



# Antinemic Metabolites of *Simarouba glauca* against Root Knot Nematode, *Meloidogyne incognita* Infesting Groundnut, *Arachis hypogaea*

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## ABSTRACT

**Background:** Groundnut, *Arachis hypogaea* is a leguminous crop grown in tropical and subtropical regions of the world. *Meloidogyne* spp., is considered to be nematode pest of economic importance for the groundnut throughout the world. Plant metabolites with nematicidal property have been in use for a long time for nematode control that offer many environmental advantages.

**Methods:** The nematicidal activity of methanolic leaf extract of *Simarouba glauca* on eggs and juveniles (Js) of root-knot nematode, *Meloidogyne incognita* infesting vegetables was evaluated at three concentrations *in vitro* (100%, 50%, 25%) with control at three exposure times.

**Result:** Results revealed that at 100% concentration, the methanolic leaf extract of *S. glauca* showed the least number of eggs hatched (7.85, 12.63, 16.30/egg mass) and the highest mortality (60.58, 70.23, 92.58/100 juveniles) at the exposure times of 24 hrs, 48 hrs, 72 hrs, respectively. The phytochemical compounds viz., Hexadecanoic acid-methyl ester, Isopropyl palmitate, Octadecanoic acid and Methyl stearate detected through GC-MS profiling of methanolic extract of *S. glauca* were found to inhibit the nematode activity. Pot and field studies proved that soil application of *Simarouba* leaf powder @ 10 g/plant and 5 kg/ha reduced the nematode infestation. This study implies that use of *Simarouba* leaf powder produces nematicidal effect against plant parasitic nematodes and reduced the nematode infestation in groundnut and is more suitable candidate in the context of biological control.

**Key words:** Antinemic, GC-MS, *Meloidogyne incognita*, Metabolites, Paradise tree, *Simarouba glauca*.

## INTRODUCTION

Groundnut (Peanut) (*Arachis hypogaea*), an important oil and food crop, is widely grown in tropical and subtropical countries, with a worldwide production estimated at 33.1 million tonnes (Mc Donald *et al.*, 2005). Several species, such as *Meloidogyne* spp., *Pratylenchus brachyurus*, *Belonolaimus longicaudatus*, *Criconebella ornate* and *Ditylenchus africanus* are considered to be pests of great economic importance for the peanut, either worldwide or in specific regions (Dickson and De Waele, 2005). These nematode species can attack the roots, pegs and hulls of the peanut. Adegbite and Adesiyun (2005) and Almohith *et al.* (2020) described nematodes as destructive parasites because the nutrients are directly taken by the nematode from the host plant's cell wall with the aid of a needle-like stylet. Depending on the severity, the losses brought on by root knot nematode might range from minimal to significant (Kavitha *et al.*, 2012). Stunting of plants results from the impeded and altered Root to Shoot (R/S) transfer of nutrients and minerals during root colonisation of nematodes (Gullino *et al.*, 2020).

Botanicals have been in use for a long time for nematode control and these compounds offer many environmental advantages. The Simaroubaceae family includes 32 genera and more than 170 species of trees and shrubs of pantropical distribution. It is characterized by its content of bitter substances, mostly responsible for its

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pharmaceutical properties (Fernando and Quinn, 1992; Muhammad *et al.*, 2004 and Babaali *et al.*, 2017). Due to the chemical diversity of the Simaroubaceae family, it is worth noting that it can be characterized as a promising source of bioactive molecules with remarkable research potential. Exploration of quassinoid chemicals is predominant in simaroubaceae family (Curcino Vieira and Braz-Filho, 2006; Kumar *et al.*, 2006; Priyadarshini *et al.*, 2023).

In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Kell *et al.*, 2005;

Robertson, 2005). The occurrence of root knot nematodes in vegetable crops is more predominant in India compared to other cultivated crops. In comparison with other nematode management strategies the phytochemicals derived from botanicals also plays a major role to control plant parasitic nematodes. Hitherto, the present study is aimed to investigate the nematicide activity of *Simarouba glauca* leaf extract and the possible bioactive chemical components against *M. incognita* by subjecting it to GC-MS analysis.

