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Research Article

Gas chromatography–mass spectrometry (GC-MS) analysis of antimicrobial compounds produced by mahua oil cake against the stem rot pathogen- *Sclerotium rolfsii*

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Abstract
The antifungal property containing oil cakes play a significant role in reducing plant disease in a wide range of soil-borne pathogens. A destructive soil-borne pathogen, *Sclerotium rolfsii* is infecting a vast range of crops worldwide. *In-vitro* efficacy of five different oil cakes *viz*. mahua cake, neem cake, pungam cake, coconut cake and castor cake extracts was tested against the stem rot pathogen of groundnut. Among the five different oil cakes, mahua oil cake extract produced the minimum mycelial growth of 1.57and1.29 cm at 5%, and 10% concentrations, respectively and showed maximum percent growth inhibition of 83.33and 86.66% respectively. Bioactive compounds in mahua oil cake were analyzed through GC-MS. From the result of GC-MS, the high retention time and peak area percentage were observed with major important bioactive compounds like n-Hexadecanoic acid (24.968) (12.22), Hexadecanoic acid, ethyl ester (23.655) (2.9), 9,12-Octadecadienoic acid (Z, Z)-(28.659) (35.61), 9-Octadecenoic acid, (E)-(28.786) (13.15), Octadecanoic acid (29.137) (33.59) and a1-Octyn-3-ol (3.023) (0.04).The bioactive compounds identified through GC-MS from mahua oil cake extract were found to be exhibiting antifungal activity against *S. rolfsii*.

Keywords: GC-MS, Mahua oil cake, Oil cake extract, *Sclerotium rolfsii*, Stem rot

INTRODUCTION

Oilseed cakes are essential organic amendments after getting from the oilseed extraction and then successfully used in an agricultural ecosystem for various purposes. Oilseed cakes belong to non-edible oilseed cakes (castor cake, neem cake, pungam cake, safflower cake, karanji cake and cottonseed cake) and edible oilseed cakes (groundnut cake, coconut cake, sesame cake, niger cake, linseed cake and rapeseed cake). The addition of the oil cake into the soil increases the cation exchange capacity water holding capacity and makes a good soil structure for plant growth and effectively reduces harmful soil-borne pathogens. In the plant protection aspect, oil cake produced high antifungal principles against soil-borne pathogens like bacteria, fungi and nematodes (Dar *et al.*, 2018). The destructive soil-borne pathogen, *Sclerotium rolfsii* is highly threatening to groundnut and other crops, which may incur 10–40% yield losses, especially under irrigated conditions. Latha and Rajeswari (2019) stated that mahua cake and neem cake highly inhibited *S.rolfsii*, *Rhizoctonia solani*, *Pythium, Fusarium* and other soil-borne pathogens. From the review of literature, many researchers

have reported that mahua cake and neem cake had major antimicrobial and disease inhibition activities. Interestingly oil cakes containing secondary metabolites like phenolic and flavonoids compounds exhibited antimicrobial activity (Karmelreetha and Muthukumar, 2020). The aim of the present research was to focus on the antifungal activities of five different oil cakes against the fast-growing pathogen *Sclerotium rolfsii* infesting stem rot in groundnut.

MATERIALS AND METHODS

Isolation of the stem rot pathogen

Sclerotium rolfsii infected groundnut plant samples were collected from Bodi of Theni districtin Tamil Nadu. The pathogen was isolated in Potato Dextrose Agar (PDA) media amended Petri plates through the tissue segmentation method. A single hyphal tip method basis pure culture of *S. rolfsii* pathogen was obtained. Morphological confirmation of the pathogen(based on mycelia and sclerotial bodies characters) and molecular confirmation as *S. rolfsii were completed (Latha and Rajeswari, 2019).* The accession number MZ277282 was received from NCBI and it was confirmed that the pathogen was *Athelia rolfsii* IS(BDI)-8.

