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Full Length Article

Antifungal Effects against *Phaeoisariopsis personata* under Greenhouse Conditions and Phytochemical Analysis of *Jatropha curcas* Leaf Extracts

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Abstract

The study was conducted to test the antifungal efficacy of *J. curcas* leaf extracts against *Phaeoisariopsis personata* (causal pathogen for groundnut late leaf spot disease**)** under *in vivo* conditions, and to identify important phytochemical constituents exhibiting antifungal properties. The results showed that the greatest reduction of late leaf spot disease incidence was achieved by all the *Jatropha curcas* leaf extracts at the highest concentration (0.5 mg mL^{-1}) as 36.89, 36.59 and 24.67% for chloroform, ethyl acetate and methanolic extracts, respectively. Subsequently, *J. curcas* leaf extracts treatments enhanced the growth and yield of groundnut compared with the control (untreated). The antifungal effects of *J. curcas* were supported by the presence of phytochemical constituents identified by GC-MS. Hexadecane; *n*-hexadecanoic acid; phenol, 2, 4 bis (-dimethylethyl); phytol and hexadecanoic methyl ester were detected as major phytocompounds in *J. curcas* leaf extracts that were possibly responsible for the antifungal activity.

Keywords: Biological control; Efficacy; Groundnut; Late leaf spot; Phytocompounds

Introduction

Groundnut (*Arachis hypogaea* L.) is a vital oil kernel crop in the tropics and subtropics countries (Pasupuleti *et al*. 2013). The groundnut is cultivated in many countries between latitudes 40˚N and 40˚S in semiarid tropics and subtropics (Kayondo *et al*. 2014). Groundnut seeds contain about 12–15% carbohydrates, 25–30% protein and 40–50% fats (Saeed and Hassan 2009). Additionally, groundnuts contain a good source of vitamin, dietary fibres, and minerals such as niacin, magnesium, iron, phosphorus, calcium, zinc, and riboflavin (Tshilenge-Lukanda *et al*. 2013). In addition, groundnut as a legume crop improves soil fertility by converting the atmospheric nitrogen to nitrates, ammonia, and organic nitrogen (Pasupuleti *et al*. 2013). Thus, groundnut production plays a great role in developing and developed nations in improving the economic status (Tsigbey *et al*. 2003).

In Tanzania, the crop is cultivated mainly by smallholder farmers in Tabora, Mtwara, Dodoma, Singida, Shinyanga and Mwanza regions. The crop acts as a good source of food, cash crop, and animal feed (Osei *et al*. 2013). Despite the importance of groundnut in Tanzania, its average yield is still low amounting to 960 kg ha^{-1} as opposed to the predictable yield potential of 2500 kg ha^{-1} in developing countries (Philipo and Nchimbi-Msolla 2019). The crop is mainly constrained by drought stress, low level of inputs, foliar fungal diseases, and insect pest attacks.

The foliar fungal diseases namely, early leaf spot, late leaf spot, and rust are among the most destructive diseases, which account for huge economic yield losses (Naidu *et al*. 1999). The late leaf spot disease is one among the three diseases, which has been identified as a major constrain wherever groundnut is cultivated in Tanzania. The late leaf spot disease infection reduces the photosynthetic area by causing intense lesions on stems, leaves, and petioles consequently leading to defoliation and hence high yield losses (Monfort *et al*. 2004; Khedikar *et al*. 2010). According to Ghewande (1989), when the late leaf spot disease attacks groundnuts, it reduces about 80 and 93% canopy carbon exchange rate and carbon uptake respectively. Late leaf spot disease also causes adverse outcomes on seed and folder quality becoming unsuitable for animal feed (Monfort *et al*. 2004).

Efforts have been made in controlling late leaf spot disease through the development and the use of synthetic

fungicides, which have been proven effective. However, the effectiveness of synthetic fungicides depends upon multiple fungicide applications; hence, smallholder farmers cannot afford the cost. Moreover, there are issues of environmental and health concerns (Jordan *et al*. 2012). The plants possess phytochemical compounds, which are effective against different pests including insects, fungi, virus, nematode, bacteria *etc*. (Khan *et al.* 2020; Javaid *et al.* 2020, 2021). Thus, plant based materials can be used as a substitute for synthetic pesticides in order to ensure the safety of human and the environment (Engindeniz and Engindeniz 2013; Khan and Javaid 2020; Banaras *et al.* 2021).

