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# In vitro efficacy of some fungicides for the management of Rice Blast Pathogen Magnaporthe oryzae

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Fungicides are used in agriculture for the management of plant diseases for effective food production. In this study, we conducted an *in vitro* experiment using three synthetic fungicides on mycelium growth of *Magnaporthe oryzae*. Among the tested fungicides, percent mycelial growth inhibition was higher in Contaf 100 % concentration (86.1%) followed by Contaf 50 % concentration (85.0 %). The least inhibition was observed in Raksha 50% concentration with 43.3% inhibition. We also evaluated the effect of seed dressing chemicals on the percentage germination of rice seeds. The results showed that all seeds treated had over 80 % germination at 7 days after sowing (DAS) compared to untreated (control) seeds which had only 65 % germination. Germination was higher (85 %) at 7 days after sowing, with seeds treated with Raksha, followed by Star-dress with 84 % while the least germination was observed with the seeds treated with Apama plus 81 %. Furthermore, the impact of seed dressing chemicals on the rice seedling vigor was also evaluated. Highest seedling vigor was observed on seeds treated with Star-dress with mean seedling length of (22.5 cm) which showed the highest vigor index of 1893, followed by Apama plus with mean seedling length of (22.1 cm) and a vigor index of 1792. Least seedling length of (18.9 cm) was observed with Raksha which was even lower than the control (20.4 cm). Our results suggested that fungicides can be effectively administered for the management of plant diseases to support seed germination and seedling vigor.

**Keywords:** seed germination, seedling vigor index, seed dressing chemicals, *Magnaporthe oryzae*

## INTRODUCTION

Rice is a major food source for people in most parts of Asia and Africa for centuries. However, annual yield losses occur due to agricultural diseases (Valent and Chumley, 1991; De Laurentiis et al., 2016). Presently, rice is the food product feeding more than half of the world population, yet its production must be doubled in the future because of the increasing demand resulting from the ever growing global population (Fisher et al., 2012). In terms of agricultural asset worldwide, rice ranks third in terms of production with 758.9 million tons in 2016, following sugarcane with 1.9 billion tons and maize with 1.0 billion tons (FAO, 2022; Abbas and Ansumali, 2010). Right now China is the leading producer of rice producing about 208.9 million tons, followed by India 163.3 million tons, Indonesia 72.7 million tons as third and Nigeria 5.3 million tonnes as 16th in the world, second in Africa and largest rice producer in West Africa (FAO, 2022).

Diseases confronted during rice production cause great yield losses and poses a huge threat to the accomplishment of global food security (De Laurentiis et al., 2016). Rice is vulnerable to a number of diseases, such as Rice blast (Magnaporthe oryzae), Brown spot (Helminthosporium oryzae), Sheath blight (Rhizoctonia solani), Bacterial leaf blight (Xanthomonas oryzae) and Sheath rot (Sarocladium oryzae), Rice yellow dwarf disease (Rice yellow dwarf virus) among others (Thakur, 1994) .One of the most serious bottlenecks to increased rice production is the presence of the rice blast fungus (Magnaporthe oryzae), which directly decreases rice yields and causes increases in production costs (Skamnioti and Gurr, 2009). It is the most important disease that affects rice production globally (Wilson and Talbot, 2009). Rice blast caused by M. oryzae is a major problem in confronting global food insecurity and accounts for nearly 30% losses encountered in rice production worldwide, this equivalent for feeding of about 60 million people (Nalley et al., 2016). The infection of M. oryzae starts with a series of morphogenetic transformation during conidial attachment and germination, resulting in the production of appressoria at the end of germ tubes for penetrating host cell surface (Talbot, 2003; Ebbole, 2007).

To reduce the losses caused by the rice blast fungus and other pathogens, fungicides are needed to suppress the activities of these pathogens. Fungicides are chemical compounds used to kill or inhibit the activities of the fungi and are used for seed treatment or for foliar application (spray) to control the diseases during seedling, vegetative growth and reproductive stages. Fungicides that are commonly used for control of rice blast are Tricyclazole, Carbendazim, Azoxystrobin, Propiconazole, Iprovalicarb, Mancozeb, Hexaconozole, Mefenoxam and Kitazin which are sprayed thrice at weekly interval starting from the initiation of the disease (Greer and Webster, 2001).

