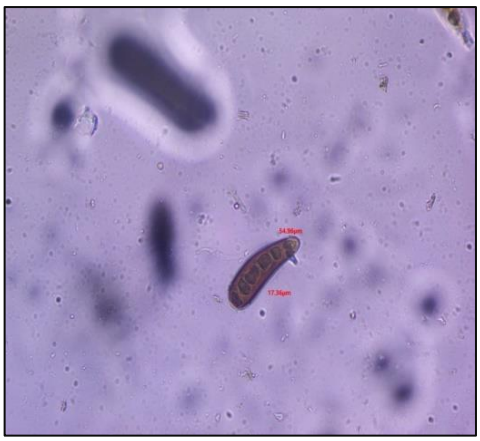


Fig 1: Length, breadth and septation of conidia of different isolates of the pathogen

Pundibari

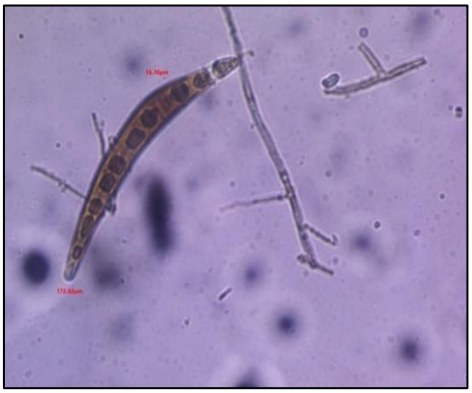


Dinhata

Kalyani

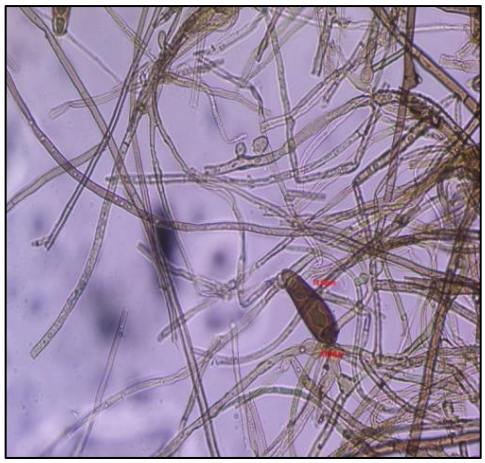
Bolpur

Tufanganj

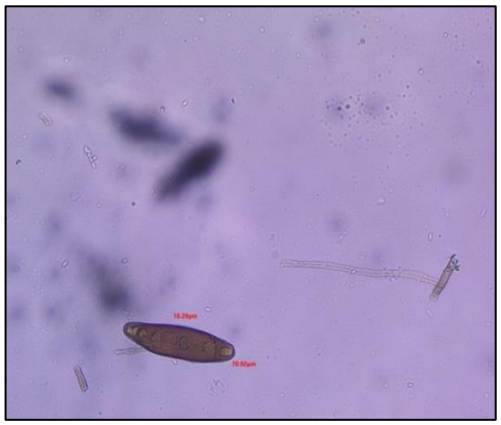


Raiganj

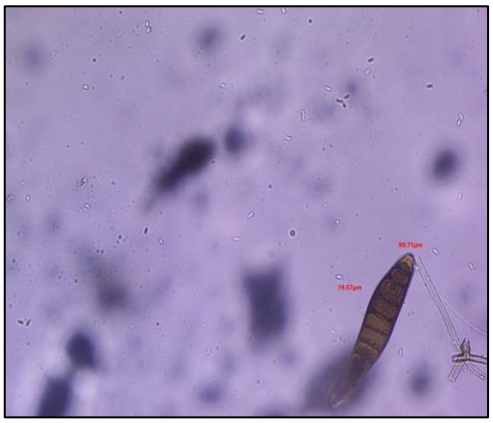
Siliguri



Malda



Bankura



Bishnupur

Plate 2: Measurement of different isolates of the pathogen

Table 3: Colony characters of different isolates of the pathogen

Isolates	Colony growth rate	Colony Colour	Margin	Colony character	Sporulation
Pundibari	Fast	Greyish Black	Regular	Suppressed	+++
Dinhata	Fast	Dark gray	Regular	Fluffy	++
Kalyani	Slow	Greyish	Irregular	Fluffy	++
Bolpur	Fast	Whitish black	Irregular	Fluffy	+
Tufanganj	Moderate	Greyish Black	Regular	Suppressed	++
Raiganj	Moderate	Greyish	Regular	Suppressed	++
Siliguri	Slow	Whitish gray	Irregular	Fluffy	++
Malda	Fast	Dark Greyish Black	Irregular	Fluffy	+
Bankura	Moderate	Dark gray	Regular	Fluffy	++
Bishnupur	Slow	Dark Greyish	Regular	Suppressed	++

'+++'- Highest Sporulation; '++'-Moderate Sporulation; '+'-Less Sporulation

The colony characteristics of different isolates collected from different locations of West Bengal was studied in Potato Dextrose Agar media. The Pundibari isolate produced highest sporulation and faster growth in Potato Dextrose Agar media with greyish black colony colour, regular margin and flat colony character at 6 days after inoculation. Among the other isolates Dinhata and Malda showed fast growth but moderate and least sporulation, respectively at 6 days after inoculation. Rest of the isolates showed moderate to slow growth rate. This result is in accordance with the findings of Kumar *et al.* (2016) who had grown different isolates on PDA and based on colony diameter those isolates were categorized into slow, moderate and fast-growing isolates. They have also found that isolates from Bihar and Jharkhand produced maximum sporulation while isolates from Gujarat produced least sporulation. Some isolates showed flat colonies while other produced fluffy or aerial mycelial growth. Greyish to grey black coloured colony colour is seen in most of the isolates. This result is in conformity with Valarmathi & Ladhalakshmi (2018) who collected 17 isolates of *B. oryzae* from different rice growing areas and they have classified those into 4 groups according to different growth characteristics. They found in some isolates there were black colouration with fluffy growth while in other isolates grey and fluffy growth was observed and, in some others, grey and suppressed growth was recorded. Most of the isolates produced regular margin and some produced irregular margins of the colony (Table 3). This result confirms the findings of Nayak and Hiremath (2019) who cultured ten different isolates of *B. oryzae* and found that some

isolates displayed regular margin while other isolates produced irregular margins of colony.

Table 4: Growth of different isolates of the pathogen in PDA

Isolates	Growth (mm)			AUMGC
	2 DAI	4 DAI	6 DAI	
Pundibari	17.01	58.77	81.58	108.03
Dinhata	15.42	49.92	86.67	100.96
Kalyani	10.47	35.18	73.80	77.31
Bolpur	7.83	46.33	88.33	94.42
Tufanganj	18.33	42.85	82.17	93.10
Raiganj	15.75	43.67	81.33	92.21
Siliguri	13.67	24.33	82.71	72.52
Malda	16.50	46.42	87.42	98.38
Bankura	18.88	40.11	76.61	87.86
Bishnupur	13.49	29.13	68.20	69.98
SE(m)±	0.735	1.787	2.380	2.189
CD (5%)	2.184	5.308	7.070	6.502
CV	8.639	7.427	5.096	4.237

Growth of all the isolates was studied in Potato Dextrose Agar media and growth was taken at 2 DAI, 4 DAI and 6 DAI and also AUMGC was calculated. The highest growth of 88.33 mm was found in Bolpur isolate at 6 days after inoculation (DAI). But if we consider the progress of mycelial growth over 6 days and calculate the Area Under Mycelial Growth Curve (AUMGC) over the 6 days then Pundibari isolate produced highest AUMGC of 108.03 with growth of 81.58 mm at 6 DAI. This is followed by Dinhata isolate with AUMGC of 100.96 with 86.67 mm growth at 6 DAI. The third highest AUMGC of 98.38 was found in Malda isolate. Pundibari isolate produced highest growth of 58.77 mm at 4 DAI among all the isolates (Fig 2) and Pundibari isolate is showing statistically *at par* growth in both 2 DAI and 6 DAI with the isolate having highest growth at 2 and 6 DAI but it is showing statistically significantly higher result in respect of AUMGC over all the other isolates (Table 4). The difference in growth rate in different isolates may be due to the fact that these isolates are quite different from one another. As these isolates are from different and distant places of West Bengal, they are forming a diverse group and so the growth is different because some of them may be genetically little different with one other which may be known if molecular characterization of those isolates would be done.

