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Research Article

Antifungal Efficacy of Chitosan against Blast Pathogenic Fungi (*Magnaporthe oryzae*) on Rice (*Oryza sativa*) Field

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Abstract

Blast disease (caused by Magnaporthe oryzae) is the most damaging disease to rice, and synthetic fungicides have been the primary means of control, however, concerns have been raised about the health risks posed, hence, the need for alternative biological crop protection strategies. This study, evaluates the antifungal efficacy of chitosan against blast pathogenic fungi on rice field. Three chitosan (High, Medium and Low Molecular Weight) were purchased while the fourth Chitosan was synthesized from crab shell (CSCS). Faro 52 rice variety was collected from National Cereal Research Institute, Badeggi and planted on a hydromorphic field. The degree of deacetylation of the synthesized chitosan was 98%. The rice field was inoculated with M. oryzae and treated with chitosan two weeks after transplanting. The blast severity and incidence and other agronomic data were taken and data were analyzed using Analysis of Variance (ANOVA) and means separated using LSD. Results reveals that blast severity of 6-(highly susceptible) 5-(moderately susceptible) and 4-(susceptible) with the incidence of 28.3%, 20%, 20% and 24.3% in rice plot before spraying with MMWC1.5%, HMWC 2.0%, LMWC2.0% and CSCS 2.0% respectively was observed. However, the severity reduced to 1(resistance) in MMWC1.5%, HMWC 2.0%, LMWC2.0% and CSCS 2.0% and incidence of 9.3%, 2.3%, 1.0% and 2.0% respectively at the end of the treatment. There was no significant difference (P≤0.05) in all other agronomic parameters except for panicle count with the highest value (8.53) in HMWC 2.0% treated plot and the lowest value (5.80) in HMWC 0.5%. Similarly, a significant difference was observed in grain yield per plot with the highest yield of 717 grams in HMWC 2.0% treated plot while the lowest yield of 190 grams was observed in MMWC 0.5% which was significantly different. It may therefore be concluded that chitosan treatment reduce the severity and incidence of blast pathogen as well as increase grain yield.

Keywords: Rice, Chitosan, Magnaporthe oryzae, Blast, Pathogen

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Introduction

Rice (*Oryzae sativa* L.) is one of the world's primary food crops mostly grown in tropical and sub-tropical climates. It is the agricultural commodity with the third highest worldwide production after sugarcane and maize (FAO, 2014). It is one of the main staple foods in Nigeria as the demand for rice is growing faster than any other staple food such as maize, sorghum and millet with consumption broadening across all socio-economic classes (Iwuagwu*et al.,* 2018). Despite efforts to reduce rice imports and encourage local production, Nigerians have been unable to meet the demand for rice through domestic production. Several factors have contributed to this failure at the farmers' level, these factors include abiotic and biotic constraints. The biotic constraints include weeds, insect pests, and diseases. Fungal pathogens that cause diseases are the major biological constraints infecting rice crops from the field to storage, and new potentially harmful species are being discovered (Islam and Ahmed, 2017). This reduces total output, availability of quality seed and grain quality for planting and processing respectively, and this also affects storage for the following planting season. Numerous fungi contaminate rice in the field, including rice blast pathogens (*Magnaporthe oryzae*), rice sheath blight pathogens (*Rhizoctonia solani*), and brown spot pathogens (*Cochliobolus miyabeanus*) (Suleiman and Akaajime, 2010). Blast disease (caused by the teleomorph *Magnaporthe oryzae* (Hebert) Barr) is the most damaging disease to rice (*Oryza sativa* L.) worldwide (Koutroubas *et al*., 2009). Because of its rapid growth on a large scale, *Magnaporthe oryzae* is one of the most serious diseases (Pham *et al.,* 2018). The pathogen is most common on leaves during the vegetative stage of growth, causing leaf blast, or on neck nodes and panicle branches during the reproductive stage, causing neck blast (Koutroubas *et al*., 2009). Furthermore, *M. oryzae* spores are extremely small and light, and can easily spread through the air, resulting in crop losses of up to 80% of total production. Over time, the use of synthetic fungicides has been the primary method of controlling this field fungal pathogen. However, serious concerns have been raised about the health risks posed by the exposure of farmers working with these fungicides and the residues on food, hence the need for the development of alternative crop protection strategies. The use of chitosan-based products as a novel approach is critical. Chitosan is a natural nontoxic biopolymer that is commercially produced by partial deacetylation of chitin, which is obtained from crustacean exoskeletons and fungi cell walls. The introduction of new biofungicides (Chitosan synthesize from shellwaste) is an alternative solution to solve this problem as it has been used in crop production and the protection of various fruits and vegetables (Zahid *et al.,* 2015). Therefore, evaluating the antifungal efficacy of chitosan against *M. oryzae* is important in achieving Integrated Pest Management (IPM).

