RESEARCH NOTE - NOTA INVESTIGATIVA

IN VITRO NEMATICIDAL ACTIVITY OF THE EXPERIMENTAL FORMULATION TEQUIL AGAINST *MELOIDOGYNE INCOGNITA* AND *HETERODERA DAVERTI*

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ABSTRACT

Giacometti R., G. d'Errico, and F. P. d'Errico. 2010. *In vitro* nematicidal activity of the experimental formulation Tequil against *Meloidogyne incognita* and *Heterodera daverti*. Nematropica 40:263-268.

A trial was conducted *in vitro* to assess the effectiveness of the experimental formulation Tequil, a blend of plant extracts (100% aqueous extract of *Quillaja saponaria*, *Yucca schidigera* and *Tagetes* spp.), on motility of second-stage juveniles (J2) of the root-knot nematode *Meloidogyne incognita* and of the cyst nematode *Heterodera daverti*. The effects of three concentrations (2.5, 5 and 10 ml/L H_2O) of Tequil were compared with those of a control and ethoprophos (1.5 ml/L H_2O) after different exposure times (2 to 24 days). All concentrations of Tequil suppressed the motility of J2 of *M. incognita*, throughout the observation period compared to water control, however, the 2.5 ml/L only reduced movement during the first 10 days. Similar effects were observed with *H. daverti*. For both nematodes, the nematicidal activity increased with the increase of the concentration of Tequil and exposure time. However, ethoprophos was more effective than Tequil during the first 18 days of exposure. Moreover, removing J2 from Tequil solutions after 6 days and transferring them to water for up to 240 h, revealed that a proportion of them resumed motility. This indicates that, at least at the tested concentrations, Tequil, besides a nematicidal activity, has a nematostatic effect.

Key words: *Heterodera daverti*, *Meloidogyne incognita*, in vitro nematicidal activity, Tequil.

RESUMEN

Giacometti R., G. d'Errico, and F. P. d'Errico. 2010. Actividad nematicida *in vitro* de la formulación experimental de Tequil contra *Meloidogyne incognita* y *Heterodera daverti*. Nematropica 40:263-268.

Se condujo un ensayo *in vitro* para evaluar la efectividad de la formulación experimental Tequil, la cual es una mezcla de extractos vegetales (100% extracto acuoso de *Quillaja saponaria*, *Yucca schidigera* y *Tagetes* spp.), sobre la movilidad de juveniles de segundo estadio (J2) del nematodo agallador *Meloidogyne incognita* y del nematodo quiste *Heterodera daverti*. Se compararon los efectos de tres concentraciones (2.5, 5 y 10 ml/L H_2O) de Tequil con controles de agua y de etoprofos (1.5 ml/L H_2O) con diferentes tiempos de exposición (2 a 24 días). Todas las concentraciones de Tequil afectaron la movilidad de J2 de *M. incognita*, pero la concentración de 2.5 ml/L sólo redujo el movimiento durante los primeros 10 días. Se observaron efectos similares con *H. daverti*. Para ambos nematodos, la actividad nematicida aumentó con el aumento de la concentración de Tequil y con el aumento del tiempo de exposición. Sin embargo, etoprofos fue más efectivo que Tequil durante los primeros 18 días de exposición. Si se transfieren los J2 de las soluciones de Tequil a agua después de 6 días, una proporción de los juveniles recupera la movilidad. Esto sugiere que, al menos para las concentraciones evaluadas, Tequil posee un efecto nematostáctico además de la actividad nematicida.

Palabras clave: actividad nematicida *in vitro*, *Heterodera daverti*, *Meloidogyne incognita*, Tequil.

The pressing demand for environmentally friendly productions and the drastic reduction in the number of synthetic pesticides available have led to a growing interest in the nematicidal activity of plant compounds. So far, over 2000 botanical species possessing biocidal activity of interest in agriculture have been reported (Ahmed *et al.*, 1984; Isman, 2000; Chitwood, 2002; Dubey *et al.*, 2010), but only a limited number of them appears promising for development as agro-chemicals.

