Applied Tropical Agriculture

Efficacy of n-hexane plant extracts in the control of rice blast disease

Adeosun, B.O[1*] and Onasanya,O.R[2]

[1]Department of Crop, Soil and Pest Magement, The Federal University of Technology, PMB 704 Akure, Ondo state, Nigeria.
[2]Department of Agriculture, Federal College of Agriculture, PMB 5029 Moor plantation, Ibadan, Oyo state Nigeria.
* Corresponding author

ABSTRACT

Studies were carried out to determine antifungal attributes of n-hexane extracts of six plant species (Leaves of African Basil; Ocimum gratissimum, Siam weed; Chromolaena odorata, Lemmon grass; Cymbopogon citratus, seeds of Clove; Eugenia aromatica, West African black pepper; Piper guineense, and nuts of bitter kola; Garcinia kola) on Pyricularia oryzae, the incitant of rice blast disease. Plant parts were separately air-dried and ground. Active ingredients were extracted with the aid of n-hexane, using soxhlet apparatus. Antifungal activity of extracts were evaluated in different concentrations (10, 20, 30, 40, 50 and 100%) of plant extracts and carbendazim at 0.5 mg. ml^2 a standard chemical, using the poisoned food technique. Mycelial growth was determined after 7 days of inoculation and incubation at each concentration and control. 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% growth inhibition, hence Cybopogon citratus, Chromolaena odorata, Ocimum gratissimum and lower concentrations (10-50%) were discarded from the study. Only the extracts from E. aromatica, G. kola and P. guineense at 100% concentration were used subsequently. Leaf blast was evaluated on Nerica and Faro-44 varieties. The rice seeds were inoculated with spores of pure culture of P. oryzae and treated with 100% extracts concentration of E. aromatica, P. guineense and G. kola each being the most promising botanicals among the six evaluated ones from the in vitro study. The untreated seeds stood as control while seeds treated with Carbendazim at 0.5 mg.ml^2 stood as negative control. Extracts from P. guineese and E. aromatica reduced leaf blast incidence by 45.8% and 37.5% respectively. Subsequently, rice seeds could be treated with 100% n-hexane extract concentration of P. guineense and E. aomatica against the growth and development of Pyricularia oryzae with a resultant control of rice blast disease.

Keywords: N-hexane, Plant, extracts, Antifungal attributes, *Pyricularia oryzae*, Leaf blast.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereals in the world, which is consumed by about 50% of the world's population (*Luo et al.,* 1998). According to Food and Agricultural Organisation FAO (2000), rice is a staple which can provide a nation's population with the nationally required food security minimum of 2,400 calories per person per day. Rice cultivation is widespread within Nigeria extending from the northern to southern parts of the country with most of the rice grown in the east to middle belt. In Nigeria, rice is planted from April to May and harvested from August to November. However, the rain-fed lowland system accounts for half of harvested area (Kassali *et al.,* 2010).

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. There are many diseases of rice, including blast disease caused by *Pyricularia oryzae* (Correll *et al.*, 2000) which is one of the most important and widely distributed seed-borne fungal diseases of rice (Agarwal *et al.*, 1989). Seed borne infections are known to decrease the germination of rice seeds (Imolehin, 1983). *Blast is the most devastating fungal disease of rice encountered by farmers in Nigeria (Kuta, 2004), causing* 40 % yield loss *(*Ou, 1985*).* Fungicides are usually useful in controlling rice diseases (Bateman *et al.*, 1986). However, fungicides are toxic to non-target organisms and cause environmental problems (Hayes and Laws, 1991).

Investigations have been made into the possible exploitation of plant products that are safe for humans and the environment (Bèlanger *et al.*, 1995). The fungal pathogens of rice, viz *Sarocladium oryzae* (sheath rot pathogen) and *Pyricularia oryzae* (blast pathogen), were controlled effectively by neem oil and neem seed kernel extracts (Mariappan *et al.* 1995). De Waard *et al.* (1993), suggested disease management through seed treatment as an integral component of rice crop production to avoid unacceptable crop losses and also to meet the challenge posed by feeding the growing world population.

