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BEATRICE CARLETTI (*) - FABIO PACI (**) - LAURA AMBROGIONI (*) - CLAUDIA BENVENUTI (*)
PIO FEDERICO ROVERSI (*)

EFFECTS OF A CLOVE OIL EXTRACT ON EGGS AND SECOND-STAGE JUVENILES OF *MELOIDOGYNE INCOGNITA* (KOFOID *ET* WHITE, 1919) CHITWOOD, 1949

(*) *C.R.A. - Centro di Ricerca per l'Agrobiologia e la Pedologia, Via di Lanciola, 12/A - 50125 Firenze, Italy;*
e-mail: beatrice.carletti@isza.it
(**) *Xeda Italia Srl, Via F. Guarini, 13/A - 47100 Forlì, Italy.*
B. Carletti, L. Ambrogioni and P.F. Roversi contributed to each phase of the research. C. Benvenuti contributed to the statistical analysis. F. Paci supplied the product.

Carletti B., Paci F., Ambrogioni L., Benvenuti C., Roversi P.F. – Effects of a clove oil extract on eggs and second-stage juveniles of *Meloidogyne incognita* (Kofoid *et* White, 1919) Chitwood, 1949.

Trials were carried out *in vitro* to evaluate the action against *Meloidogyne incognita* (Kofoid *et* White) Chitwood of the clove oil extract ABT-EU04® (Xeda International S. A.), whose active principle is derived from buds of *Syzygium aromaticum* (L.) Merrill *et* Perry. Three aqueous concentrations of the product (0.125%, 0.25% and 0.50%) were tested on *M. incognita* unsegmented and embryonated eggs and on second-stage juveniles, both free and included in the egg masses. The experiment, run twice, was carried out in small glass containers in the dark at a constant temperature of 25°C. The data from the two tests were statistically analysed. The clove oil extract stopped the embryogenesis of eggs and showed a complete nematicidal action against second-stage juveniles both free and of the egg masses. These properties make this product an interesting tool for a novel control strategy in nematode pest management.

KEY WORDS: eugenol, *Meloidogyne incognita*, clove oil, root-knot nematode, eggs, second-stage juveniles, egg masses.

INTRODUCTION

Plant extracts and agro-industrial by-products have been studied as alternatives to conventional phytoparassitic organism chemical control, which is expensive and harmful to the environment. The composition of plant residues results in the formation of substances that are active also against plant parassitic nematodes. Therefore, continuous research on new compounds is centred on natural active substances isolated from plants with nematicidal properties (FERRAZ & DE FREITAS, 2004).

Among the plant extracts the essential oils, concentrated and hydrophobic liquids containing volatile aroma compounds, have a very high economic value. In fact they have traditionally been used in folk medicines for their aseptic and anthelmintic activity (ASHA *et al.,* 2001; PESSOA *et al.,* 2002; PRABUSEENIVASAN *et al.,* 2006) and in the cosmetic and food industries as fragrances and flavouring agents (ISMAN, 2001). In recent years certain essential oils and their major constituents have shown an antimicrobial, fungicidal and insecticidal activity (COATS *et al.,* 1991; RICE & COATS, 1994; WILSON *et al.,* 1997; BOWERS & LOCKE, 2000; 2004; RHAYOUR *et al.,* 2003) and also a rapid nematicidal action (ISMAN, 2001; CHITWOOD, 2002). Indeed the essential oils, extracted from different plant species belonging to the Labiatae, Myrtaceae and Poaceae families and their major monoterpenoidal constituents were reported to possess promising nematicidal properties (SANGWAN *et al.*, 1990; ISMAN, 2000; OKA, 2001; PARK *et al.,* 2005).

Among the essential plant oils eugenol, the main constituent of clove oil, derived from buds of *Syzygium aromaticum* (L.) Merril *et* Perry (sin. *Eugenia caryophyllata* Thumb.) and leaves of *Ocimum sanctum* L. (sacred basil) and *Pimenta dioica* (L.) Merril (allspice), resulted active against pathogenic organisms including nematodes (CHATTERJEE *et al.*, 1982; SANGWAN *et al.,* 1990; GOKTE *et al.*, 1991; LEELA & RAMANA, 2000; PARK *et al.*, 2005; MEYER *et al.,* 2008a, b).

