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Research article

Genotypic variations in the inhibitory potentials of four combined botanicals on mycelia growth of *Macrophomina phaseolina* of cowpea [*Vigna unguiculata* (L) Walp.]

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Abstract: The ethanol extracts of *Ficus asperifolia, Mormordica charantia, Anacardium occidentals* and *Psidium guajava* were evaluated sole and in treatment combinations at 25, 50 and 75mg ml^{-1} concentration levels against the mycelial growth of *Macrophomina phaseolina* of Cowpea. The pathogen was cultured on plates containing botanicals amended Potato Dextrose Agar (PDA) in three replicates while only ethanol treated PDA tested plates served the control experiment. The radial growths were recorded at 4^{th}, 6^{th} and 8^{th} day after inoculation. Data obtained were analysed using the SAS software program version 9.2. The extract of *Mormordica charantia* was the most effective in the botanical treatments alone. The most significant inhibition of *Macrophomina phaseolina* were observed from the combined treatments of *Ficus asperifolia, Mormordica charantia* and *Anacardium occidentals* (3.11 cm), followed by *Mormordica charantia* and *Psidium guajava* (3.29 cm), then combination of four extracts; *Ficus asperifolia, Mormordica charantia, Anacardium occidentals* and *Psidium guajava* (3.53 cm), then *Mormordica charantia* and *Anacardium occidentals* (3.84 cm). Other treatments, either alone or in combination produced significant result compared to the control experiment (6.94 cm). However, the efficacy of botanicals increased with concentration and also significantly correlated with time and reduction in mycelia extension of the pathogen. More so, variability in the antifungicidal potentials of the botanicals on *Macrophomina phaseolina* ranges from 15.93% to 34.06% according to Eigen proportions. The treatment combinations of; *Ficus asperifolia, Mormordica charantia* and *Anacardium occidentals* at 75mg ml^{-1} concentration level produced the most inhibitory effect against *Macrophomina phaseolina* in vitro. However, the untreated plates did not show inhibitory effect on the mycelial growth of the pathogen. Therefore, combined treatments of botanicals could be a potential source in the practise of plant disease control.

Keywords: *Macrophomina phaseolina* - Mycelial growth - Correlation - Eigen proportion.

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INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. belongs to the family Botryosphaeriaceae. It is a highly polyphagus necrotrophic fungal pathogen that infects more than 500 plant hosts (Wyllie 1988). The plants invaded by this pathogen are; major food crops, pulse, fiber and oil crops (Su *et al*. 2001, Mayek-Pérez *et al*. 2001, Dinakaran & Mohammed 2001, Aly *et al*. 2007, Khan 2007). *Macrophomina phaseolina* have a wide geographical distribution, and is especially found in tropical and subtropical countries with arid to semi-arid climates in; Africa, Asia, Europe, and North and South America (Wrather *et al*. 2001). Diseases caused by *Macrophomina phaseolina* include ashy seedling blight, seedling damping-off, charcoal rot, stem rot, and root rot leads to significant yield loss (Emechebe & Lagoke 2002, Wellington *et al*. 2011). Incidences of these diseases are

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favoured at low soil moisture (Sandhu *et al*. 1999, Islam *et al*. 2012) and high temperatures (30–35°C). The comparatively high capacity of Cowpea to withstand drought stress, poor soil conditions and high temperatures made the crop very valuable especially in arid and Sub-Saharan regions of West Africa where cowpea is a crop of major economic importance for resource poor farmers (Sanginga *et al*. 2003).

The persistence of sclerotia of *Macrophomina phaseolina* in the soil and plant debris (Short *et al*. 1980) coupled with its wide host range has resulted in difficulty of the disease control especially on Cowpea where the pathogen constitutes a major yield-suppressing factor even when under drought stress (Wyllie 1993). The ineffective chemical control approach against *Mormordica phaseolina* necessitates a biological control measure (Mark & Norman 2007). However, disease caused by fungi pathogens and their control with botanicals has been found to be environmental friendly and effective against the targeted pathogen (Odebode 2006, Akanmu *et al*. 2013a, Abiala *et al*. 2013). This study investigates the efficacy of the interactive treatments of the extracts of *Ficus asperifolia, Mormordica charantia, Anacardium occidentals* and *Psidium guajava* alone and in treatment combinations against the pathogenic *Macrophomina phaseolina* of Cowpea.

