Pak. J. Biotechnol. Vol. 13 (2) 95- 100 (2016)
www.pjbt.org
ISSN Print: 1812-1837
ISSN Online: 2312-7791

SCREENING OF POTENTIAL BACTERIAL BIO-CONTROL AGENTS AGAINST THE *HELMINTHOSPORIUM ORYZAE IN-VITRO* CONDITIONS. CAUSED BY BROWN SPOT OF RICE

Ghulam Hussain Jatoi[1,5*], Manzoor Ali Abro[1**], Javed Asghar Tariq[3**], Shabana Memon[2**], Naimtullah Mangi[**2], Azhar-ul-Din Keerio[4], Muswar Ali Gadhi[1], Shahid Hussain[1] and Dewen Qiu[5]

[1]Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University Tandojam. Pakistan, [2]Department of Plant Breeding and Genitics, Faculty of Crop Production, Sindh Agriculture University Tandojam Pakistan, [3]Nuclear institute of Agriculture (NIA) Tandojam Sindh Pakistan, [4]Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University Tandojam. Pakistan. [5]Institute of Plant protection Chinese Academy of Agricultural Sciences (CAAS) Beijing China.
Email*: jatoighulamhussain@hotmail.com

Article received 5.12.2015; Revised 28.2.2016 Accepted 12.3.2016

ABSTARCT:

Brown spot of rice caused by *Helminthosporium oryzae* is one of the most destructive diseases of rice worldwide. This disease causing rotting of seeds that ultimately reduce yield by 16-43%. This study was aimed to evaluate bio-control agents under *in vitro* conditions against *H. oryzae.* The method followed was based on survey and sampling of rizhospheric soils samples from Larkana district and surrounding areas. Rhizobacteria were isolated from soil-rice plants and 25 isolates were studied for their antagonistic effect on the linear colony growth of *H. oryzae* by using PDA medium as for growth. Results of the present study showed that the use of bio-control agent (Rhizobacteria) against the tested fungus showed that, four isolates out of 24 bacterial isolates has inhibited the linear colony growth of *H. oryzae* up to 1-2 mm with different levels (-, +, ++, +++).. The use of bio-control agent Rhizobacteria like (*Agrobacterium* and *Pseudomonas*) isolates also showed antagonistic activity against the tested fungus (*H. oryzae*). This study could be helpful for researchers and farming community in future for better management of this disease.

Key worlds: Rice, Brown spot disease, *Helminthosporium oryzae*, Bacterial Bio-control agents, *Agrobacterium*, *Pseudomonas*, *Erwinia* and *Streptomyces*

INTRODUCTION

Rice (*Oryza sativa.* L) is one of the most important crops in the world and a staple food for more than half of the world's people. World's annual rice production during economic year 2014 was 741.3 million tonnes (FAO STAT, 2014). Around 90% of total rice is produced by Asia conti -nent alone (Arain, 2013). Major rice producer countries are China, India, Indonesia, Bangladesh, Philippines and Pakistan. As a cash crop, it contributes 4.49% to the foreign exchange earnings and accounts for 1.3% GDP (Anonymous, 2014). In Pakistan, rice is being cultivated on an area of 2789 thousand hectors with total production of 6798 thousand tonnes. Sindh is second most important rice producer province, which gives double yield as compared to Punjab. A few of the important cultivars sowing in the country are Super basmati, Karnal basmati, IRRI-6 and IRRI-9. Sindh province is particular in producing long grain white rice IRRI-9 and IRRI-6. Major varieties produced in this area are IRRI-9, IRRI-6 and D-98 while minor varieties include Supper basmati. Major rice growing areas in Sindh provinces are Larkana, Shikarpur, Dadu, Qamber-Shahdad kot and Jacobabad districts in upper Sindh. While, Thatta, Badin and Tando Muhammad Khan are major rice growing areas in lower Sindh. More than 100 kg of rice are consumed by 3 billion worldwide people each year individually (Nguyen *and* Ferrero, 2006). On an average, each household in Pakistan spends about 3.8% of its total food expenditure on rice.

