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Research Article

# Antifungal Activity of Three Different Ethanolic Extract against Isolates from Diseased Rice Plant

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## Abstract

One of the major fungal disease of rice that farmers are facing today were rice blast and sheath blight. These diseases cause drastic decrease in the productivity and become a problem in related to the consumption. The main objective of this study is to determine the antifungal activity of Datura metel, *Jatropha carcus* and *Ruellia tuberosa* ethanolic leaf extract against the isolated pathogen causing sheath blight and rice blast disease of rice. This study employed experimental research method with completely randomized design wherein sheath blight isolates were tested into three different concentrations (25%, 50% and 100%) of the three plant extracts while rice blast isolates were tested in 100% concentration of the three plant extracts. The food poisoned technique assay was used to access the antifungal activity of different three ethanolic extract and was done with three replications. The results showed that the ethanolic leaf extract of *D. metel* and *J. carcus* has the highest antifungal activity at 100% concentration against isolated pathogen causing sheath blight having98.611 ± 1.589% and 98.588 ± 1.589 of mycelial inhibition, respectively. Whereas, *J.carcus* and *R.tuberosa* has highest antifungal property against rice blast having 97.436 ± 0.555% and 97.115 ± 0.96% respectively. The three plant extracts exhibited high percentage of mycelial inhibition compared to mancozeb. Therefore, the extracts from these three plants have an active potential to inhibit the growth of fungus and can be used as bio fungicide to control infection of rice blast and sheath blight in rice. Since these bio fungicides came from plants, the negative effect for the environment and other organisms will be inhibited and can also support the goal of the government in finding on how to delimit the use of chemical fungicides. To assert the effectiveness on the actual field management of plant health, *in vivo* trials are recommended.

**Keywords:** Rice blast; Sheath blight; Mancozeb WP 80%; Poisoned Food Technique; Iodoform Test

**Abbreviations:** IRRI: International Rice Research Institute; SAARC: South Asian Association for Regional Cooperation; NSS: Normal Saline Solution; P.S.I: Pound per square inch; PFT: Poisoned Food Technique; PDA: Potato Dextrose Agar; ANOVA: Analysis of Variance; LSD: Least Significant Difference; DMRT: Duncan's Multiple Range Test; NaOH : Sodium Hydroxide; SPSS: Statistical Package for Social Science

## 1. Introduction

Rice (*Oryza sativa*) is one of the major cereal crops worldwide and it is the main food source for more than half of the human population around the world and 68% of the Asian country's population like India, Nepal, Pakistan, China, Bangladesh, Philippines, Thailand and Malaysia [1, 2]. It is cultivated in around 114 countries all over the world, whereas more than 90% of crop production has been occurring in Asian countries. In Philippines, most of the Filipinos are consuming rice as their daily major source of food [3, 4]. According to Ricepedia the online authority on rice [5], harvested rice area of the Philippines is still very small compared with major crop producing countries of Asia in which it has imported about 10% of its annual consumption requirement.

Nowadays, rice demand is increasing day by day, gradually because of population overgrowth worldwide and It is predicted that by 2050 the general agricultural production should increase by 60% to cover the food requirements. In particular, the rice demand of Asia is expected to be 70% higher within 30 years [1]. In other hand farmers are facing challenges to meet the demand of rice because of reducing fertile agricultural lands due to urbanization and to high prevalence of fungal rice diseases like rice blast and sheath blight [6, 7]. The rice yield was reduced by 50-60% due to a fungal disease that has been reported in recent years, which became a major problem of all farmers all over the world. Among all of the fungal diseases, rice blast and sheath blight are known to have a high prevalence in the condition in Asian countries especially in Philippines. Rice blast is caused by *Pycularia grisea* which gives a major destruction of rice yield up to 100% on the basis of severity under favorable condition [3, 8, 9]. Malicdem and Fernandez, [10] reported that between 50-85% yield losses has been seen in Philippines and this disease will attack the different parts of the plant such as collar that results death to the entire leaf blade, it also attacks the stem which turns blackish and break easily. Another major disease that farmers facing is sheath blight that is caused by *Rhizoctonia solani* which caused around 25-50% yield losses. The suitable environment for this disease to grow is high temperature between 28-32°C, high level of nitrogen, and relative humidity. Main symptoms of this disease are to have oval or ellipsoidal greenish gray lesion about 1-3cm long on the leaf sheath [11]. In Philippines, farmers are still facing challenges to reduce the effect of fungal diseases over rice crop because there aren't any rice varieties that produce total resistance to all these diseases. In the other hand, the causative agents of all these diseases are rapidly developing resistance to currently available synthetic fungicides in the market. These synthetic fungicides are non-biodegradable and can accumulate in soil, water and plant that are toxic and has undesirable effects on other organism that is present in the environment and which may affect the food chain. The development of resistance toward the currently available synthetic fungicides has a greater concern for the food and drug activity. Due to this reason, the Department of Agriculture promotes the use of alternative products such as natural products as bio

