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Biostimulants for Plant Growth Promotion and Sustainable Management of Phytoparasitic Nematodes in Vegetable Crops

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Abstract: The parasitism of root-knot nematodes, *Meloidogyne* spp., can cause heavy yield losses to vegetable crops. Plant biostimulants are often reported for a side-suppressive effect on these pests and many commercial products are increasingly included in sustainable nematode control strategies. Source materials of most biostimulants derived from plant or seaweed raw materials were documented for a reliable suppression of root-knot nematode species, whereas the suppressiveness of microbial biostimulants was found largely variable, as related to the crop and to environmental factors. Chitosan-based biostimulants were also stated for a variable phytonematode suppression, though clearly demonstrated only by a few number of studies. In a preliminary experimental case study, four commercial biostimulants based on quillay extract (QE), sesame oil (SO), seaweeds (SE), or neem seed cake (NC) were comparatively investigated for their effects against the root-knot nematode *M. incognita* on potted tomato. Soil treatments with all the four biostimulants resulted in a significant reduction of nematode eggs and galls on tomato roots, though NC and SO were significantly more suppressive than QE or SE. In addition, almost all biostimulant treatments also resulted in a significant improvement of tomato growth compared to the non-treated control. These preliminary results seem to confirm the literature data and clearly indicate the potential role of biostimulants for a safe nematode management both in organic and integrated crop systems.

Keywords: biostimulants; phytoparasitic nematodes; suppressiveness; sustainable management

1. Introduction

Phytoparasitic nematodes are among the most harmful pests of vegetable crops, responsible for an annual yield loss amounting to 9–15% of the world crop yield [1]. Most of these losses are due to root-knot nematode species, *Meloidogyne* spp., causing poor plant growth and reduced crop yield and quality and reducing plant resistance to other biotic and abiotic stresses [2]. Traditionally, control of these pests relied on soil treatments with synthetic nematicides, but the increasing demand for a higher crop safety to the environment and humans has led to a progressive dismission of these products, giving a strong impulse to the search and the implementation of control strategies based on natural mechanisms, such as the use of plant biostimulants [3].

Plant biostimulants derived from natural materials have been receiving a growing interest by researchers, farmers, and industrial companies, as considered an effective tool for improving crop productivity [4]. The previous unclear and misunderstanding legislation frame led to include among the biostimulants a large variety of products with different activities, such as growth enhancers, plant strengtheners or conditioners, resistance elicitors, as well to registration procedures variable among countries or even within the same country [5]. The uncertain legislative frame resulted in the immission in the market of a large variety of biostimulants stated for a suppressiveness on phytoparasitic nematodes, because of their content of raw materials (plants, seaweeds, microorganisms, and more) widely demonstrated for an activity against phytonematode species [6–8]. However, the recent EU Regulation 2019/1009 [9] has restricted the definition of fertilizing products and biostimulants and, therefore, many of these borderline products are destined to be classified as phytochemicals, dealing with more complex and expensive registration procedures.

Because of the increasing technical and economic relevance of these products, the aim of this study is to provide a review of the main groups of nematode-suppressive plant biostimulants actually available in the market and to indicate their potential for an effective but safe nematode management by a preliminary experimental case study on the root knot nematode *M. incognita* Kofoid et White (Chitw.) on tomato.

2. The State-of-the-Art

2.1. The Market Supply

A survey of the Italian market in 2018 revealed the presence of almost 40 different commercial plant biostimulants/strengtheners declaring a side activity on phytoparasitic nematodes on their labels (Table 1). More than 50% of these commercial products were based on plant raw materials, such as extracts, seed oils or green and seed biomasses, whereas another 25% was represented by seaweed derivatives. There was only one chitosan-based formulate, whereas the remaining others were microbial formulations. Only four products were clearly described as nematotoxic and the activity of other nine formulates was related to nematode repellence, disorientation, or antifeeding effects, whereas the remaining products were generically described as enhancers of plant resistance or of unfavorable soil conditions.

Table 1. Commercial biostimulants reporting an activity against phytoparasitic nematodes available in the Italian market at December 2018.

