OPEN ACCESS

J. Micro. Sci. Vol. 2(1), 14-20, 2023,
https://doi.org/10.38211/jms.2023.01.50

Research Article

Available on Innovative Science and Technology Publishers
Journal of Microbiological Sciences
www.isciencepress.com/journals/index.php/jms
E-ISSN- 2959-975X

Isolation of Rhizospheric Bacteria from Soil and Their Antifungal Activity Against *Helminthosporium oryzae* the Causal Agent of Brown Spot of Rice

Irfan Ali[1*], Manzoor Ali Abro[1], Rehana Naz Syed[2]

[1]Department of Plant Pathology, Sindh Agriculture University Tandojam
[2]Department of Plant Protection, Sindh Agriculture University Tandojam
*Corespondig author: Irfan Ali[1]
Article Received 17-06-2023, Article Revised 14-07-2023, Article Accepted 18-08-2023

Abstract

The brown spot of rice caused by Helminthosporium oryzae is a serious disease that affects the yield of rice crops. Brown spots of rice have been reported in almost all rice-growing countries. Therefore, the present study was to explore the management of brown spots of rice. through an antagonistic activity using rhizospheric bacteria. The method employed for soil sample collection was persuaded for bacterial isolation through the serial dilution plate technique. For the results of morphological characteristics of bacterial strain from soils of rice crop collected from Hyderabad. The results of the present study show that many colonies made by bacteria and which were consisted of different sizes, shapes, and colors. Whereas the results of the 15 isolates strains of rhizobacteria, only 06 bacterial strains IAP-1, IAP-2, IAP-04, IAP-09, IAP-12, and IAP-14 were found more effective in antagonizing *H.oryzae*, under *in-vitro* conditions. Antagonistic activity of rhizobacteria against *H. organize showed significant inhibitory* effects. The rhizobacteria showed the primordial result against against *H. oryzae* and will be helpful as a formulation not only for commercial purposes but also for beneficial biological control against the Brown spot of rice.

Keywords: Rhizospheric bacteria, antifungal, Helminthosporium oryzae

INTRODUCTION

Rice (*Oryza sativa* L.) serves humans in a variety of ways throughout the world. Rice is used by around half of the world's population, either partly or entirely. It is consumed by the vast majority 90% of the impoverished Asian populace. It also contributes to Pakistan's economy in a variety of way. It is the country's second most common food and the main sources of employment (9 percent). Unfortunately, there is a large yield gap of 1-3 t/ha between potential and actual rice output (Reni *et al*., 2016; Sudheer *et al*., 2018).

Ninety percent of the world rice crop is grown and consumed in Asia. On an average. each household in Pakistan spends about 3.8% of its total food expenditure on rice and rice flour in Sindh province specializes in producing long-grain white rice IRRI-6 and IRRI-9. Major verities produced in this area are IRRI-6, IRRI-9, and D-8 while minor verities including Super Basmati. Major rice-growing areas in Sindh province are Kashmore, Jacobabd, Larkana, Qambar Shahdadkot Shikarpur, and Dadu in Sindh, while Badin, Thatta, and Tando Muhammad Khan are Major rice-growing areas in lower Sindh.

Rice is attacked by more than 50 different diseases, including six bacterial infections, 21 fungal infections, four parasitic nematodes, and 12 viral infections. The most dangerous infections are *Pyricularia oryzae* (which causes blast), *H. oryzae* (which causes brown spots), and *Xanthomonas oryzae* (which causes bacterial blight). *H.oryzae*, casual organism brown spot of rice, the pathogen responsible for brown spot disease, stands out as the most lethal infection on a global scale. *H. oryzae* was first discovered in 1942-43 in Bengal, India. During this time, the disease wiped out 50-90% of rice crops, resulting in a terrible famine that killed two million people due to malnutrition (Padmanabhan, 1973).

The pathogen responsible for brown leaf spot disease in rice is *H.oryzae* commonly known as *Bipolaris oryzae* Breda De Hann. It is a member of the family *Dematiaceae*, the order *Moniliales*, and the sub-division *Deuteromycotina* its ideal stage is known as *Cochliobolus miyabeanus*, and it belongs to the *Ascomycotina* sub-division, especially the sac fungi. Within the host plant, *H. oryzae* brown mycelium can be found intercellularly or intracellularly (Ahlawat, 2007).