fungicides which are cheap, locally available, non-toxic and easily degradable. Recently, many researchers have shown interest in the application of plant products as bio fungicides to reduce fungal diseases as an alternative to synthetic fungicides. Bio fungicides are less toxic and they will not pose any effect on other organisms present in the environment. Unlike the chemical fungicides, bio fungicides give better protection to the crop, soil and everything present in the environment. Bio fungicides will also reduce the risk of developing the pathogen resistance. The regular use of synthetic fungicides has made resistance to the fungus. Hence it is necessary to search new antifungal compound as an alternative, safe, ecofriendly, cheap and easily degradable fungicides from plants [12-15].

The present study proposed the use of plant extracts namely *Datura metel*, *Ruellia tuberosa* and *Jatropha carcus* against the rice pathogen. *D. metel* commonly called "angels trumpet" is one of the poisonous plants here in the Philippines but proved its medicinal properties that the leaves, stem and flowers has antimycotic, antiasthmatic, antibacterial, antioxidant, antiseptic, antihyperglycemic and it is also used as a sedative and for increase appetite [16, 17]. *Ruellia tuberosa* or known as "cracker plant" has no medicial value here in the Philippines but it is widely used as traditional medicine in some countries like Sri Lanka, Suriname and Dominican Republic. The leaves of this plant is used to treat gonorrhea, stomach problems, ear problems, for scorpion bites and kidney stone disorder. Different activities of this plant were also reported to have antioxidant, antibacterial, anticancer, gastro protective and anti-inflammatory [18, 19]. Lastly, *J. carcus* commonly known as physic nut and has a major role in the treatment of various diseases including bacterial, fungal infection. Traditionally *J. carcus* leaf decoction is used for the treatment of skin diseases, stomach disorder, anti-cough and as a disinfectant after birth [20]. The ethanolic extracts of *D. metel*, *R. tuberosa* and *J. carcus* have a common chemical constituent like alkaloids, flavonoids, saponins, steroids, lignins, and flavonoids that belong to the largest group of secondary metabolites that acts as defensive compound against fungi and have a capability to inhibit the growth of pathogen through several mechanism of action involving cross linking of microbial enzyme, inhibition of pathogen cellulose, xylenes and pectinases, chelation of metal ions relevant for enzymatic activities and disrupt the cell wall [15, 21, 22]. The aim of this study is to evaluate the antifungal activity of the three ethanolic extracts of *D. metel*, *R. tuberosa* and *J. carcus* against isolates from diseased rice plant and this is the first time that these plants were evaluated for its potential to inhibit the growth of pathogenic fungi found in rice plants. However, the researchers did not perform phytochemical screening for the three plant samples and specific species of rice plant and the pathogenic organisms responsible for the disease of rice plant under study. Previous researches have shown the antifungal activities of these three plant extracts against clinically tested pathogens. If these plants proven effective, it may have a wide distribution in agricultural industries.

## 2. Materials and Methods

### 2.1 Research design

This study employs experimental design using a completely randomized design. Sheath blight was tested into three different concentrations (25%, 50% and 100%) of the three plant extracts and rice blast was tested in 100% concentration of the three plant extracts. The set-up was done in three replications.

Ethanol solvent was used in preparing all the plant extracts. The antifungal activity was assessed using a poisoned food technique and evaluated the results by the percentage mycelial inhibition. Thirty-three standard sized petri dishes were used in the experiment. In this study, three different ethanolic plant extracts, isolates from diseased rice plant like Rice blast and Sheath blight and controls (Mancozeb Wp 80% and NSS) were independent variable, whereas the percentage of mycelial inhibition of isolates from diseased rice plant were dependent variable. The experimentation was conducted at Medicine Laboratory of Lyceum Northwestern University, Dagupan city, Pangasinan.

### 2.2 Instrumentation and data collection

**2.2.1 Reagent and glassware:** Glassware like Erlenmeyer flask, graduated cylinders, stirring rods, beakers, test tubes, petri dishes, inoculating loops, rotating evaporator, autoclave, Vernier caliper was borrowed from LNU Medicine laboratory and potato dextrose agar, ethanol, normal saline solution, Mancozeb and disinfectant solution was bought from Agricultural shop and Mercury drug center at Dagupan City.

**2.2.2 Sterilization of Instruments:** At first, all instruments which were used in laboratory were made sterile, all glassware's that were used in the assay were placed in an autoclave at 121°C under 15 psi pressure for 25 min by using Autoclave and followed aseptic technique method.

