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ВЛИЯНИЕ *RUTA GRAVEOLENS* L. НА ГАЛЛОВУЮ НЕМАТОДУ (*MELOIDOGYNE* SPP.)

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Аннотация

Два эксперимента *in vitro* и два испытания в открытом грунте были проведены с целью изучения нематицидного действия *Ruta graveolens* на галловую нематоду *Meloidogyne incognita*. В первом эксперименте *in vitro* яйцевые мешки нематоды помещали на три недели в водный экстракт листьев растений руты. Затем яйцевые мешки подвергали инкубационному тесту в дистиллированной воде еще в течение пяти недель. В другом эксперименте *in vitro* яйцевые мешки *M. incognita* подвергали действию экстракта руты в течение 4, 8 и 16

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часов и затем инкубировали в дистиллированной воде в течение восьми недель. Для первого и второго эксперимента *in vitro* в качестве контроля использовали дистиллированную воду и водный раствор фенамифоса с концентрацией 5 мкг/мл и только дистиллированную воду соответственно. Оба теста на вылупление были проведены в термостате при 25±2 ^{0}C. Число личинок второй стадии (J2), появляющихся еженедельно, выражали в виде кумулятивного процента общего содержания яиц в массе яиц (инкубационные яйца + вылупившиеся J2). В первом эксперименте вылупление из яйцевых мешков, обработанных экстрактами руты и водным раствором фенамифоса, оказалось значительно ниже, чем в необработанном контроле. Во втором эксперименте *in vitro* погружение на 8 часов яйцевые мешки *M. incognita* в экстракт руты привело к тому, что конечный выход был значительно ниже, чем в дистиллированной воде. Нематицидный эффект *R. graveolens*, используемой в качестве зеленого удобрения, также был исследован в условиях открытого грунта на растениях томата и табака в Южной Италии в Монтерони (провинция Лечче, регион Апулия) в песчаной почве с естественным фоном *M. incognita*. Участки с добавлением растений руты сравнивали с необработанными и обработанными фенамифосом участками (3 т/га). Повторность – четырехкратная для каждой обработки. Семена *R. graveolens* высевали за два месяца до посадки томатов и табака. В конце полевых испытаний определяли урожайность, индекс галлообразования и плотность популяции нематод в почве, проводили статистический анализ. Внесение растений *R. graveolens* в почву значительно повысило урожайность томатов и табака и уменьшило индекс галлообразования и плотность популяции нематод в почве по сравнению с необработанными контролями.

Ключевые слова: рута, почвенные добавки, *Meloidogyne incognita*, фенамифос, борьба с паразитическими нематодами растений.

ACTIVITY OF *RUTA GRAVEOLENS* L. AGAINST ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)

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Abstract

Two *in vitro* experiments and two open field trials were carried out to investigate the nematicidal effect of *Ruta graveolens* on the root-knot nematode *Meloidogyne incognita*. In the first *in vitro* experiment the nematode egg masses were immersed for 3 weeks in aqueous leaf extract of rue plants and then subjected to an hatching test in distilled water for five more weeks. In the other in vitro experiment *M. incognita* egg masses were exposed to the rue extract for 4, 8 and 16 hours and then incubated in distilled water for 8 weeks. Distilled water and a 5 μg/mL an aqueous solution of Fenamiphos or only distilled water were used as controls in the first and second *in vitro* experiment, respectively. Both hatching tests were carried out in a growth cabinet at 25±2 ^{0}C, providing four replicates for each treatment. In the first experiment the hatching from the egg masses treated with rue extracts and Fenamiphos solution resulted significantly lower than that in the untreated control. In the second *in vitro* experiment, a 8-hour immersion of the egg masses in the rue extract resulted in a final hatch significantly lower than that in distilled water. The nematicidal effect of *R. graveolens* green manure was investigated in open field condition on tomato and tobacco. Plots amended with rue plant biomass were compared with untreated and Fenamiphos treated plots (3 t/ha). Four replications were provided for each treatment. Crop yield, root gall index and soil nematode population density were recorded at the end of each crop cycle. Soil incorporation with *R. graveolens* plant materials significantly increased both tomato and tobacco yield and reduced root gall index and soil nematode population density in comparison to the untreated controls. These experiments demonstrated a high suitability of *R. graveolens* for the development of new sustainable nematicidal products.

