

***In vitro* investigation on antifungal activity of some plant extracts against *Pyricularia oryzae*.**

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Abstract

Studies were carried out to determine the antifungal attributes of some plant extracts against *Pyricularia oryzae*. The plant species evaluated were the leaves of *Ocimum gratissimum*, *Chromolaena odorata*, *Cymbopogon citratus*, seeds of *Eugenia aromatica*, *Piper guineense*, and nuts of *Garcinia kola*. Antifungal activity was tested at concentrations of 10, 20, 30, 40, 50 and 100 % of plant extracts, using the poisoned food technique. All plant extracts reduced the growth of *Pyricularia oryzae* at all tested concentrations. Highest growth inhibition was achieved at 100 % concentration with *E. aromatica*, 100 % ; *P.guineense* 98 % and *G.kola*, 97.3 % mycelial growth inhibition. Extracts from *E. aromatica*, *G.kola* and *P. guineense* at 100 % concentration promoted significant ($P \leq 0.05$) inhibition on mycelial growth and sporulation of *P. oryzae* than the control, *O.gratissimum*, *C. odorata* and *C. citratus*. It could be inferred that extracts of *E.aromatica* *P.guineense* and *G.kola* at 100 % concentration can serve as bio-fungicides against the growth of *P. oryzae*.

Keywords: Plant extracts, Antifungal attributes, *Pyricularia oryzae*, sporulation, mycelial growth.

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Introduction

Rice (*Oryza sativa* L.) is one of the most important cereals in the world and is consumed by about 50 percent of the world's population (Luo *et al.*, 1998). Reports by West Africa Rice Development Association WARDA (1996) revealed that Nigeria has since 1980 become the largest rice producing country in West Africa and the third largest in Africa, after Egypt and Madagascar producing an average of 3.2 million tons of paddy rice.

However, blast disease is the most devastating fungal disease encountered by farmers in Nigeria and it accounts for about 50 % yield loss of rice fields (Kuta, 2004; AgricultureBusiness Week, 2008). WARDA (1995) estimated the yearly yield loss due to blast disease at more than 10 million U.S dollars. Ghazanfar *et al.* (2009) highlighted that synthetic fungicides are effective in keeping

down rice blast disease. Nevertheless, the continued use of synthetic fungicides are toxic to non-target organisms and cause environmental problems (Hayes and Laws, 1991).

However, investigations revealed that the use of bio fungicides of plant origin are safe for human and the environment (Amadioha, 2000; Chiejina, 2006). Wendorff and Wee (1997) reported significant inhibitory effect on the growth of some pathogenic molds, with *Eugenia aromatica* extract. Other well known plants with antimicrobial activities include *Ocimum gratissimum*, *Chromolaena odorata*, *Allium sativum*, *Azadirachta indica*, *Cymbopogon citratus*, *Garcinia kola* and *Piper guineense* (Malkhan *et al.*, 2011).

Plant extracts have been tested against *P. oryzae* with significant results (Mariappan *et al.*, 1995; Netam *et al.*, 2011). The efficacy of

plant extracts against *P. oryzae* has not been widely reported in Nigeria. The aim of this study however, is to evaluate the antifungal efficacy of some locally available medicinal plant extracts against *Pyricularia oryzae*.

Materials and methods

Source and preparation of plant materials: Fresh leaves of *O. gratissimum*, *C. odorata* and *C. citratus* were collected from the environment of crop, soil and pest management laboratory FUTA, Nigeria. Seeds of *E. aromatica*, *P. guineense* and nuts of *G. kola* were purchased at Oba market Akure. Voucher specimen of plant samples was deposited in the herbarium for identification. Plant samples were air dried for 14 days and ground separately using a Porex mini mill hand grinder (model number, 46175).

Preparation of active principles: Extraction thimbles were filled with 50 gram powder of each plant sample and 250ml n-hexane was added. The extract was collected after 3 hours of soxhlation and was concentrated in a rotary evaporator.

Bioassay technique: The poisoned food technique was used to determine mycelial growth inhibition and sporulation inhibition. A modified Srivastava *et al.*, 2009 method was used for herbal extract dilutions and concentrations. About 1-5 ml and 10 ml portions each of plant extracts were dissolved separately in 0.5ml Tween 80 solvent to obtain different concentrations of 10, 20, 30, 40, 50 and 100 % of plant extracts. Thereafter, about 1 ml each of plant extracts was withdrawn and then mixed with 9 ml of potatoe dextrose agar (PDA) medium. Carbendazim (0.5mg/ml) was used as standard control. Plates without the extracts or Carbendazim stood as negative control. The agar- extract / Cabendazim mixture was poured into each of three 9mm Petri dishes to make three replicates. Using a sterile cork borer, 6mm discs cut from periphery of 7 days old pure culture were placed upside down at the centre of the medium in the Petri-dishes at each specified concentrations and control. The experiment was laid out in completely randomized design (CRD) in three replications. All the plates were incubated at $27 \pm 2^{\circ}\text{C}$ and observation for mycelial growth and sporulation inhibition was

recorded at 8th day of inoculation and incubation.