**2.2.3 Collection of extract plant and diseased rice plant:** *D.metel* was picked up along the roads of Baguio City while *R.tuberosa* and *J. curcas* was picked up from Agoo, La Union. Afterwards, it was given for plant species authentication at the City Agriculture Office, Dagupan City (Plate 1) whereas diseased rice plant collected from the rice field in San Fabian, Pangasinan having the symptoms of fungal diseases such as minute spots on the coleoptile, leaf blade, leaf sheath and glume, on leaves typical spots are brown in color with grey or whitish center, cylindrical or oval in shape resembling sesame seeds usually with yellow halo while young spots are small, circular and may appear as dark brown or purplish brown spots [23], and placed it in a clean Ziploc. Then the sample was sent to office of municipal agriculturist, Sta. Barbara where three diseases namely sheath blight, rice blast and brown spot was identified on basis of their sign and symptoms by Agriculturist expert but only two diseases namely sheath blight and rice blast was tested.


**Plate 1:** Collected Plant Leaves a) *D.metel* b) *R.tuberosa* c) *J.carcus*.

**2.2.4 Preparation of Potato Dextrose Agar (PDA):** Potato Dextrose Agar media was prepared by researchers for growing of fungi inside the laboratory. The researchers used standard size (100mm× 15mm) petri dishes as required for whole experiment. For preparation of PDA, 39-gram PDA powder was mixed with 1000 ml of distilled water and stirred to obtain homogenized mixture. After which, PDA mixture was placed in Autoclave under 15 psi pressure, at 121°C for 25 min for sterilization of media. After that Researchers poured the culture media into petri dishes at ratio of 20 ml/dish and was left half covered on the table to let the agar cool down and solidify at room temperature [24].

**2.2.5 Isolation of Fungi from Diseased Rice Plant:** Isolation process carried out according to Seint San Aye et.al [25] with modification. The causative agent isolated from the sample of infected rice plant by sheath blight and rice blast, which was collected from rice field in San Fabian, Pangasinan, Philippines. The leaves were cut into small pieces (1 × 1 cm) by using sterile scissors, small pieces surface was sterilized by using 2% sodium hypochlorite for 1 minute to remove the dirt attached in it then washed with distilled water three times, then blotted with clean paper towel to dry. After drying, the plant samples were placed on Potato Dextrose media and incubated it at 25°C for 2-3 days to be able the hyphae to grow (plate 2). After growth of hyphae, petri dishes were preserved at 4°C in refrigerator until it was used.

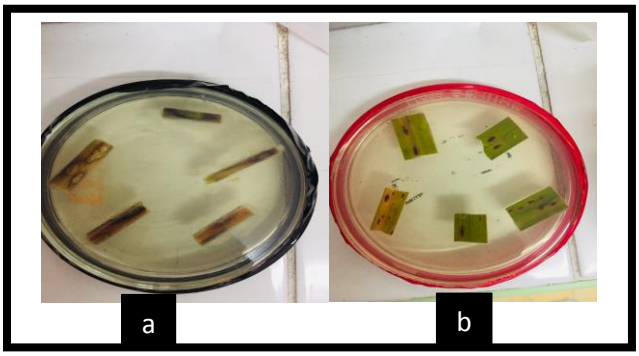


**Plate 2:** a) inoculated sheath blight Petri dish b) inoculated rice blast Petri dish.

**2.2.6 Preparation of Plant Extracts:** Plant extraction process was done accordingly to Abdulaziz et al [26] with slightly modified. The collected approximately 2 kg of each plant materials namely *D. metel, R. tuberosa and J.carcus* were washed with distilled water and then air dried separately, afterwards grounded into powder by using mechanical blender. Three hundred fifty (350) gm of each plant materials were soaked in 1000 ml of 95% ethanol and stored in dark glass bottle for 72 hours. The extract was filtered through Whatman filter paper no.4. Then extraction process was carried out by using rotatory evaporator to get final pure extracts at the pharmacy lab of Virgen Milagrosa University Foundation (VMUF) at San Carlos, Pangasinan. After getting pure extracts of plants it was stored in refrigerator at 5°C until needed.

**2.2.7 Iodoform test:** Iodoform test was followed according to Clark J. [27] methods with slight modification. A 10 drops of 1M NaOH and 25 drops of 0.5M iodine solution was added to 10 drops of plant crude extracts namely *D.metel*, *R.tuberosa* and *J.carcus*. This test was conducted to test the presence of ethanol in the extracts. A visible yellow precipitate indicates the presence of ethanol in the extracts sample.

**2.2.8 Preparation of Mancozeb:** The researchers prepared 500ppm concentration of Mancozeb wherein 625 mg of Mancozeb WP 80% was dissolved in 1000 mL of sterile distilled water, then stir gently until it was homogenized the container was covered with aluminum foil until used.