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Keywords: green manure, *Meloidogyne incognita*, rue, soil amendments, sustainable control.

Introduction. Yield of many vegetable and fruit crops can be heavily affected by the infestations of root-knot nematodes (*Meloidogyne* spp.) (Lamberti et al., 2001; Sasanelli et al., 2018). A satisfactory control of these pests can be achieved by soil treatments with synthetic nematicides, especially fumigants (Basile et al., 2003; Sasanelli et al., 2019). The increasing public attention to health and environmental risks related to the use of chemicals is evoking alternative control methods against nematode pests. Among these alternatives, use of plant derived products is gaining more and more interest among farmers and also scientists (Ntalli and Caboni, 2012; Renčo et al., 2014). Biocidal properties against phytoparasitic nematodes have been documented for a wide range of plant species compounds (Gommers, 1981; Chitwood, 2002), among which many species from *Rutaceae* family and particularly for the genus *Ruta* (Grainge and Ahmed, 1988). The extracts from *Ruta graveolens* L. have been reported for biocidal properties against insects, soil pathogens, weeds and also plant parasitic nematodes (Sasanelli, 1992; Sasanelli and D'Addabbo, 1992; Aliotta et al., 1994; Landolt et al., 1999; Oliva and Lahoz, 1999). Furanocoumarins, flavonoids, alkaloids and volatile essential oils are the main active compounds detected in this species (Kostova et al., 1999). Use of plants as green manure has been widely investigated for its beneficial effect on the soil phisico-chemical properties and organic matter content, whereas the phytosanitary potential of this agronomic practice was studied mainly on *Brassicaceae* and *Leguminosae* species (D'Addabbo et al., 2004; Avato et al., 2013; 2009; Wen-Ching, 2018) and very few on *Rutaceae* plants (Sasanelli and D'Addabbo, 1993). The aim of this work was to assess the nematicidal potential of *R. graveolens* on the root-knot nematode *Meloidogyne incognita* through both in vitro assays and field experiments on tomato and tobacco.

Materials and methods. *In vitro* tests.

In both the in vitro tests rue leaf extracts were prepared by soaking 50 g of green leaves in 200 mL of distilled water (1:4) for 24 hours, sufficient to solubilize the active principles. Leaves were then comminuted in a blender for 1 min (8,000 rpm) and the suspension filtered on a filter paper (Non Waven N° 261/A). This filtrate (pH 7.3) was stored in plastic bottles in a freezer until required, keeping only small quantities in a refrigerator (5 ^{0}C) for the immediate use.

An Italian population of the root-knot nematode *M. incognita* was reared on tomato (*Solanum lycopersicum* L.) cv. Roma VF for two months in a glass-

house at 25±2 °C. Batches of 25 egg masses of similar size (averaging 10,000 eggs/batch), collected from the infested tomato roots, were placed on 2 cm diam sieves (215 μm aperture). Each sieve was put in a 3.5 cm diam Petri dish.

In the first *in vitro* assay, three mL of the rue aqueous extract were then added to each egg mass batches. After the first three weeks, the egg masses were removed from the test solutions and the incubation continued in distilled water. In the second *in vitro* test, the *M. incognita* egg mass batches were immersed for 4, 8 and 16 hours in the rue extract and then transferred to distilled water. Distilled water and a 5 μg/mL solution of Fenamiphos or only water were used as controls in the first and second experiment, respectively (Greco and Thomason, 1980).

