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NEMATICIDAL EFFICACY OF NEW ABAMECTIN-BASED PRODUCTS USED ALONE AND IN COMBINATION WITH INDOLEBUTYRIC ACID AGAINST THE ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA*

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d'Errico G., Marra R., Vinale F., Landi S., Roversi P.F., Woo S.L. – Nematicidal efficacy of new abamectin-based products used alone and in combination with indolebutyric acid against the root-knot nematode *Meloidogyne incognita*.

Abamectin has been proven to be effective in controlling root-knot nematodes (*Meloidogyne* spp.). *In vitro* and pot experiments were conducted to test the effects of Tervigo® and two new abamectin-based products (CHA 2061-02 EW and CHA 2080 SC), emulsion-water (EW) and liquid suspension-concentrate (SC) formulations, at different doses on *M. incognita*. The activity of the EW abamectin formulations were tested alone and in combination with indolebutyric acid (IBA), to assess the effect of the products applied as a soil drench to tomato planted in sterilized and naturally nematode-infested soils. *In vitro*, both Tervigo® and CHA 2061-02 EW products mainly showed a nematostatic activity against second-stage juveniles (J2s) of *M. incognita*. For the most part, J2s that were immobilized following exposure to these products, after rinsing and transfer to water resumed mobility over time. Both formulations were also effective *in vivo* for the control of *M. incognita*-infested tomato plants. No visual symptoms of phytotoxicity were detected for either of the products. Root applications of CHA 2061-02 EW plus the addition of IBA improved overall plant growth, but not nematode control. Results demonstrated that both abamectin formulations have the potential for nematode control in tomato production. Additionally, the combination with IBA could be useful to enhance plant growth, in short-term production cycles or when nematode population densities are relatively low. Tested Abamectin-based products may be used for *M. incognita* management programs.

KEY WORDS: abamectin, indolebutyric acid, root-knot nematodes, *Solanum lycopersicum*, Tervigo®.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are among the most damaging pests to agricultural production in a wide range of crops (CHITWOOD, 2002). In Italy, tomato root-knot caused by *Meloidogyne incognita* (Kofoid & White) Chitwood is considered a very serious disease. The negative side effects to the environment and human health associated with chemicals applied for nematode control, as well as restrictions in the use of nematicidal fumigants, have prompted worldwide research towards alternative methods for nematode control (NICO *et al.*, 2004). Avermectins are a class of macrocyclic lactones, isolated from the soil bacterium *Streptomyces avermitilis* Kim & Goodfellow, that have been demonstrated to act as potential soil nematicides (PUTTER *et al.*, 1981; OMURA & SHIOMI, 2007). Several avermectin formulations are available to control mite and insect attack of plants (KHALIL, 2013). The avermectins act as Gamma-Amino Butyric Acid (GABA) mimetic, blocking the transmittance of electrical activity at the neuromuscular junctions (BLOOMQUIST, 1996; RÖDER & STAIR, 1998). Abamectin, a blend that contains about 80% of avermectin B1a and 20% of avermectin B1b, is used for a wide range of commercial formulations that exhibit good efficacy against nematodes (PITTERNA *et al.*, 2009; SAAD *et al.*, 2012; KHALIL, 2013). The success of a crop protection product depends not only on the active ingredient and its mode of action, but also on its formulation, which plays an important

role in guaranteeing homogeneity of distribution and effective penetration (BARBOSA DOS SANTOS *et al.*, 2005). Abamectin is characterized by low toxicity to non-target beneficial organisms and the environment, and is considered a valid component in integrated pest management programs (IPM) for controlling crop pests (GARABEDIAN & VAN GUNDY, 1983; KHALIL, 2013). There are several benefits associated with the use of abamectin, such as its rapid degradation by soil microorganisms, that result in lower residues in or on crops and ground water (KHALIL, 2013). Abamectin has been successfully used for several crops showing many advantages of its use as pesticide (KHALIL, 2013).