Rice crop is attacked by 50 diseases, *i.e.*, fungal, bacterial, parasitic (nematodes) and viral diseases. The three most important pathogens of rice are *Pyricularia oryzae*, *Helminthosporium oryzae* and *Xanthomonas oryzae,* which causes blast, brown spot and bacterial blight, respectively. Among them, *Helminthosporium oryze* caused by brown spot of rice is the most devastating disease in the world. The first documented case of *Helminthosporium oryzae* was reported in Bengal, India. In 1942-43 where 50-90% of the rice crops were destroyed that causes a major famine in which two million people died of starvation (Padmanabhan, 1973). The microorganisms that colonize roots are ideal use as a bio-control agent soil-borne disease *Streptomyces griseoviridis* is a good example for colonization of plant rizhosphere by *actionmycetes*. *S. griseo-virids* is an antagonistic microorganism dominant in biocontrol of plant disease such as *Helmin -thosporium oryzae* (Tahvonen, 1988). The patho-

genesis related peroxidases induced by *Pseudomonas aeruginosa* in rice were purified and demonstrated to show antifungal activity against *H. oryzae* (Saikia *et al.,* 2006). Though, use of antagonistic microbes for plant fitness managing has emerged as a viable expertise in the current history. Commercially presented antagonistic microbes, usually belonging to the genera *Pseudomonas*, can reduce the injure by straight effects on the pathogens (mycoparasitism, antibiosis, competition for iron) or by developing plant resistance (Singh *et al*., 2005). Direct antagonism has been the key factor in repression of many pathogens, while IR is active against diverse e foliar pathogens together with in cooperation bacteria and fungi (Shoresh *et al*., 2010). Keeping inview importance of rice and there losses caused by brown spot of rice disease. The present studies were conducted to check the effect of bacteria against *H.oryzae* at Nuclear Institute of Agriculture (NIA) Tandojam.

MATERIALS AND METHODS

Isolation and purification of rhizobacteria isolated from different rice soils: Isolation of rhizobacteria was also done from rice soil collected from various localities, *i.e.,* Bakhodero, Dokri, Bakrani and Rasheed Wagan. Methodologies for isolation of bacteria were followed as described by Kasimpur *et al.,* (2004). One gram of soil was mixed in the 20 ml test tube containing 9 ml sterilized water. The suspension was mixed and dilutions were made up to 10^{-8}. From each test tube 0.1 ml dilution was spread on Nutrent Agar (NA) plates and plates were incubated at 28 ± 2°C until colony development was observed and purification was done by streaking method.

Morphological charactriation of rhizobacteria: Rhyzobacterial isolates were morphologicaly charactrized on the basis of colony morphology cell morphology and Gram staining (Pandy, 2003).

Antagonistic activity of bacteria against *Helminthosporium oryzae:* Antagonistic activity of selected 24 rhizobacterail strains against *Helminthosporium oryzae* was checked by the method as described by Montesnos *et al*., (l996) with some modifications. Nutrient Agar and PDA were prepared separately then were mixed together and were poured in to petri plates. 5 mm discs of 7 to 10 days old culture of *H. oryzae* were placed on center of plates and bacterial strain were streaked around the discs. Then plates were incubated at 28°C. Data was recorded after 3 days and continued up to 7 days. Streak opposite to test fungus. Then Petri plates were incubated at 28°C. Data were recorded when growth of both the organism was initiated.

RESULTS

Isolation and purification of rhizobacteria from rhizospher of soil: A large number of bacterial colonies of different shapes, (filamenttous, circular, irregular, rhizoid) size (Small, medium and large) colors (white, off white, creamy, yellow and grey) were observed when collected soil samples were processed for isolation of bacteria by dilution plate technique. Among them, 24 purified colonies were selected for antagonism and for further studies. Details of their characteristics are as under *in-vivo* conditions.

