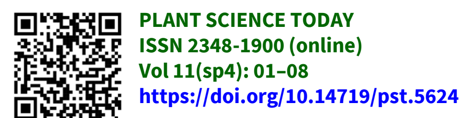

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RESEARCH ARTICLE

Exploring the potentiality of botanicals and phyllosphere microbiome for the management of anthracnose disease in Black gram (*Vigna mungo* L. Hepper)

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Abstract

Black gram (*Vigna mungo* L. Hepper) is the third most important pulse crop, and its yield is reduced due to many diseases. Among them, anthracnose is one of the devasting global fungal diseases which cause severe damage. Use of fungicide is highly effective in controlling this disease, but continuous use of fungicides is unsafe for environment, and it causes fungicidal resistance. To overcome these problems, effect of plant extracts *viz., Anisomeles malabarica*, *Azadirachta indica* leaf extracts, phyllosphere antagonist *Bacillus amyloliquifaciens,* and essential oils *viz.*, peppermint oil and lemon grass oil were evaluated against black gram anthracnose disease caused by *Colletotrichum lindimuthianum* under pot culture. Among the different treatments tested, plants sprayed with standard chemical *viz.,* Carbendazim @ 0.2 percent recorded the least percent disease index (PDI) of 13.39 followed by *A. malabarica* leaf extract (10%) treatments, which recorded a PDI of 16.62, whereas plants treated with *B. amyloliquifaciens*, lemongrass oil and peppermint oil recorded a PDI of 20.91, 21.23 and 24.0, respectively, compared to the inoculated control (77.33 PDI). The induction of defense enzymes *viz.,* phenol, peroxidase (PO), polyphenol oxidase (PPO) was recorded more (21.25 µg of catechol/g of leaf tissue, 3.82 changes in absorbance/min/g leaf tissue, 3.32 changes in absorbance/min/g leaf tissue, respectively) in the plants sprayed with *A. malabarica.* GC-MS analysis of hexane and ethanol extract of *A. malabarica* showed the presence of 26 phytocompounds. Among them, phenol 2,4- bis (1,1- dimethylethyl) phosphite from ethanol extract was a predominant compound (100%). Formulation and standardization of dosage for phenol 2,4- bis (1,1-dimethylethyl) phosphite compound will be useful for managing the disease effectively.

Keywords

Colletotrichum; botanicals; *Anisomeles malabarica*

Introduction

Black gram (*Vigna mungo* L. Hepper), the third most important pulse crop, belongs to the family *Fabaceae*. In India, it is cultivated over an area of 4.63 million hectares with a production of 2.78 million tonnes and a productivity of 600 Kg/ha. Major black gram growing states in India are Andhra Pradesh, Bihar, Karnataka, Maharashtra, Madhya Pradesh, Orissa, Rajasthan, Tamil

Nadu, Uttar Pradesh and West Bengal (1).

Urd bean (Black gram) has high nutritive value as it contains protein (24%), fat (1.4%), carbohydrates (59.6%), calcium (154 mg), phosphorus (385 mg), iron (9.1 mg), beta carotene (38 mg), thiamine (0.4 mg), riboflavin (0.37 mg) and niacin (2 mg) per 100 g seeds (2). The dehulled and defatted flour of urd bean contains 25% protein which is rich in globulins (63%) (3). Black gram thrives well in crop rotation and mixed cropping due to its short duration nature.

Regarding the climatic requirement, this legume requires hot and humid conditions for better growth. The cultivation of this crop is mainly concentrated in rainfed and rice fallow condition. Well drained loamy soil with pH of 6.5 to 7.8 is the most ideal one. Though the black gram plays a major role in food and fodder, its yield is reduced due to many diseases. Among them, anthracnose is one of the devasting global fungal disease that affects all the above aerial parts of black gram inflicting severe damage. Anthracnose disease in black gram is caused by *C. lindemuthianum*. A telomorphic stage of *Colletotrichum* is *Glomerella*. Both *C. lindemuthianum* and the *Glomerella* stage are widely prevalent in cool and humid climates. This pathogen is seed-borne, air-borne and also survives in plant debris. The host range of this fungus includes both major and minor legumes such as *Cajanus cajan* (pigeon pea), *Lablab purpureus* (hyacinth bean), *Phaseolus vulgaris* (common bean), *Vigna sinensis* (asparagus bean), *Vigna unguiculata Glycine max* (soybean), *Lens culinaris* (lentil), *Phaseolus coccineus* (runner bean), *Pisum sativum* (pea), *Vicia faba* (broad bean), *Vigna radiata* (mung bean), and *Canavalia ensiformis* (gotani bean). In black gram leaves, it produces circular, black sunken spots with dark centre and bright red to orange margins. In severe cases it produces shot hole symptom and complete defoliation.

