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Pathogenicity and in vitro extracts inhibition of fungi causing severe leaf blight in *Thaumatoccocus danielli* (Benn.) Benth

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ABSTRACT

Isolation and identification of fungi associated with severe leaf blight of *Thaumatococcus danielli* was conducted. The fungi were subjected to pathogenicity test in the screen-house experiment. Laboratory assessment of antifungicidal activities of; *Ficus asperifolia, Psidium guajava, Mormodica charantia* and *Anarcadium occidentales* prepared at 25, 50 and 75 mg/mL concentrations against the three most virulent pathogens was carried out on PDA, V8 Agar, CZA, and MEA culture media. Data gathered were subjected to ANOVA. *Aspergillus niger, A. flavus, Pythium* sp., *Penicillium* sp., *Fusarium oxysporum, Macrophomina phaseolina, Trichoderma viride, T. koniigi* and *T. harzianum* were the fungi found associated with the diseased parts and the rhizospheric soil. *M. phaseolina, A. flavus* and *A. niger* were the most virulent obtained in the pathogenicity test. Growths of these pathogens were mostly inhibited by *M. charantia* as its efficacy increased with increase in the concentration levels. Mycelia growths were mostly supported by MEA medium.

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Pathogenicity; plant extracts; media; phytofungicidal potential; mycelia growths

Introduction

Thaumatococcus danielli (Benn.) Benth. is a rhizomatous, perennial and monocotyledonous herb. The slender stalks of *T. danielli* grow to about 2–3 m in height and terminates into a single tough, almost round and versatile leaf (Makinde and Taiwo 2004; Abiodun et al. 2014). This multipurpose plant is a member of Maranthaceae family and is widely distributed in the tropical rain forest areas of West Africa (Olabanji et al. 2014). *T. danielli* is cultivated in most parts of south-western Nigeria, Ghana and Cameroon where it contributes to the economy of the rural

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population, therefore making it a very important economic plant in the West Africa sub-region (Osemeobo 2005; Arowosoge and Popoola 2006).

Different parts of *T. danielli* are of immense economic importance. The good flavour and preservative effects of the leaves make it a choice in food wrapping. It is also useful in roof thatching while the stems are used locally in artistic works such as weaving mats, baskets, bags, hats, hand fan among others (Elemo et al. 1999; Olabanji et al. 2014). Industrially, fibre are produced from the stems while the fruits of *T. danielli* are of cardinal importance because of their low-calorie protein contents named "Thaumatin" which is about 2000–3000 times sweeter than sucrose and it is neither allergic nor mutagenic or teratogenic (Yeboah et al. 2003; Waliszewski et al. 2012). Thaumatin is a suitable sweetener for diabetic patients, and is often used as flavour modifier in foods and drinks than as sweetener and does not promote teeth decay like the other sweeteners. Thus thaumatin has been approved for use in many countries as both a flavour enhancer and a high-intensity sweetener (Zemanek and Wasserman 1995; Abiodun et al. 2014; Olabanji et al. 2014).

The continuous cultivation of this plant is threatened by some phytopathogenic organisms which cause impaired health and yield loss of this important plant, as plant diseases have been reported to play a direct role in the destruction of natural resources in agriculture (Mohamed Elanwar et al. 2011). The use of chemical control measures in the management of plant diseases are recently being discouraged as a result of the environmental pollution caused, whereas biological control measures using plant extracts have been reported for its ease of application, cost effectiveness, readily availability, environmental friendliness and efficacy against many plant pathogens (Abiala et al. 2013; Akanmu et al. 2015). Therefore, biological control of plant pathogens with plant extracts appears as an attractive and realistic approach of disease control.

The occurrence of severe leaf blights on the *T. danielli* plantation at the experimental site of the Department of Botany, University of Ibadan, Nigeria during 2014/2015 planting season, necessitated this research. This study therefore investigated the fungal pathogens associated with severe leaf blight of *T. danielli* and also evaluated the phytofungicidal potential of some plant extracts on the most pathogenic fungus isolated.

Materials and methods

Research location: The experiment was carried out in the plant Pathology/Mycology laboratory and the Screen house of Botany Department, University of Ibadan Nigeria.

Preparation of media: The media used in the in vitro control of the fungi were; Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), V-8 Juice Agar and Czapedox Agar (CZA). Each of the media was prepared according to the manufacturer's specification.

Preparation of plant extracts: Fresh leaves of *Ficus asperifolia, Psidium guajava, Mormodica charantia* and *Anarcadium occidentale* obtained from the Botanical garden, University of Ibadan, Nigeria were separately rinsed in 5% NaOCI solution for one minute and rinsed again in two exchanges of sterile distilled water, then air dried on a sterilised surface. The air dried leaves were blended in an already sterilised electric blender (Super master blender, Model number: SMB-2898, made in china). After this, each of the blended leaves were weighed by grams scale into 5, 10 and 15 g, respectively, then dispensed in 200 ml of ethanol each to make a concentration of 25, 50 and 75 mg/mL. These mixtures were left for 24 h, with intermittent manual shaking as to allow the active ingredient in the leaves to dislodge into the solvent after which each extract was sieved using sterile muslin cloth.

