

EVALUATION OF RICE (*Oryza sativa*, L.) BLAST DISEASE AND THEIR CONTROL WITH INDIA HELIOTROPE (*Heliotropium indicum*) IN ANINRI LOCAL GOVERNMENT, ENUGU STATE, NIGERIA

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ABSTRACT

Rice blast has been one of the major threats to rice production in Nigeria. It is one of the main diseases affecting the yield of rice in Enugu, Nigeria. This study focused on the use of *Heliotropium indicum* for controlling rice blast disease. The diseased rice plants were collected from five fields in sample bags and taken to Central Laboratory, National Roots Crop Research Institute, Umudike, Abia State, Nigeria for isolation, and their inhibition with aqueous and ethanol extract of *H. indicum*. Data were collected on blast symptoms, percentage of occurrence, and inhibition of the isolated fungi with *H. indicum* and phytochemical screening of the extract. The data collected were subjected to analysis of variance (ANOVA). The results revealed that leaf lesion, leaf blast, sheath rot, seed discoloration and malformed seed were symptoms observed on the rice fields. The fungi species isolated from the diseased rice were *Fusarium morniliforme*, *Pyricularia oryzae*, *Curvularia lunata* and *Rhizoctonia solani*. The percentage of occurrence ranged from *R. solani* (9.52) to *F. morniliforme* (39.10). *F. morniliforme* was more prevalent in all the fields. Both aqueous and ethanol extract of *H. indicum* significantly inhibit the mycelia growth of the four isolates. As the concentration of the extract increases, the mycelia inhibition also increases. However, ethanol extract significantly inhibit the mycelia growth of the fungal isolates than the

aqueous extract. Also, phytochemical screening conducted on *H. indicum* reveals that Alkanoids, Flavinoid, Tannin, Saponin, Phenol, Steroids and Cyanogenic glucosides were present in *H. indicum*. Tannin (11.52) had the highest content while Steroid (1.09) had the least. However, it is suggested that further study should be focus on the pathogenicity of the isolated fungus on rice plants and that in vivo study should be conducted on the extract.

Key words: Rice, Blast, Symptoms, *Helitropium indicum*, Extract

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the family Poaceae, is one of the main cereal food crops in most part of Africa (Traore *et al.*, 2006). It is recognized as an important strategic food security crop and as a crucial element in the staple food economies of Sub Saharan Africa (Abdu *et al.*, 2013). It is also the most rapidly growing source of food crop in Africa, and significant importance to food security and food self-sufficiency in an increasing number of low-income food deficit countries (FAO, 2012). Rice cultivation is widespread within Nigeria, extending from the northern to southern zones with most rice grown in the Eastern and Middle Belt zones of the country (Idowu *et al.*, 2013). Nigeria is the 15th largest rice producer in the world trailing behind Egypt (FAO, 2016) with paddy rice production of 6,070,813 tonnes and paddy rice yield of 2.99 tonnes per hectare (FAO, 2016). The major problems in rice production around the world are biotic and abiotic factors (Ou, 1985). One of the biotic factors in rice crop is blast disease

caused by a filamentous, ascomycetous fungus, *Pyricularia oryzae* Cav. It is an infectious fungal disease infecting rice plants in African countries (WARDA, 2004) which is distributed worldwide and prevailing in more than 85 countries of the world (Jamal *et al.*, 2012). *Pyricularia oryzae* is otherwise known as rice blast, rice seedling blight, and rotten-neck blast, pitting disease, leaf blast, node blast, panicle blast, collar blast and Johnson's spot (NDPRB, 2011). Blast can be found on the rice plant from the seedling stage to maturity. The leaf blast phase occurs between the seedling and late tillering stages (Couch and Kohn, 2002). Several rice blast epidemics have occurred in different parts of the world, resulting in heavy yield losses ranging from 50 to 90 % of the expected crop (Jamal *et al.*, 2012). The use of chemicals has helped increase of yields obtained but one of the major problems with the constant use of chemicals is that resistance can be induced in target organisms (Okigbo and Ikediugwu, 2000). Plant extracts have been used successful to control disease in plants

(Amadioha and Obi, 1999; Okigbo and Emoghene, 2004; Okigbo and Nmeka, 2005; Yekini *et al.*, 2015).

