**Evaluation of *Datura metel* leaves extracts for their antioxidant and nematicidal properties against *Meloidogyne javanica* as a potential biopesticide.**

**Abstract**

Meeting the food needs of the world's population has become a major challenge for all countries. The availability of this food depends on a number of factors, including good crop management. The latter is under attack from several plant parasites, including root-knot nematodes, which are sometimes responsible for more than 30% of crop losses. In this study, we evaluate the nematicidal properties of *Datura metel* leaf extracts.

In this study, secondary metabolites were extracted using solvents of different polarity and phytochemical characterization was carried out using qualitative determination methods. The nematicidal activity of the extracts was evaluated on *Meloidogyne javanica* nematodes at the J2 stage of larval development.

The results showed the presence of alkaloids, flavonoids, tannins, reducing compounds and saponins in *Datura metel* leaves.

All extracts caused varying degrees of mortality depending on their type, concentration and incubation time. Polar extracts were the most active in the *in vitro* study, with 77, 85 and 99% mortality after 48 hours of incubation for crude, aqueous and ethyl acetate extracts respectively. However, in the *in vivo* study, the polar aqueous extract remained the most active with a mortality rate of 63%. This was followed by the non-polar hexane extract with 61% mortality. The differences and variations observed in these results could be explained by the nature of the compounds present and their synergistic and antagonistic effects.

From these results it can be concluded that the *Datura metel* plant could be a promising alternative to synthetic pesticides for the control of plant parasites.

**Keywords**: *Datura metel*, nematodes, Meloidogyne, biopesticides.

1. **Introduction**

The world population, which was 4 billion in 1974, is now estimated by the United Nations at 8 billion. This could rise to 9.7 billion by 2050. This increase will be greatest in Africa, where, according to the United Nations, more than half of the world's demographic growth between now and 2050 will take place [1]. A number of security, economic, health and food-related challenges therefore need to be met in order to satisfy the primary needs of these populations. As far as food needs are concerned, agriculture remains one of the top priorities. Despite the limited space and arable land available for production, agriculture is under attack from harmful plant pests. These include arthropods, viruses, bacteria, fungi and nematodes [2], [3]. The latter, which affect around 3,000 plant species, contribute to global economic losses estimated at around 173 billion dollars a year [4]. Root-knot nematodes are extremely destructive, causing yield losses of up to 10-30% in various crops, thereby exacerbating food security problems [5],[4],[6].

The main methods used to control plant-parasitic nematodes are synthetic nematicides and fumigants, which are appreciated for their effectiveness and speed of application [7]. However, these products present major risks to the environment and human health [8], particularly through the presence of residues in food and their accumulation in subcutaneous fat, which can lead to serious or even fatal effects on people in contact [9]. The market for plant protection products mobilizes large sums, up to US$1 billion a year, almost half of which is spent on controlling root-knot nematodes [10].

Growing concern about the impact of these products has led, on the one hand, to a significant reduction in their use and, on the other, to a ban in some countries on the synthetic nematicides that are most toxic to the environment and humans. Hence the urgent need to develop new alternatives for the production of nematicides [11] [12] [7]. Among these alternatives, plant-based biopesticides are proving to be an effective, promising and environmentally friendly solution [13]. Biopesticides are generally considered to be non-persistent because they are easily transformed by light, oxygen and micro-organisms into less toxic products that are easier to break down [14].

The use of plant extracts as an alternative to synthetic pesticides for the control of root-knot nematodes has become a reality in several countries [15].

Numerous studies have reported that 57 plant families, including Solanaceae, may contain nematicidal compounds [16].

It is in this context that we are interested in studying the antioxidant and nematicidal activity of leaf extracts of *Datura metel*, a herbaceous plant belonging to the Solanaceae family. Several parts of this plant (leaves and seeds) are traditionally used for a variety of purposes, including psychoactive activities [17], [18]. Other authors have demonstrated that the active constituents of *Datura metel* extract include scopolamine, atropine, hyoscyamine, withanolides, tropane alkaloids… most of which are used as sedatives, antispasmodics and mydriatics [19].

