Multi-Season Yield Enhancement Following Citrus Nematode Management in an Egyptian Citrus Orchard

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Abstract

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Chemical and biological nematicides were evaluated for the management of the citrus nematode *Tylenchulus semipenetrans* in an Egyptian lemon orchard. The experiment included 7 treatments as follows: native *Heterorhabditis indica* applied to the soil surface in aqueous suspension, *H. indica* infected cadavers applied beneath the soil surface, Abamectin®, Micronema® a product containing *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *Bacillus thuringiensis*, chemical treatments cadusafos and two rates of the chemical oxamyl. The experiment followed a randomized complete block design with 10 blocks, and treatments were applied between October 2018 and February 2019. All treatments reduced ($P \leq 0.05$) population densities of female citrus nematodes in roots between 32 and 77% for three months period following the final treatment. Average lemon yield in the summer of 2019 was 18-38% higher in treated compared to untreated plots, but only the Micronema and cadusafos treatments were significantly higher. The average yields in treated plots were 15-42% higher than that in control plots in 2020, but the increase was not statistically significant. Despite few treatment differences in either year, the mean 2019 and 2020 combined yield for each treatment, except for the *H. indica treatment,* was 18-33% higher ($P \leq 0.05$) than that of the untreated plots. The lemon yield during both years was inversely correlated ($P \leq 0.001$) with the mean nematode females/g of roots following the treatments. In August and September 2021, all the previous treatments except *H. indica* applied in aqueous suspension and Micronema continued to suppress female citrus nematodes in roots, on average of 21–69%.

Keywords: Biocontrol, citrus, crop loss assessment, nematicides, nematode management, *Tylenchulus semipenetrans.*

Introduction

Egypt is a major citrus producing country and citriculture has important socio-economic value in Egypt by providing an important source of foreign currency and local employment (Abdel-Razek & Abd-Elgawad, 2021). Although the area planted to lemon trees has steadily declined, a dramatic increase in lemon prices has sparked recent interest in replanting and in avoiding production limitations (Anonymous, 2021). Two main constraints are unsound pest management (Osman *et al.*, 2022) and under-watering trees by some growers to force them to flower off-season to sell fruits at a higher price, but such practice has a negative effect on the tree's growth (Anonymous, 2021). Stress from underwatering is compounded in the presence of pests/pathogens such as plant-parasitic nematodes (PPNs). The most widespread and economically important nematode pest of citrus worldwide is the citrus nematode *Tylenchulus semipenetrans* (Shokoohi & Duncan, 2018). It is a semi-endoparasite of fibrous (feeder) roots, establishing a feeding site within the cortical root tissue where the female nematode remains to feed throughout its life. It can decrease root function and increase root turnover rate causing 'slow decline' disease (Hamid *et al.*, 1998). The disease symptoms can be exacerbated by other factors such as water deficit, high temperature, soil salinity, and concomitant pathogens and pests (Shokoohi & Duncan, 2018).

Nematicides have still a key role in *T. semipenetrans* control, but application of their various materials has led to mixed results (Pretorius & Le Roux, 2017; Verdejo-Lucas & McKenry, 2004). Although it is formally registered for use against *T. semipenetrans,* cadusafos is seldom applied in Egypt and its effectiveness under local conditions is unclear (Agricultural Pesticides Committee, 2020). Cadusafos achieved promising results in Arizona (USA), where granular and liquid formulations reduced *T. semipenetrans* populations to undetectable levels and boosted both the lemon yield and rate of fruit maturity (McClure & Schmitt, 1996). In Australia, granular cadusafos demonstrated superior effectiveness for *T. semipenetrans* control (Walker & Morey, 1999). Pretorius & Le Roux (2017) reported that using cadusafos was reliable in breaking the *T. semipenetrans* life cycle in South Africa, whereas fenamiphos, aldicarb, and terbufos failed to adequately decrease *T. semipenetrans* population levels. Such carbamate or organophosphate post-plant nematlcides suppress nematode populations via inhibition of acetylcholinesterase. The current and pending withdrawal or stricter regulation of organophosphate/carbamate nematicides emphasizes a need to optimize their use by modifications to dosages, use of combinations, application timing and/or delivery methods. Moreover, novel bionematicides with different mechanisms are commercially emerging and their efficacy requires extensive testing. A few biocontrol agents (BCAs) have dual-purpose application.

