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Chemical Composition and Evaluation of the Nematicidal Activity of *Datura metel* Seed Oil against *Meloidogyne javanica*

Médoune Mbaye[1], Cheikh Sall[1], Djibril Djigal[2], Ousmane Faye[1], Mamadou Soumboundou[1], Awa Ndong[1], Malick Ndao[1], Serigne Modou Sylla[1], Diégane Sarr[3]

[1]Chemistry Laboratory, UMRED, UFR Santé, Université Iba Der Thiam de Thiès, Thies, Senegal
[2]Nematology Laboratory, ISRA/CDH Sangalkam Research CENTER, Sangalkam, Senegal
[3]Equipe des Matériaux, Electrochimie et Photochimie Analytique, Université Alioune Diop, Bambey, Senegal
Email: cheikh.sall@univ-thies.sn

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Abstract

Today, agricultural production is threatened by crop pathogens, including plant-parasitic nematodes. Because of their harmful effects on the environment and human health, synthetic nematicides are gradually being banned in several countries. This study evaluates the nematicidal activity of *Datura metel* oil. *Datura metel* seed oil was obtained using the Soxhlet extractor in hexane. The resulting oil was characterized by determining physicochemical parameters and molecular composition using GC-MS. The nematicidal activity of the oil was assessed by determining the number of dead nematodes. Physicochemical characterization gave an acidity index of 0.3% and a peroxide index of 10 meq.O2/Kg, while GC-MS analysis identified 30 molecules made up mainly of fatty acid esters, four of which represented over 74% of the oil's total weight. The nematicidal activity of the oil, expressed in terms of mortality rate as a function of concentration, showed mortality rates of 58; 69 and 79% over 48 hours of incubation at concentrations of 25, 50 and 100 µg/mL respectively. The activity observed could be linked to the high presence of the four compounds most commonly identified in the oil. These results suggest that *Datura metel* oil could be a promising alternative to synthetic pesticides for the control of crop pests.

Keywords

Nematodes, *Meloidogyne javanica*, *Datura metel*, Bio-Pesticides

1. Introduction

Plant-parasitic nematodes are one of the major threats to agricultural production

worldwide. Among them, root-knot nematodes cause more than $157 billion in annual crop losses worldwide [1]. *Meloidogyne spp*. is considered the world's most destructive nematode. Its juvenile stage of development (J2), corresponds to its ability to move in the soil and infect plants [2]. There are some 4100 known species of plant-parasitic nematodes, which are polyphagous in nature [3]. Among them is the genus *Meloidogyne*, which comprises a total of 105 species and stands out as one of the most common plant-parasitic nematodes.

According to a recent study, four species of root-knot nematodes, namely *Meloidogyne arenaria*, *Meloidogyne incognita*, *Meloidogyne javanica and Meloidogyne hapla*, which are accustomed to tropical and temperate conditions, together contribute to more than 90% of plant damage [4]. Plant Nematodes cause substantial yield losses by diverting nutrients, disrupting water transport, increasing susceptibility to secondary infections and acting as virus vectors [5], [6]. Most of these nematodes attack the plant's root system, while others can attack aerial parts.

Meloidogynes infect monocotyledonous and dicotyledonous plant species, inducing transcriptional reprogramming in host cells, leading to the formation of giant cells. This establishes a permanent feeding site within the host plant where the nematodes obtain nutrients while completing their parasitic life cycle [7].

Controlling these plant-parasitic nematodes requires several approaches, including the use of synthetic or natural organic pesticides. Although synthetic pesticides have brought many benefits in protecting plants and improving crop yields, they have caused many problems for human health and the environment. Indeed, products such as 1,3-dichloropropene, fenamiphos and fosthiazate have been either restricted or banned from use on crops in Europe and other developed countries [8] [9].