Seed to seedling transmission of *Alternaria padwickii*, *Bipolaris oryzae* and *Fusarium moniliforme* were controlled by seed treatment with essential oils from *Cymbopogon citratus* and *Ocimum gratissimum* (*Nguefack et al., 2008).* 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. Inspite 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). Rice cultivation is faced with biotic and abiotic constrains (Tagne *et al.*, 2,000). Among the biotic constrain factors of rice, disease is the most important factor which results in crop losses of $5 billion every year (Asghar *et al.,* 2007). Hence, in the present study, n-hexane extracts from some plants were evaluated *in vitro* against *P. oryzae* and further tested *in vivo* for their usefulness as seed treatment against rice blast disease.

MATERIALS AND METHODS

The experiment was carried out in the laboratory and screen house of Crop, Soil and Pest Management Department of the Federal University of Technology Akure (FUTA) , Nigeria.

Pant samples: Plant samples viz Seeds of *Eugenia aromatica*, *Piper guineense,* nuts of *Garcinia kola*, leaves of *Chromolaena odorata, Ocimum gratissimum* and *Cymbopogon citratus* were sourced from FUTA environment and Oba market in Akure, Ondo state.

Extraction of active components: Plant samples were air dried for 14 days and ground using a Polex mini mill hand grinder (model number, 46175). Oil was extracted separately from ground plant samples with the aid of soxhlet apparatus using n-hexane.

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 ± 2^{0}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= C – T X 100/C

Where; %I=percentage inhibition

C= fungal growth in control plates.

T= fungal growth in treated plates.

Seed inoculation and treatment: Rice seeds (Nerica and Faro 44) were kept in spore suspension from pure culture of *P.oryzae* for 24 hours and air dried overnight. The highest growth inhibition of *P.oryzae* was achieved at 100% concentration *in vitro,* with *E. aromatica*, 100%, *P.guineense* 98 % and *G.kola,* 97.3% growth inhibition, hence other extracts and lower concentrations were discarded from the study and only the extracts from *E. aromatica, G. kola* and *P. guineense* at 100 % concentration were used subsequently.

About 100 µl of *E. aromatica, G. kola* and *P. guineense* at 100 % concentration was applied per gram of rice seeds following the proceedure of Adegoke and Odesola (1996) with slight modifications. Seeds were allowed to stay in each extract for 6 hours.

Soil treatment: Sandy-loam soil was collected and sterilized in a metal drum at 100 ^{0}C for 5 hours. Soil was allowed to cool down and about 2kg each filled in polythene bags. Ten seeds were sown per pot, and laid in a 2x3 factorial experiment in Completely randomized design (CRD) with three replications, as two rice varieties and three plant extracts. Untreated seeds served as control and the seeds treated with carbendazim at 0.5 mg. ml^{-2} as standard.

Disease index evaluation: Blast incidence and severity was recorded 50 days after planting by examining 3 randomly sellected plants per pot. Leaf blast incidence was expressed as a percentage of total number of healthy rice leaves in each pot. Data on leaf blast severity was collected using disease rating scale of 0-9 developed by International Rice Research Institute (IRRI, 1996). The interpretation of the scale is as follows:

0 = No typical susceptible lesion observed
0= No typical susceptible lesion observed
1= Small brown specks (bs) of pin-point size without sporulating centre
3= Small roundish to slightly elongated, necrotic grey spots, 1-2mm in Diameter with a distinct brown margin (bg)
5= Typical susceptible blast lesions 3mm or longer, infecting less than 10% of leaf area
7= Typical susceptible blast lesions infecting 11-50% of the leaf area
9= More than 75% leaf area affected.

Blast incidence disease was computed using the formula:

Average of the disease score/9 X 100

RESULTS

Effect of n-hexane extracts on mycelial growth inhibition

Result in Table 1 show that all plant extracts at all doses significantly inhibited mycelial growth of *P. oryzae.* Complete inhibition on *P. oryzae* was found at 50 and 100% concentrations with *E. aromatica* extract. *Garcinia kola showed 75% to 97.3% inhibition while P. guineense showed 59.3% to 98% growth inhibition at 50% and 100% concentrations,* respectively. At lower concentrations of 10 to 40%, *E. aromatica* was also found to be effective showing 75 to 90% growth inhibition. *Garcinia kola* showed 61.1 to 70% growth inhibition while *P. guineense* showed

59.33 to 88% growth inhibition. Extract from *C.odorata showed 66 to7% while C. Citratus* showed 21.2 to 38.3% growth inhibition and *O. gratissimum showed 33.3 to 50% inhibition. Based on their significantly higher antifungal effects, the extracts from E. aromatica, P. guineense and G. kola were found to be more effective on P.oryzae at most of the concentrations in this study (Table1).*