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Entomopathogenic nematodes (EPNs) are aptly named as controlling insect pests, but they have also been shown to suppress PPNs on turfgrass (Grewal *et al.,* 1997), tomato (Kepenekci *et al.,* 2018), and other crops including citrus (Abd-Elgawad, 2017). Microbial compounds such as abamectin (El-Saedy *et al.,* 2019), and a mixture of pathogenic bacteria formulated as Micronema (Hammam *et al.,* 2016) exhibited efficacy against *T. semipenetrans* infecting citrus. Thus, comparative studies of current and recently developed options continue to be important. For this study, we selected an orchard in a locality characterized by the expansion of citrus production in Egypt.

We hypothesize that *T. semipenetrans* can be better managed to result in subsequent yield increases using some currently available nematicides than others. Several novel and environmentally safe materials in addition to traditional carbamate (oxamyl) and organophosphate (cadusaphos) products aimed to control the citrus nematode and mitigation of lemon yield loss were compared.

Materials and Methods

An orchard (30°30’43.2”N; 30°17’45.0”E) of eighteen-year-old Banzaheer acid lime (*Citrus aurantifolia*) trees grafted on sour orange (*C. aurantium*) rootstock in an area known to be infested by *T. semipenetrans* was selected in El-Nubaria district, El-Beheira governorate. The orchard is in a newly reclaimed desert area known for expansion of citriculture (Anonymous, 2015). The trees were spaced at 5 × 6 m (333 trees per ha), grown in sandy soil (sand 90%, silt 6%, clay 4%, pH 7.4, $CaCO_3$ 7.8% and organic matter 1.7%), and pruned every other year. The original sandy soil was reclaimed via levelling and mulching virgin soil with a portion of silty clay soil from the Nile Valley (Abd-Elgawad *et al.,* 2016). The trees are rarely flooded, but, when water is insufficient, drip irrigation with emitters at 30 cm intervals in a one-meter radius around tree trunks was applied. The radius of the tree canopy range was 1.95-2.10 m. All agricultural practices conform with the national program for enhancing citrus production guidelines (El-Barkoki & Abou-Aziz, 1989). Weed control was usually done by hand hoeing.

Two weeks prior to treatments, one kg of soil was collected (0-20 cm depth) from undercanopies of each of five randomly selected groups of five adjacent trees throughout the experimental site. In the laboratory, five 200g aliquots per sample were each baited with 5 *G. mellonella* larvae. Cadavers were placed on white traps (Hammam *et al.,* 2016) to recover and identify EPNs.

Trees with uniform and healthy appearance were selected and treatments were arranged in a randomized complete block design with 10 blocks. On October 3, 2018, one tree per block received one of following treatments: (1) an aqueous application of an Egyptian EPN strain of *Heterorhabditis indica* Hi-EG, at a rate of 25 infective juveniles (IJs)/cm^2, equivalent to 2.5×10^9 IJs/ hectare, (2) ten *H. indica* Hi-EG-infected *G. mellonella* cadavers, (3) cadusafos (Rugby® 10% G; FMC Corp., American origin and produced in Egypt by FMC Corporation) at a rate of 24 kg/feddan (= 4200 m^2), (4) Micronema® (mixture of *Serratia* sp., *Pseudomonas* sp., *Azotobacter* sp., *Bacillus circulans* and *Bacillus thuringiensis*; General Authority of the Agricultural Budget Fund of the Egyptian Ministry of Agriculture) at a rate of 200 ml/tree, (5) avermectin (Abamex® 1.8% EC; Arab Company for the Manufacturing of Pesticides and Veterinary Medicines, Jordan) applied at 3 ml/7.5 L of water per tree, and (6) untreated control (water only). On 15 January 2019, two additional treatments were added to the trial, (7) oxamyl (Vydate® 24% L; Corteva, Egypt) at 3 liters/feddan and (8) oxamyl at 4 liters/feddan. Oxamyl applications in Egypt are conventionally made in the winter months. Both oxamyl treatments were repeated three weeks later.

