International Journal of Agricultural Technology 2013 Vol. 9(2): 333-348
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Inhibitory activity of *Chaetomium globosum* Kunze extract against Philippine strain of *Pyricularia oryzae* Cavara

Emmanuel E. Gandalera[1], Cynthia C. Divina[1*] and Joselito Dg. Dar[2]

[1]Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, [2]College of Allied Health, Good Samaritan Colleges, Cabanatuan City, Nueva Ecija, Philippines

Emmanuel E. Gandalera, Cynthia C. Divina and Joselito Dg. Dar (2013) Inhibitory activity of *Chaetomium globosum* Kunze extract against Philippine strain of *Pyricularia oryzae* Cavara. International Journal of Agricultural Technology 9(2): 333-348.

Abstract This study evaluated the inhibitory activity of *Chaetomium globosum* extract against Philippine strain of *Pyricularia oryzae*. *P. oryzae* were isolated from blast infected leaves of rice in Pangasinan, Philippines. They were cultured and their identities were validated morphologically. Their pathogenecity was tested in vitro. The most virulent strain of *P. oryzae* isolated was used in the bioassay for the inhibitory effect of *C. globosum* ethanol extract in vitro. The virulent *P. oryzae* was grown in dishes with 50 ppm, 100ppm, 500 ppm and 1000 ppm ethanol extract of C. globosum. Sizes of the colony of the *P. oryzae* were observed and percent inhibition were computed and compared.
One of the three Philippine strains of *Pyricularia oryzae* Cavara was avirulent while the other two were found be virulent to susceptible and intermediate rice varieties. The in-vitro bioassay of inhibitory activity of ethanol extract of *C. globosum* against *P. oryzae* showed that colony diameters were significantly smaller and percent inhibition higher in dishes with 1000 ppm and 500 ppm extract compared to those with 100 ppm, 50 ppm and distilled water. Results implied the growth of *P. oryzae* could be inhibited with 500 to 1000 ppm of ethanol extract of *C. globosum in vitro*.

Key words: *Pyricularia oryzae, Chetomium globosum,* inhibitory activity of extract

Introduction

Rice blast is one of the most devastating fungal diseases that accounts to almost 50% yield loss of thousands of hectares of rice fields (Agriculture Business Week, 2008). The fungal rice blast disease infects all above ground parts of the plant but the leaf and panicle lesions are the most serious (Zeigler and Correa, 2000). Although there are intermediate and resistant varieties, *Pyricularia oryzae* Cavara is able to co-evolve with the changes on its host plant, therefore rice is still prone to blast infection.

[*] **Corresponding author:** Cynthia C. Divina; **e-mail:** cynthiacdivina@yahoo.com

Pyricularia oryzae is a fungus belonging to Ascomycota, class Sordariomycetes, order Magnaporthales, family Magnaporthaceae and genus *Magnaporthe.* It is the anamorph or asexual state of *Magnaporthe oryzae* established by Couch and Kohn (2002) as strains isolated from rice varieties and commonly known as the rice blast fungus. *Magnaporthe oryzae* B. Couch (anamorph *Pyricularia oryzae* Cavara) formerly *Magnaporthe grisea* (Hebert) Barr (Zeigler *et al.,* 1994 as cited by Zeigler and Correa, 2000; TeBeest *et al.,* 2007; Zhang *et al.,* 2011) has been described from the latter as a new species due to a multilocus gene genealogy concordant with host preference which indicated segregation of a new species (Couch and Kohn, 2002). It is a filamentous fungus (Citizendium, 2011), an ascomycete that produces sexual spores called ascospores inside an asci, currently under family Magnaporthaceae (TeBeest *et al.,* 2007). The fungus is characterized by three-celled conidia which are pale brown to hyaline and pyriform (pear-like) in shape. Conidia are produced from sympodially conidiogenus proliferating cells. *Pyricularia oryzae* can sporulate on the host tissues. The aerial mycelia can be present or mostly absent. In times when the aerial mycelia are present, it appears to be branched and hyaline to olivaceous. When absent, conidiophores may arise directly from the tissue surface either singly or in tiny groups or bundles according to Mew and Gonzales (2002) and Mew and Misra (1994).

When grown in pure culture, the fungal colony appears white, light gray or dark gray (Udagawa and Yaegashi, 1978 as cited by Harmon and Latin, 2003). The growth of rice blast pathogen varies on different media used in culture. Mew and Gonzales (2002), Pappas (1998), and Mew and Misra (1994) described the colony of *Pyricularia oryzae* grown in PDA with different light exposures. The colony has a septated, branched and hyaline mycelium. The rising conidiophores are simple to rarely branched that are moderately long and septated. Conidiophores are light brown in color and slight thickening at the base, denticles are also found at the tip. Conidia are found sympodially and at the tip and generally pyriform to obclavate. The color of the conidia is from pale olive to hyaline. Usually the conidia is divided into two septations while rarely one to three sepatations.