Brown spot disease is caused by a fungal pathogen that can damage the rice crop at any stage of development, affecting both seedlings and adult plants. It appears as scattered brown patches on the foliage, which might coalesce and cause the leaves to wither and yellow. Furthermore, the disease inhibits seed germination, resulting in seed and root breakdown. Significant economic losses result from impaired seed development and seedling vigor (Mia & Nahar, 2001; Naeem *et al*., 2001; Malavolta *et al*., 2002). Brown spot disease caused by *H.oryzae* has been linked to agricultural output losses ranging 16- 43 % (Datnoff *et al*., 1991). It is also acknowledged to be a severe problem for impoverished rice growers (Zasoks, 2002).

Symptoms of *H. oryzae* infection can be seen as early as the tillering stage. They begin as tiny, spherical foliar lesions that are dark brown to purple-brown in colour. These lesions range in form from round to oval, with a light brown to grey center and a reddish-brown edge. The lesions are frequently encircled by a brown or yellow-brown halo, which is a pathogen toxin (Vidyasekaran *et al*., 1980). Lesions on vulnerable rice cultivars can range in length from 6 to15 mm, causing leaf wilting (Webster & Gunnel, 1992). Lesions on tolerant cultivars, on the other hand, are smaller and brown, resembling pinheads. Lesions on leaf sheaths look similar to those on leaves. In addition to damaging the leaves and plant components, the virus *H. oryzae* can infect the grains, causing "pecky rice." The emergence of spots and discoloration on the grains is described by this phrase (Pandy, 2003). Various techniques, including the use of fungicides, are being used to manage this fungal disease. Other management measures proposed by Ribot *et al*. (2008) include using bio-control agents, resistant cultivars, agronomic practices, and biotechnology technologies. These alternate treatments can also be used to address brown spot disease in rice.

When it comes to chemical control, using pesticides to manage *H. oryzae* is seen to be the most effective method. However, it is crucial to remember that attaining effective management under high disease pressure might be difficult (Lore *et al*., 2007). Furthermore, the usage of fungicides might lead to contamination of the environment. While fungicide application is still a low-cost control tool for brown spot caused by *H.oryzae* management, depending only on host plant tolerance is a more cost-effective technique. Unfortunately, due to the establishment of new or more virulent races of the virus, host plant tolerance is limited and unstable (Katasntone *et al*., 2007). As a result of these conditions, there has been a change in emphasis towards investigating alternate management practices.

According to Harpyaree *et al*. (2010), the relevance of plant compounds discovered in medicinal plants has received substantial attention, particularly in terms of their potential in disease prevention. According to Karuppusamy *et al*. (2009), botanical extracts have been widely examined for their antibacterial activity and have been used in a variety of applications such as disease management, food preservation, pharmaceuticals, medications, and natural treatments. According to Philip *et al*. (2009), researchers are increasingly focused on natural products as a source of fresh leads for generating superior medications against microbial diseases. Numerous angiosperm plants contain useful chemotherapeutic chemicals, and biological utilisation of these plants has showed promise in treating diseases, as demonstrated by Tewari *et al*. (1988). Microorganisms colonising plant roots, such as Streptomyces griseoviridis, have showed potential as a bio-control technique for soil-borne diseases. *S. griseoviridis* is an antagonistic microbe renowned for colonising the rhizosphere and efficiently controlling disease such as *H.oryzae* (Tahvonen, 1988). Furthermore, Pseudomonas aeruginosa-induced peroxidases in rice have been shown to have antifungal action against *H. oryzae* (Saikia *et al*., 2006). These findings emphasize the possibility of antagonistic microorganisms to improve plant health and disease resistance. It is a new field in agriculture that can potentially improve crop fitness. Commercially available antagonistic microorganisms, generally belonging to the Pseudomonas genus, have been found to significantly minimize pathogen impacts via processes such as mycoparasitism, antibiosis, iron competition, and plant resistance induction (Singh *et al*., 2005). Direct antagonism is efficient in reducing multiple pathogens, whereas induced resistance (IR) is effective against a variety of foliar pathogens, including both bacteria and fungi, frequently in conjunction with other microorganisms (Shoresh *et al*., 2010). Therefore, the present studies were designed to explore rhizospheric bacteria from Sindh soil that are antagonistic to brown spot fungus *H.oryzae* The present studies were conducted on the following objectives. **Objectives**

- Sampling of rice crops for the isolation and identification of the causal fungus *Helminthosporium oryzae* from the vicinity of Tandojam
- Isolation and identification of bacteria from rhizospheric soils of rice
- To screen potential biocontrol agents against the *Helminthosporium oryzae* under *in-vitro* condition