The *Jatropha curcas* belonging to Euphorbiaceae family is a multipurpose plant that survives in both tropics and arid regions. Almost all the plant parts of *Jatropha* are reported to possess antimicrobial potential against disease causing pathogens including fungi (Prasad *et al*. 2012; Dada *et al*. 2014). Moreover, *J. curcas* leaf possesses important compounds such as sterols, terpenes, flavonoids, saponin and steroids steroids, which play a great antifungal role (Nwosu and Okafor 1995; Campa *et al*. 2008; Saetae and Worapot 2010). Many findings confirm the antifungal potential of *J. curcas* in managing fungal diseases. A study by Thangavelu *et al*. (2004) found that *J. curcas* was effective in managing banana anthracnose disease. In addition, *J. curcas* leaf extracts were found effective in managing *Azolla* disease caused by *Sclerotium* spp. (Garcia and Lawas 1990). However, little is known about the effectiveness of *J. curcas* against *P. personata* pathogen causing the late leaf spot disease on groundnuts. Thus, the present study evaluated the efficacy of *J. curcas* against the groundnut late leaf spot disease and analysed the possible compounds exhibiting antifungal properties.

Materials and Methods

Isolation of pathogen and culture preparation

Groundnut leaves showing symptoms of *P. personata* (circular spots underneath the leaflets) were obtained from the farmer's fields in Singida and Dodoma regions, Tanzania. Fungal isolation was done by adopting the method described by Kishore *et al*. (2007) with some modification. The diseased portions of leaves were cut into pieces (0.5–1 cm) and sterilized with 5% NaOCl. Thereafter, the pieces were rinsed three times in the sterilized distilled water and dried on a blotter paper in Petri dishes. Thereafter, the pieces were plated onto potato dextrose agar (PDA) medium in a laminar hood then incubated at 28 ± 2°C for 7 days. After the emergence of mycelial growth, each fungal colony was sub-cultured into fresh PDA plates and incubated at 28 ± 2°C for 7 days to obtain the pure culture of *P. personata*. The *P. personata* pathogen was identified by a single spore method under compound microscope (magnification 40X).

Preparation of *J. curcas* leaf extracts

J. curcas leaves were collected from different parts in Arusha, Tanzania. The leaves were washed thoroughly and then air-dried at room temperature. Thereafter, these were pounded into powder. The fine powdered leaf sample (1 kg) was successively extracted through chloroform, ethyl acetate, and lastly methanol (48 h in each) at standard room temperature. Then, the leaf extracts were filtered with a Whatman No.1 filter paper and concentrated by a rotary evaporator. The product, which was a dark sticky semisolid extracts, was then stored under cold condition (4°C) for further experiment.

Greenhouse experiment on antifungal assay

The study was carried out to test the effectiveness of methanol, chloroform and ethyl acetate leaf extracts of *J. curcas* against late leaf spot disease under greenhouse condition. Three groundnut seeds (Upendo variety) were grown in a plastic pots filled with a mixture of black soil (later thinned to one). The plants were artificially inoculated by *J. curcas* conidial suspension 30 days after sowing (DAS). After inoculation, the plants were shielded with plastic sheets for 48 h to maintain leaf wetness during the nights. Four foliar sprays were applied onto the plants each *J. curcas* leaf extract at 0.1, 0.25 and 0.5 mg mL^{-1} concentration, chlorothalonil (2 mL L^{-1}) (positive control) and sterile distilled water (negative control) using a hand sprayer at 14 days interval. The plants were sprayed starting from 48 DAS and completed two weeks before the harvest. The experiment was inspected often and the data on disease, growth, and yield were recorded. The trial was set in a completely randomized design replicated three times. The experiment was repeated twice. The disease incidence was assessed on each plant by evaluating the percent of the infected leaves per plant by adopting the formula by Subrahmanyam *et al*. (1995).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected leaves per plant}}{\text{Total number of leaves per plant}} \text{X } 100$$

The late leaf spot disease severity under different treatments was scored using (1–9) disease rating scale (Chiteka *et al.* 1988).

Gas chromatography mass spectroscopy analysis

The phytochemical analysis of *J. curcas* leaf extracts was done using gas chromatography-mass spectroscopy (GC-MS) on Agilent technologies 7890A GC connected to Agilent 5975 MSD (Agilent technology, USA), comprising a 30 m length and film 0.25 μm and internal diameter of 0.250 mm and temperature limit of 50°C to 340°C (360°C). The inert gas helium was used as a carrier gas with 1.2 mL min^{-1} flow rate. The inlet temperature was 250°C and the total running time was 35 min. The obtained peaks were

compared with the known compounds spectra stored in the National Institute Standard and Technology library.

Data analysis

Data were subjected to 3-way ANOVA (analysis of variance) in factorial arrangement, using STATISTICA program. The treatment means were compared by applying Fischer's least significant difference (LSD) at 5% level of significance.

Results

In this study, the effectiveness of *J. curcas* leaf extracts against the late leaf spot disease was determined by observing their effect on reducing the disease incidence and severity. Moreover, growth and yield attributes were also assessed. Three leaf extract of *J. curcas* (methanolic, chloroform, and ethyl acetate), one standard fungicide chlorothalonil (positive control) and one negative control (distilled water) as foliar spray were used as treatments against late leaf spot disease. The effects of treatments, solvents and concentrations on late leaf spot disease incidence, severity, growth and yield attributes are presented in Table 1, 2 and 3.

