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Complete inhibition of mycelial growth of fungal pathogens of maize by botanicals

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Rice husk extract (RHE), bamboo extract (BE) and wood extract (WE) at different concentration levels (0.0, 0.1, 0.5, 1.0 and 1.5%) were evaluated (in completely randomized design) *in vitro* as an antagonist to mycelial growth of selected fungal pathogens of maize. Botanical preparations were utilized singly (rice husk, bamboo and wood extracts) and in combinations (RHE x BE, RHE x WE, BE x WE and RHE x BE x WE). RHE at 1.0% concentration and in combination- RHE x BE x WE at 1.5% concentration, completely inhibited mycelial growth of *Fusarium solani, Fusarium equiseti, Fusarium verticilloides* and *Macrophomina phaseolina*. Other botanical preparations, either singly or in combinations showed significant ($p<0.05$) reduction in mycelial growth of the fungal pathogens. Thus, these botanicals have phytofungicidal potentials towards controlling pathogenic fungi of maize, hence, could be useful in the control and management of maize diseases on large scale farming.

Key words: Rice husk, bamboo, wood, extract, maize, mycelial growth, fungal pathogens.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereals in the world after wheat and rice with regards to cultivation area and total production (Akinbode, 2010). Diseases have been a major constraint to maize production, it reduce the value and quality of maize grains produced (Lamprecht et al., 2008) and may definitely increase the cost of harvesting. There are diverse diseases of maize consisting of seed rots and seedling blights (Crous et al., 2006), Northern corn leaf blight, Anthracnose, *Pythium* and *Fusarium* root rot and Southern rust (Gautam and Stein, 2011) among others. Various approaches have been used over many decades to control maize diseases such as breeding for resistance and chemical pesticides (Tagne et al., 2008).

The problems of chemical pesticides are resistance, pest resurgence, environmental pollution and risks to human health. Most of the pesticides and inorganic fertilizers are not environmentally friendly, apart from the

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fact that health hazards may loom as a result of the consumption of their residues in food, these agrochemicals are expensive and may not be available for farmers use when needed (Oyekanmi et al., 2008). In view of this, national and international bodies have raised a global call to promote maize production through biological approaches, being environmentally friendly and cost-effective (Abiala et al., 2011).

Biological control is on the increase but the use of natural bioprotectants like botanical extracts has not really received significant attention. Therefore, encouraging the use of botanical extracts as a promising alternative is a good step towards controlling and managing fungal pathogens of maize in Nigeria. Rice husk, bamboo and wood extracts have been used singly and reported to be effective on mycelial growth of *Mycosphaerella fijiensis* Morelet (Abiala et al., 2011). Similarly, rice husk extract alone was also reported by Killani et al. (2011) to be effective in the laboratory and on the field on pathogenic fungi isolated from rhizosphere soil of cowpea. To further establish the activities of rice husk, bamboo and wood extracts, we therefore focused this research work on *in vitro* effects of these botanical extracts (singly and in combination) on mycelial growth of fungal pathogens of maize prior to field application.

MATERIALS AND METHODS

Source of fungal pathogens and botanical extracts

Fusarium oxysporum, Fusarium solani and *Fusarium equiseti* were obtained from Plant Pathology Unit, Department of Botany, University of Ibadan, while *Macrophomina phaseolina*, *Curvularia lunata*, *Drechslera* sp., *Fusarium verticilloides* and *Bipolaris maydis* were obtained from the Plant Pathology Unit of Institutes of Agricultural Research and Training, Ibadan. The botanical extracts: rice husk, wood and bamboo were obtained from Dr. H. Kikuno of Plant Physiology Unit, International Institute of Tropical Agriculture (IITA) Ibadan.

Evaluation of botanical extracts

One liter potato dextrose (PD) agar (39 g/l) was prepared in media bottle and dispensed at varying volumes of 100, 99.9, 99.5, 99.0 and 98.5ml into 250-ml sterile conical flasks. The contents were sterilized in the autoclave at a temperature of 121°C for 15 min at 1.2 bars. After autoclaving, the medium was allowed to cool to the temperature of 45°C. Equal volumes of rice husk, bamboo, and wood extracts were utilized in different treatment combinations: Rice husk extract (RHE) x bamboo extract (BE), RHE x wood extract (WE), BE x WE and RHE x BE x WE. Thereafter, 0.0, 0.1, 0.5, 1.0 and 1.5 ml of each of the botanical extracts singly or in combinations were aseptically pipetted with a calibrated 250 ml pipette into sterilized PDA medium to represent concentration of 0, 0.1, 0.5, 1.0, and 1.5%, respectively. These were slowly mixed together by rolling each bottle in the palm to allow homogenous mixture of medium and the extract. Fifteen milliliters (15 ml) of this mixture was poured into 9 cm sterile disposable Petri dishes and allowed to solidify at room temperature inside the laminar flow hood. Mycelial discs of young actively growing cultures of each pathogen was cut separately with a sterile cork borer and inoculated at the center of already prepared plates containing the mixture (botanical extracts + medium) and the control plates (medium alone). The experiment was carried out in three replicates. The plates were incubated at 28±2°C and periodically observed in 3 day intervals for nine days to allow antagonist-pathogen interactions.

Data collection

Laboratory data were collected on the 3rd, 6th and 9th day. The mycelial growth diameter (cm) of each pathogen was measured and the percentage of growth inhibition was calculated according to Odebode et al. (2004) as follows:

$$\text{Growth inhibition (\%)} = \frac{(D_o - D_t) \times 100}{D_o}$$

Where D_o = Diameter of mycelial growth of fungal pathogen in the control plates; D_t = diameter of mycelial growth of fungal pathogen in the treatment plates.