In India, anthracnose of black gram was earlier considered to be of minor importance but with the intensification of black gram cultivation and the severity of this disease it is considered as a major constraint. The yield loss due to this disease is based on the stage of infection, genotypes and environmental condition (4). In India, *C. lindemuthianum* infection in black gram causes 80 -100 % yield loss if the weather conditions are suitable for the pathogen (5). The disease can be controlled by selection of resistant varieties, application of biocontrol agents and fungicides. Among these control methods, the usage of fungicide is found to be the most effective and widely recommended practice among the farmers. However, continuous use of fungicide is not only unsafe for the environment and humans, it also causes fungicide resistance to this fungus. To overcome these problems, there is an urgent need to develop an effective management practice for this disease. Nowadays, chemical fungicides are being replaced by the biocontrol agents because of its eco-friendly nature. Hence, in the present study, potentiality of phyllosphere microbiome and botanicals was explored for the management of black gram anthracnose.

Materials and Methods

Effect of plant extracts, phyllosphere antagonists and essential oils against black gram anthracnose under pot culture condition

A pot culture experiment was conducted at Agricultural College and Research Institute, Killikulam (8.7063° N, 77.8550° E) during 2022 to manage the black gram anthracnose disease. The treatment details are appended in Table1.

The above experiment was conducted on black gram KKM1 variety. The pathogen was inoculated in the black gram leaf surface by spraying conidial suspension after 30 days of sowing. All the treatments were imposed 45 days after sowing. Black gram plants inoculated with *C. lindemuthianum* alone served as positive control and the healthy plant without any inoculation served as negative control. All the treatments were replicated thrice. The experiment was conducted in Completely Randomized Design (CRD). The incidence of black gram anthracnose disease was recorded in all the treatments and the percent disease index (PDI) was calculated by using the following formula,

$$\text{Percent disease Index} = \frac{\text{Sum of individual ratings}}{\text{Total no of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

Induction of defense mechanism by plant extracts, phyllosphere antagonists and essential oils against *Colletotrichum lindemuthianum*

Induction of defense enzymes *viz.,* peroxidase (PO), polyphenol oxidase (PPO), phenol was assessed in black gram plants treated with effective plant extracts, phyllosphere antagonists, essential oils and inoculated with black gram anthracnose pathogen *C. lindemuthianum* at 2, 4, 6, 8 and 10 days after application of treatments. The activity of above-mentioned enzymes was also assessed in the black gram plants immediately after the inoculation of pathogen.

Assay of phenolic content

Phenolic content in black gram leaf was estimated using the method given by Zieslin and Ben-Zaken (6). One gram of leaf sample was collected from each treatment individually and macerated by adding 10 ml of 80 per cent ethanol in a sterile pestle and mortar and centrifuged at 10,000 rpm for 20 min. The supernatant layer was collected separately and allowed to dry. Then each residue was mixed with 5 ml of sterile water. From this, 0.2 ml was taken, and it was made up to 3 ml with sterile water and to that 0.25 ml of (1N) Folin- Ciocaltue reagent was added. After three minutes, 1 ml of sodium carbonate was added and mixed well. Then, the tubes were placed in boiling water for one minute and cooled. The absorbance of each mixture was noticed at 725 nm against blank. The phenolic content was expressed as µg of catechol g^{-1} of leaf tissue.

Peroxidase activity (PO)

The fresh black gram leaves weighing 1 gm were taken from each treatment and ground by adding 1 ml of 0.1 M

Table 1. Treatment details for the management of black gram anthracnose

T. No	Treatments	Concentrations
T1	Foliar spray of PAB1 (*Bacillus amyloliquifaciens*)	10^8 cfu/ml (0.5%)
T2	Foliar spray of leaf extract of *A. malabarica*	10%
T3	Foliar spray of *Azadirachta indica*	10%
T4	Foliar spray of Peppermint oil	1000 ppm
T5	Foliar spray of Lemongrass oil	1000 ppm
T6	Foliar spray of Carbendazim	0.2%
T7	Inoculated control	-
T8	Uninoculated control	-

phosphate buffer to a pre-chilled pestle and mortar. The extract was centrifuged at 15,000 rpm at 4°C for 15 min and the supernatant was used as enzyme source. The reaction mixture was prepared by adding 1.5 ml of pyrogallol, 0.1 ml of enzyme extract and 0.5 ml of 1% H_2O_2. Changes in absorbance were recorded in the reaction mixture at 420 nm at 30 sec intervals for 3 min at room temperature (28 ± 2°C). The boiled enzyme preparation served as blank. The enzyme activity was expressed as change in absorbance of reaction mixture $min^{-1}g^{-1}$ of leaf (7).

