Nematicidal Principles from Two Species of Lamiaceae[1]

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Abstract: Aqueous extracts of *Ocimum sanctum* and *O. basilicum* leaves contained compounds that killed *Meloidogyne incognita* larvae in 160 min. Thin layer and gas-liquid chromatography, and infrared spectrophotometry indicated that the essential oils eugenol and linalool were the active nematicidal compounds. *Key words:* root-knot nematodes, essential oils, allelochemics.

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Many plants have been shown to have nematicidal properties but the active principle(s) have been identified in only a few (5,6,7,8,11). Crude extracts of *Ocimum basilicum* L. were reported to be nematicidal to *Meloidogyne incognita* (12). We report here the isolation and identification of compounds in extracts from *O. basilicum* and *O. sanctum* which adversely affect *M. incognita* larvae.

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MATERIALS AND METHODS

Crude extract bioassay: Twenty grams of *O. sanctum* and *O. basilicum* leaves were separately extracted by squashing in 20 ml distilled water. The extracts were centrifuged at 4,000 xg for 5 min, and the supernatant fluids decanted and stored at 0 C.

Meloidogyne incognita larvae were collected by sieving infested soil supporting

lady's fingers, (*Hibiscus esculentus* (L). The collected larvae were placed in watch glasses (100/watch glass) to which 5 ml of test extract at room temperature was added. The viability of larvae after exposure to the test extracts for various lengths of time was determined by the vital staining technique of Fenner (4). The exposure time required for 100% mortality was recorded. Test extracts were also boiled for 5 min and bioassayed in the same way. The experiment was replicated five times.

Essential oil bioassay: Twenty grams of fresh leaf material from *O. sanctum* and *O. basilicum* were separately steam distilled for 1 h to extract essential oils. The distillates were each extracted three times in a separatory funnel with petroleum ether. The ether extracts were evaporated and the oil residues dried over anhydrous magnesium sulphate.

The dried residues were then suspended in gum acasia (0.02 g oil resdue/5.0 ml gum acasia) and emulsified (2). Fifty *M. incognita* larvae, suspended in water, were placed in a groved block. The water withdrawn, and immediately replaced with the emulsion of oil and gum acasia. Controls consisted of larvae treated with gum acasia. The motility of the larvae was determined every 10 min as previously described. The rates of mortality were established statistically from the percent mortality and the time of exposure (1,10). The experiment was replicated five times.

Extraction of essential oil: The oils extracted from the plants were separated by thin layer chromatography (TLC) on silica gel using benzene:ethyl acetate (95:5) and concentrated using the same system on prepretive TLC plates. The major oil fraction from each plant extract was eluted with ether from the prepretive TLC plate and the ether evaporated, the oils bioassayed, and the mortality regression determined as previously described.

Identification of essential oil: The major essential oils of *O. sanctum* and *O. basilicum* are reported to be eugenol (3) and linalool (9), respectively. Commercial standards of these oils were co-chromatographed on TLC with the extracted oils to confirm their identity. The identity of the extract oils were further confirmed by gas-liquid chromatography (GLC) (column, SE 30; injector and flame ionization detector, 220 C; oven 140 C) and by comparing the infrared (IR) spectra of the standards and the extracted oils.

Eugenol and ilnalool bioassay: The commercial standards of eugenol and linalool used to confirm the identity of the extracted oils were bioassayed as previously described.