All treatments were applied to pre-moistened soil. The agitated EPN suspension was evenly sprayed on the soil surface beneath the entire tree canopy after scraping debris from the soil surface layer. The nematodes were applied early in the morning and immediately followed with four liters water per tree via hand sprayer to help incorporate them in the soil to protect them from UV radiation and desiccation. The EPN strain was originally isolated from a citrus grove (Shehata *et al.,* 2019), maintained at 10°C, cultured, and applied within 3 days after exiting *G. mellonella* last instar larval cadavers (Kaya & Stock, 1997). The EPN in cadavers were applied six days post infection, by evenly spacing and burying of insects (5 inside and 5 outside the irrigation circuit) about 3 cm beneath the soil surface under the tree canopy followed by similar watering. Cadusafos was evenly sprinkled by hand within 75 cm on each side of the drip irrigation line, i.e. 1.50-m bands (each 9.4 m^2 area/tree) beneath the tree canopy. Four liters of water were immediately added after its application to wash the nematicide into the top layer of soil. Oxamyl, Micronema and Abamex were sprayed on the soil surface in the same pattern as that of cadusafos, followed by 4 liters of water similar to the untreated control.

The undercanopy soil and roots were sampled to assess plant-parasitic nematode population levels on 3 October 2018, prior to the initial six treatments, and then on 15 January 2019 (which was prior to the final two treatments), April 25, 2019, and August 4, 2021, for all 8 treatments. Both oxamyl treatments and the untreated controls final sampling was on September 21, 2021. Nematode population levels were assessed by digging soil with a shovel (0-30 cm depth) at three locations within the treated area for each tree, mixed to make a combined sample of ca. 1.5 kg/tree. From each thoroughly mixed sample, an aliquot of 250 g soil was processed by sieving, decanting, and sucrose-centrifugation to extract soil nematodes (Barker, 1985). Feeder roots in the samples were gently washed free of soil and an aliquot of 3 g per tree was cut into 2-cm-long pieces, placed in plastic dishes with distilled water and incubated under laboratory conditions (25±5ºC) for a week to extract *T. semipenetrans*-second stage juveniles (J_2) and males (Hammam *et al.,* 2016). An additional 3 g roots were cut into 2-cm-long pieces and blended at 3×10^3 rpm for 3 min to recover *T. semipenetrans* females (Southey, 1970). The lemon fruit yield was recorded from July to September in 2019 and 2020. The lemon yield was not collected in 2021 because of global pandemic travel restrictions.

Nematode data were transformed ($\log_n$ X+1) to normalize prior to ANOVA and mean comparisons by

Tukey's Honestly Significant Difference test. Untransformed means were reported. Fruit weight per tree in each harvest season and the combined 2-year weights were subjected to ANOVA and means were compared using Dunnet's test of all treatments against a control. The relative yield/tree for each year (yield/highest yield) were combined (n = 80) and regressed against the average number of female citrus nematodes per gram root in the two samples following the 2019 winter and spring treatments.

Results

No naturally occurring EPNs were detected in the orchard prior to initiating the experiments. *Tylenchulus semipenetrans* was the dominant PPN species in all soil and root samples. Species of *Criconemoides*, *Helicotylenchus*, and *Tylenchorhynchus* were also detected but were just 0.7% of the total PPN community, too low to be analyzed. The average *T. semipenetrans* initial population densities were 2626 second-stage juveniles (J_2) and males per 250 g soil and 1 g fresh fibrous roots as well as 264 females per 1 gm feeder roots.