Polyphenol oxidase (PPO)

The leaf sample weighing one gram was collected from each treatment and macerated in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4°C and centrifuged at 20,000 g for 15 min at 4°C. The supernatant was served as an enzyme source. Reaction mixture was prepared by adding 1.5 ml of 0.1 M sodium phosphate buffer with pH 6.5 and 200 µl of enzyme extract. The reaction was started by adding 200 µl of 0.01 M catechol and the enzyme activity was expressed as change in absorbance at 490 nm $min^{-1}g^{-1}$ of leaf tissue (8).

Analysis of antimicrobial compounds from the leaf extract *Anisomeles malabarica*

Among the different plant extracts tested against *C. lindemuthianum*, under laboratory and pot culture conditions, *A. malabarica* leaf extract effectively inhibited the pathogen. Hence, phytoconstituents from *A. malabarica* were analyzed through GC-MS.

Collection and preparation of extract

The leaves of *Anisomeles malabarica* were collected from the medicinal garden of Agricultural College and Research Institute, Killikulam. The leaves were dried in the shade for 10 days and powdered well. The powdered sample measuring 25 g was filled in the thimble and extracted using the solvent ethanol and hexane in Soxhlet extractor for 48 h. The extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle for further analysis.

GC-MS analysis

The GC-MS analysis was carried out on a Shimadzu Gas chromatography (QP 2020 with Rxi 5 MS Column) equipped with a mass detector Turbo mass gold containing an Elite- 1 (100% Dimethyl Poly Siloxane), 30 m ×0.25 mm ID employing the following conditions: Carrier gas, helium (1 ml/min). The oven temperature was programmed from 110°C (2 min) ending with a 9 min isothermal point at 280°C; injector temperature 250°C. Total GC running time was 45 minutes. Aliquots sample of 1 ml was injected into the chromatograph. The major constituents were identified with the aid of a computer driven algorithm and then by comparing the mass spectrum of the analysis to that of a National Institute of Standards and Technology (NIST) library (Version 2.0, 2005). The software used for gas chromatography mass spectroscopy (GC-MS) was Turbo mass -5.1. This work was carried out at the Centre of Innovation for Excellence, Agricultural College and Research Institute. Tamil Nadu Agricultural University, Madurai.

Results and Discussion

Effect of plant extracts *viz., A. malabarica* leaf extract, *Azadirachta indica* leaf extract, phyllosphere antagonist *B. amyloliquifaciens* and essential oils *viz.*, peppermint oil and lemon grass oil were evaluated against black gram anthracnose disease under pot culture. Among the different treatments tested, plants sprayed with standard chemical Carbendazim @ 0.2 per cent recorded the least percent disease index of 13.39 and a higher per cent reduction over disease control (82.68) followed by *A. malabarica* leaf extract (10%), which recorded a PDI of 16.62 and per cent reduction over disease control (78.50). Whereas plants treated with *B. amyloliquifaciens*, lemongrass oil and peppermint oil recorded a PDI of 20.91, 21.23 and 24.0 respectively against inoculated control (77.33 PDI) (Table 2). Induction of defense enzymes *viz.,*

Table 2. Effect of plant extracts, phyllosphere antagonists and essential oils on black gram anthracnose under pot culture.

