Nematol. medit. (1992), *20*: 57-58

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NEMATICIDAL ACTIVITY OF ESSENTIAL OIL OF *PELARGONIUM GRAVEOLENS* AGAINST THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

by
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Summary. Nematicidal activity of essential oil of *Pelargonium graveolens* L. (cv. Algerian) and its major constituents namely citronellol, geraniol and linalool was determined against the root-knot nematode *Meloidogyne incognita*. Geraniol was found to be the most effective constituent which was followed by citronellol and linalool.

Essential oil of *Pelargonium graveolens* L. is known to possess antibacterial (Nigam, 1982) and antifungal (Raghavaiah and Jayaramaiah, 1987) properties. Its nematicidal properties have not been reported so far. We have investigated the nematicidal activity of essential oil of *P. graveolens* (Cv. Algerian) and of its major constituents citronellol, geraniol and linalool against the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw.

Materials and methods

One kg of freshly harvested leaves of *P. graveolens* was hydrodistilled for four hours to extract essential oil. The distillate was separated from water using a separating funnel and dried over anhydrous sodium sulphate. One ml of the essential oil was dissolved in methanol to give a 10% (v/v) solution and one ml of this was emulsified in nine ml of 0.3% Tween-20. One ml of this emulsion was transferred to a petri dish and three drops of streptocyclin solution (25 mg/10 ml) was added to it. Two hundred freshly hatched second stage juveniles of the root-knot nematode *M. incognita* suspended in one ml distilled water were added to the petri dish which was then covered with a lid and kept at room temperature. Mortality of the nematodes was tested twenty four hours after incubation. One ml methanol in 0.3% Tween-20 served as the control. Each treatment was replicated thrice.

The major constituents of the essential oil used in the studies were determined by GLC analysis using a Hewlett Packard Gas Chromatograph equipped with FID and Stainless steel column (6' X 1/8") packed with 10% carbowax 20 M - adsorbed on chromosorb W (80-100 mesh). The operating conditions were set as follows. Detector and injector temperature 240 °C, column temperature 165-185 °C at a rate of 2 °C/min. The major constituents of the oil were identified as citronellol, geraniol and linalool by comparing their retention times with those of authentic samples under the same operating conditions. The abundance of the constituents were calculated from peak areas by area normalisation. Authentic samples of citronellol and geraniol supplied by Aldrich Co. Ltd., and linalool by Sigma Co. Ltd., were used in the present studies.

A series of concentrations (125, 250, 500, 1000, 2000 and 4000 µl/l) of the essential oil and its three constituents namely citronellol, geraniol and linalool were prepared by diluting with methanol and assayed for their nematicidal activity against the root-knot nematode *M. incognita* as described earlier.

Results and discussion

The relative abundance of the major constituents of essential oil of *P. graveolens* as determined by GLC analysis were citronellol 41.3%, geraniol 9.9%, linalool 12.7% and other minor constituents 36.1%. Based on our results on GLC analysis of the essential oil of *P. graveolens*, the effective concentration of essential oil which gave 100% mortality (2000 µl/l) will correspond to 826 µl/l of citronel-

Contribution No. 90/91.
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lol, 254 µl/l of linalool and 198 µl/l of geraniol (Fig. 1). Linalool and geraniol at these concentrations exhibited hardly any significant nematicidal activity and citronellol showed less than 85% mortality. This shows that the combined effect of these constituents also play a role in the nematicidal activity as observed in the case of essential oil of cymbopogon grasses by Sangwan *et. al.* (1985). There can also be other constituents present in the essential oil which contribute to its nematicidal activity.

Essential oils are generally considered to have low mammalian toxicity (Sangwan *et. al.*, 1990). In this connection, essential oil of *P. graveolens* and its major constituents can be effectively utilised for the design of safer nematicides by further chemical modifications.

Acknowledgements - The authors wish to express their gratitude to Dr. S. P. Angadi for providing plant material and Dr. G. S. R. Murti, for extending GLC facilities and Dr. M. V. Chandravadana for scientific guidance. They are also thankful to Dr. R. M. Pandey, Director, Indian Institute of Horticultural Research, for his keen interest and encouragement.