The mean population densities among treatments differed significantly prior to the applications; therefore, the initial estimates measured in October were used as co-variants when analyzing the treatment effects on populations measured in January. The mean citrus nematode numbers in all treatments measured 14 weeks following the October 2018 applications were numerically below those of the control (Figure 1). All treatments except the cadaver-applied EPN and abamectin, suppressed ($P \leq 0.05$) *T. semipenetrans* females in roots (Figure 1).

Cadusafos reduced the nematode females by 53%, whereas Micronema and EPNs in suspension also reduced the root infection rate. By April 2019, all treatments reduced citrus nematode in soil and roots (Figure 1). Root infections by the nematode were 23 and 32% of control levels when treated three months earlier with the low and high rates of oxamyl, respectively. Cadusafos reduced female citrus nematode by 61% in April and the remaining treatments reduced females to levels between 32 and 42% of untreated controls. More than two years later, by August 2021, long-term nematode control from several of the treatments was still evident. The soil stages were significantly lower than controls for cadusafos and the high rate of oxamyl (Figure 1-A); however, females in roots continued to be significantly reduced by as much as 24% to 59% by abamectin, cadusafos, and both rates of oxamyl (Figure 1-B). The variation in fruit yield between the individual trees was high within and between treatments during both harvest seasons (Figure 2).

Nevertheless, there was a highly significant linear relationship between the lemon fruits harvested from the trees during the two seasons and the average number of female nematodes per gram of roots measured following the treatments in 2019. The mean measured lemon fruit yield each year in 2019-2020 for all treatments ranged between 16–42% higher than those of untreated plots (Figure 3).

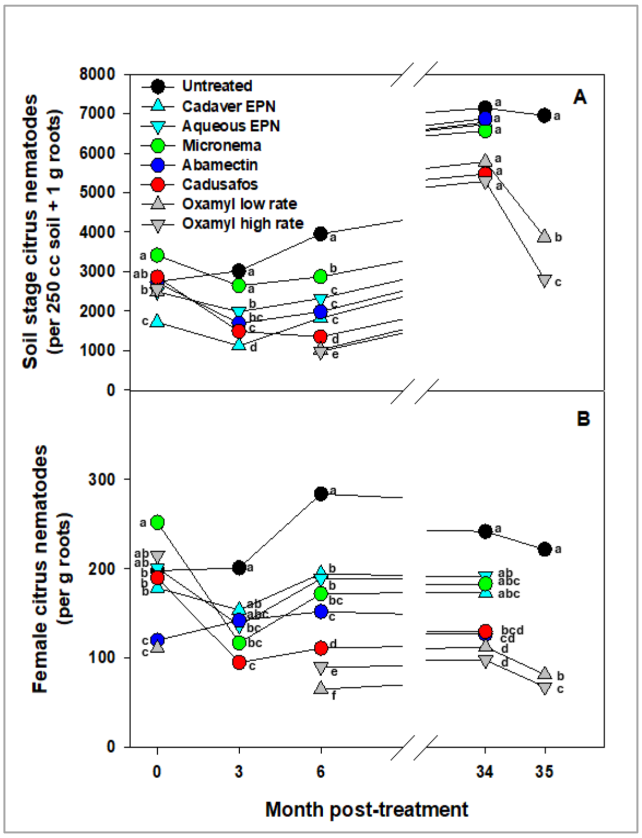

Figure 1. Population density of *Tylenchulus semipenetrans* soil stages (A) and females, (B) during 35 months following the first series of treatments with chemical and biological pesticides. All treatments were applied immediately after sampling at time 0, except oxamyl which was applied after three months.