Faced with the dangers associated with chemical pesticides, there is an urgent need to develop new, less toxic and more environmentally-friendly substitutes. Biological control methods, such as biopesticides, offer a significant and sustainable solution to this problem [10]. Biopesticides have several advantages, including limiting the use of chemical pesticides, being less toxic, reducing the risk of developing resistance, possessing greater specificity of action and not being associated with the release of greenhouse gases [11]. What's more, they improve the quality of life of farm workers, do not impose a pre-harvest delay, and provide consumers with healthier products, thus enjoying greater acceptance. Plants are an important source of biopesticides. Numerous compounds with nematicidal activity have been found in plants, including alkaloids, diterpenes, fatty acids, phenols, sesquiterpenes, isothiocyanates etc. [12]. These compounds are widely used in the current formulation of many biopesticides. According to Wiratno *et al.*, plant extracts of *Nicotiana tabacum*, *Syzygium aromaticum*, *Piper betle* and *Acorus calamus* showed EC_{50} five to ten times lower than synthetic pesticides such as chlorpyrifos, carbosulfan and deltamethrin [13]. This demonstrates their importance in nematode control.

It is in this context that we are interested in studying the nematicidal activities

of *Datura metel* oil. *Datura metel* is an herbaceous plant in the *Solanaceae* family. This plant is commonly used in Senegal and other countries of the world by traditional healers to treat various pathologies, such as asthma and bronchodilation problems [14].

Datura metel seeds are particularly popular in traditional medicine for the treatment of rheumatoid arthritis and epileptic seizures [15]. Although toxic and narcotic, *Datura metel* seeds are said to possess powerful analgesic, anthelmintic, antimicrobial and antiviral activities [14] [16].

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

Datura metel fruits were harvested in Téorou Mbaye, a village located in the Gossas department in Senegal, with the following geographical coordinates: latitude 14.48 North, longitude 16.01 West and altitude 25.00 m/82.02ft. After identification by the botanical laboratory of the Faculty of Medicine, Pharmacy and Odontology at Cheikh Anta Diop University in Dakar, the fruits were dried in the dark at room temperature for 15 days. The seeds obtained from the dried fruit are then ground using an electric grinder. The crushed seeds are stored in a refrigerator at 4˚C until use.

a. Extraction of *Datura metel* seed oil.

A 100 g mass of *Datura metel* seed powder was extracted by Soxhlet with hexane for 1 hour. The resulting mixture was then evaporated to dryness using a rotary evaporator. The resulting oil was weighed and the extraction yield calculated as a percentage (weight/weight) based on the weight of the dried seed powder.

b. Analysis of oil physicochemical parameters.

The analysis of the physicochemical parameters of the oil was carried out by determining the acidity index, peroxide, iodine, density and pH respectively described by the methods of *Boulfane et al.* [17], *Novdizro et al.* [18] and *Nasma et al.* [19].

2.1.2. Harvesting Nematodes

Plant-parasitic nematodes are uniform in appearance, invisible to the naked eye and possess hollow needles connected to a hypertrophied glandular system [20]. *Meloidogyne javanica* nematodes were extracted using the method described by Baermann [21]. It consists of extracting nematodes from infested tomato roots (Mongal variety). First, the gall roots were washed with water and then cut into small pieces about 1 cm long. Part of the resulting mixture was then sieved using 100 µm diameter sieves. The sieve was then soaked in water and incubated for a week in a petri dish. The resulting nematode solution was observed under a microscope to confirm the presence of *Meloidogyne javanica* and stored at 4˚C until use.

2.2. Methods

2.2.1. Analysis by Gas Chromatography/Mass Spectrometry

Gas chromatography (GC) (GCMS-QP2010-SE) equipped with a DB-5MS UI

column (30 m × 0.25 mm inner diameter; film thickness: 0.25 µm), used as stationary phase, and directly connected to an A0C-20I mass detector, was used to analyze the oil after the methylation phase. High-purity helium (99%) was used as carrier gas with a flow rate of 0.97 mL min^{-1} and a pressure of 70.7 kPa. A sample volume of 1 µL was injected via an autosampler in a 1:20 split ratio. The mass spectrum was acquired in full scan mode with electron ionization (EI) at 70 eV. The mass spectra bank of the Faculty of Pharmacy of the Université Paris Cité was used for compound identification by closest match of the mass fragmentation pattern.