Oil cake aqueous extract preparation

The cold water extraction method was used to get the aqueous extract of oilseed cakes, and its efficacy was assessed against the stem rot pathogen of groundnut. The oil cake extracts like mahua cake, neem cake, pungam cake, coconut cake and castor cake were prepared using well-powdered oil cakes. About 100g of oil cakes were mixed in sterile distilled water at the ratio of 1g for 1.25 ml of sterile distilled water. Mixed content was kept overnight. Finally, the content was ground with a pestle and mortar, followed by filtration through a double-layered sterilized muslin cloth. Filtered contents were centrifuged at 10,000 rpm for 15 mins. The supernatant was a standard extract solution 100 % (Dubey, 2002). From the standard extract, 5 ml and 10 ml were taken separately and mixed with 95 and 90 mlPDA medium to prepare 5% and 10% concentrated PDA media. Likewise, all the oil cake extracts were prepared with two different concentrations.

Poisoned food technique

The efficacy of five different oil cakes (mahua cake, neem cake, pungam cake, coconut cake and castor cake) at two different concentrations were screened against the *S.rolfsii* through the poisoned food technique (Schmitz, 1930). In this assay, 15 ml of oil cake extract containing PDA medium was poured into the sterile Petri plate under aseptic conditions. A nine mm plug of 5days old *S.rolfsii* was placed on the center of the Petri plate. In the case of control, PDA medium alone was used. Inoculated Petri plates were incubated at 28±2 °C. When the growth of *S.rolfsii* covered the entire dish in control, the percent growth inhibition of the pathogen was calculated by using the formula

$$PI = \frac{C-T}{T} \times 100$$

……Eq.1

PI - Percent inhibition
C - Average diameter of the fungal growth in control
T - Average diameter of the fungal growth in treatment

Solvent extraction of mahua oil cake through Soxhlet apparatus

From the mahua oil cake, bioactive compounds were extracted through the Soxhlet extraction method (Dean *et al.*, 1997). In this extraction, 10 grams of oil cake was placed in the thimble, which was then drawn into a distillation flask with 150 ml of solvent n-hexane (n-hexane had a low boiling point character, high oil solubility, and high oil extraction percentage). Finally, the solvent reached the overflow level; the solution was sucked from the thimble holder *via* a siphon pipe, releasing the solution into the distillation flask. The process was repeated until the bioactive compounds get extracted entirely into the solvent (1-6 hr).

Sample preparation for GC-MS

Extracted bioactive compounds containing the solvent were filtered through Whatman No.1 filter paper.The sample content was concentrated by running through the rotary vacuum evaporator until the solvent had completely been evaporated. The final output was diluted with 2ml of hexane and again filtered through a bacterial filter.

Gas chromatography-mass spectrum analysis (GC-MS) of mahua oil cake extract

The mahua oil cake extracts' bioactive compounds were determined with a Shimadzu Gas chromatography equipped with a mass detector Turbo mass gold containing an Elite-1 (100% Dimethyl Poly Siloxane), 30 m × 0.25 mm ID × one mM df. The conditions employed were the following: Carrier gas, helium (1 ml/min), Oven temperature program 110 °C (2 min) to 280 °C (9 min), Injector temperature (250 °C), Total GC time (45 min), The ethyl acetate extract was injected into the chromatograph in 1.0 ml aliquots. The major constituents were identified with the aid of a computer-driven algorithm and then matched the mass spectrum of the analysis with that of a National Institute of Standards and Technology (NIST) library (Version. 2.0, year-2005). The software used for gas chromatography-mass spectroscopy (GC-MS) was Turbo mass-5.1. This work was carried out in the center of innovation for excellence, Agricultural College & Research Institute, Tamil Nadu Agricultural University, Madurai.