1. **Materials and methods**
   1. **Materials**
      1. **Plant material**

*Datura metel* leaves were collected from Téorou Mbaye, a village in the Gossas department of Senegal, with the following geographical coordinates: latitude 14.48 north, longitude 16.01 west and altitude 25.00m/82.02ft. After identification by the botanical laboratory of the Faculty of Medicine, Pharmacy and Odontology at Cheikh Anta Diop University in Dakar, the leaves were dried in the dark at room temperature for two weeks. They are then ground using an electric grinder. The crushed material is stored in a refrigerator at 4°C until use.

* + - 1. **Extraction**

A mass of 100g of leaf powder was macerated in 170 mL of hexane for defatting for 72 hours at room temperature with stirring. The solid residue obtained after filtration was then macerated in a mixture of hydroethanolic mixture (70/30) for 48 hours. The resulting mixture was filtered and the filtrate evaporated to dryness using a vacuum rotary evaporator.

A portion of 1.5g of the hydroethanolic extract was fractionated in turn with two solvents of different polarity, first with dichloromethane and then with ethyl acetate. The following codes were used for extracts and fractions: hexane extract (E\_Hexane); crude extract (E\_crude); aqueous fraction (F\_Aq); dichloromethane fraction (F\_DCM); ethyl acetate fraction (F\_AE). The various extracts and fractions obtained are stored at 40C until use.

* + 1. **Obtaining nematodes**

Plant-parasitic nematodes are uniform in appearance, invisible to the naked eye, and have hollow needles connected to a hypertrophied glandular system [20]. *Meloidogyne javanica* nematodes were extracted using the method described by Baermann [21]. This involved extracting nematodes from infested tomato roots (Mongal variety). First, the gall roots were washed with water and then cut into small pieces about 1cm 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 stored at 4°C until use.

* 1. **Methods**
     1. **Phytochemical characterization**

Phytochemical characterization is a qualitative analysis based on precipitation and/or staining reactions. These are used to indicate the presence or absence of secondary metabolites that may be present in a plant organ.

In this study, it focused on the search for alkaloids, flavonoids, tannins, terpenoids, reducing compounds and saponins using the methods described by Rosette[22] and Daira[23] respectively.

* + 1. **Antioxidant activity by DPPH radical scavenging**

The anti-free radical activity of *Datura metel* extracts and leaf fractions was measured using the method described in our previous work [24]. DPPH is a stable free radical of purplish color with an absorbance band at 517 nm.

The ethanolic solution of DPPH˙ was prepared by dissolving 4 mg of this product in 100 mL of ethanol. The resulting solution is kept in the dark for 12h. Then, to 50 μL of each extract solution at different concentrations (0.0625 - 0.125 - 0.25 -0.5 -1 mg/mL), 200 μL of DPPH solution were added. The resulting mixture was incubated for 30 min, followed by absorbance readings at 517 nm using a spectrophotometer.

The ascorbic acid used as a reference was prepared under the same conditions as the extracts. Three measurements were taken for each concentration tested. Absorbances were used to calculate the inhibition percentages (IP) according to the formula below.

*A0: absorbance of DPPH; Ai: absorbance after addition of extract*.

* + 1. ***In vitro* nematicidal activity of extracts**

The nematicidal activity of the extracts was evaluated according to the method described by Yuji Oka[25]*,* with a slight modification. This method consists of several steps.

Three concentrations of 25, 50 and 100 µg/mL were prepared from *Datura metel* extracts, using a mixture of tween 40/water (0.3/9.7 v/v) as the diluting solution. In a tube, a mixture is made up of 1 mL of the nematode solution previously prepared and 500 µL of the diluted extract solutions. The resulting mixture is incubated at room temperature for 24 and 48 hours. The number of dead nematodes (immobile for 10 s) is then quantified using an optical microscope. A negative control consisting of water was carried out under the same conditions. The water mortality rate, corresponding to natural mortality, was subtracted from the extract mortality rate.

* + 1. ***In vivo* nematicidal activity of extracts**

This *in vivo* activity of extracts on nematodes was carried out according to the method described by Yuji Oka[26],with a slight modification by following the steps below:

Sand was extracted from a depth of 50 cm to prepare 50 pots for Mongal tomato plants. The sand obtained was sterilized in an oven at 100°C before each 5 kg pot was filled with sterilized sand and then watered with 500 mL of water. After 24 hours, 2 tomato plants per pot were 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 l5 mL of the previously prepared nematode solution and immediately treated with 500 µL of the solution of each extract 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[27].