India Heliotrope, (*Heliotropium indicum*, L.) belong to the family Boraginaceae (Rahmatullah *et al.*, 2009). It is one of the plants used in the traditional management of conjunctivitis (Roeder and Wiedenfeld, 2013). However, several activities have been linked to the plant scientifically. These include gastro protective activity (Adelaja *et al.*, 2008), wound healing activity (Reddy *et al.*, 2002), anti-inflammatory activity (Srinivas *et al.*, 2000), antituberculosis effect (Machinan *et al.*, 2005), antiproliferative effect (Moongkarandi *et al.*, 2004), as well as immunostimulant effect (Ashok *et al.*, 2009). Afolabi, and Afolabi, (2013) reported Saponin, Tannin, Phenol, Flavonoid and Alkaloid as phytochemical constituents in *H. indicum*. Also, Afolabi and Afolabi, (2013) further reported that *H. indicum* had the highest Tannin and Saponin content.

There has been virtually no report on the antimicrobial activity of this plant in Nigeria. This research was therefore carried out to investigate the fungal organisms associated with the diseased rice sample in Aninri Local Government, Enugu State, inhibition with aqueous and ethanol extracts of India Heliotrope, (*H. indicum*, L.) and the determination of the phytochemical constituent of the extract.

MATERIALS AND METHODS

Collection of the diseased rice plant samples

Infected rice plants were randomly collected from five rice farms within Aninri Local Government Area, Enugu State, South-eastern, Nigeria in October, 2015 cropping season. Infected rice plants with blast symptoms such as leaf spot, sheath rot, seed discoloration, malformed seeds and leaf lesion were collected and taken to the Central Laboratory, National Roots Crop Research Institute, Umudike, Abia State, Nigeria in sample bags for isolation of the fungal organisms.

Collection of plant materials

Healthy leaves of India Heliotrope (*H. indicum*) was source from the premises of Teaching and Research Farms, Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria

Isolation of fungi from infected rice plants

Infected rice Leaves, stems and seeds pieces (2 x 2 mm) were cut between the advancing edge of infected and healthy tissues, surface sterilized (1% NaOCl) for 1 min and rinsed in five changes of sterile distilled water), dried on sterile tissue paper and plated on 0.3 ml streptomycin amended PDA medium. Five replicate pieces from each of the infected leaves and seeds were plated on each

plate. The plates were incubated at temperature of 28-30°C for 5 days. Fungal growths associated with the infected tissues were observed and detailed structural features of each isolate were compared with those described in a standard manual of fungi (Barnett and Hunter, 1999).

Determination of incidence of fungal isolates

To determine the incidence of the fungal isolates, the total number of diseased plants surveyed across the five rice farms was expressed as a percentage of the number of diseased plants that each pathogen was isolated from the farms (Okigbo and Ikediugwu, 2000).

$$\% \text{ Incidence} = \frac{T}{N} \times 100$$

Where N= Total number of diseased plants surveyed

T= Number of diseased plant from which pathogen was isolated

Preparation of plant extracts

Fresh fully expanded leaves of India Heliotrope (*H. indicum*) were washed thoroughly with water and soaked in a 1% solution of sodium hypochlorite for 2 min, rinsed severally with sterile distilled water and spread out on laboratory benches at temperature of 28-30°C to dry for 10-12 days until they became crispy. Three different aqueous and

ethanol extract concentrations were prepared by weighing 25, 50 and 75 g of the leaves on Mettler-Toledo AG balance, AB104, Switzerland, and blended in a Eurosonic, ES210, Great Star, Asia, blender and 100 ml of sterile distilled water added while ethanol extraction were done by adding 100 ml of 70 % ethanol, using same weights as for aqueous above for 2 hrs. All the extracts were allowed for to settle 2 hrs and sieved through four layers of sterile cheese-cloth. Extract concentrations of 2.5, 5.0 and 7.5 % were obtained. One ml of each extract concentration was dispensed per Petri dish and 9 ml of molten PDA were added to prepare a PDA-extract mixture with corresponding 2.5, 5.0 and 7.5% extract concentrations (Sangoyomi, 2004). The plates were gently rotated to ensure even dispersion of the extracts. The agar-extract mixtures were allowed to gel and used for the inhibition of mycelia growth of the tested fungi.