Isolation and identification of fungal pathogens

***Blotter technique*:** The infected leaves of *T. danielli* were cut into pieces of 1 cm^2, surface sterilised using 2% sodium hypochlorite solution for 2 min and subjected to Standard Blotter Method (SBM) and Agar Plate Method (APM) and ISTA Rules (ISTA 1966 cited by Toma and Abdulla 2013). The plates were incubated under 12h/12h cycles of lightness and darkness for 7 days after incubation, mycelia growth of potential pathogenic fungi were picked with sterile inoculating needle on culture of PDA plates and re-subculture to have pure cultures which were further prepared in slants and stored for further studies.

***Isolation of fungi from soil*:** The sample of the air dried rhizospheric soil collected from *T. danielli* plantation was crushed with the use of sterilised mortar and pestle, after which it was sieved to obtain fine texture. The fine texture soil (0.005–0.15 g) were measured into sterile Petri dishes according to the method of Warcup (1950) cited by Sobowale (2001), after which PDA medium was dispensed into the plates containing soil samples directly as illustrated by Leslie and Summerell (2006). The process was carried out in three replicates and the plate were incubated at room temperature and observed for fungal growth.

***Identification of the fungal species*:** Pure cultures of each isolated fungal species were identified based on their morphological growth and microscopic characters. This was carried out at International Institute of tropical Agriculture (IITA), Ibadan, Oyo state, Nigeria

Category	Severity rating
Apparently infected	0
0–25% leaf area infected	1
26–50% leaf area infected	2
51–75% leaf area infected	3
>75% leaf area infected	4

***Stock Cultures*:** Pure cultures of the identified fungal species were prepared on PDA slant and stored at 4 °C in the refrigerator.

Pathogenicity test

Soil sterilisation: The top soil collected at the experimental farm of Department of Botany, University of Ibadan, Nigeria was sterilised using electric soil steriliser. After cooling, 4 kg of the sterilised soil were packed into each polythene bag and arranged in the already sanitised screen house.

Experimental set up: The experiment involving the treatments which consists of one variety of *T. danielli* and nine fungal species were arranged in a complete randomised design (CRD), and replicated three times alongside the control.

***Rhizome sanitisation and Planting*:** Prior to pathogenicity, uninfected rhizomes of *T. danielli* used for this research work were obtained from a local farm in Ekiti south west Local Government, Ekiti state, Nigeria. Each rhizome of *T. danielli* were first sanitised in 5% NaOCl for one minutes, then rinsed in three exchanges of sterile distilled water. The rhizome of approximately 5 cm in height was planted at 2 cm depth into the soil contained in the polythene bag.

***Inoculums quantification and multiplication*:** The pure cultures of each of the nine fungal species were grown on PDA and incubated till the seventh day. Each species were harvested separately by picking ten pieces of each actively growing young culture into already sterilised conical flask containing 250 mL sterile distilled water, using a sterile 5 mm cork borer. The solution was then homogenised with a blender and sieved with cheese cloth. The number of fungal spores was counted with haemocytometer. The spore suspensions of all fungal isolates were adjusted to 3.3×10^6 spore/mL.

***Inoculation*:** Both foliar and soil inoculation were carried out by spraying 10 mL spore suspension of each fungal species at the fifth week after planting while the control experiments were sprayed with 10 mL sterile distilled water. Each treated samples were covered with polythene bag for 24 h to keep samples in moist condition and as well avoid secondary contamination.

Disease Assessment.

***Scoring of Disease Severity*:** The severity of infection caused by each of the nine pathogenic fungal species on the leaves of *T. danielli* was evaluated according to the method described by Horsfall and Heuberger, 1942.

***Data Collection*:** The experiment was carried out under close observation and data were collected at seventh day interval to monitor the growth progress and possible disease infection during the period of observation. The data collected includes; plant height, stem girths, leaf length, leaf width and disease severity.

*Biocontrol experiment (*in vitro*)*

The highly pathogenic fungi in the pathogenicity test were subjected to the control measures with the use of some plant extracts; *F. asperifolia, P. guajava, M. charantia* and *A. occidentales.*

Extract of *F. asperifolia, P. guajava, M. charantia* and *A. occidentales* prepared at different concentration levels; 25, 50 and 75 mg/mL were assessed against the most pathogenic fungi of *T. danielli.* One ml of the extracts at each concentration level to 9 mL of the media were dispensed into each plate and allowed to solidify. The control experiments were prepared with 1 mL of 70% ethanol to 9 mL of media. The sterile 5 mm cork borer was used to pick each fungal mycelium from the actively growing six-day-old pure cultures and were separately placed at the centre of the solidified media in each plate based on the treatment levels. The cultures were then incubated at room temperature for 9 days and data were taken at 2 days interval, starting from day 4. The percentage mycelia inhibition was calculated according to the method of Odebode (2006).

$$\text{Percentage mycelia inhibition} = R = \frac{R_1 - R_2}{R_1} \times 100$$

where R_1 the value of radial growth of pathogen on control plate; R_2 the value of radial growth of pathogen in treatment plate.