Pyricularia oryza Cavara can infect the rice plant with distinct mode of infection. Skamnioti (2009), Betts (2007) and Kato (2001) studied the way *Pyricularia oryzae* infects the tissues of its host organism. Conidia germinate on the surface of the host tissue in the presence of a little water. A germ tube and an appressorium are produced on the bound conidia based on the study conducted by Sreenivasaprasad *et al.* (2002). According to Koga (2008), under scanning and transmission electron microscope, the conidia form an appressoria that will penetrate the epidermal cells of the host tissues within 24 hours after

the conidia have landed on the tissue. An appressorium is a melanized, dome-shaped, specialized structure that produces an infection peg. Sreenivasaprasad *et al.,* (2002) and Sneh *et al.,* (1996) reported that melanization of the appessoria is very important in providing enough turgor pressure for the penetration peg. This infection peg is rich in actin-microfilaments that provide mechanical support in penetrating the epidermal cells of the host plant's tissues (Microbe Wiki, 2010; Talbot and Wilson, 2009; Caracuel-rios and Talbot, 2007; TeBeest *et al.,* 2007). After four to five hours under favorable condition according to Kandari (2010), the pathogen already penetrated the host cell, hyphae will start to grow and differentiate into invasive hyphae that is able to infect the adjacent and nearby cells, characterized by a biothrophic invasion (Angliker *et al*., 2010). The plasmodesmata of the plant cells serve as entrance for the successive cell to cell growth of the intracellular invasive hyphae which is a virus-like movement (Czymmek *et al*., 2007). The pathogen will colonize the plant tissues and lesions will be formed. The fungus will sporulate and therefore infecting the nearby plants is possible through the same infection process according to Kandari (2010), Talbot and Wilson, (2009), Wopereis *et al.,* (2009) and Koga (2008).

Sibounnayong *et al.,* (2006) confirmed that *Chaetomium globosum* strains are saprobic organisms and their ability to suppress plant pathogens resulted to induced growth, and high yield of the plant. Pitt and Hocking (2009) reported that *Chetomium* species can be found in leguminous plants like peanuts and mungbean and also on graminous plants like rice. Liu *et al.,* (2008) reported that a gene of *Chaetomium globosum,* 46-kDa codes for an endokinase (chi46) that degrades cell walls of plant pathogens *Rhizoctonia solani, Fusarium oxysporum, Sclerotinia sclerotiorum, Valsa sordida, S. tritici*, and *Phytophthora sojae*. Pitt and Hocking (2009) reported that *Chaetomium* species produce cellulases, enzymes that can degrade cellulytic organisms. Fogle *et al.,* (2007) and Soytong *et al.,* (2001) obtained chaetoglobosins and Park *et al.,* (2006) obtained chaetoviridins from the two *Chaetomium* species. The metabolites were purified and tested and found to have antifungal activities against *P. oryzae*.

A study conducted by Jeamjitt (2007), Park *et al.,* (2006) and Soytong (1989) proved the antagonistic property of *Chaetomium globosum* against *Pyricularia oryzae.* Sibounnavong *et al.,* (2011) Kaewchai and Soytong (2010) also reported that *C. globosum* together with other fungal antagonists *Trichoderma sp*., *Emericella sp*., and other species of *Chaetomium* exhibit inhibitory mechanisms against rice blast pathogen and other phytopathogenic fungi like *Curvularia lunata, Fusarium oxysporum* f.sp. *lycopersici*, and *Rhizoctonia oryzae*. The mode of antagonism elucidated by C. *globosum*

against soil-borne and seed-borne pathogens was reported to be competition, mycoparasitism and antibiosis. A dual culture of both organisms inhibited the growth of *P. oryzae*. Seed treatment of rice seeds with *C. globosum* prevented the infection of blast pathogen. An ascospore suspension of *C. globosum* that was applied on rice leaves a day prior to inoculation prevented the early infection of *P. oryzae*. These studies proved that *C. globosum* as well as *C. cupreum* produces mycotoxins and secondary metabolites that could antagonize plant pathogens based on Kanokmedhakul *et al.* (2002).

Biocontrol is the use of an organism as an antagonist, an agent that can control, regulate or inhibit the effect of a pathogen. A biofungicide uses living organism, or substances isolated from that living organism which can kill a specific pathogen. The study uses an established biocontrol agent to different plant root and leaf pathogen that will possibly affect *Pyricularia oryzae* Cavara. Inhibiting the growth of *Pyricularia oryzae* Cavara suggests a maximum production of rice provided that the environmental conditions are favorable. Moreover, the results of this study could be used as basis for other studies against rice diseases as well as other plant-fungal diseases. The different isolated strains of *Pyricularia oryzae* Cavara can also be used for further tests of potential antagonists. The study could also serve as basic information for modern biotechnology.

This study aimed to determine the effect of *Chaetomium globosum* Kunze as potential antagonist against *Pyricularia oryzae* Cavara. Specifically, the study isolated three Philippine strains of *Pyricularia oryzae* Cavara; determine the difference on the pathogenicity of the different isolated strains; and determine the possible inhibition of *Chaetomium globosum* Kunze crude ethanol extract on the mycelial growth of *P. oryzae* Cavara. This study was conducted to provide information on the control of *Pyricularia oryzae* Cavara, the causative fungus of rice blast. The information on the antagonistic effect of *Cheatomium globosum* Kunze will enable the farmers to produce rice plants that may grow better and provide more seeds. The growth and yield of rice will be improved due to leaves free from fungal infection. The antagonist will provide resistance to susceptible rice varieties as well as improving the resistance of intermediate and resistant ones on *Pyricularia oryzae* Cavara. The use of a biocontrol agent is a contribution to organic farming which serves as an alternative to hazardous chemical fungicides. Farmers will have safer and healthier fungicide practices. Also, they will be having higher yields and income in relation to a good rice production. There would be no harm on the environment and to the farmer adapting the study. The rice industry will increase and will provide more supplies of rice to people.