Commercial Name	Formulation [1]	Raw Materials	Activity [2]
Aegis ™	P	Micorrhizal fungi	1, 4, 5
Alg-a-Mic ™	L	Seaweed extract	1, 4, 5, 7
Algafit ™	L	Seaweed extract	1, 4
Ascogreen ™	L	Seaweed extract	1, 4
Biofence ™	P	Brassica meal	1, 3, 5, 6
Biofence 10 ™	P	Brassica meal	1, 3, 5, 6
Biofence FL ™	L	Brassica extract	1, 2, 4, 6
Bioki ™	p	Neem oil	1, 3, 7
Cogisin ™	L	Plant extracts	1, 2, 4, 7
Ecoessen NP ™	P	Bone meal, neem cake	1, 3, 6
Ekoprop Nemax ™	P	Mycorrhizal fungi	1, 2, 4
Ergo Bio ™	L	Humic and fulvic acids	1, 3, 4, 5, 8
Ergon ™	L	Seaweed extract	1, 4
Fertineem ™	L	Neem oil	1, 4
Force 4 ™	L	Seaweed extract	1, 2, 4, 5
Hunter ™	L	Plant extracts	1, 4
Ilsaneem ™	P	Neem cake	1, 2, 3, 7
Kendal Nem ™	L	Plant extracts	1, 2, 4, 6
Keos Guardian ™	L	Chitosan	1, 5
Micofort ™	P	Micorrhizal fungi	1, 2, 4, 5
Micosat F ™	P	Micorrhizal fungi	1, 4, 5
Micosat Jolly ™	P	Micorrhizal fungi	1, 4, 5
Mychodeep ™	P	Micorrhizal fungi	1, 2, 4

Table 1. *Cont.*

Commercial Name	Formulation [1]	Raw Materials	Activity [2]
Neem Soil ™	**P**	**Neem cake**	**1, 3, 4, 6**
Neem Care FL ™	L	Plant extracts	1, 2, 4
Nema 300 WW ™	L	Plant oils	1, 2, 4
Nemaforce ™	L	Humic and fulvic acids, plant extracts	1, 2, 4, 5, 7
Nematec ™	**L**	**Seaweed extract**	**1, 2, 4**
Nematiller ™	L	Plant extracts	1, 2, 4
Nematon EC ™	L	Sesame oil	1, 4
NeMax ™	**L**	**Sesame oil**	**1, 4**
Nutrich ™	P	Neem and pongamia cake	1, 2, 3, 4
Propoli oleoso ™	L	Propolis oil	1, 4
Rigenera Active	L	Seaweed macerate, plant extracts	1, 2, 4
Sesamin EC ™	L	Sesame oil	2, 4, 5
Tagete ™	L	Tagetes extract	1, 2, 4, 8
Tequil Multi ™	**L**	**Quillay and yucca extracts**	**1, 2, 4, 8**
Tyson ™	L	Propolis oil	1, 4, 8
Xedaneem ™	P	Neem cake	1, 6

[1] L = liquid; D = dry meals, P = pellets, G = granules; [2] 1 = biostimulant; 2 = rooting; 3 = fertilizing; 4 = plant defense enhancement; 5 = increase of soil beneficial microflora; 6 = creation of a nematode-unfavorable environment; 7 = repellence, antifeeding, disorientation; 8 = toxicity. Products applied in the case study are reported in bold.

2.2. The Literature Review

Plant-derived biostimulants previously documented for an activity on phytonematodes were mostly liquid formulations of extracts and oils or, at a less instance, granular or powder seed meal or cake derivatives. A large number of plant biostimulants based on sesame seed oil [10], quillay water extract [11,12], or meals from biomasses or seeds of *Brassicaceae* plants and neem [13–15] were previously demonstrated for a suppressive activity on root-knot nematode populations on field and greenhouse tomato.