Morphological characteristics of bacterial micro-flora isolated from Dokri soils: Rhizobacteria strains isolated from Dokri soils were tentatively identified as belong to genus *Cocci, Streptomyces, Xanthomonas,* and *Streptomyces*. *Cocci* were found Gram positive and motile their colonies on Nutrient Agar (NA) medium were circular, raised, curled creamy, rough and round. *Streptomyces* were Gram positive motile. Their colonies on NA medium were irregular, convex, filamentous, off white in color and, smooth. Whereas, *Xanthomonas* were Gram negative and motile. Their colonies on NA media were raised, curled, yellow, smooth and small (Table - 01).

Table-1: Characterization of bacterial strains isolated from Dokri soils.

Isolates	Colony characteristics on NA plates					Cell Morphology			
	Shape	Elevation	Edge	Colour	Surface	Shape	Motility	Gram reaction	Tentative Identification
GHJ-8	Circular	Raised	Curled	Creamy	Rough	round	Non motile	+	*Cocci*
GHJ-9	Circular	Convex	Entire	White	Smooth shiny	round	Non motile	+	*Cocci*
GHJ-10	Circular	Raised	Filamentous	Off-white	Smooth	Round	Motile	+	*Cocci*
GHJ-11	Irregular	Convex	Filamenouts	Off-white	Smooth	Small to long rods	Motile	+	*Streptomyces*
GHJ-12	Irregular	Raised	Curled	Yellows	Smooth	Small to long	Motile	-	*Xanthomonas spp.*
GHJ-13	Circular	Raised	Filamenouts	Cream	Smooth	Small to long	Non motile	+	*Streptomyces*

Antagonism between Helminthosporium oryzae and bacterial biocontrol agent. (+++ very good inhibition; + + good inhibition; + poor inhibition; - no-inhibition); Montesnos et al, (l996)

Morphological characteristics of bacterial micro -flora isolated from Bakhodero soils: Rhizobacterial strain isolated from Bakhodero soils were tentatively identified as belonging to genus *Agrobacterium, Cocci* and *Xanthomonas, Agrobacterium spp.* were Gram negative and motile. Their colonies on NA medium were filamentous, raised, off white, smooth shiny and small. While, *Cocci* were Gram negative and motile their colonies on NA media were filamentous, raised, White, dry, and round in shape. *Xanthomonas* were Gram negative and non-motile their colonies on NA media were circular, convex, curled, off white, smooth and shiny (Table - 2).

Table-2: Morphological characteristics of bacterial strain isolated from Bakhodero soils.

Isolates	Colony characteristics on NA Medium					Cell Morphology			
	Shape	Elevation	Edge	Colour	Surface	Shape	Motility	Gram reaction	Tentative Identification
GHJ-1	Filamentous	Raised	Filamentous	Off-White	Smooth shiny	Small Rod	Motile	-	*Agrobacterium spp*
GHJ-2	Filamentous	Raised	Filamentous	White	Dry	Round	Non motile	+	*Cocci*
GHJ-3	Irregular	Convex	Entire	Off-White	Smooth shiny	Round	Non motile	+	*Cocci*
GHJ-4	Circular	Convex	Curled	Off-White	Smooth shiny	Rod	Motile	-	*Xanthomonas*
GHJ-5	Circular	Convex	Curled	Creamy	Smooth shiny	Cocci	Non motile	-	*Cocci*
GHJ-6	Filamentous	Convex	Filamentous	White	Dry	Round	Motile	+	*Cocci*
GHJ-7	Circular	Raised	Curled	Off-white	Dry	Round	Motile	-	*Cocci*

Antagonism between Helminthosporium oryzae and bacterial biocontrol agent. (+++ very good inhibition; + + good inhibition; + poor inhibition; - no-inhibition); Montesnos et al, (l996)

Morphological characteristics of bacterial microflora isolated from Bakrani soils: Four types of rhizobacteria strain were isolated and identified from Bakrani soil *i.e. Erwinia, Xylella, Pseudomonas* and *Streptomyces. Erwinia* were found Gram negative and motile under light microscopy. Their colonies on NA medium were circular, raised, curled, off white, smooth and shiny. *Xylella* were Gram negative and non-motile. Their colonies on NA media were circular, raised, curled, cream and dry. *Pseudomonas* was Gram positive and non-motile. Their colonies on NA medium were filamentous, convex, gray, and smooth. *Streptomyces* were Gram positive and nonmotile. Their colonies on NA media were filamentous, raised, filamentous, creamy, smooth and shiny (Table- 03).