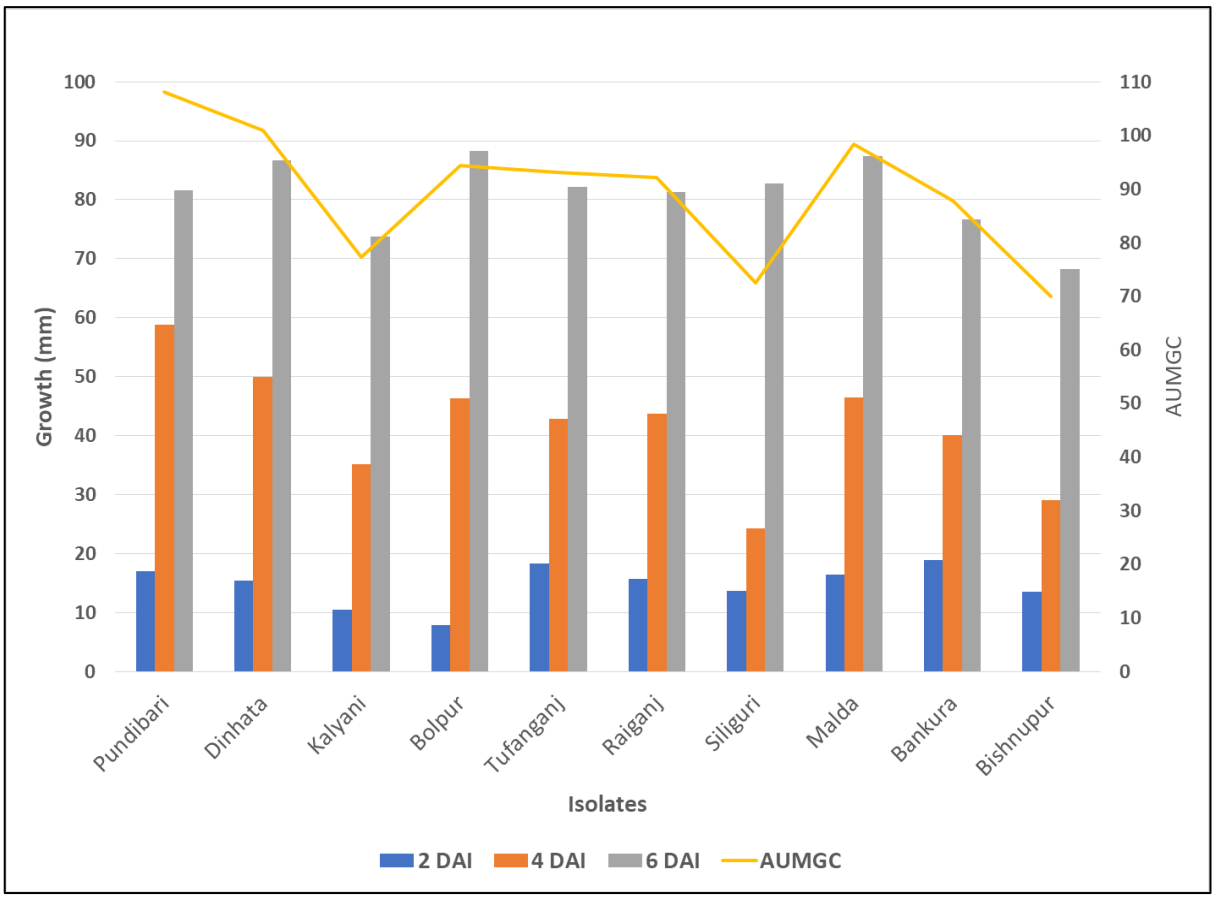


Fig 2: Growth of different isolates of the pathogen in PDA

Table 5: Colony characters of the pathogen in different media

Media	Colony growth rate	Colony Colour	Margin	Colony character	Sporulation
Potato Dextrose Agar	Moderate	Greyish Black	Irregular	Fluffy	++
Potato Sucrose Agar	Fast	Dark grey	Regular	Fluffy	+++
Malt Extract Agar	Fast	Greyish	Regular	Fluffy	+++
Czapek Dox Agar	Slow	Whitish black	Irregular	Fluffy	+
Host Extract Agar	Moderate	Greyish Black	Regular	Fluffy	+++
Corn Meal Agar	Moderate	Greyish	Irregular	Flat	++

'+++'- Highest Sporulation; '++'-Moderate Sporulation; '+'-Less Sporulation

Colony characteristics of the Pundibari isolate of the pathogen was studied in six different media. It was found that Malt Extract Agar and Potato Sucrose Agar produced highest sporulation with fast colony growth rate, fluffy colony character and regular margin. Host extract Agar also produced highest sporulation with moderate colony growth rate, fluffy colony character and regular margin. Potato Dextrose Agar and Corn Meal Agar produced good sporulation with moderate growth rate. The colony colour is different in different media like dark grey, greyish, greyish black in PSA, MEA, HEA respectively (Table 5). The reason behind the different colony characteristics in different media may be due to the fact that the isolate requires some specific nutrients for their good growth. The difference in sporulation pattern in different media indicate that the isolate loves those media for sporulation and those media can be recommended for further study of the pathogen under *in vitro* condition. As different media contains different nutrients, the difference in growth pattern in different media is obvious. Host extract agar produced good growth and highest sporulation and it may be due to the fact that since the pathogen is naturally grown on that host the growth and the sporulation of the pathogen on host extract agar media will be definitely good.

Table 6: Growth of the pathogen in different media

Media	Growth (mm)			AUMGC
	2 DAI	4 DAI	6 DAI	
Potato Dextrose Agar (PDA)	8.00	51.00	75.00	92.50
Potato Sucrose Agar (PSA)	11.00	60.33	90.00	110.83
Malt Extract Agar (MEA)	11.83	63.67	90.00	114.58
Czapek Dox Agar (CDA)	6.30	35.47	62.33	69.78
Host Extract Agar (HEA)	9.67	56.33	86.33	104.33
Corn Meal Agar (CMA)	7.58	53.00	83.33	98.46
Oat Meal Agar (OMA)	2.67	40.67	75.67	79.83
SE(m)±	0.929	3.295	1.403	3.522
CD (5%)	2.846	10.091	4.297	10.787
CV	19.752	11.083	3.023	6.371

Growth of the pathogen in different media was seen at 2 DAI, 4 DAI and 6 DAI and Area Under Mycelial Growth Curve (AUMGC) was also found out. It was noticed that Malt Extract Agar and Potato Sucrose Agar produced full plate (90 mm) growth at 6 DAI (Plate 3) with Malt Extract Agar produced highest AUMGC of 114.58 and Potato Sucrose Agar produced second highest AUMGC of 110.83. The third highest AUMGC of 104.33 was recorded by Host Extract Agar with 86.33 mm growth at 6 DAI (Table 6). This result is in conformity with the findings of Arshad *et al*. (2013) who tested five different culture media for growth of *Bipolaris oryzae* and it was noted that best growth was achieved in Malt extract Agar and Potato Dextrose Agar followed by Rice Polish Agar media. It also supports the results obtained by Valarmathi and Ladhalakshmi (2018) who found that Rice Polish Agar produced highest growth at 5 days after inoculation. Nayak and Hiremath (2019) also found that potato dextrose agar showed maximum radial growth of 89.33 mm in all the isolates followed by host extract dextrose agar with a radial growth of 87.25 mm. In the present research work Malt Extract Agar, Potato Sucrose Agar and Host Extract Agar were statistically *at par* with each other in respect of AUMGC. The lowest growth at 6 DAI of 62.33 mm was found in Czapek's Dox Agar with lowest AUMGC of 69.78 among all the tested media (Fig 3).

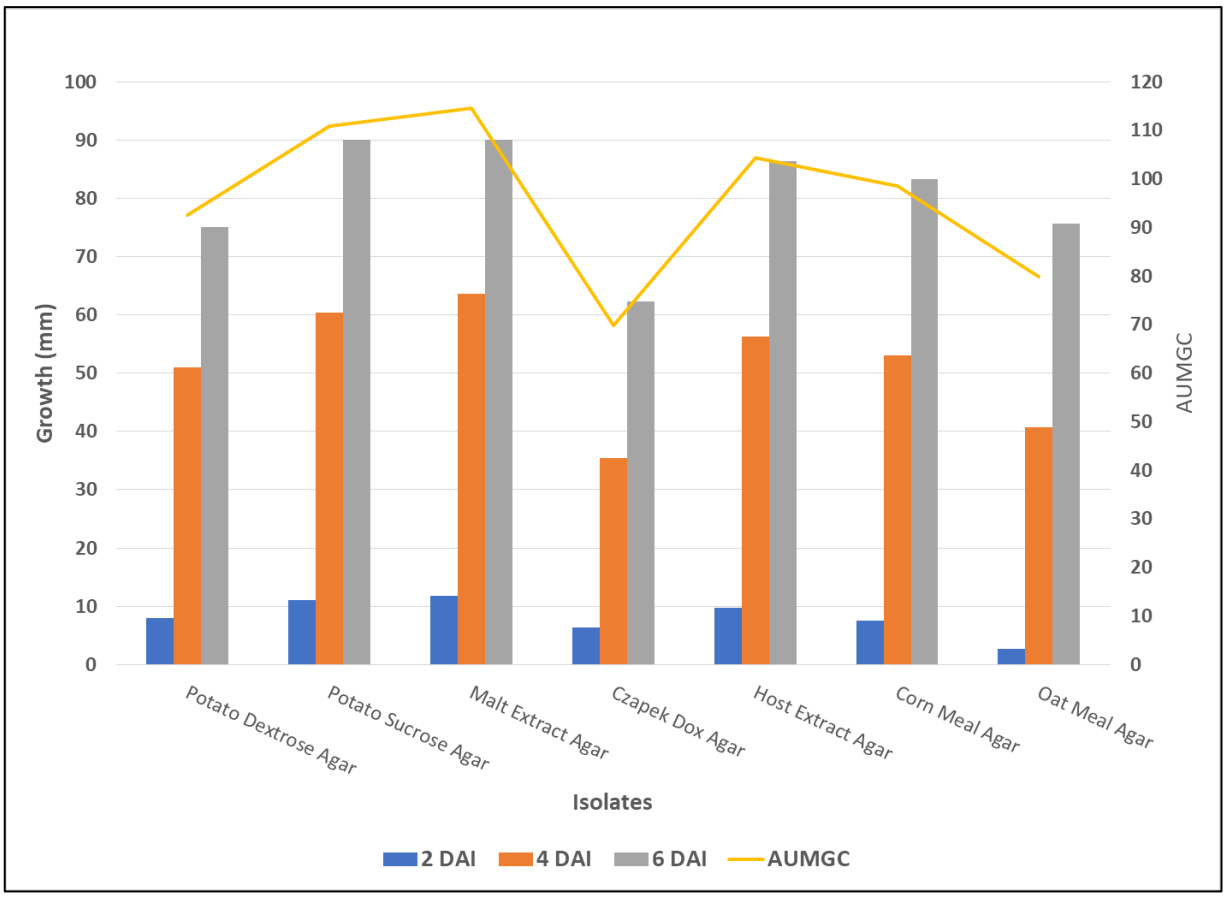
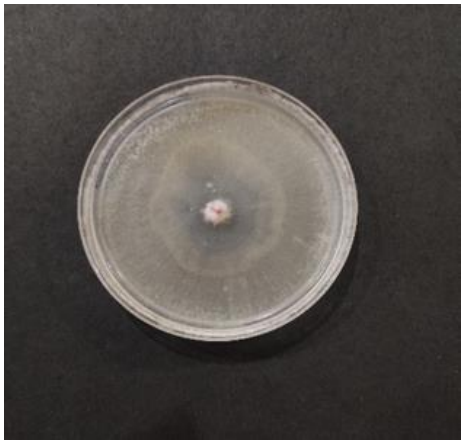
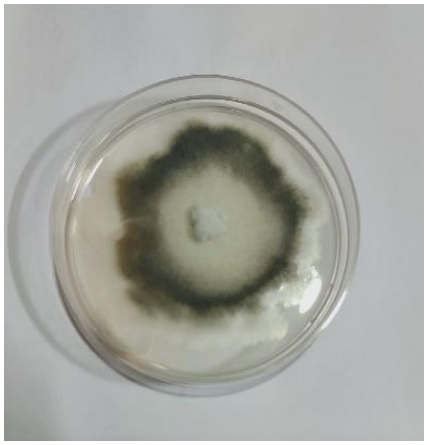