Seaweed extracts were found to cause an almost complete mortality of root-knot nematode juveniles and eggs in in vitro studies [16,17], as well as formulations of the extracts from seaweed species *Ascophyllum nodosum* L. and *Ecklonia maxima* Osbeck were reported for an effective control of root-knot nematodes also in soil experiments on tomato [18–20]. In addition to extract derivatives, a strong suppression of *Meloidogyne* spp. infestations on fruit or vegetable crops was described also for soil amendments with biomasses of seaweeds *Uva lactuca* L. and *Spatoglossus schroederi* Agardh (Kützing), may be due to their high content of phenolics and other bioactive compounds [21,22]. In addition to Meloidogyne species, suppressive activity of seaweed products was also detected on nematode parasites economically relevant to tropical or subtropical vegetable crops, such as *Helicotylenchus indicus* Siddiqui, *Belonolaimus longicaudatus* Rau, or *Radopholus similis* Cobb (Thorne) [23–26].

Literature studies are available also on the suppressive activity of chitosan and/or its derivatives, both alone or combined with other suppressive materials (agricultural wastes, plant compounds, biocontrol agents), either on root-knot nematodes [27–30] and other phytoparasitic species i.e., the soybean cyst nematode *Heterodera glycines* Ichinoe and the pinewood parasite *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle [31–33].

Most of the microbial biostimulants reported as active on phytoparasitic nematodes were formulations of arbuscular mycorrhizal fungi [34,35]. Suppressiveness to root-knot nematodes of these products, either alone or combined with other microorganisms or plant extracts, was documented both in field and greenhouse [36–39]. Moreover, their activity was demonstrated also on other phytonematode parasites, such as *Nacobbus aberrans* Thorne et Allen or *Helicotylenchus multicinctus* (Cobb) Golden on field banana and greenhouse tomato, respectively [40,41]. In addition to mycorrhizal fungi, formulations of other fungal or bacterial biocontrol agents (*Trichoderma* spp., *Bacillus* spp.) or nitrogen fixers (*Azospirillum* spp., *Azotobacter* spp.) were also reported for controlling *M. incognita* in glasshouse tomato and field sunflower [42–44], or improving crop tolerance to the cyst nematode *Heterodera schachtii* Schmidt and more generically to soil phytoparasitic nematophauna [45,46].

3. An Experimental Case Study

3.1. Materials and Methods

A sandy soil (64.4% sand,18.7% silt, 16.9% clay, 0.8% organic matter, pH 7.5; 18.2% soil average humidity, 23.5% field capacity, 12.9% wilting point), artificially infested with the root-knot nematode *M. incognita* (8 eggs and juveniles mL^{-1} soil) was poured into 2.5 L clay pots. Soil was then treated with three commercial liquid biostimulants derived from quillay (*Quillaja saponaria* Molina) extract (Tequil Multi®, Fertenia) (QE), sesame (*Sesamum indicum* L.) oil (NeMax®, Sumitomo Chemical) (SO) or brown algae (*Laminaria* spp.) extract (AgriPrime Nematec®, BioAtlantis) (SE), and a granular formulation of neem (*Azadirachta indica* Juss) cake (Neem Soil®, Serbios) (NC). QE, SO and SE were applied at transplant and 15 and 30 days later at amounts corresponding to 60, 10, and 2 L ha^{-1}, respectively, whereas NC was incorporated to the soil at a 1000 kg ha^{-1} rate two weeks before transplanting. The same treatments were also provided to pots containing non-infested soil. Soil treated with the nematicide Oxamyl (OX), applied at a 10 L ha^{-1} field rate 3 days before tomato transplant and 15 days later, and non-treated soil, both infested (NT) and non-infested (NI) by *M. incognita*, were used as controls. One-month-old tomato seedlings (cv. Harvester) were transplanted in each pot, providing five replicates for each treatment in comparison.

The pots were arranged in a randomized block design in a plastic greenhouse at 25 °C, where they were maintained for 75 days, receiving a regular irrigation but no additional pesticide or fertilizer treatment. At the end of their permanence in the greenhouse, plants were uprooted and weight of green and root biomass was recorded for each plant. Root gall formation was estimated according to a 0–10 scale [47] and nematode multiplication on tomato roots was determined by extracting eggs and juveniles by the Hussey and Barker's method [48]. Data were statistically analyzed by ANOVA and treatment means were compared by the Fisher's Least Significant Difference Test at $P \leq 0.05$, using PlotIT 3.2 (Scientific Programming Enterprises, Haslett, MI) software.