The nematicidal activity of eugenol varies according to the different nematode species. For example, the same concentration (1.5 mg oil/Kg soil) that reduced the number of galls induced on tomato by *M. arenaria (*Neal) Chitwood has no significant effect on galls on *M. incognita* infested tomato (WALKER & MELIN, 1996). Different concentrations of clove oil extract (including other components in addition to eugenol) were found to be nematoxic to other phytoparasitic nematodes such as *X. americanum* Cobb, *Longidorus* sp., *Hoplolaimus indicus* Sher, *Pratylenchus* sp. and *Helicotylenchus indicus* Siddiqi (PANDEY & DWIVEDI, 2000). Essential oil of *E. caryophyllata* at a concentration of 1000 m1/l showed good nematicidal activity, causing 100% immobility in a population of *Bursaphelenchus xylophilus* (Steiner *et* Buhrer) Nickle, whereas there was no effect at 500 m1/l (PARK *et al.,* 2005). The differing nematicidal activity of clove oil extracts could be due to the different compounds present (eugenol alone or clove oil with other components), dose, stage, sex and species of nematode considered. Clove oil has also been patented as a plant fungicide (WALTER *et al.,* 1997).

A recent study focused on the use of clove oil for *M. incognita* control and on the mode of action and dose-response effects (MEYER *et al.,* 2008a), although it did not consider the mechanism of action against different biological stages.

A new natural nematicide ABT-EU04® (Xeda International S. A.), a clove oil extract-based formulation regi-

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stered for preventive soil treatment, has been formulated in France and is now under technical evaluation by the Italian Ministry of Agriculture. Clove oil has already been included in Annex 1 of 91/414 EEC Directive Authorisation relating to the commercial inclusion of phytosanitary products. The active ingredient is the monoterpenoid phenolic eugenol, the chief constituent of clove oil (about 80%) obtained from the flower buds of *S. aromaticum*.

Here we report on trials carried out *in vitro* to determine the mechanism of action and role of different concentrations of this clove oil extract on *M. incognita* unsegmented and embryonated eggs and on second-stage juveniles, both free and of the egg masses. The choice of this nematode species, an obligate sedentary endoparasite, was based on his world wide distribution, extreme polyphagy and significant economic impact.

MATERIALS AND METHODS

All experiments were run twice, denoted as Test 1 and Test 2.

Preparation of the extract, isolation and identification of the major constituents

The essential oil was originally isolated from flower buds of *Syzygium aromaticum*. 100 g of commercial product contain 20% clove oil (eugenol is the main component at 80-92%, corresponding to 18% of the product), 35% lecithin from non-genetically modified soybean, and 45% co-formulation emulsifying alimentar. Three concentrations were prepared by diluting stock solution in sterile bidistilled water filtered through a nitrocellulose membrane with filter pore size of 0.45µm; this water was also used as the control.

Screening for nematicidal activity

The effect of this plant product was investigated under laboratory conditions on *M. incognita* unsegmented and embryonated eggs and in second-stage juveniles, both free and of the egg masses.

The four trials, for each test, were carried out *in vitro* in sterilized staining cavity blocks (3 ml capacity) covered with glass lids to prevent evaporation of the solutions. For each trial, three concentrations (0.125%, 0.25% and 0.50%) plus the control were used; all the treatments were replicated four times. Hence, 64 glass cavity blocks were used for each test, and 2 ml of the tested concentrations or sterile bidistilled water (control) were introduced in each of them. Incubation always took place in a growth chamber in the dark at 25°C.

Eggs and juveniles used in this study were collected from the same generation of *M. incognita* obtained from infected tomato roots var. Marmande reared in pots filled with a commercial compost in a greenhouse at 25°C ± 2°C.

Unsegmented and embryonated eggs

The eggs were collected manually from egg masses isolated from infected tomato roots, rinsed with sterile water and soaked in water. Approximately 102 to 108 of unsegmented eggs and 130 to 158 embryonated eggs (these last ones were at late stage of egg development) released from egg masses and selected separately (at light microscope with magnifications of 250x) using a micro-brush was added to a suspension of 10 µl of water in each cavity block. Two ml of each extract concentration or of water were then poured into each cavity block. After a week of exposure to different plant extract concentrations a few of unsegmented eggs were collected and transferred to other cavity blocks containing only water to see if the embryogenesis was inhibited or stopped.

Second-stage juveniles hatched from unsegmented eggs were counted every 2 days for 22 days, while those hatched from embryonated eggs were counted daily for 13 days. At each observation, the hatched second-stage juveniles were withdrawn with a needle for nematodes. We considered it necessary to do this difference in observation days since the time required for unsegmented egg embryogenic development is longer than the time required for embryonated egg hatching.