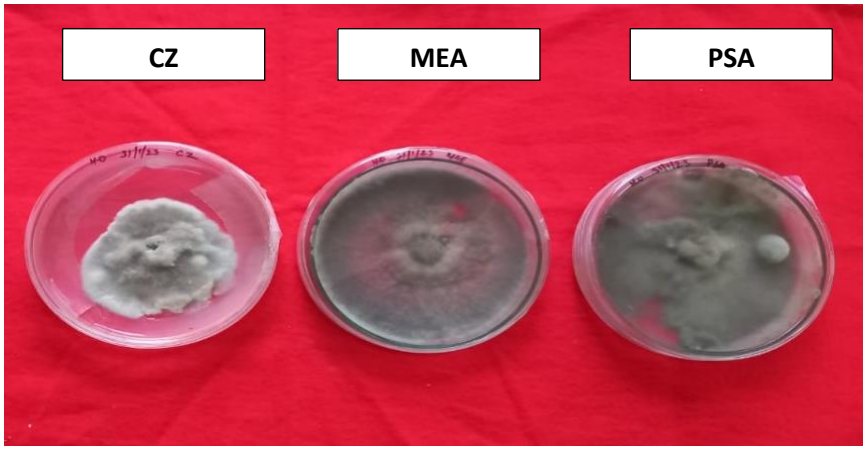
Fig 3: Growth of the pathogen in different media



Host Extract Agar



Oat Meal Agar



Czapek Dox Agar, Malt Extract Agar and Potato Sucrose Agar

Potato Dextrose Agar

Corn Meal Agar

Plate 3: Growth of the pathogen in different media

Table 7: Management of the pathogen *in vitro* by poisoned food technique

Treatments	Growth (mm)					
	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Carbendazim 50% WP	52	43	30	22	15	10
Mancozeb 75%WP	55	46	35	26	18	12
Carbendazim 12% + Mancozeb 63% WP	50	40	28	20	14	9
Difenoconazole 25% EC	28	20	9	4	0	0
Azoxystrobin 18.2%w/w	32	23	14	7	2	0
Mancozeb 66.7% + Azoxystrobin 8.3% Wg	36	24	17	11	6	2
Azoxystrobin 18.2% + Difenoconazole 11.4%SC	27	15	8	2	0	0
Control	90	90	90	90	90	90
SE(m)±	1.893	1.683	1.768	1.555	1.190	0.736
CD (5%)	5.724	5.090	5.345	4.701	3.599	2.225
CV	7.089	7.749	10.604	11.836	11.374	8.291

The pathogen was tested against different chemicals under *in vitro* condition using poisoned food technique. Under *in vitro* testing against the pathogen, it was found that Azoxystrobin + Difenoconazole was the best treatment as it produced minimum growth of the pathogen in all the concentrations tested among all the chemicals. At 300 ppm it produced least growth of 2 mm of the pathogen as against the full plate growth of the pathogen in control and Difenoconazole is the second-best treatment as it produced second minimum growth of 4 mm at 300 ppm concentration (Table 7). This result is in conformity with Arbol *et al* (2022) who found that Azoxystrobin + Difenoconazole was the most effective fungicide as it produced lowest growth of 13.15 mm at 100 ppm and at 250 ppm no growth was noticed. The present research result also supports the findings of Nayak and Hiremath (2019a) who found that Propiconazole 25% EC and Tebuconazole 50 % + Trifloxystrobin 25 % WG was the most effective fungicide against *B. oryzae* under *in vitro* condition. These two treatments are statistically *at par* with each other. All the other treatments have more growth than these two treatments. Mancozeb is the least effective treatment as it produced highest growth in all the concentrations.

Table 8: *in vitro* percent inhibition of the pathogen over control by poisoned food technique

Treatments	% inhibition of growth over control						ED 50 (ppm)
	50 ppm	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	
Carbendazim	42.22 (40.50)	52.22 (46.26)	66.67 (54.72)	75.56 (60.41)	83.33 (65.91)	88.89 (70.52)	81.64
Mancozeb	38.89 (38.54)	48.89 (44.34)	61.11 (51.40)	71.11 (57.50)	80.00 (63.43)	86.67 (68.60)	97.28
Carbendazim + Mancozeb	44.44 (41.78)	55.56 (48.17)	68.89 (56.15)	77.78 (61.86)	84.44 (66.90)	90.00 (71.61)	72.59
Difenoconazole	68.89 (56.10)	77.78 (61.87)	90.00 (71.55)	95.56 (77.86)	100.00 (90.00)	100.00 (90.00)	26.96
Azoxystrobin	64.44 (53.38)	74.44 (59.66)	84.44 (66.80)	92.22 (73.95)	97.78 (83.07)	100.00 (90.00)	33.29
Mancozeb + Azoxystrobin	60.00 (50.77)	73.33 (58.93)	81.11 (64.35)	87.78 (69.67)	93.33 (75.10)	97.78 (83.07)	36.33
Azoxystrobin + Difenoconazole	70.00 (56.77)	83.33 (65.94)	91.11 (72.76)	97.78 (83.07)	100.00 (90.00)	100.00 (90.00)	23.66
SE(m)±	1.329	1.270	1.509	1.954	1.736	1.518	-
CD (5%)	4.069	3.889	4.622	5.985	5.315	4.649	-
CV	4.768	3.997	4.180	4.892	3.938	3.264	-

Figures in parenthesis are angular transformed values

Under *in vitro* testing against the pathogen, it was found that Azoxystrobin + Difenoconazole treatment was the best as it produced highest inhibition of growth over control among all the treatments in all the concentrations. At 300 ppm concentration the inhibition of growth over control was 97.78% (Fig 4). This finding is in accordance with the findings of Monisha *et al* (2019) who reported that among the twelve fungicides tested against brown spot of rice under *in vitro* condition, Hexaconazole 5% EC and Tebuconazole 25% + Trifloxystrobin 50% WG showed complete inhibition in all six concentrations of 50, 100, 250, 500, 1000, 1500 ppm by Poison Food Technique. This Azoxystrobin + Difenoconazole treatment of the present research work also produced

lowest ED 50 value of 23.66 ppm (Fig 12). This treatment is followed by Difenoconazole treatment as it produced second highest inhibition of growth over control in all the concentrations (Table 8 & Fig 4). At 300 ppm concentration this treatment produced 95.56% growth inhibition over control and it has recorded 26.96 ppm ED 50 value (Fig 9). These two treatments are statistically *at par* with each other. This result is in agreement with the results of Chouhan and Kumar (2022) who found that lowest EC 50 value of 0.27 ppm of a.i was found in Propiconazole treatment which is followed by Pyraclostrobin + Epoxiconazole and Tebuconazole + Trifloxystrobin treatment with ED 50 values of 1.48 ppm a.i and 1.50 ppm ai., respectively. So, combination of an azole and a Strobilurin fungicide has been proved very effective in controlling brown spot pathogen of rice under *in vitro* condition. Mancozeb recorded least growth inhibition over control where it produced 71.11 % inhibition of growth over control at 300 ppm concentration (Plate 4) with highest ED 50 value of 97.28 ppm among all the treatments (Fig 7). So, it can be concluded that Azoxystrobin + Difenoconazole is the most effective treatment and Mancozeb is the least effective treatment against the pathogen under *in vitro* condition.