Materials And Methods

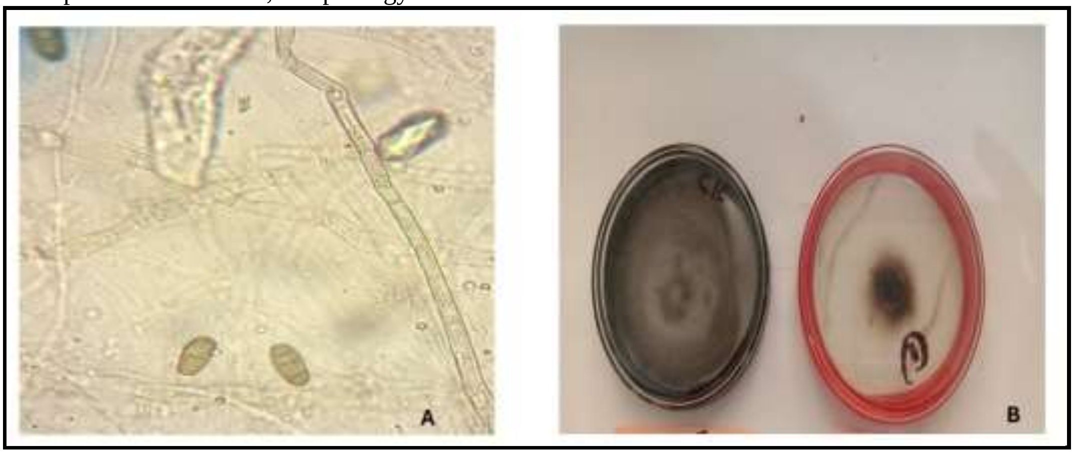
Isolation and identification of the fungal pathogen: The collected samples consisted of rice leaves of DR -82, DR-83, DR-59, and IR-8, which were collected from the district of Hyderabad surrounding areas. These rice verities are commonly cultivated in different districts of Sindh province, These samples were brought to the Laboratory, Department of Plant Pathology, FCPT, SAU Tando jam for the isolation and identification of the casual fungus of brown leaf spot of rice. These sample placed in standard blotter paper method was used for the isolation and identification of fungi with help of key as described by Mew and Gonzales (2002).

Isolation and identification of bacterial isolates: Isolation, purification, identification and morphological characterization of Rhizosphoric bacteria rice soils which were collected rice grown in the vicinity of Hyderabad, For the isolation, dilution plate techniques was used and morphological characterization was done by light microscopy. Rhizobacteria were recovered from rice soil samples obtained from vicinity Hyderabad. Kasimpur *et al*. (2004) described an isolation approach that was used. In this approach, one gramme of soil was mixed in the 20ml 0.9y Nacl solution . The suspension was carefully mixed, and diluted up to 10^{-8} were made. 0.1 ml of each dilution was spread on the nutrient agar (NA) plates for further process. The plates were then incubated at 28+2°C . The obtained bacterial colonies were purified using the streaking technique rhizobacterial isolates were 0.3/.KOH test and visual inspection of colonies, morphology

Antagonistic activity of rhizosphoeric bacteria against *H.oryzae* using dual culture method: We followed the procedure mentioned by Montensnos *et al*. (1996) with slight modification for observing the antagonistic action of 15 selected strains against *H.oryzae* casual organisam of brown spot of rice on, Potato Dextrose Agar (PDA) were kept for 7-10 days. Old culture (at least 1 week old) of *H. oryzae* was placed in the center of Petri dishes for streaking of bacterial strain around. All these were incubated at 28°C, whereas the data were collected after 3 days and last for 7 days.

RESULTS

Identification of fungul pathogen: The fungul pathogen was identified based on cultural and morphological characteristic. The mycelial colony on PDA was smooth, and the color was dark black. In addition, microscopic examinations of the conidia were slightly curved,widest at the middle and tapering towards the hemispherical apex,where their width approximate half the median width of mature conidia are brownish with a moderately thin peripheral wall Figure 1.