**2.2.9 Poisoned Food Technique Assay:** Antifungal assay using poisoned food technique was followed according to Shrestha and Tiwari [28], Zaker, et al. [29], and Mohammad, et al. [30] with slight modification. 2mL of each plant concentration were poured in sterilized petri plates followed by the addition of 20mL of sterilized melted PDA and agitated gently in circular motion to become homogenized. The same procedure was done with positive (Mancozeb 80%) and negative (NSS) controls. All of the petri dishes were allowed to solidify. Afterwards, from the advancing hyphae of 7-day old culture, a 5mm diameter mycelial disc were made using sterile 5 mm diameter cork borer. Each mycelial disc was placed aseptically at the center of each petri plates with treatments. The inoculated petri dishes were sealed using tape and incubated at room temperature for 7 days. Each treatment was replicated thrice. The growth of mycelium was measured using Vernier caliper.

**2.2.10 Tools for Data Analysis:** Percentage mycelial inhibition was calculated by using the formula:

$$\%\, inhibition = \frac{C - T}{C} x100$$

Where C was the growth of mycelium in control set subtracting the diameter of the inoculum disc and T was the growth of mycelium in treatment set subtracting the diameter of the inoculum disc.

To determine the significant differences among the percentage mycelial inhibition of the plant extracts and the control against the isolated pathogen causing rice blast and sheath blight, one way and Two-way Analysis of Variance (ANOVA) were used respectively. Least Significant Difference (LSD) was used for post hoc analysis to determine which of the treatments were significantly different against isolated pathogen causing rice blast and Duncan's Multiple Range Test (DMRT) was used for post hoc test to determine which of the treatments was significantly different against isolated pathogen causing sheath blight. All of the statistical analysis was evaluated using SPSS software.

## 3. Result and Discussion

Among all the different treatments used, results showed that *D. metel* and *J. carcus* at 100% concentration exhibited the highest response of percentage mycelial inhibition of 98.611 ± 1.589% and 98.588 ± 1.589%, respectively. Followed by Mancozeb 500 ppm which is the positive control having 86.111% of mycelial inhibition. The least effective treatment was *R. tuberosa* at 25% concentration having 7.407 ± 1.589% of mycelial inhibition (Table 1-5). The presence of the secondary metabolites might have a great contribution on the mycelial inhibition of the isolated pathogen causing sheath blight such as alkaloids, flavonoids, phenolic compounds and glycosides. *R. tuberosa* exhibited low mycelial inhibition maybe because of the structure of its secondary metabolites. Zaker M [15] Merziak, et al. [22] cited that the effectivity of flavonoids against pathogens depends on its structure whether the hydroxy or methyl groups are substituted or unsubstituted. The ability of these flavonoids as well as phenolic compounds inhibit the growth of fungal pathogens is cross-linking of microbial enzymes, inhibition of pathogen to cellular activities such as tightening of cell wall leading to formation of protective barrier to plants. Some secondary metabolites are not yet known for its mechanism in plant resistance, but it is still considered as to develop resistant in living organism against pathogen, it maybe has some similarities to plants in inhibiting pathogenic organisms. Alkaloids have the ability to change the permeability of the cell membrane and impair mitochondrial function. Saponins may act as a secondary immune system that will act as detoxifying the plants immune system [31, 32].

| **Treatment** | **Percentage Mean Mycelial Inhibition (%)** | | |
|---|---|---|---|
| | **Concentrations (%)** | | |
| | **25** | **50** | **100** |
| *D. metel* | 43.056$^{d}$ | 49.537$^{d}$ | 98.611$^{a}$ |
| *R. tuberosa* | 7.407$^{g}$ | 15.278$^{f}$ | 74.537$^{c}$ |
| *J. carcus* | 35.185$^{e}$ | 84.722$^{b}$ | 98.588$^{a}$ |
| Mancozeb | - | 86.111$^{b}$ | - |
| Pr > F(Model) | | | < 0.0001 |
| Significant | | | Yes |
| Pr > F(Treatment*Concentration) Significant | | | < 0.0001 Yes |

Means followed by the same letter is not significantly different at $p<0.05$, when analyzed using Duncan's Multiple Range Test of Two-Way ANOVA, Pr>F = significance probability associated with F statistic

**Table 1:** Response of the Different Plant Extracts in Different Concentrations and the Controls Used Against the Isolated Pathogen Causing Sheath Blight Disease of Rice.

| **Treatment** | **Mean Average mycelial growth(mm)** | | |
|---|---|---|---|
| | **Concentration** | | |
| | 25% | 50% | 100% |
| *D.metel* | 20.31 | 18.00 | 0.50 |
| *R.tuberosa* | 33.02 | 30.22 | 9.08 |
| *J.carcus* | 23.12 | 5.45 | 0.50 |
| Mancozeb | - | 4.95 | - |
| NSS | - | - | 35.67 |