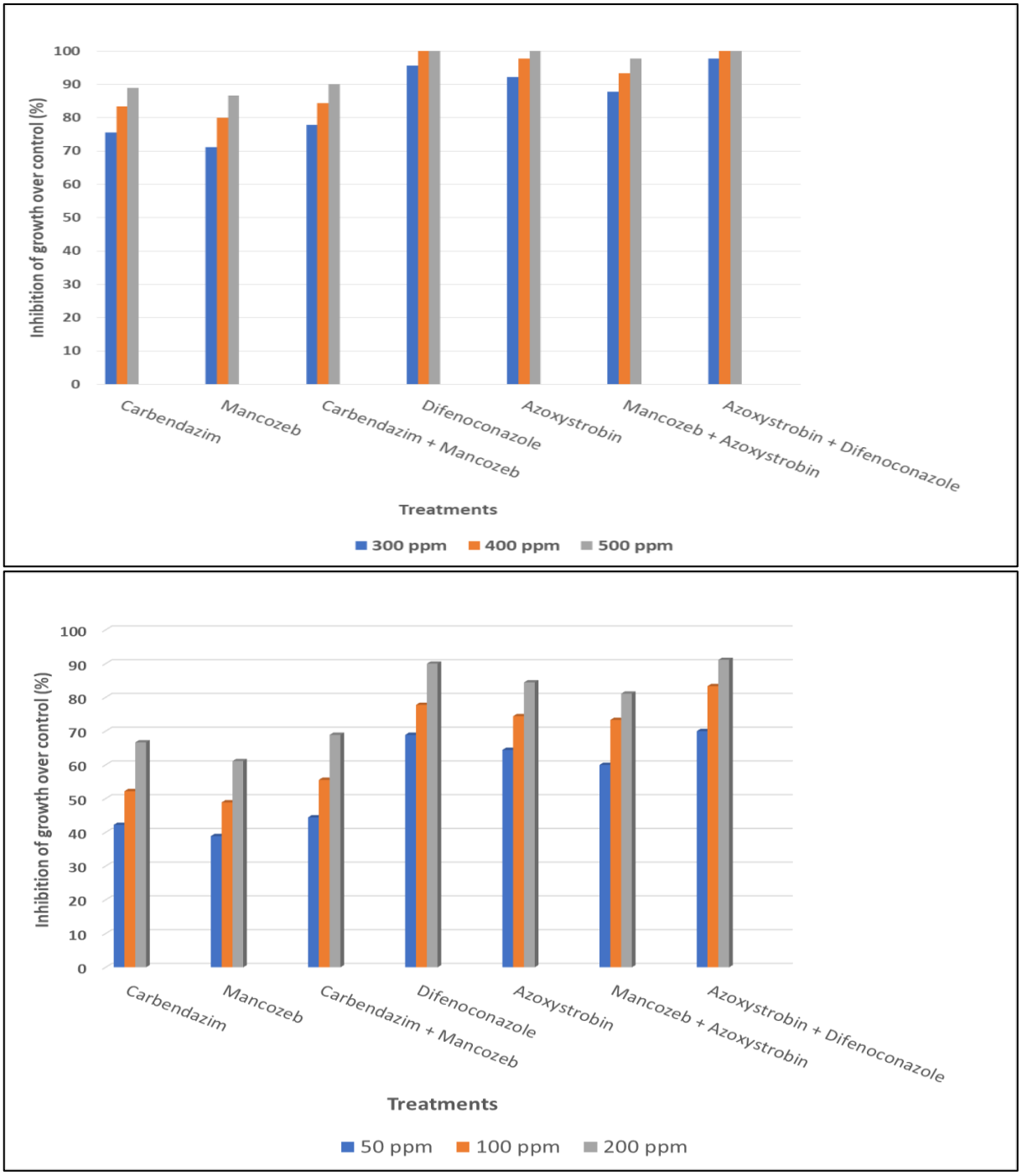


Fig 4: *in vitro* percent inhibition of the pathogen over control

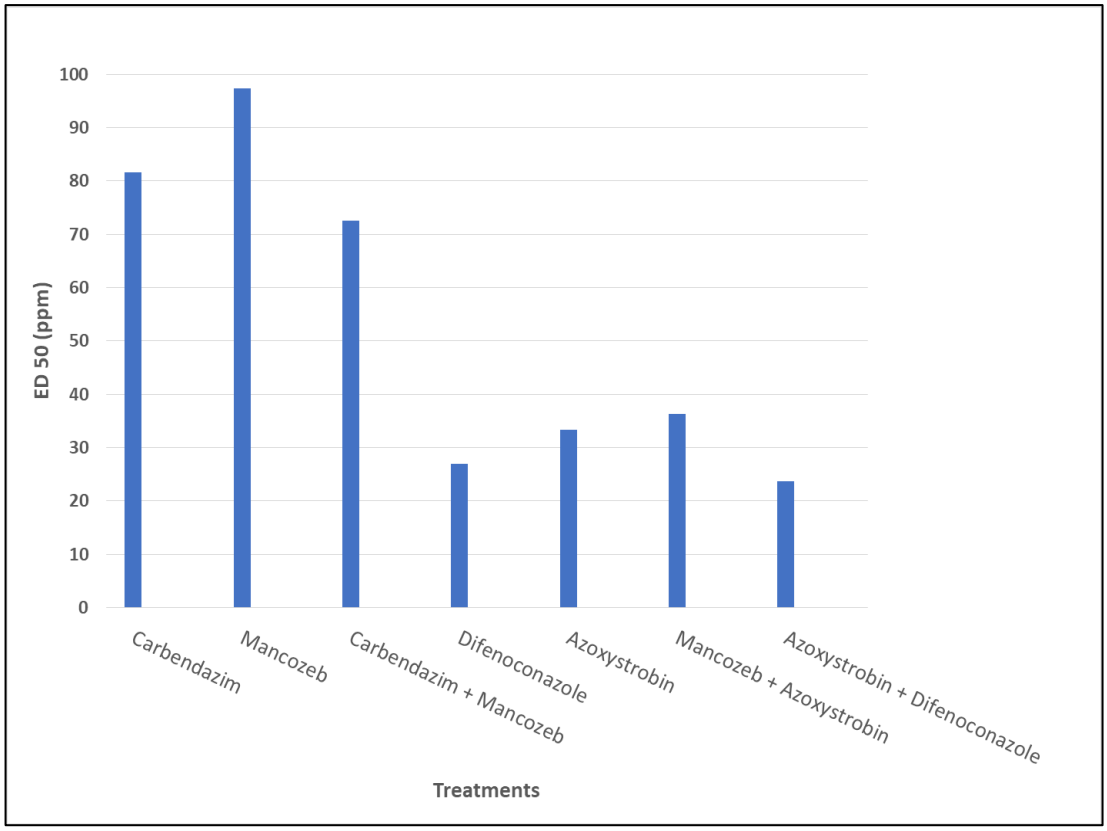


Fig 5: ED 50 values of all the treatments under *in vitro* condition

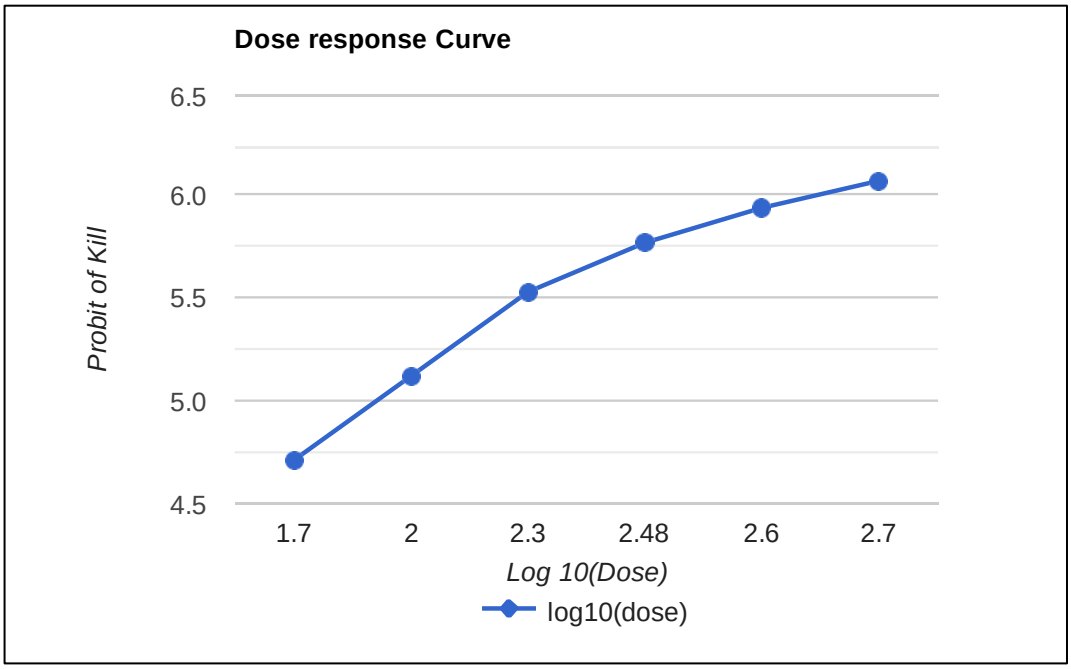


Fig 6: Dose response curve of Carbendazim

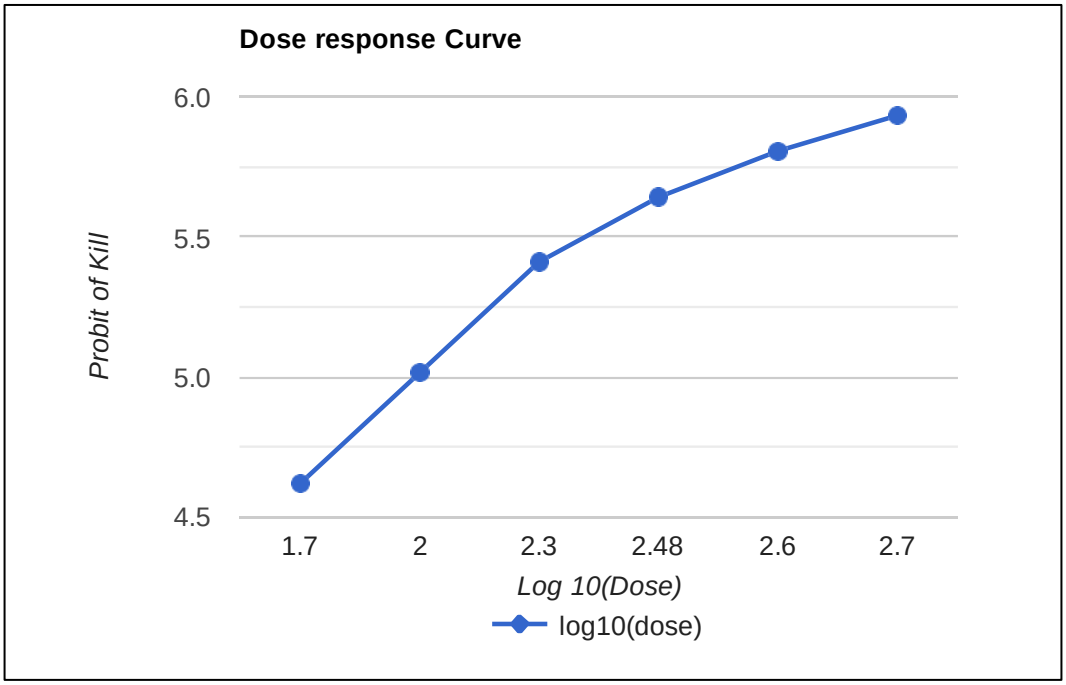


Fig 7: Dose response curve of Manocozeb

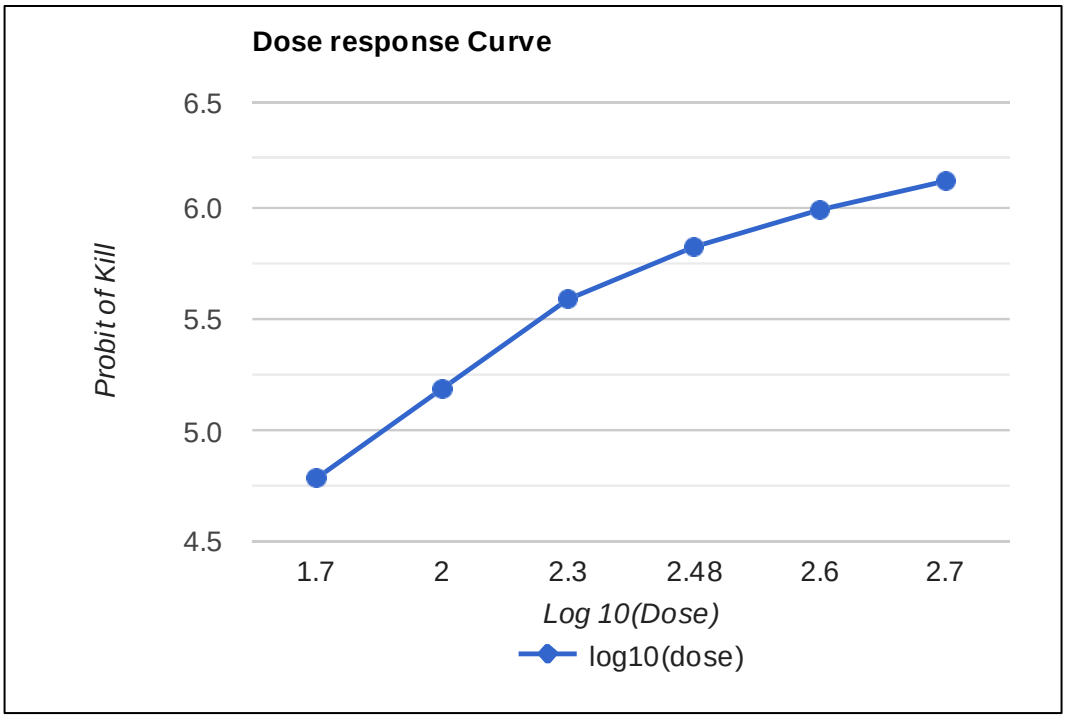


Fig 8: Dose response curve of Carbendazim+Mancozeb

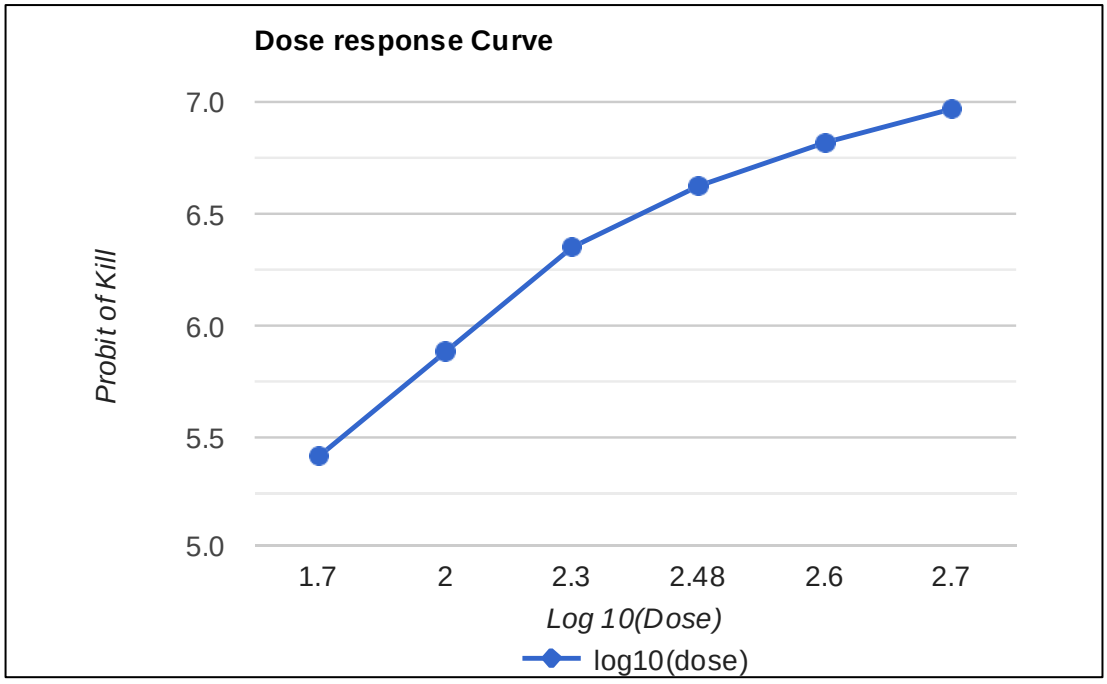


Fig 9: Dose response curve of Difenoconazole

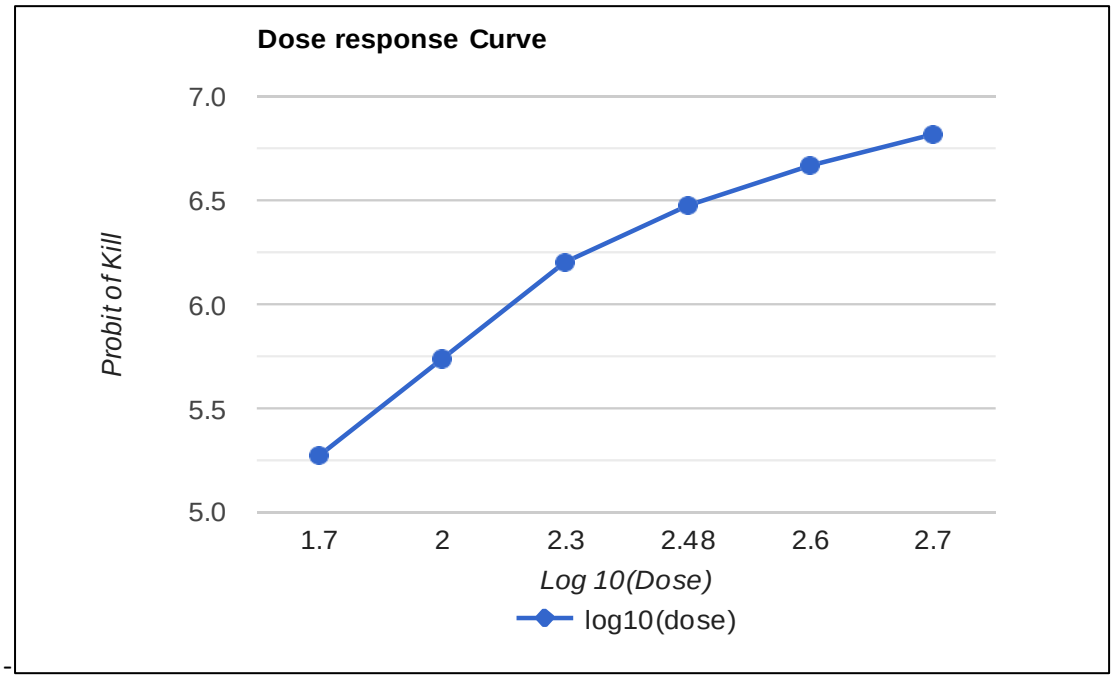


Fig 10: Dose response curve of Azoxystrobin

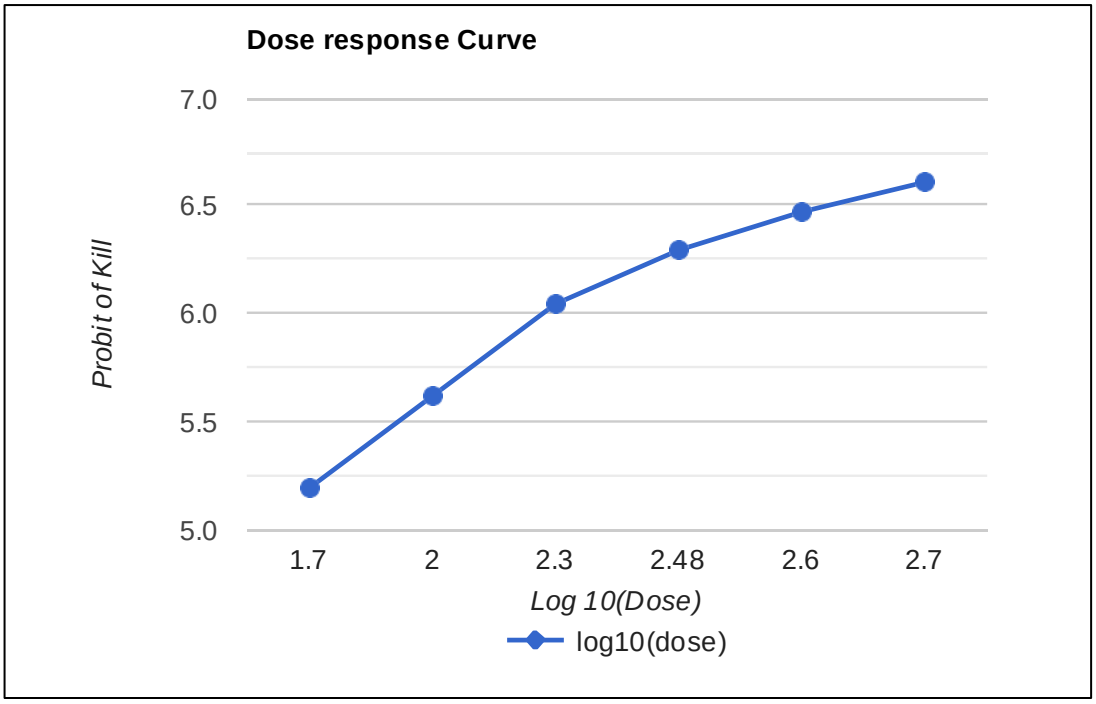


Fig 11: Dose response curve of Mancozeb+Azoxystrobin

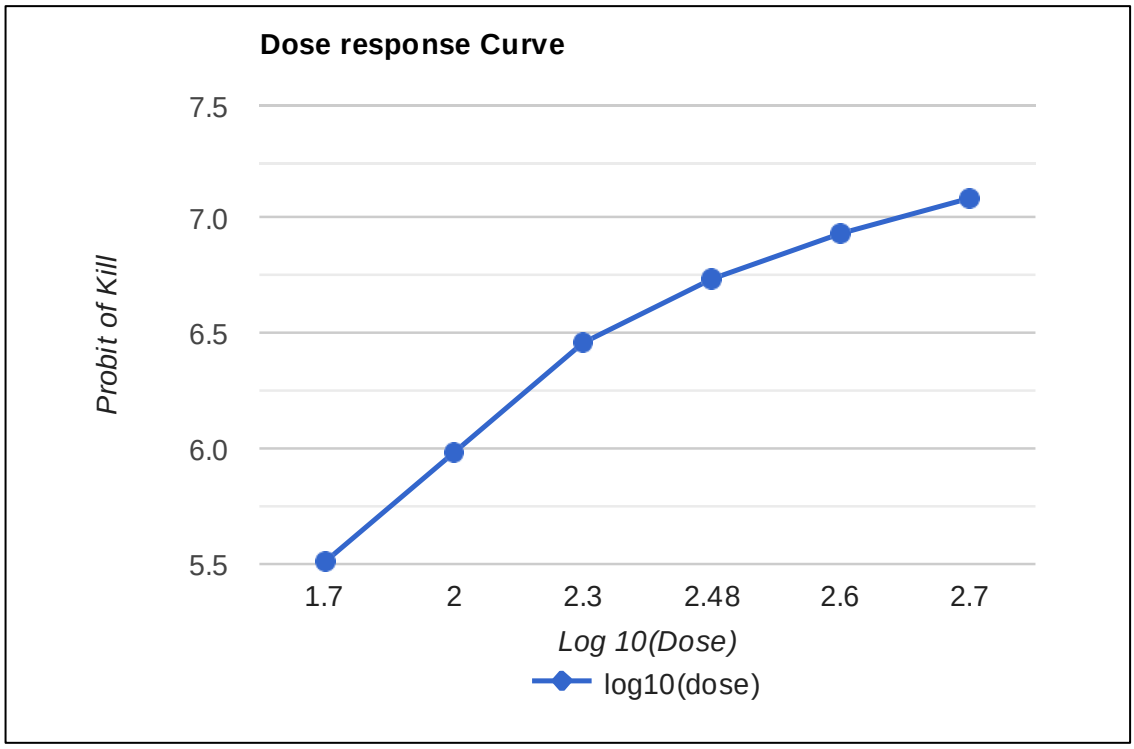
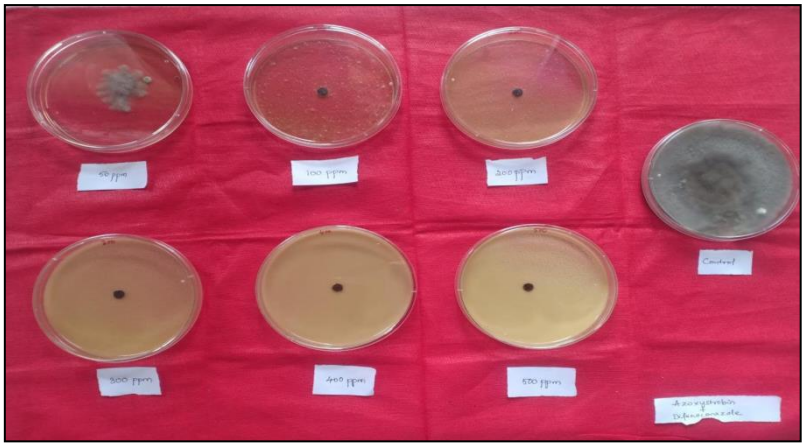
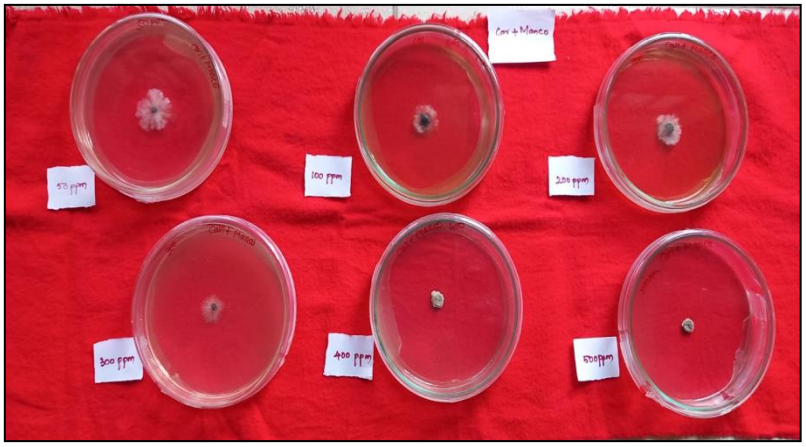


Fig 12: Dose response curve of Azoxystrobin+Difenoconazole

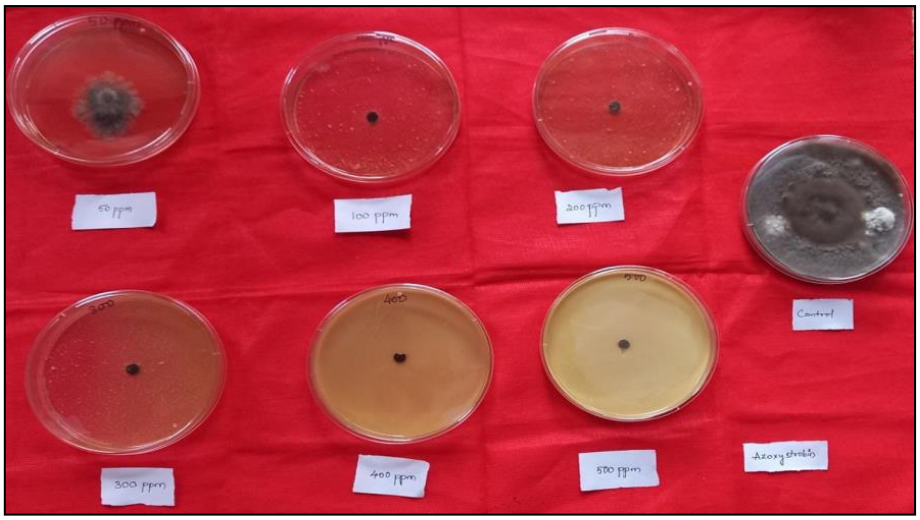


Azoxystrobin+Difenoconazole treatment

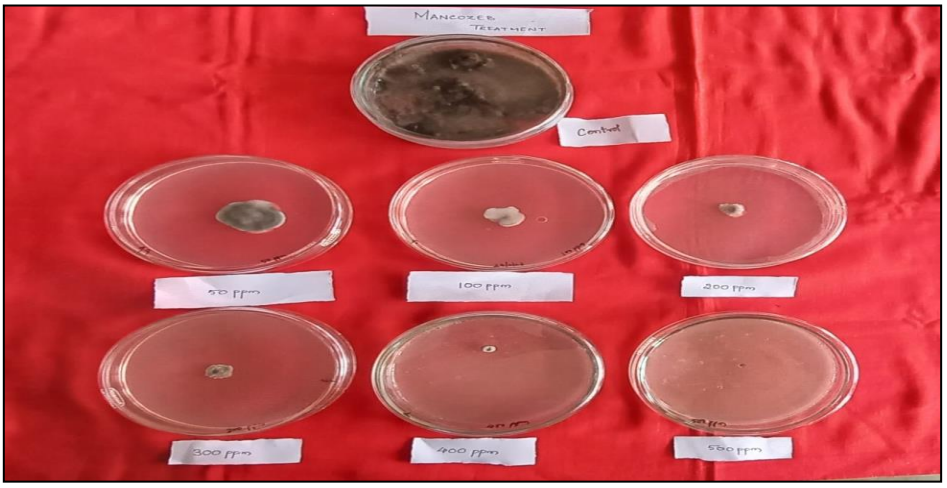
Carbendazim treatment



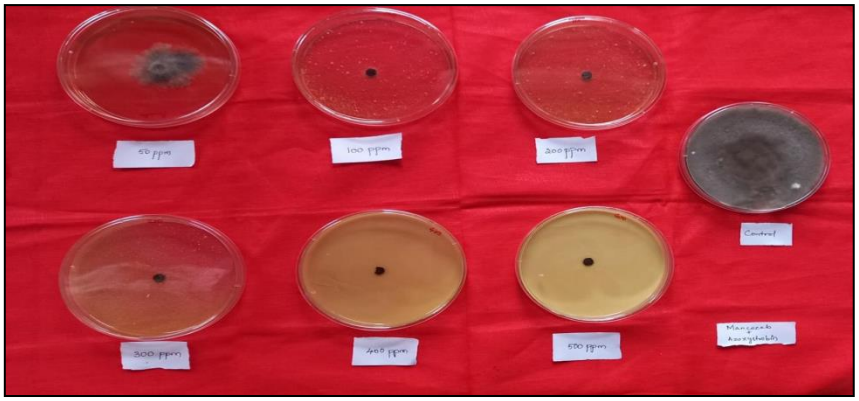
Carbendazim+Mancozeb treatment



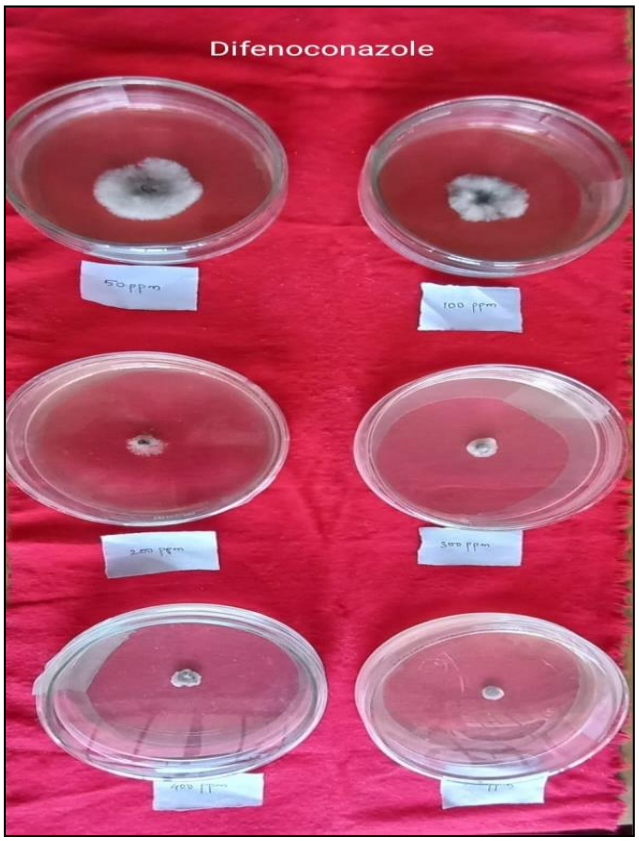
Azoxystrobin treatment