Data analysis: All the data collected in this study were subjected to analysis of variance (ANOVA) using SAS, Window Version 9.1 (SAS 2003) while the means were separated by Duncan Multiple Range Test.

Results

Pathogenicity test

The fungi isolated from the infected leaves and rhizospheric soils of *T. danielli* were; *Macrophomina phaseolina, Aspergillus flavus, A. niger, Fusarium oxysporum*, *Pythium* sp., *Penicillium* sp., *Trichoderma*

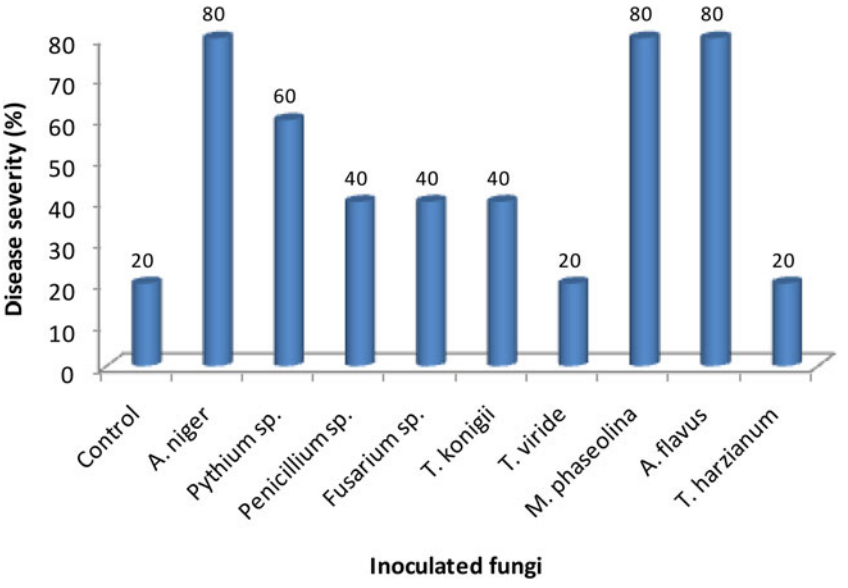

Figure 1. Percentage disease severity caused by the inoculated fungi on *Thaumatococcus danielli* plant.

harzianum, *T. koniigi* and *Trichorderma viride*. The results of pathogenicity obtained from screenhouse showed *A. niger, M. phaseolina* and *A. flavus,* followed by *Pythium* sp. as the most virulent pathogens causing the leaf blight of *T. danielli* plant. The results obtained from *T. viride* and *T. harzianum* were not significantly ($p < 0.05$) different from the control (Figure 1).

The fitted model for the pathogenicity experiment produced significant effect in all the characters measured at $p < 0.01$ except in stem girth which was significant at $p < 0.05$. The effect of fungi was significant ($p < 0.01$) with plant height (114.24 cm), leaf length (19.89 cm) and leaf width (10.34 cm). Duration of experiment measured as week after inoculation (WAI) showed significant increase in the stem girth (1.18cm), leaf length (15.97 cm) and leaf width (5.03 cm) of the *T. danielli* plant (Table 1).

In the fungi inoculated, the plant heights recorded the most significant ($p < 0.05$) growth the control experiment (30.22 cm), followed by *A. flavus* (19.77 cm) and *A. niger* (14.23 cm) while there was no significant difference among the results obtained in the remaining treatments. Results of the stem girth showed that those of *A. niger* (1.67 cm), *Pythium* sp. (1.60 cm), *T. viride* (1.81 cm), *M. phaseolina* (1.74 cm) and *A. flavus* (1.75 cm) were not significantly difference from the control (2.22 cm). In the leaf length and leaf width, only *A. flavus* (9.12 cm, 6.87 cm) showed similar level of significance with control experiment (9.22 cm, 5.89 cm) respectively.

Table 1. ANOVA effect of fungi and duration (WAI) on the growth of *Thaumatoccocus danielli* plant.

Source	*df*	Plant height (cm)	Stem girth (cm)	Leaf length (cm)	Leaf width (cm)
Model	11	93.48**	0.46*	19.18**	9.38**
Fungi	9	114.24**	0.31	19.89**	10.34**
WAI	2	0.06	1.18**	15.97**	5.03**
Error	18	222.4	2.93	47.75	13.08
Corrected total	29	1250.71	8.04	258.77	116.22

Significant ($p < 0.01$) = **, Significant ($p < 0.05$) = *, WAI = Week After Inoculation.

The effect of WAI showed no significant result with respect to the plants height. A more significant increase in the stem girth was recorded at first WAI but results at third and fifth WAI were the most significant in the leaf length (5.07 cm, 5.64 cm) and leaf width (3.28 cm, 3.83 cm), respectively (Table 2).

Mean with the different letter across the column are significantly ($p < 0.05$) different from one another with respect to each parameter. LSD = Least Significant Difference

Positive and highly significant ($p < 0.01$) correlative effect exists between plant height with stem girth ($r = 0.65$), leaf length ($r = 0.74$) and leaf width ($r = 0.71$), while the association with Fungi ($r = 0.55$) was significant at $p < 0.05$ level. Also, strong and significant ($p < 0.01$) relationship was recorded between the leaf length and leaf width ($r = 0.97$), while the fungi as well showed significant ($p < 0.05$) increase of the leaf width. However, the WAI was only found significant ($p < 0.05$) and negatively correlated with stem girth (r = –0.53) (Table 3).