Figur.1. *Helminthosporium oryzae* isolated from rice seed

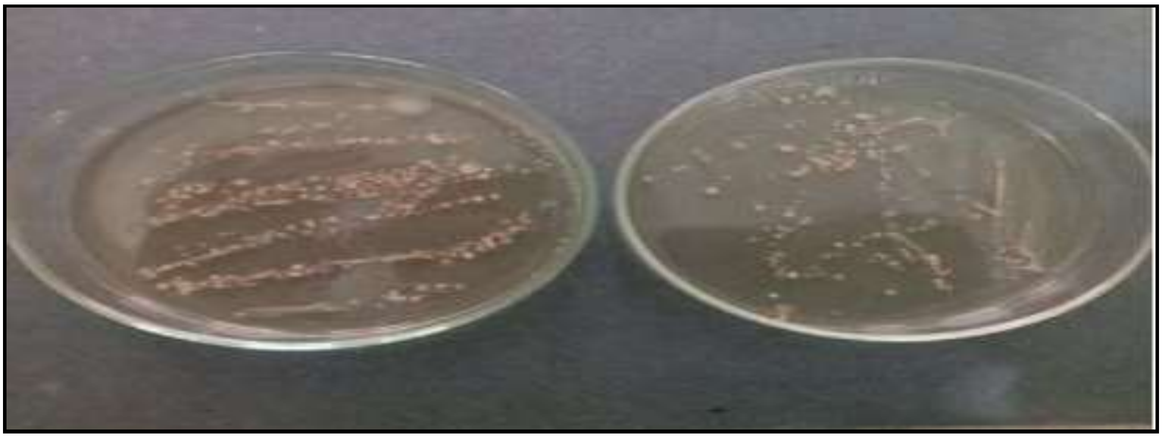


Figure. 2. Cultural characteristics of *rhizospheric* bacteria Cultural and morphological characteristics of *H.oryzaee*

Tentative identification of bacterial isolates: Based on visual inspection, all strains were rod-shaped,with varying textures. Most were off-white to cream-coloured and smooth. However, two strains (IAP-14 and IAP–15) were domed–shaped. In addition, the KOH test result revealed that all strains were gram-positive, as no bacterial mixture became gummy in Figure 2 (Table.1).

Table 1 Tentative identification Bacillus spp

S.No	Strains	Elevation	Shape	color	KOH	Tentative identification
1	IAP1	Smooth	Round	Off-white	+	Bacillus sp.
2	IAP2	Smooth	Round	Off-white	+	Bacillus sp.
3	IAP3	Smooth	Round	Off-white	+	Bacillus sp.
4	IAP4	Smooth	Round	Off-white	+	Bacillus sp.
5	IAP5	Smooth	Round	Off-white	+	Bacillus sp.
6	IAP6	Smooth	Round	Off-white	+	Bacillus sp.
7	IAP7	Smooth	Round	Off-white	+	Bacillus sp.
8	IAP8	Smooth	Round	Off-white	+	Bacillus sp.
9	IAP9	Smooth	Round	Off-white	+	Bacillus sp.
10	IAP10	Smooth	Round	Off-white	+	Bacillus sp.
11	IAP11	Smooth	Round	Off-white	+	Bacillus sp.
12	IAP12	Smooth	Round	Off-white	+	Bacillus sp.
13	IAP13	Smooth	Round	Off-white	+	Bacillus sp.
14	IAP14	Domed	Round	creamy	+	Bacillus sp.
15	IAP15	Domed	Round	creamy	+	Bacillus sp.