T. No	Treatment	Concentration	Percent Disease index*	Per cent Disease reduction over control
T1	*Bacillus amyloliquifaciens*	10^8 cfu/ml (0.5%)	20.91 (27.20)	72.96 (58.66)^c
T2	*Anisomeles malabarica*	10 %	16.62 (24.04)	78.50 (62.38)^b
T3	*Azadirachta indica*	10 %	31.91 (34.39)	58.73 (50.00)^e
T4	Peppermint oil	1000 ppm	24.00 (29.33)	68.96 (56.13)^d
T5	Lemongrass oil	1000 ppm	21.23 (27.41)	72.54 (58.42)^c
T6	Carbendazim	0.2%	13.39 (21.46)	82.68 (65.39)^a
T7	Inoculated control	-	77.33 (61.58)	0.00 (0.286)f
T8	Un inoculated control	-	0.00 (0.286)	0.00 (0.286)^f
	CD(P=0.05)		**1.90**	**2.17**
	SE(d)		**0.90**	**1.02**

phenol, peroxidase (PO), polyphenol oxidase (PPO) was estimated in black gram plants treated with different plant extracts, phyllosphere antagonists and essential oils before challenge inoculation with *C. lindemuthianum* at different time intervals. In all the treatments, phenolic content, peroxidase activity and polyphenol level were found increasing up to 6th day after application. Still, after 8th day there was a reduction in phenolic content, peroxidase activity and polyphenol level. Among the different treatments, *A. malabarica* leaf extract (10%) showed maximum phenol content, peroxidase and polyphenol oxidase activity (21.25 µg of catechol/g of leaf tissue, 3.82 changes in absorbance/min/g leaf tissue, 3.32 changes in absorbance/min/g leaf tissue respectively) at 6th day after challenge inoculation (Table 3, Table 4, Table 5). This is in agreement with the reports of Gholamnezhad (9) who reported that the application of neem leaf extract induced the defense responses against *Botrytis cinerea* in apples.

Leaf extract of *A. malabarica* showed highest mycelial inhibition against *C. lindemuthianum*, which was further analyzed through GC-MS for identification of phytoconstituents with antimicrobial activity. GC-MS analysis of hexane and ethanol extract of *A. malabarica* showed the presence of 26 bioactive compounds based on comparison of retention times and by interpretation of their mass spectra. The identified compounds and their retention time, area percent, molecular formula and molecular weight are listed in the (Table 6, Fig 1, Fig 2).

Among the 26 phytocompounds, six compounds viz., (2,3 Diphenylcyclopropyl) methylphenylsulfoxide, Tetracosane, 3, 5 ,9 -Trimethyl deca -2, 4, 8 -trien-1-ol, Cyclononasiloxane Octadecamethyl, Phytol, 1,2-Benzenedicarboxylic acid bis (2- methylpropyl) from hexane extract and Phenol 2,4- bis (1,1- dimethylethyl)

Table 3. Induction of phenol content in black gram leaves treated with different treatments under pot culture.

T. No	Treatments	*Changes in absorbance / min/ g of leaf tissue Days after inoculation						
		0	2	4	6	8	10	Mean
T1	*Bacillus amyloliquifaciens* 10^8 cfu/ml (0.5%)	11.50	12.60	18.50	19.25	17.26	14.75	15.64^b
T2	*Anisomeles malabarica* 10%	12.75	13.53	20.00	21.25	19.75	15.25	17.08^a
T3	*Azadirachta indica* 10%	10.25	11.23	16.25	17.15	15.25	12.50	13.77^c
T4	Peppermint oil 1000 ppm	8.33	9.35	11.00	12.25	10.50	9.00	10.07^e
T5	Lemongrass oil 1000 ppm	9.53	10.25	11.50	13.00	11.25	10.50	11.00^d
T6	Carbendazim 0.2%	7.16	8.11	10.25	12.50	11.15	10.52	9.94^f
T7	Inoculated control	7.06	8.20	12.45	13.00	12.25	9.300	10.37^e

*Mean of three replications.
The treatment means are compared using Duncan multiple range test (DMRT)
In a column, means followed by a common letter(s) are not significantly different (p= 0.05)

Table 4. Induction of peroxidase (PO) activity in black gram leaves treated with various treatments under pot culture experiment

T. No	Treatments	*Changes in absorbance / min/ g of leaf tissue Days after inoculation						
		0	2	4	6	8	10	Mean
T1	*Bacillus amyloliquifaciens* 10^8 cfu/ml (0.5%)	1.83	2.13	3.10	2.82	2.70	2.52	2.51^d
T2	*Anisomeles malabarica* 10%	2.20	2.75	3.19	3.82	3.20	3.15	3.05^a
T3	*Azadirachta indica* 10%	1.90	2.10	2.52	2.70	2.68	2.49	2.39^e
T4	Peppermint oil 1000 ppm	1.95	2.15	2.62	2.89	2.82	2.60	2.50^d
T5	Lemongrass oil 1000 ppm	1.92	2.18	2.50	3.42	2.79	3.11	2.72^b
T6	Carbendazim 0.2%	2.12	2.50	2.55	2.75	3.20	2.52	2.60^c
T7	Inoculated control	1.66	1.82	1.95	2.09	1.92	1.79	1.87^f

*Mean of three replications.
The treatment means are compared using Duncan multiple range test (DMRT)
In a column, means followed by a common letter(s) are not significantly different (p= 0.05)

Table 5. Induction of polyphenol oxidase (PPO) activity in black gram leaves treated with various treatments under pot culture experiment.