In both experiments the dishes were arranged in a complete randomized block design with four replications per treatment and incubated in a growth cabinet at 25±2 °C. Emerged juveniles (J2) were counted at weekly intervals, renewing the test solutions or distilled water at the same time, throughout 8 weeks (Sasanelli and Di Vito, 1991).

At the end of the two hatching tests, the egg masses were shaken for 3 min in a 1% sodium hypochlorite aqueous solution (Hussey and Barker, 1973) and the unhatched eggs were counted. Numbers of juveniles emerging weekly were expressed as cumulative percent of the total initial population (hatched + unhatched eggs). Data were arcsin square root transformed and subjected to analysis of variance (ANOVA), comparing means by Least Significant Difference's Test. Nematode mortality rates were calculated on the base of hatching results and corrected according to the Schnaider-Orelli's formula (1947).

Field trials.

The trials were undertaken in a sandy soil (sandy 64.4%, silt 18.7%, clay 16.9%, organic matter 0.8%, pH 7.5) naturally infested by *M. incognita* located at Monteroni (Lecce province, Apulia region, Southern Italy) (40° 33' 26'' N, 18° 10' 00'' E). In both trials soil was deeply ploughed, rotavated and subdivided in 12 m^2 plots, spaced 1 m each other and distributed in a randomized block design with four replications for each treatment. Rue was sown in eight plots, four for each crop, and grown for two months after plant emergence. Rue plant biomass was incorporated into the soil (6 Kg/m^2) one week before the transplant of tomato (cv. Tondino di Zagaria) or tobacco (cv. Erzegovina 6B) seedlings. Untreated or treated with 3 t/ha of Fenamiphos soils were used as controls.

During the growing season tomato and tobacco plants received all the cultural practices (weed, insect and pathogen control, fertilizer application and irrigation) commonly adopted in the area.

Crop yield, root gall index and soil nematode population density were recorded at the end of both tomato and tobacco crop cycle. Root gall index was estimated on each root according to a 0–5 scale (0 no galls and 5 root system completely deformed by large and numerous galls) (Lamberti, 1971). Nematodes were extracted by the Coolen's method (Coolen, 1979) from 500 mL soil sub-samples of composite samples collected from each plot.

Data were subjected to ANOVAand means compared by Duncan's Multiple Range Test.

All statistical analysis were performed using the PlotIT program ver.3.2 (Scientific Programming Enterprises, Haslett, MI, USA).

Results. *In vitro* tests.

In the first *in vitro* test, the egg masses exposed to the rue extract resulted in a total percentage hatch (0.4%) significantly lower than those immersed in the Fenamiphos solution or distilled water (22.7 and 75.9%, respectively) (Tab. 1). Treatment of egg masses with the *R. graveolens* extract resulted in a significantly higher egg mortality (99.5%) compared to Fenamiphos (70.1%).

Table 1

Effect of *Ruta graveolens* extract on the final hatch (8 weeks) of egg masses of *Meloidogyne incognita*

Treatment	% Hatch			% Mortality [1]
Ruta graveolens	0.4 [2]	a [3]	A	99.5
Fenamiphos	22.7	b	B	70.1
Distilled water (untreated control)	75.9	c	C	---

[1] Per cent corrected mortality, according to the Schnaider-Orelli's formula, in comparison to the untreated control;

[2] Each value is an average of 4 replications;

[3] Data flanked in each column by the same letters are not statistically different according to Least Significant Difference's Test (small letters for P = 0.05; capital letters for P = 0.01).

In the second *in vitro* test, percentage of egg mortality ranged from 4 to 41.1% after the different times of exposure to the rue extract (Tab. 2). The

highest mortality was found for the eggs exposed to the longest exposure time (16 hrs). The relationship between the per cent mortality of *M. incognita* eggs (y) and the exposure time (x) was linear ($y = 2.71x - 2.2$) with a high correlation index ($r = 0.98$).