Statistical analysis

Mean differences of the treatment were evaluated with ANOVA by using Duncan's Multiple Range Test at 5% significance (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Antifungal activity of oil cakes against *S. rolfsii*

The result of *in vitro* assay of antifungal activity of oil cake (organic amendments) against the stem rot pathogen*S.rolfsii* revealed that mahua oil cake extract produced the minimum mycelial growth of 1.57cm and 1.29cm and the maximum percent growth inhibition such as 83.33 and 86.66% at 5% and 10% concentrations respectively. This may be due to the presence of antimicrobial compounds such as saponins, flavonoids and glycosides in mahua oil cake than in other oil cakes (Yadav and sing, 2012).In contrast, coconut oil cake produced the highest mycelial growth (8.22cm and 7.92cm) and poor mycelial growth inhibition (8.88% and 12.22%) in the above said concentrations as compared to the control. The treatment efficacy was high in the 10% concentration of all the five oil cake extracts against the *S. rolfsii,* which is shown in Table 1 and Fig. 1.The present results were in accordance with the findings of Anitha *et al.* (2019), who reported that 10% concentration of mahua oil cake extract produced the maximum growth inhibition 80.11% of *S. rolfsii*. *Latha and Rajeswari (2019)* reported that six different oil cakes were tested against *S.rolfsii*. Among them, mahua oil cake recorded the maximum percent growth inhibition (83.07%). (Alice and Sundravadana, 2012)

Fig. 1. Antifungal activity of different oil cakes extract against the *S.rolfsii* (poisoned food technique)

Table 1. *Antifungal activity of different oil cakes against the S. rolfsii*

S.NO	Organic Amendments	Concentration (%)			
		5%		10%	
		Mycelial growth (cm)*	Per cent growth reduction over control (%)	Mycelial growth (cm)*	Per cent growth reduction over control (%)
1	Pungam cake	3.81^{c}	57.77	3.24^{c}	64.44
2	Neem cake	2.48^{b}	73.33	1.93^{b}	78.88
3	Coconut	8.22^{e}	8.88	7.92^{e}	12.22
4	Mahua cake	1.57^{a}	83.33	1.29^{a}	86.66
5	Caster	7.64^{d}	15.55	6.78^{d}	25.55
6	Control (Sterile distilled water)	9.00^{f}	00.00	9.00^{f}	00.00
	CD (P=0.05)	0.22	-	0.31	-

*Mean of three replications; Means with the same letter do not have significant difference according to Duncan's multiple range test at p<0.05

stated that at 10 % concentration, mahua oil cake expressed the complete inhibition of *Macrophomina* sp., which caused root rot in *Gloriosa superba.* The antifungal effect of mahua oil cake against chilli damping-off was reported by Karmelreetha *et al.* (2020), Vincken *et al.* (2007), indicating that mahua oil cake exhibited two-fold inhibition activity against *Fusarium oxysporum* than carbendazim treatment. The mahua oil cake contains steroidal and terpenoid nature of low molecular weight secondary metabolite, saponin (Wani *et al.*, 1982). Compared to the findings of other reports, the present experimental study showed mahua oil cake extract much more inhibited the growth of *S. rolfsii* due to some volatile and non-volatile compounds that arrested the mycelial growth of the pathogen at starting itself.

GC-MS analysis of mahua oil cake

The mahua oil cake extract was reported to have the following major important bioactive compounds like n-Hexadecanoic acid (24.968),(12.22), Hexadecanoic acid, ethyl ester(23.655), (2.9), 9,12-Octadecadienoic acid (Z, Z)-(28.659),(35.61),9-Octadecenoic acid, (E)-(28.786),(13.15), Octadecanoic acid (29.137),(33.59), which are shown in Fig. 2 and other antimicrobial activity containing compounds like Squalene (39.409) (0.07) and 1-Octyn-3-ol (3.023) (0.04), 9,12- Octadecadienoic acid (Z<Z)-methyl ester (27.062), (0.07), 9 - Octadecadienoic acidmethyl ester(E)- (27.196) (0.2) with their RT time, peak area, molecular formulae and molecular weightare listed in Table 2 and Fig. 3, and their chemical structures are shown in Fig. 4.Comparable findings were reported by(Ali *et al., 2017) indicating that* fatty acid methyl esters like Hexadecanoic acidethyl ester,9,12-Octadecadienoic acid (Z,Z)- ,9-Octadecenoic acid, (E)- andOctadecanoic acid had the antifungal activity against the *S.rolfsii* . Similarly (Agoramoorthy *et al.*, 2007) reported that Linoleic acid (omega-6 fatty acid) and Linolenic acid (omega-3fatty acid) nature like compounds (9, 12-Octadecadienoic acid, methyl ester, 9, 12, 15-Octadecatrienoic acid, me-

