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IN VITRO EFFECT OF ABAMECTIN FROM *STREPTOMYCES AVERMITILIS* ON THE SURVIVAL OF THE CYST NEMATODES *GLOBODERA PALLIDA, HETERODERA CAROTAE* AND *HETERODERA SCHACHTII*

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Abstract

The effect of an abamectin formulation (Vertimec© EC) was tested against the cyst nematodes Globodera pallida, Heterodera carotae and Heterodera schachtii in an in vitro hatching test. Abamectin (a mixture of two macrocyclic lactones B1a and B1b) is produced by the actinomycete Streptomyces avermitilis. Cysts of the nematodes were extracted from samples of infested soils by the Fenwick can and then subjected to different concentrations of an aqueous solution of the abamectin formulation (0, 1.125, 2.25, 4.5, 9.0, 18.0 and 36 µg/ml) and exposure times from 24 to 384 hours. For each nematode specie batches of 50 cysts were set up and arranged in a growth cabinet (20°C ± 2) according to a randomized block design. Untreated cysts were used as control. There were three replications for each treatment. As natural and artificial hatching agents for H. carotae, H. schachtii and G. pallida were used carrot root leachate, 0.3 mM zinc chloride and 0.6 mM sodium metavanadate aqueous solutions, respectively. Every week emerged juveniles were counted. At the end of the hatching test cysts were crushed and unhatched eggs and juveniles counted. The total number of juveniles emerged in the hatching test were expressed as the percentage of the total egg content of the cysts (hatched + unhatched). From percentage hatch was calculated the mortality of each treatment considering the natural death in the control. Data of percentage mortality were subjected to probit analysis to calculate values of lethal doses (LD) required for 50% egg mortality. At 24 hours exposure, values of LD_{50} for H. carotae, G. pallida and H. schachtii were 9.9, 13.2 and 796 µg/ml, respectively. For the same nematode species at 384 hours exposure LD_{50} decreased at 3.6, 2.9 and 17.6 µg/ml.

Key words*: cyst forming nematodes, lethal doses, nematicidal effect, nematode management.*

INTRODUCTION

A wide range of biologically active substances especially antibiotics and hydrolytic enzymes protecting plant growth from pathogenic fungi and pests are produced by Streptomycetes spp. (Jones and Samac, 1996; Trejo-Estrada et al., 1998; Sasanelli et al., 2016). Among these substances abamectin is known for its insecticidal, acaricidal and anthelmintic activities (Jayakumar, 2009; Poiras et al., 2013). Abamectin is a mixture of macrocyclic lactones (B1a and B1b) isolated from fermentation broths of the actinomycetes *Streptomyces avermitilis* (Burg et al.) Kim and Goodfellow. It is registered as an miticide/insecticide in many countries and it was introduced as bio-pesticide in 1985. Abamectin is commercialised under the names Affirm, Agri-Mek, Avid, Avomec, Dynamec, Vertimec EC and Zephyr depending on the formulations. Jansson and Dybas (1998) reported that avermectins blok the transmittance of electrical activity in nerves and muscle cells by the release of g-aminobutyric acid-like (GABA) neurotransmitters. Abamectin is an effective control method as bionematicide against some plant parasitic nematodes as *Pratylenchus penetrans* Cobb, Sher et Allen (Samac and

Kinkel, 2001), the reniform nematode *Rotylenchulus reniformis* Lindford and Oliveira and the root-knot nematodes *Meloidogyne incognita* (Kofoid et White) Chitw. and *M. arenaria* (Neal) Chitw. (Cayrol et al., 1993; Jayakumar, 2009; Laquale et al., 2014). Although it is known the nematicidal effect of the abamectin on these plant parasitic nematodes, few information is available on the effect of abamectin on the cyst forming nematodes. Therefore, the present in vitro investigation was undertaken to explore the potential nematicidal effect of different doses of an abamectin formulation (Vertimec© EC), applied for different exposure times, in the control of the cyst nematodes *Globodera pallida* (Stone) Behrens, *Heterodera carotae* Jones and *H. schachtii* Schmidt.