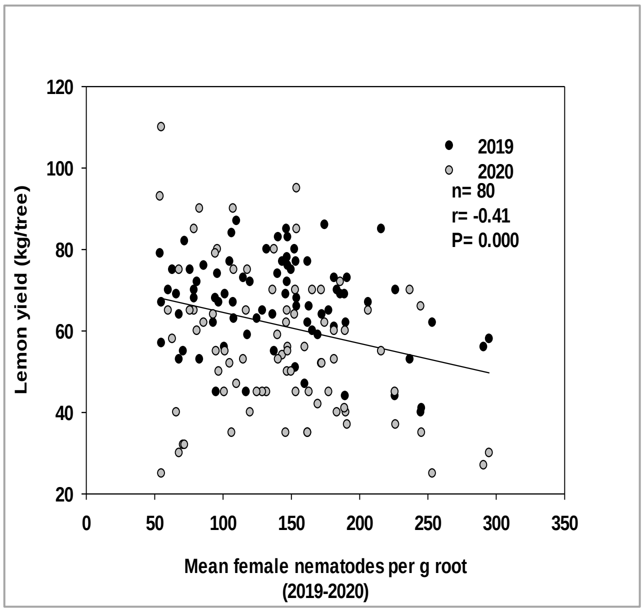

Figure 2. Relative lemon yield (calculated separately for each year). Regression was calculated for the combined 2-year yield per tree (N = 80), but each year is shown separately in the figure.

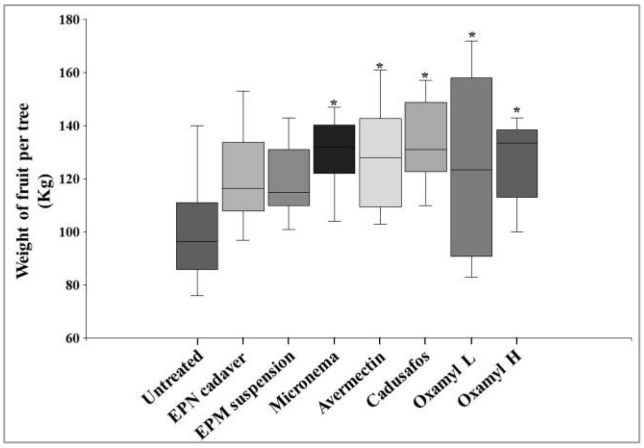

Figure 3. Two-year lemon fruit weight per tree harvested in 2019 and 2020. Treatment means that differ (P < 0.05) from the untreated control are marked by an asterisk.

Micronema and cadusafos were the only treatments to increase fruit yield significantly in 2019 (not shown) and no treatment produced significantly higher lemon yield than controls in 2020. However, the mean two-year combined yield ranged between 25-33% higher (P < 0.01) than that of controls for all but the EPN treatments (P > 0.05) (Figure 3). There were no differences (P > 0.05) between the yields of those treatments found to increase lemon yield.

Discussion

The citrus nematode population levels initially encountered in this trial were large enough in such a high value crop as lemon to justify management, based on the *T. semipenetrans* economic thresholds in this reclaimed desert region (Abd-Elgawad *et al.*, 2016). This was confirmed by the inverse relationships measured between fruit weight and nematode infection rates and by the increased fruit production by most nematicidal treatments. The cost per hectare of the commercial products used here ranged from $24 for abamectin to $950 for cadusafos. The 2019-2020 average export price paid for lemon in Egypt was $1.14/kg which represents a value of $38,277/ha based on yield in the untreated control plots. Yield increases of the magnitude measured here (28±1.4%) would return approximately $9767-$10,693/ha over two years, depending on the product used.

