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Research Article

Effect of *Pseudomonas Fluorescens* as Biocontrol of Rice Blight Disease Cause by *Curvularia Lunata* on Rice Variety Gawal R1

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Abstract

Curvularia lunata is a soil-borne fungal pathogen that attacks rice and causes significant losses in its production, while *Pseudomonas fluorescens* is one of the common biocontrol agents used for managing foliar infection in soil born pathogen. The aim of this research was to study the effect of *Pseudomonas fluorescens* as biocontrol agent against *Curvularia lunata* on rice variety Gawal r1. The isolate were tested for pathogenicity. In a pot experiment, *P. fluorescens* significantly reduced the disease severity of rice blight caused by *C. lunata*. After application of a spore suspension of *Pseudomonas fluorescens* -biofungicide and Synthetic fungicide (Mancozeb) to rice seedlings inoculated with *C. lunata*, the disease severity was reduced by 2.00, 2.55, 3.2, and 4.66, respectively. In Screen house trial, the concentrations *Pseudomonas fluorescens* concentrations at 3.0×10^8 CFU/ml gave the best results in all plant parameters, as compared to the inoculated control with *C. lunata*.

Keywords

Biocontrol, Pseudomonas Fluorescens, Curvularia Lunata, Rice

1. Introduction

Rice has been described as the most important food [23], providing 21% of the global human per capita energy, and 15% of the per capita protein [8]. Currently, almost half of the world's population rely on rice every day, being the staple food across Asia, where about half the world's poorest people live, and it is fast becoming increasingly important in Africa and America. As of 2018, two countries, China and India, together contributed more than half of the world's total rice production [5]. The main rice-growing areas include the Volta, Northern, Upper East and Upper West regions. In total, land cultivated to rice is around 239,340 hectares. However, the country is not sufficient in rice production, as domestic production currently meets only 30-34% of consumption, with the remaining 66-70% being imported. As a result, the country Nigeria spends large amounts of foreign exchange to import rice from Thailand, Vietnam and India [5].

Rice blight is one of the problems associated with rice of many varieties in Nigeria and is caused by *Curvularia lunata*. The pathogen not only infects leaves, but also infects rice seeds [10]. Other problems for rice production are pathogen resistance to chemical fungicides and their toxic residues, which can negatively impact the environment and human health. It is challenging to find alternative methods which are safe for agricultural inputs like bio-fertilizer and

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bio-pesticides to be used instead of toxic chemicals on rice production representing of good agricultural practice (GAP) and organic agriculture [21].

The objectives of this research were to study biological control of rice blight caused by *C. lunata*, and pot experiment using cultivated rice variety Gawal r1. *Pseudomonas fluorescens* is one of the common biocontrol agents used for managing foliar infection, and is among the most potential rhizosphere bacteria, as they not only suppress disease, but also promote plant development [17]. Rice blight disease caused by *C. lunata* has led to large scale yield losses in China (50%), Japan (40%), and (India 75%), where intensive agricultural practices are being followed, while under favorable environmental conditions, blight fungus can reduce yield by up to 50% [13].

In Nigeria, rice blight is an emerging disease that can cause up to 75% yield loss [4]. In 2019, rice farmers in Gusau, Talata Marafa, Maradum and Bakura, all in Zamfara State, reported significant decline in rice yield due to an unidentified disease (rice blight), of which *C. lunata* was found to be the causative agent [3]. According to the American Plant Health Instructor [2], rice blight has never been eliminated from a region in which rice is grown, and a single change in the way in which rice is grown, or in the way resistance genes are deployed, can result in significant disease losses even after years of successful management. Millions of people in Nigeria and the world over depend on rice as a staple food, thus failure or decrease in rice production, for any reason, poses a real threat of starvation. This disease is a model that demonstrates the seriousness, elusiveness, and longevity of some plant diseases, therefore, there is need to investigate on the most important rice disease, the blight, in order to obtain base-line information on the status of rice blight disease in Nigeria with the aim of preventing its epidemic [1]. There is a need for sourcing for biocontrol agents against *Curvularia lunata* to avoid frequent spraying of chemicals, reduce high cost of production, produce safe rice grains without chemical residuals for end user, and safeguard the environment [10]. *Pseudomonas spp* are excellent candidates for use as biocontrol agents against this devastating disease (rice blight). They can achieve biological control through competition, antibiosis, and hyper parasitism [6], more so, they are natural products which are safe to the environment, have low toxicity to living organisms, and are now gaining interest as important ingredients for use in the development of fungicides, and these may hopely serve as effective substitutes for synthetic fungicides.