## MATERIALS AND METHODS

The leaf and seed of *Simarouba glauca* were collected from Tamil Nadu Agricultural University, Coimbatore. The plant materials were washed under running tap water and dried for 1 week at room temperature in a shaded area. The dried samples were finely powdered by using an electric blender and stored in a plastic bag container for further use at room temperature.

Fifty gram of finely ground leaf and seed samples of *Simarouba* were packed separately in a porous bag. The solvent (250 ml of methanol) was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser on an isomantle. Extraction was carried out separately for leaf and seed samples. Finally the leaf and seed extracts were evaporated and stored in a refrigerator at 0-4°C in an air tight container for further use. The egg masses of root knot nematode, *M. incognita* infested Groundnut plants were uprooted from the infested field and used for the collection of egg masses and infective juveniles. The species level confirmation of root knot nematode as *Meloidogyne incognita* was confirmed based on morphological characters of perineal pattern. Five mature females of *Meloidogyne* spp. were taken out of the root tissue using forceps, placed in a drop of warm lactophenol on a glass slide and examined under a light microscope to determine perineal patterns (Eisenback *et al.*, 1981).

### In vitro bioassay

Each treatment consisted of 5 replicates of egg mass in 25%, 50%, 100% concentrations of each extract. The experiment was conducted in a 24-well plate maintained in an incubator in the dark at 24°C. Hatched Juveniles were counted after 24, 48 and 72 hours in treatments. The effect of methanolic extracts on 100 Juveniles of *M. incognita* at

concentrations of 25%, 50%, 100% obtained from the stock solution was evaluated for 24, 48, 72 hours and repeated the each treatment 5 times. Using a double counter, 100 Juveniles between dead and live juveniles were counted after 24, 48 and 72 hours of exposure, considering a young straight or motionless juvenile as dead, which was confirmed by touching them with a fine needle. To compare the results, natural mortality was counted in distilled water which serves as control (Costa *et al.*, 2003).

### Preliminary phytochemical screening

Extraction is the separation of bioactive portions of plant using selective solvents of increasing polarity such as ethanol. The purpose of extraction is to separate the soluble plant metabolites, leaving behind the insoluble residue. The initial crude extracts contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids and flavonoids. The methanol extract was tested for alkaloids, anthroquinones, flavonoids, phenols, steroids, tannins, terpenoids, glycosides, saponins and volatile oils using GC-MS analysis (Table 2).

### GC-MS (Gas chromatography-mass spectrometry) analysis

Volatile compounds like nonpolar components and essential oil, fattyacids, lipids and alkaloids that have antimicrobial property are identified by the GC-MS analysis. The methanolic extract of the leaf and seed of *Simarouba* were used for the GC-MS analysis. 1 µl of the methanolic extract of the leaf and seed of *Simarouba* were dissolved separately in HPLC grade methanol and subjected to GC and MS facility available in the Department of Microbiology, TNAU, Coimbatore. The Clarus SQ 8C Gas Chromatography - Mass Spectrometer from Perkin Elmer, were engaged for analysis (Tables 3 and 4).

The pot and field experiment were conducted in the Department of Nematology, Centre for Plant Protection studies, TNAU, Coimbatore during 2021-2023 using different botanical formulations of *Simarouba glauca*. The treatments were applied to 15 days old Groundnut plants at two different time intervals @ 15 days and 45 days after transplantation. The observations includes No. of adult females/5 g root, No. of egg masses/5 g root, Gall index, Root population (5 g of root) and Soil population (250 cc soil) were recorded at 60 days after transplantation.

**Table 1:** Nematotoxic potential of crude leaf extract of *Simarouba glauca* on egg hatching and juvenile mortality of *Meloidogyne incognita*.

Crude leaf extract (%)	Exposure time (hrs)					
	No. of eggs hatched/egg mass			No. of juveniles dead/100 juveniles*		
	24	48	72	24	48	72
100	7.85 (2.78)	12.63 (1.61)	16.30 (4.02)	60.58 (1.78)	70.23 (1.84)	92.58 (1.96)
50	12.72 (3.37)	29.02 (5.45)	36.54 (6.03)	38.20 (1.88)	48.11 (1.68)	62.44 (1.78)
25	34.13 (5.83)	38.22 (6.18)	42.39 (6.51)	30.13 (1.47)	40.73 (1.60)	46.46 (1.66)
Control (Distilled water)	75.45 (8.78)	167.21 (12.93)	286.53 (6.92)	00.00 (0.28)	00.00 (0.28)	4.01 (0.64)
CD (0.05)	0.3712	0.2740	0.1802	0.0400	0.0334	0.5467

Figures in the paranthesis are arsine transformation values.