Determination of mycelia growth inhibition

The method of Amadioha and Obi (1999) was used to determine the effect of the extracts on fungal growth using completely randomized design (CRD) with three replications. This involved creating a four equal section on each Petri-dish by drawing two perpendicular lines at the bottom of the plate, the point

of intersection indicating the center of the plate. This was done before dispensing PDA into each of the plates. About 1 ml of the extract was separately introduced into the Petri-dish containing the media (PDA). A disc (4 mm diameter) of the pure culture of each isolate about 7 day old were placed on the extract just at the point of intersection of the two lines drawn at the bottom of the Petri dish. The negative controls were set up using blank agar plates (no extracts) and the positive control consisted of the fungicide Mancozeb (ethylene bisdithiocarbamate) which was prepared according to manufacturer directions by mixing 0.5 g in 100 ml of sterile distilled water. Mancozeb is a multipurpose, preventive, contact, broad spectrum fungicide. Three replicates plates of PDA-extract per isolate were incubated at 27°C and radial growth were measured daily for 5 days. Colony diameters were taken as the means along two directions on two perpendicular lines drawn on the reverse of the plates.

Fungi toxicity of the extracts were recorded in terms of percentage colony inhibition and calculated according to Pandey *et al* (1982):

$$\text{Growth inhibition (\%)} = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100$$

Where: DC -Average Diameter of control and

DT -Average diameter of fungal colony with treatment.

Phytochemical screening of plant extracts

Twenty gram of the plant leaves was air dried at a temperature of 28-30°C to dry for 10-12 days until they became crispy before being processed to powdered and stored in an air tight container prior to analysis.

Determination of Tannin content

The percentage composition of tannin in the plants was determined using the AOAC methods (1990) with some modifications. Folin-Denis reagent and saturated sodium carbonate were prepared in accordance with the procedure to analyze the tannin content. Standard solution of tannic acid was freshly prepared by dissolving 10 mg of tannic acid in 100 ml water. A series of tannic acid standard were prepared in the range of 0 - 2.5 ml aliquots in 25 ml volumetric flasks then added with 1.25 ml Folin-Denis reagent and 2.5 ml sodium carbonate solution. The mixture was made up to the volume and the colour was measured after 30 min at 760 nm using a spectrophotometer (Perkin Elmer). The samples were prepared by boiling 1 g of their dried powder in 80 ml of water for 30 min. The samples were cooled,

transferred into a 100 ml volumetric flask and diluted to mark. The solution was filtered to get a clear filtrate and analyzed as in the standard. Tannin content was determined by a tannic acid standard curve and expressed as milligrams of tannic acid equivalence (TAE) per 100 g of dried sample.

Determination of Steroids

This was determined by the method described by Okeke and Elekwa (2003). A measured weight of tested sample (5 g) were dispersed in 100 ml freshly distilled water and homogenized in a laboratory blender. The homogenate were filtered and the filtrate was diluted with normal ammonium hydroxide solution (pH 9). One mill of the elute was put into a flask (250 ml) and mixed with 2 ml of Chloroform. Ice-cold acetic anhydride (3 ml) was added to the mixture in the flask and 3 drops of concentrated H_2SO_4 was cautiously added to cool. Standard sterol solution were prepared and treated as described by Okeke and Elekwa (2003) as standard and blank. The absorbance of standard and prepared sample was measured in a spectrophotometer at 420 nm wavelength, using the reagent blank to calibrate the instrument at zero. The steroids content were calculated as shown below;

$$\% \text{ Steroid} = \frac{100 \times Au \times C \times Vf}{W \times As \times 1000 \times Va}$$

