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Exploitation and identification of antifungal compounds of botanicals through gas chromatography-mass spectrometry (GC-MS) against *Bipolaris oryzae* in rice

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Abstract
Bipolaris oryzae, the pathogen which incessantly infecting rice crop which causes at any growth stage. It greatly infects grains, lower seed germination, seed quality that reduces the yield up to 60%. Various chemicals have been used for controlling this disease but it is very parlous to human being due to its toxicity. Besides, some eco-friendly management practices also followed to control this disease using various aspects. Henceforth, plant leaf extracts were screened against this fungus by poison food technique. Among the 24 different leaf extracts, zimmu (*Allium cepa* L. × *Allium sativum* L.) and henna (*Lawsonia inermis*) showed the maximum level of inhibition 77.78% and 75.56% respectively. It is the first report that zimmu extract showed maximum inhibition against *B. oryzae.* The present investigation revealed that zimmu leaf extract contains biologically active compounds of phytol (16.03%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-(15.13%) and henna leaf extract contains n-Hexadecanoic acid (19.05%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (14.46%).

Keywords: *Bipolaris oryzae,* zimmu, henna, GC-MS

Introduction
Bipolaris oryzae causing brown leaf spot is a devastating, most commonly occurring disease in rice which have the impact of 5 to 45% loss and in certain areas 90% loss in the crop yield. It also causes more than 50% seedling mortality [1]. It is well known that still fungicides were used against various plant pathogens and pests for the management practices. Many of the chemical fungicides are too expensive, to make the farmer's perspective and eco-friendly in nature, an alternative to chemical fungicides, plant extracts were used for the management of wide range of plant pathogens. Plant extracts contains alkaloids, tannins, phenolic compounds, coumarins, quinines and phytoalexins etc. which are known for antifungal property [2, 3]. *In vitro* studies of two leaf extracts, *Nerium oleander* and *Pithecolobium dulce* showed the highest inhibition on mycelial growth and spore germination of *B. oryzae* [4]. Alcohol leaf extract of *Azadiracta indica* was the most effective in reducing radial growth of the pathogen *Cochliobolus miyabeanus* [5]. Neem extracts reduced dead heart and white head by 38.38% and 58.08% yellow stem borer (*Scirpophaga incertulas*) in rice [6]. Brown plant hopper feeding enhances sterol biosynthetic pathway and strengthens wax biosynthesis and phytol metabolism in rice plants that elucidating the resistance genes [7]. The main chemical constituents of essential oils of *Premna angolensis* and *Premna quadrifolia* leaves contains phytol showed the insecticidal and repellent effects on *Sitotroga cerealella* an insect pest of rice [8]. The botanical extract of *Caesalpinia gilliesii* showed high efficiency against *Sitophilus oryzae* and the compounds were identified as tetradecanoic acid, pentadecanoic acid, loliolide, octadecanoic acid, n-hexadecanoic acid, and phytol.
The leaf extract of zimmu (*Allium cepa* x *Allium sativum*) effective in inhibiting the growth of some important foliar and soil borne fungal plant pathogens [9-11]. Likewise, henna (*Lawsonia inermis*) leaf extract also have antimicrobial activity against various plant pathogens [12, 13]. Akila and Mini 2020 [14] tested eight botanicals against the mycelial growth of *B. oryzae* by poison food technique, the leaf extract (10%) of Maruthani (*L. inermis*) showed maximum per cent inhibition. The protein fractions of henna exhibited four to five times more percentage inhibition of mycelial growth of *B. oryzae* than the no protein fractions [15].

Natarajan and Lalithakumari 1987 [13] reported that the antifungal activity of *L. inermis* against *B. oryzae* was due to the presence of lawsone (2-hydroxy1,4-naphthoquinone). Hence, the study was carried out for screening the effective plant leaf extracts against *B. oryzae* and identification of the antifungal compounds present in it.

Materials and Methods

Isolation of the pathogen

The heavily infected paddy leaf showing typical symptoms of brown spot of rice disease collected from the field was used for isolation of the pathogen. The leaves with typical symptoms were cut into bits, surface sterilized with 0.1% mercuric chloride and placed on sterilized PDA medium. The plates were incubated at room temperature (28 ± 2 ºC) for 5 days and observed for fungal growth. The growing fungal colony of each plant piece was sub cultured and purified by single hyphal tip method [16]. The pure culture of the pathogen was maintained on PDA slants for further use in this study.