**Table 2:** Average mycelial growth of the isolated pathogen causing sheath blight disease.

| Treatments | Concentration | Mean Average growth of Mycelium(mm) | % inhibition of Mycelium |
|---|---|---|---|
| *D.metel* | 100% | 0.50 | 98.60 |
| *J.carcus* | 100% | 0.50 | 98.59 |
| Mancozeb | 500ppm | 4.95 | 86.12 |
| *J.carcus* | 50% | 5.45 | 84.72 |
| *R.tuberosa* | 100% | 9.08 | 74.54 |
| *D.metel* | 50% | 18.00 | 49.54 |
| *D.metel* | 25% | 20.31 | 43.06 |
| *J.carcus* | 25% | 23.12 | 35.18 |
| R.tuberosa | 50% | 30.22 | 15.28 |
| R.tuberosa | 25% | 33.02 | 7.43 |
| NSS | - | 35.67 | 0 |

*% of inhibition of mycelium growth = C-T/C*100; C=NSS

**Table 3:** Percentage mycelial inhibition for sheath blight.

| Source | DF | Sum of squares | Mean squares | F | Pr > F |
|---|---|---|---|---|---|
| Model | 9 | 28090.072 | 3121.119 | 412.21 | < 0.0001 |
| Error | 17 | 128.720 | 7.572 | - | - |
| Corrected Total | 26 | 28218.792 | - | - | - |

Computed against model Y=Mean(Y)

**Table 4:** Summary of Two-Way Analysis of Variance.

| Source | DF | Sum of squares | Mean squares | F | Pr > F |
|---|---|---|---|---|---|
| Treatment | 2 | 7160.96 | 3580.48 | 472.873 | < 0.0001 |
| Concentration | 2 | 14519.2 | 7259.62 | 958.776 | < 0.0001 |
| Treatment*Concentration | 4 | 2079 | 519.751 | 68.643 | < 0.0001 |

Based on the Type III sum of squares, the following variables bring significant information to explain the variability of the dependent variable Response: Treatment, Concentration, Treatment*Concentration.

**Table 5:** Type III Sum of Squares analysis (Response).

Among the explanatory variables, based on the Type III sum of squares, variable Concentration is the most influential. Mean percentage inhibition of the different plant extracts with different concentrations shows significantly different with Mancozeb ($F$=412.206, $P_{26}$ = 0.001) against the isolated pathogen causing sheath blight disease of rice. It means that the mean percentage mycelial inhibition was dependent by the treatment and the concentrations of the extracts. To determine which among the treatments are significantly different, DMRT were employed. Based on DMRT results, the three treatments are significantly different among themselves ($p<0.05$). *D. metel* and *J. carcus* at 100% concentrations have the mean percentage inhibition of 98.611 ± 1.589% and 98.588 ± 1.589%, respectively, were significantly different as compared to Mancozeb having 86.111 ± 0.001% mycelial inhibition. Whereas, *J. carcus* at 50% has 84.722 ± 1.589% mycelial inhibition was not significantly different as

compared with Mancozeb. The least effective inhibitory activity was *R. tuberosa* at 25% concentration having 7.407 ± 1.589% mycelial inhibition was significantly different in all of the treatments. It was clearly seen that in 100% concentration of *D. metel* and *J. carcus* inhibits the growth of pathogenic fungi responsible for sheath blight disease compared to Mancozeb 500 ppm (Figure 1). Whereas, 25% and 50% of *R. tuberosa* extracts has almost similar inhibition compared to NSS.