A suspension concentrate (SC) of abamectin (Tervigo® SC, Syngenta) has been recently registered in Italy for Root Knot Nematode (RKN) control at the dose of 5 L ha⁻¹, applied at 10-14 d intervals to different crops. In the present study, this commercial product was tested and compared to other nematicides, as well as formulations of an abamectin emulsion in water (CHA 2061-02 EW) and an abamectin SC (CHA 2080 SC), which are pending registration as nematicides. The effects of these two products on *M. incognita* are currently unknown. Additionally, no data is yet available on the interaction of abamectin with phytohormones belonging to the auxin family, such as Indole-3-Butyric Acid (IBA). IBA is a plant growth regulator widely used to promote growth and development, to contrast biotic and abiotic stresses and to increase crop

yields (HARTMANN *et al.*, 1997; TOGNETTI *et al.*, 2010). Given the growing interest in using non-toxic compounds for nematode control, additional data are needed to determine their potential applications. The objectives of this study were to evaluate: 1. the effect of Tervigo® SC and CHA 2061-02 EW at different doses, on the mobility of second-stage juveniles (J2s) *in vitro* bioassays; 2. the efficacy of CHA 2061-02 EW and CHA 2080 SC used at different doses, compared to commercial Tervigo® SC used at the recommended doses on nematodes *in vivo* bioassays; and 3. the effects of CHA 2061-02 EW alone and in combination with IBA on plants grown in nematode-infested and sterilized soils.

MATERIALS AND METHODS

CHEMICALS

Abamectin 18 g L⁻¹ EW (CHA 2061-02 EW; Cheminova), as an emulsion water at final doses of 5, 7.5 and 10 L ha⁻¹ was used. The highest application dose (10 L ha⁻¹) of abamectin 18 g L⁻¹ EW was mixed with IBA (Hormon L; Cheminova) at a dose of 2 L ha⁻¹. Abamectin 180 g L⁻¹ SC (CHA 2080 SC; Cheminova) was suspended in water at doses of 1 and 5 L ha⁻¹. Other commercial nematicidal products were used as a reference for comparison: Fosthiazate (Fost) 150 g L⁻¹ (Nemathorin 150 EC; Syngenta), oxamyl (Oxam) 100 g L⁻¹ (Vydate 10 L; DuPont) and abamectin 20 g L⁻¹ (Tervigo® SC 1; Syngenta), were used at the label recommended doses for a field application in 15.000 L (H₂O) ha⁻¹. Chemicals are presented and identified by specific treatment codes as reported in Table 1.

IN VITRO EXPERIMENTS

The nematode population was previously morphological and molecular identified (D'ERRICO *et al.*, 2014) and reared on tomatoes planted in infested soil in the greenhouse of the University of Naples Federico II. Eggs of *M. incognita* were collected from infested roots of tomato *Solanum lycopersicum* L. cv. Naxos, using NaOCl (HUSSEY & BARKER, 1973). J2s were collected from eggs spread in hatching boxes using a modified Jenkins method (JENKINS, 1964). Only freshly hatched J2s (24 hours old) were used for the experiments. Twenty-five *M. incognita* J2s were added to individual wells, each containing the chemical solutions

under assessment, wrapped in parafilm and incubated at 25±2°C, in the dark. The J2s exposed to water served as controls. Nematode mobility was evaluated every 24h, whereby the reversibility or irreversibility of immobilized J2s was determined by rinsing and shifting the motionless nematodes to distilled water (GIACOMETTI *et al.*, 2010), and subsequently counting the number of immobile J2s that became motile. J2s that did not recover their motility in water and confirmed no movement after poking them with a needle during an observation period of 10 s were considered dead. The experiment was performed twice and each treatment was replicated 4 times. The nematicidal potential of abamectin-based products was compared to Fost and Oxam.