The late leaf spot disease varied significantly ($P \leq 0.001$) with the effect to treatments. The plants treated by *Jatropha* leaf extracts had lower late leaf spot disease incidence (13.33%) similar to the standard fungicide chlorothalonil (5.41%). Moreover, the late leaf spot disease incidence differed significantly ($P \leq 0.01$) with the type of solvents used for extraction. Methanolic leaf extract of *J. curcas* had lower late leaf spot disease incidence (24.7%) as compared to chloroform (36.89%) and ethyl acetate (36.59%,) extracts (Table 1). Moreover, the results showed that the late leaf spot disease incidence and severity differed significantly ($P \leq 0.001$) from the effect of *J. curcas* leaf extracts concentration. *J. curcas* leaf extracts at the highest concentration (0.5 mg mL^{-1}) significantly reduced the late leaf spot disease incidence as compared to the lowest concentration (0.1 mg mL^{-1}) (Table 1).

Growth parameters varied significantly ($P \leq 0.001$) with the effect of treatments, solvents and concentrations of *J. curcas* leaf extracts (Table 2). The plants treated with chlorothalonil and *J. curcas* had taller shoots and big number of leaves per plant both at flowering and maturity (38.59 cm, 8.93) (47.26 cm, 12.89), (27.78 cm, 6.74) and (36.07 cm, 9.48) respectively compared with the control. Similarly, growth parameter differed significantly ($P \leq 0.05$) from the type of solvents used for extraction. The methanolic and chloroform leaf extracts of *J. curcas* had taller shoots (30.48 cm, 29.56 cm), (38.63 cm, and 36.96 cm) at both flowering and maturity respectively compared with ethyl acetate extracts (27.52 cm, 34.93 cm). Additionally, shoot length and big number of leaves per plant differed significantly ($P \leq 0.001$) at different *J. curcas* leaf extracts concentrations. The plants treated with plant extracts at the highest concentration (0.5 mg mL^{-1}) had taller shoots and bigger number of leaves per plant at flowering and maturity (31.89 cm, 7.88), (39.9 3 cm, 9.93), respectively compared with the control (25.91 cm, 5.82), (33.48 cm, 7.81) at both flowering and maturity (Table 2).

Likewise, yield attributes components varied significantly ($P \leq 0.001$) with the treatments where *J. curcas* leaf extracts had bigger number of pods per plant, seeds per plant and seed yield (ton ha^{-1}). *J. curcas* was less similar to the standard fungicides (chlorothalonil) that is, the number of pods per plant (32.9), the number of seeds per plant (61.2) and seed yield (1.6 ton ha^{-1}) (Table 3). Yield data did not differ significantly with the effect to solvents, similar results were observed. The yield attributes differed significantly ($P \leq 0.01$) with the effect to *J. curcas* leaf extracts concentration. *J. curcas* leaf extracts at the highest concentration had bigger number of pods $plant^{-1}$ (34.59), the number of seeds per plant (63.19), 100 seed weight (53.09 g) and seed yield (1.85 ton ha^{-1}) (Table 3).

The GC-MS results led to the identification of different phytochemical constituents from fractions of ethyl acetate, methanolic, and chloroform leaf extracts of *J. curcas*. The mass spectra of the detected compounds from methanolic, chloroform and ethyl acetate leaf extracts of *J. curcas* were compared with the spectra of the recognized compounds in the NIST library. The name of compound, molecular weight, retention time, and molecular formula of the compounds contained in these leaf extracts are presented in Tables 4, 5 and 6. The following phytoconstituents with antifungal properties were recognized by GC-MS from the chloroform leaf extract of *J. curcas*; dodecane; 2,6,11-trimethyl-2-tetradecene; tetradecane; pentadecane; octacosane; sulfurous acid butyl decyl ester; 2-bromo heneicosane; phenol 2,4-bis (1, 1-dimethylethyl); hexadecane; heptadecane; heptacosane; 2,4-dimethyldodecane; n-hexadecanoic acid; ethanol 2-(octadecyloxy)-; hentriacontane; geranylgeraniol; octadecane; 12-methyl-E-E-2 13-octadecadien-1-ol; tetradecanal and cyclotetracosane (Table 4). Among them *n*-hexadecanoic acid (7.89%); phenol 2,4-bis (1,1-dimethylethyl) (4.04%); cyclotetracosane (1.23%); hexadecane (1.20%) and octacosane (1.02%) were the major identified phytoconstituents compounds (Table 4).