Mancozeb treatment



Mancozeb+Azoxystrobin treatment



Difenoconazole treatment

Plate 4: Treatment with different chemicals against *H. oryzae* under *in vitro* condition

Table 9: Management of the disease with chemicals under field condition (*in vivo*)

Treatments	Disease Severity (%)		
	PDI 1	**PDI 2**	**PDI 3**
Carbendazim	12.22 (20.44)	19.69 (26.33)	29.11 (32.63)
Mancozeb	14.44 (22.32)	22.36 (28.20)	31.19 (33.91)
Carbendazim + Mancozeb	14.20 (22.12)	19.26 (25.99)	29.61 (32.95)
Difenoconazole	6.82 (15.05)	9.71 (18.10)	13.33 (21.39)
Azoxystrobin	9.41 (17.85)	10.13 (18.38)	14.23 (22.14)
Mancozeb + Azoxystrobin	9.11 (17.54)	10.56 (18.90)	14.78 (22.56)
Azoxystrobin + Difenoconazole	5.41 (13.25)	7.96 (16.23)	10.43 (18.79)
Control	20.56 (26.94)	31.54 (34.15)	45.51 (42.40)
SE(m)±	0.823	1.170	0.865
CD (5%)	2.519	3.583	2.650
CV	7.330	8.702	5.287

Figures in parenthesis are angular transformed values

All the fungicides which were tested under *in vitro* condition was applied in the field condition to manage the disease. It was found that the similar result that was found under *in vitro* condition was followed here too. From Table No. 9, it was noted that Azoxystrobin + Difenoconazole treatment produced lowest PDI of 5.41, 7.96 and 10.43 at first, second and third disease scoring time, respectively (Fig 13). This result is in accordance with the findings of Kumar *et al* (2019) who found that Nativo 75 WG (Tebuconazole 50% + Trifloxystrobin 25%) @ 0.04% resulted lowest brown spot of rice disease intensity of 6.5% in the farmer's field situation. This treatment is closely followed by followed by Difenoconazole treatment with disease severity of 6.82, 9.71 and 13.33 at PDI 1, PDI 2 and PDI 3 disease scoring time, respectively. This result is in conformity with the results found by Poudel *et al* (2019) where Propiconazole treatment was found to be best with least brown spot disease severity of 12.99 at the last disease recording at 113 days after transplanting. These two treatments are statistically *at par* at PDI 1 and PDI 2 but they are showing statistically significant difference at PDI 3. Among the fungicides Mancozeb treatment was the least effective as it recorded 14.44, 22.36 and 31.19 disease severity at first, second and third disease scoring days, respectively. The highest disease of

20.56, 31.54 and 45.51 at PDI 1, PDI 2 and PDI 3 disease scoring time, respectively was recorded in control.