Efficacy of crude plant extract against *B. oryzae in vitro*

Preparation of plant extracts [17]

Fresh plant tissues of various plant species were used for the preparation of extracts. The plant materials (leaves/bulbs) were separately washed in fresh water and finally with sterile water. These were ground in sterile water @ one ml g^{-1} of tissue in a pestle and mortar. The macerate was squeezed through sterilized cotton wool to express the extract. The extract was strained through two layers of muslin cloth further with Whatman No.1 filter paper and finally filtered through Seitz filter (0.2µm) to free the extract from bacterial contamination. This formed the standard plant extract (100%).

Efficacy against the mycelial growth (poison food technique) [18]

The efficacy of plant extracts on the growth of *B. oryzae* was studied by poison food technique. From the standard plant extract 10 ml were added to 90 ml of sterilized and cooled (warm) PDA medium and thoroughly mixed by shaking for making 10% concentration. This was plated into sterile Petri plates (9 cm dia) at 10 ml quantities and allowed to solidify. A nine mm diameter actively growing culture disc of *B. oryzae* was aseptically placed onto the medium at the centre of the plate. Three replications were maintained for each treatment. The PDA medium without incorporating the plant extract served as control. The plates were incubated at room temperature (25±2 °C). The diameter of the colony was measured after the control plate has been fully covered and expressed as% growth reduction over control.

$$PI = \frac{Dc\ Dt}{Dc} \times 100$$

Dc = Average diameter of fungal growth (cm) in control
Dt = Average diameter of fungal growth (cm) in treatment
PI = Per cent inhibition

Extraction of principle compounds using Soxhlet apparatus [19]

The finely powdered 15 to 20g air dried leaf sample (zimmu and henna) was filled in thimble and it was placed in a Soxhlet extractor. The plant material was extracted with organic solvent methanol (200 mL) with continuous extraction of 3 to 4 hours. The crude extract was collected after redistillation. It was concentrated by rotary vacuum evaporator and it is diluted for further use.

In vitro testing of principle compounds

The effect of extracted compounds (Secondary metabolites) from zimmu and henna by Soxhlet extraction were tested by above described method (poison food technique) with different concentration (100, 500 and 1000 ppm) and Percent inhibition was assessed using the formula (PI).

Analysis of antifungal compound through gas chromatography mass spectroscopy (GC-MS)

Based on the growth inhibition studies, zimmu and henna leaf extract were selected and chemical constituents were determined with a Shimadzu Gas chromatography equipped with a mass detector Turbo mass gold containing a Elite-1 (100% Dimethyl Poly Siloxane), 30 m × 0.25 mm ID × 1 mM df. Conditions employed were the following: Carrier gas, helium (1 mL/min); oven temperature program e 110 °C (2 min) to 280 °C (9 min); injector temperature (250 °C); total GC time (45 min). The methanol 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 by matching the mass spectrum of the analysis with that of a National Institute of Standards and Technology (NIST) library (Version. 2.0, year-2005). Software used for gas chromatography mass spectroscopy (GC-MS) was Turbo mass-5.1. This work was carried out in Center of Innovation for excellence, Agricultural College & Research Institute, Tamil Nadu Agricultural University, Madurai.

Statistical analysis

The data were statistically analyzed using the SPSS (Statistical Package for the Social Sciences) version 16.0. Data were subjected to analysis of variance (ANOVA) at significant levels ($P<0.05$) and means were compared by Duncan's Multiple Range Test (DMRT) [20].

Results

Identification of the pathogen

Identification of isolated pathogen was done by microscopic examination based on their unique hyphal and conidial characters of *B. oryzae*. Molecular confirmation of the isolate *B. oryzae* was confirmed by amplification of 18S rRNA polymerase chain reaction (PCR). The amplified sequence was submitted in National Center for Biotechnology Information (NCBI) and got accession number MN796085.

Mycelial growth inhibition test

Among the twenty-four plant extracts tested, zimmu showed the maximum 77.78% inhibition over control. This was statistically on par with henna showing 75.56% inhibition over control. Minimum inhibition was recorded in tulsi showing 9.26% inhibition over control (Table 1 and Fig. 1).

