Efficacy of Bio-Fungicide at Varying Concentrations against Rice Blast (*Magnaporthe grisea*)

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Abstract

Various researches have already reported the efficacy of bio-fungicides against rice blast (*Magnaporthe grisea*). However, studies on the optimum concentration that will inhibit the growth of the fungus were not yet conducted. This study aimed to determine the optimum concentration of bio-fungicide formulated by the Free Farmers Federation against rice blast (*Magnaporthe grisea*). It was undertaken using paper disc method and slide germination technique laid out in Completely Randomized Design (CRD). Results showed that among the concentrations of bio-fungicide (bf), 0.009 ppm was proven to be more effective than the other treatments with 19.5 mm average zone of inhibition on the mycelial growth of M. grisea that is 5%, 12%, 15%, and 100% higher than 0.005 ppm bf, 0.007 ppm bf, 0.00375 ppm Dithane, and negative control, respectively. Highest percentage kill is also exhibited by the same treatment at a rate of 53.8% as shown in the results on slide germination technique. Therefore, 0.009 ppm bio-fungicide can be the optimum concentration of bio-fungicide to control rice blast.

Keywords: rice production, disease control, bio-fungicide, antifungal properties

Introduction

Rice is the staple crop of the Asia-Pacific Region. Over 90% of the worlds rice is produced and consumed in the Region. In the last ten years, the Region produced 91.37% of the worlds rice production (FAO, 2005). Data on rice production poses a projection by the Food and Agriculture Organization to decrease due to the 50% increase of consumers in 2025. Hence, production has to keep pace with the growing population. Status of paddy rice (palay) production in the Philippines in CY2014 reached a record of 19 million tons, up 2.87 percent from the CY2013 level (18.4 million tons) despite a marginally lower area harvested of 2.64 million. Yields also improved to 4.01 tons per hectare from 3.86 tons per hectare during the same period. The increase in production is accounted to favorable weather conditions, wider area harvested, and better yields (PSA, 2015). Nevertheless,the said increase also aggravates the deterioration of environmental quality and human health due to massive use of chemical fertilizers and synthetic pesticide especially in controlling major insect pests like green leafhopper, brown planthopper, black bug, and stemborer, and diseases like blast, bacterial leaf blight, and sheath blight (Heong and Escalada, 1998).

Several rice blast epidemics have occurred in different parts of the world, resulting in heavy yield losses in these areas ranging from 50 to 90% of the expected crop (Chaudhary et al., 1994). Under usual conditions, yield losses due to blast ranged from 1-50% in different rice growing regions of the world depending upon the type of cultivars grown and upon environmental conditions that prevailed (Greer et al., 1997). Rice yield losses caused by blast were reported to be 5-10%, 8%, and 14% in India (1960-1961), Korea (mid-1970s), and in China (1980-1981), respectively. In the Philippines, yield losses ranging from 50-85% were reported (Leung, 2010).

Chemical fungicides are commonly applied inr controlling rice blast disease. However, the frequent use of synthetic fungicides on crops may cause hazards to human beings, plant health, ben-

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eficial micro-organisms, and develop fungicide resistance into the pathogens and residual toxicity in plant parts (Weller et al., 2014). On the other hand, some botanical pesticides and bio-control agents have proved to be most secure and have no adverse impact on the environment (Iftikhar et al., 2010). Several research papers have already reported the efficiency of chemical fungicides in controlling blast. Few researches have been conducted to evaluate the efficacy of bio-fungicides. Hence, this paper presents the efficacy of bio-fungicide formulation of the Free Farmers Fedearation (FFF) of Mayantoc, Tarlac. The main goal is to establish the optimum amount of concentration of bio-fungicide which will inhibit the growth of blast on rice.

Methodology

Experimental Site

The study was conducted at the plant pathology laboratory of Philippine Rice Research Institute (PRRI)- Central Experiment Station in Brgy. Maligaya, Science City of Muoz, Nueva Ecija.

Experimental Set-up

In determining the optimum concentration of fungicide, the following methods were done:

Culture Media Preparation

Oat meal media was used in blast (*Magnaporthe grisea*) isolation. Fifty grams (50g) of rolled oats was boiled in 500 ml distilled water. Constant stirring was done. Twenty grams (20 g) of agar was also diluted in water and then heated to dissolve. Oat meal juice was strained through a fine strainer. Then, oat meal juice was poured to the agar solution adjusting it to 1 L volume by adding distilled water. Afterwards, five grams (5g) of sucrose was mixed. The resulting solution was placed in duran bottles, autoclaved twice for 40 minutes at 15 psi, and poured into plated petri dishes (about 10-15 ml to each plate).