T. No	Treatments	*Changes in absorbance / min/ g of leaf tissue						
		Days after inoculation						
		0	2	4	6	8	10	Mean
T1	*Bacillus amyloliquifaciens* 10^8 cfu/ ml (0.5%)	1.90	2.68	2.83	3.20	3.06	3.01	2.78[b]
T2	*Anisomeles malabarica* 10%	1.97	2.72	2.93	3.32	3.21	3.03	5.72[a]
T3	*Azadirachta indica* 10%	1.88	2.35	2.80	2.79	2.45	2.62	2.48[c]
T4	Peppermint oil 1000 ppm	1.84	2.20	2.70	2.76	2.37	2.45	2.38[c]
T5	Lemongrass oil 1000 ppm	1.55	1.64	1.80	2.90	2.64	2.40	2.15[d]
T6	Carbendazim 0.2%	1.50	1.52	1.79	2.88	2.56	2.24	2.08[d]
T7	Inoculated control	1.32	1.46	1.57	2.14	1.65	1.44	1.59[e]

*Mean of three replications.
The treatment means are compared using Duncan multiple range test (DMRT)
In a column, means followed by a common letter(s) are not significantly different (p= 0.05)

Table 6. Detection of phytocompounds from *A. malabarica* leaf extract by GC-MS analysis

Peak #	Retention time (min)	Compounds identified through hexane extract	Molecular formula	Molecular weight	Area %
1	5.024	o- Xylene	C8H10	106	0.67
2	20.755	2,4 -Di-tert-butylphenol	C14H22O	338	1.69
3	25.433	Eicosane	C20H42	282	0.89
4	26.445	Eicosane	C20H42	282	0.43
5	28.604	2-Pentadecanone,6,10,14- trimethyl	C18H36O	268	1.14
6	29.010	1,2- Benzenedicarboxylic Acid, bis (2- methyl propyl)	C16H22O4	278	7.31
7	29.985	1-Pentadecanamine,N,N- dimethyl	C17H37N	255	1.16
8	30.043	7,9-Di-ter-butyl-1-oxaspiro (4,5) deca-6,9-dien	C17H24O3	276	2.61
9	30.109	Eicosane	C20H42	282	1.04
10	30.195	Cyclodecasiloxane,eicosamethyl	C20H60010Si10	740	2.72
11	30.366	Hexadecanoic acid, methyl ester	C17H34O2	270	2.46
12	31.003	Tetracosane	C24H50	338	0.63
13	32.611	3,5,9- Trimethyl-deca-2,4,8- trien-1-ol	C13H22O	194	1.78
14	33.298	Cyclooctasiloxane, hexadecamethyl	C16H48O8Si8	592	2.90
15	34.343	Phytol	C20H40O	296	8.14
16	34.630	2-Methylhexacosane	C27H56	380	1.43
17	34.706	Methyl stearate	C19H38O2	298	1.71
18	36.671	Cyclononasiloxane, octadecamethyl	C18H54O9Si9	666	3.98
19	36.821	3,5,9-Trimethyl-deca-2,4,8- trien-1-ol	C13H22O	194	1.67
20	39.614	Tetracosane	C24H50	338	0.71
21	39.776	Cyclononasiloxane, octadecamethyl	C18H54O9Si9	666	3.37
22	42.076	(2,3- Diphenylcyclopropyl) methyl phenyl sulfo	C22H20OS	332	43.21
23	42.531	Cyclononasiloxane, octadecamethyl	C18H54O9Si9	666	4.05

24	43.664	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	0.51
25	44.974	Cyclononasiloxane, octadecamethyl	$C_{18}H_{54}O_9Si_9$	666	3.77
		Compound identified through ethanol extract			
26	31.868	Phenol, 2,4 -bis (1,1 – dimethylethyl)- phosphite	$C_{42}H_{63}O_3P$	646	100

