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Nematicidal Activity of Aqueous Leaf Extracts of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens*

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Abstract: The present study was aimed to screen the phytochemicals and to evaluate nematicidal activity of leaf of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens*. Phytoconstituents such as alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, tannins, cardiac glycosides, anthroquinone glycosides, saponins and triterpenes were analyzed by qualitatively in aqueous leaf extracts of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens*. The extracts were showed positive results for phytocompounds like alkaloids, steroids, flavonoids, terpenoids, phenolic compounds, tannins, saponins and triterpenes in leaf of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens.* The cardiac glycosides and anthroquinone glycosides were absent in all three leaf extracts. The nematicidal potential of aqueous extracts of leaf of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* against the most devastating root-knot nematode *Meloidogyne incognita* was studied. The leaf samples were subjected to nematicidal activity at different concentrations like 0.5%, 1%, 1.5% and 2%. The *in vitro* nematicidal activity showed that the aqueous leaf extract of *Brugmansia suaveolens* possessed maximum mortality on second stage juveniles of *Meloidogyne incognita* when compared with *Datura metel* and *Datura innoxia*. The concentrations of extract at 1.5% and 2% were found more effective against nematodes when compared to 0.5% and 1%. The mortality rate was also increased with increasing exposure time with leaf extracts. From these results, this study concluded that the nematicidal activity may be due to the presence of phytocompounds in leaf of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens.* This information would be useful for further characterization and purification of individual nematicidal compounds from these plants and it may also be helpful to find new bionematicidal compounds.

Keywords: *Datura metel*, *Datura innoxia*, *Brugmansia suaveolens*, Phytochemicals, *Meloidogyne incognita*

1. Introduction

Plants have limitless ability to synthesize aromatic substances, mainly secondary metabolites such as alkaloids, tannins, saponins, flavonoids and phenolics, which play defensive role in plants and therefore they protect the plants from their invaders like fungi, bacteria, viruses and nematodes [1]. Agricultural countries study the agricultural productivity, which is appropriately protected from pests and diseases caused by insects, nematodes, fungi, viruses and bacteria [2]. Plant-parasitic nematodes are the major pests in many countries, particularly in tropics and subtropics, where they are recognized as the cause of serious yield losses on the wide range of crops [3]. Global crop loss caused by plant parasitic nematodes is estimated to be more than $100 billion annually [4]. *Meloidogyne* spp., the root-knot nematodes are the most damaging nematodes in agriculture [5]. The most destructive species of root-knot nematodes is *Meloidogyne incognita*, which causes serious problems to a number of economically important agriculture and green house crops [6, 7].

The nematodes may be controlled by cultural practices, chemical nematicides and the use of resistant cultivars. Although, the chemical nematicides hold major promise in the nematode control system but high costs, non-availability at the time of need and the hazards they pose as environmental pollutants discourage the most potential users

[8]. So, there is an urgent need for alternative nematode control measures. The pesticides of natural origin are relatively safer but equally effective compared with their chemically synthesized predecessors [9]. The plant extracts are easily degraded, pollution free, leave no harmful residues, are cheaper and not toxic to host plants and humans [10]. In recent years, the use of plant materials and animal manures are in the forefront of nematode control research [11]. Different plant parts have been tested to identify the sources of nematicidal substances. More attention is being given to natural nematicides from plant extracts, because of these are more environmental friendly. Several plants have more environmentally and toxicologically safe, selective and efficacious nematicidal potential [12].

These botanicals not only control nematodes but also improve soil productivity and crop yield by several folds. The botanical extracts that contain alkaloids and flavonoides were found to have ovicidal property against *Meloidogyne* eggs [13]. The impact of aqueous plant extracts on plant parasitic nematodes has been reported by several researchers [14, 15]. Plant extracts of *Tagetes erecta* has nematicidal effect on root-knot nematodes [16-19] and the extract of *Origanum majarana* containing substances reduce the nematode populations [20, 21]. Some of the plant species and their parts antagonistic to *Meloidogyne* spp. [22]. The effect of *Azadirachta indica*, *Calotropis procera* and *Datura alba* on larval mortality of citrus nematode (*Tylenchulus semipenetrans*) [23]. *Calotropis procera*, *Datura stramonium* and *Tagetes erecta* are commonly found in many countries and they have been reported to possess nematicidal properties [24].