Because several of the treatments appeared highly profitable, non-target effects on soil food webs, groundwater pollution and human health also become important considerations when choosing between the products. The more environmentally benign EPN and bacterial biological controls were very effective against *T. semipenetrans*, even compared to the more toxic chemical products. Several previous studies evaluated EPNs for management of citrus nematode. Duncan *et al.* (2007) reported a significant reduction in the seasonal increase of citrus nematode populations in mature orange trees in Florida nine months after applying the EPN *Steinernema riobrave* to manage a root weevil pest; however, the effect on the nematode was brief and inconsistent and did not increase fruit yield, significantly. Abo-Korah (2020) reported reducing *T. semipenetrans* on orange trees in a pot study by 50% following application of *Steinernema feltiae*. *Heterorhabditis bacteriophora* applications maintained citrus nematode populations below half those on non-treated orange trees consistently for 27 weeks, but without increasing fruit yield in an orchard in the Nile Basin (Hammam *et al.*, 2016). In the present experiment, nematode suppression was measurable eight months following application of both formulations of *H. indica*, and fewer females of citrus nematodes were recovered from roots of trees treated with the cadaver formulation for almost 3 years. The consistent, effective management of citrus nematode in Egypt orchards, contrasts with that reported from Florida and may be due to the climatic and edaphic differences, EPN species employed, or many other factors. Following early reports of EPN antagonism to plant-parasitic nematodes in pot and field trials (Grewal *et al.*, 1997; Lewis *et al.*, 2001), numerous studies, primarily focused on root-knot nematode, have shown similar variability in responses by the PPN and the host plants (Damascena *et al.*, 2019; Felicitas et al., 2021; Jagdale *et al.*, 2009; Li et al., 2023). The most common mechanisms investigated for EPN effects on PPN involve allelopathy (Kenney & Eleftherianos, 2016) or the induction of plant defenses (Jagdale *et al.*, 2009). Kamali *et al.* (2022) proposed that plants mistake EPNs for herbivores. They demonstrated that EPNs induce both the systemic acquired resistance and the induced systemic resistance pathways, thereby reducing populations of PPN belowground, and arthropod leaf miner aboveground.

Much like the EPN treatments, Micronema provided similar results against *T. semipenetrans* in this reclaimed desert orchard as were reported previously for an orange orchard in the Nile Basin infested with citrus nematode (Hammam *et al.*, 2016). More importantly, in this and the previous study, compared to untreated trees, Micronema increased the fruit yield to levels equivalent to those of the more conventional nematicide treatments. El-Nagdi *et al.* (2015) found that Micronema increased harvested bananas by suppressing *Meloidogyne incognita* and *Fusarium* root rot. Micronema also increased the yield of sugar beet by controlling *M. incognita* (Youssef *et al.*, 2017). An increasing number of reports showed effective nematode control and consequent crop responses by such biocontrol bacteria as used in this study (Migunova & Sasanelli, 2021). Bacterial activity against nematodes depends on their capacity to compete for ecological space by settling on the root surface and generating antimicrobial/nematicidal toxins, hydrolytic enzymes, antibiotics, and/or siderophores. As reported for EPNs, these bacteria and/or their metabolites can also influence the plant host in part by stimulating induced systemic resistance (Migunova & Sasanelli, 2021).

Abamectin is a metabolite of *Streptomyces avermitilis*. The commercial product has a chelated formulation that is required to increase the soil penetration of the active ingredient (avermectin B1a:B1b, 80%:20%). Abamectin blocks the transmission of electrical activity in the nematodes and invertebrate nerve/muscle cells leading to hyperpolarization and paralysis of neuromuscular systems (Abd-Elgawad, 2020a). It has a short half-life in the soil, a low affinity for mammalian nervous systems and is safe to management – hence the disease name "slow decline" (Le Roux *et al.*, 1996; 1998; 2000). Here the mean nematode densities of all treatments were lower than that of untreated trees at every post-application sampling date. The females in roots remained significantly lower for abamectin, cadusafos and oxamyl for nearly 3 years. The long-lasting efficacy of both chemical and biological control products in this trial combined with the availability of threshold population densities for management decisions (Abd-Elgawad *et al.*, 2016), would appear to make citrus nematode IPM especially feasible in Egypt. The responses to biological control treatments in these single-tree plots emphasize the importance of working with growers to validate the efficacy and profitability of products such as Micronema and Avermectin in large plot trials in commercial citrus orchards.

use. In Egypt it has been shown to control PPNs and increase yields of potato, tomato, pepper and citrus (Abd-Elgawad, 2020a; 2020b; 2020c; 2020d), respectively. It was the cheapest product used in this trial, an especially important factor in developing countries, where the other nematicides may not be affordable.