From ethyl acetate leaf extract of *J. curcas*, the following phytoconstituents with antifungal activity were identified by GC-MS; 1,2,3-ropanetriol; monoacetate; 2,5-pyrrolidinedione; thiomorpholine; methyl salicylate; triacetin, 1-naphthalenol; 8-hexadecenal; 14-methyl-, (Z)-, undecane; phenol, 2,4-bis (1,1-dimethylethyl); hexadecane; heptadecane; 1H-indene 1-ethylideneoctahydro-7 a-methyl-*cis*-, E-14-hexadecenal; 1-tetradecene; tetramethyl-2-hexadecen-1-ol; 9,12-octadecadienoic acid (Z,Z); 5-eicosene, (E); hexadecanoic acid ethyl ester; 2-methyl-Z,Z-3,13-octadecadienol,; (Z)-; *n*-hexadecanoic acid; phytol; 9,12,15-octadecatrienoic acid ethyl ester; (Z,Z,Z)-;

Table 1: Late leaf spot disease incidence and severity as affected with treatments, solvent and concentration

Factors	Incidence	Severity
Treatments		
Jatropha curcas	13.33 ± 2.02^{b}	2.26 ± 0.31^{b}
Chlorothalonil	5.41 ± 1.07^{a}	1.33 ± 0.22^{a}
Control	89.41 ± 0.92^{c}	8.96 ± 0.04^{c}
Solvents		
Chloroform	36.89 ± 7.49^{b}	3.89 ± 0.63^{b}
Ethyl acetate	36.59 ± 7.59^{b}	3.00 ± 0.59^{b}
Methanol	24.67 ± 7.60^{a}	2.67 ± 0.63^{a}
Concentrations		
0.1 mg mL^{-1}	41.89 ± 7.36^{c}	5.42 ± 0.53^{c}
0.25 mg mL^{-1}	35.93 ± 7.39^{b}	4.00 ± 0.59^{b}
0.5 mg mL^{-1}	20.33 ± 7.76^{a}	2.07 ± 0.69^{a}
3-WAY ANOVA (F-value)		
Treatments	4332.52***	512.564***
Solvents	2390.38**	1.14**
Concentrations	1.62***	20.34***
Treatments*Solvents	37.11ns	4.555**
Treatments*Concentrations	1.57ns	8.04***
Solvents*Concentrations	7.751***	0.38ns
Treatments*Solvents*Concentrations	0.381ns	0.56ns

Means with the same letter(s) were considered statistically not significant at ($P = 0.05$), Fischer's least significant difference (LSD) test

Table 2: Growth attributes of groundnut Upendo genotype as affected by treatments, solvents and concentrations

Factors	Shoot length (cm) flowering	Number of branches at flowering	Shoot length at maturity	Number of branches at maturity
Treatments				
Jatropha curcas	27.78 ± 1.16^{b}	6.74 ± 0.27^{b}	36.07 ± 1.22^{b}	9.48 ± 0.43^{b}
Chlorothalonil	38.59 ± 1.10^{c}	8.93 ± 0.35^{c}	47.26 ± 1.19^{c}	12.89 ± 0.47^{c}
Control	21.19 ± 0.72^{a}	4.85 ± 0.17^{a}	27.19 ± 0.71^{a}	4.48 ± 0.14^{a}
Solvents				
Chloroform	29.56 ± 1.76^{b}	6.96 ± 0.42^{a}	36.96 ± 2.00^{b}	9.33 ± 0.85^{b}
Ethyl acetate	27.52 ± 1.63^{a}	6.59 ± 0.40^{a}	34.93 ± 1.79^{a}	8.15 ± 0.71^{a}
Methanol	30.48 ± 1.75^{b}	6.69 ± 0.45^{a}	38.63 ± 1.93^{c}	9.37 ± 0.73^{b}
Concentrations				
0.1 mg mL^{-1}	25.91 ± 0.95^{a}	5.82 ± 0.31^{a}	33.48 ± 1.53a	7.81 ± 0.62^{a}
0.25 mg mL^{-1}	29.58 ± 1.10^{b}	6.81 ± 0.39^{b}	$37. 11 \pm 1.95^{b}$	9.11 ± 0.72^{b}
0.5 mg mL^{-1}	31.89 ± 1.18^{c}	7.88 ± 0.47^{c}	39.93 ± 2.07^{c}	9.93 ± 0.89^{c}
3-Way ANOVA (F-value)				
Treatments	153.15***	108.23***	179.32***	252.32***
Solvents	4.56*	1.190ns	6.098**	6.819**
Concentrations	17.54***	28.012***	18.49***	15.994***
Treatments*Solvents	10.29***	4.226**	8.58***	7.72***
Treatments*Concentrations	2.251ns	3.51*	3.15*	4.74**
Solvents*Concentrations	0.15ns	1.048ns	0.09ns	0.18ns
Treatments*Solvents*Concentrations	1.20ns	0.896ns	0.923ns	0.96ns

Means with the same letter(s) were considered statistically not significant at ($P = 0.05$), Fischer's least significant difference (LSD) test

heptadecanoic acid ethyl ester and eicosane (Table 5). The major phytoconstituents were phytol (9.31%); thiomorpholine (4.83%); hexadecanoic acid ethyl ester (3.97%); phenol 2,4-bis (1,1-dimethylethyl) (3.37%); 9,12,15-octadecatrienoic acid ethyl ester, (Z,Z,Z)- (2.75%); 5-eicosene, (E)- (2. 11%) and 1-heneicosyl (1.92%) (Table 5).