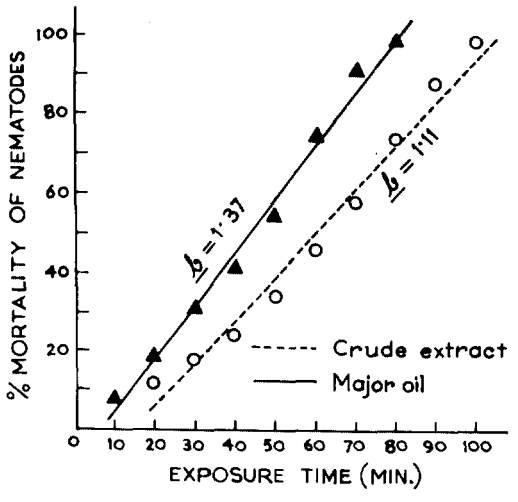
RESULTS

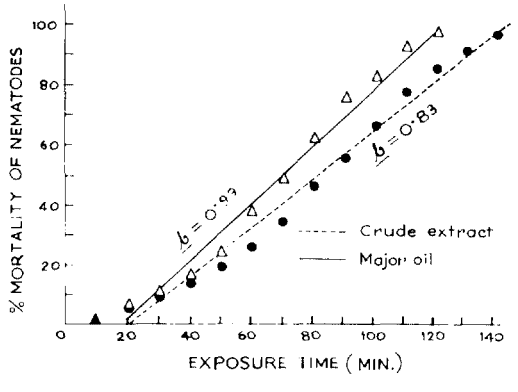
Bioassays: The vital staining technique indicated that all larvae were killed within 120 and 160 min by exposure to crude extracts of *O. sanctum* and *O. basilicum,* respectively. Boiled extracts from both plants killed less than 10% of the larvae exposed for 120 min.

The regression lines, and the coefficients of regression are shown for the mortality of *M. incognita* exposed to the oil extract and its main constituent from *O. sanctum* (Fig. 1) and *O. basilicum* (Fig. 2). The regression coefficients of the oil extracts and their major constituents were 1.11 and 1.37, respectively, for *O. sanctum,* and 0.83 and 0.99, respectively, for *O. basilicum.* The percentage mortality of *M. incognita* larvae exposed to commercial eugenol and linalool for various lengths of time closely paralleled those from the major oil constituents of *O. sanctum, O. basilicum.* No mortality was observed for larvae exposed to gum acasia for 120 min.

Chemical identification: Commercial eugenol and the major oil constituent from *O. sanctum* had the same Rf (0.56) when co-chromatographed. Similarly, commercial linalool and the major oil constituent from *O. basilicum* had the same Rf (0.44) when co-chromatographed. The retention times on GLC of eugenol and the major oil constituent of *O. sanctum* were identical (6 min); linalool and the major oil constituent from *O. basilicum* also had identical retention times (8 min) on GLC.

The IR absorption spectra of the major oil constituent from *O. sanctum* had peaks at 3,500 cm^{-1} (phenolic OH), 1,640 cm^{-1} (double bond), 1,600 cm^{-1} (aromatic ring), and was identical to eugenol. Similarly, the major oil constituent from *O. basilicum* had IR absorption peaks at 3,200–3,580 cm^{-1} (phenolic OH), 2,960 cm^{-1} and 2,915 cm^{-1}





Figs. 1–2. Effect on the mortality of *Meloidogyne incognita* larvae exposed for various times to the crude essential oils and the major constitutive oil extracted from leaves of *Ocimum sanctum* or *O. basilicum*. 1) Extract from *O. sanctum;* major constituent identified as eugenol. 2) Extract from *O. basilicum;* major constituent identified as linalool.

(dimethyl group), and 910 cm^{-1} (double bond), and was identical to linalool.

DISCUSSION

Bioassay of crude aqueous extracts from *O. sanctum* and *O. basilicum* leaves clearly showed that the plant possessed strong nematicidal properties. The volatile nature of the active principle was suggested by the reduced nematicidal activity of boiled crude extract. The volatile essential oils eugenol and linalool were known to be major constituents of *O. sanctum* and *O. basilicum,* respectively, and were prime candidates for the nematicidal principles. This suspicion was confirmed by TLC, GLC, and IR spectrophotometric analysis. Additionally, a commercial standard of eugenol had essentially the same nematicidal efficacy as did the major constituent of the oil from *O. sanctum;* commercial linalool and the major oil constituent from *O. basilicum* had essentially the same nematicidal efficacy.

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