### Statistical analysis

The data were analysed using (ANOVA) with IBM SPSS version 20.00 software (SPSS Inc., Chicago, IL, USA). Duncan's Multiple Range Test (DMRT) with 0.05 probability assessed the significant differences between treatments. With the help of probit analysis, the  $LC_{50}$  values of the compound determined.

### RESULTS AND DISCUSSION

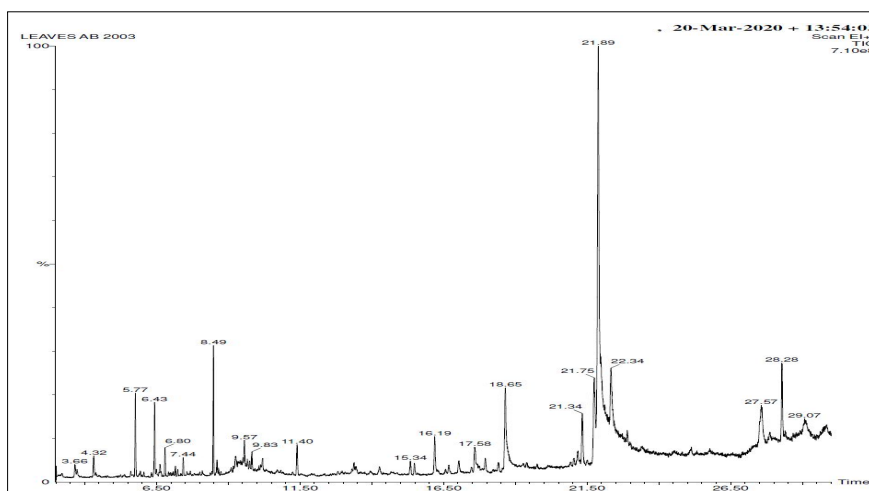
The crude leaf extract of *S. glauca* (100%) exposure after 24 hrs on *M. incognita* observed the least eggs hatched (7.85/egg mass) and juveniles mortality was 60.58/100 juveniles, followed by 50% crude leaf extract on the number of eggs hatched (12.72/egg mass) and juveniles mortality (38.20/100 juveniles). The maximum number of eggs hatched (34.13/egg mass) and the lowest juveniles mortality (30.13/100 juveniles) were recorded in the 25% crude leaf extract compared to control had no juvenile mortality and the number of eggs hatched (75.45/egg mass).

The crude leaf extract of *S. glauca* (100%) exposure after 48 hrs on *M. incognita* observed the least eggs hatched (12.63/egg mass) and juveniles mortality was 70.23/100 juveniles, followed by 50% crude leaf extract on the number

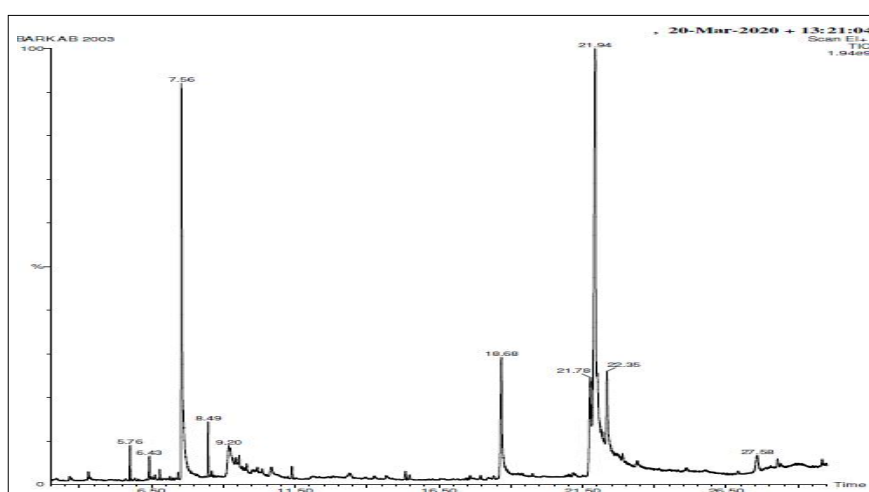
**Table 2:** Preliminary phytochemical screening of methanolic extracts of *S. glauca*.