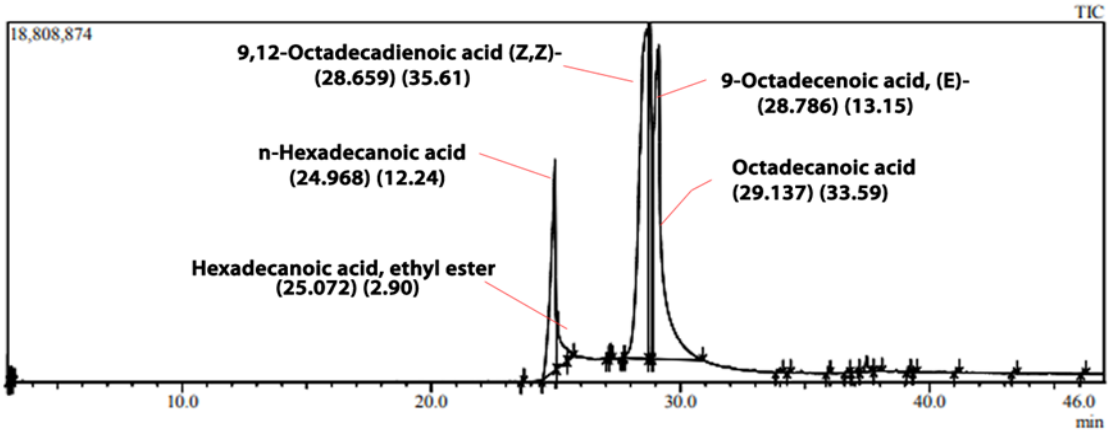

Fig. 2. *Gas chromatogram of antifungal compounds identified from mahua oil cake extract*