The phytoconstituents with antifungal property identified by GC-MS in *J. curcas* methanolic leaf extract were; 1,2,3-propanetriol monoacetate; methyl salicylate; 2-undecanone; decanoic acid methyl ester; 2-methoxy-4-vinylphenol; tert-hexadecanethiol; phenol 2,6-dimethoxy; tetradecane; cyclotetradecane; pentanoic acid ethyl ester; 2-propenoic acid 3-phenyl- methyl ester; diphenyl ether; pentadecane; tridecane; hexadecane; heptadecane; 17-pentatriacontene, 1-nonadecene; E-15-heptadecenal; 8-hexadecenal 14-methyl; cyclopentadecane; hexadecanoic acid methyl ester; 1-octadecene; 2-methyl-Z, Z-3, 13-octadecadienol; oleic acid, 9,17-octadecadienal, (Z); 2-methyl-Z,Z-3,13-octadecadienol; 9, 12-octadecadienoic acid (Z,Z)-methyl ester; phytol; octadecanoic acid methyl ester; behenic alcohol; octadecanoic acid ethyl ester; 3,7,11,15-tetramethyl-2-hexadecen-1-ol; 9,17-octadecadienal; eicosane and docosanoic acid methyl ester (Table 6). Phytol (26.75%); hexadecanoic acid methyl ester (14.32%); octadecanoic acid methyl ester (2.79%) and 9,12-octadecadienoic acid (Z,Z)- methyl ester (2.33%) were identified as major phytoconstituents (Table 6).

Table 3: Yield attributes of groundnut Upendo genotype as affected by treatments, solvents and concentrations

Factors	Number of pods/plant	Number of seeds/plant	100 kernel weight (g)	Seed yield (tonnes/ha)
Treatments				
Jatropha curcas	32.96 ± 1.13[b]	61.15 ± 2.25[b]	49.00 ± 2.30[b]	1.59 ± 0.09[b]
Chlorothalonil	40.30 ± 1.27[c]	75.81 ± 2.49[c]	54.18 ± 1.50[c]	2.49 ± 0.10[c]
Control	16.0 ± 0.95[a]	24.41 ± 1.67[a]	33.85 ± 1.75[a]	0.49 ± 0.03[a]
Solvents				
Chloroform	30.07 ± 2.40[b]	53.96 ± 5.04[a]	50.07 ± 1.79[a]	1.54 ± 0.19[a]
Ethyl acetate	28.59 ± 2.18[a]	51.33 ± 4.56[a]	50.82 ± 1.75[a]	1.50 ± 0.18[a]
Methanol	30.59 ± 2.25[b]	56.07 ± 4.62[a]	51.13 ± 1.57[b]	1.54 ± 0.17[a]
Concentration				
0.1 mg mL^{-1}	24.59 ± 2.00[a]	43.85 ± 4.15[a]	49.16 ± 1.52[a]	1.20 ± 0.14[a]
0.25 mg mL^{-1}	30.07 ± 2.18[b]	54.33 ± 4.55[b]	49.78 ± 1.71[a]	1.52 ± 0.17[b]
0.5 mg mL^{-1}	34.59 ± 2.23[c]	63.19 ± 4.76[c]	53.09 ± 1.82[b]	1.85 ± 0.20[c]
3-Way ANOVA (F-value)				
Treatments	282.57***	353.15***	38.74***	248.92***
Solvents	1.96ns	2.84ns	0.135*	0.15ns
Concentrations	45.63**	47.17**	0.47*	25.883***
Treatments*Solvents	1.707ns	1.92ns	0.44ns	0.63ns
Treatments*Concentrations	1.179ns	1.47ns	0.43ns	3.136*
Solvents*Concentrations	4.033*	3.669*	0.45ns	1.774ns
Treatments*Solvents*Concentrations	0.238ns	0.212ns	1.20ns	1.482ns

Means with the same letter(s) were considered statistically not significant at (P = 0.05), Fischer's least significant difference (LSD) test

Table 4: Phytochemical compounds with antifungal activity obtained from chloroform leaf extract of *J. curcas*