Materials and Methods

Collection of Chitosan

Four different chitosan were used for this trial, three already prepared and one extracted.

The three already made different molecular weight chitosans (Low molecular weight chitosan (LMWC) (MW 50 kDa; 75–85% deacetylated), Medium molecular weight chitosan (MMWC) (MW 400 kDa; 75–85% deacetylated) and High molecular weight chitosan (HMWC) (MW 760 kDa; ≥ 85%)) were purchased from Chitin-Chitosan BioChemika and Sigma- Aldrich Company USA. Also crab shells were also obtained from sea shores in Warri, Delta State Nigeria for chitosan extraction.

Extraction of Chitosan from Crab Shell (CSCS)

Crab (*Callinectesamnicola*) shells waste were washed and dried in a hot air oven at 60^{o}C for 24 hrs. Dried shells waste were packed in a

polyethylene bag and stored at -4 ^{o}C. Dried shells were pulverized manually using mortar and pestle. The modified extraction procedure of Gaikwad *et al*., (2015) was followed which include the basic steps of deproteinization, demineralization, decolouration and deacetylation. Pulverized shells were deproteinized by treating with 3.5% (w/w) NaOH solution for 2 hrs at 65^{o}C with constant stirring at a solid to solvent ratio of 1:10 (w/v), demineralized with 1N HCL for 30 min at ambient temperature in a solid to solvent ratio of 1:15 (w/v) for 15 min and decolourized with acetone for 10 min and dried for 2 hrs under hood, followed by bleaching with 0.32 % (v/v) solution of sodium hypochloride (containing 5.25% available chlorine). After each step, the chitin was filtered, washed with distilled water to neutral pH. Chitin deacetylation was carried out at 15 psi/121^{o}C using 50 % sodium hydroxide (NaOH) solution for 15 min. The samples were filtered off, washed with distilled water to neutral pH and dried in an oven at 60 ^{o}C for 24 hrs.

Determination of the degree of deacetylation

Fourier transform infrared (FTIR) analysis was used to determine the degree of deacetylation of the synthesized chitosan. Chitosan solution was prepared as a pellet in potassium bromide (KBr) at a 1:99 chitosan sample to KBr ratio, and the sample mixture was then subjected to an infrared (IR) radiation spectroscopy (machine-Model-ABB FTLA 2000-100 Quebec, Canada) at a resolution limit of 16 cm^{-1} (Sneha *et al.,* 2014). The degree of deacetylation of chitosan was calculated using IR results and the ratio of peak areas at wavelengths 1655cm^{-1} and 3450cm^{-1}

Preparation of chitosan solution

Chitosan solutions were made by weighing 0.5, 1.0, 1.5, and 2.0g of each chitosan and dissolving them in 100 mL of sterile water containing 0.5 mL (v/v) glacial acetic acid. An overhead stirrer was used to dissolve the mixture. Depending on the pH reading, the solution was adjusted to 5.6 by adding either 1N NaOH or 1N HCl using a digital pH meter (Madushani*et al.,* 2012).

Study Area

The research was carried out at the National Cereals Research Institute's hydromorphic field (latitude N9^{o}.04"-02.05 and longitudes E6^{o}.01"-30.31) in Badeggi, Niger State, Nigeria during the cropping season 2021.

Experimental Design

A randomized complete block design (RCBD) was used with three replicates of four different molecular weights chitosan at four different concentrations (0.5, 1.0, 1.5, and 2.0%) which was applied at 2 weeks after transplanting. FARO 52 rice variety was used for the experiment. The same plant population was used throughout the plot, with 20cm by 20cm spacing between rows and between plants. The disease incidence was scored four times using the International Rice Research Institute (IRRI) standard evaluation system (SES) at the onset of symptoms, 42, 63, and 90 days after transplanting (DAT) to monitor disease progression and chitosan efficacy (Quazi *et al.,* 2021), and data were taken accordingly

The following data were collected from the experiment; disease incidence at the appearance of symptoms, disease incidence at 42 days after transplanting, disease incidence at 63 days after transplanting, disease incidence at 90 days after transplanting and the disease Severity at the appearance of symptoms and at 90 days after transplanting

Fertilizer application rates were 80, 40, 40kg per ha of N, P_2O_5, and K_2O with NPK fertilizer applied as basal and subsequently top-dressed with N at 21 and 42 days after planting. Weeds were controlled with 4 litres per hectare of Orizo plus, with supplementary hand weeding.