Datura and *Brugmansia* are closely related genera because the *Brugmansia suaveolens* was under the *Datura* genera and then separated into different genera as *Brugmansia* that produce tropane alkaloids [25]. *Datura metel, Datura innoxia and Brugmansia suaveolens* belongs to the family of Solanaceae and they are distributed in worldwide [26]. There are many studies on *Datura metel, Datura innoxia and Brugmansia suaveolens,* but there is no study on controlling nematodes of crop plants. The use of synthetic nematicidal chemicals for the management of nematodes is an expensive and highly toxic. Due to the above facts, there is a need for the search of cheaper and less toxic alternative control measure on nematodes of crop plants. So, the above mentioned reasons the present study was aimed to evaluate the nematicidal activity of leaf of selected plants *Datura metel, Datura innoxia and Brugmansia suaveolens* against root-knot nematode *Meloidogyne incognita*.

2. Materials and Methods

2.1. Collection and Preparation of Plant Materials

Fresh plant leaves of *Datura metel* and *Datura innoxia* were collected from Orathanadu Village, Thanjavur District, Tamilnadu, India and *Brugmansia suaveolens* was collected from Ooty, Nilgiris District, Tamilnadu, India. The collected plants were identified by Rev. S. John Britto, Director, Rabinet Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India and deposited in the herbarium (Voucher specimen number ANK 001, ANK 002 and JN 001). The leaves were washed thoroughly in running tap water and then finally washed with distilled water. The plant materials were dried under shade and then ground well into fine powder. The powdered materials were stored in air-tight containers at 4°C until the time of use.

2.2. Preparation of Aqueous Extract for Phytochemical Screening

50g of leaf powder of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* were soaked in 500ml of water separately and then kept in orbital shaker for 48hrs at room temperature. After 48hrs, the mixture was filtered through a clean muslin cloth. The filtrate again filtered by using Whatmann No.1 filter paper and then the extracts were concentrated and dried in a rotary evaporator at 37°C till a sticky mass was obtained. After evaporation, the dried extracts were stored at 4°C until further use [27].

2.3. Phytochemical Analysis

Phytochemical tests for the screening and identification of bioactive constituents in leaf extracts of *Datura metel, Datura innoxia and Brugmansia suaveolens* were carried out by using the standard procedures [28-30].

2.4. Preparation of Aqueous Extract for Nematicidal Activity

2g of leaf powder of plants were soaked in 100ml of distilled water for 72 hours at room temperature. The extract was filtered through Whatmann No.1 filter paper and centrifuged at 2000g for 5 minutes. The supernatant was collected and stored at 4°C prior to use.

2.5. Assay of Nematicidal Activity

Meloidogyne incognita juveniles were collected from infested banana roots under field condition at Experimental Farm of ICAR-National Research Centre for Banana and identified by Dr. P. Sundararaju, Principal Scientist, ICAR-National Research Centre for Banana, Tiruchirappalli, Tamilnadu, India. From this, the 2^{nd} stage larval suspension in distilled water prepared for bioassay. The nematicidal activity was carried out using 6 well sterile plates and were poured of 20µl of 2^{nd} stage juveniles of *M. incognita* suspension ($50J_2$/20µl) and then added 2ml of aqueous extract of leaf of *Datura metel, Datura innoxia* and *Brugmansia suaveolens* separately at different concentrations like 0.5%, 1.0%, 1.5% and 2.0% and the 2ml of distilled water alone without extracts served as control and then incubated at room temperature. Each treatment was replicated three times. Mortality of 2^{nd} stage juveniles of *M. incognita* was recorded after 24, 48 and 72hrs. Mortality was confirmed by touching larva with a fine needle for the

determination of movements [31].

2.6. Statistical Analysis

The results of this study were subjected to statistical analysis and the results were expressed as mean percentage ± standard deviation of three replicates. Mortality percent value of nematicidal activity was calculated and significancy was determined by using Duncan's Multiple Range Test (DMRT) at 5% level ($P \leq 0.05$).

3. Results and Discussion

In traditional medicinal practices, many plant extracts are used as medicine. Based on the literature survey the medicinal plants *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* were selected for the present study. The screening of phytochemicals and nematicidal activity of aqueous leaf extract of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* against *Meloidogyne incognita* were carried out. The results of qualitative analysis of phytochemicals of aqueous leaf extract of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* were reported in Table 1. The secondary metabolites alkaloids, steroids, terpenoids, triterpenes, saponins, total phenolics, tannins and flavonoids were present and cardiac glycosides and anthroquinone glycosides were absent in leaf of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens*. Phenolic compounds are characterized by the presence of several phenol groups and act as chemical barriers against invading pathogens and thus protecting the plant species [32]. Alkaloids are involved in protective function and used as medicine especially the steroidal alkaloids [33].