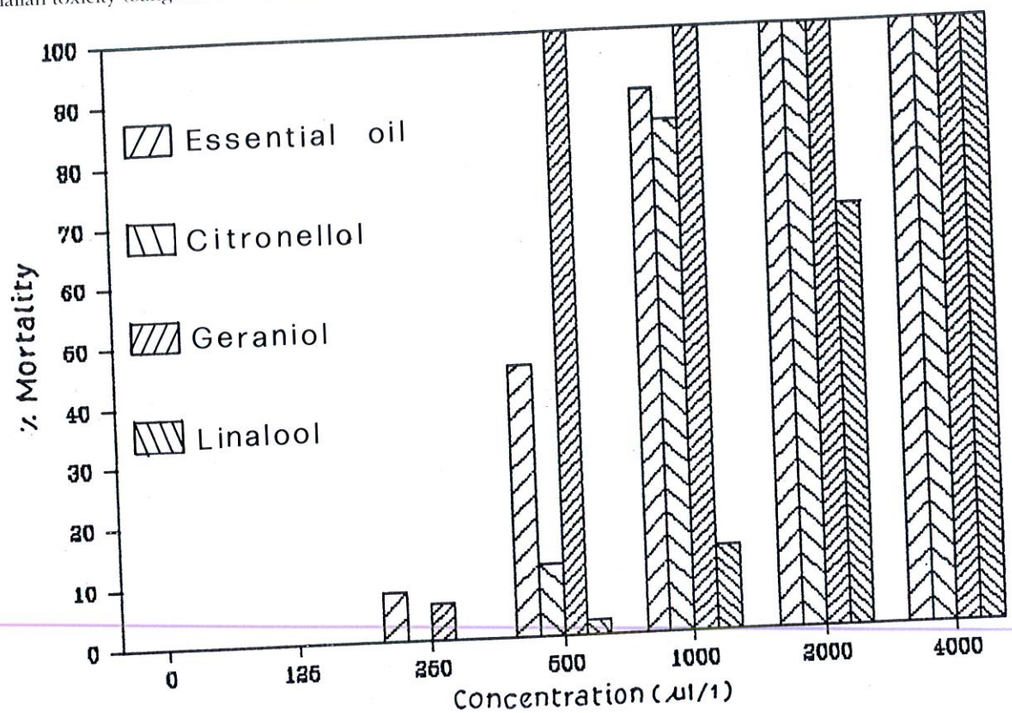

Fig. 1 - Nematicidal activity of essential oil of *Pelargonium graveolens* and its major constituents against the second stage juveniles of *M. incognita*.

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Accepted for publication on 14 January 1992.

Nematol. medit. (1992), *20*: 59-61

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ON THE ORIGIN AND SPREAD OF HETERODERA AVENAE IN AUSTRALIA

by
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Summary. *Heterodera avenae* is widely established in south eastern Australia and in recent years has been recorded in discrete infestations at far-distant northern locations. The pattern of its detection in Australia and data on host range, relation of hatch to temperature and pathotype reactions are consistent with introduction to southern Australia from Europe in the late nineteenth century.

The generally accepted explanation of the origin of cereal cyst nematode, *Heterodera avenae* Woll. in Australia is the hypothesis of Meagher (1972, 1977) and Brown (1984) that the nematode was introduced to southern Australia, probably South Australia, in the late nineteenth century, from Europe where it probably originated as a parasite of oats or rye (Meagher, 1977).

Origin

Introduction of the nematode first to South Australia and in the late 1800s is historically plausible. Between 1850 and 1890 wheat production expanded dramatically in South Australia, making it the main wheat producing state (Macindoe, 1975). Wheat was grown close to seaports on Gulf St. Vincent and Spencer Gulf and export of wheat from Australia began from these ports. One possibility is that cysts came with ships' ballast which was dumped on wheat land near ports. Solid ballast was often carried by ships sailing from Europe and was dumped at ports where cargo was loaded (Wace, 1985).

Introduction initially into South Australia and spread from there is consistent with the pattern of detection of *H. avenae*. Herbarium material showing *H. avenae* on wheat grown in South Australia in 1904 provides the earliest evidence of the nematode in Australia (Meagher, 1972). In 1930 it was described as a pest of cereals in South Australia (Davidson, 1930) and by 1938 it was recognized as a pest in the Wimmera districts of Victoria (Millikan, 1938). In 1958, Meagher (1958) described it as widespread in both the Wimmera and Mallee districts of Victoria. Recognition of it at Geraldton in Western Australia, 1800 km from previously known infestations, dates from the late 1960s (Meagher, 1977), indicating that a long-distance spread event had occurred by then.