Phytochemical constituents	Test reagents	Reaction
Alkaloids	Wagner test	+
Anthroquinones	Borntrager's test	+
Flavonoids	Shinoda test	+
Phenols	Phenol test	+
Steroids	Liebermann test	+
Tannins	Braemer's test	+
Terpenoids	LB Salkowski test	+
Protein/Aminoacids	Biuret test	+
Volatile oils	Strin test	+

+ - indicated as positive.



**Fig 1:** GC-MS chromatogram of the methanolic extracts of *S. glauca* leaf.



**Fig 2:** GC-MS chromatogram of the methanolic extracts of *S. glauca* seed.

**Table 3:** Metabolites identified in the methanolic extracts of *S. glauca* leaf.

RT	Compound name	MF	MW	Area %	Probability	Activity
3.013	3-Hydroxypyridine	C <sub>5</sub> H <sub>5</sub> NO	95	2.846	63.4	Antioxidant
4.174	Thymine	C <sub>5</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>	126	0.664	31.7	Antimetabolite
5.109	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C <sub>14</sub> H <sub>16</sub> O <sub>3</sub>	232	1.051	94.9	Compound with flavonoid fraction, is an important bioactive chemical which exhibited antifungal activity to inhibit growth or spore germination
6.625	6-Hydroxy-4-methyl-3-phenylcoumarin	C <sub>10</sub> H <sub>8</sub> O <sub>3</sub>	176	0.237	24.4	Antioxidant activities inhibit aflatoxin formation
8.581	3- [N'- (3H-Indol-3-ylmethylene)-hydrazino]-5-methyl- [1, 2, 4] triazol-4-ylamine	C <sub>12</sub> H <sub>13</sub> N <sub>7</sub>	255	0.309	29.9	Antioxidant
9.486	Melezitose	C <sub>18</sub> H <sub>32</sub> O <sub>16</sub>	504	1.102	13.1	Anti-viral, anti-fungal, anti-inflammatory and wound healing and antiemetic properties
9.961	2,4-Di-tert-butylphenol	C <sub>14</sub> H <sub>22</sub> O	206	0.216	41.0	Anti-inflammatory
10.071	Methyl vanillate, 2-methylpropyl ether			0.254	12.9	Antimicrobial, anti-inflammatory and antioxidant/anticancer properties
12.697	1b,4a-Epoxy-2H-cyclopenta [3, 4] cyclopropano [8, 9] cycloundec [1, 2-b] oxiren-5 (6H) one, 7 (acetyloxy) decahydro-2, 9, 10-trihydroxy-3,6,8,8,10a-pentamethyl-	C <sub>28</sub> H <sub>38</sub> O <sub>11</sub>	550	0.247	24.7	Antimicrobial, Anti-inflammatory
14.938	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2 (4H)-one	C <sub>11</sub> H <sub>16</sub> O <sub>3</sub>	196	0.623	75.1	Antioxidant and anti-inflammatory properties of 80% methanolic
18.199	Hexadecanoic acid, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	8.170	72.5	potential antioxidant, antitumor, anti-inflammatory, antibacterial and antifungal activities
18.655	Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	0.298	17.3	Antimicrobial activity against gram positive and gram negative
18.960	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	6.402	79.7	potential antioxidant, antitumor, anti-inflammatory, antibacterial and antifungal activities
19.070	Ethyl 9-hexadecenoate	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	0.529	23.8	Anti-breast-cancer and antiviral activities
19.515	Hexadecanoic acid, ethyl ester	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	2.063	69.1	Potential antioxidant, antitumor, anti-inflammatory, Antibacterial and antifungal activities
20.085	Isopropyl palmitate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	0.240	53.3	An additive for imparting bactericidal and antimicrobial properties
21.316	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C <sub>22</sub> H <sub>42</sub> O <sub>3</sub> Si	382	18.065	29.3	Anti-inflammatory, cholesterol lowering and have anticancer property
21.456	9-Octadecenoic acid, methyl ester, (E)-	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296	25.261	11.3	Anti-inflammatory, cholesterol lowering and have anticancer property
21.961	Methyl stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	4.811	72.8	Antifungal, antioxidant
22.126	Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	1.377	13.2	Anti-nutritional properties
22.636	(E)-9-Octadecenoic acid ethyl ester	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	4.278	21.3	Anti-inflammatory, cholesterol lowering and have anticancer property

Table 3: Continue...