* + 1. **Statistical analysis of data**

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

1. **RESULTS AND DISCUSSION**
   1. **Phytochemical characterization**

The results of phytochemical characterization tests carried out on the various extracts are given in Table 1 below.

# Table 1: Chemical groups identified in *Datura metel* leaf extracts

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Alkaloids** | **Flavonoids** | **Tannins** | **Saponins** | **Reducing**  **compounds** | **Terpenoids** |
| **E. Hexane** | + | - | - | - | - | - |
| **E. Hydro** | + | + | + | + | + | - |
| **F. Ac Eth** | + | + | + | - | - | - |
| **F. DCM** | + | - | - | - | - | - |
| **F. Aqueous** | + | + | + | + | + | - |
| **Present (+)** ; |  | **Absent (-)** |  |  |  |  |

The results of phytochemical characterization show that alkaloids are present in all extracts, while flavonoids and tannins are absent in the nonpolar extracts (hexane and dichloromethane). Saponins and reducing compounds are present in the two most polar extracts (aqueous and hydroalcoholic). We note the total absence of terpenoids in all extracts. These results are in agreement with the work ofOkwu[28]**,** who obtained almost similar results apart from the presence of saponins in the ethyl acetate fraction. On the other hand, Ali Jaber and Moustapha confirmed the presence of alkaloids, tannins, flavonoids and saponins in *Datura metel* leaves [29],[30].

* 1. **Antioxidant activity**

Results for antioxidant activity by DPPH radical reduction are expressed as a percentage of inhibition as a function of extract concentration.



*Linéaire (Raw Extract)*

*Linéaire (F. Dichloro)*

*Linéaire (F. Ethyl acetate)*

*Linéaire (F. Aqueous)*

**Concentration (mg/mL)**

1,2

1

0,8

0,6

0,4

0,2

0

5

0

**y = 4,1529x + 10,409 R² = 0,8749**

10

**y = 7,4359x + 7,8146 R² = 0,7607**

20

15

**y = 10,212x + 10,259 R² = 0,9854**

30

25

**y = 25,382x + 12,195 R² = 0,9819**

40

35

**Inhibition percentage**

**Figure 1:** Percentage inhibition curve as a function of extract concentration.

Inhibitory concentrations (IC50) were calculated from linear regression equations resulting from inhibition percentages as a function of extract and ascorbic acid concentration. The results obtained are given in Table 2.

**Table 2**: **Inhibitory concentration of extracts and vitamin C**

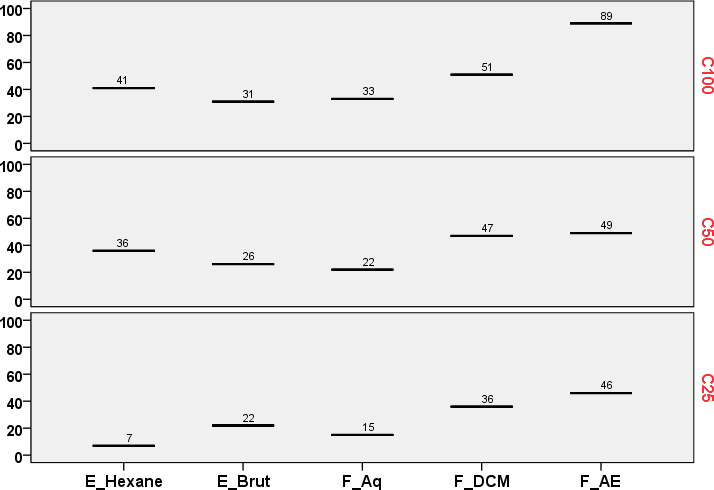
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **F A. Eth** | **F Aqueous** | **E raw** | **F DCM** | **Vitamin C** |
| **IC50 (mg/mL)** | 1,49 | 3,89 | 5,69 | 9,53 | 0,022 |