MATERIALS AND METHODS

The populations of *G. pallida*, *H. carotae* and *H. schachtii* used in the *in vitro* experiments were obtained from soil samples collected in infested fields at Conversano (Province of Bari, Apulia region, Italy) (40°.57'N, 17°.09'E), Zapponeta (Province of Foggia, Apulia region, Italy) (41°.45'N, 15°.96'E) and Luco dei Marsi (Province of L'Aquila, Abruzzo region, Italy) (41°.96'N, 13°.48'E), respectively. The cysts were collected by the Fenwick can from dried soil (Figure 1).

Figure 1. Apparatus of Fenwick for cysts extraction

For each nematode specie identification was based on morphological parametrs and on the shape of vulva and ano of ten cysts. Slides of vulval cones and juveniles were prepared for nematode identification by microscope observation (Figure 2).

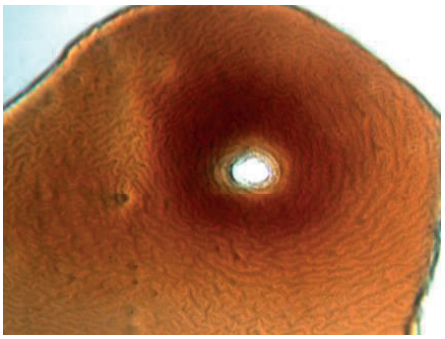

Figure 2. Vulval cone of *Globodera pallida*

Batches of 50 cysts of each nematode specie were placed in 2 cm diam sieves (215 µm aperture). Each sieve was put in a 3.2 cm diam Petri dish (Shepherd, 1986) and all dishes were arranged according to a complete randomised block design in a growth cabinet at 20°C with three replications for each treatment (Figure 3).

Figure 3. Batches of *Heterodera carotae* cysts for the hatching test

A commercial formulation of abamectin Vertimec© EC (a.i. abamectin 18 g/l), normally registered as insecticide and acaricide on stone fruit, citrus plants, flowers and vegetables, was used to prepare different concentrations from 0.0625 to 2 ml c.p./l, in a geometric series, corresponding to from 1.125 to 36 µg a.i./ml. The different concentrations were obtained by dissolving the largest rate of
the commercial formulation in distilled water. Four ml of each test solution, sufficient to cover cysts, were then added to each batch of cysts for different exposure times (0, 24, 48,

96, 192 and 384 hours). Untreated cysts were used as controls. After treatments cysts in each batch were removed from the test solutions and rinsed for 3 times in distilled water and then subjected to an hatching test to verify the nematicidal effect of each treatment (concentration x exposure time).

As hatching agents were used 0.6 mM sodium metavanadate aqueous solution, 3 mM zinc chloride aqueous solution and carrot root leachate for *G. pallida*, *H. schachtii* and *H. carotae*, respectively (Clarke and Sheperd, 1966; Sasanelli and D'Addabbo, 1995). Carrot root leachate was collected from 40 days-old actively growing carrot plants (cv. 92) (30 plants/pot) cultiva 2,500 ml clay pots, by drenching the soil with excess tap water. The leachate was then centrifuged at 1,300 rpm for 5 minutes, stored in plastic bottles and kept in a freezer until required. Only small amounts were kept at room temperature for immediate use (Figure 4).

Figure 4. Preparation of the carrot root leachate for the hatching test of *Heterodera carotae*

Juveniles (J2) emerging from cysts were counted and removed every week over a 10 week period renewing the hatching agents every week, according to an already described methodology (Sasanelli and Di Vito, 1991; Sasanelli and D'Addabbo, 1992). At the end of the experiments cysts were crushed according to Seinhorst and Den Ouden's technique (1966), and juveniles and unhatched eggs were counted. Numbers of J2 emerging weekly were expressed as cumulative percentages of the total egg content of the cysts (hatched + unhatched eggs). The number of extracted J2 per treatments were also expressed as percentage of those in the control and the difference to 100 as percentage mortalities, according to the following formula:

% Mortality = 100 - % hatched J2 where % hatched J2 = (% hatched J2 in the treatment/% hatched in the control) x 100.