Similarly, the researchers reported that the medicinal plant *Brugmansia arborea* contain three tropane alkaloids with a significant spasmolytic activity have been isolated and characterized [34-35]. The leaves of *Datura innoxia*, *Calotropis procera* and *Eichhornia crassipes* are used in traditional medicines for their bioactive compounds potential and used to cure the bacterial infectious disease [36]. The leaves of *Datura stramonium*, bark of *Terminalia arjuna* and root of *Withania somnifera* are used for their therapeutic potentials in traditional medicine. These plants are potential sources of serotonin, histamine and prostaglandins, which are used as polyherbal formulation with significant anti inflammatory and analgesic activities [37].

Table 1. *Phytochemical analysis of aqueous leaf extracts of Datura metel, Datura innoxia and Brugmansia suaveolens.*

Name of the phytocompounds	Aqueous leaf extract		
	Datura metel	***Datura innoxia***	***Brugmansia suaveolens***
Alkaloids	+	+	+
Steroids	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Phenolic compounds	+	+	+
Tannins	+	+	+
Cardiac glycosides	–	–	–
Anthroquinone glycosides	–	–	–
Saponins	+	+	+
Triterpenes	+	+	+

+ Present:- Absent

Table 2. *Nematicidal activity of aqueous leaf extract of Datura metel.*

Concentration of aqueous leaf extract (%)	Nematicidal activity (%)		
	24hrs	**48hrs**	**72hrs**
0.5	66.00 ± 6.00^{g}	72.00 ± 5.29^{fg}	86.66 ± 6.11^{cd}
1.0	72.00 ± 3.46^{fg}	78.00 ± 2.00^{ef}	93.33 ± 6.42^{abc}
1.5	83.33 ± 4.61^{de}	89.33 ± 3.05^{cd}	100.00 ± 0.00^{a}
2.0	92.66 ± 3.05^{bc}	97.33 ± 1.15^{ab}	100.00 ± 0.00^{a}
Control	2.00 ± 2.00^{i}	4.00 ± 0.00^{hi}	8.66 ± 3.05^{h}

Values are expressed as mean ± SD of three replicates
Mean values with common letter are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test (DMRT)

The aqueous leaf extracts of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* were subjected to nematicidal activity against the mortality of young 2nd stage juveniles of root-knot nematode, *Meloidogyne incognita* under laboratory condition. The nematode mortality was recorded at different concentrations such as 0.5%, 1.0%, 1.5% and 2.0% of aqueous leaf extracts of *Datura metel, Datura innoxia* and *Brugmansia suaveolens* when exposed to different time intervals like 24, 48 and 72hrs. It is seen from the data presented in Tables 2 to 4 revealed that all three tested leaf extracts were showed nematicidal activity against root-knot nematode, *Meloidogyne incognita.* The nematicidal activity of aqueous leaf extracts of *Datura metel* was reported in Table 2. The 100% per cent mortality of *Meloidogyne incognita* was recorded in leaf extract of *Datura metel* at 1.5% and 2% concentrations when exposed to 72hrs.

The aqueous leaf extract of *Datura innoxia* showed nematicidal activity and the results were presented in Table 3.

The 100% mortality of *Meloidogyne incognita* was recorded at 0.5%, 1.0%, 1.5% and 2.0% concentrations when exposed for 72hrs in aqueous leaf extracts of *Datura innoxia* except 2.0% concentration which exhibited 100% mortality at 48hrs of exposure time.

Table 3. *Nematicidal activity of aqueous leaf extract of Datura innoxia.*

Concentration of aqueous leaf extract (%)	Nematicidal activity (%)		
	24hrs	48hrs	72hrs
0.5	74.00 ± 3.464 ^{e}	85.33 ± 5.033 ^{c}	100.00 ±0.000 ^{a}
1.0	78.67 ± 2.309 ^{d}	95.33 ± 1.155 $^{a\,b}$	100.00 ±0.000 ^{a}
1.5	86.00 ± 5.292 ^{c}	98.00 ± 3.464 ^{a}	100.00 ± 0.000 ^{a}
2.0	91.33 ± 4.163 ^{b}	100.00 ± 0.000 ^{a}	-
Control	2.00 ± 2.000 ^{g}	4.00 ± 0.000 ^{g}	8.67 ± 3.055 ^{f}