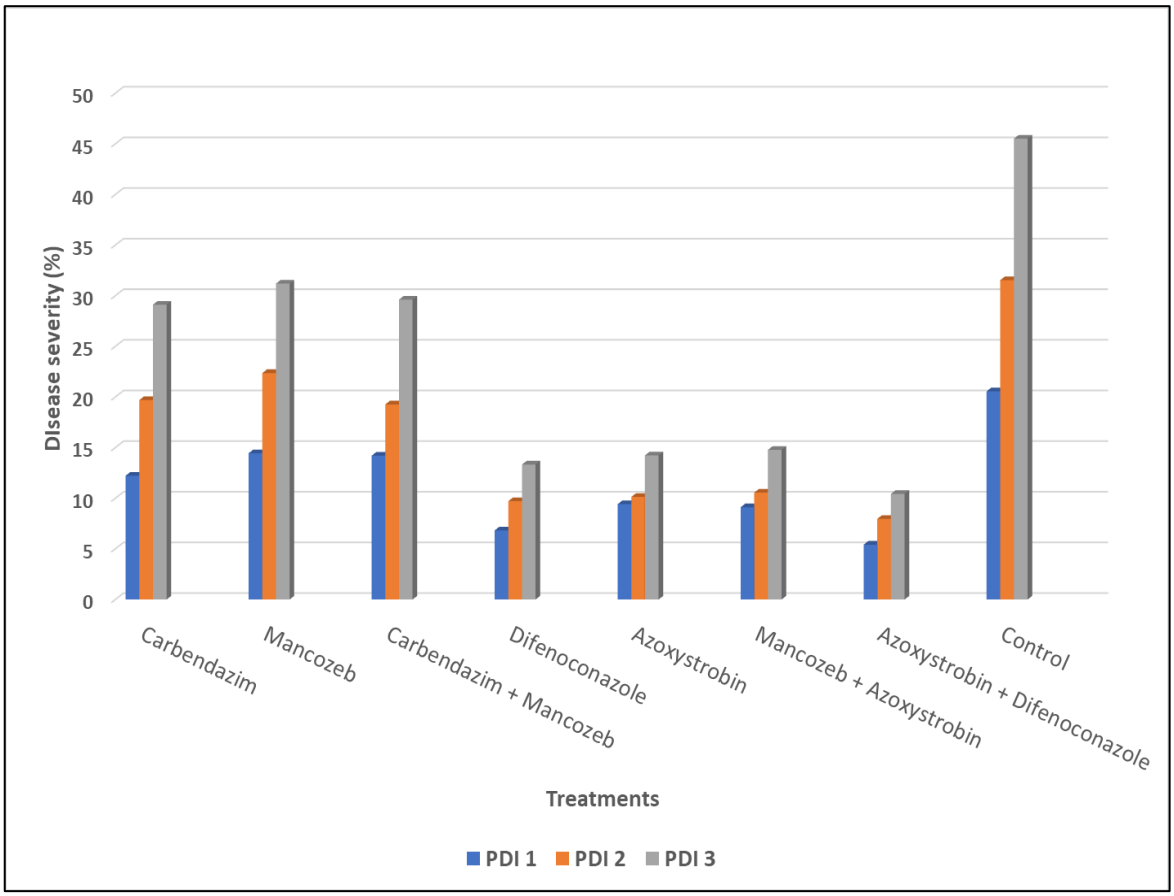


Fig 13: Disease severity by different treatments under field condition

Table 10: Area under disease progress curve (AUDPC) and yield as influenced by different treatments under field condition (*in vivo*)

Treatments	AUDPC	% reduction over control	Yield (t/ha)	% increase in yield over control
Carbendazim	201.77	37.51	4.30	9.97
Mancozeb	225.88	30.05	4.07	4.09
Carbendazim + Mancozeb	205.81	36.26	4.63	18.41
Difenoconazole	98.91	69.37	5.12	31.20
Azoxystrobin	109.75	66.01	5.06	29.41
Mancozeb + Azoxystrobin	112.50	65.16	5.04	28.90
Azoxystrobin + Difenoconazole	79.40	75.41	5.44	39.13
Control	322.89	-	3.91	-
SE(m)±	6.953		0.258	-
CD (5%)	21.293		0.790	-
CV	7.100		9.516	-

From Table No. 10 it was seen that Azoxystrobin + Difenoconazole is the best treatment as it has recorded the lowest AUDPC of 79.40 and highest disease reduction over control (75.41%). This treatment was followed by Difenoconazole treatment which recorded AUDPC of 98.91 and 69.37% disease reduction over control (Fig 14). These two treatments are statistically *at par* with each other. This result is in agreement with the research findings of Poudel *et al* in 2019 who reported significantly lowest AUDPC value of 415.7 in the field treated with the Propiconazole which was statistically *at par* with Azoxystrobin + Tebuconazole with an AUDPC value of 464.3. Azoxystrobin + Difenoconazole treatment is statistically significantly superior than all other treatments (except Difenoconazole treatment). This result also confirms the results obtained by Kamei *et al* (2022) who recorded lowest pooled AUDPC of 2 years with a value of 142.02 among all the treatments. As in this experiment Difenoconazole was found to be *at par* with the best treatment and as Difenoconazole and Propiconazole are having the same mode of action (Sterol Biosynthesis Inhibitor) with same FRAC Code 3, so the result found by Kamei *et al* is very much similar with the present research finding. Among the fungicide treatments, the highest AUDPC of 225.88 was recorded in Mancozeb treatment whereas the highest AUDPC of 322.89 was recorded in control among all the treatments.

Similarly, the highest yield was recorded in the same Azoxystrobin + Difenoconazole treatment with a yield of 5.44 t/ha which is closely followed by

Difenoconazole treatment recording 5.12 t/ha yield (Table 10). These two treatments are statistically *at par* with each other. This treatment is in accordance with Kumar *et al* (2019) who found similar combination of one azole and one strobilurin fungicide (Tebuconazole 50%+Trifloxystrobin 25%) @ 0.05% resulted highest mean grain yield of 5.23 t/ha in rice crop. Azoxystrobin + Difenoconazole treatment produced 39.13% increase in yield over control whereas Difenoconazole treatment recorded 31.20% increase in yield over control (Fig 15). This result is similar with the result obtained by Hossain *et al* (2011) where they found that Azoxystrobin @ 1 ml/litre of water at tillering stage recorded highest rice yield of 6.08 t/ha and Propiconazole @ 1 ml/litre water application at tillering stage produced second highest yield of 5.83 t/ha. They also reported that application of Azoxystrobin and Propiconazole at tillering stage increased yield by 32.17% and 26.74%, respectively over control. Among fungicide treatments, Mancozeb treatment recorded the lowest yield of 4.07 t/ha whereas the lowest yield of 3.91 t/ha was recorded in control among all the treatments.

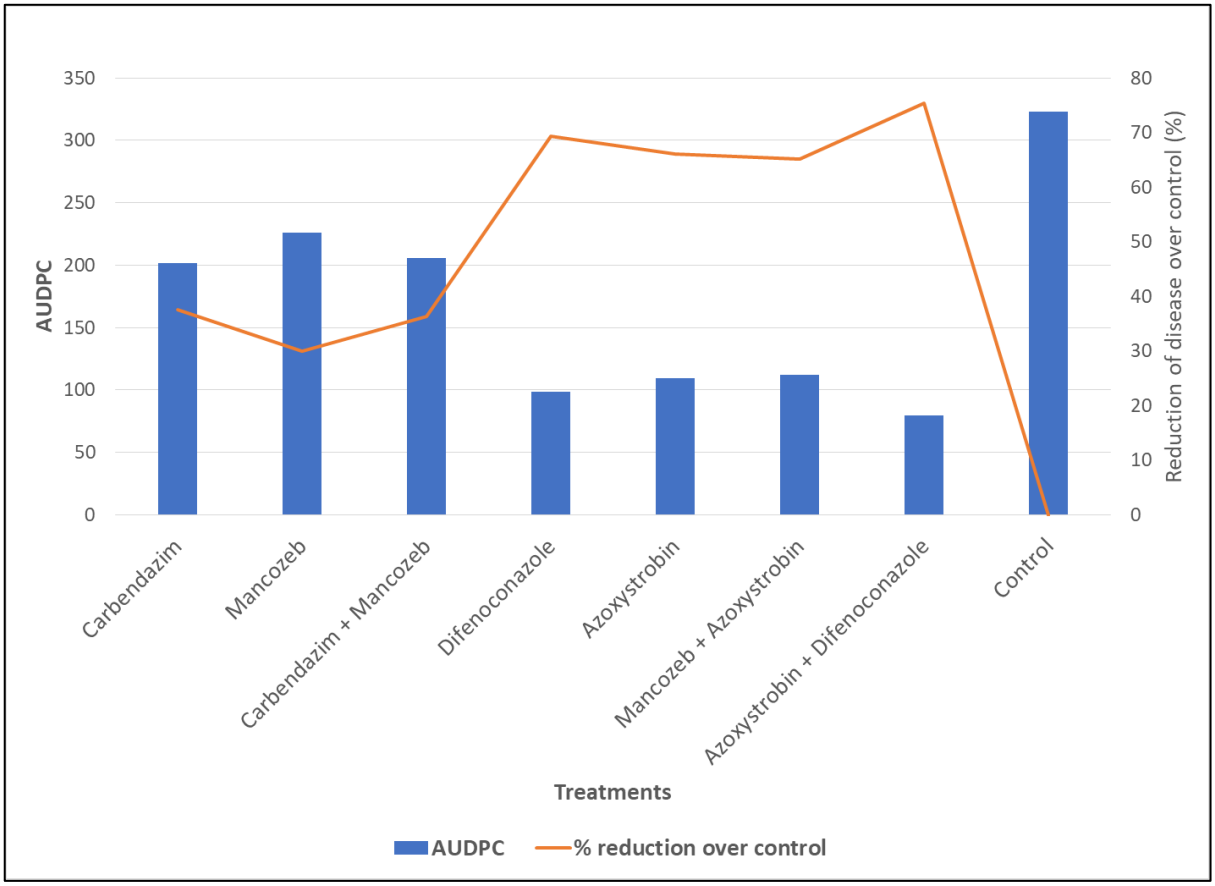


Fig 14: AUDPC and percent reduction of disease over control for different treatments under field condition