The overall demand for rice is increasing at an alarming rate and will continue for decades to come, and Nigeria is facing various diseases that limit rice production particularly rice blast. Therefore, there is need to evaluate the effect of some fungicides and seed dressing chemicals in the management of the disease, because the global annual demand for rice is expected to be around 800 million tons by 2025 (David et al., 2011). In view of the expected increase in population and significant important of rice to the food security, coupled with the greatest possibility of developing a rice industry in coming years, primarily driven by government promotion and changes in rice production and closed boarder policy to boost food security, and enhances consumption of locally produced rice as enforced by Nigerian government. This necessitates the reason for conducting this research to evaluate the in vitro efficacy of some selected fungicides on mycelium growth of Magnaporthe oryzae with aim of finding the best fungicide for the management of the rice blast fungus and determine the seed dressing chemicals on seed viability and vigour on the treated rice seeds.

# MATERIALS AND METHODS

**Sample collection**

Diseased samples with Pyricularia oryzae; Syn. Magnaporthe oryzae symptoms were collected from five rice fields across Panshekara District of Kumbotso Local Government Area, Kano State- Nigeria during the 2023 growing season. The rice fields selected for sampling represent the most intensive rice production areas in Kumbotso local government, due to the nature of the areas that can support rice production for both rainy and irrigation periods. In all the collections, samples of infected leaves were collected at random from rice plants in a quadrant of 3 × 3 meters apart and transported in separate plastic bags to the laboratory the same day for Magnaporthe oryzae isolation.

**Isolation and Identification of the pathogen**

Isolation and identification of the pathogen was carried out at the Plant pathology laboratory, Department of Crop Protection, Faculty of Agriculture, Bayero University Kano-Nigeria. To isolate the fungus, infected leaves were first rinsed gently using running tap water and distilled water respectively. A small portion of tissue (5 mm) was cut from the advance margin of a leaf lesion and placed abaxial side up on 2.0 % sodium hypochlorite for two minutes. The samples were then dried with sterile filter paper and aseptically transferred to Potato dextrose agar (PDA) plate supplemented with streptomycin (100 µg/mL) and rifampicin (10 µg/mL), incubated at 28 °C as described by Talbot et al. (1993). Purification was performed by two sequential transfers of a single spore collected from mycelium hyphae to a fresh PDA plate to grow for 14 days in the dark to develop more colonies, which will be used for this study and kept for further use.

To identify the fungus, it was viewed under a microscope and identified according to the morphology using illustrated genera of imperfect fungi, fourth edition identification manual as a guide (Barnett and Hunter, 2006). Based on the morphology, Magnaporthe oryzae was identified to be associated with the deterioration and infection of rice leaves which were believed to be rice blast pathogen.

**In-vitro evaluation of fungicides on mycelial growth of rice blast Magnaporthe oryzae**

The mycelial inhibitory effect of this devastating fungus was tested in vitro using three different synthetic fungicides. These includes Contaf Plus (Hexaconazole) TATA Africa Services (Nigeria) Limited, Ridomil Gold (Metalaxyl-m + Mancozeb) Syngenta Group Company and Raksha (Mancozeb 80% WP) WACOP Limited. Mycelial plugs (3 mm in diameter) were taken from the margin of revived 14 days old colonies and inoculated onto a new Potato dextrose agar plates (9 cm diameter) with the treatments (amended with fungicide) or without (control). Prior to that, stock solution of the fungicides was constituted using a ratio of fungicide and sterile distilled water (1 mL: 100 mL) for liquid fungicide and (1 g: 100 mL) of powder or dust (WP) fungicide to sterile distilled water respectively. A solution was further formed from the stock solution as 50 % and 100 % concentration; 5 mL each from the constituted fungicides solution were added to the media respectively. The experiment was laid out in a completely randomized design (CRD) using four replications for all treatments and the control incubated at room temperature 28 ± 2° C.

The colony sizes of the fungus on both treated and control plates were measured using a meter ruler at three, five and seven days after inoculation, the percent growth inhibition of the pathogen was calculated according to the formula used by Pandey (1995) as;

$$\text{Percentage inhibition} = (DC - DT)/ DC \times 100$$

Where: DC = is the average colony diameter of the fungus in the control, DT = is the average colony diameter of the fungus in treatment.

**Data analysis**

Data collected were subjected to analysis of variance (ANOVA) using GenStat software version 17, and treatment means were separated at 5% level of significance to determine the least significant difference (LSD).