In Italy, many nematodes damage crop plants. Of them the most severe and widespread are *Meloidogyne* spp. Moreover, *Heterodera daverti* Wouts *et* Sturhan has been reported as a severe pest of carnation in the Campania region (Ambrogioni *et al.*, 1986; Ambrogioni and d'Errico, 1994). Therefore, preliminary tests were conducted to assess the nematicidal effectiveness of Tequil, an experimental mixture of plant extracts, against these two nematodes.

The trials were conducted *in vitro* using the root-knot nematode *Meloidogyne incognita* (Kofoid *et* White) Chitw. and the cyst nematode *Heterodera daverti*. *Meloidogyne incognita* was isolated from roots of tomatoes and *H. daverti* from roots of carnations. Both the plant species were increased in pots in unheated greenhouses located in the Campania region.

To obtain second stage juveniles (J2's) of the two nematode species, infected roots of both plant species were washed free of adhering soil, minced and placed on a cotton wool filter for 48 hours (Oostenbrink, 1960). The experimental formulation Tequil (containing 100% aqueous extract of *Quillaja saponaria* Molina (80%), *Yucca schidigera* Roezl (10%) and *Tagetes* spp. (10%)), produced and marketed by the Italian company Fertenia s.r.l., was used at three concentrations (2.5 ml/L, 5 ml/L and 10 ml/L) obtained by serial dilutions in distilled water. Aliquots of 2 ml of each solution were poured in each well of 4 ml-well plates with lid to prevent evaporation of the solutions. Each well received 100 hand-picked motile J2's of either nematode species. The effects of Tequil concentrations were compared with that of J2's in distilled water (control) and in the nematicide ethoprophos (19% a.i.) at the field application recommended rate (1.5 ml/L). The pH of all solutions was adjusted to 5 ± 0.1 by adding citric acid. Each treatment was replicated four times and arranged in a completely randomized design. Plates were maintained in the dark at 20°C. The test was run for 24 days and motile and non-motile juveniles were counted every other day.

To ascertain whether the tested solutions had nematicidal or nematostatic activity, after 6 days 32 non-motile J2, except those in distilled water, were removed from the four replicated wells of every test solution of the previous experiment and transferred to four separate wells (8 J2's per each of the four replicates) containing distilled water and observed up to 240 hours for resumption of the motility. Motile and non-motile J2's were observed under 16X magnification on a stereo microscope. Juveniles were considered non-motile if their bodies were straight and did not move even after mechanical prodding.

All data were subjected to analysis of variance and treatments means compared with the water control using Fisher's LSD test at $P < 0.05$.

Tequil significantly suppressed the motility of J2's of both nematode species compared to water control (Tables 1 and 2). The number of motile J2's of *M. incognita* in the solution of Tequil at 5 and 10 ml/L was significantly less than in the control since the first observation

Table 1. Effect of different concentrations of and exposure times to Tequil on the motility of second-stage juveniles of Meloidogyne incognita.

	Exposure time (days)											
	2	4	6	8	10	12	14	16	18	20	22	24
Treatment	Number of motile second-stage juveniles											
Ethoprophos 1.5 ml/L	23.0 c[x]	6.5 c	3.5 c	1.0 c	1.0 c	0.5 d	0.5 d	0.0 d	0.0 e	0.0 c	0.0 b	0.0 b
Tequil 2.5 ml/L	99.0 a	92.0 a	92.0 a	87.0 a	83.0 a	73.5 b	58.5 b	35.5 b	23.5 b	5.5 b	0.5 b	0.0 b
Tequil 5 ml/L	64.5 b	47.5 b	49.0 b	31.0 b	31.0 b	29.5 c	28.0 c	18.0 c	7.5 c	1.0 bc	0.0 b	0.0 b
Tequil 10 ml/L	60.5 b	50.5 b	38.0 b	30.5 b	25.0 b	25.0 c	22.0 c	15.5 c	3.0 d	0.5 bc	0.0 b	0.0 b
Untreated	100.0 a	100.0 a	100.0 a	99.5 a	100.0 a	99.5 a	99.5 a	99.0 a	98.0 a	98.0 a	91.0 a	59.5 a
LSD (0.05)	6.5	10.3	12.5	16,3	19.3	10.1	12.5	6.7	2.3	5.3	61.7	35.2

[x]Means with same letter in the same column are not significantly different based on Fisher's LSD test at $P < 0.05$.