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
10.629	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212.41	(Zhang *et al.* 2015)
11.745	2-Tetradecene	$C_{14}H_{28}$	196.37	(Shirani *et al.* 2017)
11.905	Tetradecane	$C_{14}H_{30}$	198.39	(Begum *et al.*2016)
12.460	Pentadecane	$C_{18}H_{38}$	254.49	(Zhang *et al.* 2015)
12.958	Octacosane	$C_{28}H_{58}$	394.76	(Zhang *et al.*2018)
13.192	Sulfurous acid butyl decyl ester	$C_{16}H_{34}O_3S$	306.50	(Sharma *et al.* 2019)
13.267	Heneicosane	$C_{21}H_{44}$	296.57	(Ebrahimabadi *et al.* 2016)
13.461	Phenol 2,4-bis(1, 1-dimethylethyl)	$C_{14}H_{22}O$	206.32	(Manikandan *et al.* 2017)
14.011	2-Bromo dodecane	$C_{12}H_{25}Br$	249.23	(Manikandan *et al.* 2017)
14.503	Hexadecane	$C_{16}H_{34}$	226.44	(Zhang *et al.* 2015)
15.041	Heptadecane, 9-octyl-	$C_{25}H_{52}$	352.68	(Musa *et al.*2015)
15.401	Heptacosane	$C_{27}H_{56}$	380.73	(Bouzabata *et al.*2018)
16.002	2,4-Dimethyldodecane	$C_{14}H_{30}$	198.38	(Begum *et al.* 2016)
16.488	Pentadecane	$C_{15}H_{32}$	212.41	(Yuan *et al.*2012; Zhang *et al.* 2015)
17.009	Ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314.50	(Mohy and Mohyeldin 2018)
18.067	Octacosane	$C_{28}H_{58}$	394.76	(Zhang *et al.*2018)
18.142	Hentriacontane	$C_{31}H_{64}$	436.84	(Ruban and Gajalakshmi 2012)
18.457	Geranylgeraniol	$C_{20}H_{34}O$	290.48	(Ashraf *et al.* 2017)
18.542	Octadecane	$C_{18}H_{38}$	254.49	(Zhang *et al.* 2018)
18.869	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	(Omoruyi *et al.* 2014)
19.584	12-Methyl-E-E-2, 13-octadecadien-1-ol	$C_{19}H_{36}O$	280.00	(Vijayabaskar and Elango 2018).
20.013	Tetradecanal	$C_{14}H_{28}O$	212.37	(Passos *et al.* 2003)
29.037	Cyclotetracosane	$C_{24}H_{48}$	336.64	(Bughio *et al.* 2017)

Discussion

The *in vivo* studies confirmed the efficacy of *J. curcas* by lowering the disease incidence and severity as the concentration increased. The lowest late leaf spot disease incidence and severity were achieved with both *J. curcas* leaf extracts, similar to the standard fungicide (chlorothalonil). This corresponds with the findings of Thangavelu *et al*. (2004), who revealed that the leaf extract of *J. curcas* effectively controlled *Colletotrichum musae* and *Sclerotium* spp. causal agents for anthracnose disease in banana and *Azolla*, respectively. Methanolic extracts showed the lowest late leaf spot disease incidence and severity compared to ethyl acetate and chloroform extracts. This suggests that more polar compounds extracted by methanol had antifungal property slightly greater than had those extracted by ethyl acetate and chloroform. This finding is consistent with the findings by Igbinosa *et al*. (2009) who revealed that, the stem bark methanolic extract of *J. curcas* inhibited the growth of *Escherichia coli*, *Bacillus subtilis* and *Proteus vulgaris.* Moreover, according to Kalimuthu *et al*. (2010) the methanolic extract of *J. curcas* inhibited *Pseudomonas*, *Klebsiella*, *E. coli* and *Staphylococcus aureus.* Moreover, the *J. curcas* leaf extracts at the highest concentration significantly reduced late leaf spot disease incidence and

Table 5: Phytochemical compounds with antifungal activity obtained from ethyl acetate leaf extract of *J. curcas*