Values are expressed as mean ± SD of three replicates
Mean values with common letter are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test (DMRT)

The results presented in Table 4 clearly showed that per cent mortality of *Meloidogyne incognita* at 0.5%, 1.0%, 1.5% and 2.0% concentrations of leaf extracts of *Brugmansia suaveolens* when exposed to 24, 48 and 72hrs. The 1.5% and 2.0% concentrations of aqueous leaf extracts of *Brugmansia suaveolens* showed 100% mortality against *Meloidogyne incognita* at 24hrs of exposure time. But the 0.5% and 1.0% concentrations of leaf extracts of *Brugmansia suaveolens* showed 100% mortality against *Meloidogyne incognita* at 72hrs of exposure time.

Table 4. *Nematicidal activity of aqueous leaf extract of Brugmansia suaveolens.*

Concentration of aqueous leaf extract (%)	Nematicidal activity (%)		
	24hrs	48hrs	72hrs
0.5	78.00 ±2.000 ^{d}	92.00 ±4.000 ^{c}	100.00 ±0.000 ^{a}
1.0	94.67 ±5.033 $^{b\,c}$	98.00 ±3.464 $^{a\,b}$	100.00 ±0.000 ^{a}
1.5	100.00 ±0.000 ^{a}	-	-
2.0	100.00 ±0.000 ^{a}	-	-
Control	2.00 ±2.000 ^{f}	4.00 ±0.000 ^{f}	8.67 ±3.055 ^{e}

Values are expressed as mean ± SD of three replicates
Mean values with common letter are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test (DMRT)

Figure 1a showed the live position of *Meloidogyne incognita* before aqueous leaf extract treatment and Figure 1b showed the dead position of *Meloidogyne incognita* after aqueous leaf extract treatment. So, the figures clearly showed the nematicidal activity of aqueous leaf extracts of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* on treatment against root-knot nematode *Meloidogyne incognita.*

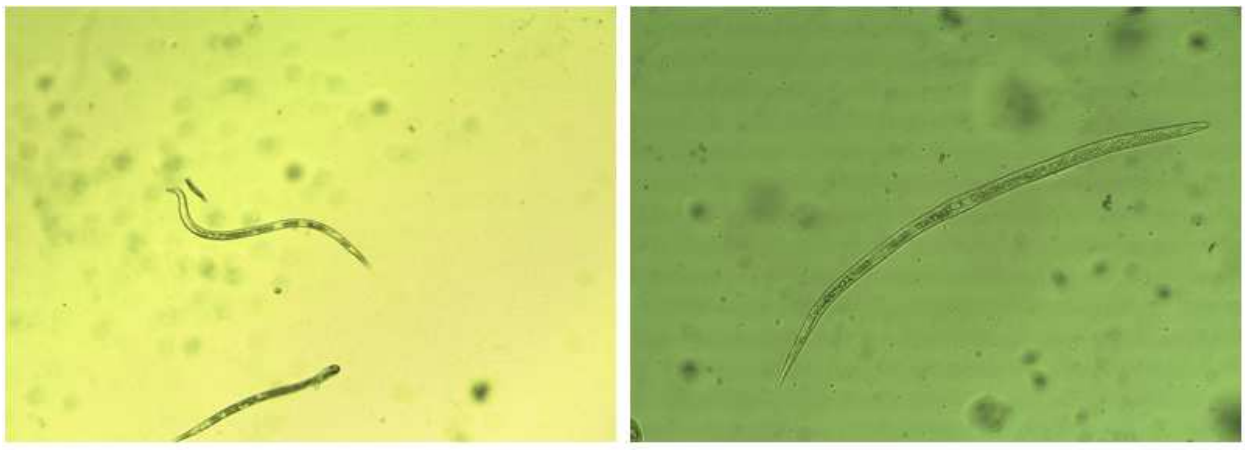

Figure 1. *Meloidogyne incognita; (a) Live position - before aqueous leaf extract treatment and (b) Dead position - after aqueous leaf extract treatment.*

In general, the mortality rate of *Meloidogyne incognita* was minimum at 24hrs in all three aqueous leaf extracts treatment when compared with 48 and 72hrs of exposure time. In this study, the analysis of variance regarding plant extracts, increased time of exposure, increased concentration and their interaction was highly significant among the different treatments. These results are in agreement with the findings of several researchers in that they reported the *Datura* possess nematode mortality and inhibition of egg hatching [38-39]. Similarly, Haseeb and Butool [40] were

suggested that exposure time and concentration of extracts can increase the larval mortality of nematode.