Where, W= Weight of the sample analyzed, Au = Absorbance of test sample, As = Absorbance of standard sterol solution, C = Concentration of standard solution in mg/ml, Vf = Total volume of extract, Va = Volume of extract analyzed

Determination of Saponin content

Saponin determination was done using the method of AOAC (1990). Saponin extraction was done using two different solvents. The first solvent, acetone, was used to extract crude lipid from the samples while the second solvent (methanol) was used for the extraction of the saponin proper. Two gram of the sample was folded into a thimble and put in a soxhlet extractor and a reflux condenser fitted on top. Extraction was done with acetone in a 250 cm³ capacity round bottomed flask for 3 hrs, after which the apparatus was dismantled and another 150 cm³ capacity round bottomed flask containing 100 cm³ of methanol was fitted to the extractor and extraction was carried on for another 3 hrs. The weight of the flask was taken before and after the second extraction in order to make the change in weight. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove any remaining solvent in the flask. The flask was then allowed to cool and the weight of the flask taken.

The saponin content of the sample was calculated thus:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W_s \times 100}$$

Where; W_2 = Weight of beaker extract, W_1 = Weight of beaker 1 and W_s = Weight of sample

Determination of Phenol

This was determined by the folin-ciocateau spectrophotometer method (AOAC, 1990). The total phenols were extracted in 0.2 g of the sample with 10 ml concentrated methanol. The mixture was shaken for 30 min at room temperature. The mixture was centrifuged at 500 rpm for 15 min and the supernatant (extract) were used for the analysis. One mill portion of the extract from each sample was treated with equal volume of folin-ciocateau reagent followed by the addition of 2 ml of 2 % Na_2CO_3 solution. Standard phenol solution were prepared and diluted to a desired concentration. One mill of the standard solution also was treated with the folin-ciocateau reagent and Na_2CO_3 solution. The intensity of the resulting blue coloration was measured (absordance) in a colorimeter (Jenway 6051) at 540 nm wavelength.

Determination of Alkaloid Content

The gravimetric method of Harborne (1973) was used in the determination of total alkaloid contents of the leaves. Five

gram of the dried samples was dispersed into 50 ml of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 hrs before filtering. It was evaporated to one quarter of its original volume and drop wise concentration of ammonium hydroxide was added to precipitate the alkaloids. The precipitate was filtered off with a pre-weighed filter paper and washed with 1 % ammonium hydroxide solution. The precipitate was oven dried for 30 min at 60 °C and re-weighed. The alkaloid contents of the samples were determined by difference using the equation:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{w} \times 100$$

Where W = weight of sample; W_1 = Weight of empty filter paper; W_2 = Weight of paper + precipitate.

Determination of Flavonoid content

Ten grams of the plant samples were extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Kocipai, 1994). The Flavonoid contents of the samples were determined by difference using the equation:

$$\% \text{ Flavonoids} = \frac{W_2 - W_1}{W_s \times 100}$$

Where, W_2 = Weight of crucible + extract, W_1 = Weight of crucible and W_s = Weight of sample

Determination of Cyanogenic glucosides content

The alkaline picrate method (Onwuka, 2005) was employed for the determination of cyanogenic glycosides. A portion (5 g) of each sample was grinded into paste and dissolved in 50 ml distilled water in a corked conical flask. The extraction was allowed to stay overnight (12 hrs). The sample was filtered and the filtrate was used for the cyanide determination. To 1 ml of the sample filtrate in a corked test tube, 4 ml of alkaline picrate was added and incubated in a water bath for 5 min and the absorbance was read at 490 nm. The absorbance of the blank which contained only 1 ml distilled water and 4 ml alkaline picrate solution was read and used to stabilize the spectrophotometer before taking the absorbance of the samples. The cyanide content was extrapolated from a cyanide standard curve. The cyanide content was calculated using the formula:

$$\text{Cyanogenic glucoside} \left(\frac{\mu\text{g}}{\text{g}} \right) =$$

$$\frac{\text{Absorbance} * \text{Gradient factor} * \text{Dilution factor}}{\text{Weight of sample}}$$

Statistical design and Analysis

The experimental design used was Completely Randomized Design (CRD) with three replications. Treatments means was subjected to analysis of variance (ANOVA) using Minitab Version 17 and the means separated using Turkey at $p \leq 0.05$.