Statistical analysis

All statistical analyses were performed using SAS System for Windows Version 9.1 (2009). The data collected were analyzed using the analysis of variance (ANOVA) procedures and the least significant difference test (LSD) at p=0.05 was used to compare treatment means for each parameter.

RESULTS

RHE significantly ($p<0.05$) reduced the mycelial growth of *F. verticillioides*, *M. phaseolina*, *F. equiseti* and *F. oxysporium* in comparison with the control. At day 3, RHE at 1.5% concentration was less effective on mycelial growth of *B. maydis* as compared to other fungal pathogens that were completely inhibited, though varied at days 6 and 9 with respect to mycelial growth of *C. lunata* (1.33 cm) and *Dreschlera* sp. (5.13 cm) (Table 1). BE was not effective on all the fungal pathogens at both 0.1 and 0.5% concentration levels. Furthermore, their mycelial growth at 0.5 and 1.0% were almost the same with their control at days 3, 6 and 9 of observation. The effect of BE on *C. lunata* was extremely discouraging at concentration levels of 0.5, 1.0 and 1.5%. BE was only effective at higher concentration (1.5%) on mycelial growth of *Dreschlera* sp., *F. verticillioides*, *M. phaseolina*, *F. equiseti* and *F. solani* (Table 2). Similarly, WE was not effective on the fungal pathogens at lower concentrations (0.1, 0.5 and 1.0%). The effect of WE at 1.0% was not different from that of 1.5% concentration. Observation at day 6 revealed that there was no comparative effect of WE on the fungal pathogens. Even at day 9, WE had no significant ($p<0.05$) effect on *B. maydis*, *C. lunata, Dreschlera* sp., *M. phaseolina*, *F. solani* or *F. oxysporium* (Table 3).

Table 1. Effect of rice husk extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	3.23±0.58^{a}	2.10±0.00^{b}	1.97±0.06^{b}	0.53±0.46^{c}	0.53±0.46^{c}
	Curvularia lunata	3.33±0.06^{a}	2.03±0.12^{b}	1.87±0.15^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Dreschlera sp.	4.23±0.55^{a}	3.80±0.20^{a}	2.50±0.10^{b}	1.67±0.15^{c}	0.00±0.00^{d}
	Fusarium verticilloides	1.37±0.35^{a}	1.00±0.10^{b}	0.90±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Macrophomina phaseolina	2.03±0.06^{a}	1.83±0.06^{a}	1.60±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium equiseti	2.27±0.12^{a}	1.80±0.10^{b}	1.50±0.00^{b}	0.30±0.52^{c}	0.00±0.00^{c}
	Fusarium solani	0.87±0.12^{a}	0.23±0.40^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
	Fusarium oxysporum	2.23±0.56^{a}	1.97±0.58^{b}	1.77±0.06^{c}	0.90±0.00^{d}	0.00±0.00^{e}
	LSD	0.42	0.30	0.12	0.44	0.28
Day 6	*Bipolaris maydis*	5.63±1.39^{a}	3.20±0.00^{b}	2.90±0.10^{bc}	1.63±0.55^{c}	1.63±0.55^{c}
	Curvularia lunata	6.80±0.10^{a}	3.80±0.20^{b}	3.80±0.20^{b}	0.53±0.46^{c}	0.53±0.46^{c}
	Dreschlera sp.	7.97±0.42^{a}	7.67±0.32^{a}	5.27±0.12^{b}	4.17±0.12^{b}	2.27±0.15^{c}
	Fusarium verticilloides	4.90±0.10^{a}	3.47±0.06^{b}	1.77±0.21^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Macrophomina phaseolina	3.67±0.58^{a}	3.83±0.06^{b}	3.40±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium equiseti	4.37±0.06^{a}	3.23±0.23^{b}	2.73±0.12^{c}	1.83±0.21^{d}	0.00±0.00^{e}
	Fusarium solani	2.20±0.26^{a}	1.93±0.15^{a}	0.60±0.52^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium oxysporum	4.9±0.10^{a}	4.03±0.06^{a}	3.67±0.15^{b}	2.40±0.1.^{c}	0.00±0.00^{d}
	LSD	0.91	0.29	0.39	0.47	0.31
Day 9	*Bipolaris maydis*	7.13±0.35^{a}	4.83±0.06^{b}	4.83±0.47^{b}	2.70±0.78^{b}	2.70±0.78^{b}
	Curvularia lunata	8.50±0.00^{a}	6.27±0.21^{b}	4.83±0.47^{c}	1.03±0.06^{d}	1.33±0.49^{d}
	Dreschlera sp.	8.40±0.17^{a}	8.03±0.15^{a}	8.00±0.10^{a}	6.37±0.47^{b}	5.13±0.15^{c}
	Fusarium verticilloides	8.07±0.15^{a}	6.67±0.12^{b}	5.03±0.15^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Macrophomina phaseolina	6.10±0.20^{a}	5.93±0.06^{d}	5.47±0.06^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium equiseti	7.77±0.06^{a}	5.33±0.23^{b}	4.43±0.12^{c}	3.43±0.25^{d}	0.00±0.00^{e}
	Fusarium solani	3.07±0.12^{a}	2.80±0.44^{a}	2.27±0.35^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium oxysporum	8.07±0.15^{a}	7.70±0.15^{b}	6.50±0.10^{c}	4.97±0.15^{d}	0.00±0.00^{e}
	LSD	0.31	0.36	0.49	0.59	0.57