**Effect of seed treatment on seed germination and Vigour Index**

**Germination test**

To determine seed germination and vigour after treating seeds with seed dressing chemicals an experiment was conducted at Plant Pathology Laboratory, Department Crop protection, Bayero University Kano. Four hundred (400) healthy seeds of JIF (local rice variety) which is known for its highest germination percentage (Bolanle et al., 2019). Three different seed dressing chemicals were used, these includes; Raksha (Mancozeb 80 % WP), Apama-plus 42 WS (20 % metalaxyl, 15 % Carboxin, and flurathlocarb) and Star-dress 45 WS (Imidacloprid 25 % and Thiram 20 %).

One hundred (100) seeds each were rinsed with distilled water prior to chemical seed dressing to facilitate absorption of the chemicals. The seed were treated with 1g of the chemical and sown on germination test trays containing sterilized loamy soil (Oven sterilization at 100 °C for 30 minutes). In each tray, 100 seeds were sown per each treatment (with seed dressing) and without (control) in a completely randomized design replicated four times. The seedling trays containing sterilized soil were moistened with sterile distilled water for 3 days before sowing, and every other day after sowing to the end of the experiment.

**Seedling vigour test**

Vigour test was done in accordance to ISTA standards (2001). Length of shoot and root were measured using a metre rule at 2 weeks after sowing. A total of eighty (80) seedlings were selected at random (20 from each of the four treatments) for the measurement of shoot and root length. The seedling vigour was determined using formula described by Abdul-baki and Anderson (1972):

Vigour Index = (mean of root length + mean of shoot length) × % seed germination

# RESULTS

**In vitro evaluation of fungicides on mycelial growth of Magnaporthe oryzae**

Significant difference ($P \leq 0.05$) was observed on mycelial growth of the rice blast pathogen at 3, 5, and 7 days after inoculation (Table 1). Percent mycelial growth inhibition was higher in plate treated with (Contaf 100% concentration at 5DAI recording 86.1% growth inhibition), followed by (Contaf 50% at 7DAI with growth inhibition of 85.0%). The least inhibition percentage was observed in plate treated with (Raksha 50% concentration with inhibition of 43.3% at 3DAI).

**Effect of seed treatment on seed germination and vigour index**

**Percentage seed germination**

Result on germination percentage showed that all the treatments had over 80 % germination at 7 days after sowing (DAS) compared to untreated (control) seeds which had only 65 % germination, with Raksha seed dressing chemical having highest germination percentage of 85 % at same day respectively (Figure 1c). The overall germination percentage is increasing with days after sowing, lowest at 3DAS and highest at 7DAS (Figure 1 a, b & c).

**Seedling vigour test**

The results obtained from the seedling vigour index showed that Star-dress seed dressing chemical has the highest vigour index of 1893, followed relatively by Apama-plus 1792 while the control treatment recorded the least seedling vigour of 1184 (Table 2).

# DISCUSSION

In this study, effects of different fungicides were evaluated on rice blast pathogen Magnaporthe oryzae using in vitro approach (Haq et al., 2002; Surapu, 2017), and significant differences observed between the fungicides ($P \leq 0.05$). Mycelial growth of the pathogen (Magnaporthe oryzae) on the tested fungicides was taken at 3, 5 and 7 days after inoculation (DAI). Percent mycelial growth inhibition was higher in plate treated with Contaf 100 % concentration at 5DAI recording 86.1 % growth inhibition, followed by Contaf 50 % concentration at 7DAI with growth inhibition of 85.0 % (Table 1). The least inhibition percentage was observed in plates treated with Raksha 50% concentration with inhibition percentage of 43.3 at 3DAI. Contaf fungicide (Hexaconazole) had a highest inhibition percentage across all the tested fungicides used in the study. In a similar work conducted by Magar (2015), a maximum control of fungal diseases of rice were achieved using tricyclazole 22% and Hexaconazole 3% respectively. In similar to our findings, Surapu (2017) also reported 86.3% inhibition of M. oryzea by Carbendazim + Hexaconazole and Vijayawada et al. (2012) reported 78.1% inhibition of the pathogen by Tricyclazole. In the present study, the lowest percentage inhibition was observed in Raksha fungicide (Mancozeb 80% WP). This result is in line with the work of Gohel et al., (2015) in the control of rice blast in Waghai region, India, where lowest percentage inhibition was observed with Mancozeb. This is consistent with previous reports involving M. oryzae-fungicide in-vitro evaluation (Haq et al., 2002).