In the pathogenicity experiment Principal Component Axis (PCA) showed Prin 1 with Eigen value of 2.86 as the most important component which contributed 71.58% to the total variation and increased with increasing plant height, leaf length and leaf width. Prin 2 contributed 23.32% of the variation especially with increasing stem girth while Prin 3 recorded an inverse association with plant height. Prin 4 accounted for increasing leaf width but decreasing leaf length (Table 4).

Bio-control experiment

The fitted model for the interaction of extracts, concentration, media and the fungi was significant across the days of observation. The individual effect of extract, media and fungi were significant ($p < 0.01$) through the period of this study. Similarly, the interaction; $E \times M$, $E \times F$ and $M \times F$ produced significant ($p < 0.01$) mycelia growths. Also, the interaction, $E \times M \times F$, $E \times F \times C$ and $E \times M \times F$ were significant ($p < 0.01$) across the days of observation while $M \times F \times C$ recorded no significant result at day 5, 8 and 9 (Table 5).

Table 2. Effect of fungi and duration (WAI) on the growth of *Thaumatoccocus danielli* plant.

Parameters	Variables	Plant height (cm)	Stem girth (cm)	Leaf length (cm)	Leaf width (cm)
Fungi	*A. niger*	14.23bc	1.67ab	5.13b	3.11b
	Pythium sp.	11.81c	1.6ab	2.08b	1.14d
	Penicillium sp.	11.28c	1.18b	2.05b	1.35cd
	Fusarium oxysporum.	10.78c	1.42b	3.60b	2.68bcd
	Trichoderma koniigi	10.52c	1.16b	4.06b	2.83bc
	Trichoderma viride	10.74c	1.81ab	3.23b	1.78bcd
	M. phaseolina	15.83c	1.74ab	4.59b	3.41b
	A. flavus	19.77b	1.75ab	9.12a	6.87a
	Trichoderma harzianum	12.23c	1.43b	3.34b	2.73bcd
	Control	30.22a	2.22a	9.22a	5.89a
	LSD	6.03	0.69	2.79	1.46
Week after inoculation	1	14.76a	1.98a	3.22b	2.43b
	3	14.66a	1.52b	5.07a	3.28a
	5	14.80a	1.30b	5.64a	3.83a
	LSD	3.3	0.38	1.53	0.8
R-square		0.82	0.63	0.82	0.89
Error mean square		12.36	0.16	2.65	0.73

Table 3. Extent of relationship between the growth characters, fungi and WAI.

Correlation	Plant height	Stem girth	Leaf length	Leaf width	Fungi	WAI
Plant height						
Stem girth	0.65**					
Leaf length	0.74**	0.19				
Leaf width	0.71**	0.19	0.97**			
Fungi	0.55*	0.28	0.49	0.57*		
WAI	0.002	−0.53*	0.34	0.29	0.00	

Significant ($p < 0.01$) = **, Significant ($p < 0.05$) = *, WAI = week after inoculation.

Table 4. Contribution of PCA to the growth of *T. danielli* plant.

PCA	Prin 1	Prin 2	Prin 3	Prin 4
Plant height	0.53	0.31	−0.78	0.12
Stem girth	0.40	0.73	0.55	−0.02
Leaf length	0.54	−0.39	0.10	−0.74
Leaf width	0.52	−0.46	0.27	0.66
Eigen value	2.86	0.93	0.17	0.04
Eigen proportion (%)	71.58	23.32	4.18	0.92

In the first five days of the in vitro experiment, *A. niger* was the least inhibited pathogen, followed by *M. phaseolina*, then *A. flavus*. However, from day 6 to day 9, *A. niger* became the mostly inhibited followed by *A. flavus* while *M. phaseolina* was the least inhibited by the extracts used. Variation was observed in the support of each media type to the fungal growths. Among the four media evaluated, PDA recorded the most significant ($p < 0.05$) growth in the first 3 days of observation, followed by CZA, MEA, then V8 agar. From the fourth day till the ninth day, MEA showed the most significant support, this was followed by CZA, then PDA while V8 showed the least support for the fungal growths (Table 6).

Table 5. ANOVA of the interactive effects of plant extracts, concentration and media in the mycelia inhibition of the pathogenic fungi of *Thaumatoccocus danielli*.