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

Considering combinations of botanical extracts, there was no complementary effect of RHE x BE at 0.1 and 0.5% levels on mycelial growth of *M. phaseolina*, *F. equiseti* and *F. solani* in comparison with *B. maydis* and other fungal pathogens. At day 6, a clear distinction on the effectiveness of RHE x BE at 0.5 and 1.0% concentration with respect to mycelial growth of all the fungal pathogens was observed. Further observation showed that at day 9, RHE x BE at 1.5% concentration completely inhibited the mycelial growth of *C. lunata*, *F. verticillioides*, *F. equiseti* and *F. oxysporium* (Table 4). Activity of RHE x WE significantly ($p<0.05$) varied on the fungal pathogens most especially at 0.1, 0.5 and 1.0% concentration. RHE x WE at 1.5% concentration, completely inhibited the mycelial growth of all the fungal pathogens with the exception of *F. solani*. Observation also showed that, RHE x WE at 1.0% concentration consistently maintained complete mycelial growth inhibition of *Dreschlera* sp. and *F. equiseti* from days 3 to 9 (Table 5). The effect of BE x WE on *C. lunata* were significantly ($p<0.05$) similar at concentrations of 0.5, 1.0 and 1.5%. Observation at day 6 and 9 showed that *F. oxysporium* defiled BE x WE at all the concentration levels. However, an outstanding mycelial growth reduction was recorded for *C. lunata* (3.40 cm) as compared to the control (7.30 cm) (Table 6). The effect of RHE *x* BE *x* WE on the fungal pathogens was highly encouraging most especially at 1.0 and 1.5% concentration levels.

Table 2. Effect of bamboo extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	3.83±0.06^{a}	2.90±0.10^{b}	2.80±0.17^{b}	2.27±0.12^{c}	2.08±0.06^{d}
	Curvularia lunata	2.67±0.15^{a}	2.47±0.06^{b}	2.47±0.06^{b}	2.33±0.06^{bc}	2.23±0.06^{c}
	Dreschlera sp.	2.63±0.58^{a}	2.30±0.20^{b}	2.27±0.15^{b}	1.63±0.25^{c}	1.23±0.06^{d}
	Fusarium verticilloides	1.93±0.57^{a}	1.80±0.10^{b}	1.70±0.00^{b}	1.47±0.06^{c}	1.23±0.06^{d}
	Macrophomina phaseolina	2.40±0.10^{a}	2.33±0.21^{a}	2.00±0.06^{b}	1.87±0.06^{b}	1.53±0.21^{c}
	Fusarium equiseti	2.10±0.10^{a}	1.90±0.10^{b}	1.63±0.06^{c}	1.17±0.06^{d}	1.00±0.10^{e}
	Fusarium solani	2.33±0.56^{a}	2.37±0.06^{a}	2.33±0.21^{a}	2.01±0.20^{a}	1.53±0.12^{b}
	Fusarium oxysporum	2.87±0.06^{a}	2.63±0.06^{b}	2.60±0.00^{b}	2.47±0.12^{c}	2.23±0.06^{d}
	LSD	0.15	0.21	0.19	0.23	0.18
Day 6	*Bipolaris maydis*	5.70±0.17^{a}	5.07±0.25^{b}	4.73±0.12^{c}	4.43±0.06^{d}	3.60±0.10^{e}
	Curvularia lunata	4.70±0.20^{a}	4.77±0.15^{a}	4.03±0.12^{b}	3.97±0.12^{b}	3.97±0.15^{b}
	Dreschlera sp.	4.90±0.10^{a}	4.47±0.15^{b}	4.33±0.21^{b}	2.77±0.25^{c}	2.80±0.17^{c}
	Fusarium verticilloides	4.27±0.12^{a}	4.10±0.10^{ab}	3.80±0.34^{bc}	3.47±0.15^{c}	2.80±0.10^{d}
	Macrophomina phaseolina	4.57±0.21^{a}	4.47±0.25^{a}	4.30±0.87^{a}	4.17±0.06^{a}	3.73±0.12^{b}
	Fusarium equiseti	3.35±0.15^{a}	3.20±0.20^{ab}	2.97±0.06^{b}	2.43±0.12^{c}	1.90±0.30^{d}
	Fusarium solani	4.63±0.06^{a}	4.67±0.21^{a}	4.33±0.21^{ab}	4.27±0.15^{b}	2.83±0.21^{c}
	Fusarium oxysporum	4.93±0.06^{a}	4.83±0.06^{a}	4.60±0.00^{b}	4.37±0.12^{c}	3.97±0.15^{d}
	LSD	0.25	0.32	0.61	0.24	0.30
Day 9	*Bipolaris maydis*	7.87±0.25^{a}	7.10±0.17^{b}	6.80±0.10^{c}	6.13±0.00^{d}	2.57±0.15^{e}
	Curvularia lunata	6.40±0.10^{a}	6.43±0.49^{a}	5.57±0.15^{b}	5.37±0.12^{b}	5.23±0.06^{b}
	Dreschlera sp.	7.10±0.20^{a}	6.80±0.17^{a}	6.43±0.12^{b}	5.07±0.31^{c}	4.77±0.12^{c}
	Fusarium verticilloides	7.00±0.10^{a}	6.80±0.10^{a}	6.10±0.95^{ab}	6.07±0.49^{ab}	2.27±0.21^{b}
	Macrophomina phaseolina	7.80±0.20^{a}	7.57±0.25^{ab}	7.00±0.52^{bc}	6.63±0.06^{bc}	6.53±0.12^{c}
	Fusarium equiseti	5.80±0.10^{a}	5.63±0.15^{a}	5.43±0.06^{a}	4.43±0.12^{b}	3.10±0.50^{c}
	Fusarium solani	6.93±0.06^{a}	6.83±2.51^{a}	6.43±0.12^{b}	6.20±0.20^{b}	4.97±0.15^{c}
	Fusarium oxysporum	6.97±0.12^{a}	6.80±0.10^{b}	6.60±0.00^{c}	6.03±0.06^{d}	5.83±0.12^{e}
	LSD	0.27	0.42	0.86	0.46	0.38

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

RHE x BE x WE completely inhibited all the fungal pathogens at both the lower (0.1 and 0.5%) and higher (1.0 and 1.5%) levels of concentration throughout the days of observation (Table 7).

