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NEMATICIDAL CONSTITUENTS IN *MUCUNA ATERRIMA* AND ITS ACTIVITY ON *MELOIDOGYNE INCOGNITA* RACE 3

by

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Summary. The nematicidal activity of roots and aerial parts of hexane, chloroform, ethyl acetate/acetone and ethanol/water crude extracts of *Mucuna aterrima* (velvetbean) was tested on *Meloidogine incognita*. The bioactive substances of these extracts were isolated and identified by fractionation in a chromatographic column, providing fractions that were purified and subjected to *in vitro* and *in vivo* tests with the nematode. Out of the twelve substances isolated, five presented activity for the nematode, HM16, ACM7, ACM63, CHR58 and ACMR17. Among these, two were identified as ACM7 and HM16, being the alcohol 1-Triacontanol ($C_{30}H_{62}O$) and the ester Triacontyl tetracosanate ($C_{54}H_{108}O_2$).

In Brazil the practice of green manuring to improve soil condition has being studied extensively. One of the plants preferred by farmers for green manuring is velvetbean *Mucuna aterrima* Piper *et* Tracy (mucuna preta) which besides enriching the soil with organic matter, has the capacity to control phytonematodes (Miyasaka *et al.*, 1983).

There is scarse information on secondary metabolites in plants of the *Mucuna* type. Its seeds are rich in the non-essential amino acid L-Dopa (L-3,4-Diidroxiphenylalanine), whose concentrations reach upto 5% of the dry weight of the seeds (Bell and Nulu, 1971). This plant is also rich in protein, with an average content of 18.5% in relation to its dry weight (Miyasaka *et al.*, 1983).

In this study the isolation and identification of nematicidal compounds responsible in part for the antagonistic effects of green manuring with velvetbean are described.

Materials and methods

Seeds were obtained from the Agriculture Institute of Campinas in São Paulo, Brazil and planted at the Coffee Farm of the Viçosa Federal University (MG), Brazil.

Plants were separated into aerial parts (leaves and stems) and roots. These were air dried at 35 °C and then powdered to produce 1.2 kg of powdered leaves and stems and 500 g of powdered roots. Both parts were successively extracted at low temperature (Costa, 1986) with hexane, chloroform, ethyl acetate/acetone 4:1 v/v and ethanol/water 4:1 v/v. The crude extracts were dried in a rotatory evaporator at low pressure.

To evaluate the nematicidal properties of the extracts, egg masses obtained from *Meloidogyne incognita* (Kofoid *et* White) Chitw., race 3 were used. The extracts were suspended in distilled water at a concentration of 1% together with two drops of Tween 20.

Three ml of crude extract were added to hatching chambers made from Petri plates with 1 mm i.d. nylon screens covered with facial tissue upon which ten egg masses were deposited.

Hatched juveniles moving through the tissue were counted at 48, 96 and 144 hours after exposure to extracts, using a Peters counting chamber. The hatching chambers were kept at 25 °C in the dark.

There were five replicates *per* treatment.

All crude extracts were submitted to silicagel (70-230 mesh) columm chromatography. Then the fractions were chromatographically collected from preparative TLC plates and reunited according to their behaviour in groups for tests with *M. incognita.*

From the hexane extract of the aerial part a white amorphous substance of melting point 71-74 °C was isolated. According to spectral data of IR (Infrared) (Nakanishi, 1982), MS (Mass Spectrometry), NMR ^{1}H (Nuclear Proton Magnetic Resonance) and NMR ^{13}C (Carbon 13 Nuclear Magnetic Resonance) (Silverstein *et al.*, 1981) this substance appeared to be a long chain ester ($C_{54}H_{108}O_2$. From the ethyl acetate/acetone extract obtained from the aerial part, a white amorphous substance of MP 76-78 °C was isolated. According to spectral data of IR, MS, NMR ^{1}H and NMR ^{13}C this substance appeared to be a long chain alcohol $C_{30}H_{62}O$.

Results and discussion

Tests with extracts from the aerial parts showed a slight reduction in numbers of living juveniles in the hexane and chloroform extracts compared to the control. These juveniles appeared to move normally. In the ethanol/water and ethyl acetate/acetone extracts reduction in numbers of living nematodes was significant. Some juveniles showed reduced movements and others were paralyzed (Table I). Similar patterns were obtained with the crude extracts obtained from the roots. In the hexane extract the hatching of juveniles was about the same as in the control. In the chloroform and ethyl acetate/acetone extracts no hatching of juveniles occurred and in the ethanol/water extract the hatching of juveniles remained low, with reduced movements of the juveniles (Table II).