Blast Isolation and Culture production

Blast infected leaves of rice were collected from the blast greenhouse. They were washed in distilled water and were cut into small pieces. Pieces of leaves were disinfected using sodium hypochlorite and were then placed in petri plates with oatmeal agar using forceps. Forceps was continuously sterilized as one piece of blast infected leaf is placed to the culture medium by simply burning the ends of the equipment. Isolates were afterwards incubated to promote mycelial growth of the fungi. In addition, grown blast isolates were mass produced in plates filled with oat meal media. Meanwhile, blast stocks previously prepared were re-isolated to redeem the viability of their spores that were reserved for emergency use. Stock culture was used in the induced inoculation process.

Fungicides Bio-assay

Two (2) fungicides were tested for fungicidal activity against *Magnaporthe grisea* pure culture using paper disc method. The fungicides used were Dithane (Mancozeb) and the Bio-fungicide formulated by Free Farmers Federation (FFF) of Mayantoc. Sterilized water was used to serve as control. Treatments were prepared using the dilution process in view of the following treatments:

- T_1- 0.005 ppm bio-fungicide
- T_2- 0.007 ppm bio-fungicide
- T_3- 0.009 ppm bio-fungicide
- T_4- 0.00375 ppm Dithane (ai Mancozeb)
- T_5- sterile water

Bio-fungicide used

The bio-fungicide used in this study is manufactured by the FFF Mayantoc, Tarlac. It has the following components: fermented noni fruit juice, wood, bamboo vinegar, fermented kakawate leaf, neem tree leaf, eucalytptus leaf extracts, kuhol amino acid, water, and molasses. Components were mixed at a ratio of 1:1:1:1:1:1. The bio-fungicide is not yet commercially available in the market but it is already being used by members of the FFF.

Paper Disc Method

Spore suspension of *M. grisea* was prepared. This was done by putting 20 ml of sterile water on 10 day old culture of *M. grisea* . Spores from the culture were dislodged by carefully scraping the surface of the culture media. One (1) ml of the spore suspension was transferred into each sterile plate poured with approximately 10 ml oatmeal agar, then mixed thoroughly for uniform distribution of spores in the plates. Sterile filter paper discs (6mm) were then immersed in each of the fungicide concentrations and laid out in the spored plates. For the untreated control, paper discs were soaked in sterile water. Plates were incubated at room temperature (28 ° - 32 ° C) for 24 hours. Zone of inhibition was measured in four (4) quadrants and was measured in millimeters indicating fungal toxicity to the fungicides.

Slide Germination Technique

Prepared solutions of fungicides (Table 1) were sprayed using atomizer (25 ml capacity) into the surface of sterile glass slides and allowed to dry for 10 minutes. During the drying process, spore suspension of *M. grisea* was prepared. This was done by putting 20 ml of sterile water on 10 day old culture of *M. grisea*. Spores from the culture were dislodged by carefully scraping the surface of the culture media and poured out in 20 ml sterile water to get a 40 ml suspension. One drop of suspension was placed on the 1cm x 1 cm area surface of the sprayed glass slide. Slides sprayed with water served as control. Each treatment was replicated four times. Treated slides were placed in petri plates with moistened tissue paper at the bottom and incubated under room temperature (28° - 32° C) for 24 hours. The slides were examined under a compound microscope for the germinated spores. Spores of *M. grisea* were considered germinated with the emergence of a germ tube. Percent germinated spores and corrected percentage was determined using the formula used by PRRI.

Data gathered

The following data were gathered after the experiment was conducted:

1. **Average Fungicide Inhibition on Mycelial Growth of Blast** was determined by measuring the zone of inhibition of the fungicide concentration that was immersed in filter paper disc and by getting its average.

2. **Percentage Germination of Blast Spores** was measured by dividing the number of spores germinated to the total number of spores multiplied by 100.

3. **Corrected Percentage Germination of Blast Spores** was determined by dividing the percentage germination of blast spores in treatments to the percentage germination of control multiplied by 100.

Data Analysis and Experimental Design

Gathered data from 20 experimental units (5 treatments x 4 replications) were subjected to analysis using Analysis of Variance (ANOVA) in Completely Randomized Design (CRD). Data were analyzed using the Statistical Tool for Agricultural Research (STAR) software.

Results and Discussion

Paper Disc Method

Table 1 shows the average zone of inhibition of concentrations of fungicides to*Magnaporthe grisea* pure culture in millimeters. Based on calculated means, Treatment C exhibited the highest zone of inhibition among the treatments with a mean of 19. 5 mm. Treatment A followed followed by Treatment B and D with means of 18.75 mm, 17.25 mm and 16.25 mm, respectively. Treatment E (control) exhibited no inhibition effect to *M. grisea* as expected.