To determine the seed germination and seed vigour, three different seed dressing chemicals were used. Result on germination percentage showed that all the treatments had over 80 % germination at 7 days after sowing (DAS) compared to untreated (control) seeds which had only 65 % germination (Figure 1c). The result of the effect of the seed dressing chemicals on the percentage germination of the treated rice seed was evaluated at 3, 5 and 7 days after sowing (Figure 1 a,b,c). Germination was higher (85 %) at 7 days after sowing, with seeds treated with Raksha. This was followed by seeds treated with Star-dress with 84% while the least germination was observed in the seeds treated with Apama plus with 81% which is much higher than that of control (untreated seeds) with 65% (Figure 1c). In similar to our findings, Bolanle et al., (2019) reported higher germination of rice seeds treated with Zeb-care (Mancozeb 80%WP) and lowest with seed treated with Apron star.

The effect of the seed dressing chemicals on the rice seedling vigor was also evaluated (Table 2). Highest seedling vigor was observed on seeds treated with Star-dress with seedling length of (22.5 cm) which also showed the highest vigor index of 1893 (Table 2), followed closely by Apama plus with mean seedling length of (22.1 cm) and a vigour index of 1792. Raksha seed dressing chemical showed the least mean seedling length of (18.9 cm) which was even lower than control (20.4 cm). However, in terms of vigour index, it was significantly higher than the control with a vigor index of 1565 compared to the control with vigour index of 1184.

Based on this study, it is reasonable to believe that Raksha seed dressing chemicals may enhances the germination percentage, but it does not have much impact on the seedling vigour of the seedling. In a similar report, rice seeds treated with Zeb-care (Mancozeb 80% WP), increased seedlings vigor index over untreated by 62.8 % (Bolanle et al., 2019). Inconsistent with our findings, seedlings vigor index increased in vegetable treated seed using BAU Bio-fungicide (Bhuiyan et al., 2005; Hossain and Hossain, 2012). Furthermore, Shultana et al., (2009) also evaluated wheat seeds treated with Bavistin and showed higher vigor index of 2843 followed by BAU Bio-fungicide treated seed 2661 respectively.

# CONCLUSION

In conclusion, our results indicate that fungicides could be used effectively in the control of rice blast pathogen M. oryzae, even though there are high possibility of toxicity to humans, animals and environment than other control methods. Despite all the aforementioned problems of using pesticides as a control measure, fungicide application remain one of the most effective approaches to control plant diseases (Kessel et al., 2018; Rekanović et al., 2012; Lurwanu et al., 2021), but resistance to fungicide such as metalaxyl can quickly develop (Chen, et al., 2018; Matson et al., 2015).

In our study, Star-dress seed dressing chemicals which is often neglected by farmers, significantly increased seed germination and seedling vigor. Treated seeds with Star-dress (80% WP), increased their vigor index over untreated by 62.8% and can be recommended as seed dressing chemical for optimum control of rice seed borne pathogens. Therefore, effective control of the disease can be achieved by using appropriate fungicides for seed dressing and foliar sprays respectively.

# REFERENCES

Abbas A., Ansumali S. (2010). Global potential of rice husk as a renewable feedstock for ethanol biofuel production. Bioenergy Research, 3: 328-334.

Abdul-Baki A.A., Anderson J.D. (1972). Physiological and Bio-chemical deterioration of seeds, In: Seed Biology, Vol. 2: Kozlowski, T. T. (Ed.), Academic Press, New York, pp. 283-315.

Barnett H.L., Hunter B.B. (2006). Illustrated genera of imperfect fungi. American Phytopathological Society (APS Press).

Bolanle, T. E., Yahuza, L., Mustapha S., Ali A.S. (2019). Seed health, quality test, and control of seed-borne fungi of some improved and local cultivars of rice (Oryza sativa L.) in Kano, Northwestern Nigeria. Journal of Tropical Crop Science, 6: 142-152.

Chen F., Zhou Q., Qin C., Li Y., Zhan J. (2018). Low evolutionary risk of iprovalicarb resistance in Phytophthora infestans. Pesticide Biochemistry and Physiology, 152: 76–83.

David T., Christian B., Jason H., Belinda L.B. (2011). Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences, 2011. 108: 20260-20264.