IN VIVO EXPERIMENTS

Pot experiments were conducted in an unheated greenhouse at Portici (Naples, Italy), using 14 cm pots containing 1400 mL of soil (total 800) placed on soil surface covered with a polyethylene film. The soil composition was 4.0% clay, 52.0% silt and 44.0% sand, with a soil texture classified as a silt loam (USDA), pH 7.8 and organic matter of 19 g kg⁻¹. The natural initial nematode population was extracted by cotton-wool filter method for 48 h at 22±2°C and counted (1480 J2s per 100 cm³ soil). Certified tomato seedlings cv. Naxos, RKN susceptible, were grown in polystyrene alveolate planting trays (2 cm) to a two true-leaf stage, transplanted to 14 cm pots and, watered with 100 mL of the test solutions. Control plants received only water. Irrigation and fertilization were homogeneously supplied according to crop need. The final nematode population in soil was determined as described above. Two independent experiments (400 pots each) were conducted. Each trial included 10 treatments (Aba EW 10; Aba EW 7.5; Aba EW 5; Aba SC 1; Aba SC 0.5; Aba SC 5; Aba EW 10+IBA; Fost; Oxam and water control) arranged in a completely randomized block design with four replicates per treatment and ten plants for each block. The first trial was performed using sterilized soil (autoclaved for 1 h, 2 d before use), to check for possible phytotoxicity of different treatments on seedlings and/or later developmental stages. Treatments were applied to soil at time of transplant (AT) plus 15 d after transplanting (DAT), with the exception of Fost that was applied only AT according to the label. The first trial was concluded after one month.

Table 1 – Treatment code, commercial product, active ingredient and doses used against J2s of *Meloidogyne incognita*.

Treatment code	Commercial product	Active ingredient	Dose L/ha ⁻¹	Dose mL/5 L ⁻¹
Aba SC 5	Tervigo®	Abamectin 20 g L ⁻¹	5.0L	1.66 mL
Aba SC 0.5	CHA 2080 SC	Abamectin 180 g L ⁻¹	0.5L	0.17 mL
Aba SC 1	CHA 2080 SC	Abamectin 180 g L ⁻¹	1.0L	0.33 mL
Aba EW 5	CHA 2061-02 EW	Abamectin 18 g L ⁻¹	5.0L	1.66 mL
Aba EW 7.5	CHA 2061-02 EW	Abamectin 18 g L ⁻¹	7.5L	2.5 mL
Aba EW 10	CHA 2061-02 EW	Abamectin 18 g L ⁻¹	10.0L	3.33 mL
Aba EW 10 + IBA	CHA 2061-02 EW + IBA	Abamectin 18 g L ⁻¹ + IBA 2 g L ⁻¹	10.0L + 2.0L	3.33 mL + 0.66 mL
IBA	Hormon L®	indole-3-butyric acid 0.4%	2.0L	0.66 mL
Fost	Nemathorin 150 EC®	Fosthiazate 150 g L ⁻¹	10.0L	3.33 mL
Oxam	Vydate 10 L®	Oxamyl 100 g L ⁻¹	20.0L	6.66 mL

The second trial was performed using naturally nematode infested soil in order to determine the effect of treatments on the nematodes and plants. Pots were filled with uniformly mixed infested soil, comprised of the same *M. incognita* population used for the *in vitro* experiments.

Treatments were applied to soil AT, then 15, 30 and 45 DAT, with the exception of Fost that was only applied AT. The experiment was terminated after two months and repeated twice.

DATA ANALYSIS

Data from the *in vitro* experiments were subjected to analysis of variance (ANOVA) and Fisher's least significant difference (LSD) tests using SPSS (SPSS Statistics, v. 21 for Windows). For all *in vivo* experiments, plant height, fresh shoot weight, fresh root weight, root galling index (RGI) and final population density of nematodes were determined at 30 DAT (sterilized soil) and 60 DAT (non-sterilized soil) from all plants. Root galling was evaluated using a 0-10 scale whereby, 0 = no galling visible, 1 = 10% of the root system galled, 2 = 20% of the roots galled, etc., and 10 = 100% galled roots (BRIDGE and PAGE, 1980). After gall rating had been performed, fresh root and shoot weights were recorded. The effect of different treatments were examined using ANOVA and means were compared using Student Newman Keuls multiple comparison test. The level of significance was set at $p < 0.05$ in all analyses.