Table 1: Efficacy of crude botanical extracts against *B. oryzae*

S. No	Botanicals	Scientific name	Mycelial growth (cm) Mean*	Per cent Inhibition (PI)
1.	Zimmu	*Allium cepa L. x Allium sativum*	2.00^{m}	77.78
2.	Henna	*Lawsonia inermis*	2.20^{m}	75.56
3.	Hibiscus	*Hibiscus rosa-sinensis*	4.67^{l}	48.15
4.	Thuthi	*Abutilon indicum*	4.87^{l}	45.93
5.	Datura	*Datura stramonium* L.	4.93^{l}	45.19
6.	Eucalyptus	*Eucalyptus globulus*	5.40^{k}	40.00
7.	Indian mint	*Coleus aromaticus*	5.47^{jk}	39.26
8.	Neem	*Azadirachta indica*	5.63^{jk}	37.41
9.	Nerium	*Nerium oleander*	5.73^{ijk}	36.30
10.	Aswagandha	*Withania somnifera*	5.83^{hij}	35.19
11.	Papaya	*Carica papaya* L.	5.87^{hij}	34.81
12.	Castor	*Ricinus communis* L.	6.10^{ghi}	32.22
13.	Notchi	*Vitex negundo* L.	6.17^{gh}	31.48
14.	Pirandai	*Cissus quadrangularis* L.	6.43^{fg}	28.52
15.	Periwinkle	*Vinca minor* L.	6.70^{ef}	25.56
16.	Lemon grass	*Cymbopogon schoenanthus* L.	6.90^{e}	23.33
17.	Ponnanganni	*Alternanthera sessilis*L.	6.93^{e}	22.96
18.	Karisalankanni	*Eclipta prostrata* L.	6.93^{e}	22.96
19.	Pungam	*Millettia pinnata* L.	6.97^{e}	22.59
20.	Tuduvalai	*Solanum trilobatum* L.	7.60^{d}	15.56
21.	Basil	*Ocimum basilicum* L.	7.67^{cd}	14.81
22.	Parthenium	*Parthenium hysterophorus* L.	7.87^{bcd}	12.59
23.	Prosopis	*Prosopis spicigera* L.	8.07^{bc}	10.37
24.	Tulsi	*Ocimum tenuiflorum* L.	8.17^{b}	9.26
25.	Control	-	9.00^{a}	0.00
CD (0.05%)			0.38	

*Mean of three replications
PI-Per cent Inhibition

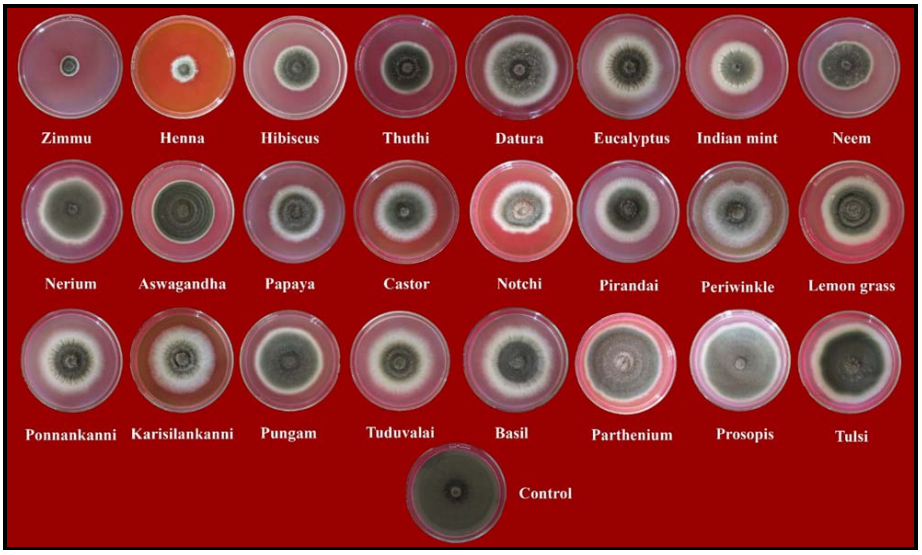

Fig 1: Efficacy of crude botanical extracts against *B. oryzae*

Efficacy of secondary metabolites on the growth of *B. oryzae*

Secondary metabolites of zimmu showed the maximum per cent inhibition recording 57.22% at 100 ppm, 70.56 at 500 ppm, 76.11% at 1000 ppm and 95.00% at 1500 ppm. This was followed by henna which recorded 55.00% at 100 ppm, 61.67% at 500 ppm, 68.33% at 1000 ppm and 93.06% at 1500 ppm inhibition over control (Table 2).