Free second-stage juveniles

The egg masses isolated from infected tomato roots and rinsed with sterile water were placed in a suitable cotton-wool milk filter soaked in water (Oostenbrink, 1960) for 48 hours to collect only vital juveniles. A suspension of 10 µl of water, with an approxymately number of 98 to 103 active juveniles, was transferred to each cavity block containing 2 ml of each extract concentration or of water. The activity of juveniles was recorded daily for 12 days.

Since the juveniles contacted with the product are at once appeared inactive, a few of these juveniles were collected after 2, 4 and 6 days of exposure to the different plant extract concentrations, and transferred to other cavity blocks containing only water, to see if they would regain motility. This was done to determine if the different concentrations of the product had nematostatic and/or nematicidal action. The juveniles were observed after 24-48 hours to record their percentage revival.

Second-stage juveniles of the egg masses

Five egg masses of uniform size and of the same age were isolated from infested roots using a pair of diamond tweezers rinsed with sterile water and then transferred to 10 µl of water per cavity block for a total number of 80 egg masses per test (5 egg masses x 4 treatments x 4 replications). Then 2 ml of each extract concentration or of water were added.

The observations and the counting of hatched juveniles were carried out every 2 days for 22 days, and the hatched juveniles were always removed with a needle. A final count of eggs and juveniles remaining in the egg masses together with the already hatched juveniles was performed on the 22nd day to calculate the mean initial number of five egg masses for each treatment. During this count, we considered all eggs and dead and alive juveniles, also observing their aspect and if they showed any kind of alteration. The mean initial number of eggs and juveniles per cavity block fluctuated between 847 and 1100.

Data analysis

The statistical analysis was carried out with Statistica Soft. Percentage data were transformed using degree-arcsine square-root before being subjected to the statistical analysis. Initially the data from the two Tests were analysed separately with Student's t test to evaluate differences between the concentrations used. They were then subjected to analysis of variance (ANOVA) followed by the Tukey HSD test. Moreover, the results from the two tests using the same concentration were compared by Student's t test.

RESULTS

UNSEGMENTED EGGS (Table 1)

No juveniles hatched from unsegmented eggs during the first 4 days of observation. In the following days, the cumulative percentage of hatched juveniles increased gradually in the controls, reaching mean values of 76.72% in Test 1 and 76.29% in Test 2 on the 22th day. From eggs exposed to the 0.125% extract, only 0.96% of juveniles hatched after 6 days in Test 1 and 0.69% in Test 2; in Test 1 and in Test 2 the value remained unchanged until the last day. Only a few eggs showed some development. No juveniles hatched from eggs kept in the more concentrated solutions of 0.25% and 0.50% in either test, the eggs remained unembryonated. The product acted stopping the embryogenesis indeed the unsegmented eggs transferred in water remained at a non-differentiated development stage (did not showed any stage of egg development). The treated eggs seemed disintegrated internally.

EMBRYONATED EGGS (Table 2)

At the end of the 1st day, juvenile hatching was observed in all the treatments, especially in the controls (mean 14.65% eggs hatched in Test 1 and 15.38% in Test 2); in the other treatments, hatching fluctuated between 0.78 and 2.36% in Test 1 and between 0.76 and 1.11% in Test 2. In the controls, hatching increased gradually until the 13th day, reaching mean values of 78.08% in Test 1 and 78.64% in Test 2. In the other treatments, hatching ceased after the 1st day in Test 1 and essentially after the 3rd day in Test 2. It was very difficul to recognize the original shape of embryos.

FREE SECOND-STAGE JUVENILES (Table 3)

At the end of the 1st day, juvenile motility was completely inhibited by all the clove oil treatments, whereas on the 12th day in the controls juvenile motility gradually decreased to mean values of 82.18% of still active juveniles in Test 1 and 82.44% in Test 2.

Transfer to water of inactive juveniles soaked in the three clove oil concentrations confirmed the nematicidal action of the product even at the lowest concentration. In fact, no juveniles regained motility in either test. The treated juveniles appeared extremely vacuolized internally.