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
7.539	1,2,3-Ropanetriol, monoacetate	$C_5H_{10}O_4$	134.13	(Teoh and Mashitah 2012)
8.460	2,5-Pyrrolidinedione	$C_8H_{13}NO_2$	331.32	(Takayama *et al*. 1982)
8.826	Hexadecane	$C_{16}H_{34}$	226.44	(Adeleye *et al*. 2010)
9.273	Methyl salicylate	$C_8H_8O_3$	152.15	(Pawar and Thaker 2006)
11.321	Triacetin	$C_9H_{14}O_6$	218.21	(Osuntokun and Olajubu 2014)
11.813	Heptadecane	$C_{17}H_{36}$	240.5	(Zhang *et al*., 2015)
11.899	8-Hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$	252.4	(Osuntokun and Olajubu 2014)
12.952	Undecane	$C_{11}H_{24}$	156.31	(Wanxi *et al*. 2013)
13.467	Phenol,2,4-bis(1,1-dimethylethyl)	$C_{17}H_{30}OSi$	278.50	(Jun *et al*. 2018)
13.993	1-Naphthalenol	$C_{10}H_8O$	144.17	(Kumar *et al*. 2012)
14.503	Hexadecane	$C_{16}H_{34}$	226.41	(Oliveira *et al*. 2014)
15.658	Heptadecane	$C_{17}H_{36}$	240.48	(Zhang *et al*. 2015)
16.889	E-14-Hexadecenal	$C_{16}H_{30}O$	238.41	(Devakumar *et al*. 2017)
17.106	1-Tetradecene	$C_{14}H_{28}$	196.37	(Tayung and Jha 2014)
17.896	Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.50	(Mohy and Mohyeldin 2018)
18.868	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	(Tyagi and Agarwal 2017)
18.983	9,12-Octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	280.40	(Mohy and Mohyeldin, 2018)
19.109	5-Eicosene, (E)-	$C_{20}H_{40}$	280.50	(Adibe *et al*. 2019)
19.172	Hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.47	(Mohy and Mohyeldin 2018)
19.338	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.50	(Adibe *et al*. 2019)
20.179	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.40	(Adibe *et al*. 2019)
20.413	Phytol	$C_{20}H_{40}O$	296.54	(Pejin *et al*. 2014)
21.008	9,12,15-Octadecatrienoic acid ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.48	(Mohy and Mohyeldin 2018)
21.186	Heptadecanoic acid ethyl ester	$C_{19}H_{38}O_2$	298.50	(Bashir *et al*. 2019)
23.869	Eicosane	$C_{20}H_{42}$	282.50	(El-Naggar *et al*. 2017)

severity as compared to the lowest concentration. This finding corresponds with the finding of an investigation by Amah and Aliero (2009) who revealed that disease incidence and severity were reported as being low in plants treated with plant extract at the highest concentration.

Growth parameters varied significantly with the effect of treatments, solvents and concentration. The plants treated with Chlorothalonil and *J. curcas* had taller shoots and bigger number of leaves per plant at both flowering and maturity compared with the control. In addition, the plants treated with methanolic and chloroform extracts had taller shoots and bigger number of leaves per plant, at both flowering and maturity compared with ethyl acetate extracts. Moreover, yield attributes components varied significantly with *J. curcas* leaf extracts concentrations, where *J. curcas* leaf extracts at the highest concentration influenced groundnut yield similar to the standard fungicides (Chlorothalonil). This observation is consistent with the results by Ghewande (1989) who found leaf extracts of *Azadirachta indica* and *Lawsonia inermis* effective in managing both groundnut late leaf spot and rust diseases and increased yield by 15–40% under field conditions. For this case, methanolic, ethyl acetate and chloroform leaf extracts of *J. curcas* were found effective against late leaf spot disease LLS subsequently improved the growth and yield of groundnuts compared with the control treatments.

GC-MS analysis was performed on chloroform, ethyl acetate and methanolic extracts of *J. curcas* since they exhibited the antifungal activity under *in vivo* experiment. The GC-MS identified the presence of different phytoconstituents from chloroform), ethyl acetate and methanolic leaf extracts of *J. curcas*. The qualitative differences of phytochemical constituents observed in this study may be attributed by different solvents employed for extraction. This observation corresponds with the findings by Kordali *et al*. (2009), who reported that, the spectra solubility of phytochemicals depends on the type of solvent used for extraction. In addition, phytochemical differences could be the result of the habitat for plant growth. This is consistent with Farooq *et al*. (2007) finding that, the phytochemical compounds composition depends on the plant habitat. The phytochemical analysis revealed the existence of octadecanoic acid; hexadecanoic acid methyl ester (palmitic acid); 9, 12-octadecadienoic acid (Z,Z) methyl ester and phytol in *J. curcas* leaf extracts. Amongst them hexadecanoic acid; octadecanoic acid methyl ester, and 9, 12-octadecadienoic acid (Z, Z) methyl ester are fatty acids, with the exception of phytol which is diterpene alcohol (Hema *et al*. 2011; Banaras *et al*. 2017). According to studies (Belakhdar *et al*. 2015; Chukwunonye *et al*. 2015), fatty acids possess antifungal property against diverse mycological pathogens. Since the fungal tissue is lipophilic in nature the fatty acids will attract the absorption of the fungus more easily (Inouye *et al*. 1999). Moreover, even the minor phytochemical components possibly contributed to antifungal effect by working synergistically with major compounds as reported by (Marino *et al*. 2001). The possession of these important phyto-compounds with antifungal properties in

Table 6: Phytochemical compounds with antifungal activity obtained from methanolic leaf extract of *J. curcas*