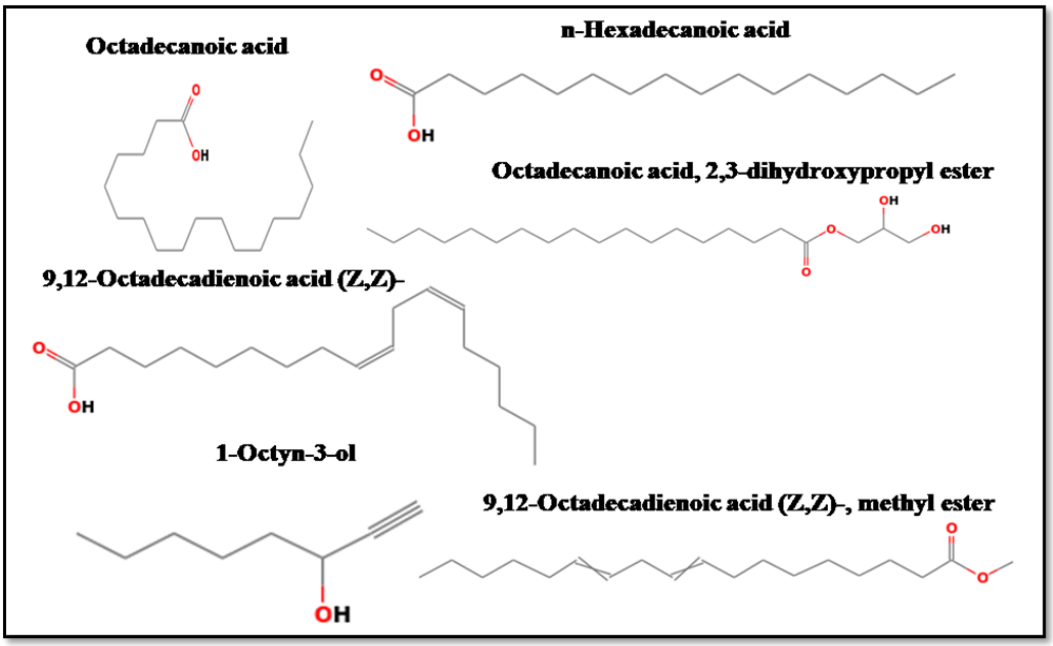

Fig. 3. *Chemical structure of important antifungal compounds of mahua oil cake*

Table 2. Mahua oil cake extracts' GCMS compounds and their nature

S. No.	Name of the compound	RT	Peak area %	MW g/mole	Molecular Formula	Specific role	Reference
1	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	27.062	0.07	294.4721	C19H34O2	Antibacterial	(Lima *et al.*, 2011)
2	9 - Octadecadienoic acid ,methyl ester,(E)-	27.196	0.2	296.4879	C19H36O2	Antimicrobial, Nematicida	(Chandrasekaran *et al.*, 2008)
3	n-Hexadecanoic acid	24.968	12.22	256.4241	C16H32O2	Antifungal, Antibacterial	(Akpuaka *et al.*, 2013)
4	Hexadecanoic acid, ethyl ester	23.655	2.9	284.4772	C18H36O2	Antifungal, Antioxidant, Antibacterial	(Agoramoorthy *et al.*, 2007)
5	9,12-Octadecadienoic acid (Z,Z)-	28.659	35.61	280.4455	C18H32O2	Antimicrobial Nematicidal	(Chandrasekaran *et al.*, 2008; Lima *et al.*, 2011)
6	9-Octadecenoic acid, (E)-	28.786	13.15	282.4614	C18H34O2	Antimicrobial, Nematicidal	(Chandrasekaran *et al.*, 2008)
7	Octadecanoic acid	29.137	33.59	284.4772	C18H36O2	Antifungal	(Akpuaka *et al.*, 2013; Frahm, 2004)
8	Octadecanoic acid, 2,3 dihydroxypropyl ester	37.862	0.17	358.5558	C21H42O4	Antioxidant	(Kumari *et al.*, 2019)
9	Tetrapentacontane, 1,54-dibromo-	37.472	0.53	759.4512	C54H110	Antioxidant, Antiviral	(Abdelhamid *et al.*, 2015)
12	6-Ethyl-3-decanol, TMS derivative	36.712	0.08	186.33	C12H26O	Antimicrobial	(Kumari *et al.*, 2019)
13	Methyl stearate	27.703	0.09	298.5	C19H38O2	Antibacterial	(MubarakAli *et al.*, 2012)
15	Squalene	39.409	0.07	410.7180	C30H50	Anti-bacterial antioxidant	(Lalitharani *et al.*, 2009; Sudha *et al.*, 2013)
16	1-Octyn-3-ol	3.023	0.04	126.1962	C8H14O	Antifungal Antibacterial	(Xiong *et al.*, 2017)

thyl ester, (z, z, z)-)having antifungal activity and these compounds are present in the mahua oil cake. Pinto *et al.* (2017) also stated that Linoleic acid methyl ester has antifungal and antibacterial activity, which was obtained from the cold extraction of mahua oil cake. Cold extraction mahua oil cake containing stearic acid also exhibited the antimicrobial activity against different soil-borne pathogens (Jubie *et al.*, 2012). Here, mahua oil cake extract having significant antifungal compounds against many pathogens was reported by different authors. These functional compounds are also found in the present GC-MS results. Based on the literature, other oil cakes do not have the above mentioned antifungal compounds, specifically in mahua oil cake. Due to these antifungal compounds (volatile and non volatile) mahua oil cake extract successfully inhibited the *S. rolfsii* mycelial growth. According to Chandrasekharan *et al*. (2008), the plant *Salicornia brachiata* of the *Chenopodiaceae* family is found to have methyl esters of fatty acid exhibiting the highest antifungal activity. It coincides with our findings that mahua oil cake extract also possesses the same fatty acids and ethyl esters having antifungal activity. Lima *et al.* (2011) documented that fatty acid methyl esters of seeds of *Annona cornifolia* mainly methyl esters of oleic acid, linoleic acid and palmitic acid have inhibited the growth of 12 strains of clinical pathogenic fungus *Paracoccidioides brasiliensis*