After symptoms appeared, five plants from each plot were used for data collection; the plants were treated with all of the different molecular weights of the chitosan solution applied as spray until run off. For three months, the development of foliar symptoms with blast necrotic lesions was monitored at one-month intervals. Disease incidence was calculated as the percentage of plants with necrotic symptoms in each treatment divided by the total number of inoculated plants.

Other parameters taken were days to 50% flowering, panicle count, panicle length tiller count, plant height,1000 seed weight and yield per plot.

Data Analysis

The collected data were subjected to ANOVA with Statistical Tools for Agricultural Research (STAR) and mean separation with LSD at the 5% (0.05) level of probability

Results and Discussion

The Fourier Transform InfraRed (FTIR) spectrum of the Chitosan showed major absorption bands ranges from 3444.72, 2966.17, 2512.60, 2144.84, 1429.74, 1258.12, 1160.05, 1025.2, 869.92, 710.50, 608.40 to 559.36 (Figure 1).

The Degree of Deacetylation of Synthesized chitosan

The degree of Deacetylation was determined using a standard formula (Gaikwad *et al*., 2015).

DDA = $100 - \frac{A_{1429}}{A_{3444}} \times 1.15$, where the Area of peak of 1439.7=21.628T and Area of peak of 3444=34.188T

$A3444 = \frac{-\log T}{100} = -\frac{\log 21.628}{100}$ = 0.66498

$A_{1439.5} \equiv \frac{-\log T}{100} = -\frac{\log 34.188}{100}$ = 0.8080

$\therefore DDA \equiv 100 - \frac{0.8080}{0.66498} \times 1.15$ DDA= 98.6%. The degree of deactylation (DDA) of the chitosan synthesize from crab shells (CSCS) was 98.6%.

The result of the blast screening of Faro 52 rice variety with chitosan treatments (Table 1) shows a reduction in the severity and incidence of blast in the plots treated with chitosan as a foliar spray. Rice plot with blast severity score of 6- (highly susceptible) and incidence of 28.3 %, severity score of 5-(moderately susceptible and incidence of 20%, severity of 5- (Moderately susceptible) and incidence of 20%, severity score of 4-(susceptible), and incidence of 24.3% before spraying with MMWC 1.5%, HMWC 2.0%. LMWC 2.0% and CSCS 2.0% respectively had incidence reduced to 9.3%, 2.3%, 1.0% and 2.0% respectively with severity 1-(highly resistance) after (90) days of application (Table 1). After chitosan application, the incidence of the blast disease reduces as the number of days increases as shown in (Table 1). It was also observed that the molecular weight of the different chitosan affect the antifungal activity of the chitosan. Blast severity also reduced to 1(resistance) in many of the treated plots. The fungicidal activity of chitosan against rice blast pathogen could be attributed to direct antifungal activity such as the destruction of mycelium and indirectly induced resistance such as defense-related enzymes' activity in rice plants as supported by the reports of FNCA (2016).Chitosan application impairs the growth of the blast fungus *M. oryzae* and has a pronounced effect on appressorium-mediated plant infection (Lopez-Moya*et al.*, 2021)

The result of other agronomic data of the chitosan treated Faro 52 rice variety (Table 2) shows that there was no significant difference (P≤0.05) in all other agronomic parameters such as days to 50% flowering, panicle length, tiller count, plant height, and 1000 seed weight except for panicle count with the highest value (8.53) in HMWC 2.0% being significantly different (P ≤ 0.05) from the lowest value (5.80) in HMWC 0.5% concentration. Similarly, a significant difference was observed in grain yield per plot with the highest yield of 717 grams in the HMWC 2.0% treated plot while the lowest yield of 190 grams was observed in MMWC 0.5% which was significantly different (P ≤0.05). It was observed that 1.5% and 2.0% concentration of HMWC increase the grain yield of the rice varieties significantly (P≤0.05).). The result further validates the hypothesis that chitosan is known to act as an elicitor with plants showing high content of chitin enzyme having a good chance of disease resistance to the pathogen. The result is in line with the work of Boonlertnirun *et al*. (2008),

who reported that the foliar spray of chitosan decreases disease incidence but do not affect plant height, tiller per plant, panicle number, 1000 seed weight but increases the average yield per plot. Therefore, chitosan does not only affect pathogenic fungi but also exhibits growth promoting effect.