Table 2: Efficacy of secondary metabolites against *B. oryzae*

Secondary metabolites	Mg (cm) 100 ppm*	PI	Mg (cm) 500 ppm	PI	Mg (cm) 1000 ppm	PI	Mg (cm) 1500 ppm	PI
Zimmu	3.85	57.22	2.65	70.56	2.15	76.11	0.45	95.00
Henna	4.05	55.00	3.45	61.67	2.85	68.33	0.63	93.06
Control	9.00	-	9.00	-	9.00	-	9.00	-
CD (0.05%)	0.37		0.16		0.16		0.16	

*Mean of four replication
Mg- Mycelial growth
PI-Per cent Inhibition

GC-MS analysis

On the basis of performance of plant extract in the preceding *in vitro* studies, zimmu and henna leaf extract was tested to determine the nature of chemical compound present in the extract. The results revealed that 22 compounds were present in Zimmu leaf (Fig. 2 and Table 3). The molecular weight, name of the compound, chemical formula, retention time and peak area percentage were given in Table 3 and 4. This analysis revealed that zimmu leaf extract contains biologically active compounds of phytol (16.03%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-(15.13%) and henna leaf extract contains n-Hexadecanoic acid (19.05%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z) (14.46%) (Table 4 and Fig. 3). Among these, n-Hexadecanoic acid which was closely related to 9, 12-Octadecadienoic acid may be responsible for the inhibition of the growth of *B. oryzae*.