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	5.024	5.010	5.050	445855	0.67	374232	2.21	1.19	o-Xylene
2	20.755	20.695	20.840	1133421	1.69	363491	2.14	3.12	2,4-Di-tert-butylphenol
3	25.433	25.385	25.490	597318	0.89	233990	1.38	2.55	Eicosane
4	26.445	26.400	26.500	287938	0.43	99501	0.59	2.89	Eicosane
5	28.604	28.550	28.690	764766	1.14	256237	1.51	2.98	2-Pentadecanone, 6,10,14-trimethyl-
6	29.010	28.935	29.110	4892078	7.31	1499425	8.84	3.26	1,2-Benzenedicarboxylic acid, bis(2-methylprc
7	29.985	29.920	30.005	777780	1.16	255683	1.51	3.04	1-Pentadecanamine, N,N-dimethyl-
8	30.043	30.005	30.085	1750384	2.61	517460	3.05	3.38	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
9	30.109	30.085	30.150	698461	1.04	252529	1.49	2.77	Eicosane
10	30.195	30.150	30.325	1821059	2.72	195753	1.15	9.30	Cyclodecasiloxane, eicosamethyl-
11	30.366	30.325	30.460	1647852	2.46	480355	2.83	3.43	Hexadecanoic acid, methyl ester
12	31.003	30.955	31.060	419151	0.63	148581	0.88	2.82	Tetracosane
13	32.611	32.545	32.690	1194332	1.78	340685	2.01	3.51	3,5,9-Trimethyl-deca-2,4,8-trien-1-ol
14	33.298	33.220	33.390	1940572	2.90	441142	2.60	4.40	Cyclooctasiloxane, hexadecamethyl-
15	34.343	34.260	34.440	5453093	8.14	1424724	8.40	3.83	Phytol
16	34.630	34.565	34.665	957368	1.43	257050	1.52	3.72	2-Methylhexacosane
17	34.706	34.665	34.785	1147500	1.71	298247	1.76	3.85	Methyl stearate
18	36.671	36.605	36.765	2668088	3.98	590153	3.48	4.52	Cyclononasiloxane, octadecamethyl-
19	36.821	36.765	36.910	1116062	1.67	276545	1.63	4.04	3,5,9-Trimethyl-deca-2,4,8-trien-1-ol
20	39.614	39.555	39.680	472561	0.71	117072	0.69	4.04	Tetracosane
21	39.776	39.680	39.870	2259362	3.37	643794	3.80	3.51	Cyclononasiloxane, octadecamethyl-
22	42.076	41.945	42.220	28932036	43.21	6360031	37.50	4.55	(2,3-Diphenylcyclopropyl)methyl phenyl sulfo:
23	42.531	42.460	42.615	2714937	4.05	809714	4.77	3.35	Cyclononasiloxane, octadecamethyl-
24	43.664	43.620	43.745	339214	0.51	100565	0.59	3.37	Bis(2-ethylhexyl) phthalate
25	44.974	44.890	45.095	2525857	3.77	623457	3.68	4.05	Cyclononasiloxane, octadecamethyl-
				66957045	100.00	16960416	100.00		