This aims at accessing the effect of *Pseudomonas fluorescens* as biocontrol of rice blight disease cause by *Curvularia lunata* on rice variety Gawal r1.

2. Materials and Methods

2.1. Plant Samples Collection

Rice infected with rice blight disease (identified by some of the symptoms of the disease such as minute spots on the coleoptile, leaf blade, leaf sheath, brown leaf spot and leaf scald) were collected during the rainy season from rice fields in Kura local government area, Kano state, situated within the Sudan Savanna of North-West Nigeria between longitude 11.7701 ℃N and latitude 8.439 ℃E.

2.2. Collection of Rice Variety

Seeds of GAWAL R1 rice variety were obtained from National Cereal's Research Institute (NCRI) Badeggi, Niger State, and used in all trials.

2.3. Isolation of *Curvularia Lunata* from Diseased Plant

Leaves of the infected rice plant were cleaned under running tap water and cut into 1 × 1 cm segments [10]. Surface sterilization was done by washing with 70% ethanol for 5 min, followed by five rinses in sterile distilled water. The sterilized diseased leaves were placed on tissue paper moistened for 5m. The leaves were then placed on potato dextrose agar (PDA), and the final rinsing water was spread onto the PDA medium to check the effectiveness of surface sterilization [11]. The absence of microbial growth on the PDA medium confirmed that the surface sterilization procedure was effective in removing the surface bacteria [11]. Inoculated plates were incubated at 28 ±2 ℃ for 7 days, and the fungal colony growing out from the plant tissues were transferred onto fresh PDA medium until a pure culture of the organisms was obtained.

2.3.1. Cultural Features of the Fungal Isolate

Cultural features of fungal isolate were carried out on PDA by incubated at 28±2 ℃ for 7 days. Features like colony growth rate, mycelial color, shape and colonies reverse side were observed [11].

2.3.2. Micro morphological Features of the Fungal Isolate

A plug of mycelium of isolate was placed in the middle of a PDA plate and incubated for 7 days. The nature of mycelium of isolate was recorded to aid in their identification. After that, hyphae and spores were fixed on a slide and observed under the microscope. The nature of the hyphae and spores were also recorded base on the following features such as conidium color, conidiophore shape and cell wall to further aid in identification of fungal isolate [11].

2.4. Pathogenicity Test of the Rice Pathogen

The isolate tested for pathogenicity followed Koch's Postulates. The pathogen inoculums was prepared as a spore suspension of 1 x 10^6 spores ml^{-1}. Twenty-days-old rice

seedlings planted in pots was inoculated by spraying then covered with plastic bags to maintain moisture content. The pathogen was re-isolated from symptomatic tissue, returned to pure culture and identified morphologically to confirm the species [11].

2.5. Source of PGPR (*Pseudomonas fluorescens*) Isolate

The bacterial isolate (*Pseudomonas fluorescens*) was obtained from International Institute of Tropical Africa (IITA) Ibadan, Oyo State Nigeria.

2.6. Culture Media of Antagonist

Pure cultures of *Pseudomonas fluorescens* were cultivated on nutrient agar and maintained at −4°C for further work [9].

2.7. Pot Preparation

A 25 cm-diamter pot with a depth of 22cm was used. Clay-loam soil was collected, weighed, and sterilized for 1 hr at 121 ℃ using autoclave. Each pot was sterilized using sodium hypochlorite, and 7 Kg of the soil were measured and added to each pot. The pots were then irrigated (2-3 times) before sowing to ensure proper germination of seeds. A total of 0.5 g of the seeds was used per pot during sowing, and the pots were regularly irrigated throughout the crop growth [7].