Table-3: Morphological characteristics of bacterial strain isolated from Bakrani soils

Isolates	Colony characteristics on NA medium					Cell Morphology			
	Shape	Elevation	Edge	Colour	Surface	Shape	Motility	Gram reaction	Tentative Identification
GHJ-14	Circular	Raised	Curled	Off White	Smooth shiny	Medium Rod	Motile	-	*Erwinia*
GHJ-15	Circular	Raised	Curled	Creamy	Dry	Small to long rod	Non motile	-	*Xylella*
GHJ-16	Filaments	Convex	Filamentous	Gray	Smooth	Small to long rod	Non motile	-	*Pseudomonas*
GHJ-17	Filaments	Raised	Filamentous	Creamy	Smooth shiny	Small to long rod	Non motile	+	*Streptomyces*

Antagonism between Helminthosporium oryzae and bacterial biocontrol agent. (+++ very good inhibition; + + good inhibition; + poor inhibition; - no-inhibition); Montesnos et al, (l996)

Morphological characteristics of bacterial microflora isolated from Rashed Wagan soils: *Streptomyces, Erwinia, Clavibacter* and *Cocci.* Type of bacteria were identified from Rashid Wagon soils. *Streptomyces* were Gram negative and motile. Their colonies on NA media were rhizoid, convex, filamentous, off white, smooth and shiny. *Erwinia* were Gram negative and motile. Their colonies on NA media were irregular, raised, entire, off white, dry and small. *Clavibacter* were Gram possitive and motile. Their colonies on NA medium were irregular, convex, and filamentous, off white, *Cocci* were Gram negative and motile. Their colonies on NA medium were irregular, raised, filaments, yellows and smooth (Table - 04).

Table-4: Morphological characteristics of bacterial strain isolated from Rashed Wagan soils

Isolates	Colony characteristics on Nutrient Agar (NA) Medium					Cell Morphology			
	Shape	Elevation	Edge	Colour	Surface	Shape	Motility	Gram reaction	Tentative Identification
GHJ-18	Rhizoid	Convex	Filamentous	Off White	Smooth shiny	small Rod	motile	-	*Streptomyces*
GHJ-19	Irregular	Raised	Entire	Off White	Dry	Small rod	Motile	-	*Erwinia*
GHJ-20	Circular	Convex	Curled	Cream	Smooth shiny	Small rod	Non motile	+	*Streptomyces*
GHJ-21	Circular	Convex	Curled	Off White	Dry	Small to long rod	Non motile	+	*Streptomyces*
GHJ-22	Irregular	Convex	Entire	yellows	Dry	Small to long rod	Non motile	+	*Streptomyces*
GHJ-23	Irregular	Convex	Filamentous	Off White	Smooth	Small to long rod	Motile	+	*Clavibacter*
GHJ-24	Irregular	Raised	Filamentous	Yellows	Smooth	Round	Motile	-	*Cocci*

Antagonism between Helminthosporium oryzae and bacterial biocontrol agent. (+++ very good inhibition; + + good inhibition; + poor inhibition; - no-inhibition); Montesnos et al, (l996)

Screening of potential bio-control agents against *H. oryzae:* Among tested 22 bacterial strains against *Helminthosporium oryzae* were found to antagonize brown spot fungus *(H. oryzae*) Therefore at 3 days to 7 days check the activity of isolated Rhizobacteria. Out of 22 antagonistic bacteria, four antagonistic bacteria had done the linear colony growth of *H. oryzae.* Poor inhibition reduced linear colony growth of Rhizobacteria locations from (No. 1 B.D. bacteria) good inhibition (No. 4 B.D bacteria), very good inhibition (No. 13 B.K. bacteria) and poor inhibition linear colony growth (No. 17. R.W bacteria) and other all isolated 18 Rhizobacteria no inhibition linear colony growth of *Helminthosporum . oryzae* (Table - 5).