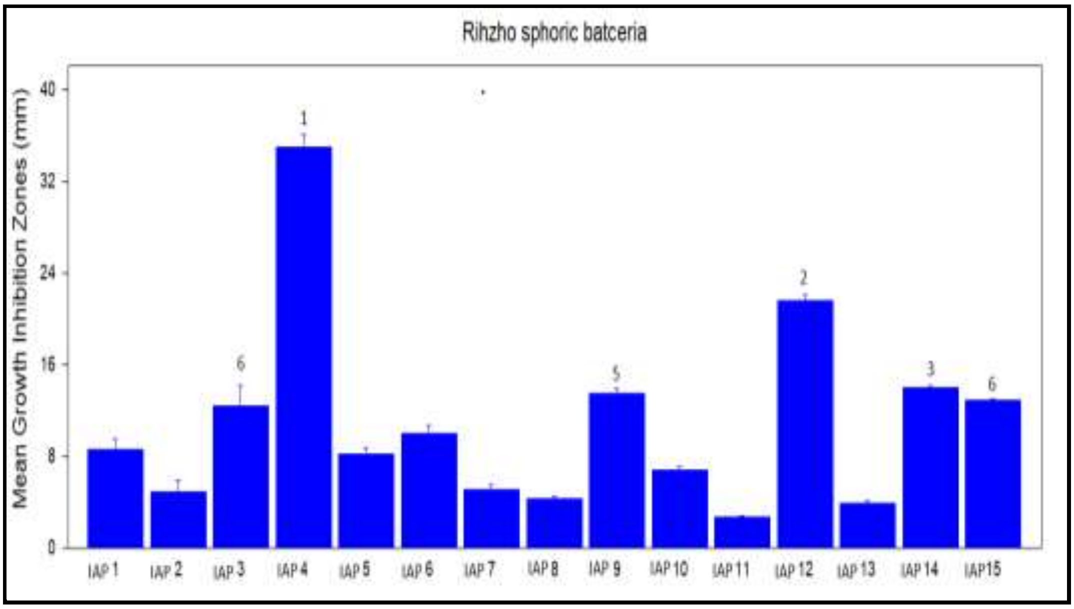


Figure 3. Mean growth inhibition zones (mm)

Potential antagonistic activity of selected strains: Out of 15, 6 strains such as IAP 04,(36.2) IAP 12,(22.5) IAP 03,(15.8) IAP 14, (14.9) IAP 09(14.0), IAP14 (13.0) were able to antagonize *H.oryzae* with mean growth inhibition zones of strains with IAP 04, (36.2) by the strain followed IAP 12, (22.5), IAP 03, (15.8), IAP 14, (14.9), IAP 09 (14.0), IAP14 (13.0) respectively. (Figure.3)

Discussion

In the present study, total 15 strain of bactertia were isolated, purified and identified the rice crop as investigated by Ashrafuzzaman *et al*. (2009) who successfully isolated ten bacteria from rice crop rhizosphoric bacteria which were designated as PGB1, PGB2, PGB3, PGB4, PGB5, PGT-1, PGT-2, PGT-3, PGG-1 and PGG-2. Rice roots are thought to be an excellent source of bio-control agents for soil-borne disease. *Streptomyces griseoviridis*, an actinomycete that colonises the rhizosphere of plants, is one example. *S. griseoviridis* has antagonistic qualities and is useful in the bio-control of plant diseases such as *H.oryzae* casual organism brown spot of rice (Tahvonen, 1988). Furthermore, Pseudomonas aeruginosa-induced peroxidases in rice have been isolated and shown to exhibit antifungal activity against *H.oryzae* (Saikia *et al*., 2006). These findings show the potential for using microbial agents and their enzymatic capabilities to manage plant diseases, notably in rice agriculture. In recent years, the use of antagonistic microorganisms to improve plant fitness has emerged as a potential subject of study. Commercially accessible antagonistic microorganisms, generally belonging to the Pseudomonas genus, have demonstrated efficacy in minimizing pathogen-caused harm. They do this through a variety of methods, including mycoparasitism, antibiosis, iron competition, and plant resistance induction (Singh *et al*., 2005). Many pathogens are suppressed by direct antagonism, but induced resistance (IR) has shown efficiency against a wide range of foliar pathogens, sometimes in conjunction with other bacteria and fungi (Shoresh *et al*., 2010). These findings emphasize the possibility of using antagonistic microorganisms in agricultural practices as a feasible technique for plant protection and disease management.

From samples taken in Hyderabad, 15 bacterial strains were isolated and purified. These strains took on a variety of forms, including round and smooth. The colonies' height were elevated and convex, and their colors ranged from creamy and white to off-white.These features offer a preliminary description of the isolated bacterial colonies from the various locales. Whereas, shape was round, Out of 15 bacterial strains isolated found all strains were +ve They exert antagonistic effects by producing a variety of antimicrobial chemicals, including antibiotics such as *bacilysin* and *siderophores*. Several investigations, including those by Jabeen (2012), Arshad *et al*. (2015), and Kala (2015), have identified bacteria isolated from rice fields as *X. oryzae* causes bacterial blight However, additional biochemical tests or molecular analyses utilising PCR are needed to identify whether these bacteria are *X. oryzae* causes bacterial blight the causative agent of bacterial blight in rice. *X. oryzae* is one of the most deadly viruses infecting rice crops globally, wreaking havoc on rice-growing regions all over the world (Ishiyama, 1990; Swing *et al*., 1992). There has recently been an increase in the occurrence of bacterial leaf blight caused by *X. oryzae* causes bacterial blight in all rice-growing regions of Pakistan. Japanese farmers initially recorded the disease in 1884-85 and has subsequently been detected in other rice-growing locations across the world, including Australia, Bangladesh, India, Sri Lanka, Thailand, the Philippines, the United States, West Africa, and Vietnam (Ezaku & Kuka, 2006). Mew and Majid (1977) first recorded the disease's existence in Pakistan, and later investigations have proven its presence on a larger scale throughout the country (Akhtar *et al*., 2003). The disease poses a severe danger to rice farming in Pakistan's different rice-growing zones.