A comparison of the activity of the hexane extracts obtained from the aerial parts with those obtained from the roots verified that there is no significant difference between these extracts with regard to the hatching of juveniles (Table III). Comparing nematicidal effects of the ethanol/water obtained from the aerial parts with those obtained from the roots, the root extracts were more active, even though not statistically significant (Table IV).

The extracts that appeared more active, as well as the less active extracts in relation to the phytonematode were subjected to column chromatography and then reunited according to their

TABLE I - *Numbers of juveniles of* Meloidogyne incognita *hatched in crude extracts obtained from the aerial parts of* Mucuna aterrima.

Extracts	Hours		
	48	96	144
Hexane	478	497	691
Chloroform	702	576	818
Ethyl acetate/acetone 4:1 v/v	–	230	341
Ethanol/water 4:1 v/v	663	229	180
Control	848	530	1165

TABLE II - *Numbers of juveniles of* M. incognita *hatched in crude extracts obtained from the roots of* M. aterrima.

Extracts	Hours		
	48	96	144
Hexane	597	323	1158
Chloroform	–	–	–
Ethyl acetate/acetone 4:1 v/v	–	–	–
Ethanol/water 4:1 v/v	164	43	14
Control	848	530	1165

TABLE III - *Numbers of juveniles (NJ) of* M. incognita, *hatched in hexane extracts obtained from the aerial parts and from the roots of* M. aterrima.

Crude extracts	NJ[a/]
Hexane aerial parts	2481.60 A
Hexane roots	2278.20 A
Control	1667.00 A

a/Averages followed by the same letter do not differ at P = 0.05 by Tukey's test.

TABLE IV - *Numbers of juveniles (NJ) of* M. incognita, *race 3, hatched in the ethanol/water extracts obtained from the aerial parts and from the roots of* M. aterrima.

Crude extracts	NJ[a/]
Ethanol/water aerial parts	1037.20 A
Ethanol/water roots	212.00 A
Control	2481.60 B

a/Averages followed by the same letter do not differ at P = 0.05 by Tukey's test.

behaviour in TLC. These groups were once again subjected to *in vitro* tests with the nematode.

Twelve substances were tested, five of which presented activity, namely (ACM7 and ACM63 isolated from the ethyl acetate/acetone extract (aerial part), CHR58 isolated from chloroform extract (root), ACMR17 isolated from ethyl acetate/acetone extract (root) and HM16 isolated from hexane extract. These were subjected to biological tests to verify the nematostatic and/or nematicidal activity, using the tomato plant *Lycopersicon esculentum* (Mill (Table V).

The results were assessed using the Taylor and Sasser scale for number of galls (Taylor and Sasser, 1978). The substances did not inhibit the eclosion of juveniles compared with the control (Table VI). Nevertheless they can be considered as nematicidal due to the effect of paralysis and death of juveniles.

TABLE V - *Numbers of galls per root system of tomato cv. Rutgers induced by* M. incognita *thirty days after exposition to substances isolated from the aerial parts and roots of* M. aterrima.

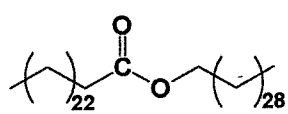
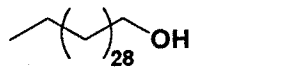
Substances	Number of galls	Index of galls
HM 16	5	2
ACM 7	10	2
ACM 63	14	3
CHR 58	9	2
ACMR 17	6	2
Control	166	5

TABLE VI - *Numbers of juveniles of* M. incognita *hatched in substances isolated from the aerial parts and roots of* M. aterrima.

Substances	Hours		
	48	96	144
HM 16	1244	875	177
ACM 7	1209	–	–
ACM 63	1748	1067	410
CHR 58	1670	1024	337
ACMR 17	1514	856	332
Control	2227	1680	1643

Of the isolated substances of the extracts obtained from the aerial parts and roots that presented activity, two were identified (Table VII) and their structures elucidated by spectral data.

TABLE VII - *Active substances of* M. aterrima *isolated and identified.*

Code	Formula	Structure	Name
HM 16	$C_{54}H_{108}O_2$		Triacontyl tetracosanate
ACM 7	$C_{30}H_{62}O$		1-Triacontanol

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