2.7.1. Treatment and Experimental Design

The treatment comprised four different concentrations (3.0×10^8, 3.0×10^7, 3.0×10^6, 3.0×10^5 CFU/ml) of *Pseudomonas fluorescens* regarded as the positive control, and distilled water as the negative control, which were used to treat three varieties of rice plant infested with a conidial suspension (1 x 10^6 spores per ml) of *Curvularia lunata* by foliar application [14]. The experimental design was conducted following a Completely Randomized Design (CRD) with a total number of 54 pots representing six treatments (including the controls), with three replications. Manual weeds control was employed in all treatments when needed. Proper water management was maintained for rice cultivation.

Inoculation of Curvularia lunata

At 14 days post-sowing, the plants were inoculated with a conidial suspension of *C. lunata* (1 x 10^6 spore/ml), and a volume of 20 ml/pot was applied until run-off using a low volume sprayer [14].

2.7.2. Application of Treatments

At 20 days post-inoculation, rice variety infected with *Curvularia lunata* were treated with *Pseudomonas fluorescens* at four concentrations of (3.0×10^8, 3.0×10^7, 3.0×10^6, 3.0×10^5 CFU/ml) at the rate of 25 ml per poi every 20 days until harvest [21]. Plant data, such as height, number of tillers and disease severity were recorded. Analysis of variance (ANOVA) was performed, and the treatment means were compared using the least significant difference (LSD) test at 5% probability level using the GenStat statistical software package.

3. Results

3.1. Cultural Morphological Features of *Curvularia Lunata* Isolate from Infected Rice Plant

The fungal colonies grew up to 7 cm (diameter) when the tissue was incubated on PDA at 25 ℃ in the dark for 7 days. The mycelia were black with velvety texture and smooth margins (Figure 1). the colonies shape ere hairy and colonies reverse side were with dark pigmentation (Table 1).

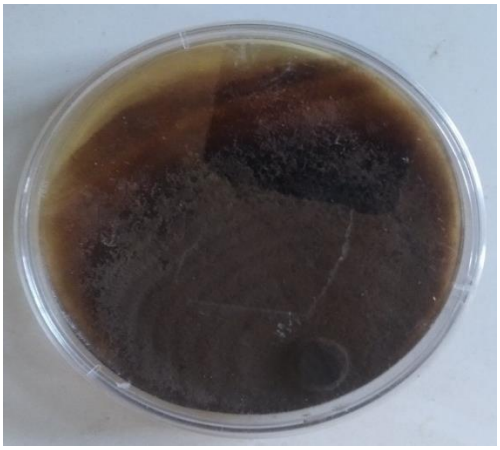

Figure 1. *Pure culture of Curvularia lunata (pigmentation on reverse plate) isolated from infected rice plant leaf.*

Table 1. *Cultural morphological feature of Curvularia lunata isolate from infected rice plant.*

Features	Cultural morphological
Mycelia color	black with velvety texture and smooth margins
Colony shape	Hairy
Colony growth	7 cm (diameter)
colonies reverse side	dark pigmentation

3.2. Micromorphological Feature of *Curvularia Lunata* Isolate from Infected Rice Plant

Hyphae were dark in color (Figure 2). Conidiophores were brown, unbranched, septate, and geniculate at the apical region. Conidia were straight to pyriform in shape, smooth-walled, transversely septate with mostly three septa. The conidia were of the dimensions 15.5 to 25.5 × 8.0 to 13.0 µm (Table 2).

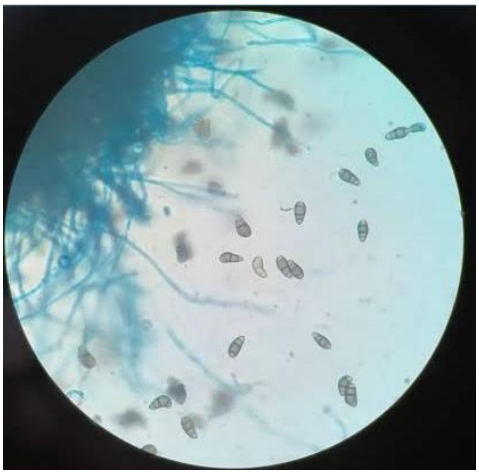

Figure 2. *Microscopic structure of Curvularia lunata (fusiform conidia with three septate) isolated from infected rice plant leaf.*

Table 2. *Micromorphological feature of Curvularia lunata isolate from infected rice plant.*

Features	Micromorphological
Colour	Conidiophores were brown
Cell wall	smooth-walled
Shape	Conidia were straight to pyriform in shape
Conidiophore	unbranched, septate, and geniculate at the apical region

3.3. Pathogenicity Test of the Rice Pathogen

C. lunata was tested for pathogenicity to 20 days old seedlings by inoculating with a spore suspension at a concentration of 1 x 10^6 spores ml^{-1}. The rice seedlings showed clear symptoms of rice blight and the pathogen was re-isolated to confirm species. The most virulent isolate was used for further experiments.