RESULTS

IN VITRO EXPERIMENTS

After one day exposure to the tested chemical compounds, at different concentrations, more than 50% of J2s were immobilized (Fig. I). The effect on the nematodes was greatest with the Fost treatment where all J2s were immobilized in one day, followed by the Oxam treatment with immobility slightly less than 100%. The other four abamectin-based products demonstrated a significantly lower percentage of immobile J2s after one day exposure (51-69%) in comparison to Fost or Oxam. After two days exposure, the percentage of immobile J2s in Aba EW 10 was significantly higher than those in Aba EW 5, Aba EW

7.5 and Aba SC 5. After three days exposure, Aba EW 10 and Aba SC 5 were not statistically different from Oxam or Fost, since the nematode mobility was rapidly reduced to almost zero. The slowest effect on mobility was reported for J2s exposed to Aba EW at the doses of 5 and 7.5 L ha⁻¹. All J2s were immobilized within six days after treatments. No J2s died in the water control treatment during two weeks of observation.

Subsequently, one day after transfer of the immobile J2s to water, none of the nematodes previously treated with Fost recuperated mobility (Fig. II). In contrast, immobile J2s pre-exposed to the other five treatments recovered a higher percentage of movement within three days after transfer. The greatest but slowest (> 7 d) recovery of J2s mobility was observed with J2s pre-exposed to Aba EW 5, with a gradual mortality over two weeks. In parallel, the majority of the J2s pre-exposed to the other solutions, that became mobile in water, demonstrated a shortened lifespan compared to untreated J2s (water control) that were still alive after 14 days of observation (data not shown).

IN VIVO EXPERIMENTS

Chemical treatments, applied to tomato plants grown in *M. incognita* naturally infested soil, reduced the soil nematode levels in comparison to untreated conditions (Table 2). The best effects for overall reduction in nematodes and attack symptom were demonstrated with the commercial formulations of Fost and Oxam treatments, whereby Fost performed slightly better than Oxam. Both formulations of abamectin applied at the highest doses (Aba EW 10, Aba SC 1 and Aba SC 5) were more effective in lowering RGI in comparison to the other abamectin treatments and the control. However, the RGI was not improved by the combination of Aba EW 10 with IBA in comparison to Aba EW 10 alone. Tomato plants grown in the untreated pots had the highest RGI. Although, the root weight of treated plants were statistically similar to those untreated, the roots of the water controls were less developed but more infested by nematodes. The reduction in *M. incognita* infestation caused by treatments, resulted in an increase of all plant growth parameters. The greatest fresh shoot weight and height were obtained in pots treated with Aba EW 10 + IBA.

Fig. I – Percentage of *Meloidogyne incognita* second-stage juveniles immobilized after exposure to the chemical solutions (see Table 1): Aba EW 5 (abamectin 18 g L⁻¹ EW, dose of 5 L ha⁻¹), Aba EW 7.5 (abamectin 18 g L⁻¹ EW, dose of 7.5 L ha⁻¹), Aba EW 10 (abamectin 18 g L⁻¹ EW, dose of 10 L ha⁻¹), Aba SC 5 (abamectin 180 g L⁻¹ SC, dose of 5 L ha⁻¹), Oxam (oxamyl 100 g L⁻¹, dose of 20 L ha⁻¹) or Fost (fosthiazate 150 g L⁻¹, dose of 10 L ha⁻¹) over time (days). Values are means of four replicates $\pm$ Standard Deviation.

Fig. II – Daily percentage of *Meloidogyne incognita* second-stage juveniles, re-mobilized in distilled water after exposure to the chemical solutions (see Table 1): Aba EW 5 (abamectin 18 g L⁻¹ EW, dose of 5 L ha⁻¹), Aba EW 7.5 (abamectin 18 g L⁻¹ EW, dose of 7.5 L ha⁻¹), Aba EW 10 (abamectin 18 g L⁻¹ EW, dose of 10 L ha⁻¹), Aba SC 5 (abamectin 180 g L⁻¹ SC, dose of 5 L ha⁻¹), Oxam (oxamyl 100 g L⁻¹, dose of 20 L ha⁻¹) or Fost (fosthiazate 150 g L⁻¹, dose of 10 L ha⁻¹) over time (days). Data are means of four replicates ± Standard Deviation.