Antioxidants are chemical compounds capable of scavenging free radicals and help prevent damage at cellular level and thus protect against a several diseases [31]. *In vitro* determination of the antioxidant activity of *Datura metel* leaf extracts by DPPH radical reduction showed that the ethyl acetate and aqueous fractions are the most active, with IC50 values of 1, 49 and 3.89 mg/mL respectively. But they are less active than ascorbic acid (IC50 = 0.022mg/mL) used as a reference. These activities are more significant than those obtained in the work of Diallo et *al* [32] and Jithu[33], who showed antioxidant

of *Datura metel* leaf extracts of 8.2 and 15 mg/mL respectively. This higher antioxidant activity of the ethyl acetate fraction could be linked to the presence of total polyphenols such as flavonoids and tannins, which are more significantly present in this fraction than in the other extracts. Indeed, these compounds carrying hydroxy groups can easily give up an electron or proton to neutralize free radicals. Fatima[34] and Belayneh [35] reported that the hydromethanolic extract of roots and ethyl acetate extract of the stem of *Datura metel* have better antioxidant activity, with respective IC50 of 13.47 and 19.34 µg/mL compared to the ethyl acetate fraction of the leaves.

* 1. **In vitro nematicidal activity of extracts**

*In vitro* nematicidal activity results for *Datura metel* leaves are expressed as mortality rates as a function of extract concentrations (Figures 2 and 3).

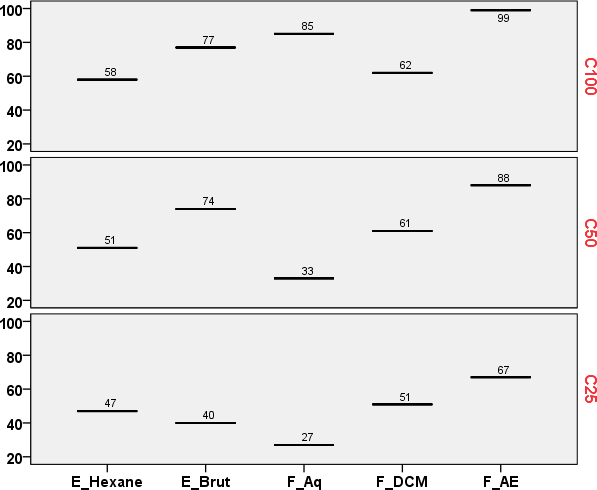


Concentration C (µg/mL)

Mortality rate (%)

**Figure 2:** *In vitro* nematicidal activity of *Datura metel* leaf extracts in 24h

Mortality rate (%)



Concentration C (µg/mL)

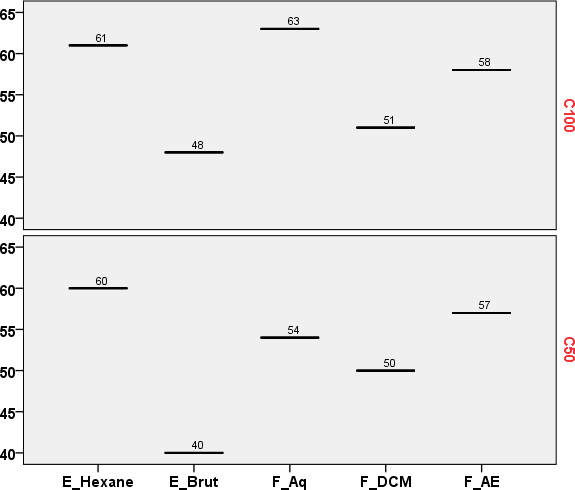
**Figure 3:** *In vitro* nematicidal activity of *Datura metel* leaf extracts in 48h