H. avenae was first found in New South Wales in 1967 at Koraleigh in the southern Riverina district, bordering infestations in Victoria (McLeod, 1968). In 1980, Southwell and McLeod (1981) reported it on two farms in northern New South Wales, 800 km north east of previously known infestations. These are still the most northly known infestations in eastern Australia. In 1984 it was detected 250 km south west of the northern infestations (McLeod, 1986a).

The biological data now available support the views that *H. avenae* in Australia is a recent introduction to Australia and that it is closely similar to populations of *H. avenae* in Europe. Fisher (1987) has noted that it has not been found on native Australian plants. The known and preferred hosts of *H. avenae* in Australia are in the genera *Avena, Hordeum, Lolium, Phalaris, Secale* and *Triticum* and all of these have been introduced to Australia (Simons, 1983).

The relation of egg hatch to temperature is similar to that reported for *H. avenae* in Europe (Fisher, 1987). This relationship, which relies on low temperature to match hatching to host availability, seems irrelevant to natural Australian conditions where host availability is governed by rainfall rather than temperature.

Pathotype testing within Australia (Brown, 1969, 1974; McLeod, 1976, 1986b) indicates a high degree of homogeneity, consistent with limited and recent introductions. It is considered that the pathotype present in Australia is not identical to any single pathotype found in Europe (Brown, 1982). However, these and other tests (Cook and McLeod, 1980; Andersen and Andersen, 1986) show that of the resistances tested, those in *Avena sterilis* L., *A. strigosa* Shreb, barley CVS KVL 191 and Morocco, wheat cv. Loros and the

Nematol. medit. (1992), *20*: 193-195

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INFLUENCE OF PLANT AGE ON ROOT-KNOT NEMATODE DEVELOPMENT IN CARDAMOM

by
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Summary. Two, 12 and 24 months old seedlings and mature vegetative suckers of cardamom (*Elettaria cardamomum* Maton) plants when inoculated with 500 second stage juveniles of *Meloidogyne incognita* showed varying responses. The highest multiplication rate, gall and egg mass indices were observed in 24 months old seedlings while the per gram root population was highest in 12 months old seedlings. The galls on the root system of mature plants were comparatively small and supported fewer nematodes. In general, young cardamom seedlings were more susceptible to root knot nematodes than mature plants.

Root knot nematodes (*Meloidogyne* spp.) are important pathogens of small cardamom, *Elettaria cardamomum* Maton (D'souza *et al.*, 1970; Kumar *et al.*, 1971; Koshy *et al.*, 1976; Sundararaju *et al.*, 1979) and widespread in cardamom nurseries and plantations in Kerala, Karnataka and Tamil Nadu (Ali, 1984, 1986; Ali and Koshy, 1982). The common aerial symptoms of nematode infestation are stunting, poor tillering, yellowing and drying of leaf tips and margins; below ground roots are galled with abnormal branching. On older seedlings and mature plants in the field, instead of typical galling, excessive branching (witches-broom type) of roots near the root tips with milky white rootlets devoid of hairs has been reported (Ali, 1984, 1987).

The present study was undertaken to understand the influence of plant age on root-knot nematode development and the effect of nematode infestation on the growth and morphology of roots of cardamom plants.