Materials and methods

In this study, cultures of *P. oryzae* were isolated from rice plants in Malasiqui, Pangasinan, Philippines and their identify were validated and pathogenicity evaluated before their response to the extract of *C. globosum* was evaluated. The protocol of the Philippine Rice Research Center for collection, monoconidial isolation for pure culture and pathogenicity test from Hayashi *et al.* (2009) were adapted with modifications.

Infected leaves of rice showing a diamond-shaped lesion were cut from the host plant. Cut leaves were placed in between coffee filter or newspapers as an alternative, stored inside a plastic bag or a plastic container, and were brought to the laboratory inside a cooler. Information of the field such as the date, place, ecosystem, and rice cultivar were also gathered. Collected leaves were dried at room temperature, and incubated at 4^{o}C upon storage.

Choi *et al.* (1999) devised a technique for the single spore isolation of fungi due to common contamination of the prepared cultures. Hayashi *et al.* (2009) used the single spore isolation technique in preparing a pure culture of *Pyricularia oryzae* Cavara. The dried leaves were cut in 3-5 cm advancing margin which were placed in a sterilized moist filter paper on Petri dish. The plates were incubated at room temperature for 24 hours. A dissecting microscope was prepared for the examination of the lesions. Bent Pasteur pipette was used in picking single spores of blast pathogen. Three percent water agar plate was prepared and placed on the stage inside out. *Pyricularia oryzae* Cavara was identified according to the descriptions of Suparyono *et al.* (2009), International Seed Testing Association (2008), Bussaban *et al.* (2005), Mew and Gonzales (2002) and Mew and Misra (1994) with the assistance of a rice blast specialist at the Philippine Rice Research Institute. Spores of *Pyricularia oryzae* Cavara were focused, using the bent Pasteur pipette, single conidia of *P. oryzae* Cavara was picked and placed on the agar plate. The plates were incubated at room temperature for one day. The germination of the spore and presence of contamination were confirmed on the other day following its inoculation on prune agar slants. Plates could be further incubated for two to three days at room temperature to see the mycelium. The pure culture on slants was used for the identification of *P. oryzae* before inoculation for the pathogenicity test. Potato dextrose agar plates were inoculated with the fungus from the slants. Using three point culture technique and single point technique, plates were inoculated and observed for 5 days.

Inoculum Production and Preparation of Plant Materials for Inoculation

The surface of the fungal growth was scraped using a sterilized toothbrush. The scraped plates were left open, only covered with cling wrap pitted with holes. Open plates were exposed under fluorescent light for three to four days which induced sporulation. Before scraping and harvesting conidia, the appearance of each plate of each isolate was recorded. Also, during counting of spores, the appearance of conidia was also noted.

Ten to twenty milliliters of distilled water was poured into the Petri dish. The sporulated plates were gently scraped using sterilized paintbrush or toothbrush. The conidial suspension was filtered using four layers of sterilized gauze bandage. The conidial suspension was approximated at 10^5 conidia per ml using hemacytometer. Drops of Tween 20 or 0.05% gelatin were added on the final concentration of the inoculum which aided the adhesion of conidia on the surface of the leaf.

Rice varieties PSB Rc82 or Peñaranda, NSIC Rc160 or Tubigan14, and NSIC Rc154 or Tubigan11 were used. Seeds that were not dormant were pre-germinated prior to sowing. Sterilization of seeds was done with soaking in 10% sodium hypochlorite for ten minutes and rinsed in sterile distilled water three times. Seeds were placed on Petri dish with distilled water for two to three days at about 28^{o}C. The water in the Petri plates was changed to supply oxygen to the seeds. Sterilized soil and trays were used for the sowing of plant materials. Ten seeds of each rice variety were sown with five replications. The sown rice seeds were incubated at 28^{o}C for two days for uniform emergence of leaves. The rice plants were inoculated after growing for three weeks inside a greenhouse. A three week old rice plant was already on its 5-6 leaf stage which included the imperfect leaf.

Pathogenicity Tests for *Pyricularia oryzae* Cavara strains. The rice blast facility at the Philippine Rice Research Institute was used for the pathogenicity test f or the three different strains of *Pyricularia oryzae* Cavara The test was performed inside a greenhouse which followed the principles of Koch's Postulate. Ten to twenty milliliters of inoculum with known conidial concentration per strain was prepared. The test plants were inoculated at approximately 21 days after sowing. Test plants were placed in a turn table or rotating rack for uniform spraying of inoculum. The conidial suspension was sprayed on the test plants until runoff. After inoculation, the test plants were placed inside a dew chamber for 20hours then transferred into the green house with high humidity level 25-30^{o}C. The degree of infection of rice blast on the leaves was assessed after seven days of incubation.

After the pathogenicity test, the blast pathogen was isolated from the lesions of the leaf. The diseased leaves were cut from the plant and brought to

the laboratory. Following monoconidial isolation, the conidia of *Pyricularia oryzae* Cavara were isolated and grown in pure culture. The blast pathogen was verified using triple point culture technique. The identified and verified most virulent strain of rice blast pathogen was used for the inhibitory test of *Chaetomium globosum* Kunze.

Treatments: Treatments were laid out using Randomized Complete Block Design (RCBD). Seedlings in control were treated with sterilized distilled water without inoculum. Three replications per strain of the pathogen with ten plants per replicate were used in the study. Inoculated rice plants were incubated at 25-32 °C after inoculation of pathogen.