2.2.2. Antioxidant Activity of *Datura metel* Oil

The antiradical activity of *Datura metel* oil was measured using the method described by Molyneux *et al.* (2004), described in our previous studies [22]. It involves reducing the stable, violet-colored free radical of the DPPH molecule to a yellow color visible at 517 nm.

Concretely, to a volume of 50 µL of a solution of the oil at different concentrations (6.25 - 12.5 - 25 - 50mg/mL), is added 200 µL of a DPPH solution (0.04 mg/mL). The resulting mixture is then incubated for 30 min before absorbance readings are taken at 517 nm using a spectrophotometer. Inhibition percentage (IP) is then calculated from the absorbances using the formula below (**Equation (1)**).

$$PI = \frac{A0 - Ai}{A0} \times 100 \quad (1)$$

$A0$ = absorbance of DPPH; Ai = absorbance after addition of extract.

Ascorbic acid, used as a reference, was tested under the same conditions as the oil. Three measurements were taken for each concentration of product tested.

2.2.3. *In vitro* Nematicidal Activity of *Datura metel* Oil

The *in vitro* nematicidal activity of the oil was evaluated according to the method described by Yuji Oka *et al.* (2000) with a slight modification [23].

Three concentrations of 25, 50 and 100 µg/mL were prepared from *Datura metel* oil using a mixture of tween 40/water (0.3/9.7 w/w) as the dilution solution.

A mixture of 1 mL nematode solution and 500 µL diluted oil solution is placed in a tube. The resulting mixture is incubated at room temperature for 24 and 48 hours. The number of dead nematodes (inert for 10 s) is then quantified using an optical microscope. A negative control consisting of water was produced under the same conditions. The water mortality rate, corresponding to natural mortality, was subtracted from the oil mortality rate. All experiments were conducted in triplicate.

2.2.4. *In vivo* Nematicidal Activity of *Datura metel* Oil

Sand was extracted from a depth of 50 cm, sterilized to prepare 50 pots for Mongal tomato plants. Each pot was filled with 5 kg of sand, then sprinkled with 500 mL of water. After 24 hours, 2 tomato plants per pot are transplanted and watered with the same volume of water. After 5 days of growth, the sand is enriched by

adding 0.6 g of urea and 0.7 g of NKP fertilizer (10-10-20) for each pot of tomatoes. This last operation is repeated every 10 days for 1 month.

After one month's growth, tomato roots are infected with 15 mL of the previously prepared nematode solution and immediately treated with 500 µL of the oil solution at concentrations of 50 and 100 µg/mL. The treated pots are then incubated for 15 days before the roots are analyzed under a magnifying glass to determine the nematode mortality rate using the following formula described by *Ghareeb et al.* (**Equation (2)**) [24].

$$\%\ \text{of mortality} = \frac{\text{Treatement mortality} - \text{Control mortality}}{\text{Control mortality}} \times 100 \qquad (2)$$

2.3. Statistical Analysis of Data S

Collected data were entered into Excel to design tables and graphs for analysis. Statistical analysis was performed using ANOVA variance ($p < 0.05$).

3. Results and Discussion

3.1. Physicochemical Parameters of *Datura metel* Oil

The physicochemical parameters of *Datura metel* oil are given in Table 1.

Table 1. Physicochemical parameters of *Datura metel* oil.

Physicochemical parameters	Results
Acid index	0.3%
Peroxyde index	10 meq.O2/Kg
Iodine index	57.105 mg I2 100/L
Density	0.935
Ph	5

Extraction of *Datura metel* seed powder using a Soxhlet in hexane allowed to obtain an oil with 15% yield. The oil yield extract may depend on a number of factors, including soil and climatic conditions, the nature of the solvent and the extraction time [25]. However, oil extraction using hexane and Soxhlet gave yields of 29; 22 and 20% from seeds of white mustard, caraway and coriander respectively [26]. The physicochemical parameters described in Table 1 show that *Datura metel* seed oil is a good quality, stable and slightly acidic oil (pH = 5). In fact, this oil has acidity and peroxide values of 0.3% and 10 meq.O2/Kg respectively, slightly similar to those of olive oil, often used as a reference oil [27]. This means that the oil has not undergone significant oxidation and that it is suitable and has a shelf life. The peroxide value confirms that the oil is extra virgin.