Table 1: Percentage mycelial growth inhibition by n-hexane extracts

Medicinal plants	Concentrations					
	10%	20%	30%	40%	50%	100%
E. aromatica	75.0^{a}	78.0^{a}	85.0^{a}	90.0^{a}	100.0^{a}	100.0^{a}
O. gratissimum	33.3^{c}	43.4^{c}	50.0^{c}	55.3^{c}	65.0^{c}	80.0^{b}
C. odorata	58.1^{b}	61.4^{b}	66.2^{b}	70.0^{b}	71.7^{b}	80.0^{b}
G .kola	61.1^{b}	65.0^{b}	68.2^{b}	70.0^{b}	75.0^{b}	97.3^{a}
C .citratus	21.2^{d}	25.0^{d}	38.3^{d}	53.7^{c}	62.0^{c}	75.0^{b}
P .guineense	59.3^{b}	65.0^{b}	85.0^{a}	88.0^{a}	92.0^{a}	98.0^{a}
Carbendazim	100					
Control	0					

Means followed by different letter (s) are significantly different ($P \leq 0.05$)

Data on leaf blast severity and leaf blast incidence are presented in Table 2. Lowest leaf blast incidence (13%) was recorded on tretments with *P. guineense* extract and Carbendazim each. Seeds treated with *E. aromatica* and *G. kola* showed 37.5 % and 29.2% reduction on leaf blast incidence respectively. Treatment with *P. guinense* extract was most effective among extracts with 45.8% reduction on leaf blast incidence. Thus the botanicals were effective in reducing leaf blast incidence. Treatments with *P. guineense*, *E. aromatica* and *G. kola* reduced leaf blast severity by 17.2%, 17.6% and 7.8%, respectively. Nevertheless, Carbendazim reduced leaf blast severity by 39.4%. Botanicals showed promise in the control of rice blast disease, showing values ranging from 7.8% to 45.8% leaf blast severity and leaf blast incidence reduction.

Table 2. Effects of botanicals on development of rice blast

Botanicals/ Concentration	% Leaf blast incidence	% Leaf blast severity
P. guineense 100 %	13.0^{a}	20.8 ^{a}
E. aromatica 100 %	15.0^{a}	20.7 ^{a}
G. kola 100 %	17.0^{ab}	23.1^{ab}
Carbendazim 0.5mg ml^{-2}	13.0	15.2
Control	24.0	25.1

Means followed by different letters are significantly different ($P \leq 0.05$).

DISCUSSION

The *in vitro* antifungal activity of n-hexane extracts revealed that *E. aromatica* extract was the most effective compared to the extracts of the other species studied. Meena and Sethi (1994) reported the efficacy of clove extract against some food-borne microorganisms, especially pathogenic molds. Investigation by Martini *et al (*1996) on *E. aromatica* idicated 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*.

Activities of *G. kola* extract under *in vitro* and *in vivo* conditions in this study revealed its promising biopesticide prospects.This is similar to the submission of Onyeke and Ugwuoke (2011), on the *in vitro* antifungal efficacy of *G.kola* against S*phenostylis stenocarpa.* Suleman and Emua (2009) also found *G. kola* extract effective in the control of root rot disease of cowpea. Furthermore, significant reduction on leaf blast incidence was recorded on treatments with *E. aromatica* when compared with the control. This alligns with the report of Suprapta and Khalim (2009) that extract from *E. aromatica* was useful in the suppression of stem rot disease of vanilla seedlings. Other scientists have published results on the usefulness of plant extracts against rice blast disease, which is similar to ours Choi *et al.* (2004) reported that extracts from roots of *Chloranthus japonica* and stem *of Paulownia coreana* were effective in the control of rice blast disease.

This study revealed that treatments with *P.guineense* and Carbendazim had comparable leaf blast suppression effects. Netam et al. (2011), found garlic extract more effective as an alternative to conventional chemical fungicide in the control of rice blast disease. Enyiukwu (2011), Enyiukwu and Awurum (2012) indicated the potency of *P. guineense* seed extracts in the inhibition of spore germination, mycelial elongation, development and spread of *Collectotrichum destructivum* the causal organism of cowpea anthracnose disease, in culture and glass house.