Inhibitory activity on rice blast pathogen (Pyricularia oryzae Cavara)

Extraction of Crude Extract*:* The crude extraction of the antagonistic fungi was done following the procedure of Kanokmedhakul *et al.* (2006) as cited by Kaewchai and Soytong (2010) with modifications. Biological control agent, *Chaetomium globosum* Cg5 was obtained from Dr. Kasem Soytong, King Mongkut's Institute of Technology Ladkrabang, Faculty of Agricultural Technology, International Biotechnology Research Laboratory, Ladkrabang, Bangkok, Thailand. *Chaetomium globosum* was cultured in PDB at room temperature (28-30˚C) with periodic agitation within a period of 30 days. Approximately twenty milliliters of PDB was inoculated with four to five approximately 3mm of mycelia block using sterile inoculating needle. After growing the biocontrol agent, filtration of fungal biomass was done to remove them from the culture media using cheesecloth or nylon mesh and air-dried overnight. A total of 65.1 g fresh mycelial mat and total of 20.92g dry mycelia of *C. globosum* were weighed separately on the laboratory using a laboratory weighing scale. The crude was extracted with 200 ml ethanol and shaken for 24 hour at room temperature. The biomass was again separated by filtration through Whatman No. 4 filter paper. The filtrates were evaporated in *vacuo* which yielded the crude extract. Crude extract was weighed, and kept in refrigerator at 5 ℃ until use.

In-vitro Assay. The most aggressive *P. oryzae* strain isolated from the pathogenicity test was used to test for inhibition of rice blast pathogen. The experiment was laid out by using Completely Randomized Design (CRD) with five replications. Crude extract was dissolved in 2% dimethyl sulfoxide (DMSO), and mixed into sterilized PDA which was autoclaved at 121 ℃, 15 psi for 30 minute duration. The different concentrations analyzed were 10 ppm. 50 ppm, 100 ppm, 500 and 100pm. Distilled water, ethanol and funguran were used as control.

The rice blast pathogen was cultured on PDA and incubated at room temperature for 7-10 days, then colony margin was cut by 3 mm diameter sterilized cork borer. Agar plug of the pathogen was transferred to the middle of PDA plate (5.0 cm diameter) in each concentration and incubated at room temperature (28-30 ℃) for five days or until control attained maximum growth. Colony diameter, number of conidia and normal and abnormal conidia served as data to be gathered. The number of conidia was counted using hemacytometer while the abnormal conidia was documented and compared to the normal conidia following the descriptions of Arase *et al*. (2008) and Truong (2012). Percentage of inhibition was also computed.

Data gathered was analyzed using Analysis of Variation to test for difference and Duncan's Multiple Range Test (DMRT) to compare means. Level of significance was set at .05.

Results

Isolation and Identification of Pyricularia oryzae Cavara Strains

Strains of *Pyricularia oryzae* Cavara were isolated from three different Barangays in Malasiqui, Pangasinan. The cultural characteristics and morphology of *Pyricularia oryzae* Cavara was used as bases for identification. Three strains of *Pyricularia oryzae* Cavara were identified as follows:

Pyricularia oryzae strain 1(*PO1*), Strain 1 was found to have the biggest diameter with a mean of 28 mm after five days of inoculation at room temperature. The colony was even to uneven, filamentous with white, thinly gown mycelia, and azonated. On the reverse side of the plate, black pigmentation was visible with zonation. It was black which became lighter towards the margin. The conidia were typically hyaline, obclavate with two septations.

Pyricularia oryzae strain 2(*PO2*), the second strain was also filamentous with the mean diameter of 26.73 mm after five days at room temperature. It was second in the diameter of the colony. It was white due to white, thick-grown aerial mycelia, also without zonation, even to uneven. The reverse side of the plate was also pigmented black that turns lighter towards the margin, zonated. Its conidium was obclavate with two septations which was hyaline.

Pyricularia oryzae strain 3(*PO3*), the third strain was found to have the smallest colony with a mean diameter of 26.73 mm after five days at room temperature. The colony was filamentous, even and white and found to have the thickest growth of white aerial mycelia, azonated. The reverse side of the plate was not pigmented with black, instead it was brown becoming white towards

the margin. Its conidium was typically hyaline, obclavate with slight thickening at the base, and two septations could be seen.

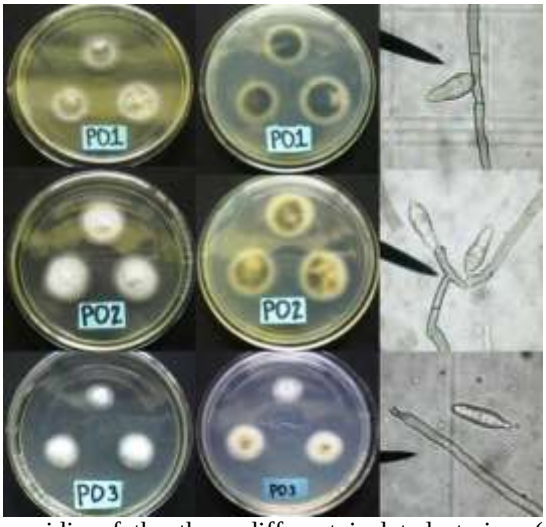

Fig. 1. Colonies and conidia of the three different isolated strains. (a) *Pyricularia oryzae* Cavara strain 1, (b) *Pyricularia oryzae* Cavara strain 2, and (c) *Pyricularia oryzae* Cavara strain 3 showing the (d) top view and the (e) reverse side of the plate;(f) showing conidia and conidiophore respectively under compound microscope.