In vivo determination of antifungal activity of Pseudomonas fluorescens to Control rice blight of rice variety GAWAL R1 caused by C. lunata in a Pot Experiment at 60 days.

Effect of Pseudomonas fluorescens on Plant Height (cm)

and number of tillers of rice variety GAWAL R1 infected with Curvularia lunata

The results of the effect of *P. fluorescens* on plant height and number tillers of rice variety Gawal r1 infected with *Curvularia lunata* at 60 days post-treatment are presented in (Table 3). The result showed that all the concentrations of *Pseudomonas fluorescens* enhanced the growth of rice. Highest results, with mean values of 90.52 cm (plant height) and 12.00 (number of tillers) were obtained from 3.0 x 10^8 CFU/ml concentration of *P. fluorescens.* While, in contrast, the inoculated control with *C. lunata* gave the least results for plant height (35.06 cm) and number of tillers (7.00) at 60 days post-treatment. There were significant differences ($P \leq 0.05$) among the concentrations (as shown in Table 3).

Table 3. *Efficacy of Pseudomonas fluorescens on Plant Height and Number Tillers of Rice Variety GAWAL R1 Infected with Curvularia lunata at 60 Days Post-Treatment in a Pot Experiment.*

Treatments	Plant Height (cm) ***	Number of Tillers***
Concentrations (ml)		
Inoculated control with *C. lunata*	35.06^{d}	7.00^{b}
Spore susp. of *P. fluorescens* (3.0 x 10^8 CFU/ml)	90.52^{a}	12.00^{a}
Spore susp. of *P. fluorescens* (3.0 x 10^7 CFU/ml)	79.60^{b}	11.78^{a}
Spore susp. of *P. fluorescens* (3.0 x 10^6 CFU/ml)	74.54^{c}	9.89^{ab}
Spore susp. of *P. fluorescens* (3.0 x 10^5 CFU/ml)	73.48^{c}	10.44^{ab}
Synthetic fungicide (Mancozeb) (2g/L)	73.10^{c}	9.56^{ab}

Means with the same letter(s) in the same column are not statistically significant different at 5% probability level.
Keys; *** = significant at 1%.

3.4. Effect of *Pseudomonas Fluorescens* on Disease Severity of Rice Variety GAWAL Infected with *Curvularia Lunata*

The result of *Pseudomonas fluorescens* on disease severity was effective at different concentration against Gawal r1 variety of rice infected with *Curvularia lunata*. It is evident from (Table 4), which show that, the concentration of 3.0x10^8 Cfu/ml significantly reduced the disease severity of the pathogen with a mean value of (6.66) at 2 WAT and 4 WAT (2.00) respectively over inoculated control with *C. lunata* at 2WAT (9.00) and 4 WAT (9.00) Table 4.

Table 4. *Effect P. fluorescens on disease severity of rice varieties infested with Curvularia lunata.*

Treatments	Disease severity at 2WAT ^{NS}	Disease severity at 4WAT **
Concentrations (ml)		
Inoculated control with *C. lunata*	9.00^{a}	9.00^{a}
Spore susp. of *P. fluorescens* (3.0x10^8 Cfu/ml)	6.66^{c}	2.00^{c}
Spore susp. of *P. fluorescens* (3.0x10^7 Cfu/ml)	8.88^{a}	2.55^{c}
Spore susp. of *P. fluorescens* (3.0x10^6 Cfu/ml)	8.88^{a}	3.22^{c}
Spore susp. of *P. fluorescens* (3.0x10^5 Cfu/ml)	8.88^{b}	3.22^{c}
Synthetic fungicide (Mancozeb) (2g/L)	9.00^{a}	4.66^{b}

Means with the same letter(s) in the same column are not statistically significant different 5% probability level.
Keys; WAT=Weeks after treatment, NS=not significant. *=significant at 10%, **=significant at 5%, ***=significant at 1%.