MATERIALS AND METHODS

Source of pathogen

The pathogenic strain of *Macrophomina phaseolina* isolated from Cowpea was obtained from the plant pathology laboratory, International Institute of Tropical Agriculture (IITA) Ibadan, Oyo state, Nigeria.

Source of plant extracts

Fresh leaves of *Ficus asperifolia, Mormordica charantia, Anacardium occidentales* and *Psidium guajava* were obtained from Botanical garden and authenticated at the herbarium laboratory, both of the Department of Botany, University of Ibadan, Ibadan, Nigeria.

Preparation of plant extracts

The fresh leaves were washed in clean water to be freed from possible dirt and debris. The cleaned leaves were soaked in 5% Sodium hypochlorite solution for 10 minutes and rinsed in three exchanges of distilled water to neutralize the effect of Sodium hypochlorite. The leaves were then air dried for two week weeks after which they were blended separately into powdery form. The ethanolic extraction was carried out by weighing 2.5 g, 5.0 g and 7.5 g of each powdered extracts separately into 100ml of 75% ethanol, to achieve 25 mg ml^{-1}, 50 mg ml^{-1} and 75 mg ml^{-1} concentration levels respectively.

In vitro control of Macrophomina phaseolina with plant extracts

The *Macrophomina phaseolina* culture obtained was asceptically subcultured on Potato Dextrose Agar (PDA) and incubated for 7 days. The biocontrol experiment using plant extracts was carried out by adding 1ml of the extracts to 9 ml of PDA poured on each plate. This was observed on each concentration level of the four plant extracts used in this study. For the interactive treatment which involves the combination of two extracts, 0.5 ml of each extracts were added to 9ml of PDA poured per plate. The interaction of three extracts were prepared by adding 0.33 ml of each extract to 9 ml of PDA while for the different four extracts used, 0.25 ml of each were added to 9 ml of PDA. The extracts amended molten PDA was initially swirled gently for homogenization before the plates were poured and allowed to solidify. Using a 5 mm cork borer, the mycelia growth from the advancing edge of the 7 day old pure culture of *Macrophomina phaseolina* were picked and inoculated at the centre of extracts treated PDA plates. The control experiment consisted of the treatments of 1 ml 75% ethanol with 9 ml of PDA. The plates were then incubated for 8 days, during which data on the radial growth of the pathogen in each plate were taken at the 4^{th} day, 6^{th} day and 8^{th} day of the experiment.

Percentage inhibition of mycelial growth by the leaf extracts was calculated using the formula:

$$\%MG_I = \frac{D_C - D_T}{D_C} X\ 100$$

Where: $\%MG_I$ = % Inhibition of mycelial growth
D_C = diameter of control
D_T = diameter of test

Data analysis

Data obtained from the radial growths were subjected to the Analysis of Variance (ANOVA) using System

Analysis Software package (SAS) 9.1 2009 version, while means were separated by Duncan Multiple Range Test (DMRT).

RESULTS

Highly significant ($p<0.0$) result was obtained on the radial growths of *Macrophomina phaseolina* by activities of the plant extracts, with respect to period (day) of the study, also, in the interactions involving; concentration x day, extracts x concentration, extracts x day, extracts x replicates, extracts x concentration x day, also, extracts x replicate x concentration. While significant ($p<0.05$) effects were produced in the interactions of concentration x day and extract x replicates x day (Table 1).

Table 1. Interactive effect of extract, replicate, concentration and day of data collection on the mycelia growth of *Macrophomina phaseolina*.

Source	df	Radial Growth
Concentration	2	1.46^{ns}
Day	2	1624.83**
Extracts	15	33.01**
Replicates	3	0.13^{ns}
Concentration x Day	4	1.32*
Extracts x Concentration	30	8.10**
Replicates x Concentration	6	0.19^{ns}
Extracts x Day	30	3.61**
Replicates x Day	6	0.03^{ns}
Extracts x Replicates	45	1.33**
Extracts x Concentration x Day	60	1.23**
Replicate x Concentration x Day	12	0.51^{ns}
Extract x Replicate x Concentration	90	1.18**
Extract x Replicate x Day	90	0.82*
Error	180	101.21
Corrected total	575	4527.88

Note: ** = Highly significant ($p<0.01$), * = Significant ($p<0.05$), ns = not significant.