Conclusions

Helminthosporium .oryzae casual organism brown spot of rice was found to be most predominate fungus among rice associated fungi. From the 15 strain isolates of rhizobacteria, only 06 bacterial strains , IAP-4, IAP-12, IAP-14, IAP-3, and IAP-9, were found more effective to antagonize *H. oryzae in-vitro* conditions. Rhizospheric bacteria's antagonistic activity against *H. oryzae* casual organism brown spot of rice has yielded good results in Hyderabad. These Rhizospheric strains have shown tolerance to environmental conditions, making them appropriate for commercial product development. Because of their antagonistic qualities, they are efficient biological control agents against Brown leaf spot disease in rice, which is a major danger to rice production. Using these bacteria's hostile activity in the rice sector can assist build effective solutions to safeguard and secure rice harvests against this deadly disease.

Recommendations

Keeping in view the results of present research work it can be recommended thatl.among bio-control agent IAP-4, IAP-12, IAP-14, IAP-3 and IAP-9 may be used for the control of brown spot of rice in future

References

Ahlawat, Y.S. (2007). Crop diseases and their management. *Division of Plant Pathology Indian Agricultural Research Institute of New Delhi*, 110012.

Akhtar, M. A., Zakria, M., & Abbasi, F. M. (2003). Inoculum build up of bacterial blight of rice in rice-wheat cropping area of Punjab in relation to zero tillage. *Asian Journal of Plant Science, 21*(3), 548-550.

Akhtar, M. A., Zakria, M., & Abbasi, F.M. (2003). Inoculum buildup of bacterial blight of rice in rice wheat cropping area of Punjab in relation to zero tillage. *Asian Journal of Plant Science*, 2, 548-550.

Arain, G. N. (2013). Crop manager-agronomy center pivot irrigation system valley irrigation Pakistan (private), Limited. P-1-17.

Arshad, H., Imran, M., Naureen, S., Saleem, K., Ali, S., Jabeen, T. & Babar, M.M. (2015). Morphological and biochemical characterization of *Xanthomonas oryzae* pv. oryzae isolates collected from Punjab. *Advance Journal of Life Science*, *72*(3), 125-130.

Arumugam, K., Ramalingam, P., & Appu, M. (2013). Isolation of *Trichoderma viride and Pseudomonas fluorescens organism* from soil and their treatment against rice pathogens. *Journal of Microbiology and Biotechnology Research*, *34*(6), 77-81.

Barnwal, M. K., Kotasthane, A., Magculia, N., Mukherjee, P. K., Savary, S., Sharma, A. K., & Zaidi, N. (2013). A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps. *European Journal of Plant Pathology*, *36*(3), 443-457.

Chaerani, R., & Voorrips, R. E. (2006). Tomato early blight (*Alternaria solani*): the pathogen, genetics, and breeding for resistance. *Journal of general plant pathology*, *72*, 335-347.

Chakrabarti, N.K. (2001). Epidemiology and disease management of brown sport of rice in India. In: Major fungal disease of Rice: recent Advances. *Journal of Crop Research,* 80(5), 293-306.

Chaudary, T., Shahid, A. A., Asif, M., Asghar, F., Majeed, R. A., Ali, M., & Anwar, W. (2016). First report of *Curvularia lunata* causing leaf spot disease of *Solanum melongena* (Eggplant) in Lahore, Pakistan. *Plant Disease*, *100*(11), 2326-2326.

Datnoff, L.E., Raid, R.N., Snyder, G.H. & & Jones, D.B. (1991). Effect of calcium silicate on blast and Brown spot intensities and yields of rice plant disease. *Journal of Agriculture Research and Management*, *75*(4), 729-735.

Ezaku, A., & Kuka, H. (2006). A historical review of bacterial blight of rice. *National Institute geobiology. Journal of Research Bulletin*, *80*(4), 207-218.

Ginnis, M. C, M.R., Rinalidi M.G.& Winn. R.E. (1986). Emerging agents of phaeohy-phomycosis pathogenic species of *Bipolaris* and *Exserohilum*. *Journal Clement Microbiology*, *24*(3), 250-259.