The three different isolated strains were evaluated for its virulence on resistant, intermediate and susceptible rice responses to rice blast. Conidial suspension of known concentration of each strain was sprayed on a four leaf stage of rice plant. The formation of leaf blast was assessed to determine its pathogenicity.

The comparison among the mean lesion scores of *Pyricularia oryzae* Cavara on the different treatments was shown in Table 1. Results showed a significant difference among the treatments. The highest mean lesion score was visible on PO2 vs NSIC RC160 (T4 = 0.667) followed by PO1 vs NSIC Rv 160 (T2 =0.167) and PO2 vs. NSIC Rc160 (T5 = 0.067). Analysis of data showed that PO2 vs NSIC Rc 154 (T4) was found to be significantly higher than the other treatments.

Table 1. Mean score on the pathogenicity test of the three isolated *Pyricularia oryzae* strains

Treatment	Rice Variety	Strain[a]	Score[b]
Treatment 1	NSIC Rc 154 (S)	*PO1*[a]	0[a]
Treatment 2	NSIC Rc 160 (I)		0.167[a]
Treatment 3	PSB Rc 82 (R)		0[a]
Treatment 4	NSIC Rc 154 (S)	*PO2*[b]	0.667[b]
Treatment 5	NSIC Rc 160 (I)		0.067[a]
Treatment 6	PSB Rc 82 (R)		0[a]
Treatment 7	NSIC Rc 154 (S)	*PO3*[a]	0[a]
Treatment 8	NSIC Rc 160 (I)		0[a]
Treatment 9	PSB Rc 82 (R)		0[a]
Treatment 10	NSIC Rc 154 (S)	DW[a]	0[a]
Treatment 11	NSIC Rc 160 (I)		0[a]
Treatment 12	PSB Rc 82 (R)		0[a]

a=Strains with the same letter superscript are not significantly different at 5% level of significance, b =Mean scores with the same letter superscript are not significantly different at 5% level

Of significance. Difference on the treatments inoculated with *PO2* was due to the different reactions of the used rice varieties which were established by Philippine Seed Board (PSB)/NSIC Rice Varieties (2011, 2009), National Seed Industry Council (2006), Bureau of Plant Industry (2008).

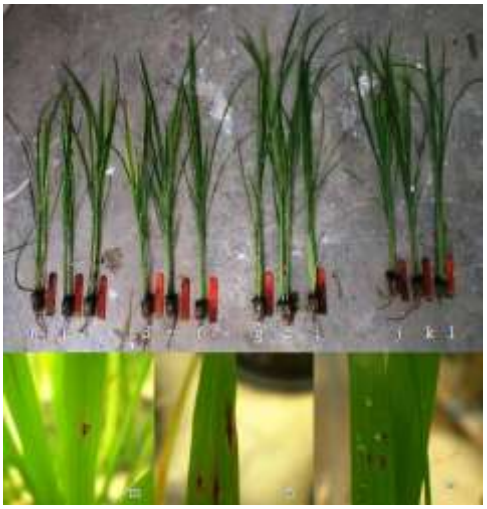

Fig. 2. Twelve treatments used for pathogenicity test of *Pyricularia oryzae* Cavara Treatments 1(a.-susceptible), 2(b.-intermediate), and 3(c.-resistant) were inoculated with *PO*1, treatments 4 (d-susceptible), 5(e.-intermediate), 6(f. resistant) were inoculated with *PO*2 and treatments 7(g- susceptible), 8(h-intermediate), 9(i-resistant) were inoculated with *PO*3. Distilled water was used as control on treatments 10(j-susceptible), 11(k-intermediate), 12(resistant). Typical leaf lesions on the scale of 3(m, n) and 2(o) found on treatments 2(b, m), 4(d, n) and 5(e, o).

Among the different rice blast symptoms, leaf blast was found seven days after inoculation. Lesions formed were characterized by diamond shape with gray center and dark brown to necrotic margin (TeBeest *et al*., 2007). Only few of the treatments formed lesions on the leaf surface. According to the disease assessment of Hayashi *et al*. (2009), the reaction of the three rice varieties on the three *P. oryzae* strains could be considered as resistant for *PO1* and *PO3* while susceptible for *PO2*. The highest scale read was at scale number three which were observed at treatments 2, 4 and 5. Strain 2 of *P.oryzae* inoculated at treatment 4 was used as the most virulent strain among the three. After the pathogenicity test, the infected leaves were cut from the rice plant and brought to the laboratory for reisolation.

Inhibitory Activity on *Chaetomium globosum* Kunze on *Pyricularia oryzae* Cavara. Ethanol crude extract of the potential biocontrol agent *Chaetomium globosum* Kunze was used to test for inhibiting the growth of *P. oryzae* Cavara. Table 4 shows the mean diameter of the growth of *P. oryzae* Cavara exhibited by the different concentrations of the crude extract in comparison to negative controls (distilled water, ethanol) and positive control (funguran) on the fifth day after inoculation.

Table 4. Mean diameter of the growth of *Pyricularia oryzae* Cavara

Treatment	Mean Diameter(mm)[a]	Mean Percent Inhibition
1000ppm	28.90[b]	15.87%[c]
500ppm	29.35[b]	14.56%[bc]
100ppm	32.93[cd]	4.13%[a]
50ppm	31.59[c]	8.02%[ab]
10ppm	32.11[c]	6.51%[a]
Distilled Water(-)	34.35[d]	0.00%[a]
Ethanol(-)	32.91[cd]	4.19%[a]
Funguran(+)	11.93[a]	65.28%[d]

Means with the same letter superscript are statistically not significant at 5% level of significance.

