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DATURA METEL L. A PLANT WITH NEMATICIDAL POTENTIAL

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Nematicidal activities of *Datura metel* L. leaves extracted in various solvents were tested. A sample of total alkaloids content and a sample of hyoscine extracted from *D. metel* leaves were also assayed for their nematicidal action. *Hoplolaimus indicus* (Sher, 1963), *Helicotylenchus multicinctus* (Cobb, 1893; Golden, 1956) and *Meloidogyne incognita* (Kofoid and White 1919; Chitwood, 1949) were selected as test nematodes. It has been observed that total alkaloids showed 90-100% mortality for all the test nematodes, whereas hyoscine was effective only against *Hoplolimus indicus*, showing 90% mortality.

Key words: *Datura metel* L., Total alkaloids, Nematodes.

Introduction

Datura metel L. syn., *D. fastuosa* L. Var., *D. alba* Nees family *Solanaceae* is an important medicinal plant. In Pakistan two species of this plant grow abundantly. i.e. *D. stramonium* found in northern region is not rich in alkaloids [1] and *D. metel* grows wildly in Sindh region particularly in Karachi and is rich in alkaloids. Since no work has been reported on this species in Pakistan, preliminary studies have been carried out regarding the amount of total alkaloids as well as that of hyoscine (Scopolamine), an important active ingredient present in the leaves of this plant [2]. Hyoscine is the most important antispasmodic and analgesic used in very small dosage. It is still known as a wonder drug for its high and rapid therapeutic action. No work has been reported so far on its nematicidal properties. Singh *et al.* [3] observed anthelmintic properties of leaf, seed and flower extracts of various medicinal plants and found these extracts to be toxic to nematodes. Ellenby [4] has reported allyl isothiocyanates (Oil of *Brassica nigra*) for control of potato root eelworm.

Horn and Lamberton [5] isolated nematicidal principles of *Tagetes* roots, Wada and Munakata [6] isolated nematicidal polyacetylenes from *Carthamus tinctorius* and tested the activities of these compounds against rice white tip nematode *(Aphelenchoides besseyi)* and observed 80-90% mortality after 48 hr.

Sangwan *et al.* [7] tested nematicidal activities of essential oils of cymbopogan grasses against seed gall nematode (*Anguina tritici*), citrus nematode (*Tylenchulus semipentrans*), root knot nematode (*Meloidogyne incognita*) and cyst nematode (*Heterodera avenae*) and observed that all the essential oils and their isolates were toxic to varying extent against four nematodes tested at different concentrations.

Mangel *et al.* [8] worked on phytochemical and nematicidal properties of *Chenopodium* species. The samples were tested against seed gall nematodes (*Anguina tritici*), citrus nematodes (*Tylenchulus semipentrans*), root knot nematode (*Meloidogyne javanica*) and cereal cyst nematode (*Heterodera avenae*). All the compounds showed 80-95% mortality against all nematodes.

Nematicidal principles from neem kernel fractions were tested against *Meloidogyne incognita* by Davakumar *et al.* [9]. Effect of certain chemical extracts from plants have been studied on the mortality of root knot nematodes by Shabana and Husain [10]. Percentage mortality of nematodes was directly proportional to the concentration. Ahmad *et al.* [11] tested the effect of some indigenous plant extracts on juvenile mortality of *M. javanica*. Sasanelli and Addabbu [12] noted the effect of *Cineraria maritina, Ruta graveolens* and *Tagetes erecta* extracts on hatching of *Heterodera schachtii*. They also noted the effect of these plants on Italian population of *Meloidogyne* species [13].

Recently, some preliminary work has been started concerning the nematicidal activities of leaf extracts of various indigenous plants [14], among which *Datura metel* L. appeared promising.

Materials and Methods

Preparation of plant extract. 100 g chopped leaves were soaked separately in 500 ml solvents, i.e. petroleum ether, ethyl acetate, methanol, chloroform and ethyl alcohol for three days at room temperature. Solvents filtered and second extract was obtained after soaking plant material for another three days in the above solvents. The extracts were pooled, filtered, and the solvents removed under vacuum.