Guri, F., Kapaj, I., Musabelliu, B., Meço, M., Topulli, E., Keco, R., Hodaj, N., Domi, S., Mehmeti, G., Gomez & Paloma, S. (2015). Characteristics of farming systems in Albania. *Journal of Technical Research, 60*(3), 1172-1183.

Habib, A. M., Richards, P., Cairns, L. S., Rogers, G. J., Bannon, C. A., Parker, H. E., & Gribble, F. M. (2012). Overlap of endocrine hormone expression in the mouse intestine revealed by transcriptional profiling and flow cytometry. *Journal of Endocrinology*, *53*(7), 3054-3065.

Habib, A., Javed, N., Sahi, S. T., & Waheed, M. (2012). Detection of seed borne mycoflora of different coarse and fine rice varieties and their management through seed treatments. *Pakistan Journal of Phytopathology, 24*(2), 133-136.

Hafeez Abdul. (1995). A guide to plant disease and there control. Food Agriculture Council, Pakistan Ministry of Agriculture Government of Pakistan Karachi. Book P-6.

Haripyaree, A., Gneshwor, K., Damayanti, M., (2010). *Electronic Journal of Environmental, Agriculture Food Chemistry*, 9(2), 337-344.

Haripyaree, Gneshwor A.K, Damayanti, M. (2010). Efficacy of some fungicides on the development *Pyricularia grisea* and *Heliminthosporium oryzae* Electronic. *Journal Environment, Agriculture Food Chemistry, 19*(2), 337-334.

Ishaq, A., Rehman, A., Zakria, M, & Munir, A (2015). Biochemical characterization of *Xanthomonas oryzaepv*. *Oryzae* population from Kallar Belt of Punjab. *Pakistan International Journal of Agriculture Innovations and Research, 38*(2) 257-266.

Ishiyama, S. (1922). Study on bacterial blight of rice. In Report of Agriculture. *Experimental Station, Tokyo, 45*, 233-261.

Jabeen, R., Iftikhar, T., & Batool, H. (2012). Isolation, characterization, preservation and pathogenicity test of *xanthomonas oryzae*. *oryzae* disease in rice. *Pakistan Journal of Botany*, *44*(1), 261-265.

Jay Parkash, Joshi, M.M., & Mishra, B. (2005). More crop per drop. The Hindu Survey of Indian Agriculture, Ram N. (Ed.), Kasturi and sons Ltd., *National press, Chennai,* 2, 41146.

Jiskani. (2001). Disease of rice. Industry and economy Pakistan leading business Magazine P-1-4.

Kala, A., Soosairaj, S., Mathiyazhagan, S., & Raja, P. (2015) Isolation and Identification of *Xanthomonas oryzae* pv. *oryzae* the causal agent of rice bacterial leaf blight and its activities against of six medicinal plants. *Asian Journal of Plant Science and Research*, *15*(3) (6), 80-83.

Kamal, T., & Mia. M.A.T. (2009). Diversity and pathogen city of the rice brown spot pathogen, *Bipolaris Oryzae* (Breda de haan) shoem. In Bangladesh assessed by genetic fingerprint analysis. *Bangladesh. Journal Botanical, 38*(2), 119-125.

Karupasamy, S., Muthuraja, G., & Rajasekaran, K.M., (2009). *Advances Journal of Biological Research*, *13*(4), 196-200.

Lawrence, E., Dantoff, R., & Lentini, S. (1994). Brown Spot in Florida Rice. The Plant Pathology Department, Florida Cooperative extension service, Institute of Food Science and agriculture Science, *University of Florida Original publication*. 1-3.

Lore, B.P. (2007). Plant Pathology Pathogen and plant Disease book, 122-123.

Malavolta, V.M.A., Parisi, J.J.D., Takada, H.M., & Martins, M.C. (2002). Effect of different incidence levels of *Bipolaris oryzae* in rice seeds on physiological aspects: seed-seedling transmission and production. *Journal of Phytopathology, 28*(4), 336-340.

Mia, M. A. T., & Nahar, M. A. (2001). Status of rice seed health in Bangladesh and farmers' seed production and management scenario. *Journal of Research Science, 10*(1), 81-86.

Mondal, S., Singh, R.P., Mason, E.R., Huerta, E. J., Autrique, E., & Joshi, A.K. (2016). Grain yield, adaptation and progress in breeding for early-maturing and heat-tolerant wheat lines in South Asia. *Journal of Field Crops Research*, 192, 78-85.