Source	*df*	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Model	119	0.24**	0.59**	1.11**	2.22**	4.06**	5.99**	7.19**	8.36**
Extracts (E)	4	1.84 **	5.15**	9.17**	11.51**	16.94**	18.54**	26.18**	30.66**
Concentration (C)	2	0.05	0.02*	0.07	0.05	0.07	0.22	1.13*	1.27*
Media (M)	3	1.19 **	2.95**	4.69**	19.88**	49.29**	69.34**	79.77**	71.85**
Fungi (F)	2	0.51 **	0.37**	0.68**	0.60**	8.25**	39.98**	64.56**	111.15**
E × C	6	0.01**	0.03**	0.10*	0.27*	0.48*	0.34	0.31	0.36
M × C	6	0.01**	0.02**	0.05	0.13	0.75**	1.17**	0.4	0.98**
F × C	4	0.05	0.02**	0.04	0.19	0.66*	0.56	0.86*	1.14**
E × M	12	0.74**	2.02**	2.82**	4.13**	4.54**	5.39**	5.42**	7.45**
E × F	6	0.03**	0.05**	0.38**	0.23	0.16	0.36**	0.45	1.67**
M × F	6	0.19**	0.42**	1.49**	3.51**	7.85**	9.69**	8.26**	5.08**
E × M × F	18	0.01 **	0.03**	0.13**	0.21*	0.40*	0.74**	0.56*	1.08**
E × F × C	12	0.01 **	0.04**	0.21**	0.40**	0.53**	0.77**	0.64*	1.61**
M × F × C	12	0.01 **	0.02**	0.11**	0.21	0.62**	0.82**	0.53	0.42
E × M × F	18	0.04**	0.09**	0.33**	0.56**	0.75**	1.12**	1.42**	3.83**
Error	191	0.65	0.99	6.75	23.35	40.71	55.71	62.82	62.97
Corrected total	310	29.07	71.71	138.37	288.1	523.73	768.16	918.11	1057.83

Significant ($p < 0.01$) = **, Significant ($p < 0.05$) = *, WAI = week after inoculation.

Table 6. Influence of media on the mycelia growth of pathogenic fungi of *Thaumatoccocus danielli*.

Parameters	Variables	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Fungi	*A. niger*	0.502a	0.69a	0.72a	1.02a	1.36a	1.58b	1.87c	2.42c	3.12c
	A. flavus	0.500b	0.54c	0.61b	0.84c	1.20b	1.62b	2.21b	2.91b	3.85b
	M. phaseolina	0.500b	0.55b	0.62b	0.92b	1.42a	2.20a	3.17a	4.09a	5.27a
Media	PDA	0.50a	0.64a	0.73a	0.86b	1.11b	1.37b	1.74c	2.37c	3.58c
	V8 Agar	0.50b	0.57b	0.54d	0.71c	0.91c	1.26b	1.80c	2.40c	3.17d
	CZA	0.50b	0.64a	0.70b	0.91b	1.08b	1.38b	1.99b	2.76b	3.77b
	MEA	0.50b	0.51c	0.64c	1.23a	2.20a	3.19a	4.16a	5.03a	5.80a
R-squared		1.00	0.98	0.98	0.95	0.92	0.92	0.93	0.93	0.94
Error mean square		0.00	0.003	0.005	0.04	0.122	0.21	0.29	0.33	0.33

Mean with the different letter across the column are significantly ($p < 0.05$) different from one another with respect to each parameter.
PDA = potato dextrose agar; MEA = malt extract agar; CZA = Czapedox agar; V-8= vitamins juice agar.

All the extracts used showed significant ($p < 0.05$) reduction in the mycelia inhibition of the fungi across the days observed, compared to the control. Inhibitory effects of *F. asperifolia, M. charantia* and *P. guajava* showed no significant ($p < 0.05$) difference in their mycelia growths while *A. occidentals* recorded a significantly higher efficacy. At the ninth-day of observation, *F. asperifolia* recorded the most significant inhibition, followed by *M. charantia* and *P. guajava* which were not significantly different from each other.

Extract concentration level 25 mg/mL showed the least inhibition across the days of observation while up to day 6 concentration, 50 and 75 mg/mL were not significantly ($p < 0.05$) different from one another, However, from day 7 to day 9 all the concentration levels recorded similar level of significance (Table 7).

The in vitro effect of the plant extracts on *A. niger*, *M. phaseolina* and *A. flavus* as illustrated in Table 8 showed that the extracts were more

Table 7. Influence of extract and concentration on the mycelial growth of pathogenic fungi of *Thaumatoccocus danielli*.

Parameters	Variables	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Extracts	Control	0.51a	1.21a	1.68a	2.29a	2.85a	3.59a	4.21a	5.14a	6.10a
	F. asperifolia	0.50b	0.52c	0.55c	0.86b	1.13c	1.61c	2.24c	2.96c	3.98c
	M. charantia	0.50b	0.54c	0.56c	0.77c	1.22bc	1.68bc	2.19c	2.76c	3.55d
	P. guajava	0.50b	0.54c	0.56c	0.82bc	1.18bc	1.53c	2.10c	2.73c	3.67d
	A. occidentales	0.50b	0.57b	0.61b	0.83bc	1.28bc	1.82b	2.10c	3.48b	4.49b
Concentration (mg/mL)	25	0.502a	0.62a	0.68a	0.99a	1.40a	1.91a	2.43a	3.20a	4.14a
	50	0.500b	0.57b	0.67a	0.92b	1.32ab	1.75b	2.47a	3.17a	4.09a
	75	0.500b	0.58b	0.62b	0.88b	1.26b	1.75b	2.36a	3.05a	4.02a
R-squared		1.00	0.98	0.98	0.95	0.92	0.92	0.93	0.93	0.94
Error mean square		0.00	0.003	0.005	0.04	0.122	0.21	0.29	0.33	0.33