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
7.539	1,2,3-Propanetriol monoacetate	$C_5H_{10}O_4$	134.13	(Teoh and Mashitah 2012)
9.273	Methyl salicylate	$C_8H_8O_3$	152.15	(Essien *et al*. 2015)
10.549	2-Undecanone	$C_{11}H_{22}O$	170.29	(Bisht and Chanotiya 2011)
10.841	Indole	C_8H_7N	117.15	(Sumiya *et al.* 2017)
10.898	Decanoic acid methyl ester	$C_{11}H_{22}O_2$	186.29	(Belakhdar *et al*. 2015)
11.121	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	(Guo *et al*. 2008)
11.287	Tert-hexadecanethiol	$C_{16}H_{34}S$	258.50	(Yang *et al*. 2016)
11.653	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154.16	(Yang *et al*. 2016)
11.813	Tetradecane	$C_{14}H_{30}$	198.39	(Begum *et al*. 2016)
11.905	Cyclotetradecane	$C_{14}H_{28}$	196.37	(Afrouzan *et al*. 2018)
11.991	Pentanoic acid ethyl ester	$C_7H_{14}O_2$	130.18	(Sumiya *et al.* 2017)
12.248	2-Propenoic acid 3-phenyl-, methyl ester	$C_{10}H_{10}O_2$	162.18	(Umaiyambigai *et al.* 2017)
12.334	Diphenyl ether	$C_{12}H_{10}$	170.21	(Zhang *et al.* 2018)
13.198	Pentadecane	$C_{15}H_{32}$	212.41	(Zhang *et al.* 2015)
13.272	Tridecane	$C_{13}H_{28}$	184.36	(Yuan *et al*. 2012)
14.503	Hexadecane	$C_{16}H_{34}$	226.44	(Oliveira *et al*. 2014)
16.706	Heptadecane	$C_{17}H_{36}$	240.47	(Musa *et al*. 2015)
16.797	17-Pentatriacontene	$C_{35}H_{70}$	490.93	(Zhang *et al.* 2015)
16.889	1-Nonadecene	$C_{19}H_{38}$	266.50	(Asong *et al*. 2019)
17.015	E-15-Heptadecenal	$C_{17}H_{32}O$	252.43	(Begum *et al*. 2016)
17.192	8-Hexadecenal 14-methyl-,	$C_{17}H_{32}O$	252.40	(Aja *et al.* 2014)
17.787	Cyclopentadecane	$C_{15}H_{30}O$	210.40	(Nakashima *et al*. 2014)
18.474	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	(Belakhdar *et al.* 2015)
18.777	1-Octadecene	$C_{18}H_{36}$	252.48	(Omoruyi *et al.* 2014)
18.868	2-Methyl-Z, Z-3, 13-octadecadienol	$C_{19}H_{36}O$	280.49	(Phatangare *et al.* 2017; Adibe *et al*. 2019)
18.983	Oleic acid	$C_{18}H_{34}O_2$	282.46	(Walters *et al*. 2004)
19.486	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.40	(Adibe *et al*. 2019)
19.836	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.28	(Adibe *et al*. 2019)
20.288	9, 12-Octadecadienoic acid (Z,Z)-methyl ester	$C_{19}H_{34}O_2$	294.47	(Chukwunonye *et al*. 2015)
20.413	Phytol	$C_{20}H_{40}O$	296.0	(Hema *et al*. 2011)
20.556	Octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.50	(Banaras *et al.* 2017)
21.129	Behenic alcohol	$C_{22}H_{46}O$	326.60	(Chandrasekaran *et al.* 2011)
21.186	Octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	312.53	(Mohy and Mohyeldin 2018)
21.380	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	(Mohy and Mohyeldin 2018)
22.096	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.40	(Chukwunonye *et al*. 2015)
23.875	Eicosane	CH	282.50	(Shirani *et al*. 2017)
24.241	Docosanoic acid methyl ester	$C_{23}H_{46}O_2$	354.61	(Aida *et al*. 2016)

J. curcas leaf extracts signifies they are effective against fungal pathogens including *P. personata*.

Conclusion

The study concludes that the methanolic, ethyl acetate and chloroform leaf extracts of *J. curcas* contain important antifungal phytoconstituents such as hexadecane; *n*-hexadecanoic acid; phenol, 2,4 bis (-dimethylethyl); phytol and hexadecanoic methyl ester, which are responsible for the control of late leaf spot disease. Hence, methanolic, ethyl acetate and chloroform leaf extracts of *J. curcas* can be used as substitute bio-pesticides for inhibiting late leaf spot disease on groundnut.

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Author Contributions

MF developed and planned the study, MF, EM and MC statistically analysed the data MF, PN and EM, interpreted the results and MF made write up.

Conflict of Interest

Authors declared no conflicts of interest.

Data Availability

The research data can be obtained through concerting the corresponding author.

Ethics Approval

The ethical approval was obtained from the Tropical Pesticide Research Institute under Herbarium section, Arusha.

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