The results of this study are in agreement with other reports, in that the neem (*Azadirachta indica*) was certified as a bionematicide and the product of this plant Azadirachtin as a nematicide in various parts of the world [41-43]. *Meloidogyne incognita* eggs were exposed to root extracts of *Melia azadirach*, *Azadirachta indica, Ricinus communis*, *Datura alba,* Neem and Dharek exhibited 100% inhibition of egg hatching and larval mortality. In the present study, larval mortality decreased with an increase in the dilution of the extracts and reduced the exposure time. Similarly with an increase in exposure time, juvenile mortality was increased [44]. Ahmad and Alam [45] reported that the leaf extracts of *Azadirachta indica, Melia azedarach, Datura alba* and *Ricinus communis* were highly toxic to *Meloidogyne incognita.* Among these plants *Azadirachta indica* showed 100% inhibition on egg hatching and larval mortality followed by *Melia azedarach, Datura alba* and *Ricinus communis*. Inhibition of egg hatch and larval mortality were significantly affected by the concentration of aqueous leaf extract and the exposure time. Similarly in the present study, the nematode mortality was increased with the increase of the concentration of leaf extracts and exposure time. Similarly, the larval mortality was reported using *Azadirachta indica, Calotropis procera* and *Datura alba* [46] and *Allium sativum, Capsicum frutescents, Datura innoxia, and Foeniculum vulgare* [47]. Joymati *et al*. [48] reported the nematicidal effect of aqueous extracts of different parts of *Momordica dioica* on egg hatching and larval mortality of *Meloidogyne incognita.* In that study, they reported the seed extract had the greatest nematicidal activity than leaf and stem extracts.

The nematicidal activity of present findings are also in agreement with the results of Vijayalakshmi [49], who reported that aqueous extracts of neem seed, neem cake and achook by root dip treatments were effective in reducing *Meloidogyne incognita* infestation in tomato plants. The *invitro* treatments with aqueous leaf extracts of *Murraya koenigii, Jasminum sambac, Citrus aurantifolia, Rauvolfta serpentine, Zizyphus jujuba, Hisbiscus rosa-sinensis* and *Justicia gandurosa* on second stage juveniles of *Meloidogyne incognita* indicated a reduction of egg hatching and an increase on nematode mortality [50]. The present investigation is also correlated with the earlier work in that they reported the nematicidal effect of aqueous extracts of leaves of *Calotropis procera* on second stage juveniles of *Meloidogyne incognita, Meloidogyne exigua* and *Tylenchulus semipenetrans* [51]. The present study results were also corroborated with previous study, in that the ethanolic extracts of leaf of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* showed nematicidal activity against *Meloidogyne incognita* [52]. Similarly, the aqueous extracts of leaf and fruits of *Ageratum conyzoides* and *Coccinia grandis* were showed the inhibition of egg hatchability and juvenile mortality of *Meloidogyne incognita* [53]. This study was also correlated with the results of Nandi [54], in that the aqueous extracts of leaf of *Artemisia vulgaris*, *Artemisia dubia*, *Leucas cephalotes*, *Leucas aspera*, *Syzigium aromaticum*, *Pandanus unguifer* and *Agave americana*, fruit of *Terminalia chebula* and bark of *Nyctanthes arbortristis* and *Vitex negundo* were showed nematicidal properties against *Meloidogyne incognita* like inhibition of egg hatchability and juvenile mortality.

4. Conclusion

The present study concluded that the medicinal plants *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* contain phytochemicals and their leaf extracts possessed nematicidal activity against root-knot nematode, *Meloidogyne incognita.* The maximum mortality of root-knot nematode was recorded in aqueous leaf extracts of *Brugmansia suaveolens* in all tested concentrations when exposed to 24, 48 and 72hrs than the extracts of *Datura metel* and *Datura innoxia*. The present findings are important in controlling plant parasitic nematodes without use of synthetic chemical nematicides in view of the environmental pollution likely to cause. However, the present study is continued to find the active principle involved in nematicidal activity and to find the effect of these leaves on controlling nematodes when they are using as green manure. This information would be useful in agro product industry for isolation, purification and characterization of nematicidal bioactive compounds from the leaf of *Datura metel*, *Datura innoxia* and *Brugmansia suaveolens* against nematodes.

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