Considering the sole treatment of the four botanicals tested, *Mormordica charantia* (4.34 cm) had the highest mean inhibitory effect of 4.34 cm on the mycelial growths of *Macrophomina phasolina*, while F. asperifolia (5.69 cm), *Anacardium occidentals* (5.59 cm) and *Psidium guajava* (5.55 cm) which showed slight inhibitory effect on the pathogen, were not significantly ($p>0.05$) different from the control (5.69 cm). A more significant ($p<0.05$) control of the pathogen was achieved by the effects of the combined treatments of the four extracts, with the extracts of *Mormordica charantia* and *Psidium guajava* (3.29 cm) as well as *Ficus asperifolia, Mormordica charantia* and *Anacardium occidentales* (3.11 cm) on *Macrophomina phaseolina* (Table 2).

Table 2. Inhibitory effect of interaction of extracts on the mycelia growth of *Macrophomina phaseolina.*

Extracts	Radial growth (cm)
Anacardium occidentals	5.59b
Psidium guajava	5.55b
Ficus asperifolia and *Mormordica charantia*	4.79c
Ficus asperifolia and *Anacardium occidentals*	4.61dc
Ficus asperifolia and *Psidium guajava*	4.63dc
Mormordica charantia and *Anacardium occidentals*	3.84gh
Mormordica charantia and *Psidium guajava*	3.29ij
Anacardium occidentals and *Psidium guajava*	4.76c
Ficus asperifolia, Mormordica charantia and *Anacardium occidentals*	3.11j
Ficus asperifolia, Mormordica charantia and *Psidium guajava*	4.36de
Mormordica charantia, Anacardium occidentals and *Psidium guajava*	4.16efg
Ficus asperifolia, Anacardium occidentals and *Psidium guajava*	3.95fg
Ficus asperifolia, Mormordica charantia, Anacardium occidentals and *Psidium guajava*	3.53hi
Control	6.94a

Note: The significant difference ($p<0.05$) is indicated by different letters along each column.

The growth inhibition at 75 mg ml^{-1} concentration produced significant ($p<0.05$) effect, followed by 25 mg ml^{-1}, while the concentration of 50 mg ml^{-1} had the least mycelia inhibition. The radial growth of the pathogen

increases with time in which the most significant growth was recorded on the 8th day of data collection (7.32 cm), followed by the 6th day (4.63 cm) while the least was recorded on the fourth day (Table 3).

Table 3. Mean performance of botanical concentration, days of observation and replicated treatments on the mycelia growth of *Macrophomina phaseolina*.

Observed characters	Variables	Radial growth (cm)
Extract concentration (mg ml^{-1})	25	4.57a
	50	4.49ab
	75	4.40b
Days	4	1.51c
	6	4.63b
	8	7.32a
Replicates	1	4.50a
	2	4.51a
	3	4.48a
	4	4.45a

Note: The significant difference ($p<0.05$) is indicated by different letters along each column.

The day of observation is positive and significantly ($p<0.05$) correlated with concentration of the extract and mycelia growth with r=0.15 and 0.85. There was negative association between the extracts and the mycelia growth with r=0.22 while non-significant and negative association occurs between the radial growth and replicate as well as concentration, no association exists between extracts and days of observation, concentration and the replicates (Table 4).

Table 4. Effect of Correlation of the extracts; extract concentration, days of observation and replicates on the radial mycelia growth of *Macrophomina phaseolina.*

Correlation	Extracts	Replicates	Concentration	Day
Replicates	0.00^{ns}			
Concentration	0.00^{ns}	0.00^{ns}		
Day	0.00^{ns}	0.00^{ns}	0.15*	
Radial growth	-0.22*	-0.01^{ns}	-0.01^{ns}	0.85**

Note: ** = Highly significant ($p<0.01$), * = Significant ($p<0.05$), ns = not significant.

The contribution of principal component analysis (PCA) in tables 5 showed variations in the Eigen values and proportion of the mycelial growth. Prin 1 accounted for the highest variation with the highest Eigen vector for extract, concentration and day of experimental observation at proportion of 34.06%. The first and fourth PCA showed positive and more relatedness for the extracts treatments. The extract concentration of the second PCA had the highest Eigen value which accounted for 25.01% of total variation while the third PCA had the highest vector for the replicates (Table 5).

Table 5. Contribution of principal component analysis (PCA) to the variation in the radial growth of *Macrophomina phaseolina*.