RESULTS

Blast symptoms and fungi isolated from the infected rice plants

The blast symptoms observed on the rice farms includes leaf lesion, brown spot, leaf blast, sheath rot, seed discoloration and malformed seeds. The commonly cultivated rice includes FARO-44, FARO-45, and Mass (Local foreign). The fungi species isolated from the diseased rice plants from the five farms were *Fusarium moniliforme*, *Pyricularia oryzae*, *Curvularia lunata* and *Rhizoctonia solani* (Table 1). *F. moniliforme* (39.10) had the highest frequency of occurrence, followed by *P. oryzae* (28.57) while *R. solani* (9.52) had the least.

Inhibition of mycelia growth of the five isolated fungi with India Heliotrope (*H. indicum*) extracts at 5 days after incubation

Both the aqueous and ethanol extract of *H. indicum* reduced the mycelia growth of the fungal species (Table 2). At 2.5 % aqueous extracts concentration; *P. oryzae* (43.50) had highest mycelia

inhibition, followed by *F. moniliforme* (31.31) while *R. solani* (12.50) had the least. Also in ethanol extracts concentration, the inhibition follows the same patterns with highest inhibition of the mycelia with *P. oryzae* (48.79), followed by *C. lunata* (37.83) while *R. solani* (22.83) had the least. However, at 5.0 % aqueous extracts concentration; *P. oryzae* (61.48) had highest mycelia inhibition, followed by *F. moniliforme* (44.83) while *R. solani* (32.67) had the least. Also, similar trend to effect of 2.5 % ethanol extracts concentrations was observed but with a higher ranged on mycelia inhibition (39.17 to 94.99 %) across all the fungal organisms. At 7.5 % extracts concentration, the pattern of mycelia growth inhibition was similar to that of lower concentration in both aqueous and ethanol. However, in all the concentrations, ethanol extract consistently inhibit the mycelia growth of all the four isolated fungus.

Phytochemical analysis of India Heliotrope (*H. indicum*)

The results of quantitative analysis of *H. indicum* leaves constituents investigated were summarized and shown in Figure 1. The result reveals that Tannin (11.52) had the highest content, followed by Saponin (7.85) while Steroids (1.09) had the least.

DISCUSSION

The presence of blast like symptoms on rice plants which includes leaf lesion, leaf blast, sheath blight, collar blast, seed discoloration and malformed seeds indicates that none of the varieties grown in all the rice farms surveyed were totally immune or highly resistant to the isolated fungus. The commonly cultivated rice includes FARO-44, FARO-45, and Mass (Local foreign). The fungi species isolated from the diseased rice plants from the five farms were *F. moniliforme*, *P. oryzae*, *C. lunata* and *R. solani*. Several fungal pathogens have been isolated from rice seeds, and have been reported to be responsible for a number of diseases right from the nursery to the field Ibiyam *et al.*, (2006). Ibiyam *et al.*, (2008) reported *F. moniliforme*, *Bipolaris oryzae*, *Fusarium oxysporum*, *Chaetomium globosum*, *C. lunata* and *Trichoderma harzianum* from the seeds of the three varieties from the field in Afikpo North L.G.A, of Ebonyi State. The percentage of occurrence ranged from *R. solani* (9.52) to *F. moniliforme* (39.10). Ibiyam *et al.*, (2008) reported *F. moniliforme* more prevalent than the other fungi both in the field and storage. Both aqueous and ethanol extract of *H. indicum* significantly inhibit the mycelia growth of the four isolates. However, ethanol extract significantly inhibit the mycelia growth of the fungal isolates than the aqueous extract. This justifies