The results of *in vitro* nematicidal activity against *Meloidogyne javanica* J2 stage parasites after 24h incubation (fig 2) showed that the ethyl acetate fraction exhibited the highest activity at all the concentrations studied. This increased from 46, 49 and 89% nematode mortality at concentrations of 25, 50 and 100 ug/mL respectively. It is followed by the dichloromethane fraction with a maximum mortality of 51% at 100ug/mL. The aqueous fraction and the crude extract have fairly similar activities, with mortalities ranging from 15 to 33%. We also note that the activity was dose-dependent, the higher the concentration, the higher the mortality rate. The same trends were observed if the incubation time was extended to 48 hours (fig. 3). In this experiment, the ethyl acetate fraction remains the most active, with 99% mortality at 100ug/mL. Here, however, the crude extract and aqueous fraction became more active than the dichloromethane fraction, with mortality rates of 85 and 77% respectively, versus 62 for dichloromethane. These results show that *Datura metel* leaf extracts are toxic against *Meloidogyne javanica*. This toxicity depends on the nature and concentration of the extract, but also on the incubation time. On the whole, polar extracts remain the most active and this could be linked to the polyphenolic compounds strongly present. The toxicity of *Datura metel* leaves has been confirmed by Moustapha et *al*, who showed that the total extract caused over 83% mortality after 48h incubation, in larvae of *Artemia salina*, a saltwater crustacean [30]. In their studies, Sharma et al showed that alkaloids such as atropine and scopolamine present in *Datura stramonium* leaves were partly responsible for the observed activities. These are thought to possess anticholinergic activities and consequently influence the synthesis of the neurotransmitter acetylcholine [31]. Nandakumar showed that aqueous leaf extract of *Datura metel* caused 97-100% mortality of *Meloidogyne incognita* after 48 and 72 hours incubation respectively [36].

In this study, statistical analyses by *in vitro* ANOVA concerning extracts, increasing exposure time, increasing concentration and their interaction, showed that concentration and extract nature have a significant effect on nematode mortality rate with a p <0.0001. But the interaction between concentration and nature of extract was not significant on mortality rate (p˃0, 05).

* 1. ***In vivo* nematicidal activity of extracts**

*In vivo* results on nematicidal activity, expressed as mortality rates as a function of extract concentration, are given in the following figure 4.



Concentration C (µg/mL)

Mortality rate (%)

**Figure 4:** *In vivo* nematicidal activity of Datura metel leaf extracts over 15 days.

The *in vivo* nematicidal activity of *Datura metel* leaf extracts were carried out on tomato plants (Mongol variety) whose roots were infested with a solution of *Meloidogyne javanica* at the J2 stage. The results of spraying the gall roots with the extracts after 15 days showed that the aqueous fraction was the most active, with a mortality rate of 63% at 100ug/mL. This was followed by the hexane extract and the ethyl acetate fraction with 61 and 58% mortality respectively. However, we note that at 50ug/mL, the hexane extract is more active, with 60% mortality. These effects are linked to the toxicity of the extracts, the synergistic effects of the compounds present and the incubation time. The high toxicity of the hexanic extract may be related to the presence and content of alkaloids. The effect of alkaloids on insects has been demonstrated in the work of Butnariu[37] and Pavela[38] on *Datura metel* extracts. These effects are linked to tropane-type alkaloids (atropine, hyoscyamine and scopolamine), and these types of alkaloids are found mainly in the leaves of this plant. It is therefore highly probable that the alkaloids significantly present in the polar fractions contribute to their toxic effects. These findings are similar to those of Babaali[39], who indicated that the tropane alkaloids of *Datura metel* are responsible for the significant activity against *Meloidogyne* nematodes. Wen[40] reported that the mode of action of alkaloids is mainly that of protease inhibitors in nematodes. The results of this study showed a dose-dependent activity of the extracts on nematodes, but at more significant mortality rates than the work of Fayoum[41]***.*** They showed that petroleum ether extracts of *Neem*, *Moringa*, *Lantana* and *Licorice* leaves could induce a mortality rate of 94%, 90%, 83% and 78% respectively, of *Meloidogyne* nematodes with a concentration of 4000 ppm over 48h of treatment. The synergistic and antagonistic effects of the compounds in the extracts could explain the differences observed in their activities [42].

1. **CONCLUSION**

This study, which investigated *in vitro* and *in vivo* the effect of *Datura metel* leaf extracts on *Meloidogyne javanica*, produced interesting results. Indeed, all leaf extracts of this plant are active on *Meloidogyne javanica*, with mortality rates varying depending on the nature and concentration of the extract studied but also on the incubation time. This study therefore concluded that the *Datura metel* plant possesses powerful nematicidal activity. However, further studies will need to assess in greater detail the usefulness of *Datura metel* leaf extracts in integrated pest management programs, particularly for the control of plant- parasitic nematodes. These studies will make it possible to determine, on a larger scale, the efficacy, safety and environmental impact of using *Datura metel* leaf extracts as a nematicide.

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