Conclusion

The present study indicated that the identified bioactive compounds in mahua oil cake, such as n-Hexadecanoic acid, Hexadecanoic acid ethyl ester, 9,12-Octadecadienoic acid (Z, Z), 9-Octadecenoic acid (E) and Octadecanoic acid possessed the antifungal activity against the *Sclerotium rolfsii*. These compounds are well-known for the mycelial growth inhibition of *S. rolfsii*. It is expected that the field application of mahua oil cake may work better under field conditions to man-

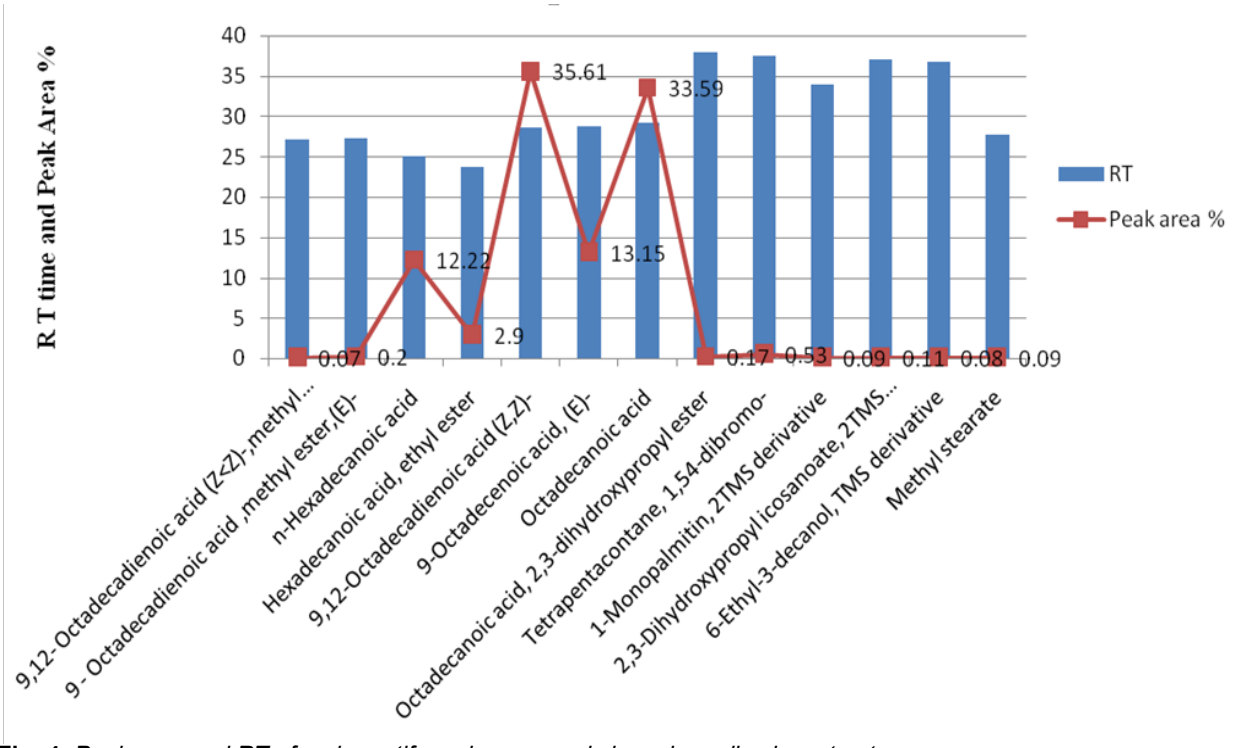

Fig. 4. *Peak area and RT of major antifungal compounds in mahua oil cake extract*

age various soil-borne pathogens.

Conflict of interest

The authors declare that they have no conflict of interest.

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