Generally, the concentration levels (0.1, 0.5, 1.0 and 1.5%) of the botanical extracts were significantly ($p<0.05$) effective on the fungal pathogens in order of effectiveness RHE > BE > WE. Complete mycelial inhibition was recorded for RHE at 1.5% concentrations on *M. phaseolina*, *F. solani* and *F. verticillioides* (Table 8) and justified that RHE alone and in combinations: RHE x BE, RHE x WE and RHE x BE x WE were observed as the best botanical extracts.

DISCUSSION

Eco-friendly approaches for plant disease management have been exploited worldwide as observed in this study. The bio-assay test at different levels of concentration (0.1, 0.5, 1.0 and 1.5%) is not far-fetched from a number of reports showing the efficacy of botanical extracts (Joshi et al., 2011; Manasathein et al., 2011). This may as well be similar to the report of Odebode et al. (2004) that plants are known to produce a variety of secondary metabolites, which are bioactive and thus may have inhibitory effects on bacteria, fungi, insects and other microorganisms. The effectiveness of the botanical extracts was observed to be dependent on the concentration used. This agreed with the work of Anamika and Simon (2011) that showed botanical extracts were effective at higher concentrations on *Alternaria alternata* of *Aloe vera* dry rot.

The rice husk extract alone showed inhibitory effect on mycelial growth of the fungal pathogens even at low

Table 3. Effect of wood extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	3.13±0.06^{a}	3.10±0.10^{a}	2.57±0.06^{b}	2.47±0.06^{b}	2.23±0.06^{c}
	Curvularia lunata	2.97±0.15^{a}	2.90±0.10^{ab}	2.63±0.15^{bc}	2.43±0.21^{c}	2.07±0.21^{d}
	Dreschlera sp.	2.83±0.06^{a}	2.77±0.15^{a}	2.70±0.26^{a}	2.37±0.23^{b}	2.00±0.10^{c}
	Fusarium verticilloides	2.37±0.15^{a}	2.43±0.06^{a}	2.43±0.06^{a}	1.97±0.23^{b}	1.67±0.21^{c}
	Macrophomina phaseolina	2.63±0.06^{a}	2.53±0.15^{ab}	2.43±0.06^{b}	2.07±0.06^{c}	1.90±0.10^{c}
	Fusarium equiseti	2.33±0.06^{a}	2.17±0.06^{a}	2.17±0.06^{a}	1.90±0.10^{a}	0.70±0.66^{b}
	Fusarium solani	2.50±0.00^{a}	2.43±0.06^{a}	2.43±0.06^{a}	2.10±0.10^{b}	2.03±0.06^{b}
	Fusarium oxysporum	2.43±0.06^{a}	2.40±0.00^{b}	2.17±0.06^{b}	2.00±0.10^{c}	1.80±0.10^{d}
	LSD	0.15	0.17	0.20	0.26	0.44
Day 6	*Bipolaris maydis*	5.60±0.17^{a}	5.53±0.25^{a}	4.83±0.06^{b}	4.73±0.12^{b}	4.37±0.12^{c}
	Curvularia lunata	5.00±0.20^{a}	4.87±0.32^{ab}	4.77±0.15^{ab}	4.73±0.38^{ab}	4.37±0.32^{b}
	Dreschlera sp.	5.00±0.10^{a}	4.93±0.15^{a}	4.73±0.31^{ab}	4.47±0.23^{b}	4.07±0.15^{c}
	Fusarium verticilloides	5.93±0.21^{a}	4.20±0.00^{ab}	4.77±0.06^{b}	4.33±0.29^{c}	3.63±0.15^{d}
	Macrophomina phaseolina	5.57±0.12^{a}	5.47±0.21^{a}	5.37±0.06^{a}	4.80±0.00^{b}	4.20±0.30^{c}
	Fusarium equiseti	4.30±0.10^{a}	4.03±0.06^{a}	4.04±0.06^{a}	3.80±0.20^{a}	2.43±1.16^{b}
	Fusarium solani	5.90±0.00^{a}	4.47±0.21^{b}	5.37±0.06^{b}	4.37±0.12^{c}	3.90±0.10^{d}
	Fusarium oxysporum	5.27±0.12^{a}	5.20±0.00^{a}	4.97±0.06^{a}	4.03±0.55^{b}	3.30±0.10^{c}
	LSD	0.24	0.32	0.23	0.49	0.78
Day 9	*Bipolaris maydis*	7.70±0.17^{a}	7.70±0.20^{a}	6.87±0.06^{b}	6.43±0.12^{c}	6.03±0.06^{d}
	Curvularia lunata	7.23±0.31^{a}	7.17±0.32^{a}	7.10±0.17^{a}	7.07±0.25^{a}	6.07±0.84^{b}
	Dreschlera sp.	7.03±0.25^{a}	6.93±0.15^{a}	6.90±0.10^{a}	6.17±0.15^{b}	5.97±0.15^{b}
	Fusarium verticilloides	7.33±0.21^{a}	7.53±0.06^{a}	6.90±0.10^{b}	6.13±0.12^{c}	5.50±0.10^{d}
	Macrophomina phaseolina	7.87±0.06^{a}	7.67±0.21^{ab}	7.57±0.06^{b}	6.63±0.06^{c}	5.87±0.25^{d}
	Fusarium equiseti	7.57±0.49^{a}	6.67±0.12^{b}	6.53±0.06^{b}	5.77±0.15^{c}	4.57±0.61^{d}
	Fusarium solani	8.00±0.00^{a}	7.67±0.21^{b}	7.57±0.06^{b}	6.33±0.21^{c}	5.73±0.21^{d}
	Fusarium oxysporum	7.63±0.15^{a}	7.53±0.06^{a}	7.13±0.12^{a}	6.46±0.67^{b}	5.40±0.10^{c}
	LSD	0.43	0.32	0.17	0.48	0.68