**Table 3: Continue...**

23.146	Octadecanoic acid, ethyl ester	$C_{20}H_{38}O_2$	310	0.213	42.2	Anti ageing and antimicrobial
25.592	4,8,12,16-Tetra methylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.215	47.1	Anti-inflammatory
25.672	Ethyl Oleate	$C_{20}H_{38}O_2$	310	0.225	30.6	Antioxidant
26.012	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	0.272	26.9	Anti-inflammatory
28.123	Tetradecylphosphonate	$C_{19}H_{38}O_2$	298	0.787	73.6	Antioxidant properties
28.293	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{34}H_{68}O_3$	524	49.9	1.986	Antioxidant activity
28.523	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	0.683	43.8	Anti-androgens

RT- Retention time, MW- Molecular weight, MF- Molecular formula.

**Table 4: Metabolites identified in the methanolic extracts of *S. glauca* seed.**

RT	Compound name	MF	MW	Area %	Probability	Activity
7.910	2, 2, 6, 7-Tetramethyl-10-oxatricyclo [4.3.0.1 (1,7)] decan-5-one	$C_{13}H_{20}O_2$	208	0.187	21.1	Anticancer activity
9.541	Sucrose	$C_{12}H_{22}O_{11}$	342	3.828	20.2	Antioxidant
10.011	2, 4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	0.347	45.2	Anti-inflammatory
10.131	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	$C_7H_6O_3$	138	0.325	52.5	<i>In vitro</i> anticancer activity
10.271	Methyl 3, 4-di-O-acetyl-2-O-methyl-6-deoxy- $\alpha$ -D-mannopyranoside	$C_{15}H_{26}O_9$	350	0.722	13.1	Anticancer
13.327	Flumetralin	$C_{16}H_{12}ClF_4N_3O_4$	421	0.156	22.5	Anti-budding agent, is applied topically on tobacco
14.998	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	$C_7H_6O_3$	138	0.318	61.6	Antioxidant;
18.254	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	9.409	69.0	Antimicrobial antioxidant
18.705	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.271	18.3	Antimicrobial activity against gram positive and gram negative
18.970	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	2.915	84.6	Potential antioxidant, antitumor, anti-inflammatory, antibacterial and antifungal activities
20.130	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	0.226	57.8	An additive for imparting bactericidal and antimicrobial properties

**Table 4: Continue....**

Table 4: Continue....

21.261	9,10-Anthracenedione, 2-methyl-	C <sub>14</sub> H <sub>8</sub> O <sub>2</sub>	208	0.140	62.2	Anti-inflammatory
21.361	9,12-Octadecadienoic acid (Z, Z) -, methyl ester	C <sub>22</sub> H <sub>42</sub> O <sub>3</sub> Si	382	20.804	29.4	Antioxidant
21.511	9-Octadecenoic acid (Z) -, methyl ester	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	29.244	10.9	Antioxidant
22.006	Methyl stearate	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	298	6.539	73.6	Antifungal, antioxidant
22.161	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	0.717	12.3	Anti-nutritional properties
22.626	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	0.737	67.0	Potential antioxidant, antitumor, anti-inflammatory, antibacterial and antifungal activities
23.777	Octadecanoic acid, 2-hydroxy-1, 3-propanediyl ester	C <sub>36</sub> H <sub>72</sub> O <sub>3</sub>	552	1.145	22.3	Antibacterial, Antitumor, Antioxidant
25.452	Methyl 18 methylInonadecanoate	C <sub>22</sub> H <sub>42</sub> O <sub>3</sub> Si	382	0.417	50.3	Antitumor and antioxidant
28.328	Glycerol 1-palmitate	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330	3.415	35.7	Antioxidant and Antimicrobial
28.633	Dimethoxycurcumin	C <sub>16</sub> H <sub>15</sub> Cl <sub>3</sub> O <sub>2</sub>	344	0.722	46.1	Superior inter-related pro-oxidant and anti-cancer
29.114	à-Tocospiro B	C <sub>29</sub> H <sub>50</sub> O <sub>4</sub>	462	1.301	48.6	Antibacterial activity against 6 <i>Staphylococcus aureus</i>

RT- Retention time.