De Laurentiis V., Hunt D.V., Rogers C.D. (2016). Overcoming food security challenges within an energy/water/food approach. Sustainability, 8: 95.

Ebbole D.J. (2007). Magnaporthe as a model for understanding host-pathogen interactions. Annual Review of Phytopathology, 45: 437-456.

FAO – Food and Agriculture Organisation (2022). Rice market mornitor, volume xx Issue No. 1. (April 2022).

Fisher M.C, Henk D.A., Briggs C.J., Brownstein J.S., Madoff L.C., McCraw S.L., Gurr S.J. (2012). Emerging fungal threats to animal, plant and ecosystem health. Nature, 484: 186.

Greer C.A., Webster R.K. (2001). Occurrence, distribution, epidemiology, cultivar reaction, and management of rice blast disease in California. Plant Disease, 85: 1096-1102.

Haq I.M., Adnan M.F., Jmil F.F., Rehman A. (2002). Screening of rice germplasm against Pyricularia oryzae and evaluation of various fungitoxicants for control of disease. Pakistan Journal Phytopathology, 14: 32-35.

ISTA (2001). International Rules for seed testing and Association, 31: 107-115.

Kessel G.J., Mullins E., Evenhuis A., Stellingwerf J., Cortes V.O., Phelan S., van den Bosch T., Förch M.G., Goedhart P., van der Voet H. (2018). Development and validation of IPM strategies for the cultivation of cisgenically modified late blight resistant potato. European Journal of Agronomy, 96: 146–155.

Lurwanu Y., Wang Y.P., Wu E.J., He D.C., Waheed A., Nkurikiyimfura O., Wang Z., Shang L.P., Yang L.N., Zhan J. (2021). Increasing temperature elevates the variation and spatial differentiation of pesticide tolerance in a plant pathogen. Evolutionary Applications, 14: 1–12.

Magar P.B. (2015). Use of chemical fungicides for the management of rice blast (Pyricularia Grisea) at Jyotinagar, Chatwan, Nepal. International Journal of Applied sciences and Biotechnology, 3: 474-478.

Matson M.E., Small I.M., Fry W.E., Judelson H.S. (2015). Metalaxyl resistance in Phytophthora infestans: Assessing role of RPA190 gene and diversity within clonal lineages. Phytopathology, 105: 1594–1600.

Nalley L., Tsiboe F., Durand-Morat A., Shew A.,Thoma G. (2016). Economic and environmental impact of rice blast pathogen (Magnaporthe oryzae) Alleviation in the United States. PloS one, 11: e0167295.

Pandey R.R., Arora D.K., Dubey R. (1995). Antagonistic interaction between fungal pathogen and phyloplane fungi of Guava. Mycopathology, 124: 31-39.

Rekanović E., Potočnik I., Milijašević-Marčić S., Stepanović M., Todorović B., Mihajlović M. (2012). Toxicity of metalaxyl, azoxystrobin, dimethomorph, cymoxanil, zoxamide and mancozeb to Phytophthora infestans isolates from Serbia. Journal of Environmental Science and Health, 47: 403–409.

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

Surapu Rani, Arani J. (2017). In Vitro Evaluation of Fungicides against Rice Blast Isolates. International Journal of Current Microbiology in Applied Sciences, 4: 53-60.

Talbot N.J. (2003). On the trail of a cereal killer: exploring the biology of Magnaporthe grisea. Annual Reviews in Microbiology, 57: 177-202.

Talbot N.J., Ebbole D.J., Hamer J.E. (1993). Identification and characterization of MPG1, a gene involved in pathogenicity from the rice blast fungus Magnaporthe grisea. The Plant Cell, 5: 1575-1590.

Thakur J. (1994). Rice production constraints in Bihar. In: Proceedings of the workshop on Rice Research Prioritization in Asia. IRRI, Philippines, pp. 21-22.

Valent B., Chumley F.G. (1991). Molecular genetic analysis of the rice blast fungus, Magnaporthe grisea. Annual Review of Phytopathology, 29: 443-467.

Vijayawada K, Orissa J., Hirata M. (2012). New fungicide launched in west Bengal. PVT Ltd, India, pp 65.

Wilson R.A., Talbot N.J. (2009). Under pressure: investigating the biology of plant infection by Magnaporthe oryzae. Nature Reviews Microbiology, 7:185.

# References