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = Least Significant Difference.

Table 4. Combined effect of rice husk and bamboo extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	3.20±0.00^{a}	2.80±0.00^{b}	2.43±0.06^{c}	1.27±0.06^{d}	0.00±0.00^{e}
	Curvularia lunata	2.93±0.12^{a}	2.67±0.12^{a}	1.83±0.40^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Dreschlera sp.	3.03±0.25^{a}	2.90±0.17^{a}	2.10±0.10^{b}	1.47±0.15^{c}	0.00±0.00^{d}
	Fusarium verticilloides	2.80±0.10^{a}	2.87±0.12^{a}	1.73±0.38^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Macrophomina phaseolina	2.20±0.10^{a}	2.20±0.10^{a}	2.17±0.06^{a}	1.63±0.06^{b}	0.97±0.15^{c}
	Fusarium equiseti	2.17±0.06^{a}	2.13±0.12^{a}	1.77±0.15^{a}	0.27±0.46^{b}	0.00±0.00^{b}
	Fusarium solani	2.23±0.38^{a}	2.10±0.00^{a}	2.03±0.15^{a}	1.13±0.11^{b}	0.00±0.00^{c}
	Fusarium oxysporum	2.23±0.06^{a}	2.10±0.10^{b}	2.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	LSD	0.30	0.18	0.37	0.31	0.09

Table 4. Contd.

Day 6	*Bipolaris maydis*	5.47±0.06^{a}	5.27±0.12^{a}	4.37±0.31^{b}	2.93±0.12^{c}	0.00±0.00^{d}
	Curvularia lunata	3.97±0.25^{a}	4.27±0.12^{a}	3.73±0.15^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Dreschlera sp.	5.23±0.25^{a}	5.13±0.32^{a}	4.20±0.10^{b}	3.43±0.15^{c}	0.67±0.61^{d}
	Fusarium verticilloides	6.10±0.26^{a}	5.70±0.17^{a}	4.67±0.15^{b}	1.40±0.69^{c}	0.00±0.00^{d}
	Macrophomina phaseolina	5.57±0.06^{a}	5.30±0.26^{ab}	4.97±0.25^{b}	4.10±0.10^{c}	3.17±0.15^{d}
	Fusarium equiseti	5.67±0.06^{a}	5.33±0.06^{a}	4.47±0.06^{b}	2.47±0.72^{c}	0.00±0.00^{d}
	Fusarium solani	5.97±0.55^{a}	5.33±0.67^{ab}	5.07±0.12^{b}	3.70±0.26^{c}	2.43±0.40^{d}
	Fusarium oxysporum	5.53±0.06^{a}	5.03±0.06^{b}	5.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	LSD	0.44	0.50	0.26	0.65	0.46
Day 9	*Bipolaris maydis*	7.33±0.21^{a}	7.50±0.10^{a}	6.83±0.12^{a}	5.47±0.38^{b}	1.63±1.42^{c}
	Curvularia lunata	7.80±0.40^{a}	7.63±0.06^{a}	7.00±0.17^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Dreschlera sp.	7.30±0.20^{a}	6.87±0.45^{ab}	6.20±0.20^{b}	6.20±0.36^{b}	2.57±0.95^{c}
	Fusarium verticilloides	7.47±0.49^{a}	7.60±0.10^{a}	6.80±0.10^{a}	2.93±0.93^{b}	0.00±0.00^{c}
	Macrophomina phaseolina	7.37±0.06^{a}	7.30±0.10^{a}	6.87±0.32^{b}	6.07±0.12^{c}	4.90±0.20^{d}
	Fusarium equiseti	7.27±0.32^{a}	7.00±0.17^{a}	6.70±0.62^{a}	3.93±1.35^{b}	0.00±0.00^{c}
	Fusarium solani	7.67±0.72^{a}	7.37±0.67^{a}	7.30±0.26^{a}	5.93±0.42^{b}	4.37±0.50^{c}
	Fusarium oxysporum	7.30±0.10^{a}	6.70±0.10^{a}	6.60±0.10^{a}	1.17±1.01^{b}	0.00±0.00^{c}
	LSD	0.65	0.52	0.50	1.25	1.11