4. Discussion

4.1. Isolation and Identification of *Curvularia Lunata*

This study showed that C. lunata is the causal agent of the rice blight disease on rice varieties. This is not the first report of any Curvularia species being associated with a plant disease in Nigeria. Several species of Curvularia have been associated with members of the grass family worldwide. This is in agreement with [10] who discovered C. lunata causing blight disease on rice in Uttar Pradesh in India.

4.2. Cultural and Micromorphological Features of *Curvularia Lunata*

Cultural and morphological study indicate that conidium of Curvularia lunata were black with velvety texture and smooth margins, grew up to 7 cm (diameter) when the tissue was incubated on PDA at 25 ℃ in the dark for 7 days. the colonies shape was hairy and colonies reverse side was with dark pigmentation. Hyphae were dark in color. Conidiophores were brown, unbranched, septate, and geniculate at the apical region. Conidia were straight to pyriform in shape, smooth-walled, transversely septate with three septate. The cultural and micromorphological features of the isolated fungus suggest it belonged to the genus Curvularia. Conidia morphology of C. lunata (Cochliobolus lunatus) is what similar to typical features of Curvularia species according to [19].

4.3. Effect of Pseudomonas Fluorescens on Plant Height of Rice Variety GAWAL R1 Infected with *Curvularia Lunata*

The effect of P. fluorescens on plant height (cm) of rice variety Gawal r1 infected with Curvularia lunata reveal that the spore susp. of P. fluorescens at the concentration of (3.0x108 Cfu/ml) give the highest result on plant height at 30 days after treatment (90.52 cm) as compared to other treatments including control. The results of the experiments on plant height are quite confirmative with the results of [16] who revealed that all the strains of P. fluorescens promoted plant growth. The increase in plant height may be due to higher water and nutrient uptake, phytohormone production i.e., indole acetic acid and siderophore production.

4.4. Effect of *Pseudomonas Fluorescens* on Number of Tillers of Rice Variety GAWAL R1 Infected with *Curvularia Lunata*

The spore. susp of *P. fluorescens* at the concentration of ($3.0x10^8$ Cfu/ml) prove to be effective on number of tillers at 60 days after treatment (12.00 tillers). This may due to the infection of pathogen and production of lesion reduced the photosynthetic area of leaves leading to less tillering nodes and different tested variety [12, 20].

4.5. Effect of *Pseudomonas Fluorescens* on Disease Severity of Rice Variety GAWAL R1 Infected with *Curvularia Lunata*

Foliar spray of *Pseudomonas fluorescens* at the concentration of $3.0x10^5$ Cfu/ml with a mean value of (6.66) at 2 week after treatment and (2.00) at 4 week after treatment proved to be effective in reducing disease severity over inoculated control with *C. lunata* with a mean value of (9.00) at 2 and 4 week after treatment. Similar result were found by [15] who study the evaluation of Plant Growth Promoting Rhizobacteria as Biocontrol Agents for the control of blast disease in rice. The results are in agreement with those of [18], who reported that minimum% disease severity was recorded in *P. fluorescens* (8%) and maximized the yield of paddy. The production of siderophore is the one that is capable to suppress the growth of the *Curvularia lunata*/soil borne disease and also capable for the antagonistic effect of *Curvularia lunata* from the findings of [22].

5. Conclusion

From the present study it is clear that *Curvularia lunata* is the causative agent of rice blight of rice disease through cultural and micro morphological identification.

The rice blight was reduced by foliar spray with a spore suspension of biofungicide (*P. fluorescens*) at the concentration of $3.0x10^8$ Cfu/ml to rice seedlings inoculated with *C. lunata* in pot experiment.

Foliar spray with *P. fluorescens* concentration the concentration of $3.0x10^8$ Cfu/ml prove to be effective on plant height and number of tillers of rice variety Gawal r1 infected with *C. lunata*.

Author Contributions

Yabagi Abdullahi Muhammad is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

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