Conclusion

The efficacy of chitosan on the field shows that higher concentration of chitosan treatment reduces the severity and incidence of blast pathogen. However, the treatment did not affect other agronomic parameters, except for increase in grain yield per plot

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Table 1: Blast Severity and Incidence before and after treatment with Chitosan

Chitosan Concentration Plant Tag	BSSA	BIAS	BI@42	BI@63	BI@90	BSSAT
HMWC 0.5	2^{b}	8.0^{b}	2.0^{b}	3.3^{b}	2.0^{b}	1^{bc}
HMWC 1.0	3^{ab}	14.3^{ab}	3.3^{b}	7.0^{ab}	2.7^{b}	2^{ab}
HMWC 1.5	3^{ab}	11.7^{b}	2.0^{b}	2.7^{b}	3.3^{b}	1^{bc}
HMWC 2.0	5^{a}	20.0^{a}	9.0^{a}	2.7^{b}	2.3^{b}	1^{bc}
MMWC 0.5	2^{b}	6.7^{b}	2.0^{b}	1.7^{b}	1.0^{b}	1^{bc}
MMWC1.0	3^{ab}	8.3^{b}	5.0^{ab}	1.3^{b}	1.7^{b}	1^{bc}
MMWC1.5	6^{a}	28.3^{a}	10.3^{a}	15.0^{a}	9.3^{a}	3^{ab}
MMWC2.0	5^{a}	13.3^{ab}	5.0^{ab}	4.3^{b}	4.0^{b}	2^{ab}
LMWC 0.5	3^{ab}	8.3^{b}	4.3^{ab}	3.0^{b}	3.3^{b}	1^{bc}
LMWC 1.0	4^{a}	10.0^{b}	2.7^{b}	6.3^{ab}	2.3^{b}	1^{bc}
LMWC 1.5	3^{ab}	8.3^{b}	2.7^{b}	4.7^{b}	2.3^{b}	1^{bc}
LMWC 2.0	5^{a}	20.0^{a}	7.3^{ab}	4.0^{b}	1.0^{b}	1^{bc}
CSCS 0.5	4^{a}	16.7^{ab}	5.0^{ab}	1.3^{b}	2.7^{b}	1^{bc}
CSCS 1.0	2^{b}	9.3^{b}	3.0^{b}	3.0^{b}	3.3^{b}	1^{bc}
CSCS 1.5	3^{ab}	10.0^{b}	2.3^{b}	6.3^{ab}	4.7^{b}	2^{ab}
CSCS 2.0	4^{a}	24.3^{a}	11.0^{a}	6.3^{ab}	2.0^{b}	1^{ab}
Control	8	35.6^{a}	34.8^{a}	34.8^{a}	34.8^{a}	7^{a}

Means with the same letter along the rows are not significantly different at 0.05 percent probability level. BSSA-Blast score at the appearance of symptoms, BIAS-Blast incidence at the appearance of symptoms, BI@42- Blast incidence at 42days after transplanting, BI@63- Blast incidence at 63days after transplanting, BI@90- Blast incidence at 90days after transplanting, BSSAT- Blast severity score after Treatment, HMWC- High molecular weight chitosan, MMWC-Medium molecular weight chitosan, LWMC-Low molecular weight chitosan, CSCS-Chitosan Synthesis from Crab Shell

Table 2: Agronomic Parameters of Faro 52 Rice Varieties after Treatment with Chitosan