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

Table 5. Combined effect of rice husk and wood extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	**0.1**	**0.5**	**1.0**	**1.5**
Day 3	*Bipolaris maydis*	2.47±0.06^{a}	2.27±0.06^{a}	2.17±0.15^{b}	1.23±0.06^{b}	0.00±0.00^{c}
	Curvularia lunata	2.53±0.12^{a}	2.40±0.00^{a}	2.30±0.00^{a}	0.73±0.64^{b}	0.00±0.00^{c}
	Dreschlera sp.	2.63±0.06^{a}	2.23±0.12^{b}	2.23±0.06^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium verticilloides	2.17±2.07^{a}	2.07±0.06^{b}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}
	Macrophomina phaseolina	2.67±0.06^{a}	2.40±0.00^{b}	1.70±0.00^{c}	1.47±0.06^{d}	0.00±0.00^{e}
	Fusarium equiseti	3.17±0.15^{a}	2.83±0.1^{b}	0.30±0.52^{c}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium solani	2.80±2.47^{a}	2.47±0.25^{b}	2.20±0.10^{c}	2.03±0.06^{c}	1.00±0.10^{d}
	Fusarium oxysporum	2.83±0.0^{a}	2.53±0.06^{a}	2.30±0.20^{a}	0.77±0.67^{b}	0.00±0.00^{c}
	LSD	0.15	0.21	0.36	0.57	0.06
Day 6	*Bipolaris maydis*	5.03±0.06^{a}	3.77±0.15^{b}	3.43±0.06^{c}	2.23±0.12^{d}	0.00±0.00^{e}
	Curvularia lunata	5.17±0.15^{a}	4.50±0.00^{b}	4.40±0.00^{b}	2.77±0.68^{c}	1.37±0.06^{d}
	Dreschlera sp.	5.43±0.06^{a}	4.87±0.06^{b}	4.57±0.12^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium verticilloides	4.67±0.15^{a}	4.40±0.10^{a}	2.27±0.21^{b}	1.50±0.87^{c}	0.00±0.00^{c}
	Macrophomina phaseolina	5.43±0.32^{a}	4.90±0.00^{b}	3.90±0.00^{c}	3.57±0.06^{d}	0.00±0.00^{e}
	Fusarium equiseti	5.83±0.32^{a}	5.37±0.15^{b}	1.63±0.32^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium solani	4.93±0.32^{a}	4.70±0.26^{a}	4.13±0.15^{b}	4.00±0.10^{b}	2.07±0.21^{c}
	Fusarium oxysporum	5.43±0.06^{a}	5.46±0.06^{a}	5.03±0.15^{a}	2.87±1.01^{b}	2.67±0.06^{b}
	LSD	0.34	0.22	0.28	0.92	0.14
Day 9	*Bipolaris maydis*	6.87±0.12^{a}	5.30±0.10^{b}	4.87±0.12^{c}	4.33±0.12^{d}	0.00±0.00^{e}
	Curvularia lunata	6.97±0.15^{a}	6.30±0.00^{b}	5.80±0.00^{c}	4.03±0.31^{d}	3.40±0.10^{e}
	Dreschlera sp.	7.83±0.06^{a}	6.73±0.12^{b}	6.07±0.12^{c}	0.00±0.00^{d}	0.00±0.00^{d}

Table 5. Cont.

Fusarium verticilloides	6.90±0.10[a]	6.47±0.06[a]	1.73±0.23[b]	0.90±1.01[b]	0.00±0.00[c]
Macrophomina phaseolina	7.10±0.10[a]	6.50±0.00[b]	5.43±0.06[c]	4.37±0.12[d]	0.00±0.00[e]
Fusarium equiseti	7.83±0.21[a]	7.10±0.26[b]	2.57±0.38[c]	0.00±0.00[d]	0.00±0.00[d]
Fusarium solani	7.00±0.10[a]	6.20±0.26[b]	5.73±0.21[c]	5.47±0.12[c]	2.93±0.35[d]
Fusarium oxysporum	7.10±0.10[a]	6.80±0.10[a]	6.30±0.20[a]	4.30±0.95[b]	4.33±0.12[b]
LSD	0.21	0.26	0.34	0.88	0.23