According to the results of the assay, the smallest colony diameter was found on plate with Funguran (11.93 mm) followed by those with 1000 ppm (28.90 mm) and 500 ppm (29.35mm) while those grown with distilled water had the largest colony diameter of 34.35mm. Analysis of data showed significant difference in colony size. Based on Duncan's Multiple Range Test, 1000 ppm was comparable with 500 ppm but significant with the other treatments. On the other hand, Funguran was found to be significantly lower than those two treatments as well as the remaining treatments.

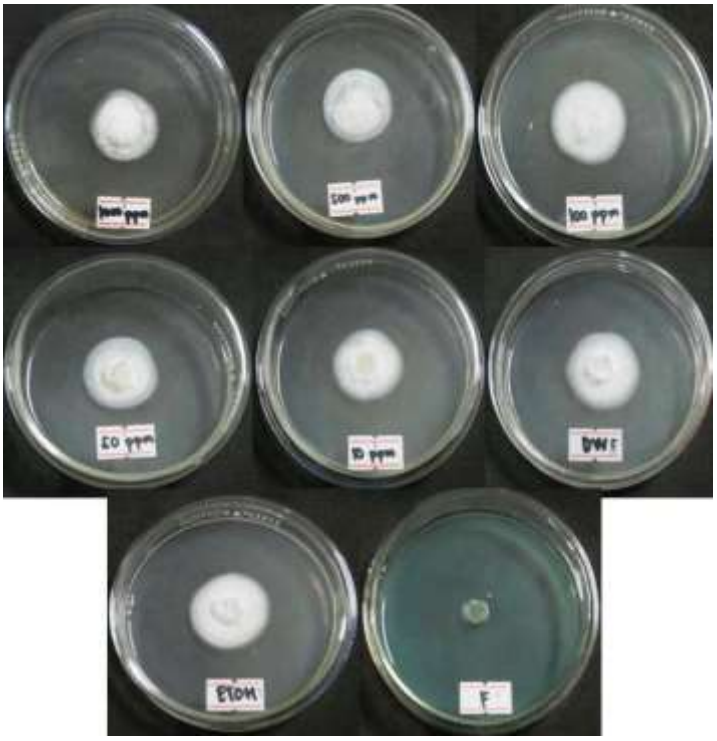

Fig. 4. Colony growth of *P. oryzae* grown in different concentration of ethanol extract of *C. globosum*

The percent inhibition of the different concentrations of *C. globosum* Kunze crude extract towards the growth of *P. oryzae* Cavara was shown in Table 5. The highest percent inhibition was found in Funguran at 65.28% followed by 1000 ppm at 15.87% while the negative control was the least with 0% inhibition. Analysis of variation showed that there was a significant difference among the treatments. Similarly, analysis using DMRT showed significant differences among the means. One thousand parts per million was found to be significantly higher than the other treatment concentrations including the negative group but significantly lower than the positive control.

Discussion

The description of the three strains of *Pyricularia oryzae* Cavara isolated from rice fields conformed with those off Mew and Gonzales (2002), Pappas (1998), and Mew and Misra (1994). *Pyricularia oryzae* can be identified by the morphological characteristics provided by Suparyono *et al.* (2009), International Seed Testing Association (2008), Bussaban *et al.* (2005), Mew and Gonzales (2002) and Mew and Misra (1994). Viewed under stereoscopic

microscope, conidia were sympodially-borne, singly, mostly obpyriform or obclavate, pale olive, grayish or dark brown. Usually 2 septated and rarely three septated with pointed apex truncated and have a short tooth at the base. Conidiophores are moderately long, simple, light brown to hyaline. Conidiophore base is slightly swollen and tapers towards the tip. According to Bussaban *et al.* (2005), the characteristics of the conidia alone can be used in identifying the pathogen upon running morphological and molecular analization of the blast fungus.

The results revealed that ethanol extract of *Chaetomium globosum* Kunze has the potential to inhibit the growth of *Pyricularia oryzae* Cavara using crude ethanol extract which in parallel with other studies. Fogle *et al.* (2007), Park *et al.* (2006) and Soytong *et al.* (2001) identified chaetoglobosins and chaetoviridins from crude extract of *Chaetomium globosum* Kunze which were found to have antifungal activities against *Pyricularia oryzae* Cavara. Kanokmedhakul *et al.* (2002) reported that *Chaetomium globosum* Kunze could produce metabolites which were according to Sibounnavong *et al.* (2011) Kaewchai (2010) and Soytong (2010) could inhibit *Pyricularia oryzae* Cavara and other phytopathogenic fungi like *Rhizoctonia oryzae*. On the other hand, the effect of *Chaetomium globosum* Kunze was not comparable with Funguran. It shows that *Chaetomium globosum* Kunze could not outrank Funguran on its antifungal capability but it could still be used as a resource for alternative biofungicide and biofungicide formulations.

There were no observed conidia on the different concentrations as well as the positive and negative controls. With this result, the effect of *Chaetomium globosum* Kunze crude extract on the production of conidia of *Pyricularia oryzae* Cavara could not be compared to Funguran. This could be due to the alterations that happened on the pathogen. Originally, the pathogen had black pigmentation on the reverse side of the plate which in time turned to white. Dos Santos *et al*. (2010) worked on the influence of period, subplanting and colony color on the sporulation of *Magnaporthe oryzae*. They found that alterations on the fungus could be caused by varying conditions when maintaining pure culture at room temperature. There was reduction on the sporulation capacity of *M. oryzae* right after the second subculturing. They also found that white colony of *M. oryzae* significantly produced the least number of conidia. Generally, the researcher made multiple successive subculturing of the pathogen on maintaining a pure culture. Due to this, the pathogen could have lost its capability to sporulate after successive subculturing and exposure to varying conditions. The number of normal and abnormal conidia was not counted due to the absence of conidia on the treatments.