Information on natural ravaging pesticides would be of great benefits to peasant farmers who find synthetic chemical control an expensive option. In addition naturally occuring plant products are non phytotoxic, biodegradable and environment friendly. Having found out that extracts from *P. guineense* and *E. aromatica* could be used to inhibit the development of rice blast disease, would enable farmers to produce rice that can grow better and produce more seeds at lower cost of production. Extracts from *P. guineense* and *E. aromatica* were effective in suppressing leaf blast. Subsequently, rice seeds be treated with n- hexane extracts of *P. guineese* and *E. aromatica* at 100% concentration. These botanicals can serve as bio-pesticides to inhibit the development of *P. oryzae.* Rice seed treatment with extracts of *E. aromatica and P. guineense* could also be integrated with other cultural control methods such as varietal resistance and or early sowing. It is therefore recommended that this experiment be repeated under field conditions to further confirm the bio-efficacy of the botanicals against rice blast disease.

REFERENCES

Asghar, A. H., Ashraf, M. R., Khan, M. H. and Chaudhry, A. Z. (2007) Improvement of basmati rice, against fungal infection through gene transfer technology, *Pakistan Journal of Botany*. 39 (4):1277-83.

Adegoke, G.O and Odesola, B.A. (1996) Storage of maize and cowpea and inhibition of biodeterioration using powder and essential oil lemon grass (*Cymbopogon citratus*). International Journal of Plant Biodeterioration and Biodegradation, 62: 81-84

Agarwal, P.C., Carmen, N. M. and Mathur, S.B. (1989) Seed-borne diseases and seed health testing of rice. Technical Bulletin. Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark and CAB International Mycological Institute, Kew, United Kingdom, 106 pp.

Bateman, G. L., Ehle, H. and Wallace, H. A. H. (1986) Fungicidal treatment of cereals seeds. In: Seed treatment Jeffs (Ed) British Crop Protection Council Publications, Thornton Heath, pp 83-106.

Bèlanger, R. R., Daayf, F. and Schnitt, A. (1995) The effects of plant of Reymoutria sachalinensis on powdery mildew development and leaf physiology of long English cucumber. Plant Disease Sciences, 79:577- 580.

Barnett, H. L. and Hunter, B. B. (1999) *Illustrated Genera of Imperfect Fungi*. (4th Edition). The American Phytopathological Society. St. Paul, Minneosta, USA, 218 pp.

Choi, G. J., Jang, K. S., Kim, J. S., Lee, S. W., Cho, K. Y. & Kim, J. C., (2004). *In vivo* fungal activity of 57 plant extracts against six plant pathogenic fungi. Plant Pathololgy Journal, 20:184-191.

Correll, J.C., Harp, T. L., Guerber, J. C., Zeigles, R. S., Liu, B. and Cartwright, R. D. (2000) Characterization of *Pyricularia grisea* in the United States using independent genetic and molecular markers. *Phytopathology*, 90: 1396-1404.

Enyiukwu, D. N. (2011) Evaluation of the fungitoxic activity of some phytochemicals on the causal agent of anthracnose of cowpea *Colletotrichum destructivum* O'Gara Unpublished MSc Thesis, Department of Plant Health Management, Michael Okpara University of Agriculture Umudike, pp 85-120.

Enyiukwu, D. N. and Awurum, A. N. (2012) Comparative fungitoxicity of benomyl and extracts of *Carica papaya* roots and seeds and *Piper guineense* seeds on *Colletotrichum destrucivum* O'Gara. Continental Journal of Biological Sciences 6 (1):38-42.

Food and Agricultural Organization (FAO), 2000. Agriculture towards 2015/30. Technical Interim Report. April, 2000 Rome.

De Waard, S.K., Georgopoulos, S.G., Hollomon, D.W., Ishii, H ., Leroux, P. N., Ragsdale, N. and Schwinn, F.J. (1993) Chemical control of plant diseases: problems and prospects. Annals of Reviews Phytopathology, 31: 403-421.

Hayes, W. J. and Laws, E. R. (1991) *Handbook of Pesticide Toxicology*, New York, Academic Press, 1576pp.

Imolehin, E.D. (1983) Rice seed-borne fungi and their effect on seed germination. Plant Disease Research, 67: 1334-1336.

IRRI (1996) Standard Evaluation system for Rice, 4th Edition, INGER Genetic Resources Center, International Rice Research Institute, Philippines, 52 pp.