The iodine value of *Datura metel* oil is 57.105 mg I2/100g, placing it among the non-drying oils for which the iodine value is between 0 and 110 mg I2/100g oil [18].

3.2. Chemical Composition of *Datura metel* Oil

Determination of the chemical composition of *Datura metel* seed oil by gas chromatography-mass spectrometry (GC-MS) revealed the presence of 30 molecules in the oil. These molecules are listed in Table 2 below, along with their retention times and weight percentages. Among these compounds, the most significant was detected after 17.93, 21.335, 21.487 and 21.734 minutes of retention, corresponding to at least 74% of the oil weight. These compounds, known respectively as Eicosanoïc acid, methyl ester; Methyl-10-trans-12-cis-Octadecadienoate; 9-octadecenoic, methyl ester (E) and Methyl stearate, are fatty acid esters. The measured iodine unsaturated fatty acids are Methyl-10-trans-12-cis-octadecadienoate; 9-octadecenoic, methyl ester (E) and 9,12-octadecandienoic acid (Z,Z)-2-hydroxy-1-(hydroxymethyl) ethyl ester with 22.4; 23.04 and 3.24%, respectively, of total oil weight. *Datura metel* oil also contains small quantities of steroids: Stigmata-5,24-(28)-dien-3-ol, (3-beta-24E); Ergost-5-en-3-ol, (3-beta.); Stigmasterol; Stigmasta-5,24 (28)-dien-3-ol, (3.beta-24Z) but also other polycyclic fatty acid esters such as: Olean-12-en-3-ol, acetate, (3-beta.) and Olean-12-en-3-ol, acetate, (3-beta.). These results show the heterogeneity of molecules present in *Datura metel* seed oil.

Table 2. Chemical composition of *Datura metel* seed oil.

N°	Compounds	R. Time	Area %	heigh %
1	Methyl tetradecanoate	13.64	0.38	1.62
2	1-Hexadecanol	16.87	0.32	0.86
3	9-Hexadecanol acid methyl ester (Z)	17.30	0.72	2.54
4	Eicosanoic acid, methyl ester	17.93	14.98	16.71
5	Methyl-10-trans-12-cis-octadecadienoate	21.33	47.05	22.14
6	9-Octadecanoic acid, methyl ester (E)	21.48	15.20	23.04
7	Methyl stearate	21.73	5.96	11.77
8	ND	23.15	0.19	3.73
9	ND	23.38	0.62	4.95
10	Methyl-9.cis,11.trans,13.trans-octadecatrier	24.26	0.34	0.51
11	Cis-11-Eicosanoic acid, methyl ester	24.59	0.46	0.90
12	Methyl18-methylnonadecanoate	25.06	0.75	2.51
13	ND	26.11	0.34	0.53
14	ND	26.77	0.21	0.18
15	ND	26.95	0.38	0.84
16	Octadecanoic acid,9,10-dihydroxymethyl ester	27.43	0.20	0.20
17	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)	27.98	0.31	0.59
18	Docosanoic acid, methyl ester	28.31	0.38	1.24
19	9,12-Octadecandienoic acid(Z,Z)-2-hydroxy	30.71	3.50	3.24

Continued

20	Tetracosanoic acid, methyl ester	31.32	0.36	1.11
21	Stigma-5,24(28)-dien-3-ol, (3 beta)	38.77	0.52	0.82
22	Ergost-5-en-3-ol, (3 beta)	38.97	0.29	0.40
23	Stigmasterol	39.56	0.19	0.30
24	Gamma-sitosterol	41.04	0.47	0.66
25	Stigmasta-5,24(28)-dien-3-ol, (3 beta, 24Z)	41.44	0.32	0.44
26	Beta-amyrin	41.92	0.24	0.26
27	ND	44.55	0.27	0.27
28	Olean-12-en-3-ol, acetate, (3 beta)	48.28	2.83	2.08
29	9-Octadecanoic acid (Z)-Octadecy esterl	50.13	0.91	0.78

R. Time: retention time; ND: Not determined.

3.3. Anti-Free Radical Activity of *Datura metel* Seed Oil

The results of free radical scavenging activity by DPPH radical reduction are given in Table 3 below. Inhibitory concentrations (IC_{50}) are calculated from linear regression equations resulting from inhibition percentages as a function of oil concentration. The IC_{50} of the oil indicates a low antiradical activity (222.31 mg/mL) compared with that of ascorbic acid, used as a reference (0.022 mg/mL).