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

Table 6. Combined effect of bamboo and wood extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	**0.1**	**0.5**	**1.0**	**1.5**
Day 3	*Bipolaris maydis*	2.60±0.10[a]	2.56±0.15[a]	1.90±0.10[a]	1.43±0.12[b]	1.73±0.12[c]
	Curvularia lunata	2.43±0.12[a]	2.27±0.12[a]	1.50±0.17[b]	1.33±0.15[b]	1.36±0.06[b]
	Dreschlera sp.	3.47±0.12[a]	3.20±0.20[a]	2.37±0.06[b]	2.20±0.10[bc]	2.07±0.21[c]
	Fusarium verticilloides	3.73±0.12[a]	3.60±0.10[a]	2.33±0.21[b]	1.90±0.00[c]	1.17±0.12[d]
	Macrophomina phaseolina	2.30±0.10[a]	2.20±0.00[a]	2.80±0.20[b]	2.67±0.25[b]	1.77±0.06[c]
	Fusarium equiseti	2.80±0.10[a]	2.50±0.10[b]	2.40±0.10[b]	2.40±0.00[b]	1.43±0.06[c]
	Fusarium solani	2.60±0.10[a]	2.47±0.15[a]	2.10±0.10[b]	2.00±0.10[b]	1.73±0.12[c]
	Fusarium oxysporum	2.73±0.21[a]	2.57±0.12[a]	3.17±1.42[a]	3.20±1.48[a]	1.93±0.06[b]
	LSD	0.21	0.22	0.89	0.93	0.19
Day 6	*Bipolaris maydis*	4.70±0.10[a]	4.63±0.46[a]	3.90±0.30[b]	3.73±0.12[b]	2.90±0.20[c]
	Curvularia lunata	4.93±0.12[a]	3.73±0.01[a]	3.70±0.17[b]	3.33±0.15[b]	3.67±0.06[b]
	Dreschlera sp.	5.90±0.00[a]	5.87±0.90[b]	5.23±0.12[c]	5.07±0.15[c]	4.13±0.25[cd]
	Fusarium verticilloides	5.53±0.12[a]	4.57±1.01[a]	4.90±0.26[a]	4.33±0.15[ab]	3.03±0.15[c]
	Macrophomina phaseolina	5.53±0.06[a]	5.37±0.15[a]	5.17±0.21[a]	4.50±0.30[b]	3.80±0.44[c]
	Fusarium equiseti	5.70±0.10[a]	5.45±0.25[a]	5.37±0.06[a]	5.37±0.06[a]	3.20±0.26[b]
	Fusarium solani	5.27±0.06[a]	5.23±0.06[a]	4.90±0.26[ab]	4.73±0.23[bc]	4.47±0.25[c]
	Fusarium oxysporum	5.77±0.15[a]	5.67±0.83[a]	5.13±0.85[a]	5.48±0.96[a]	4.47±0.25[a]
	LSD	0.17	1.20	0.62	0.66	0.44
Day 9	*Bipolaris maydis*	6.90±0.10[a]	6.93±0.12[a]	5.87±0.31[b]	5.53±0.31[c]	5.43±0.15[c]
	Curvularia lunata	7.30±0.17[a]	5.63±0.15[b]	5.47±0.12[b]	5.13±0.15[c]	3.40±0.10[c]
	Dreschlera sp.	7.90±0.00[a]	6.57±0.25[b]	6.23±0.12[d]	6.90±0.36[b]	6.00±0.10[d]
	Fusarium verticilloides	5.57±0.06[c]	6.67±0.12[a]	6.27±0.23[b]	5.90±0.17[d]	4.97±0.15[e]
	Macrophomina phaseolina	7.23±0.12[a]	7.23±0.21[a]	7.03±0.15[ab]	6.43±0.15[b]	4.93±0.67[c]
	Fusarium equiseti	7.43±0.12[a]	7.33±0.12[a]	6.97±0.40[a]	7.10±0.10[a]	4.37±0.40[b]
	Fusarium solani	7.00±0.10[a]	6.97±0.06[a]	6.27±0.23[b]	6.00±0.02[b]	6.07±025[b]
	Fusarium oxysporum	7.40±0.10[a]	6.63±1.03[a]	6.37±0.95[a]	7.03±0.68[a]	6.03±0.38[a]
	LSD	0.18	0.68	0.70	0.48	0.57

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

concentration. Similar observation of rice husk extract has been reported by Abiala et al. (2011) on *Mycosphaerella fijensis*. This suggests that the rice husk extract may contain some acidic compounds (Killani et al., 2011)

Table 7. Combined effect of rice husk, bamboo and wood extract on pathogenic fungi of maize.

Days	Treatment	Mycelial mean growth (cm)				
		Concentration (%)				
		0.0	0.1	0.5	1.0	1.5
Day 3	*Bipolaris maydis*	2.47±0.06^{a}	2.33±0.06^{b}	2.03±0.12^{c}	1.53±0.06^{d}	0.00±0.00^{e}
	Curvularia lunata	2.60±0.10^{a}	2.20±0.00^{b}	1.57±0.06^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Dreschlera sp.	2.53±0.12^{a}	2.13±0.12^{b}	1.90±0.10^{b}	0.17±2.25^{c}	0.00±0.00^{d}
	Fusarium verticilloides	2.33±0.06^{a}	2.06±0.12^{a}	1.73±0.31^{a}	0.83±0.76^{b}	0.00±0.00^{c}
	Macrophomina phaseolina	2.07±0.31^{a}	1.80±0.20^{a}	1.47±0.12^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	Fusarium equiseti	2.93±0.12^{a}	2.67±0.12^{a}	1.70±0.20^{b}	0.83±0.72^{c}	0.00±0.00^{d}
	Fusarium solani	2.47±0.06^{a}	2.07±0.06^{b}	1.83±0.06^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium oxysporum	2.57±0.15^{a}	2.57±0.15^{a}	2.17±0.12^{b}	0.00±0.00^{c}	0.00±0.00^{c}
	LSD	0.25	0.19	0.27	0.66	0.00
Day 6	*Bipolaris maydis*	4.73±0.21^{a}	4.37±0.21^{b}	3.80±0.26^{c}	2.70±0.17^{d}	0.00±0.00^{e}
	Curvularia lunata	5.47±0.12^{a}	4.90±0.00^{b}	3.73±0.12^{c}	0.30±0.52^{d}	0.00±0.00^{d}
	Dreschlera sp.	5.10±0.17^{a}	4.67±0.12^{b}	3.90±0.10^{c}	3.30±0.36^{d}	0.00±0.00^{e}
	Fusarium verticilloides	3.53±0.06^{a}	3.27±0.15^{a}	2.97±0.15^{b}	2.03±0.25^{c}	0.00±0.00^{d}
	Macrophomina phaseolina	4.10±0.26^{a}	3.70±0.20^{a}	3.93±0.15^{a}	0.27±0.46^{b}	0.00±0.00^{b}
	Fusarium equiseti	4.60±0.10^{a}	4.23±0.15^{a}	2.63±0.60^{b}	1.87±0.06^{c}	0.00±0.00^{d}
	Fusarium solani	5.53±0.15^{a}	4.07±0.15^{b}	3.33±0.06^{c}	0.00±0.00^{d}	0.00±0.00^{d}
	Fusarium oxysporum	4.67±0.15^{a}	3.60±0.44^{b}	3.30±0.43^{b}	1.73±0.23^{c}	0.00±0.00^{d}
	LSD	0.28	0.37	0.51	0.53	0.00
Day 9	*Bipolaris maydis*	7.10±0.26^{a}	6.87±0.12^{a}	5.80±0.30^{d}	4.13±0.15^{c}	0.00±0.00^{d}
	Curvularia lunata	7.20±0.17^{a}	6.97±0.06^{a}	5.60±0.00^{b}	1.40±0.70^{c}	0.00±0.00^{d}
	Dreschlera sp.	7.20±0.17^{a}	6.17±0.12^{b}	5.93±0.12^{b}	5.00±0.36^{c}	0.00±0.00^{d}
	Fusarium verticilloides	6.00±0.10^{a}	5.77±0.15^{a}	5.40±0.20^{b}	2.97±0.31^{c}	0.00±0.00^{d}
	Macrophomina phaseolina	7.10±0.20^{a}	6.47±0.15^{a}	5.77±0.21^{a}	1.03±1.79^{b}	0.00±0.00^{b}
	Fusarium equiseti	6..73±0.15^{a}	6.40±0.20^{a}	4.03±0.90^{b}	2.83±0.12^{c}	0.00±0.00^{d}
	Fusarium solani	6.33±0.12^{a}	5.80±0.17^{b}	5.50±0.10^{c}	1.63±0.15^{d}	0.00±0.00^{e}
	Fusarium oxysporum	7.10±0.10^{a}	6.57±0.25^{b}	5.90±0.10^{c}	2.47±0.38^{d}	0.00±0.00^{e}
	LSD	0.29	0.28	0.62	1.24	0.00