Radial growth	Prin1	Prin2	Prin3	Prin4
Extracts	0.706	-0.067	-0.011	0.705
Replicates	0.022	0.389	0.920	0.003
Concentration	0.035	0.919	-0.390	0.047
Day	0.707	0.010	0.002	-0.707
Eigen values	1.360	1.000	1.000	0.637
Proportion (%)	34.06	25.01	25.00	15.930

DISCUSSION AND CONCLUSION

Research has demonstrated that biological control is a potentially feasible alternative to the use of pesticides (Jacobsen *et al*. 2004, Adandonon *et al*. 2006, Sobowale *et al*. 2008, Olawuyi *et al*. 2011, Akanmu *et al*. 2012, 2013a, Olawuyi *et al*. 2013). The combined use of biocontrol agents in the control of some important pathogens affecting crops of economic importance is a new trend in plant disease control experiments. The integrated approach of biocontrol research adopted in this research using botanicals of; *Ficus asperifolia*, *Mormordica charantia*, *Anacardium occidentals* and *Psidium guajava* at different concentrations levels in sole and in combinations towards the control of the mycelial growth of *Macrophomina phaseolina* of cowpea is in line with the observations earlier made (Odebode 2006, Adandonon *et al*. 2006).

Macrophomina phaseolina had been reported as the cause of charcoal rot disease which is a major biotic factor that limits cowpea productivity worldwide (Ma *et al*. 2010, Zveibil *et al*. 2012, Arshad *et al*. 2012). This was demonstrated in the reaction of cowpea varieties to infection of *Macrophomina phaseolina* obtained from six different leguminous plants in Nigeria, as was earlier investigated by Amusa *et al*. (2007). The possible control of this pathogen using botanicals as investigated in this research showed the efficacy of all the extracts tested when compared to the results obtained in the control experiment. The effectiveness of the botanicals against *Macrophomina phaseolina* could be attributed to their antifungal properties as was also discovered by Arshad *et al*. (2012) who reported the efficacy of the antifungal properties of different parts of *Sorghum halepense* Pers. in the control of *Macrophomina phaseolina* of cowpea.

The efficacy of the extract of *Mormordica charantia* which showed the most inhibitoriest potential on the growth of *Macrophomina phaseolina* had earlier been reported in the treatment of human and plant diseases (Virdi *et al*. 2003, Burger *et al*. 2010, Jonathan *et al*. 2012). However, the most significant mycelial inhibition of *Macrophomina phaseolina* which was recorded in the combination of the extracts of; *Ficus asperifolia*, *Mormordica charantia* and *Anacardium occidentals*, compared to the control experiment conformed to the findings of Akhter *et al*. (2006) who observed 91% to 100% inhibition of conidial germination of *Bipolaris sorokiniana* by plant extracts amended with cow dung and cow urine. The efficacy of combined treatment of plant extracts was also affirmed in the combined treatments of botanicals and antibiotics against some emerging drug-resistance microorganisms investigated by Rakholiva & Chanda (2012).

The significant result in interactions of the biological agents with the pathogen is in accordance with findings of Sobowale *et al*. (2009), Akanmu *et al*. (2013b), Olawuyi *et al*. (2011, 2013). More so, the efficacy of botanicals increases with concentration while it was also significantly correlated with time and the reduction in the mycelia extension of the pathogen as similar reported by Akinbode (2010).

The significant correlation of the days of observation with extracts concentration and mycelia growth is an indication of the positive contribution of the extract and their concentration in inhibition of *Macrophomina phaseolina* as similarly observed by Akanmu *et al*. (2013a) and Olawuyi *et al*. (2014). The contribution of the principal component analysis in this study is an indication of variability in the inhibitory potential of the botanical extracts on the mycelia growth of the pathogen in accordance with the findings of Olowe *et al*. (2013). The mycelial growths of *Macrophomina phaseolina* showed variability which ranges from 15.93% to 34.06% due to the interactive treatments of botanicals at varying concentration over a period of time according to Eigen proportion. The variations in the extract performances could be attributed to differences in their anti-fungicidal properties, as similarly confirmed by Burger *et al*. (2010), Olawuyi *et al*. (2010) and Fapounda *et al*. (2011).

This suggests that there are variations in bioactive of antifungal compounds with varying characteristics and potentials in their modes of action. However, the effectiveness of botanicals in field condition could subsequently affirm the findings of this study to prove further their potentials in plant disease control.

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