Materials and methods

Cardamom plants of four age groups viz., two, 12 and 24 months old seedlings and mature vegetative suckers were raised in nematode-free soil in a screenhouse. From these, plants of uniform size from each age category were transplanted into plastic pots (22 çm diameter) containing sterilised soil. Once established, a nematode suspension of 500 active second stage juveniles of *Meloidogyne incognita* (Kofoid *et* White) Chitw. was poured on to the root zone in five pots of each age group of plants; the inoculum was collected from root-knot nematode cultures maintained on cardamom. A few pots without nematodes were retained to see the root branching pattern in them. After three months, the plants were uprooted and the root systems were removed, washed thoroughly and their fresh weights were recorded. The entire root system was carefully observed under a microscope and rated for galling and egg masses based on Taylor and Sasser's (1978) scale. Egg masses were stained with Phloxine-B for easy detection (Daykin and Hussey, 1985). Comparison was made with uninoculated plants to identify any change in root morphology due to nematode infestation. Root-knot nematode galls, root-knot and root diameters of ten randomly selected galls from each plant were measured at the point of greatest girth along a line parellel to the root and root diameter at a point one cm above the gall. To assess the total nematode populations, roots were cut into small pieces and stained with acid fuchsin (Byrd *et al.*, 1983). These roots were macerated in an electric mixer and three 1 ml aliquots per sample were removed from the suspension for counting. The average of the counts was used to calculate the total nematodes per plant and per gram of root.

Results and discussion

Plants of all age groups were susceptible to nematode attack as is evident from the final nematode population (Table I). However, the highest root-knot index (RKI), egg mass index (EMI) and final nematode population were observed in 24 months old seedlings. Plants of other ages had similar RKI and EMI but supported greatly different nematode populations. The lowest multiplication rate in two

Table I - *Mean gall, egg mass indices and number of nematodes in the roots of cardamom plants of different ages inoculated with* Meloidogyne incognita *(mean of five replications).*

Plant age	Root knot index	Egg mass index	Final population (Pf)	Per 'g' root population
2 - months	2.8 a*	2.6 a	811.33 a**	467.7 a**
12 - months	2.8 a	2.8 a	2860.22 b	849.2 a
24 - months	4.2 b	4.0 b	4543.60 b	307.6 ab
Suckers	3.5 ab	3.5 ab	1828.10 ab	93.5 b

* Means with different letters are significantly different, P = 0.05 according to Duncan's multiple range test; ** nematode counts were log transformed prior to statistical analysis and the weighted means were used for comparison.

month old seedlings probably was due to the small root mass available for nematode colonisation. The high nematode population in roots (per g) of two months old seedlings which is on par with that of 12 months and 24 months old seedlings also supports this. Suckers had a mean RKI and EMI of 3.5 each, even at a low nematode level in the roots. In suckers, adult females were embedded in the outer periphery of roots with their egg masses exposed, hence the high EMI in suckers. Also, there were more galls but of smaller size than in seedling plants.

Gall size was compared in different age groups of plants by measuring the difference in gall diameter with the corresponding root diameter as the root diameters varied considerably in various age groups of plants. Significantly bigger galls were observed in two months old seedlings (Table II). Gall size is reported to be related to the number of nematodes in the tissue (Dropkin, 1954). It is also observed that small galls are induced by juveniles continuously feeding at the root surface without actually entering the roots (Lowenberg *et al.*, 1960).

The low number of nematodes in suckers may be due to low penetration or because of the low multiplication rate of nematodes. Tissue maturation and tissue senescence are two important factors influencing the susceptibility of a plant to pathogens (Bruehl, 1987). Weiser (1955) reported that actively elongating roots were more attractive to nematode juveniles than roots in which extension had slowed.

In contradiction to the earlier reports (Ali, 1984, 1987), no consistent pattern of root branching caused by nematode invasion was associated with any of the age categories of plant. In the present study young cardamom seedlings were more susceptible to root-knot nematodes than older seedlings or mature plants. The inverse relationship between increasing plant age and decreased root galling and population development has been observed in alfalfa (Griffin and Hunt, 1972), in tea (Sivapalan, 1972) and in sugar beets (Olthof, 1983).

The study shows that more emphasis has to be given for early protection of cardamom seedlings as the age of the seedling at the time of exposure to the pathogen is critical.

Table II - *Comparison of root and root-knot diameters of cardamom plants of various ages exposed to* M. incognita *(mean of five replications).*

Plant age	Mean root diameter (mm)	Mean gall diameter (mm)	Gall diameter as % of root diameter
2 - months	0.52 a*	1.04 a	269.2 a
12 - months	1.06 b	1.36 b	129.3 b
24 - months	0.95 b	1.21 ab	129.1 b
Suckers	0.71 ab	0.95 a	135.1 b

* Means with different letters are significantly different, P = 0.05 according to Duncan's multiple range test.

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Accepted for publication on 16 May 1992.