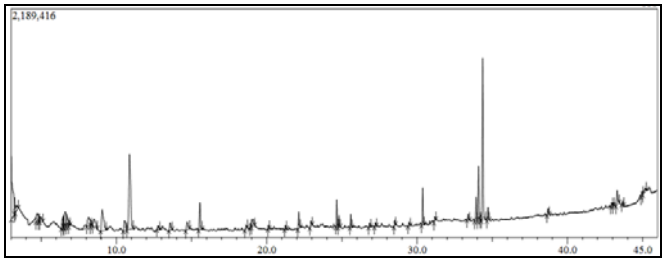

Fig 2: Chromatogram of GC-MS analysis of zimmu leaf extract

Table 3: Phyto-components identified by GC-MS analysis of zimmu leaf extract

R. Time	Area%	A/H Name	Molecular formula	Molecular weight
3.02	8.5	Hydroperoxide, 1-methylbutyl	$C_5H_{12}O_2$	104.15
3.223	1.35	n-Hexane	C_6H_{14}	86.18
3.421	1.09	1,3-Pentanediol, 4-methyl-2-nitro-	$C_6H_{13}NO_4$	163.17
4.736	1.71	Butanoic acid, 2-ethyl-3-oxo-, methyl ester	$C_7H_{12}O_3$	144.17
4.835	0.5	2-Heptanol, acetate	$C_9H_{18}O_2$	158.24
4.971	1.02	Acetamide, N-(2-hydroxyethyl)-	$C_4H_9NO_2$	103.12
6.445	1.37	Glycerin	$C_3H_8O_3$	92.09
6.485	1.14	Dimethyl trisulfide	$C_2H_6S_3$	126.26
6.611	2.9	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on	$C_6H_8O_4$	144.13
6.685	1.94	Sorbitol	$C_6H_{14}O_6$	182.17
6.895	0.67	Dimethyl trisulfide	$C_2H_6S_3$	126.26
8.154	2.18	Octane, 2-methyl-	C_9H_{20}	128.26
8.31	0.71	Piperidine, 1,2-dimethyl-	$C_7H_{15}N$	113.20
8.531	1.98	2,5-Dimethylfuran-3,4(2H,5H)-dione	$C_6H_8O_3$	128.13
9.058	4.94	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126.12
10.545	2.09	Butanoic acid, 2-methyl-3-oxo-, ethyl ester	$C_7H_{12}O_3$	144.17
10.871	15.13	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144.13
12.777	0.59	Isosorbide	$C_6H_{10}O_4$	146.14
13.578	1.09	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.13
14.706	1.27	(S)-(-)-1,2,4-Butanetriol, 2-acetate	$C_6H_{12}O_4$	148.16
15.559	2.99	4-Hydroxy-2-methylacetophenone	$C_9H_{10}O_2$	150.17
18.629	0.42	Benzeneethanamine, N-(3-methylbutylidene)-	$C_{13}H_{19}N$	189.3
18.96	0.4	1,10-Decanediol, 2TMS derivative	$C_{10}H_{22}O_2$	174.28
19.076	1.63	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	151.12
20.124	0.28	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	$C_{15}H_{22}$	202.34
21.253	0.27	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-m ethylene	$C_{15}H_{24}$	204.35
22.131	1.52	Dodecanoic acid	$C_{12}H_{24}O_2$	200.32
22.946	0.56	Ethyl N-(o-anisyl) formimidate	$C_{10}H_{13}NO_2$	179.22
24.659	2.15	aR-Turmerone	$C_{15}H_{20}O$	216.32
24.798	1	Tumerone	$C_{15}H_{22}O$	218.33
25.59	1.07	Curlone	$C_{15}H_{22}O$	218.33
26.838	0.38	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37
27.241	0.33	(E)-Atlantone	$C_{15}H_{22}O$	218.33
28.516	0.41	Neophytadiene	$C_{20}H_{38}$	278.52
29.466	0.4	Oleyl alcohol, trifluoroacetate	$C_{20}H_{35}F_3O_2$	364.49
30.377	2.84	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45
31.198	0.37	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
33.394	0.58	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	280.45
33.928	2.28	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47
34.085	5.45	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.49
34.236	0.52	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.49
34.36	16.03	Phytol	$C_{20}H_{40}O$	296.53
34.717	1.24	Methyl stearate	$C_{19}H_{38}O_2$	298.50
38.709	0.58	9-Octadecenoic acid, 12-hydroxy-, methyl este	$C_{19}H_{36}O_3$	312.49
42.941	0.37	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667.39
43.08	0.32	Eicosane	$C_{20}H_{42}$	282.55
43.301	1.79	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.50
43.67	0.31	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56
44.97	0.5	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	667.39
45.164	0.85	Dodecanoic acid, 1,2,3-propanetriyl ester	$C_{39}H_{74}O_6$	639.00