Table 2. Effect of different concentrations of and exposure times to Tequil on the motility of second-stage juveniles of *Heterodera daverti*.

	Exposure time (days)											
	2	4	6	8	10	12	14	16	18	20	22	24
Treatment	Number of motile second-stage juveniles											
Ethoprophos 1.5 ml/L	19.5 c[x]	7.5 d	1.5 c	0.5 d	0.5 d	0.0 d	0.0 e	0.0 d	0.0 d	0.0 c	0.0 b	0.0 b
Tequil 2.5 ml/L	94.5 a	93.0 a	91.0 a	80.5 b	78.5 b	75.5 b	55.5 b	21.0 b	19.5 b	4.5 b	0.0 b	0.0 b
Tequil 5 ml/L	63.5 b	64.0 b	47.5 b	34.5 c	32.5 c	26.5 c	28.0 c	20.5 b	6.5 c	2.5 bc	0.5 b	0.0 b
Tequil 10ml/L	59.0 b	49.5 c	36.5 b	27.0 c	25.5 c	20.5 c	18.5 d	7.0 c	4.5 c	1.0 bc	0.0 b	0.0 b
Untreated	100.0 a	100.0 a	100.0 a	100.0 a	99.0 a	99.0 a	98.5 a	98.5 a	98.0 a	97.5 a	89.5 a	64.5 a
LSD (0.05)	7.3	10.3	15.3	10.1	9.3	15.6	6.2	5.7	3.5	4.0	45.7	37.4

[x]Means with same letter in the same column are not significantly different based on Fisher's LSD test at $P < 0.05$.

and continued so throughout the observation period. After 2 days, nematode mobility was reduced by 40% in the Tequil 5 and 10 ml/L treatments as compared to the water control; however, ethoprophos reduced mobility by 77%. Motility in Tequil at the lowest concentration (2.5 ml/L) was also suppressed but only by 26% and only from the 12th day of exposure onwards. In general, the number of motile J2's was reduced with the increase of the concentration of Tequil from 2.5 to 5-10 ml/L and with the increase of the exposure time and no significant difference occurred between the two higher concentrations. Ethoprophos was significantly more effective than Tequil. Motile J2's were negligible (below 6%) or absent in ethoprophos solution starting from an exposure of 6 days onwards and in solutions of Tequil only after 18 days in 10 ml/L, 20 days in 5 ml/L and 22 days in 2.5 ml/L.

The effect of Tequil on *H. daverti* was similar to that on *M. incognita*. However, the motility of the J2 of this nematode in 2.5 ml of Tequil/L was significantly suppressed after 8 days of exposure. Also, after an exposure of 14 days, significantly less motile J2 were observed in 10 ml/L than in 5 ml/L. In the water control, between the day 22 and 24, the number of motile J2's of both nematode species was reduced.

When second-stage juveniles of both nematodes, exposed for 6 days to the nematicide solutions, were transferred to distilled water (Tables 3 and 4), no resumption of motility was observed of J2's previously exposed to ethoprophos. Of the J2's exposed to Tequil 60% of those previously incubated in 2.5 ml and 50% of those in 5 ml resumed motility during the first 12 hours in distilled water, but thereafter motility was reduced until 96 hours in distilled water and completely lost later on. In 10 ml Tequil/L resumption of J2 motility was significantly less (37%) after 8 hours in distilled water and again completely lost after 72 hours. Recovery of motility in water of J2's of *H. daverti* was similar to that of J2's of *M. incognita*.