The experiments were performed twice. Percentage mortality data were subjected to analysis of variance (ANOVA) and means compared by the Least Significant Difference's test (LSD's Test). Data were also subjected probit analysis (Finney, 1971) to estimate LD_{50} and LD_{90} rates for each exposure time. All statistical analysis were performed using the PlotIt software V. 3.2.

RESULTS AND DISCUSSIONS

The nematicidal effect of the different abamectin concentrations, in a range of exposure time (24-384 hours), on *G. pallida*, *H. carotae* and *H. schachtii* is reported in the Tables 1, 2 and 3, respectively.

For all cyst nematodes a significant increase of mortality was observed with the increase of abamectin dose (at the same exposure time) or exposure time (at the same concentration).

The use of an aqueous abamectin solution of 2.25 µg a.i./ml resulted in a significant increase of *G. pallida* mortality in comparison to that noted the lowest abamectin concentration at 24, 48 and 384 hours exposure (Table 1). An abamectin dose of 4.5 µg a.i./ml was effective for a significant increase of *G. pallida* mortality at 96 and 192 hours exposure compared to the previous used abamectin doses. The highest dose (36 µg a.i./ml) resulted in a *G. pallida* mortality ranging between 66.4 and 93.3% at the different exposures times resulting significantly higher than those observed for 18 µg a.i./ml at 24 and 48 hours (Table 1).

Heterodera carotae egg mortality for an exposure time of 24 hours was not affected by the used abamectin doses with the exception of the highest rate (Table 2). Nematode mortality ranged between 18.5-82.5, 23.4-90.3, 30.1-93.3 and 17.0-91.1% at 48, 96, 192 and 384 hours, respectively (Table 2). More than 50% *H. carotae* mortality was observed from 4.5 µg a.i./ml at 192 hours exposure time (Table 2). At the highest abamectin dose mortality of *H.*

carotae resulted higher than 80% just also at the lowest exposure time (Table 2).

Heterodera schachtii resulted less sensible than the two other cyst nematodes to abamectin aqueous solutions. Fifty % mortality was achieved only at the highest abamectin dose at 384 hours exposure (Table 3). The highest *H. schachtii* egg mortality (75.7%) was observed at 36 µg a.i./ml x 384 hours exposure time (Table 3).

Abamectin doses required to kill 50 and 90% vitality of *Globodera pallida*, *Heterodera carotae* and *Heterodera schachtii* eggs inside cysts treated with different concentrations of abamectin aqueous solutions (1.125, 2.25, 4.5, 9, 18 and 36 µg a.i./ml) were also calculated by probit analysis (Table 4). In the considered range of the exposure times, LD_{50} varied between 2.9-13.2 and 2.5-9.9 µg a.i./ml for *G. pallida* and *H. carotae,* respectively (Table 4). For *H. schachtii* it was not possible to calculate accurate LD values because for the exposure times from 24 to 192 hours mortality was lower than 50% (Table 3 and 4). For the sugar beet nematode 17.5 µg a.i./ml was calculated as LD_{50} at 384 hours exposure time (Table 4).

Based on results *H. schachtii* resulted less sensitive than *G. pallida* and *H. carotae* to abamectin treatments. The carrot cyst nematode resulted the most sensible to the bio-pesticide because its LD_{50} was lower than that calculated for *G. pallida* at each exposure time.