SECOND-STAGE JUVENILES OF THE EGG MASSES (Tables 4 and 5)

The egg masses soaked in water (controls) showed mean hatching rates of 12.27% in Test 1 and 12.30% in Test 2 after the first 2 days; the values gradually increased until the end of the tests, reaching means of 57.18% in Test 1 and 57.25% in Test 2. In contrast, there was negligible emergence (0.14-0.41% in Test 1 and 0.13-0.39% in Test 2) from the egg masses soaked in the different clove oil concentrations and hatching ceased at once in both tests (Table 4).

Table 1 – Effect of different clove oil extract concentrations on the hatching of *M. incognita* unsegmented eggs: cumulative percentages of hatched second-stage juveniles.

	Conc. %	Exposure time (days)										
		2	4	6	8	10	12	14	16	18	20	22
Test 1	0.000	0.00	0.00	12.57	20.16	33.72	45.45	58.21	74.29	75.85	76.72	76.72
	0.125	0.00	0.00	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96
	0.250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Test 2	0.000	0.00	0.00	11.95	20.73	30.64	43.04	55.99	72.13	74.44	75.83	76.29
	0.125	0.00	0.00	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.69	0.6
	0.250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Data are means of 4 replications.

Table 2 – Effect of different clove oil extract concentrations on the hatching of *M. incognita* embryonated eggs: cumulative percentages of hatched second-stage juveniles.

	Conc. %	Exposure time (days)												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Test 1	0.000	14.65	23.44	30.94	38.81	43.30	47.06	51.55	58.00	65.65	72.64	77.54	77.89	78.08
	0.125	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
	0.250	2.36	2.36	2.36	2.36	2.36	2.36	2.36	2.36	2.36	2.36	2.36	2.36	2.36
	0.500	1.97	1.97	1.97	1.97	1.97	1.97	1.97	1.97	1.97	1.97	1.97	1.97	1.97
Test 2	0.000	15.38	22.57	31.16	38.20	42.19	47.83	51.69	57.42	65.81	72.83	77.73	78.35	78.64
	0.125	0.76	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92
	0.250	1.03	1.72	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09	2.09
	0.500	1.11	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70

Data are means of 4 replications.

Table 3 – Effect of different clove oil extract concentrations on the juvenile motility of *M. incognita*: cumulative percentages of active second-stage juveniles.

	Conc. %	Exposure time (days)											
		1	2	3	4	5	6	7	8	9	10	11	12
Test 1	0.000	97.19	95.48	93.81	93.53	93.25	92.97	91.71	89.22	85.02	84.00	82.95	82.18
	0.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Test 2	0.000	97.22	95.70	93.90	93.14	92.36	91.86	91.35	90.59	86.78	84.76	82.70	82.44
	0.125	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.250	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Data are means of 4 replications.

Table 4 – Effect of different clove oil extract concentrations on the juvenile hatching from *M. incognita* egg masses: cumulative percentage of hatched second-stage juvenile.

	Conc. %	Exposure time (days)										
		2	4	6	8	10	12	14	16	18	20	22
Test 1	0.000	12.27	24.11	28.52	37.92	45.49	49.04	51.53	53.40	55.32	57.18	57.18
	0.125	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
	0.250	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
	0.500	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Test 2	0.000	12.30	24.11	28.50	37.95	45.50	49.07	51.55	53.25	55.41	57.25	57.25
	0.125	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
	0.250	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
	0.500	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31

Data are means of 4 replications.

Table 5 – Contents of egg masses exposed to different clove oil extract concentrations on the 22nd day: percentages of eggs and second-stage juveniles.

	Conc. %	Hatching juveniles	Eggs and juveniles remaining in the egg masses		
			Eggs	Alive juveniles	Dead juveniles
Test 1	0.000	57.18	41.13	0.00	1.69
	0.125	0.41	99.59	0.00	0.00
	0.250	0.14	99.86	0.00	0.00
	0.500	0.32	99.68	0.00	0.00
Test 2	0.000	57.25	41.14	0.00	1.61
	0.125	0.39	99.61	0.00	0.00
	0.250	0.13	99.87	0.00	0.00
	0.500	0.31	99.70	0.00	0.00

Data are means of 4 replications.

The final count (Table 5) to evaluate the number of eggs and alive or dead juveniles remaining inside of the egg masses showed mean control values of 41.13% in Test 1 and 41.14% in Test 2 for eggs and 1.69% in Test 1 and 1.61% in Test 2 for dead juveniles (there were no alive juveniles). In the other treatments the entire initial amount of eggs remained in the egg masses at the end of the tests and there were no traces of hatched juveniles. The eggs seemed disintegrated internally and in the one containing an embryo it was impossible to recognize the shape of the juvenile.