Results also showed that Treatment A was 8% and 13% higher in zone of inhibition than Treatments B and D but showed 4% less than Treatment C. Treatment B showed 6% greater zone of inhibition than Treatment D but showed 8% and 12% less than Treatments A and C, respectively. Treatment C exhibited greater inhibitions to all treatments with registered 5%, 12% and 15% greater zone of inhibition than Treatments A, B, and D, respectively. All treatments brought

Table 1. Average Fungicide Inhibition on Mycelial Growth of *Magnaporthe grisea*

Treatment (Fungicide Concentration)	Mean (mm)
A – Bio-fungicide (5 ml in 100 ml water)	18.75^{ab}
B – Bio-fungicide (7 ml in 100 ml water)	17.25^{b}
C – Bio-fungicide (9 ml in 100 ml water)	19.50^{c}
D – Mancozeb (Dithane) (0.375 g in 100 ml water)	16.25^{cd}
E – Sterilized water (5 ml)	0.00^{e}

out 100% higher zone of inhibition to Treatment E (control).

All treatments statistically showed differences. Treatment A is significantly different with Treatments B, D, and E but shows no variation to Treatment C. Treatment C and D also show no significant differences with each other. The significant differences among all the treatments are due to the level of toxicity that each concentration of fungicide exhibits. This conforms to the findings of Khan et al. (1987) which state that some leaf extracts including those from neem had a characteristic effect on fungi especially on low polar extracts over the high polar ones. Their findings also suggested that one possible explanation for this is the flavonoid quercetin contained in the extracts. Singh et al. (1980) also reported that the fungicidal and bactericidal properties of extracts from neem leaves either in vitro or in vivo trials is attributed to the presence of several antimicrobial active ingredients such as desactylimbin, quercetin, and sitosterol. Furthermore, research findings by Zaker and Mosallanejad (2010) on the methanolic extracts of eucalyptus extracts showed inhibition on the mycelial growth and spore germination of the fungus *Alternaria alternata* using poisoned food technique and spore germination assay. Hence, it can be attributed that the combined fungicidal effects of neem tree and eucalyptus extracts present in the bio-fungicide formulated by the FFF is responsible for strong anti-fungal activities.

Slide Germination Method

Table 2 shows the results in the slide germination method. As shown in the number of blast spores before treatment application, Treatment E exhibited the highest number in terms of blast spores population. It was followed by Treatments B, C, A with mean averages of 30.3, 29 and 28.8, respectively. Treatment D resulted to having the lowest number of blast spores with a registered mean of 26.8.

Results also revealed that Treatment E (control) has 26%, 22%, 25%, and 31% higher number of spores before germination. Treatment A projected 7% higher number of spores, but is 2% and 1% lower than Treatments B and C, respectively. Treatment B resulted to having 5%, 4%, and 11% higher number of spores than Treatments A, C, and D, respectively. Meanwhile, Treatment C projected 1% and 8% higher number of spores than Treatments A and D. However, it resulted to having 4% lower number of spores than Treatment B.

Analysis of variance (ANOVA) on the number of blast spores before treatment application showed no significant differences at 5% and 1% level of significance among treatments. Treatments B and E revealed significant differences among other treatments. Treatments A, B, and D were found to be statistically the same.

Table 2 also shows the number of germinated spores of blast after treatments application. Result revealed that Treatment E has the highest number of germinated blast spores with a mean of 24.3. While, treatments B, A, and C ranked 2nd, 3rd and 4th with mean averages of 12.8, 12.3, and 8.5, respectively. Lastly, Treatment D projected a mean average of 7.8 that means it has the lowest number of germinated blast spores.

High number of germinated spores mean low toxicity. In this case, Treatment A is 4% more toxic than Treatment B, 37% more toxic than Treatment E, but is 31% and 37% less toxic than Treatments C and D, respectively. Treatment B showed 5%, 34%, and 37% less toxicity than Treatments A, C, and D, respectively. However, it showed 47% greater toxicity than Treatment E (control). Meanwhile, Treatment C exhibited greater toxicity than Treatments A, B, and D at 37%, 34% and 65%, respectively but showed 8%

Table 2. Average Number of Blast Spores Before and 24 Hours After Treatment Application

Treatment (Fungicide Concentration)	***Number of Blast Spores Before treatment application**	***Number of Germinated Spores 24 hours after treatment application**	**% Reduction**
A – Bio-fungicide (5 ml in 100 ml water)	28.8	12.30^a	57.3
B – Bio-fungicide (7 ml in 100 ml water)	30.3	12.80^a	57.8
C – Bio-fungicide (9 ml in 100 ml water)	29	8.50^b	70.7
D – Mancozeb (Dithane) (0.375 g in 100 ml water)	26.8	7.80^a	70.9
E – Sterilized water (5 ml)	38.8	38.80^a	0

less toxicity than Treatment D. Treatment D exhibited greater toxicity level than all treatments. It showed 37% higher toxicity level than Treatment A, 39% than Treatment B, 8% than Treatment C, and 68% than Treatment E.