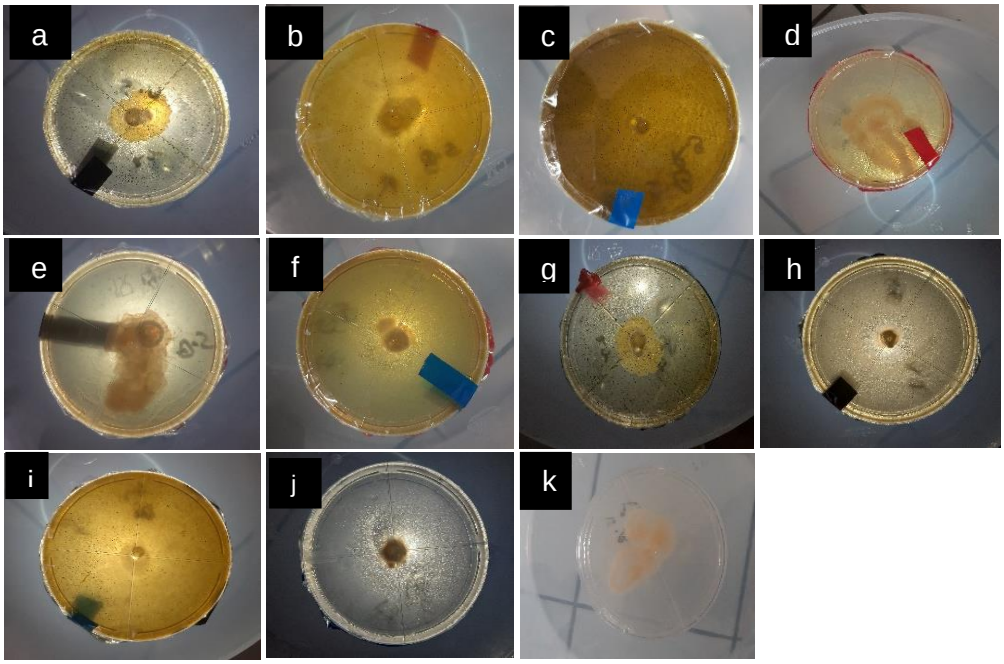


**Figure 1:** Inhibition of mycelial growth results for the three ethanolic leaf extracts at different concentrations and the controls against sheath blight. a) *D. metel* at 25% b) *D. metel* at 50% c) *D. metel* at 100% d) *R. tuberosa* at 25% e) *R. tuberosa* at 50% f) *R. tuberosa* at 100% g) *J.carcus* at 25% h) *J.carcus* at 50% i) *J.carcus* at 100% j) Mancozeb 500ppm k) NSS

Similar study made by Dasgupta et al. [33], Shamsi and Chawdhary [34], Shehab et al. [35] and Bashir et al. [36] wherein they evaluated antifungal efficacy of *D.metel* and *J.carcus* have proven that the *D. metel* and *J. carcus* have strong potential to inhibit the plant fungal pathogens. The higher the concentration of the extract, the higher percentage of mycelial inhibition. The results of this study were similar to the results of Hussein, et al. [37] and Jagessar, et al. [38] where ethanolic extracts of the different extracts showed good inhibitory effect for mycelial growth of *R. solani,* a pathogen responsible for sheath blight disease. One of the similar studies was made by Bhawana Sharma et al. [39] in which one genus of *D. metel* was tested for antifungal activity for *R. solani*, *D. stramonium* that showed the maximum inhibition percentage for *Rhizoctonia solani*, was 88%, where as another study done by Srinivas et al. [40] to determine the efficacy of Mancozeb against *R.solani* where result showed 0.1% Concentration Mancozeb was 100% mycelial inhibition using Food Poisoned Assay whereas in this study Mancozeb 500 ppm was showed only 86.111% against isolated pathogen causing sheath blight, It seems that dose dependent

mode of action of Mancozeb might be responsible for such type of result against sheath blight in this study. So, it means efficacy of Mancozeb as fungicide against sheath blight also depend upon concentration.

*D. metel* and *J. carcus* showed highly significant effect against the pathogen causing sheath blight this may be due to the presence of the secondary metabolites present in the two extracts. The secondary metabolites found in these plant extracts were alkaloids, flavonoids, glycosides, tannins, saponins and phenolic compounds [20, 35, 41-44]. The presence of these compounds has the major role in inhibiting the growth of pathogenic fungi by loss membrane integrity, interruption to cellular respiration and inactivation of pathogenic adhesion [15, 22, 31]. In this study result showed that *D. metel* and *J. carcus* have high percentage of mycelial growth than *R. tuberosa* in respective concentration against the isolated pathogen that causes sheath blight disease. This may be because of low concentration as well as the structure of the secondary metabolites present in *R. tuberosa* extract at 25% concentration [15, 22]. Another fungal disease that our farmers are facing today was rice blast. The responsible organism was *Pycularia grisea* synonymous to *Magnaporthe oryzae.*

| **Treatments** | **Mean Mycelial Inhibition (%)** |
|---|---|
| *J. carcus* | 97.436 |
| *R. tuberosa* | 97.115 |
| *D.metel* | 89.744 |
| Mancozeb | 65.705 |

**Table 6:** Response of the Different Plant Extracts in Different Concentrations and the Controls Used Against the Isolated Pathogen Causing Rice Blast Disease of Rice.

| | ***D.metel*** | ***R. tuberosa*** | ***J.carcus*** | **Mancozeb** | **NSS** |
|---|---|---|---|---|---|
| | 91.34615385 | 96.153846 | 97.11538462 | 67.30769231 | -10.5769230 |
| | 89.42307692 | 97.115385 | 98.07692308 | 67.30769231 | -8.65384615 |
| | 88.46153846 | 98.076923 | 97.11538462 | 62.5 | -1.92307692 |
| **Replication Count** | 3 | 3 | 3 | 3 | 3 |
| **Average** | 89.74358974 | 97.115385 | 97.43589744 | 65.70512821 | 0 |
| **Std. Dev.** | 1.469 | 0.962 | 0.555 | 2.776 | 9.759 |
| **Variance** | 2.157 | 0.925 | 0.308 | 7.705 | 95.229 |
| **Alpha** | 0.05 | | | | |