Table 3. Anti-free radical activity of *Datura metel* oil.

Inhibitory concentration	*Datura metel* oil	Ascorbic acid
IC_{50} (mg/mL)	222.317	0.022

Although this activity is low compared to ascorbic acid, it proves more interesting than some essential oils tested on the DPPH radical. Indeed, studies by Rachid *et al.* (2017), *Samah et al.* (2015) gave antioxidant activities of essential oils ranging from 1000 to 2260 mg/mL [28] [29]. Natural antioxidants are currently the subject of numerous studies in the preservation of food and phytosanitary products. They are thought to play an important role in the treatment of diseases in which oxidative stress is involved [28].

3.4. *In vitro* Nematicidal Activity of *Datura metel* Oil

In vitro results for nematicidal activity are expressed as mortality rates as a function of oil concentration (Figure 1). After 24 hours, the oil showed mortality rates of 11%, 46% and 47% at concentrations of 25, 50 and 100 µg/mL respectively. These mortality rates are more significant after 48 hours of incubation.

An *in vitro* study of the nematicidal activity of *Datura metel* oil showed significant toxicity to *Meloidogyne javanica* nematodes at the J2 stage. The oil caused 11, 46 and 47% mortality within 24 hours, and 58, 69 and 79% mortality within 48 hours of incubation with oil concentrations of 25, 50 and 100 µg/mL respectively.

This highly significant mortality is dose-dependent and could be linked to the high content of eicosanoic acid, methyl ester; methyl (10*E*,12*Z*)-Octadecadienoate; methyl ester 9-octadecenoic acid and methyl (E)-stearate, which together account for over 74% of the total oil's weight. These fatty acid esters have also shown significant nematicidal activity against *Meloidogyne incognita* on bananas [30]. Other saturated fatty acid esters such as methyl pelargonate and ethylene glycol pelargonate caused mortality rates on *Meloidogyne incognita* of up to over 90% [31]. Further work by *Ramadan et al.* on four commercial oils obtained from *Cytrullus colocynthis*, *Simmondsia chinensis*, *Moringa oleifera* seeds and marjoram oil showed interesting activities in inhibiting egg hatching and high mortality of Meloidogyne incognita. The longer the incubation period, the greater the effect [32].

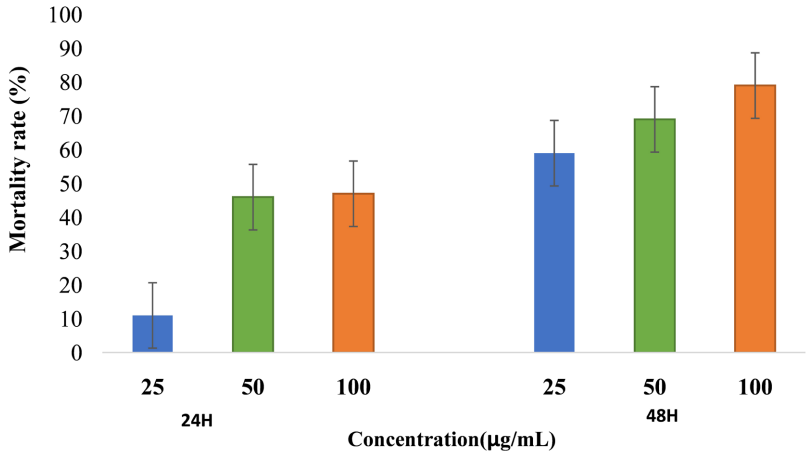

Figure 1. *In vitro* nematicidal activity of *Datura metel* oil.