1. Ethyl alcohol extract = 6.9200g (T_1)
2. Methanol extract = 3.9405g (T_2)
3. Chloroform extract = 4.3881g (T_3)
4. Ethylacetate extract = 1.6299g (T_4)
5. Pertroleum ether extract = 1.2905g (T_5)

Preparation of total alkaloidal extract (T_6). 500g air dried (two days under shade) leaves were chopped and soaked in 3 litre ethyl alcohol for three days. Extract filtered off and material was soaked for another three days in ethyl alcohol. Pooled extracts were filtered and the solvent was removed under vacuum.

The alcoholic extract (33.862 g) was extracted with N10 hydrochloric acid. The combined clear, reddish brown solution was made alkaline with a dilute 20% solution of ammonium hydroxide (pH 8-9) and immediately extracted with successive portions of chloroform. The chloroform extracts were combined, dried with anhydrous sodium sulphate, filtered and dried under vacuum, 3.225g extract of total alkaloids was obtained (0.645% in the leaves).

TABLE 1.NEMATICIDAL ACTIVITY OF EXTRACTS/ISOLATES FROM *DATURA METEL* L. LEAVES AGAINST *HOPLOLAIMUS INDICUS* (SHER 1963).

Extracts/	Percent mortality / Concentration after 48 hr			
Isolates	0.5%	0.25%	0.125%	0.00 (Control)
T_1	28.66	20.33	10.66	2.00
T_2	75.33	78.33	6.33	1.33
T_3	51.66	50.36	45.00	3.00
T_4	53.00	50.66	40.33	4.33
T_5	60.33	60.33	20.66	2.66
T_6	90.66	90.33	80.00	1.00
T_7	90.33	80.00	70.66	1.33
T_8	100.00	100.33	80.33	2.66
T_9	-	-	-	1.00
SD	22.87	24.27	28.32	1.05

SD = Standard deviation.

TABLE 2. NEMATICIDAL ACTIVITY OF EXTRACTS/ISOLATES FROM *DATURA METEL* L. LEAVES AGAINST (*HELICOTYLENCHUS MULTICINCTUS* (COBB 1993, GOLDEN 1956).

Extracts/	Percent mortality / Concentration after 48 hr.			
Isolates	0.5%	0.25%	0.125%	0.00 (Control)
T_1	90.33	80.66	60.33	1.66
T_2	90.66	60.33	50.66	2.33
T_3	10.33	20.66	20.66	1.00
T_4	2.66	20.33	18.33	2.33
T_5	50.33	60.66	30.66	4.22
T_6	100.33	90.66	90.66	6.00
T_7	25.66	20.33	10.00	1.00
T_8	100.66	90.66	75.00	2.00
T_9	-	-	-	3.00
SD	38.98	29.24	27.35	82.42

SD = Standard deviation.

Isolation of hyoscine from total alkaloids (T_7). The alkaloidal extract (T_6) was dissolved in chloroform 20 cc citrate buffer pH (6.0) was added, mixed with 2.0g Kieselguhr and dried at room temperature. The powdered material was placed on Kieselguhr column 60 cm long having an internal diameter 2.5 cm. Kieselguhr used (40 g) was previously mixed with 20 cc citrate buffer at pH 6.0 [16]. The column was eluted with ether saturated with citrate buffer at pH 6.0. Thirty fractions, 5 cc each, were collected and afterwards last fractions were eluted with chloroform saturated with above buffer. The characterization of alkaloids in all the eluents was done by TLC and paper chromatography. Hyoscine was determined in the fractions eluted by following methods [16]:
(i) As hyoscine reineckate m.p. 171-172°C (decompn.).(ii) Quantitative estimation by titration using 0.005 N sulphuric acid and bromocresol green as indicator.(iii) By GLC on a column 3% OV 17 on gaschrom Q. Out of 3.225g of total alkaloids (T_6), the amount of hyoscine determined quantitatively was 0.005418g. The percentage of hyoscine determined from T_6 = 0.168%.

Thin layer chromatography. Ascending, 32°C, aluminium oxide (E. Merck) Solvent system: Chloroform: Diethylamine (9:1). Dectection by Munier Mcheboeuf Dragendorff reagent [13].

Hyoscine Rf = 0.80

Atropine Rf = 0.90

Paper chromatography. (i) Ascending Whatman No.1 filter paper saturated with citrate buffer pH 6.0 solvent system: Ether saturated with buffer.