**Table 7:** Summary for Percentage Mycelial Inhibition for Rice Blast.

| ***Source of Variation*** | ***SS*** | ***df*** | ***MS*** | ***F*** | ***P-value*** | ***F crit*** |
|---|---|---|---|---|---|---|
| Between Groups | 20388.68 | 4 | 5097.171 | 239.7 | 6.97E-10 | 3.47805 |
| Within Groups | 212.6479 | 10 | 21.26479 | | | |
| Total | 20601.33 | 14 | | | | |

**Table 8:** Summary of One-Way ANOVA for Rice Blast.

| Family Conf. Int.=75.51%, Individual Conf. Int.=95% | | | | | |
|---|---|---|---|---|---|
| **Comparisons** | **Diff. in Means** | **LSD** | **LCon** | **UCon** | **Sig Diff.?** |
| *D.metel* - *R. tuberosa* | -7.37179 | 8.389 | -15.761 | 1.018 | No |
| *D.metel* – *J.carcus* | -7.69231 | 8.389 | -16.082 | 0.697 | No |
| *D.metel* – Mancozeb | 24.03846 | 8.389 | 15.649 | 32.428 | Yes |
| *D.metel* – NSS | 89.74359 | 8.389 | 81.354 | 98.133 | Yes |
| *R. tuberosa* – *J.carcus* | -0.32051 | 8.389 | -8.710 | 8.069 | No |
| *R. tuberosa* – Mancozeb | 31.41026 | 8.389 | 23.021 | 39.800 | Yes |
| *R. tuberosa* – NSS | 97.11538 | 8.389 | 88.726 | 105.505 | Yes |
| *J.carcus* – Mancozeb | 31.73077 | 8.389 | 23.341 | 40.120 | Yes |
| *J.carcus- NSS* | 97.4359 | 8.389 | 89.047 | 105.825 | Yes |
| Mancozeb – NSS | 65.70513 | 8.389 | 57.316 | 74.094 | Yes |
| There is evidence that some pairs of means are different. | | | | | |

**Table 9:** Fisher Least Significant Difference (LSD) Method for Rice Blast.

The ethanolic leaf extracts of *J. carcus*, *R. tuberosa* and *D. metel* showed a maximum inhibitory effect having 97.44 ± 0.56%, 97.12 ± 0.96% and 89.74 ± 1.47%, respectively, compared to Mancozeb that has 65.71% ± 2.78 of mycelial inhibition against the isolated pathogen that causes rice blast disease (Table 6-9). The results of this study may be due to the secondary metabolites present, wherein saponin was lacking on the two plant extracts. Saponins were ineffective against the isolated pathogen causing rice blast since it was a filamentous oomycete due to lack of hydroxyl sterols in their cell membranes [45]. The presence of alkaloids, flavonoids, coumarins and sterols in *J. carcus* and *R. tuberosa* played an important role to have the maximum inhibitory of mycelial growth these four secondary metabolites inhibit both mycelial growth by cell wall disruption and interference of cellular respiration and cell division [15, 22].

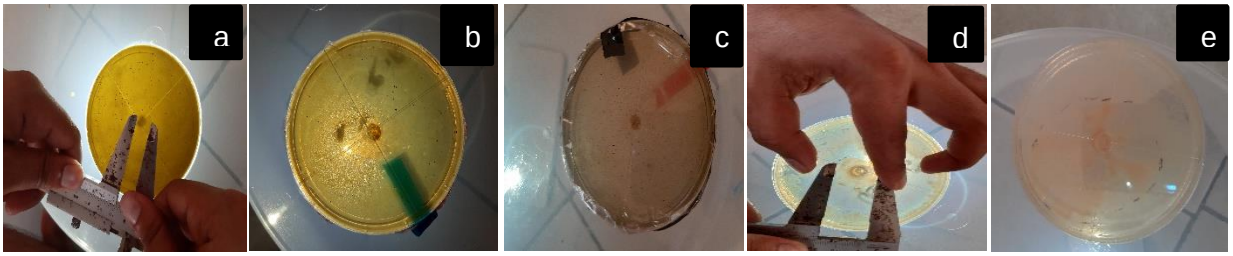


**Figure 2:** Inhibition of mycelium results in different ethanolic leaf extracts and the controls against rice blast. a) *D. metel* b) *R. tuberosa* c) *J.carcus* d) Mancozeb e) NSS.