Table - 5: Screening of potential bio-control agents against *Helminthosporium oryzae*

Isolates	Location	01-12-14	02-12-14	03-12-14	04-12-14	05-12-14	06-12-14	07-12-14
GHJ-1	Bakhodero	+	+	+	+	+	+	+
GHJ-2	Bakhodero	-	-	-	-	-	-	-
GHJ-3	Bakhodero	-	-	-	-	-	-	-
GHJ-4	Bakhodero	-	+++	+++	+++	+++	+++	+++
GHJ-5	Bakhodero	-	-	-	-	-	-	-
GHJ-6	Bakhodero	-	-	-	-	-	-	-
GHJ-7	Bakhodero	-	-	-	-	-	-	-
GHJ-8	Dokri	-	-	-	-	-	-	-
GHJ-9	Dokri	-	-	-	-	-	-	-
GHJ-10	Dokri	-	-	-	-	-	-	-
GHJ-11	Dokri	-	-	-	-	-	-	-
GHJ-12	Dokri	-	-	-	-	-	-	-
GHJ-13	Dokri	-	-	-	-	-	-	-
GHJ-14	Bakrani	++	++	++	++	++	++	++
GHJ-15	Bakrani	-	-	-	-	-	-	-
GHJ-16	Bakrani	-	-	-	-	-	-	-
GHJ-17	Bakrani	-	-	-	-	-	-	-
GHJ-18	R. Wagan	+	+	+	+	+	+	+
GHJ-19	R. Wagan	-	-	-	-	-	-	-
GHJ-20	R. Wagan	-	-	-	-	-	-	-
GHJ-21	R. Wagan	-	-	-	-	-	-	-
GHJ-22	R. Wagan	-	-	-	-	-	-	-
GHJ-23	R. Wagan	-	-	-	-	-	-	-
GHJ-24	R. Wagan	-	-	-	-	-	-	-

Antagonism between Helminthosporium oryzae and bacterial biocontrol agent. (+++ very good inhibition; + + good inhibition; + poor inhibition; - no-inhibition); Montesnos et al, (l996)

DISCUSSIONS

The results of our studies showed that when we isolate the Rhizobacteria from Bakhodero, Dokri, Bakrani and Rashed wagan soils the size of Rhizobacteria were from (1-2 mm) size. Their shape was 12 circular, 7 irregular, filaments 5 and 1 razed; their elevation was 12 raised, 12 convex. As for their edge in cause there were 12 Filaments 9 curled and 4 entire. Their color was cream, yellow off white but in some case there is gray color. As same time, shape were smooth in same case there are surface are smooth shiny but only some were dry.

During present studies, showed Rhizobacteria was isolated from Bakhodero, Dokri, Bakrani and Rasheed wagan soils, the host of Rhizobacteria were from rice soils. Morphologically, it was seen that the bacteria was small to long rod and cocci but in some cases there is small rod shape. Predominantly non-motile but in some cases it was observed as motile. Both Gram positive and gram negative in their reaction. Our results were found to be in close agreement to Montesinos *et al.* (1996).

Due to their ability to antagonize the pathogen by changed modes of action and to efficiently colonize distinct plant habitats and solution are used around the globe (Raaijmakers *et al*., 2002). Most concentration has been determined on the use of Gram-negative bacteria belonging to genera *Erwinia or Pseudomonas*. Gram-positive *Bacillus* species however have some advantages that make them good candidates for use as bio-control agents (BCAs) (Cartwright *et al*., 1995; Braun-Kiewnick *et al.,* 2000; Shoda, 2000: Costa *et al*., 2001). First, their antagonistic effect is caused by their ability to produce different types of antimicrobial compounds, such as antibiotics (e.g. bacilysin, iturin, mycosubtilin) and siderophores (Shoda, 2000). Second they are able to induce development and defence responses in the host plant (Raupach and Kloepper, 1998). Also Bacillus is able to produce spores resistant to UV light and desiccation, which allows them to resist adverse ecological conditions and permits easy formulation for commercial purposes (Raaij-makers *et al*., 2002). A basic principle for the achievement of a biocontrol agenda is a good adaptation of a given BCA to the local ecological conditions in which it is supposed to work. In this sense, the aim of this work was to evaluate the possibilities of exploitation of bacteria in the bio-control of *H. oryzae* being a devastating fungus to rice industry.