Table 2

Egg mortality of *Meloidogyne incognita* from egg masses exposed to aqueous leaves extract of *Ruta graveolens* for different exposure times (4, 8 and 16 hours)

Treatment	Exposure time (h)	% Hatch			% Mortality [1]
Ruta graveolens	4	69.8 [2]	bc [3]	B	4.0
	8	56.6	b	AB	22.1
	16	42.8	a	A	41.1
Distilled water (untreated control)	---	72.7	c	B	---

[1] Per cent corrected mortality, according to the Schnaider-Orelli's formula, in comparison to the untreated control;

[2] Each value is an average of 4 replications;

[3] Data flanked in each column by the same letters are not statistically different according to Least Significant Difference's Test (small letters for $P = 0.05$; capital letters for $P = 0.01$).

These results confirm the high nematicidal activity of *R. graveolens* leaf extract, due to the presence of highly active compounds, such as furanocoumarins, flavonoids, alkaloids and volatile essential oils. However, the content of these bioactive compounds can be variable among the different plant tissues, as well as according to the crop season in which they are produced (Waghorn and McNabb, 2003).

Field trials.

Tomato yield from plots amended with *R. graveolens* biomass was significantly higher than that from the nontreated soil and not statistically different from the treatment with fenamiphos (Tab. 3). Soil incorporation of rue plant biomass and treatment with fenamiphos significantly reduced gall formation on tomato roots in comparison to the untreated control, whereas nematode population density was significantly reduced only in soil treated with fenamiphos ($P = 0.05$) (Tab. 3).

Tobacco yield was significantly higher than the untreated control only for the treatment with Fenamiphos which, however, was not statistically different

from the rue soil amendment (P = 0.05) (Tab. 4). Both root gall index and soil nematode population density were significantly reduced by Fenamiphos and rue amendment in comparison to the untreated control (Tab. 4).

Table 3

Effect of *Ruta graveolens* biomass incorporation into the soil on the root-knot nematode *Meloidogyne incognita* on tomato (cv. Tondino di Zagaria)

Treatments	Dose (t/ha)	Yield (t/ha)			Root gall index (0–5)			Eggs and juveniles/mL soil		
Control (untreated)	-	25.4 [1]	a [2]	A	4.5	a	A	19.8	a	A
Ruta graveolens	60	35.3	b	B	2.3	b	A	12.2	ab	A
Fenamiphos	0.3	40.5	c	B	2.7	b	A	10.6	b	A

[1] Each value is the average of four replications;

[2] Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (small letters for P = 0.05; capital letters for P = 0.01).

Table 4

Effect of *Ruta graveolens* biomass incorporation into the soil on the root-knot nematode *Meloidogyne incognita* on tobacco (cv. Erzegovina 6B)

Treatments	Dose (t/ha)	Yield (t/ha)			Root gall index (0–5)			Eggs and juveniles/mL soil		
Control (untreated)	-	14.8 [1]	a [2]	A	4.6	a	A	35.1	a	A
Ruta graveolens	60	18.8	ab	A	2.7	b	B	5.2	b	B
Fenamiphos	0.3	20.7	b	A	3.8	b	AB	5.7	b	B

[1] Each value is the average of four replications;

[2] Data flanked in each column by the same letters are not statistically different according to Duncan's Multiple Range Test (small letters for P = 0.05; capital letters for P = 0.01).

Conclusion. Data from this study demonstrate a strong biocidal activity of *R. graveolens* leaf extract, mainly attributable to the high content of furanocumarins (bergapten, isopimpinellim, psoralen and xanthotoxin) in plant green tissues. These compounds were previously reported for antibacterial, antiviral, insecticidal and fungicide activities and their content is influenced by the vegetative plant stage (Wangchuk et al., 2014). Results also indicated

the potential of *R. graveolens* green manure as an alternative to chemicals for an environmentally safe phytonematode control both in organic and conventional crops.

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