Montesiones, V.V, Ambikapathy & Panneerselvam. A. (1996). Antifungal potentiality of some medicinal plants extracts against *Bipolaris oryzae*. *Asia. Journal Plant Science Research*, *1*(3), 77-80.

Motaher, M.D., Farjana, S., Abu, H. D., & Asadur, R. (2013). A comparative screening of hybrid, modern varieties and local rice cultivar for brown leaf spot disease susceptibility and yield performance. *Journal Archives of Phytopathology and Plant Protection*, 3, 01-8.

Motlagh, M. S., & Kaviani, B. (2008). Characterization of new Bipolaris spp.: the causal agent of rice brown spot disease in the north of Iran. *International Journal of Agriculture and Biology*, *10*(6), 638-642.

Naeem, K., Anwar, S. A., Haque, M. I., Riaz, A., & Khan, M. A. (2001). Seed-borne fungi and bacteria of rice and their impact on seed germination. *Pakistan Journal of Phytopatholgy*, *13*(1), 75-81.

New, T.W., Vera, C.M. & Medulla, E.S. (1992). Changes in race frequency of *Xathanus Oryzaepv. Oryzae* in response to rice cultivators planted in Philippines. *Journal of Plant Disease, 76*(2), 1029-1032.

Ou, S.H. (1985). Rice Diseases (2nd Ed.) CMI, Kew, England, 370.

Padmanabhan, S.Y. (1973). The great Bengal Famine. Annual. Review of . *Phytopathology*. *11*,11-26.

Pandy. B.P. (2003). Plant Pathology Pathogen and plant disease book P-145-148.

Philip, K., Malek, S. N., Sani, W., Shin, S. K., Kumar, S., Lai, H. S., & Rahman, S. N. (2009). Antimicrobial activity of some medicinal plants from Malaysia. *American Journal of Applied Sciences*, *6*(8), 1613.

Rafi, A., Hameed, A., Akhtar, M. A., Shah, S. M. A., Junaid, M., Shahid, M., & Shah, S. F. (2013). Field based assessment of rice bacterial leaf blight in major rice growing zones of Pakistan. *Sarhad Journal of Agriculture*, *29*(3), 415-422.

Ribot, C., Hirsch, J., Balzergue, S., Tharreau, D., Nottéghem, J. L., Lebrun, M. H., & Morel, J. B. (2008). Susceptibility of rice to the blast fungus, Magnaporthe grisea. *Journal of Plant Physiology*, *165*(1), 114-124.

Roy, M., Shrma, G. D., & Ramana, C. V. (2013). *Psudomonads:* Potential bio-control agents of rice disease. *Research Journal Agriculture and Forestry Science*, *70*(4), 19-25.

Shoresh, M., Harman, G. E., & Mastouri, F. (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Journal of Phytopathology*, *48*(2), 21-43.

Sikia, M., Qing, D. W., Decatur, T., Maurilli, J. Fournier, L., & Tao, P. (2015). A Systematic, Ligation-based approach to stud RAA modifications. *Published by Cold Spring Harbor Laboratory Press*, *12*(1), 2025-2033.

Singh, R. K., Singh, C. V., & Shukla, V. D. (2013). Phosphorus nutrition reduces brown spot incidence in rainfed upland rice. *Journal of Rice Research*, *30*(2), 31-32.

Swings, J., Mooter, M. V. D., Vauterin, L., Hoste, B., Gillis, M., Mew, T. W., & Kersters, K. (1990). Classification of the causal agents of bacterial blight and bacterial leaf streak of rice as Pathovars of *Xanthomonas oryzae* (ex *Ishiyama*) sp. *International Journal of Systematic & Evolutionary Microbiology*, *40*(3), 309-311.

Tahvonen, R. (1988). Mycostop-a biological control agent against fungal diseases. *Journal of Diseases Research*, *49*(1), 86-90.

Tewari, S.N., Sukla, H.S., Biswal, M.K., & Nayak, (1988). *National Acadmy Science*., *40,* 369-373.

Vidhyasekaran, K. P. N., Ramanatha, A., Rajappan, K., Paranidharan, V., & Velazhan. (1980). Evaluation of a powder formulation of *Pseudomonas fluorescens* Pf1 for control of rice sheath blight roots. *Journal of Bio-control Science & Technology*, *9*(1), 67-74.

Webster, R.K., & Gunnell, P.S., (1992). Compendium of rice diseases. St. Paul. *American Phytopalogical Society*.

Zadok, J. C. (2002). Fifty years of crop protection. (1950-2000). *Neth Journal Agriculture Sciences*, 181-193.