Table 1. Percentage mortality of *Globodera pallida* at different abamectin doses after a range of exposure times

Exposure time (hours)	Abamectin dose (µg a.i./ml)											
	1.125		2.25		4.5		9.0		18.0		36.0	
24	11.0[1]	a[2]	26.3	b	27.3	b	49.0	c	49.7	c	69.0	d
48	9.7	a	24.4	b	33.2	bc	39.5	c	39.1	c	66.4	d
96	13.5	a	28.8	ab	35.1	b	38.9	b	51.9	bc	69.2	c
192	21.4	a	30.0	a	55.0	b	75.5	c	76.4	c	77.3	c
384	9.8	a	50.6	b	75.0	c	84.1	cd	89.0	cd	93.3	d

[1]Each value is an average of six replications from two independent *in vitro* experiments;
[2]Data flanked in each row by the same letters are not statistically different according to Least Significant Difference's test (P≤0.05).

Table 2. Percentage mortality of *Heterodera carotae* at different abamectin doses after a range of exposure times

Exposure time (hours)	Abamectin dose (µg a.i./ml)											
	1.125		2.25		4.5		9.0		18.0		36.0	
24	25.0[1]	a[2]	31.3	a	39.1	a	38.7	a	49.0	a	81.0	b
48	18.5	a	36.0	ab	48.9	bc	52.4	bc	72.9	cd	82.5	d
96	23.4	a	45.0	abc	42.3	ab	73.7	bcd	76.2	cd	90.3	d
192	30.1	a	45.9	ab	62.7	b	86.7	c	90.4	c	93.3	c
384	17.0	a	45.7	b	60.2	bc	72.1	bcd	80.2	cd	91.1	d

[1]Each value is an average of six replications from two independent *in vitro* experiments;
[2]Data flanked in each row by the same letters are not statistically different according to Least Significant Difference's test (P≤0.05).

Table 3. Percentage mortality of *Heterodera schachtii* at different abamectin doses after a range of exposure times

Exposure time (hours)	Abamectin dose (µg a.i./ml)											
	1.125		2.25		4.5		9.0		18.0		36.0	
24	2.9[1]	a[2]	0.0	a	1.2	a	3.6	a	6.8	a	10.7	a
48	2.8	a	5.0	a	5.9	a	11.6	a	31.3	b	33.8	b
96	4.3	a	6.3	a	7.5	a	17.7	ab	30.9	b	32.6	b
192	1.4	a	5.7	ab	12.3	abc	19.2	bc	27.8	bc	34.7	c
384	1.0	a	7.7	ab	11.6	b	55.5	c	57.7	c	75.7	d

[1]Each value is an average of six replications from two independent *in vitro* experiments;
[2]Data flanked in each row by the same letters are not statistically different according to Least Significant Difference's test (P≤0.05).

Table 4. Abamectin doses required to kill 50 and 90% vitality of *Globodera pallida*, *Heterodera carotae* and *Heterodera schachtii* eggs inside cysts treated with different abamectin concentrations (1.125, 2.25, 4.5, 9, 18 and 36 µg a.i./ml)

Exposure time (hours)	LD_{50} (µg/ml)			LD_{90} (µg/ml)		
	Globodera pallida	*Heterodera carotae*	*Heterodera schachtii*	*Globodera pallida*	*Heterodera carotae*	*Heterodera schachtii*
24	13.2	9.9	---[1]	231.0	325.0	---
48	18.1	5.7	---	444.0	75.6	---
96	13.2	3.9	---	316.0	40.9	---
192	4.5	2.5	---	58.3	17.9	---
384	2.9	3.6	17.5	15.5	30.0	61.1

[1]For *Heterodera schachtii* it was not possible to calculate accurate LD values because for the indicated exposure times (24-192 hours) mortality was lower than 50%.

CONCLUSIONS

Results from the *in vitro* experiments clearly demonstrate the efficacy of the abamectin solutions in the control of cysts nematodes, although *H. schachtii* seemed to be less sensible in comparison to *G. pallida* and *H. carotae.* The use of abamectin solutions appears to be a promising tool to use in Integrated Pest Management programs and organic farming. The reduction of eggs viability inside cysts could help growers to reduce soil nematode population density for the following susceptible crops.

However, further studies are suggested to investigate the effect of abamectin in field condition with different types of soils and nematode genera and species.

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