CONCLUSION: Among the 24 isolates of Rhizobacteria, only 04. GHJ-1, GHJ-4, GHJ-14, and GHJ-18 were found more effective to antagonize *Helminthosporium oryzae* in *in-vitro* conditions.

REFERENCE

Anonymous, Agriculture Statistics of Pakistan. Govt. of Pak. Ministry of food, Agriculture and Livestock Division (Economic Wing), Islamabad. Pp. 29 (2014).

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

Braun-Kiewnick,A., B.J.Jacobsen and D.C.Sands, Biological control of *Pseudomonas* syringae PV syringae, the causal agentof basal kernel blight of barley by antagonistc Pantoea agglomerans. Phytopathology **90**: 368–375 (2000).

Cartwright, D.K., W.S. Chilton and D.M.Benson, Pyrrolnitrin and phenazine production by *Pseudomonas-cepaciastrain* 5.5b, a biocontrol agent of *Rhizoctonia-solani*. Appl Microbiol Biotechnol. **43**: 211–216 (1995).

Costa,E., N.Teixido, J.Usall, E.Atarés and I.Viñas, Production of the biocontrol agent *Pantoea aglomerans* strain CPA-2 using commercial products and by-products. Appl. Microbiol. Biotechnol. **56**: 367–371 (2001).

FAO Statistical Year Book. Country Profiles-WEB Edition, Statistics Division FAO Food and Agriculture Surfed on NOV 12, at http://www.fao.org/ statistics /yearbook. 22(2): 6 (2014).

Kasimpur, M., G. E. Harman and F. Mastouri, Induced systemic resistance and plant responses to fungal biocontrol agents. Annual Review of Phytopath. **48**:21–43 (2004).

Montesinos,V., V.Ambikapathy and A. Panneerselvam, Antifungal potentiality of some medicinal plants extracts against Bipolaris oryzae (Breda de Haan). Asia. J. Plant. Sci. Res. **1**(3): 77-80 (1996).

Nguyen, N.V. and A. Ferrero, Meeting the challenges of global rice production. Paddy Water and Environ. **4**: 1-9 (2006).

Padmanabhan, S.Y., The great Bengal famine. Ann. Rev. Phytopathology **11**:11-26 (1973).

Raaijmakers, J.M., M. Vlami and J.T. de Souza, Antibiotic production by bacterial biocontrol agents. Antonie van Leeuwenhoek 81: 537–547 (2002).

Pandy, B.P., Plant Pathology Pathogen and plant Disease book Pp-122-123 (2003).

Raupach, G. S. and J.W. Kloepper, Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology **88**: 1158–1164 (1998).

Saikia Mridusmita, Qing Dai, Wayne A. Decatur, Maurille J. Fournier, Joseph A. Piccirilli and T. Pan, A systematic, ligation -based approach to stud RNA modifications. RNA **12**(11): 2025-2033 (2006).

Shoda, M., Bacterial control of plant diseases. J. Biosci. Bioeng. **89**: 515–521 (2000).

Shoresh, M., G. E. Harman and F. Mastouri, Induced systemic resistance and plant responses to fungal biocontrol agents. Annual Review of Phytopath. **48**: 21–43 (2010).

Singh, R. K., C. V. Singh and V. D. Shukla, Phosphorus nutrition reduces brown spot incidence in rainfed upland rice. International Rice Research Notes **30**(2): 31–32 (2005).

Tahvonen, R.T., Microbial control of plant disease with *streptomuces* spp. EPPO. Bull. **18**:55-59(1988).