Mean with the different letter across the column are significantly ($p < 0.05$) different from one another with respect to each parameter.

effective on *A. niger* at day 4, day 6 and day 8 after inoculation. This was followed by *A. flavus* which also shared similar level of significance ($p < 0.05$) with *A. niger* while *M. phaseolina* showed different inhibitory activity. At day 8 after inoculation, the best inhibition of *A. niger* and *M. phaseolina* were recorded for *M. charantia* extracts, followed by *P. guajava*, then *A. occidentals* and *F. asperifolia*. Slight variation occurred in the order of efficacy of plant extracts on *A. flavus*; *M. charantia*, *P. guajava*, *A. occidentals* and *F. asperifolia*, although no significant ($p < 0.05$) differences occurred among the extracts. Generally, at all concentration levels the extracts were tested, result showed significant ($p < 0.05$) inhibition of mycelia growth of the pathogens. The efficacy of the extracts increases with increase in concentration and the most significant inhibition was recorded across the extracts at 75 mg/mL concentration. MEA amended with each type of extract was observed to enhance fungal growth as observed in day 4, day 6 and day 8 of the experiment, followed by CZA and PDA which showed consistent growth promotion of the fungi in *P. guajava* and *A. occidentales* amended samples, while V8 agar which appeared as the least supported mycelial growth on V8 agar amended with *P. guajava*.

Mean with the different letter across the column are significantly ($p < 0.05$) different from one another with respect to each parameter. PDA = Potato Dextrose Agar; MEA = Malt Extract Agar; CZA = Czapedox Agar; V-8 = Vitamins Juice Agar; F. asp = *Ficus asperifolia*, M.char = *Mormodica charantia*, P.guaj = *P. guajava*, A. occid. = *Anacardium occidentales*

The scatter plot showed variations of the Principal Component Axis in the phytofungicidal activities of the extracts. The PC 1 which explained 97.56% of the total variations recorded positive association with *M. charantia, P. guajava* and Control while *F. asperifolia and A. occidentales* showed negative relationship with Prin 1 with *A. occidentales* expressing

Table 8. Effects of plant extracts, concentration and media type on the growth inhibition of the pathogenic fungi of *Thaumatoccocus danielli*.

		Day 4				Day 6				Day 8			
Parameters	Variables	*F. asp.*	*M. char.*	*P. guaj.*	*A. occid.*	*F. asp.*	*M. cha*	*P. guaj.*	*A. occid.*	*F. asp.*	*M. cha*	*P. guaj*	*A. occid.*
Fungi	*A. niger*	1.83^{b}	1.06^{b}	0.96^{b}	1.20^{b}	2.77^{c}	1.64^{c}	1.63^{c}	1.98^{c}	3.49^{c}	1.82^{c}	2.23^{c}	2.57^{c}
	A. flavus	1.86^{b}	1.11^{b}	1.06^{b}	1.24^{b}	3.20^{b}	1.91^{b}	1.93^{b}	2.43^{b}	4.27^{b}	2.52^{b}	2.67^{b}	3.07^{b}
	M. phaseolina	2.57^{a}	1.62^{a}	1.44^{a}	1.58^{a}	4.50^{a}	2.83^{a}	2.59^{a}	3.36^{a}	5.70^{a}	3.66^{a}	3.35^{a}	4.42^{a}
Extract concentrations (mg/mL)	25	1.52^{b}	1.70^{b}	1.63^{b}	1.64^{c}	3.13^{b}	2.82^{b}	2.88^{ab}	3.54^{b}	4.13^{b}	3.54^{b}	3.75^{b}	4.70^{b}
	50	1.53^{b}	1.64^{b}	1.45^{b}	1.99^{b}	2.79^{c}	2.68^{b}	2.72^{b}	3.58^{b}	3.88^{bc}	3.51^{b}	3.77^{b}	4.50^{bc}
	75	1.71^{b}	1.71^{b}	1.53^{b}	1.73^{c}	2.90^{bc}	2.78^{b}	2.60^{c}	3.25^{c}	3.85^{c}	3.60^{b}	3.48^{b}	4.24^{c}
	Control	3.59^{a}	3.59^{a}	3.59^{a}	3.59^{a}	5.13^{a}	5.13^{a}	5.13^{a}	5.13^{a}	6.10^{a}	6.10^{a}	6.10^{a}	6.10^{a}
Media	PDA	2.29^{b}	0.80^{b}	0.58^{c}	0.80^{c}	3.37^{b}	1.34^{c}	1.05^{c}	1.93^{c}	4.73^{b}	2.40^{b}	1.94^{c}	2.56^{c}
	MEA	3.03^{a}	2.47^{a}	2.34^{a}	2.53^{a}	5.06^{a}	3.70^{a}	3.75^{a}	3.87^{a}	5.83^{a}	4.05^{a}	4.50^{a}	4.47^{a}
	CZA	1.52^{c}	0.99^{b}	0.85^{b}	1.13^{b}	2.91^{b}	1.77^{b}	1.78^{b}	2.51^{b}	4.02^{c}	2.09^{c}	2.39^{b}	3.76^{b}
	V8	1.51^{c}	0.81^{b}	0.84^{b}	0.90^{bc}	2.61^{b}	1.49^{c}	1.62^{a}	2.05^{b}	3.36^{d}	2.11^{c}	2.16^{bc}	2.64^{c}
Error means square (EMS)		0.22	0.19	0.10	0.17	0.25	0.17	0.22	0.23	0.20	0.19	0.28	0.26

stronger association. The PC 2 explained 2.23% of the total variation and showed only the Control experiment in favour of the direction of mycelial growth with respect to days of observation while all the extracts treatments were negatively associated with PC 2 (Figure 2).