The organophosphate cadusafos and both rates of the carbamate oxamyl provided the greatest and most persistent level of nematode control but provided a similar yield boost compared to the biocontrol products. Both products have safety and environmental profiles that have resulted in deregistration of many other pesticides, like these, that target acetylcholinesterase. Numerous studies report citrus yield increases within 1-3 years following application of organophosphate or carbamate nematicides (Duncan, 1989; Greco *et al.*, 1993; McClure & Schmitt, 1996; Verdejo-Lucas & McKenry, 2004).

A striking feature about citrus nematode is that, unlike many plant-parasitic nematodes species, *T. semipenetrans* migrate exceptionally slowly within orchards (Duncan *et al.*, 1995) and their populations rebound slowly following

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الملخص

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تمّ تقييم مبيدات نيماتودية كيميائية وحيوية لمكافحة نيماتودا الحمضيات/الموالح (*Tylenchulus semipenetrans*) في بستان ليمون حامض بجمهورية مصر العربية. تضمنت التجربة 7 معاملات: نيماتودا الحشرات *Heterorhabditis indica* المحلية على سطح التربة بصورة معلق مائي، جثث الحشرات الملقحة بنيماتودا *H. indica* المطبقة تحت سطح التربة، مبيد الأبامكتين (Abamectin®)، Micronema® منتج تجاري يحتوي على *Serratia* sp.، *Pseudomonas* sp.، *Azotobacter* sp.، *Bacillus circulans*، *Bacillus thuringiensis*، مبيد كيميائي Cadusafos وجرعتين مختلفتين من مبيد الأوكساميل الكيميائي. استخدمت التجربة تصميم القطاعات العشوائية الكاملة المكونة من 10 قطع، وتمّ تطبيق المعاملات في الفترة ما بين تشرين الأول/أكتوبر 2018 وشباط/فبراير 2019. أدت جميع المعاملات إلى خفض ($P \geq 0.05$) أعداد إناث نيماتودا الحمضيات/الموالح في الجذور بنسبة تراوحت ما بين 32 و 77% خلال ثلاثة أشهر بعد آخر معاملة. كان متوسط إنتاج الليمون الحامض في صيف عام 2019 أعلى بنسبة 18–38% في الأراضي المعاملة مقارنة بالأراضي غير المعاملة (الشاهد)، وأعطت المعاملات Micronema و Cadusafos أعلى إنتاج، وكان متوسط العائد في قطع الأراضي المعالجة أعلى بنسبة 15–42% مقارنةً بقطع الأراضي غير المعاملة في عام 2020، لكن الزيادة لم تكن ذات دلالة إحصائية. على الرغم من الاختلافات القليلة في العلاج في أيّ من العامين، كان متوسط العائد المشترك لعامي 2019 و 2020 لكل علاج، باستثناء تلك التي تحتوي على *H. indica*، أعلى بنسبة 18–33% ($P \geq 0.05$) مقارنةً بالشاهد غير المعامل. وارتبط محصول الليمون الحامض بعد المعاملات خلال العامين كليهما عكسياً ($P \geq 0.001$) مع متوسط أعداد إناث النيماتودا لكل غرام من الجذور. استمرت جميع المعاملات المطبقة، باستثناء *H. indica* في معلق مائي و Micronema، بتثبيط إناث نيماتودا الموالح في الجذور، بمعدل 21–69% خلال شهري آب/أغسطس وأيلول/سبتمبر 2021.

كلمات مفتاحية: مكافحة حيوية، حمضيات/موالح، تقييم فاقد المحصول، مبيدات النيماتودا، إدارة النيماتودا، *Tylenchulus semipenetrans*.

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