3.2. Results

The number of nematode eggs and juveniles on tomato roots were always significantly lower in the soil treated with the four biostimulants or OX than in NT soil (Figure 1A). Moreover, the multiplication of *M. incognita* in pots treated with NC or SO was not statistically different from OX and significantly lower than the treatments with QE and SE. Finally, QE resulted to be significantly more suppressive than SE.

Treatments with the four biostimulants and OX also resulted in a significantly lower number of root galls in comparison with NT (Figures 1B and 2). As for nematode eggs and juveniles, the formation of galls in soil treated with NC and SO was statistically lower than QE and SE, though only NC was significantly not different from OX. No statistical difference occurred between the number of galls from QE and SE.

Tomato plant biomass in soil infested by *M. incognita*, either non-treated and treated with the biostimulants or OX, was always significantly lower than NI (Figure 3A). Green biomass from plants in soil treated with QE was significantly larger compared to all the other treatments and NT. Adversely, weight of green biomass from pots treated with the other three formulates was not significantly different from NT and statistically lower than OX.

Weight of the tomato roots from all the treatments but NC was significantly higher than the NT (Figure 3B). Moreover, QE resulted in a root biomass significantly heavier than the other three biostimulants and OX and not different from NI. Finally, SE resulted in a root growth statistically not different from OX but higher compared to NC and SO.

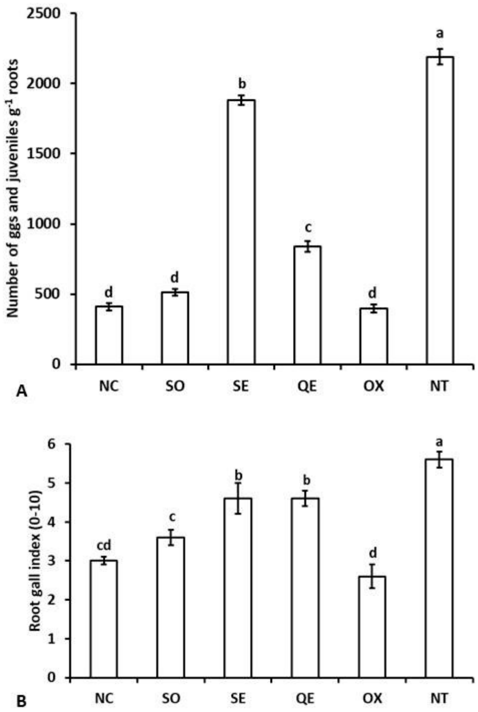

Figure 1. Multiplication of the root-knot nematode *Meloidogyne incognita* (**A**) and gall formation (**B**) on the roots of tomato cv. Harvester in soil non-treated (NT) or treated with commercial biostimulants based on neem cake (NC), sesame oil (SO), seaweed extract (SE), and quillay extract (QE) or with nematicide Oxamyl (OX). Bars tagged with the same letters are not statistically different ($P \leq 0.05$) according to the Least Significant Difference's Test.

Figure 2. Roots of tomato plants cv. Harvester from soil treated with commercial biostimulants based on neem cake (NC), sesame oil (SE), seaweed extract (SE) and quillay extract (QE) or with nematicide Oxamyl (OX) and from non-treated soil (NT).