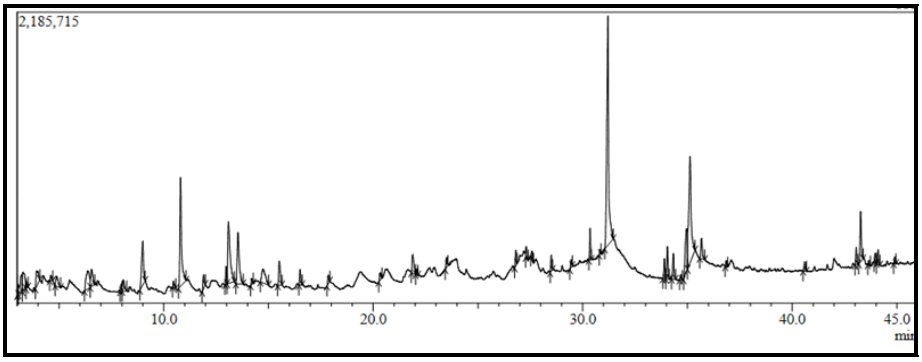

Fig 3: Chromatogram of GC-MS analysis of henna leaf extract

Table 4: Phyto-components identified by GC-MS analysis of henna leaf extract

R. Time	Area%	Name	Molecular formula	Molecular weight
3.02	0.51	Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl)-	$C_7H_{14}N_2O$	142.2
3.2	1.79	2-Propenoic acid, ethenyl ester	$C_5H_6O_2$	98.09
3.29	2.03	Acetic acid, methyl ester	$C_3H_6O_2$	74.08
3.465	0.35	2,3-Butanediol	$C_4H_{10}O_2$	90.12
3.931	1.62	Glyceraldehyde	$C_3H_6O_3$	90.08
4.618	0.36	Acetic acid, (1-methylethoxy)-, 1-methylethyl	$C_9H_{18}O_4$	190.24
4.881	1.08	dl-Glyceraldehyde dimer	$C_6H_{12}O_6$	180.16
6.374	2.83	Glycerin	$C_3H_8O_3$	92.09
6.557	1.64	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	144.13
7.969	0.37	Pantolactone	$C_6H_{10}O_3$	130.14
8.069	0.96	Pentanoic acid, 4-oxo-	$C_5H_8O_3$	116.12
9.004	3.72	1,3,5-Triazine-2,4,6-triamine	$C_3H_6N_6$	126.12
10.47	0.39	Pentanoic acid, 4-oxo-	$C_5H_8O_3$	116.12
0.806	8.4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.13
11.905	0.82	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	$C_6H_6O_4$	142.11
12.982	0.9	Benzofuran, 2,3-dihydro-	C_8H_8O	120.15
13.094	6.84	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11
13.543	4.97	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.13
14.207	0.3	dl-Mevalonic acid lactone	$C_6H_{10}O_3$	130.14
14.742	2.33	Heptanoic acid, 6-oxo-	$C_7H_{12}O_3$	144.17
15.517	1.77	Benzamide,3-nitro-N-(1H-1,2,4-triazol-3-yl)-	$C_9H_7N_5O_3$	233.18
16.513	0.84	1(3H)-Isobenzofuranone	$C_8H_6O_2$	134.13
17.858	0.49	Dimethyl(bis [(2Z)-pent-2-en-1-yloxy]) silane	$C_{12}H_{24}O_2Si$	228.40
20.34	0.47	2-Acetylbenzoic acid	$C_9H_8O_3$	164.16
21.888	1.59	1,4-Naphthalenedione, 2-hydroxy-	$C_{10}H_6O_3$	174.15
22.072	0.29	Dodecanoic acid	$C_{12}H_{24}O_2$	200.32
23.506	0.57	Benzoic acid, 4-formyl-, 4-nitrophenyl (ester)	$C_{14}H_{10}O_3$	226.23
26.806	0.66	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37
27.306	0.64	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.54
27.571	0.41	2-Methylresorcinol, diacetate	$C_{11}H_{12}O_4$	208.21
28.501	0.69	Neophytadiene	$C_{20}H_{38}$	278.52
29.45	0.4	Oleyl alcohol, trifluoroacetate	$C_{20}H_{35}F_3O_2$	364.49
30.356	1.27	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45
30.824	0.18	Isophytol	$C_{20}H_{40}O$	296.53
31.205	19.12	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42
33.903	0.99	9,12-Octadecadienoic acid (Z,Z)methyl ester	$C_{19}H_{34}O_2$	294.47
34.042	1.77	9,12,15-Octadecatrienoic acid, methyl ester,	$C_{19}H_{32}O_2$	292.46
34.328	1.45	Phytol	$C_{20}H_{40}O$	296.53
34.686	0.28	Methyl stearate	$C_{19}H_{38}O_2$	298.50
34.955	4.32	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280.45
35.122	11.98	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$	278.43
35.676	1.41	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48
36.854	0.29	Phytol, acetate	$C_{22}H_{42}O_2$	338.57
40.595	0.49	Dodecanoic acid, hex-3-enyl ester	$C_{18}H_{34}O_2$	282.5
43.059	0.8	Heneicosane	$C_{21}H_{44}$	296.57
43.265	3	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.50
43.664	0.22	Henicosanal	$C_{21}H_{42}O$	310.56
43.96	0.4	Z,E-7,11-Hexadecadien-1-yl acetate	$C_{18}H_{32}O_2$	280.4
44.105	0.73	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.43
44.901	0.28	Heneicosane	$C_{21}H_{44}$	296.57