Hyoscine Rf = 0.271

Atropine Rf = 0.0556

(ii) Whatman No.1 filter paper, accending solvent system: BuOH: AcOH: H_2O (4:1:5) [14].

TABLE 3.NEMATICIDAL ACTIVITY OF EXTRACTS/ISOLATES FROM DATURA METEL L. LEAVES AGAINST *MELOIDOGYNE INCOGNITA* (KOFOID & WHITE 1919, CHITWOOD 1949).

Extracts/	Percent mortality / Concentration after 48 hr			
Isolates	0.5%	0.25%	0.125%	0.00 (Control)
T_1	25.00	20.33	20.33	3.11
T_2	30.66	30.66	20.66	2.00
T_3	50.33	40.33	30.33	1.33
T_4	25.66	25.33	20.66	3.22
T_5	40.33	40.00	35.33	1.00
T_6	90.66	90.33	80.00	1.33
T_7	10.33	20.00	20.00	2.66
T_8	90.33	8.66	75.66	1.66
T_9	-	-	-	2.00
SD	25.76	26.85	23.68	0.25

SD = Standard deviation.

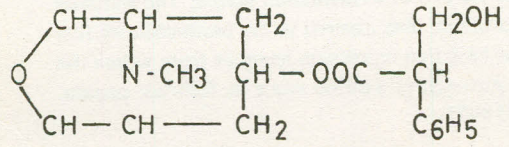
Hyoscine Rf = 0.73

Altropine Rf = 0.83

Munier and Machebocuf Dragendorff reagent has been used for location of alkaloids.

Hyoscine is a syrupy compound having $(£)^{20}_{D}$-28° (H_2O, c=2.678), $(£)^{20}_{D}$-18° (EtOH, c=2.835). When mixed with alkali, it recemises to the (t) optically inactive form. Hyoscine is soluble in chloroform and alcohol, sparingly soluble in benzene petroleum ether.

Screening for nematicidal activities. Nematicidal activities of extracts/isolates were tested against lance nematode (*Hoplolaimus indicus*), spiral nematode *(Hilicotylenchus multicinctus)* and root knot nematode (*Meloidogyne incognita*).



Hyoscine (Scopolamine, atrosine) ($C_{17}H_{21}O_4N$). Molecular weight = 303.36

Scheme 1

For *Hoplolaimus indicus* 20 mature nematodes were taken in 10 cc of distilled water in 8 cm diameter sterilized petridishes. In case of *M. incognita*, 100 larvae of the same age were taken in 5 cc of distilled water. Measured amount of stock solution was added to make the dilution 0.5, 0.25 and 0.125%. Standard nematicide Furadon was taken for comparison, only distilled water was taken as control. Each treatment was replicated thrice. The petridishes were kept at room temperature 28° ± 2°C for 48 hr. After 48 hr the number of active and inactive nematodes was counted under stereoscopic binocular microscope. Death of nematodes was confirmed by keeping them in distilled water for 24 hr. Percent mortality was calculated.

Results and Discussion

Nematicidal activities of *Datura metel* L. leaves were tested in various solvents namely ethanolic extract (T_1), methanolic extract (T_2), chloroform extract (T_3), ethyl acetate extract (T_4), petroleum ether extract (T_5), total alkaloids (T_6) and hyoscine (T_7). Conventional nematicide. Furadan (T_8) was used for comparison. Distilled water (T_9) was used as control against *Hoplolamius indicus, Helicotylenchus and Meloidogyne incognita* (Table 1-3).

It was observed that methanolic extract (T_2) of *Datura metel* L. leaves showed 75% mortality at 0.5% concentration whereas total alkaloids (T_6) and hyoscine (T_7) showed 90% mortality against *Hoplolaimus indicus* as compared to Furadan showing 100% mortality at the same concentration. In the case of *Helicotylenchus multicinctus* both T_1 and T_2 showed 90% mortality and total alkaloids (T_6) showed 100% mortality similar to Furadan.

All the extracts showed a very low order of activity against *Meloidogyne incognita* except T_6 and T_8 showing 90% mortality at 0.5% concentration. At all the concentrations all the samples showed higher mortality against the tested nematodes as compared to that observed in control (T_9) but lower activity as compared to conventional nematicide Furadan.

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