Fig. 1. GCMS spectral chromatogram for hexane extract of *A. malabarica*

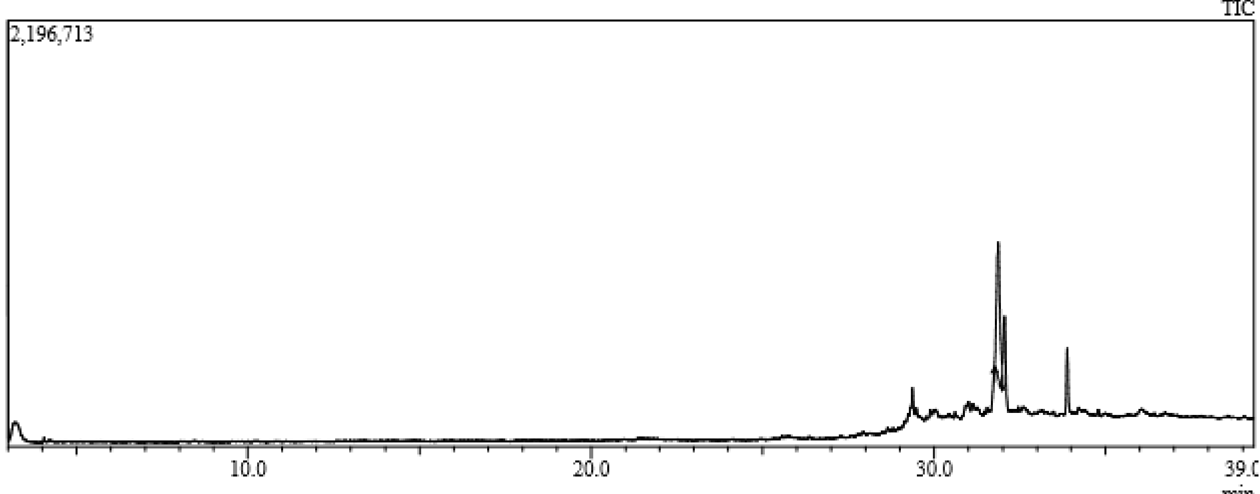

Fig. 2. GCMS spectral chromatogram for ethanol extract of *A. malabarica*

phosphite from ethanol extract, showed biological activity. In total seven compounds showed biological activity. Among the seven compounds, Phenol 2,4- bis (1,1-) phosphite from ethanol extract was found as a predominant compound (100%) followed by (2,3 Diphenylcyclopropyl) methyl phenyl sulfoxide (43.21 %) from hexane extract. The reported literature clearly demonstrated the antimicrobial, antioxidant, antibacterial, insecticidal activity of these compounds (Table 7).

Table 7. Biological activity for some of the phytocompounds identified in the hexane and ethanol extract of *A. malabarica* by GC-MS.

S.no	Compound	Area %	Biological activity	Chemical structure
1	(2,3Diphenylcyclopropyl) Methyl phenyl sulfoxide	43.21	Antimicrobial activity	
2	Tetracosane	0.63	Antioxidant and antibacterial	
3	3,5,9 – Trimethyl- deca – 2,4,8 -trien-1-ol	1.67	Antimicrobial	
4	Cyclononasiloxane, octadecamethyl.	3.77	Insecticidal activity	
5	Phytol	8.14	Antimicrobial, antibacterial	
6	1,2- Benzenedicarboxylic Acid, bis (2- methyl propyl)	7.31	Antimicrobial	
7	Phenol,2,4-bis (1,1- dimethylethyl) phosphite	100	Antibacterial, antioxidant and anti-microbial activity	

Rajarajan *et al.* (10) reported that methanolic extract of *A. malabarica* showed the antimicrobial activity against fungal pathogens and reported the phytoconstituent 3,5,9 – Trimethyl- deca – 2,4,8 -trien-1-ol which was identified in the present investigation.

Ojekale *et al.* (11) documented the antioxidant and insecticidal activity of *Thaumatococcus danielli* and found out that Cyclononasiloxane, octadecamethyl and phytol were the major constituent in the essential oil of the plant extract. Similarly, potential antibacterial, antioxidant and anticancer effects of phytol, a key a cyclic diterpene alcohol were reported in *Pergularia daemia* by Rukshana *et al.* (12). The antibacterial, antioxidant activity of ethanolic extract of *Anisomeles malabarica* against bacterial pathogens have been already reported by Krishna *et al* (13) who also identified phenol, 2,4 -bis (1,1 – dimethylethyl) phosphite as one of the important constituents in GC-MS analysis. In the present study too, Phenol 2,4 -bis (1,1 – dimethylethyl) phosphite was identified in the ethanolic extract as the major constituent.

Conclusion

Spraying of *A. malabarica* leaf extract (10%) effectively reduced blackgram anthracnose disease incidence. A total of 25 phytocompounds from hexane extract and one compound from ethanol extract of *A. malabarica* leaf extract were identified. Among them, phenol 2,4- bis (1,1- dimethylethyl) phosphite from ethanol extract of *A. malabarica* was found as a predominant compound (100%). But further research is needed to standardise the dosage and application method of phenol 2,4- bis (1,1- dimethylethyl) phosphite for effective management of black gram anthracnose. A study of interaction between the phytocompounds from plant extracts and pathogen will be useful in developing an eco-friendly management strategy for the disease.

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Authors' Contributions

Author NR Designed the study and did antimicrobial compound identification from botanicals, EV Carried out pot culture experiments and estimation of defense enzymes, KE and ES assisted in the estimation of defense enzymes, RK and JS compiled the manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest among them.

Ethical issues: None

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