Philippine strains of *Pyricularia oryzae* Cavara were isolated and identified. One of the three strains was avirulent while the other two was found to have virulence on susceptible and intermediate rice varieties. Moreover, *Chaetomium globosum* Kunze has the potential to inhibit the growth of *Pyricularia oryzae* Cavara using crude ethanol extract.

References

Agriculture Business Week, (2008). Rice Disease Series (Part 2) – Rice Blast. Retrieved on http://www.agribusinessweek.com/rice-disease-seriespart-2-rice-blast/ last 8-31-2011

Betts, M.F. (2007). Identification of the new pathogenicity genes in *Magnaporthe oryzae* through the construction of an *Agrobacterium tumafaciens*-mediated insertion mutant library. A published doctoral dissertation. Graduate College of the University of Arizona, Arizona. Proquest information and learning Company. Retrieved from http://books.google.com.ph/books?id=AzjCghzUeK0C&printsec=frontcover&hl=tl#v=onepage&q&f=false on December 10, 2012

Arase, S., Katano, Y., Li, X., Honda, Y. and Nozu, M. (2008). Morphological Variation in Spores of *Pyricularia oryzae* Cavara. Journal of phythopathology. 142(3) 253-257 Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1439434.1994.tb04537.x/abstract?systemMessage=Wiley+Online+Library+will+be+disrupted+4+Feb+from+102+GMT+for+month+maintenance on February 3, 2012.

Bussaban, B., Lumyong, S., Lumyong, P., Seelana, T., Park, D.C. and Mckenzie, E.H.C. (2005). Molecular and morphological characterization of *Pyricularia* and allied genera. Mycologia 97(5):1002-1111. Retrieved from http://www.mycologia.org/content/97/5/1002.full on December 26, 2011.

Caracuel-rios, Z. and Talbot, N. (2007). Cellular differentiation and host invasion by the rice blast fungus *Magnaporthe grisea*. Current opinion in microbiology 10(4):339-345 Retrieved from http://cogeme.ex.ac.uk/talbot/pdf/2007_Caracuel-Rios_Curr_Opin_Micro_blast.pdf last August 31, 2011.

Citizendium (2011). Magnaporthe grisea. Retrieved from http:// dbg.citizendium.org/wiki/Magnaporthegrisea on 06-03-11

Couch, B.C. and Kohn, L.M. (2002). A multilocus gene genealogy concordant with host preference indicates segregation of a new species, Magnaporthe oryzae, *Mycologia* 94:683-693.

Czymmek, K., Kankanala, P. and Valent, B. (2007). Roles for rice membrane dynamics and plasmodesmata fungi during biotrophic invasion by the blast fungus. The Plant Cell Online 19(2): pp. 706.

Choi, Y.W., Hyde, K.D. and Ho, W.H. (1999). Single spore isolation of fungi. Fungal Diversity 3:29-38.

Fogle, M.R., Douglas, D.R., Jumper, C.A. and Straus, D.C. (2007). Growth and mycotoxin production by *Chaetomium globosum*. Mycopathologia 164(1):49-56.

Harmon, P.F. and Latin, R. (2003). Gray leaf spot of perennial ryegrass. Online. plant health Progress

Hayashi, N., Kobayashi, N., Vera Cruz, C.M. and Fukuta, Y. (2009). Protocols for the sampling of diseased specimens and evaluation of blast disease of rice. Japan international research center for agricultural science. Working report no. 63:7-28.

International Seed Testing Association (2008). Detection of *Pyricularia oryzae* on *Oryza sativa* (Rice). International Rules for Seed Testing Annexe to Chapter 7: Seed Health Testing Methods. International Seed Testing Association (ISTA): Bassersdorf, Switzerland.

Jeamjitt, O. (2007). Diversity of coprophilous fungi, antagonism against plant pathogenic fungi, and secondary metabolites of *Ascodesmis macrospora* and *Sordaria fimicola*. Published doctoral dissertation. Graduate School, Kasetsart University. Kasetsart University. Office of the University Library.

Kaewchai, S. and Soytong, K. (2010). Application of biofungicides against Rigidoporus microporus causing white root disease of rubber trees. Journal of Agricultural Technology 6(2):349-363.

Kaewchai, S., Soytong, K. and Hyde, K.D. (2009). Mycofungicides and fungal biofertilizers. Fungal Diversity 38:25-50.

Kanokmedhakul, S., K., Kanokmedhakul, N., Phonkerd, K., Soytong, K., Kongsaeree, P. and Suksamrarn, A. (2002). Antimycobacterial anthraquinone-chromanone compound and diketopiperazine alkaloid from the fungus *Chaetomium globosum* KMITL-N0802. Planta Medica. 68:832-836.

Kato, H. (2001). Rice Blast Disease. Pesticide outlook. 21:23-25.

Koga, H. (2008). An Electron Microscopic Study of the Infection of Spikelets of Rice by *Pyricularia oryzae.* Journal of Phytopathology 143(7):439-445.