Although less effective than ethoprophos, Tequil was somewhat effective in suppressing the motility of *M. incognita* and *H. daverti*. The resumption of the motility of a proportion of J2's of both nematode species transferred to distilled water after 6 days exposure to Tequil, indicates that at least at the tested concentrations, Tequil, has a nematostatic effect. These findings (nematostatic and nematicidal proprieties) agree with those in other substances such as those in *Azadirachta indica* Adr. Juss. plant extracts (Paruthi *et al.*, 1977; Ambrogioni *et al.*, 2003) and with those of several researches carried out, in vitro and in field, to verify the effect of aqueous extracts of *Tagetes* spp., *Quillaja saponaria* and *Yucca schidigera*, utilized individually, on different species of nematodes (Sainz, 1999; Insunza *et al.*, 2001; D'Addabbo *et al.*, 2005; San Martin and Magunacelaya, 2005).

A peculiar characteristic of Tequil is the various molecules from different plant species, which may synergistically interact increasing their effectiveness and stability. It is known that pest face greater difficulty neutralizing a group of molecules than a single molecule (Feng and Isman, 1995). This mixture could also reduce the chance of the pest to select populations resistant to the pesticide.

This preliminary trial suggests Tequil, a natural bionematicide, may be an optional alternative to synthetic chemicals for suppressing nematode populations. Further investigations are needed to confirm the efficacy of Tequil under field conditions and to determine the most appropriate rating and timing of applications.

Table 3. Recovery of motility of second-stage juveniles of *Meloidogyne incognita* exposed to test solutions for 6 days and then transferred to distilled water.

	Observation time (hr)												
	4	8	12	24	48	72	96	120	144	168	192	216	240
Test solution	Number of second-stage juveniles recovering motility												
Ethoprophos 1.5 ml/L	0.0 c[x]	0.0 c	0.0 c	0.0 b	1.0 b	1.0 b	0.0 c	0.0 b	0.0 b	0.0 b	0.0 b	0.0 a	0.0 a
Tequil 2.5 ml/L	5.5 a	5.5 a	5.0 a	3.0 a	3.0 a	2.5 a	2.5 a	1.5 a	1.0 a	1.0 a	1.0 a	0.0 a	0.0 a
Tequil 5 ml/L	5.0 a	5.5 a	4.0 a	2.5 a	2.5 a	1.5 b	1.0 b	1.0 a	0.0 b	0.0 b	0.0 b	0.0 a	0.0 a
Tequil 10 ml/L	3.0 b	3.0 b	1.5 b	1.0 b	1.0 b	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b	0.0 b	0.0 a	0.0 a
LSD (0.05)	1.7	1.9	1.3	1.2	1.1	0.8	0.7	0.8	0.5	0.5	0.5	—	—

[x]Means with same letter in the same column are not significantly different based on Fisher's LSD test at $P < 0.05$.

Table 4. Recovery of motility of second-stage juveniles of *Heterodera daverti* exposed to test solutions for 6 days and then transferred to distilled water.

	Observation time (hr)												
	4	8	12	24	48	72	96	120	144	168	192	216	240
Test solution	Number of second-stage juveniles recovering motility												
Ethoprophos 1.5 ml/L	0.0 c[x]	0.0 c	0.0 c	1.0 c	0.0 c	0.0 b	0.0 b	0.0 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Tequil 2.5 ml/L	6.5 a	6.5 a	5.0 a	5.0 a	3.0 a	1.5 a	1.5 a	1.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Tequil 5 ml/L	3.0 b	3.0 b	2.5 b	2.5 b	1.5 b	1.0 a	1.0 a	0.0 b	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Tequil 10 ml/L	4.0 b	3.0 b	1.5 b	1.5 c	1.0 b	1.0 a	1.0 a	1.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
LSD (0.05)	1.5	2.5	1.3	1.3	0.7	0.8	0.8	0.5	—	—	—	—	—

[x]Means with same letter in the same column are not significantly different based on Fisher's LSD test at $P < 0.05$.

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