In this study, three ethanolic extracts at 100% concentration was tested and based on the results, the three plant extracts have high mycelial inhibition compared to the controls (Figure 2). M.N.A. Uda et al. [46], Amadioha, Anderson [47] and Chee, et al. [48] proved the effectivity of ethanolic plant extracts against the responsible pathogen that causes rice blast disease of rice and found out the high inhibition of mycelial growth of all the plant extracts. These were similar to the results of this study, wherein the three ethanolic leaf extracts of *D. metel*, *R. tuberosa* and *J. carcus* showed significantly high difference compared to Mancozeb may be due to low concentration of Mancozeb 500 ppm against isolated pathogen causing rice blast. The previous studies performed to evaluate antifungal efficacy of Mancozeb showed the lower concentration; less percentage of mycelial inhibition and higher concentration; maximum percentage of mycelial inhibition, the studies showed that more than 1000ppm of Mancozeb exhibited 100% mycelial inhibition [49]. In this result, the efficacy of Mancozeb against isolated pathogen causing rice blast showed less effective than previous study result reflected that might be due to its low concentration means the Mancozeb efficacy dependent upon its concentration.

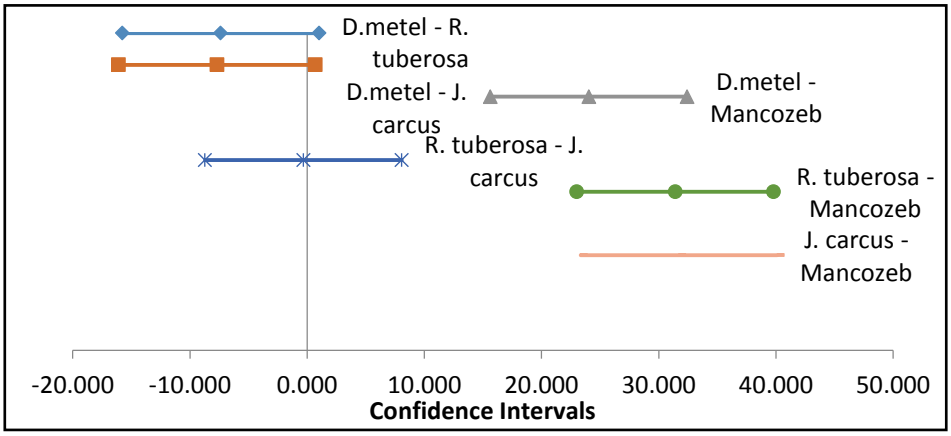


**Figure 3:** Fisher's LSD Method Confidence Interval.

The mean mycelial inhibition of the three plant extracts showed significantly different with Mancozeb (F = 239.7, $P_{14, 0.05}$ = 0.001) against the isolated pathogen causing rice blast disease of rice. To determine which of the following treatments are significantly different, LSD was conducted to evaluate the results. Based on the results, *J. carcus* and *R. tuberosa* were highly significant having difference in means of 31.73 and 31.41, respectively, as compared to Mancozeb followed by *D. metel* has the difference in means of 24.04, the three ethanolic leaf extracts have no significant difference between each other (Figure 3). The antifungal activities of the tested plant extracts maybe due to the presence of the secondary metabolites such as alkaloids, flavonoids, glycosides, and steroids which are proven by some researchers that the tested plant exhibited these compounds [20, 35, 43-44, 50-52]. The mechanism of these compounds to inhibit the growth of the pathogen causing rice blast was cross linking of microbial enzyme, inhibition of pathogen cellulose, xylenes and pectinases, chelation of metal ions relevant for enzymatic activities and disrupt the cell wall [15, 21, 22].

The result of this study was exhibited that Mancozeb is more effective against sheath blight than rice blast may be due to pathogenicity of both fungi are different causing disease in rice [53]. Most of the previous studies made a combination treatment of mancozeb and other chemical fungicides against sheath blight and rice blast [40, 49, 54]. Thus, chemical constituents present in Mancozeb has more potential in inhibiting the mycelial growth of sheath blight than rice blast because of the different morphology of the causal organism to rice diseases. Farmers can use all these extract as bio fungicide to control the effect of sheath blight and rice blast over their crops as alternative of synthetic fungicide which is biodegradable, cheaper and less harmful as well as agricultural industries can produce fungicide by using active secondary metabolites of the plant to maintain the quality and quantity of the crop.

## 4. Conclusion

Biological control has attained importance in modern agriculture to reduce the hazards use of chemical from disease control. Based on the above finding's researchers concluded that these three different plant extracts have an antifungal effect against rice blast and sheath blight due to presence of active secondary metabolites like flavonoids, alkaloids, saponins and tannins, Due to presence of these active secondary metabolites in three different plant extracts which exhibited the maximum fungal growth. The concentration of the different plant extracts is directly proportional to the percentage of mycelial inhibition and therefore, 100% concentration of three different plants extracts ha shown great results for inhibition of mycelial growth compared to Mancozeb. Therefore, the extracts from these three plants have an active potential to inhibit the growth of fungus and can be used as bio fungicides to control infection of rice blast and sheath blight in rice. Researcher recommended to further researcher to conduct future researcher to conduct studies and series of experiment using different assay such as MIC, plate diffusion method and Agar well diffusion methods. Conduct *in vivo* trials to assert the effectiveness on the actual field management of plant health.

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