Table 2 - Effects of treatments on second stage juveniles (J2s) of *Meloidogyne incognita* on height, fresh shoot and root weights and root galling index (RGI) of tomato plants cv Naxos grown in nematode-infested soil, 60 d after treatments.

Treatments	J2s 100 cm ³ soil ^a	Plant height (cm)	Fresh shoot weight (g)	Fresh root weight (g)	RGI ^b
Aba SC 5	3769 b	81.4 ab	55.3 b	5.8 b	2.8 a
Aba SC 0.5	5091 c	81.1 ab	54.2 b	5.9 b	5.0 ab
Aba SC 1	4011 b	81.7 ab	55.1 b	6.0 b	2.5 a
Aba EW 5	4940 c	80.6 ab	52.9 b	5.8 b	4.7 ab
Aba EW 7.5	4403 bc	80.9 ab	51.8 b	5.5 b	4.5 ab
Aba EW 10	4362 bc	80.6 ab	54.2 b	5.6 b	2.8 a
Aba EW 10+IBA	3879 b	85.2 a	61.8 a	7.2 a	5.2 ab
Fost	2389 a	83.2 a	55.9 b	6.3 b	1.9 a
Oxam	3021 ab	83.7 a	58.0 ab	6.3 b	2.2 a
Control	6646 d	73.2 b	46.2 c	6.2 b	6.8 b

^a *Meloidogyne incognita* (Kofoid & White) Chitwood were determined from 100 cm³ soil using a standard sieving procedure.

^b RGI 0 = no galls and 10 = 100% of roots galled.

Data are arithmetic means of 40 plants per treatment, and letters indicate means that are significantly different as determined with the Student Newman Keuls test ($p < 0.05$).

In the second experiment, the majority of the chemical treatments applied to the plants grown in sterilized soil demonstrated no phytotoxic effects to the plants in comparison to those from the water control (Table 3). The greatest increase in growth was noted with plants treated with Aba EW 10 + IBA, that were taller, and had more shoot and root development than those from other treatments (Table 3). However, only plants treated with Fost were reduced in growth and development relative to the other chemical treatments and the water control, as a consequence of moderate phytotoxic effect at early developmental stages.

DISCUSSION

Application of abamectin-based products at different doses and formulations affected mobility of the J2s of *M.*

incognita. Increased concentration of the active ingredient corresponded to an increased nematode immobility, suggesting a dose effect of abamectin for nematode control, as previously reported (QIAO *et al.*, 2012). After three days in the *in vitro* experiment, J2s exposed to Aba EW 10 and Aba SC 5 were immobilized up to 90%, similar as Oxam and Fost, with Aba SC 5 being slightly more effective than Aba EW 10. The difference on J2s mobility with the various concentrations of abamectin treatments could be due not only to the active ingredient concentration but also to the adjuvants in test solutions as noted in previous studies (NTALLI *et al.*, 2009). In general, ISMAM (2006) suggests that the emulsifiable concentrate formulations are promising due to their high storage stability. However, our *in vitro* experiments indicate that the SC formulation was marginally better than EW. The immobility of J2s, when treated with the abamectin-based products, and their

Table 3 – Effects of treatments on height, fresh shoot and root weights of tomato plants cv Naxos growth and development in sterilized soil, 30 d after treatments.

Treatments	Plant height (cm)	Fresh shoot weight (g)	Fresh root weight (g)
Aba SC 5	33.9 ab ^a	7.1 ab	1.3 ab
Aba SC 0.5	34.8 ab	7.5 a	1.2 ab
Aba SC 1	34.6 ab	7.0 ab	1.2 ab
Aba EW 5	34.7 ab	7.3 a	1.3 ab
Aba EW 7.5	33.2 ab	7.7 a	1.2 ab
Aba EW 10	34.3 ab	6.9 ab	1.2 ab
Aba EW+IBA	38.5 a	7.8 a	1.5 a
Fost	28.7 b	6.2 b	1.0 b
Oxam	34.8 ab	7.6 a	1.3 ab
Control	35.5 ab	7.7 a	1.3 ab

Data are arithmetic means of 40 plants per treatment and the letters indicate that the means are significantly different as determined with the Student Newman Keuls test ($p < 0.05$).

mobility recovery upon transfer to distilled water indicated that the paralysis on *M. incognita* was reversible, and primarily a nematostatic effect. However, the J2s mobility recovery also reduced the lifespan mainly when they were pre-exposed to higher abamectin doses. HAYDOCK *et al.* (2006) suggested that although nematodes may recover under laboratory conditions, they are likely too weak to locate host plant root systems in the field, and thus not able to reproduce.