Discussion

The mycelial growth of *B. oryzae* was inhibited with the concentration @ 10% plant extract amended with the medium, zimmu showed maximum of 77.78%. This is the first report the extract of zimmu showed maximum inhibition against *B. oryzae*. No reports were available in previous literature about the use of this extract under *in vitro* against *B. oryzae* in Tamil Nadu. But, some findings of researchers revealed that zimmu were tested against various plant pathogens (fungi and bacteria) showed inhibition at different concentration. Thangavelu and Ganga Devi *et al*., 2013 [11] reported that interspecific hybrid *Allium cepa* x *Allium sativum* (zimmu) provided 100% inhibition on spore germination and 1.7-2.0 cm zone of inhibition of mycelial growth was observed with Zimmu leaf extract under *in vitro* conditions. Muthukumar and Eswaran *et al*., 2010 [9] reported zimmu leaf extract showed the highest inhibition of mycelial growth of *P. aphanidermatum* (13.7 mm). Satya and Radhajeyalakshmi *et al*., 2005 [10] reported that zimmu exhibited maximum antifungal activity against *R. solani* with the inhibition zone of 12mm and it also effective against other plant pathogens *Alternaria solani, Aspergillus flavus, Curvularia lunata, Xanthomonas oryzae* pv. *oryzae, Xanthomonas campestris* pv. *Malvacearum* and *Xanthomonas axonopodis* pv. *citri.*

In this study, henna also showed the inhibition level (75.56%) on mycelial growth of the tested pathogen. The results were more similar with the study carried by Akila and Mini 2020 [14] with the 10% leaf extract of *L. inermis* were highly effective against *B. oryzae* and showed 76.40% reduction over control. Jayaraj and Rubin Jose *et al*., 2018 [21] investigated four medicinally important plant species, 80% leaf extract of *L. inermis* recorded complete inhibition of *Helminthosporium oryzae* mycelial growth. Ramachandra Naik and Akila *et al*., 2016 [22] tested 15 botanicals, among them *L. inermis* with 10% concentration resulted in the reduction of maximum mycelial growth of *B. oryzae* with 65% reduction over control. In contrast, the study carried out by Harish and Saravanakumar *et al*., 2007 [4] 50 plant species were tested, among them leaf extracts of *Nerium oleander* and *P. dulce* showed the highest inhibition of mycelial growth 77.4 and 75.1% against *B. oryzae* respectively.

The principle compound leaf was extracted by Soxhlet extraction method was tested with different concentration against *B. oryzae* that showed reducing mycelial growth with increasing concentration of the extracted compound. Methanol extract of zimmu was examined in GC-MS, compound phytol (16.03%) had highest peak followed by 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (15.13%). Phytol, is widely distributed as a constituent of chlorophyll. Lee and Woo *et al*., 2016 [23] confirmed the antibacterial activity of phytol and its mechanism inducing oxidative cell death in *Pseudomonas aeruginosa*. Muthukumar and Eswaran *et al*., 2010 [9] also reported the presence of 22 compounds in Zimmu, the lipid compound n-Hexadecanoic acid is responsible for the inhibition of the growth of the pathogen *Pythium aphanidermatum*. Akila and Mini 2020 [14] reported the antifungal activity of protein fractions of crude plant extracts of *L. inermis* on *B. oryzae* inhibited 90% over control on mycelial growth. 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP), a compound with flavonoid fraction, is an important bioactive chemical which exhibited antifungal activity [24]. Some scientists reported that 9-Octadecenoic acid, methyl ester, (E) the presence of antimicrobial activity [25-27]. Abubakar and Majinda 2016 [28] tested the preliminary antimicrobial assay for the chloroform extracts of *Albizia adianthifolia* (commonly known as flat-crown) against a fungus/yeast (*Candida albicans*) strains and also showed best activity against *E. coli* with minimum inhibition quantity (MIQ) of 1 µg. Rahman and Ahmad *et al*., 2014 [29] identified the antimicrobial activity of 9-Octadecenoic acid, methyl ester, (E) that reduces the bacterial count. GC-MS analysis of henna showed that chemical compound n-Hexadecanoic acid (19.12%) had highest peak followed by 9, 12,15-Octadecatrienoic acid, (Z,Z,Z) (11.98%). n-hexadecanoic acid was isolated from *Canthium parviflorum* leaves were significantly effective against both gram-positive, gram-negative and fungi organisms [30].

Conclusion

The use of natural substance for plant protection strategies, regulates with organic farming practice. Nevertheless, phytol and n-Hexadecanoic acid seems to offer a promising alternative to the use of synthetic compounds. Hopefully the future will see the increased development of successful plant protection strategies based on natural products.

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