Table 1: Percentage of occurrence of the isolated fungi in Aninri Local Government, Enugu State

Isolated fungi	Number of occurrence	Percentage of occurrence (%)
<i>P. oryzae</i>	6	28.57
<i>F. moniliforme</i>	8	39.10
<i>C. lunata</i>	5	23.81
<i>R. solani</i>	2	9.52

Table 2: Effects of aqueous and ethanol extracts of India Heliotrope (*H. indicum*) on the mycelia growth of the isolated fungus

Organisms	2.5%		5.0%		7.5%		0.5g of fungicide (Mancozeb)	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
<i>P. oryzae</i>	43.50 ^a	48.79 ^a	61.48 ^a	94.99 ^a	66.85 ^a	97.10 ^a	99.33 ^a	100.00 ^a
<i>F. moniliforme</i>	31.31 ^{ab}	35.28 ^b	44.83 ^b	59.08 ^b	70.92 ^a	74.26 ^b	100.00 ^a	100.00 ^a
<i>C. lunata</i>	28.33 ^{ab}	37.83 ^b	40.00 ^{bc}	46.02 ^{bc}	58.33 ^a	66.73 ^b	97.50 ^a	99.17 ^a
<i>R. solani</i>	12.50 ^b	22.83 ^c	32.67 ^c	39.17 ^c	41.40 ^b	43.67 ^c	95.67 ^a	100.00 ^a
Mean	28.91	36.18	44.74	59.81	59.38	70.44	98.13	99.79
SE	3.72	2.85	3.36	6.64	3.57	5.99	0.88	0.21
CV	44.62	27.28	25.99	38.44	20.81	29.46	3.10	0.72

Means in the same column with different superscripts are significantly different ($P < 0.05$).

SE means Standard Error while CE means Coefficient of Variation

the report of Bharathajothi *et al*, (2015) that the chloroform extract of *H. indicum* showed better inhibition zone formation against all the tested fungal strains than the aqueous extract. The results obtained also showed that both extracts of *H. indicum* were most effective in higher concentration compared to the other concentrations used. The antifun-

gal activity revealed that the chloroform extract had better inhibitory zone than the aqueous extract Bharathajothi *et al*, (2015). Qualitative phytochemical analyses reveal the presence of alkaloids, tannins, phenols, saponin, flavonoids, cyanogenic glucoside and steroids. It is in lines with Stauth, (2007) who also detected the presence of saponins, tannins and alkaloids in the methanol

leaf extract of this plants. Tannin was more in content followed by saponin than other constituents of the extracts. This is in agreement with the report of Afolabi and Afolabi, (2013) that alkaloids, tannins, phenols, saponin and flavonoids were present in *H. indicum* and that *H. indicum* had the highest Tannin and Saponin content. Tannins have high potency for the treatment of intestinal disorders such as diarrhoea and dysentery Akinpelu, and Onakoya, (2006). The presence of alkaloids and saponins explains the reason why these medicinal plants are used to treat hypertension because alkaloids and saponins prevent the excessive intestinal absorption of cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension Akinpelu, and Onakoya, (2006). The presence of secondary metabolites in plants, produce some biological activity in man and animals and it is responsible for their use as herbs. Tannin and phenols are known to possess antimicrobial properties. Alkaloid and flavonoids had antimicrobial activities and phenols present in the plants are known to be toxic for microorganisms.

Conclusion and recommendations

In conclusion, the use of *H. indicum* for the control of rice blast gave a relative value for all the organisms when compared with the systemic fungicide.

The potentials of *H. indicum* leaf extracts on the inhibition of rice blast diseases are worthy. It further buttresses the potential of natural plant products in the management of rice blast disease on the field. Further study is needed to determine pathogenicity of the associated fungal species on rice seedlings. Also, the promising potential of these extract in the *in vitro* trials calls for further evaluation of the extract efficacy *in vivo* in the management of rice blast diseases. It can however be combined with other extracts in future studies at a more higher rate than the one used in this study to control rice blast disease in Nigeria and the world at large.

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