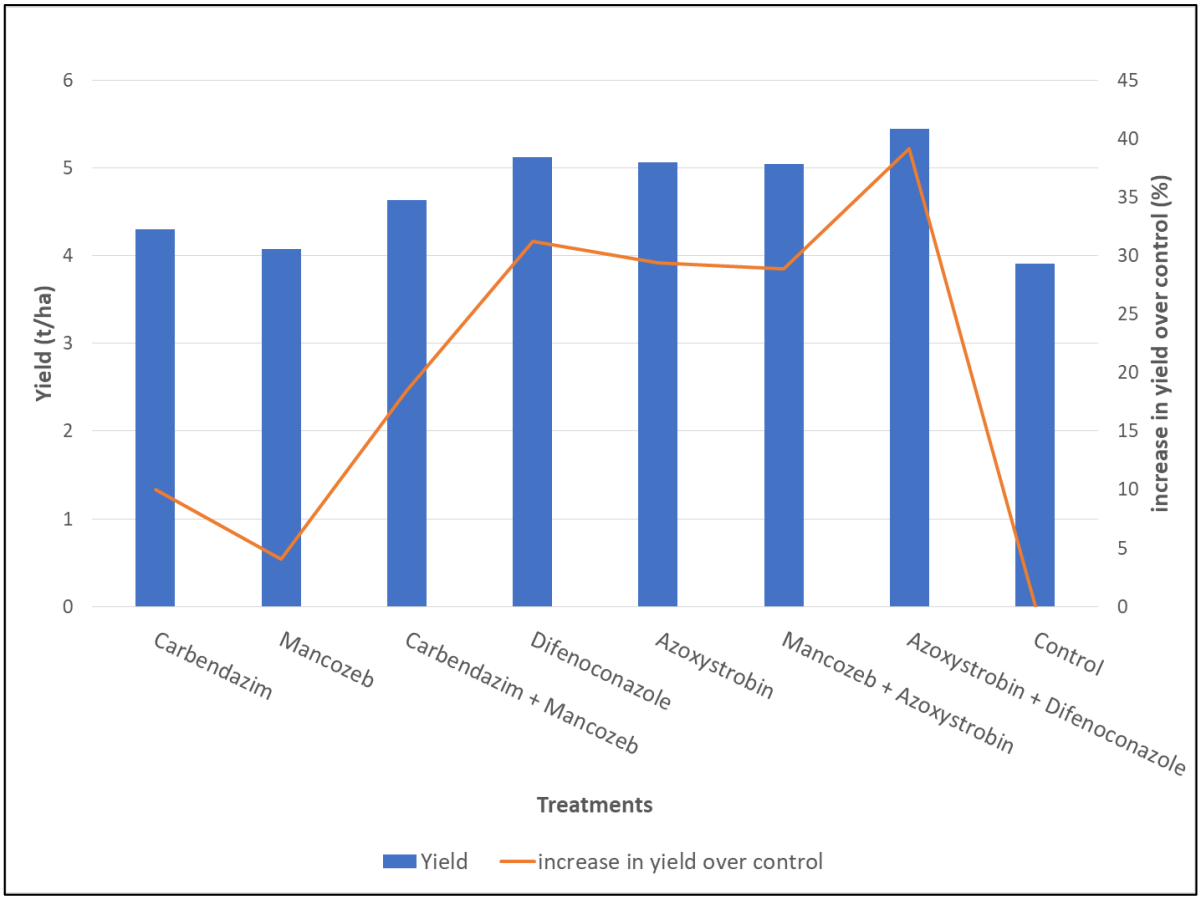


Fig 15: Yield and percent increase in yield over control for different treatment

Table 11: Incremental Benefit Cost ratio for different treatments over control per hectare

Treatments	Incremental cost over control (Rs.)	Incremental yield over control (t/ha)	Incremental return over control (Rs.)	Incremental cost benefit ratio
Carbendazim	4500	0.39	7800	1.73
Mancozeb	4800	0.16	3200	0.67
Carbendazim + Mancozeb	6000	0.72	14400	2.40
Difenoconazole	9000	1.21	24200	2.69
Azoxystrobin	10000	1.15	23000	2.30
Mancozeb + Azoxystrobin	7800	1.13	22600	2.90
Azoxystrobin + Difenoconazole	9000	1.53	30600	3.40

Incremental benefit cost ratio over control was found out for all the treatments. From Table 11, it was found that highest incremental return over control of Rs. 30,600/- per ha with incremental cost benefit ratio of 3.40 was recorded by Azoxystrobin +

Difenoconazole treatment. This result is in agreement with the results obtained by Kumar et al (2019) who got highest net profit of Rs 28,900 / ha with B:C Ratio as 2.24 with the application Trifloxystrobin+tebuconazole treatment in Rice. This treatment is followed by Mancozeb + Azoxystrobin getting Rs. 22,600/- per ha incremental return and incremental cost benefit ratio of 2.90 and Difenoconazole treatment recorded Rs. 24,200/- per ha incremental return over control with incremental cost benefit ratio of 2.69, respectively. Similar result was also found by Asghar *et al* (2019) who found that rice crop sprayed with Difenoconazole @ 315 $mlha^{-1}$ + NPK (20:20:20) @ 500gha^{-1} gave the highest additional income of Rs. 26305 ha^{-1}. The lowest incremental return and incremental cost benefit ratio of Rs. 3,200/- per ha and 0.67, respectively was recorded by Mancozeb treatment among all the treatments.

CHAPTER- 5
SUMMARY & CONCLUSION

5. SUMMARY AND CONCLUSION

Rice (*Oryza sativa*) is the most important and predominant crop over other crops. It is the staple food for nearly half of world's population. Globally it is grown nearly in about 11% of total cultivated area. This crop is affected by number of diseases. To increase the rice production to feed the increasing global population these diseases must be reduced. Brown spot of rice is among on of the diseases which affects Rice crop. Although this disease does not cause serious damage every year, it can cause severe damage at any time if weather condition and the virulent pathogenic strain of the pathogen coincide. So, some older molecules along with some new molecules and their combination product have been tried both in the laboratory condition and at field level to see their effect on the disease. Along with that number of isolates of the pathogen i.e. *Helminthosporium oryzae* have been collected and isolated from different locations of West Bengal and they have been culturally characterized to some extent.

Ten isolates were collected from ten different locations of West Bengal. The locations were Pundibari, Dinhata, Kalyani, Bolpur, Tufanganj, Raiganj, Siliguri, Malda, Bankura and Bishnupur. The pathogen was named as HO 1 to HO 10, respectively.

Koch Postulate of the Pundibari isolate of the pathogen was established and pathogenicity test for that isolate is done.

The length, breadth and number of septa of conidia of all those isolates were counted. It was found that Raiganj isolate has the highest average length of conidia (173.42 μm) and Bishnupur isolate has the highest average breadth (19.19 μm). Raiganj and Pundibari isolate has the highest no. of average septa (9.10).

Different colony characteristics and sporulation of all those isoltes were tested in Potato Dextrose Agar media. Different isolates produced similar or different colony colour like grey, black or white with regular or ireegular margin and either fluffy or suppressed growth. Among all the isolates Pundibari isolate produced highest sporulation and fast growth in Potato Dextrose Agar media.

Growth of all the isolates were studied in Potato Dextrose Agar media. The highest growth of 88.33 mm was found in Bolpur isolate at 6 days after inoculation. But highest Area Under Mycelia Growth Curve (AUMGC) from 2^{nd} to 6^{th} day of growth was found highest in Pundibari isolate with AUMGC reading of 108.03 among all the isolates.

Colony characteristics of the Pundibari isolate of the pathogen was studied in six different media. It was found that Malt Extract Agar and Potato Sucrose Agar produced

highest sporulation with fast colony growth rate, fluffy colony character and regular margin. Host extract Agar also produced highest sporulation with moderate colony growth rate, fluffy colony character and regular margin.

Growth of the pathogen in different media was seen. It was noticed that Malt Extract Agar and Potato Sucrose Agar produced full plate (90 mm) growth at 6 Days After Inoculation (DAI) with Malt Extract Agar producing highest AUMGC of 114.58 and Potato Sucrose Agar produced second highest AUMGC of 110.83 at 6 DAI.

The pathogen was tested against different chemicals under *in vitro* condition using Poisoned food technique. It was found that Azoxystrobin + Difenoconazole was the best treatment as it produced minimum growth of the pathogen in all the concentrations tested among all the chemicals. At 300 ppm concentration this treatment produced highest inhibition of growth of 97.78% over control. This treatment also produced lowest ED 50 value of 23.66 ppm among all the treatments.

All the fungicides were tested for the management of brown spot disease of rice under field situation. Azoxystrobin + Difenoconazole treatment was best here too as it produced lowest PDI of 5.41, 7.96 and 10.43 at first, second and third disease scoring time, respectively. This treatment also recorded the lowest AUDPC of 79.40 and highest disease reduction over control (75.41%). The highest yield was also recorded in the same Azoxystrobin + Difenoconazole treatment with a yield of 5.44 t/ha and 39.13% increase in yield over control.

Incremental benefit cost ratio over control was found out for all the treatments. It was again found that highest incremental return over control of Rs. 30,600/- per ha with incremental cost benefit ratio of 3.40 was recorded by Azoxystrobin + Difenoconazole treatment. So, this treatment can be recommended for the management of Brown spot disease of Rice in the field.

CHAPTER- 6
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6. BIBLIOGRAPHY

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INTRODUCTION Rice (Oryzae sativa) is the most important and predominant crop over other crops. It is the staple food for nearly half of world's population. Globally it is

grown nearly in about 11% of total cultivated area (Rout and Tewari, 2012).

Bandyopadhyay 07.09.2023

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