The results of the ANOVA followed by the Tukey HSD test are reported in Table 6 while those of Student's t test are in Table 7.

Table 6 – ANOVA results.

Developmental stage	Conc. %	Test 1	Test 2
Unsegmented eggs	0.000	61.55 A	61.16 A
	0.125	4.80 B	4.12 B
	0.250	0.00 B	0.00 B
	0.500	0.00 B	0.00 B
		F = 249.11	F =341.91
Embryonated eggs	0.000	62.25 A	62.49 A
	0.125	4.26 B	5.47 B
	0.250	7.13 B	8.60 B
	0.500	7.98 B	7.47 B
		F = 189.42	F = 5221.33
2nd stage juveniles	0.000	65.08 A	65.22 A
	0.125	0.00 B	0.00 B
	0.250	0.00 B	0.00 B
	0.500	0.00 B	0.00 B
		F = 4460.60	F = 301210.8
Egg masses	0.000	49.15 A	49.19 A
	0.125	3.08 B	3.55 B
	0.250	1.49 B	1.74 B
	0.500	2.62 B	2.98 B
		F = 334.70	F = 506.62

Percentage data are means of four replications; they were transformed with degree-arcsine ($x^{0.5}$) before being subjected to analysis of variance. Values in each column followed by different letters are significantly different (Tukey HSD test, P=0.05).

Table 7 – Test t di Student results.

Developmental stage	Test	Concentration %			
		0.000	0.125	0.250	0.500
Unsegmented eggs	1	61.55	4.80	0.00	0.00
	2	61.16	4.12	0.00	0.00
		p value = 0.85	p value = 0.38	–	–
Embryonated eggs	1	62.25	4.26	7.13	7.98
	2	62.49	5.47	8.60	7.47
		p value = 0.89	p value = 0.41	p value = 0.65	p value = 0.53
2nd stage juveniles	1	65.08	0.00	0.00	0.00
	2	65.22	0.00	0.00	0.00
		p value = 0.89	–	–	–
Egg masses	1	49.15	3.08	1.49	2.62
	2	49.19	3.55	1.74	2.97
		p value = 0.73	p value = 0.66	p value = 0.85	p value = 0.57

Percentage date are means of 4 replications; they were trasformed with degree-arcsine ($x^{0.5}$) before being subject to statistical analysis.

DISCUSSION

Commercialization of pesticides based on plant essential oils and their major constituents may represent an alternative source for control products, since they have a broad spectrum of activity against important insects, plant pathogenic fungi, bacteria and a range of phytoparasitic nematodes. Terpenoids are the major components of essential plant oils and those with strong nematicidal properties are resulted eugenol, linalool and geraniol (CHATTERJEE *et al.*, 1982; CHITWOOD, 2002), their effect appeared to be irreversible.

In the present study potent nematicidal activity was observed in eugenol-clove oil, in fact even the lowest concentration (0.125%) stopped the embryogenesis of unsegmented eggs, only 0.96% of second-stage juveniles hatched from eggs in Test 1 and 0.69% in Test 2 after 4 days, their development stopped permanently. The more

concentrated solutions (0.25% and 0.50%) induced immediately a total stop of embryogenesis in both tests.

Each concentration of the product also acted quickly on embryonated eggs: in Test 1, juveniles hatched only on the 1st day of observation, with very low percentage values of 0.78-2.36, while in Test 2, they hatched only in the first 3 days, with percentage values of 0.92-2.09.

Clove oil extract also had a very significant action on juvenile motility. All concentrations had a complete nematicidal action after 24 hours exposure in both tests, and inactive juveniles transferred to water failed to regain motility.

Finally, the different concentrations of clove oil had a highly significant effect on eggs and juveniles of the egg masses. As soon as the egg masses were exposed to the extracts, the embryogenesis of eggs and the hatching of juveniles were stopped. Our results revealed the efficacy of the extract on *M. incognita* unsegmented and embryonated eggs, free second-stage juveniles and second-stage juveniles of the egg masses. The eggs and embryos in our study seemed disintegrated internally and it was impossible to recognize their original shape, furthemore the inactive juveniles appeared internally vacuolized.

The statistical analysis showed highly significant differences between the control value and the values obtained with the three clove oil concentrations in each test and for all the developmental stages (Table 6). In addition there are not statistically significant differences between the results of two tests, as shown by the results Student's t test (Table 7).