The percentage inhibition of mycelial growth was obtained as;

$$\% I = \frac{C - T \times 100}{C}$$

Where ; %I= percentage inhibition
C= fungal growth in control plates.
T= fungal growth in treated plates.

Spore counts was done by cutting 1cm³ agar disc each from treated and control plates, dislodging the spores separately into 50 ml beaker containing 10 ml distilled water, with the aid of camel's hair brush. The suspension was stirred vigorously with the aid of a glass rod. A drop of spore suspension from each concentration as well as the control was counted with the hemacytometer slide and a light microscope. The percentage inhibition of sporulation was obtained as;

$$\frac{\text{Spore number control} - \text{Spore number treatment}}{\text{Spore number control}}$$

$$\times \frac{100}{1}$$

Isolation and identification of rice blast pathogen: Rice leaves showing typical symptoms of blast disease were collected from rice farm in Akure, Ondo state. The leaves were cut into small sections, rinsed in distilled water and surface sterilized in 70 % alcohol for 5 seconds. The cut leaves were thereafter rinsed in several changes of sterile water and aseptically plated in sterile 9 mm Petri dishes containing potato dextrose agar (PDA). Fungal growths from points of inoculation were subsequently transferred to new PDA. The PDA plates were later incubated at $27 \pm 2^{\circ}\text{C}$. Identification of the isolated fungus was done macroscopically and microscopically. Macroscopic identification was based on observed culture growth. Microscopic identification was based on mount culture. Fungal identification was confirmed with the aid of book by Barnett and Hunter (1999).

Results

Effects of plant extracts on Sporulation of Pyricularia oryzae: Table 1 shows plant extracts effect on sporulation. Result showed that the extracts promoted significant ($P \leq 0.05$) inhibition of sporulation in *P. oryzae* at all the concentrations tested. On the whole, result showed that sporulation inhibition is usually minimal at lower concentrations (10 to 30 %) as compared with higher concentrations (40 to 100 %) of plant extracts, which showed maximal effect on sporulation. *E. aromatica* was most effective against *P.oryzae*, inhibiting sporulation between 77 and 85 % at lower concentrations and 90 -100 % at higher concentrations respectively. *P. guineense* was effective with inhibition varying from 60 – 85

% at lower concentrations and 79 - 90% at higher concentrations respectively . At 10 % concentration, result showed that *C. odorata* inhibited sporulation by 73 % and not significantly different from *E.aromatica* (77 %). Extracts from *O. gratissimum* and *C.citratu*s were the least effective with 38 % and 25 % sporulation inhibition respectively. However, at 100% concentration, *E.aromatica* completely (100 %) inhibited sporulation but not significantly different from *P.guineense* (90 %). Extracts from *G.kola*, *O.gratissimum*, *C.citratu*s and *C.odorata* were not significantly different from one another but less effective than *E.aromatica* and *P.guineense* at 100% concentration.

Table 1: Percentage sporulation inhibition of *Pyricularia oryzae* by the extracts.

Medicinal plants	Concentrations					
	10%	20%	30%	40%	50%	100%
<i>E.aromatica</i>	77.00 ^a	80.00 ^a	85.00 ^a	90.00 ^a	100.00 ^a	100.00 ^a
<i>O.gratissimum</i>	38.00 ^c	50.00 ^d	56.00 ^c	85.6 ^b	60.00 ^c	85.00 ^b
<i>C.odorata</i>	73.00 ^a	60.00 ^c	69.22 ^b	70.00 ^c	72.66 ^b	79.00 ^b
<i>G.kola</i>	61.00 ^b	65.00 ^c	70.22 ^b	69.00 ^c	60.00 ^c	92.00 ^a
<i>C.citratu</i> s	25.00 ^d	30.00 ^e	40.9 ^d	53.00 ^d	92.00 ^a	75.00 ^b
<i>P.guineense</i>	60.30 ^b	70.00 ^b	85.00 ^a	79.00 ^a	67.00 ^c	90.00 ^a
Carbendazim (0.05%)						100
Control						0

Means followed by the same letter or letters in each column do not differ significantly at $p \leq 0.05$ by Duncan’s Multiple Range Test.