Koga, H., Dohi, K., Nakayachi, O. and Mori, M. (2004). A novel inoculation method of *Magnaporthe grisea* for cytological observation of the infection process using intact leaf sheaths of rice plants. Physiological and Molecular Plant Pathology 64(2):67-72

Liu, Z.H., Yang, Q., Hu, S., Zhang, J.D. and Ma, J. (2008). Cloning and characterization of a novel chitinase gene (chi46) from *Chaetomium globosum* and identification of its biological activity. Applied Microbiology and Biotechnology 80(2):241-252

Mew, T.W. and Gonzales, P. (2002). A handbook of rice seed borne fungi. Los Baños, Laguna: IRRI ; Enfield, N.H. : Science Publishers, Inc. p 27-31ISBN9712201740 p. 27-31.

Mew, T.W. and Misra, J.K. (1994). A manual of rice seed health testing. International Rice Research Institute: Manila, Philippines. ISBN 971-22-0049-3 p.83

MicrobeWiki, (2010). Magnaporthe. Retrieved from http://microbewiki.kenyon.edu/index.php/ Magnaporthe last August 31, 2011.

Pappas, A.C. and Paplomatas, E.J. (1998). Pyricularia leaf spot: A new disease of ornamental plants of the family Marantaceae. Plant Dis. 82:465-469.

Retrieved from http://apsjournals.apsnet.org/doi/pdf/10.1094/PDIS.1998.82.5.465

Park, J.H., Choi, G.J., Jang, K.S., Lim, H.K., Kim, H.T. and Cho, K.Y. (2006). Antifungal activity against plant pathogenic fungi of chaetoviridins isolated from *Chaetomium globosum*. FEMS Microbiology letters. 252(2):309-313.

Retrieved from http://onlinelibrary.wiley.com/doi/10.1016/j.femsle.2005.09.013/full on January 31, 2012

Pitt, J. and Hocking, A.D. (2009). Fungi and Food Spoilage (3rd Ed.). Springer Dordrecht Heidelberg London New York: Heidelberg London New York.

Sibounnavong, P., Charoenporn, C., Kanokmedhakul, S. and Soytong, K. (2011). Antifungal metabolites from antagonistic fungi used to control tomato wilt fungus *Fusarium oxysporum f. sp. lycopersici*. African Journal of Biotechnology 10(85):19714-19722.

Sibounnavong, P., Sysouphanthong, P., X.L., Phoutasay, P., Promrin, K. and Pongnak, W., (2006). Application of biological products for organic crop production of Kangkong (Ipomoea aquatica). Journal of Agricultural Technology 2(2):177-189.

Skamnioti, P. and Gurr, S.J. (2009). Against the grain: safeguarding rice from rice blast disease. Trends in Biotechnology 27(3):141-150.

Sneh, B., Jabaji-Hare, S., Neate, S. and Dijst, G. (1996). *Rhizoctionia* species: Taxonomy, Molecular biology, Ecology, Pathology and Disesae Control. Kluwer Academic Publishers. Po.O. Box 17, 3300 AA Dordrecht, The Netherlands.

Soytong, K., Kanokmedhakuf, S., Kukongviriyapa, V. and Isobe, M. (2001). Application of *Chaetomium* species (Ketomium®) as a new broad spectrum biological fungicide for plant disease control: A review article. Fungal Diversity 7:1-15

Soytong, K. (1988). Identification of species of *Chaetomium* in the Philippines and screening for their biocontrol properties against seed borne fungi of rice. Agris.

Sreenivasaprasad, S., Johnson, R. and Manibhushan Rao, K. (2002). Major fungal diseases of rice recent advances. P.O. Box 17, 3300 AA Dordrecht, The Netherlands. Kluwer Academic Publishers

Suparyono, Catindig, J.L.A. and Oña, I.P. (2009). Rice Blast. Rice Doctor. Rice Knowledge Bank. Retrieved from http://www.knowledgebank.irri.org/ricedoctor/index.php/information-sheets-mainmenu-2730/diseases-mainmenu-2735/rice-blast mainmenu-2767 on January 2, 2012

Talbot, J. and Wilson, R. (2009). Under Pressure: Investigating the biology of Plant infection by *Magnaporthe oryzae*. *Nature reviews* 434(7036):185-195. Retrieved from http://cogeme.ex.ac.uk/talbot/pdf/2009_Wilson_Moryzae_infection.pdf last August 31, 2011.

TeBeest, D.O., C. Guerber and M. Ditmore (2007). Rice blast. The plant health instructor. Retrieved from http://www.apsnet.org/edcenter/intropp/lessons/fungi/ascomycetes/Pages/RiceBlast.aspx last 8-31-2011.

Wopereis (2009). Reference 4 major diseases in rice. PLAR–IRM Curriculum: Technical Manual. www.warda.cgiar.org/publications/PLAR /techmanual/reference24.pdf.

Zeigler, R.S. and Correa, F.J. (2000). Applying *Magnaporthe grisea* population analyses for durable rice blast resistance. 2000. APS*net* Features. Online. doi: 10.1094/APSnetFeature-2000-0700A Retrieved on http://www.apsnet.org/publications/apsnetfeatures/Pages/ApplyingMagnaporthe.aspx last 8-31-2011

Zhang N., Zhao, S. and Shen Q. (2011). A six-gene phylogeny reveals the evolution mode of infection in the rice blast fungus and allied species. DOI: 10.3852/11-022 Retrieved from http://www.mycologia.org/content/early/2011/06/03/11-022.abstract last 8-31-2011.

(Received 15 Febuary 2012; accepted 28 Febuary 2013)