Data are presented as means for three replicates, followed by standard deviation within replicates. Values followed by the same letters in rows are not significantly different at P = 0.05. LSD = least significant difference.

as reported by Yoshida et al. (2000) that it contains pyroligeneous acid which is the bioactive secondary metabolite that has inhibitory effects on fungal pathogens. The significant effect of the rice husk extract on different fungal pathogens is therefore an indication that the mechanisms of interaction of the pyroligeneous acid and the fungal pathogens should be given attention.

Bamboo and wood extracts showed the least inhibitory effects on all the fungal pathogens even at 1.5% concentration. This indicates that the effect of the botanical extracts as a phytofungicide also depends on the concentration levels used on the pathogenic fungi. Abiala et al. (2011) reported that bamboo and wood extracts completely inhibited mycelial growth of *Mycosphaerella fijensis* at higher (5%) concentration level as compared to mycelial growth reduction at low (1.5 and 2.5%) concentration levels. Correlating this with our study suggests that, increase in concentration levels may likely increase the effectiveness of bamboo and wood extracts on mycelial growth of fungal pathogens of maize. Also, the significant differences and variation in the effectiveness of bamboo and wood extracts may be as a result of their unknown active ingredient. This is in agreement with Maobe et al. (2013) that evaluated eight medicinal plants and proposed that the crude extracts may contain lots of phytochemical compounds that may be responsible for their effect on the clinical pathogens. With respect to this, the inhibitory activity of botanical extracts may vary with the virulence of the pathogens and most likely with the chemical components of the botanicals. This also conforms to the work of Odebode et al. (2004) carried out on two annonaceous plants *Isolana cualifora* verdc and

Table 8. Mean percentage growth inhibition of fungal pathogens at 1.5% concentration (day 9).

Pathogenic fungi	RHE	BE	WE	RHE *x* BE	RHE *x* WE	BE *x* WE	RHE *x* BE *x* WE
B. maydis	42.00	22.04	16.45	77.73	100.0	21.26	100.0
C. lunata	56.62	16.16	4.60	100.0	51.99	55.42	100.0
Dreschlera sp.	24.20	28.63	11.89	64.84	100.0	24.05	100.0
F. verticilloides	100.0	13.33	16.36	100.0	100.0	10.78	100.0
M. phaseolina	100.0	15.00	13.98	39.49	100.0	31.80	100.0
F. equiseti	55.80	23.57	16.02	100.0	100.0	41.25	100.0
F. solani	100.0	13.07	20.88	43.04	58.10	14.29	100.0
F. oxysporum	38.43	13.41	15.28	100.0	38.97	18.47	100.0

Cleistochlamys krikii Berth (Oliv), the crude extract and pure compounds isolated from both plants inhibited both bacterial and fungal pathogens.

Evaluation of botanicals individually has been the norm in plant pathology with respect to biological control of plant diseases. We hypothesized in this study that the significant effect of botanical extracts in their combinations on pathogenic fungi of maize is possible and may likely perform far better than individually. The combination of RHE x BE x WE, RHE x BE and RHE x WE extracts significantly inhibited the mycelial growth of the pathogenic fungi. Although, increase in concentration levels favours BE x WE combination. This conforms to the report of Webster et al. (2008) that crude extracts are generally a mixture of active and non-active compounds (crude fusions) and therefore higher minimum inhibitory concentration are expected. Observed variation of antifungal activities of these botanical extract combinations suggests that there may be differences in the nature and chemical composition of the plants.

The *in vitro* antifungal properties of the extract either singly or in combinations reveal its efficacy in the control of at least one of the pathogenic fungi. The rice husk extract at low concentration levels performed excellently well, followed by bamboo extract while the least was wood extract. The complete mycelial growth inhibition observed in this study may likely correlate with what is expected *in vivo*. Taking advantage of these botanical extracts most especially in their combinations will be of significant importance to sustainable crop production and thus, support ecofriendly-based agricultural management systems. Therefore, proper management coupled with good formulations of these botanicals will be of significant effect for total elimination of these fungal pathogens on maize field.

Conflict of interest

The authors did not declare any conflict of interest.

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