The scatter plot showed variations in the support of different media to the mycelia growth of fungi evaluated. The PC 1 with Eigen proportion of 99.09% showed PDA and Czapek as positively related while MEA and V8 agar were negatively associated with PC 1. The PC 2 which explained 0.71% of the total variation recorded positive relationship with MEA which also supported the direction of growth from day 2 to day 9. While other media increased with decreasing PC 2 (Figure 3).

Phyto-fungicidal potential of thaumatoccocus danielli

The antifungicidal potential of *T. danielli* plant was investigated on *A. niger*, *A.flavus* and *M. phaseolina* in the course of this research. The extract of *T. danielli* significantly ($p < 0.05$) inhibited the mycelial growth of the fungi with the increase in concentration of *T. danielli* extract. *A. niger* and *A. flavus* were the most inhibited at day 3 and day 5, while *A. flavus* was the most significantly ($p < 0.05$) inhibited at day 7, followed by *M. phaseolina* then *A. niger*. The phytofungicidal activities of *T. danielli* extracts were significantly increased with increase in concentration levels while varying growth rates was demonstrated with the extracts of *T. danielli* amended in different media. PDA was observed to best support the mycelial growth, followed by MEA and CZA which were not

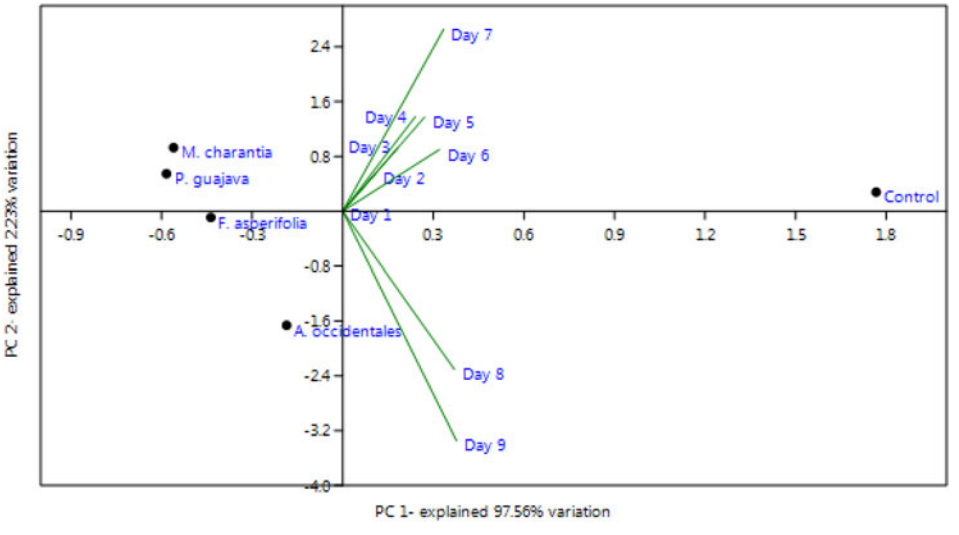

Figure 2. Contribution of PC 1 and PC 2 to the variations in the inhibitory effects of the extracts with respect to control.

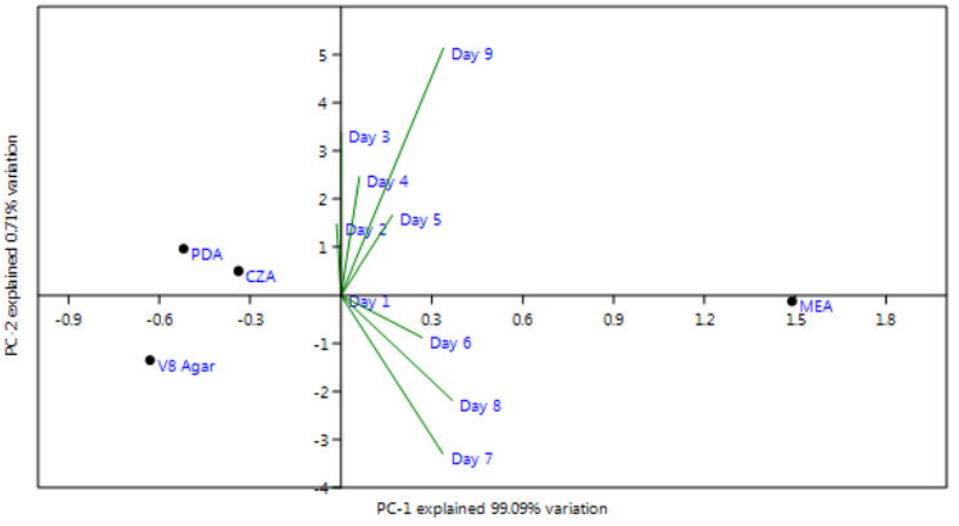

Figure 3. Contributions of PC 1 and PC 2 to the variations in the mycelia growth of fungi on different media with respect to days.

significantly different from one another, while V8 agar showed the least support of the pathogens as observed in days 3, 5 and 7 (Table 9).