Analysis of variance on the number of germinated spores after 24 hours of treatment application showed significant differences among treatments.

Percentage reduction also showed that Treatment D exhibited the highest percentage at 70.9% that is 0.2%, 13.1%, and 13.6% higher than Treatments C, B, A, respectively. Treatment C, B, A exhibited 70.7%, 57.8%, and 57.3% reduction, respectively. Treatment C showed 12.9% and 13.4% higher than Treatments B and A, respectively. Treatment B 0.5% had higher reduction than Treatment A. And Treatment E exhibited zero percent reduction.

Table 3 shows the percentage germination of blast spores after treatments. Treatment E registered the highest mean at 62.5. Treatment A exhibited a mean average of 42.9 while treatment B registered 41.5. Treatment C resulted into a mean average of 29.8. Finally, Treatment D projected the lowest average at 28.9.

Results signify that the higher the mean that each treatment projects, the lower the toxicity level. Treatment D exhibited greater toxicity than treatments A, B, C and E at 33%, 30%, 3% and 54%, respectively. Treatment A exhibited 3%, 31%, and 23% less toxic than treatments A, C and D, respectively but is 31% more toxic than treatment E. Meanwhile, treatment B resulted to 28% less toxic than treatment C and 30% less toxic to treatment D.However, it exhibited 3% and 34% greater toxicity than treatments A and E, respectively. Results also revealed that treatment C is 3% less toxic than treatment D, but it also showed that it has 28%, 31%, and 33% greater toxicity than treatments A, B, and E, respectively. Finally, Treatment E exhibited less toxicity level than treatments A, B, C, and D at 31%, 34%, 33%, and 54%, respectively.

Results also showed that the highest percentage kill is exhibited by Treatment D with a registered % kill of 53.8. It revealed a 1.5%, 21.2%, 22.4%, and 53.8% higher percentage kill than treatments C, B, A, and E, respectively. Treatment C also registered higher percentage kill than treatments B, A, and E at 18.7%, 20.9%, and 52.3%. While Treatment B exhibited 2.2% and 33.6% higher percentage kill than treatments A and E, respectively. Moreover, Treatment A also showed 31.4% higher percentage kill than treatment E. Lastly, zero % kill was exhibited by Treatment E.

Analysis of variance displayed highly significant difference among treatments. However, some treatments resulted in non-significance. Treatment A and E were significantly different to all others treatments. Treatment B is significantly different among other treatments but not to Treatment A. Treatments C and D showed non-significant differences that means, statistically, they are the same. Significant differences among all treatments are due to the level of toxicity that each fungicide concentration exhibits. In this

Table 3. Percentage Germination of Blast Spores After Treatment Application

Treatment (Fungicide Concentration)	% Germination	% Corrected Germination	% Kill
A – Bio-fungicide (5 ml in 100 ml water)	42.90^{b}	68.6	31.4
B – Bio-fungicide (7 ml in 100 ml water)	41.50^{bc}	66.4	33.6
C – Bio-fungicide (9 ml in 100 ml water)	29.80^{d}	47.7	52.3
D – Mancozeb (Dithane) (0.375 g in 100 ml water)	28.90^{de}	46.2	53.8
E – Sterilized water (5 ml)	62.50^{a}	100	0

case, Treatment C and D are the most toxic to blast. These findings are in agreement with the bio-assay findings of Olufolaji (2006) using slide germination technique stating that bio-fungicide of plants origin are proven to be effective against *M. grisea* and can be inferred as alternatives for chemical fungicides.

Conclusions

After undertaking the study, it was learned that the bio-fungicide used in varying concentrations was proven to be effective against rice blast. In addition, the results on paper disc method revealed that the bio-fungicide at 0.009 PPM is comparable to synthetic fungicide check Dithane (Mancozeb). Likewise, slide germination method projected the same results. Hence, these results indicate the optimum concentration of bio-fungicide to control blast. Moreover, the results projected in this study may also serve as guide in the development of accurate concentration for the bio-fungicide to establish its great effect against rice blast.

Furthermore, the bio-fungicide used in the study can be used by famers as an alternative way to control blast instead of using costly and hazardous synthetic fungicides. What is remarkable is that the formulation of bio-fungicide only requires less costs and that materials in formulating such are readily available in the market and in the environment and can be easily done by farmers. Finally, the effect of the bio-fungicide against blast may not cause any hazardous effects to the environment and to the human health and that the resulting concentration may serve as basis in the conduct of future experiments.

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