Table 5: Evaluation of botanical formulations of *S. glauca* against root knot nematode, *Meloidogyne incognita* in groundnut under glass house conditions.

Treatment details	Nematode population/incidence				
	No. of adult females/5 g root	No. of egg masses/5 g root	Gall index	Root population (5 g of root)	Soil population (250 cc soil)
T <sub>1</sub> - Soil application with <i>Simarouba</i> leaf powder @ 5 g/plant	65.8 (8.12)	37.5 (6.16)	2.7	186.3 (13.63)	235.7 (15.32)
T <sub>2</sub> - Soil application with <i>Simarouba</i> leaf powder @ 10 g/plant	37.9 (6.16)	21.5 (4.8)	1.6	85.4 (9.21)	109.6 (10.6)
T <sub>3</sub> - Soil application with <i>Simarouba</i> seed oil @ 2.5 ml/plant	85.6 (9.27)	33.9 (6.32)	2.3	108.6 (10.39)	196.5 (14.0)
T <sub>4</sub> - Soil application with <i>Simarouba</i> seed oil @ 5.0 ml/plant	58.4 (7.68)	45.0 (6.7)	2.6	153.5 (12.36)	283.0 (16.82)
T <sub>5</sub> - Soil application with <i>Simarouba</i> oil cake @ 10 g/plant	72.0 (8.48)	35.8 (5.91)	2.2	126.6 (11.22)	178.2 (13.34)
T <sub>6</sub> - Soil application with <i>Simarouba</i> oil cake @ 20 g/plant	45.3 (6.70)	29.0 (5.38)	1.8	96.7 (9.84)	121.4 (11.0)
T <sub>7</sub> - Carbofuran 3G @ 3 g/plant	46.5 (6.78)	29.5 (5.4)	1.6	95.5 (9.6)	116.0 (10.6)
T <sub>8</sub> - Fluensulfone 2% GR-3 g/plant	40.1 (5.9)	28.0 (5.1)	1.5	81.5 (8.91)	107.6 (10.31)
T <sub>9</sub> - Control	112.3 (10.5)	48.0 (6.92)	3.6	267.8 (16.34)	321.4 (17.91)
CD (0.05)	1.10	3.33	0.42	0.96	1.64

Treatments given at two intervals i.e., 15 and 45 days after planting.

Figures in parentheses are square root transformed values.



**Table 6:** Evaluation of botanical formulations of *S. glauca* against root knot nematode, *Meloidogyne incognita* in Groundnut under field conditions.

Treatments	Nematode population (250 cc soil)			No. of galls/5 g of root			No. of egg masses/5 g root		
	Initial	15 days	45 days	Initial	15 days	45 days	Initial	15 days	45 days
T <sub>1</sub> - Soil application with <i>Simarouba</i> leaf powder @ 2.5 kg/ha	152.43 (12.34)	150.62 (12.29)	143.54 (12.01)	25	20	16	21	17	14
T <sub>2</sub> - Soil application with <i>Simarouba</i> leaf powder @ 5.0 kg/ha	120.24 (11.03)	106.89 (10.41)	92.65 (9.78)	27	18	12	25	12	10
T <sub>3</sub> - Soil application with <i>Simarouba</i> seed oil @ 1.0 l/ha	172.53 (13.12)	165.32 (12.85)	160.53 (12.72)	32	27	21	27	21	19
T <sub>4</sub> - Soil application with <i>Simarouba</i> seed oil @ 2.0 l/ha	230.29 (15.23)	201.30 (14.21)	188.69 (13.78)	40	32	29	36	29	25
T <sub>5</sub> - Soil application with <i>Simarouba</i> oil cake @ 5 kg/ha	170.50 (13.10)	163.89 (12.78)	152.35 (12.34)	26	23	20	21	19	18
T <sub>6</sub> - Soil application with <i>Simarouba</i> oil cake @ 10 kg/plant	132.73 (11.56)	125.34 (11.17)	123.65 (11.15)	29	20	17	25	18	13
T <sub>7</sub> - Carbofuran 3G @ 3 g/plant	146.54 (12.09)	132.36 (11.94)	125.67 (11.22)	24	21	18	23	19	18
T <sub>8</sub> - Fluensulfone 2% GR-3 g/plant	149.61 (12.27)	137.32 (11.81)	129.45 (11.41)	27	24	19	23	20	18
Untreated control	196.35 (14.03)	198.62 (14.11)	200.53 (14.25)	29	35	39	24	25	27
CD value	0.11	0.10	0.08						