Our results confirm the nematicidal activity exhibited *in vitro* by eugenol, the most active compound of clove oil, reported from several groups of researches. Indeed this compost was active against the second-stage juveniles of *Anguina tritici*, Steinbuch, *Tylenchulus semipenetrans*, Cobb, *Heterodera cajani,* Koshy and *Meloidogyne javanica* (Treub) Chitwood (SANGWAN *et al.*, 1990). The concentration of eugenol providing 50% mortality (LC_{50}) of juvenile populations of *M. javanica* was the highest 1240 µm/ml, while the lowest value was 33 µm/ml for *A. tritici*. Previously a commercial standard of eugenol, extract from *Ocimum basilicum* L. leaves, was able to kill *M. incognita* juveniles in 160 minutes (CHATTERJEE *et al.*, 1982). Very recently a good nematicidal activity against a mixture of juveniles and adults of *B. xylophilus* was achieved with essential oil of *E. caryophillata* at 1000 µm ml/l causing 100% immobility at 24 h of exposure after treatment (PARK *et al.*, 2005). Lethal activity was observed always using essential oil of clove bud a 10 mg/ml solution against *B. xylophilus* adults (KONG *et al.*, 2006). After 24 h of exposure 100% mortality was obtained, the treated nematodes were dead within 3 hours and their bodies showed usually an extended shape without movement.

In microwell tests the mean effective concentrations of clove oil required to kill 50% of second stage juvenile population of *M. incognita* or to reduce egg hatch by 50% was 0.145% and 0.097% respectively (MEYER *et al.*, 2008a). These Authors besides in greenhouse studies, using a clove oil formulation previously resulted to be toxic to *M. incognita*, found that the concentrations not phytotoxic to tested vegetable crop seedlings, did not reduce consistently the *M. incognita* populations (MEYER *et al.*, 2008b). In fact current informations indicate that the essential oils, that are most efficacious against pests, are often the most phytotoxic (ISMAN, 2000).

The mode of action of essential oils and their components in unclear. In insects several essential oils inhibit acetylcholinesterase activity (RYAN & BIRNE, 1988). Since several essential oils have realed nematicidal and insecticidal activity and acetylcholine is a neurotransmitter in nematodes, it may suppose that they are involved in interrupting the nematode nervous system (OKA, 2001). Another possibility is that these natural products may disrupt the cell membrane of the nematode and change its permeability, mechanism suggested to explain the fungicide activity (OKA, 2001).

In conclusion, clove oil extract could have interesting applications as a novel control strategy in nematode pest management. It is economical, its effects against different developmental stages of the root-knot nematodes appear rapidly at the lowest concentration, and as far as we know it is a good fungicide (WALTER *et al.*, 1997). Therefore, this substance could be an effective mean of reducing nematode populations in crop systems. Soil treatment with clove oil extract could serve as potential alternative to used currently nematode control agents. However further study is necessary to develop formulations, concentrations, doses and application times to improve the clove oil efficacy and to reduce its phytotoxicity to vegetal crop seedlings.

RIASSUNTO

EFFETTI DI UN ESTRATTO DI OLIO DI CHIODI DI GAROFANO SULLE UOVA E SULLE LARVE DI SECONDO STADIO DI MELOIDOGYNE INCOGNITA *(KOFOID* ET *WHITE) CHITWOOD*

Sono state condotte prove *in vitro* per valutare l'azione dell'estratto di olio di chiodi di garofano ABT-EU04® (Xeda International S.A.), il cui principio attivo è derivato da gemme di *Syzygium aromaticum* (L.) Merrill *et* Perry, nei confronti del nematode *Meloidogyne incognita* (Kofoid *et* White) Chitwood. Tre concentrazioni del prodotto (0.125%, 0.25% e 0.50%) sono state testate su uova insegmentate, uova embrionate, forme giovanili di secondo stadio libere e incluse negli ovisacchi di *M. incognita*. L'esperimento, ripetuto due volte, è stato eseguito in piccoli contenitori di vetro tenuti al buio e a una temperatura costante di 25°C. I dati sono stati analizzati statisticamente. L'estratto di chiodi di garofano ha bloccato l'embriogenesi delle uova e ha mostrato un'azione nematocida nei confronti delle forme giovanili di secondo stadio sia libere che racchiuse negli ovisacchi. Queste proprietà rendono questo prodotto un interessante mezzo per una nuova strategia di controllo del nematode fitoparassita.

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