Effect of plant extracts on mycelial growth of Pyricularia oryzae: Different doses (10, 20,30,40,50 and 100 %) of plants extracts (*E.aromatica*, *P.guineense*, *G.kola*, *C.citratu*s, *C.odorata* and *O.gratissimum*) were tested against *P. oryzae* to determine their antifungal activity *in vitro*. At lower concentrations (10 to 30 %), the

least effective among the plant extracts was *C.citratu*s promoting inhibition of *P.oryzae* by 21.22 to 38.29 %. Nevertheless, at 100 % concentration the antifungal activity of *C.citratu*s (75 %) was not significantly different from *O.gratissimum* and *C.odorata* with 85% and 79 % growth inhibition respectively.

Table 2: Percentage mycelial growth inhibition of *Pyricularia oryzae* by the extracts.

Medicinal plants	Concentrations					
	10%	20%	30%	40%	50%	100%
<i>E.aromatica</i>	75.00 ^a	78.00 ^a	85.00 ^a	90.00 ^a	100.00 ^a	100.00 ^a
<i>O.gratissimum</i>	33.33 ^c	43.44 ^c	50.00 ^c	55.33 ^c	65.00 ^c	80.00 ^b
<i>C.odorata</i>	71.11 ^a	61.44 ^b	66.22 ^b	70.00 ^b	71.66 ^b	80.00 ^b
<i>G.kola</i>	61.11 ^b	65.00 ^b	68.22 ^b	70.00 ^b	75.00 ^b	97.33 ^a
<i>C.citratus</i>	21.22 ^d	25.00 ^d	38.29 ^d	53.66 ^c	92.00 ^a	75.00 ^b
<i>P.guineense</i>	59.333 ^b	65.00 ^b	85.00 ^a	88.00 ^a	92.00 ^a	98.00 ^a
Carbendazim						100
Control						0

Means followed by the same letter or letters in each column do not differ significantly at $p \leq 0.05$ by Duncan's Multiple Range Test.

Discussion

Results revealed that all plant extracts studied, promoted significant ($P < 0.05$) inhibition on mycelial growth and sporulation of *P.oryzae*. This conforms with the findings of Choi *et al.*, 2004 and Amadioha, 2000 on the control of *P. oryzae* with some plant extracts. This study revealed that *E. aromatica* was most effective compared to the extracts of other species studied. *E. aromatica* at 100 % concentration displayed the same high effect as Carbendazim. Similarly, Meena and Sethi (1994) reported that *E. aromatica* completely inhibited the growth of some food- borne pathogenic molds. Investigation by Martini *et al.* (1996) on *E. aromatica* indicated that eugenol was the active compound in clove responsible for strong antimicrobial activities. It is suspected that this eugenol could also be responsible for the high antifungal activity displayed in this study against *P. oryzae*. Extracts of *Piper guineense* displayed strong antifungal activity against *P. oryzae*. Similarly, Enyiukwu and Awurum (2011) highlighted strong antifungal activity of *P. guineense* against sporulation of *Colletotrichum destructivum*. Results also revealed that *G. kola* disallowed growth of *P. oryzae*. This conforms with the report of Onyeke and Ugwuoke (2011) on the antifungal activity of *G. kola* extract against the development of seed-borne fungi of African yam bean, *Sphenostylis stenocarpa*. Furthermore, *Chromolaena odorata* extract exhibited considerable antifungal properties against *P. oryzae*. Similar effect was recorded by Poornima *et al.* (2011) on the suppression of *Cercospora beticola*. Extracts from *O. gratissimum* and *C. Citratus* were the least effective at lower concentrations, but *O.*

gratissimum and *C. Citratus* exhibited considerable inhibitory effects against the isolated pathogen at higher concentrations. This is similar to the effect observed by Nguefack, *et al.* (2008) on the suppression of some seed-borne fungi of rice viz *Alternaria padwickii*, *Bipolaris oryzae* and *Fusarium moniliforme*.

Efforts have been made at boosting rice production in Nigeria and this has led to a tremendous increase in area planted, output and productivity of paddy rice production in the last two decades. In spite of these improvements, rice production has not kept up with domestic consumption demands of the Nigerian populace and consequently, rice is still imported (Singh *et al.*, 1997). Information on antagonistic effects of medicinal plants provided in this study would enable farmers produce rice plants that may grow better and produce more seeds at lower cost of production and consequently, increase domestic rice production. It is therefore recommended that this experiment be further studied under field conditions to confirm bio-efficacy of the extracts against rice blast disease. It could be inferred that extracts from *E.aromatica*, *P.guineense* and *G.kola* at 100% concentration could serve as bio-fungicides against the growth of *P.oryzae*.

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