Kuta, D.D (2004) Pathogenic variability and effective management of blast in Nigeria, Rice blast in West Africa: Characterization of pathogen diversity, key screening sites and host resistance. *WARDA Proceedings, p,*15

Kassali, R., Kareem, R.O., Oluwasola, O. and Ohaegbulam,O.M. (2010) Analysis of demand for rice in Ile Ife, Osun State, Nigeria Journal of Sustainable Development in Africa 12 (2): 63-7

Luo, Y. P. S., Tang, N.G., Febellar, D.O. and TeBeest (1998) Risk analysis of yield losses caused by rice leaf blast associated with temperature changes above and below for five Asian countries. *Agricultural Ecosystem and Environment* 68:1970-205.

Mew, T.W., Shahjahan, A. K. M. and Mariappan,V (1986) Diseases and disease management of rainfed lowland rice. In: Progress in rainfed lowland rice, IRRI Los Banos, Laguna, pp 339-348.

Martini, H., Weidenborner, M., Adams, S. and Kunz, B. (1996). Eugenol and carvacol: the main fungicidal compounds in clove and savory. Intercontinental Journal of Food Science, 81:63-67.

Mariappan,V., Rajeswari, E. and Kamalakannan,. A.(1995) Management of rice blast, *Pyricularia oryzae* by using neem (*Azadirachta indica)* amd other plant products. *In:* Mariappan,V. [Ed.] Neem for the Management of Crop Diseases. Associated Publishing Co., New Delhi, India, pp 3-10.

Meena, M.R. and Sethi, V. (1994) Antimicrobial activity of essential oils from spices. Journal of Food. Science. Technology. 31 (1):68-70.

Nguefack, J., Leth,V., Lekagne Dongmo, J.B., Torp, J., Amvam Zollo, P.H. and Nyasse,S. (2008) Use of Three Essential Oils as Seed Treatments against Seed-borne Fungi of Rice (Oryza sativa L.). American-Eurasian Journal of Agriculture and Environmental Sciences, 4 (5): 554-560.

Netam, R.S., Bahadur, A.N., Tiwari, U. and Tiwari, R.K.S. (2011) Efficacy of plant extracts for the Control of (Pyricularia grisea) Blast of Rice under Field Condition of Bastar, Chhattisgarh. Research Journal of Agricultural Sciences, 2 (2): 269-271.

Ou S H. (1985) Rice diseases. 2nd edition. Commonwealth Mycological Institute Kew, Survey, England, 380 pp.

Onyeke, C.C and Ugwuoke, K.I. (2011) Effects of botanical extracts on the mycelial growth of seed-borne fungi of the african yam bean, Sphenostylis stenocarpa (Hochst ex a. Rich) Harms. Nigerian Journal of Biotechnology, 22: 1- 7.

Suprapta, D. N. and Khalim, K. (2009) Effects of plant extract formulation to suppress stem rot disease of vanilla seedlings. ISSA Journal. (15):34-41.

Singh, B. N., Fagade, S., Ukwungwu, M. N., Williarn, L., Jagtap, S. S., Oladimeji, O., Effisue, A. and Okhidievbie, O. (1997) Rice growing environments and biophysical constraints in different agroecological zones of Nigeria. Metrology Journal, 2 (1): 35-44.

Suleman, M. N. and Emua, S. A. (2009) Efficacy of four plant extracts in the control of root rot disease of cowpea. African Journal of Biotechnology, 8 (16):3806-3808.

Srivastava,B.,Singh,P.,Shukla,R.,Dubey,N.K.(2009) Alatoxin inhibition by *Arbabotrys odoratissimus*.Advances in Environmental Biology.,3(3):219-225.

Tagne, A., Nguefack, J., The, C. and Amvam Zollo, P.H. (2000) Natural control of fungi and mycotoxin grains- means of reducing human and animal contamination. Journal of Applied Science. Southern Africa, 6: 37-44.

Wokocha, R.C and Uchendu, B.E. (2009) Antifungal activity of *Garcinia kola* extract and Benlate on *Cercospora arachidis,* incitant of the early leaf spot disease of groundnut in Umudike. Nigerian Journal of Plant Protection.23:162-167.

WARDA (1996) Rice trends in sub Saharan Africa: A synthesis of statistics on rice production, trade and consumption (1973 – 1992), West Africa Rice Development Association, Bouake, Cote d' Ivoire.