Chitosan Concentration Plant Tag	Days 50%FLW	Panicle Count	Panicle length (cm)	Tiller Count	Plant height (cm)	1000Seed Weight (gram)	Grain yield/plot(gram)
HMWC 0.5	76^{a}	5.80^{b}	24.9^{a}	9.6^{b}	94.9^{a}	20.4 ^{a}	363^{b}
HMWC 1.0	77.0^{a}	7.93^{a}	23.5^{a}	11.5^{ab}	91.5^{a}	19.9 ^{a}	443^{ab}
HMWC 1.5	79.7^{a}	6.60^{ab}	25.4^{a}	10.8^{ab}	88.3^{a}	21.1 ^{a}	621^{a}
HMWC 2.0	76.3^{a}	8.53^{a}	23.1^{a}	10.5^{ab}	82.3^{a}	20.4 ^{a}	717^{a}
MMWC 0.5	78^{a}	7.27^{a}	24.3^{a}	13.4^{a}	83.6^{a}	19.0 ^{a}	190^{c}
MMWC1.0	76.7^{a}	7.53^{a}	24.5^{a}	9.7^{b}	87.5^{a}	20.2 ^{a}	454^{ab}
MMWC1.5	79.7^{a}	7.93^{a}	25.1^{a}	12.3^{a}	86.7^{a}	20.7 ^{a}	471^{ab}
MMWC2.0	74.7^{a}	7.60^{a}	25.1^{a}	10.5^{ab}	94.2^{a}	20.6 ^{a}	522^{ab}
LMWC 0.5	77.7^{a}	8.33^{a}	24.2^{a}	10.4^{ab}	82.6^{a}	19.8 ^{a}	298^{b}
LMWC 1.0	76.3^{a}	7.47^{a}	24.6^{a}	15.4^{a}	85.1^{a}	20.0 ^{a}	333^{b}
LMWC 1.5	77.0^{a}	7.93^{a}	23.2^{a}	10.5^{ab}	85.5^{a}	19.0 ^{a}	432^{ab}
LMWC 2.0	75.3^{a}	6.40^{ab}	24.8^{a}	12.5^{a}	83.9^{a}	20.3 ^{a}	512^{ab}
CSCS 0.5	78^{a}	6.73^{ab}	25.6^{a}	10.8^{b}	82.5^{a}	20.4 ^{a}	344^{b}
CSCS 1.0	74.7^{a}	7.40^{a}	24.6^{a}	12.9^{a}	87.1^{a}	19.8 ^{a}	373^{b}
CSCS 1.5	75.7^{a}	7.60^{a}	23.9^{a}	11.7^{ab}	85.4^{a}	19.8 ^{a}	479^{ab}
CSCS 2.0	78.3^{a}	8.07^{a}	24.9^{a}	12.7^{a}	81.7^{a}	19.7 ^{a}	520^{ab}
Control	74.5^{a}	7.60^{a}	24.3^{a}	12.5^{a}	80.5^{a}	19.6^{a}	280^{b}

Means with the same letter along the rows are not significantly different at 0.05 percent probability level. 50%FLW- Days to 50% flowering, BSSAT- Blast severity score after Treatment, HMWC- High molecular weight chitosan, MMWC-Medium molecular weight chitosan, LWMC-Low molecular weight chitosan, CSCS-Chitosan Synthesis from Crab Shell

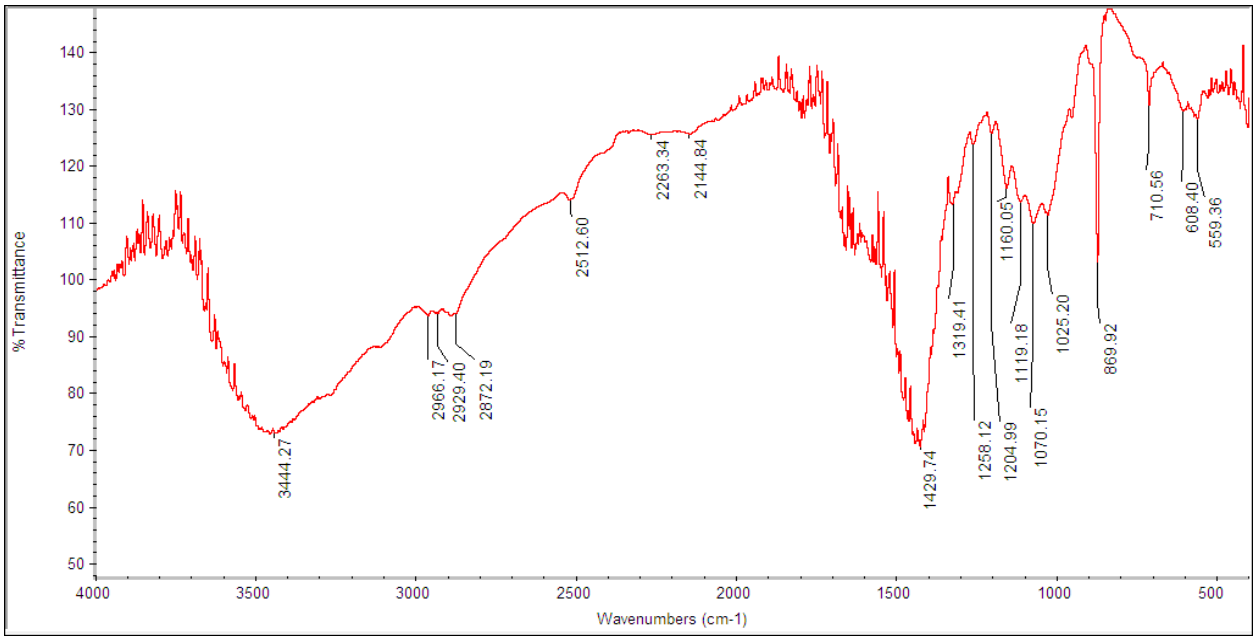

Figure 1 : Fourier Transform Infrared (FTIR) spectra graph of Synthesize Chitosan for determination of DDA