Statistical analysis showed that concentration of oil had a significant effect on nematode mortality. Indeed, the degrees of freedom are 8 and 3; the f-values are 6.1729 and 37.0374 and the p-values < 0.05 for oil and concentration respectively.

On the other hand, the interaction between concentration and oil had no significant effect on mortality (degree of freedom 24, F value 1.1225 and p value > 0.05).

The *in vivo* results of the nematicidal activity of *Datura metel* oil on *Meloidogyne javanica* nematodes at the J2 stage, expressed as infection rate as a function of concentration, are shown in **Figure 2**.

These results show that *Datura metel* oil also has protective activity against infection of the tomato roots studied. Indeed, after 15 days of treatment, nematode mortality remained at 54% for concentrations of 50 and 100 ug/mL, showing that this activity is not dose-dependent.

Statistical analysis by ANOVA indicates that oil has a significant effect on infection rate. Indeed, the degrees of freedom were 6 and 1, the f-values were 778.7 and 97.8, and the p-values were <0.05 for oil and concentration respectively. However, the interaction between concentration and oil had no significant effect on the infection rate (p > 0.05).

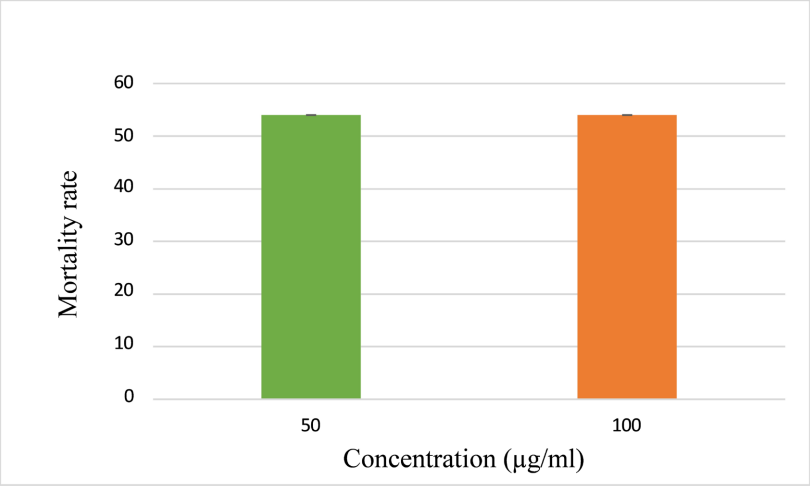

Figure 2. *In vivo* nematicidal activity of *Datura metel* oil.

Due to their complex composition, oils extracted from plants can affect several targets simultaneously, reducing the likelihood of target organisms developing resistance or adapting to the treatment. The mode of action of essential oils against nematodes is not very well defined, but in insects, several essential oils inhibit acetylcholinesterase activity and consequently affect the insect nervous system [23]. Other authors stipulate that essential oils act on nematodes via a variety of mechanisms, including disrupting their nervous system, inducing adverse effects on plasma membrane permeability, penetrating the gelatinous matrix of nematode eggs and disrupting intracellular redox status [33].

4. Conclusion

The aim of this study was to determine the chemical composition of *Datura metel* oil and evaluate its nematicidal properties on *Meloidogyne javanica*, *a* parasite of tomato plants. *Datura metel* seed oil is essentially made up of saturated and unsaturated fatty acid esters and a few steroids. The diverse composition of this oil justifies its significant nematicidal activity in *vitro* and *in vivo*, through the synergistic or antagonistic action of its component molecules. The results of this study show that *Datura metel* oil is a stable oil and would constitute an alternative to the use of synthetic pesticides in the field of crop parasite control. *Datura metel* oil, a natural biodegradable compound, is said to have minimal toxicity and environmental impact. Unlike synthetic pesticides, it poses no danger to human health. However, it would also be necessary to test the effect of this oil on the development of other crop nematodes in order to obtain a broader spectrum of action.

Conflicts of Interest

The authors declare that they have no conflict of interest in relation to this article.

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