Treatments given at two intervals i.e., 15 and 45 days after planting.

Figures in parentheses are square root transformed values indicates the percent decrease in nematode population.

of eggs hatched (29.02/egg mass) and juveniles mortality (48.11/100 juveniles). Recorded the maximum number of eggs hatched (38.22/egg mass) and the lowest juveniles mortality (40.73/100 juveniles) in the 25% crude leaf extract compared to control had no juveniles mortality and the number of eggs hatched (167.21/egg mass) (Table 1).

After 72 hrs, the exposure of *S. glauca* crude leaf extract (100%) on *M. incognita* had the highest juvenile mortality rate and the lowest number of eggs hatched was 92.58/100 juveniles and 16.30/egg mass, followed by 50% crude leaf extract on juveniles mortality (62.44/100 juveniles) and the number of eggs hatched (36.54/egg mass). Recorded the lowest juveniles mortality (46.46/100 juveniles) and the maximum number of eggs hatched (42.39/egg mass) in the 25% crude leaf extract compared to control had juveniles mortality (4.01/100 juveniles) and the number of eggs hatched (286.53/egg mass). They have inhibited the egg hatching by increasing the concentration of the extracts from 25 to 100%. The present study verified the efficacy of methanolic leaf extract for *M. incognita* egg hatch inhibition and juvenile mortality (Table 1).

The phytochemical tests showed the presence of alkaloids, cardiac glycosides, flavonoids, phenols, phlobatannin, reducing sugars, saponins, steroids, tannins, terpenoids, volatile oils, carbohydrates and protein/amino acids in methanolic extract of *S. glauca* (Table 2) as described by (Robertson, 2005). The GC-MS analysis has shown the presence of different phytochemical compounds in the methanolic extract of *Simarouba glauca*. A total of 21 compounds were identified representing 84.49% of total methanolic extract composition. The GC-MS spectrum confirmed the presence of various components with different retention times of *Simarouba* leaf and seed as illustrated in Fig 1 and 2, Table 3 and 4.

Pot and field experiments revealed that *Simarouba* leaf powder applied @ 10 g/plant in pot and 5 kg/ha under field conditions showed a significant reduction in the incidence of number of female nematode, egg masses, number of galls, total soil population and root population (Table 5 and 6).

## CONCLUSION

From the results, it is evident that *Simarouba glauca* contains various phytocomponents that are having antifungal, antimicrobial, nematocidal property and can be effectively formulated and employed for integrated biotic stress management for agricultural crops. The presence of various bio-active compounds with antimicrobial properties detected after GC-MS analysis using the methanolic extract of *S. glauca* justifies the use of its leaves and seed against agricultural insects, pest and nematodes for their effective control. However, the biological activity of individual compounds against plant pathogens especially nematodes will subjected to understand their biocontrol potential. Evaluation of the nematocidal property of bioactive compounds of *Simarouba glauca* against root knot nematode, *Meloidogyne incognita* infesting groundnut

revealed that *Simarouba* leaf powder seed oil cake applied in the soil significantly reduced the soil and root population of root knot nematode in Groundnut. This study provide an insight in development and application of botanical formulations that replace the hazardous chemical compounds in the context of integrated nematode management.

### Conflict of interest

The authors are declaring that there is no conflict of interest in the publication of the paper.

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