In the soil naturally infested by *M. incognita*, all chemical treatments were effective in lowering soil population levels in comparison to untreated controls. The lowest RGI values were obtained in pots treated with the nematocides Oxam and Fost, and the higher doses of abamectin (Aba EW 10, Aba SC 1 and Aba SC 5). In accordance to other studies conducted under greenhouse conditions, *M. incognita* galling index decreased with increasing concentrations of abamectin (FASKE & STARR, 2006; MONFORT *et al.*, 2006; QIAO *et al.*, 2012). The greatest plant height was obtained in Aba EW 10+IBA, Oxam and Fost treatments, whereas plants from the other abamectin-based treatments were intermediate and the untreated control had the lowest height. As a consequence of nematode reduction, all chemical treatments improved plant growth and development in comparison to the water control, the Aba EW 10+IBA treatment resulted in a greater increase in shoot and root biomass. Likely, the increase in root weight and RGI values after Aba EW 10 + IBA treatment in comparison to Aba EW 10 alone and control, could be associated to better soil colonization by roots but also a greater root area exposed to nematode attack. The addition of IBA was useful to promote all plant growth parameters and also rooting as reported by Husain *et al.* (2008). Additionally, in late stages, the efficacy of abamectin present in Aba EW 10 + IBA treatment may be reduced owing to the natural degradation of abamectin in soil and subsequent increase in nematode infestation. The plant response to phytohormone treatments was also dependent on its concentration (MAILA & MASHELA, 2013). Some studies reported that IBA is effective in reducing *M. javanica* (Treub) Chitwood, populations and inhibiting the development of *M. graminicola* Golden & Birchfield (IBRAHIM, 1977; PRASAD & RAO, 1976). Indolic compounds, including IBA, are located in the vegetative

tissues, as well as in egg masses and juveniles of different nematode species, therefore it has been reported that this close interaction may have an inhibitory effect on nematodes (HUTANGURA *et al.*, 1999; GOVERSE *et al.*, 2000; KHANNA & YADAV, 2004; LONG *et al.*, 2006; GRUNEWALD *et al.*, 2009). Our results indicate that nematode suppression was not improved by the abamectin and hormone combination, in agreement with results reported by other researchers (ESMENJAUD *et al.*, 1993). However, conflicting results on the action of these hormones on plant nematode resistance and susceptibility have been reported (KOCHBA & SAMISH, 1971; CAROPPO *et al.*, 1985; HUETTEL & HAMMERSCHLAG, 1986; ESMENJAUD *et al.*, 1993; 1995). Overall, the auxin IBA and Aba EW could be successfully mixed. This blend could be useful in short-term crops or when nematode population densities are low. The pot experiments presented here should be followed by field experiments with different nematode-crop combinations, application methods and soil types to confirm results. In general, formulation plays an important role in spreading an active ingredient. Differences in the formulations may have effect on the control activity. *In vitro* experiments, Aba SC 5 (SC formulation) was found to be slightly more effective than EW formulation, whereas, in pot experiments no relevant differences at equal concentration of active ingredient in product were detected.

Tomato plants grown in sterilized soil maintained regular growth, whereby most of the nematocide applications did not significantly affect plant growth parameters when compared to the untreated controls. Only those plants treated with Fost, showed lower fresh shoot weight due to a slightly phytotoxic effect at early growth stages. The absence of phytotoxicity with the other compounds confirmed that soil application of the tested abamectin-based products were safe to tomato production. In conclusion, the new proposed commercial abamectin-based products obtained an adequate nematode control against *M. incognita*.

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