Discussion

The fungi found associated with leaves and rhizospheric soil of *T. danielli* were; *A. niger, Pythium* sp., *Penicillium* sp., *T. konigii, T. viride, Fusarium* sp., *M. phaseolina, A. flavus* and *T. harzianum.* These fungi have been reported in several studies as associated with the diseases of many crop plants worldwide (Couch and Kohn 2002; Odebode et al. 2004; Akanmu et al. 2015). The high virulence of *M. phaseolina, A. flavus* and *A. niger,* followed by *Pythium* sp. among the isolated fungi evaluated in the screenhouse experiment agreed with some earlier reports of the pathogenic effect of *M. phaseolina* (Taylor 1995; Bandamaravuri et al. 2007), *Aspergillus* species (Perrone et al. 2007; Ruchi, 2012) and *Pythium* sp. (Weiland et al. 2013) reported on other crops. Whereas, the *Trichoderma* species recorded either slight or no significant disease severity and this could be attributed to their biocontrol potentials (Cumagun 2014).

Relationship was established in the dependency of the growth parameters on one another, but the fungi activities were pronounced on the plant height while the stem girth decreased significantly with increase in the period of study. Similar observation had been reported that positive and strong correlation between the morphological characters indicate that genotypic make-up of maize affects the morphological characters,

Table 9. Evaluation of phytofungicidal potentials of *Thaumatoccocus danielli* leaves.

		Days of observation		
Treatments	Variables	3	5	7
Fungi	*A. niger*	1.38^{b}	3.07^{b}	7.42^{a}
	A. flavus	1.33^{b}	3.00^{b}	4.39^{c}
	M. phaseolina	2.20^{a}	6.24^{a}	4.85^{b}
Concentrations (mg/mL)	Control	2.27^{a}	4.59^{a}	6.18^{a}
	25	2.10^{a}	4.69^{a}	6.13^{a}
	50	1.41^{b}	4.30^{b}	5.79^{b}
	75	0.80^{c}	3.81^{c}	5.15^{c}
Media	PDA	2.10^{a}	5.01^{a}	6.43^{a}
	MEA	1.66^{b}	3.94^{b}	5.38^{b}
	CZA	1.47^{bc}	3.93^{b}	5.51^{b}
	V8	1.30^{c}	3.52^{c}	4.89^{c}
	Error mean square	0.18	0.22	0.27

which invariably influences the quality of yield products (Olawuyi et al. 2015). More so, the highest proportion and Eigen vector accounted for the variation of *T. danielli* characters in Prin 1 which should be considered for the crop improvement.

The significance of the interactive effect of the extracts, concentration, media and fungi evaluated across the day of observation explained the condition best suitable for the optimum performances of the plant extracts in suppressing the pathogenic Fungi of *T. danielli* as was similarly demonstrated in an earlier study (Akanmu et al. 2013; Olowe et al. 2013; Olawuyi et al. 2015). The fungal growths were best supported on the media; MEA followed by CZA, PDA then V8 agar. Variations recorded in the performances of fungi on different media could explained most mycologists' preferences for certain types of media based on peculiarities of the type of fungi that are routinely grown, since media will affect colony morphology and color, whether particular structures are formed or not, and may affect whether the fungus will even grow in culture (Stevens 1981; Ayodele et al. 2009). *A. niger* was the mostly inhibited pathogen by the extracts followed by *A. flavus,* then *M. phaseolina* while efficacy of plant extracts; *M. charantia, P. guajava, F. asperifolia* and *A.occidentales* were arranged in decreasing order of inhibiting the fungi mycelia growth. Several studies have reported the efficacy of plant extracts in inhibiting fungi growth in-vitro, this has also been attributed to their phytochemical constituents and the phyto-fungicidal potentials (Pandey and Shweta 2011; Mohamed et al. 2013; Abiala et al. 2013; Akanmu et al. 2015; Wang et al. 2016).

With respect to the media, the PC-1 which accounted for 99.09% variation recorded increase with increasing activities of PDA and CZA, while the PC-1 which accounted for 97.56% variation on the extracts contributed positively and also increased with increase in the inhibitory activities of *M. charantia* and *P. guajava*. Thus, amendment of PDA and

CZA with extracts of *M. charantia* and *P. guajava* best supported the fungi inhibition. This was in agreement with an earlier report which stated that fungal nutrition, metabolism, growth and reproduction generally relates to interaction of fungi with their biotic and abiotic environment (Anjisha and Vrinda 2012).

Disclosure statement

No potential conflict of interest was reported by the authors.

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