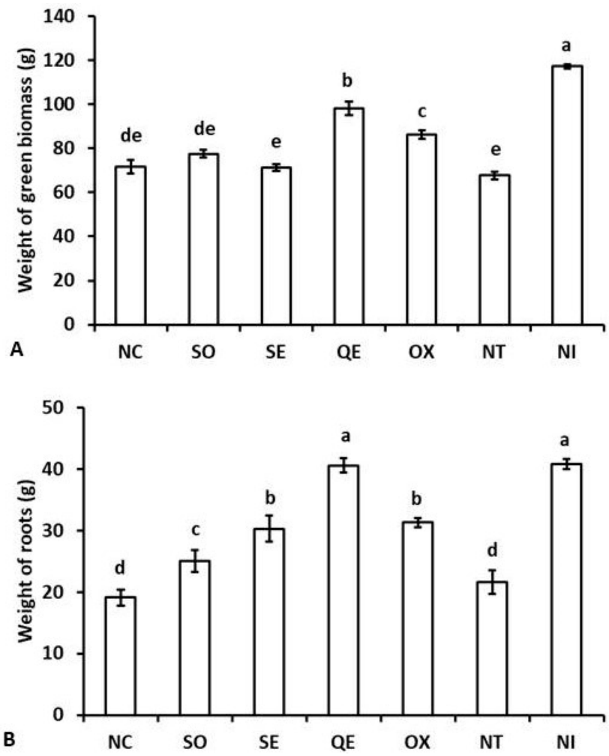

Figure 3. Weight of green biomass (**A**) and roots (**B**) of tomato plants cv. Harvester in soil non-treated (NT) or treated with commercial biostimulants based on neem cake (NC), sesame oil (SO), seaweed extract (SE), and quillay extract (QE) or with nematicide Oxamyl (OX). Bars tagged with the same letters are not statistically different ($P \leq 0.05$) according to the Least Significant Difference's Test.

4. Discussion

The experimental case study indicated that biostimulants can also provide a satisfactory nematode suppression, as confirming previous findings from literature studies. However, these results aim to be only indicative of the potential use of biostimulants in nematode management and need to be validated by future trials in field conditions, as well as different combinations of biostimulants should be also tested to verify a potential synergism among different products.

The mechanisms of biostimulants suppressiveness to nematodes are only partially known or simply hypothesized. Seaweed activity on phytoparasitic nematodes was generally attributed to their content of secondary metabolites, such as steroids, triterpenoids, alkaloids, and phenols, known for a nematicidal activity or as plant resistance elicitors [49,50]. Analogously, the suppressiveness to phytonematode populations of plant-based biostimulants is mainly related to nematotoxic metabolites both preformed in raw plant material (saponins, fatty acids, alkaloids and more) or released during the plant materials degradation in soil [51,52]. Induction of a systemic plant resistance to nematode penetration has been also documented for some active compounds of plant-derived biostimulants, such as neem azadiractin or chestnut (*Castanea sativa* Mill.) tannins [53,54]. Nematode suppression by microbial biostimulants was generally attributed to the induction of crop defense responses to nematode invasion [55,56]. Additional or alternative mechanisms, such as a competition for nutrients and space or the synthesis of nematicidal microbial metabolites have been also suggested [57–59]. The nematicidal effectiveness of chitosan products was generally attributed to the induction of a local or systemic plant resistance [60], though an enhancement of nematode-suppressive rhizospheric bacteria and fungi has been also hypothesized [36,40].

In our study, only QE was confirmed for a biostimulant effect on tomato growth, as limited only to the root biomass for SO and SE or nil for NC. The growth effect of QE can be attributed to the high content of triterpenic saponins, widely acknowledged for significant plant growth regulating properties [61], in *Q. saponaria* extracts.

Chemical composition of plant-based biostimulants can change according to a range of environmental and agronomic factors [62], as well as the nematode suppressiveness of microbial formulations may vary according to microbial strains, crop species/varieties, and environmental conditions [63]. Variable effects on soil phytonematode populations were also documented for chitosan products, as strictly dependent on the molecular weight of raw materials [32,64]. The unstable composition is a serious constraint to the full implementation of biostimulants in nematode management strategies, as leading to a fluctuating activity in field and, consequently, to a difficult certification of nematicidal performances and registration of commercial products [51]. A preliminary standardization of source raw materials and manufacturing processes should ensure constant suppressive performances and a successful market presence to the future commercial plant biostimulants addressed to nematode management. Moreover, preliminary toxicological screenings should be provided for any new biostimulant, as to exclude the presence of compounds with an unknown toxicological profile or the persistence of human pathogens in materials of animal origin [51].

In conclusion, plant biostimulants can also play a relevant role in the future nematode management strategies, as providing an acceptable nematode suppression in addition to their main activity of plant growth and ensuring a full safety to the other biotic soil components. It may be reasonably expected that the Regulation 2019/1009 [9] will lead to the disappearance of products with a direct toxicity to nematodes activity, because of the high costs of their registration as pesticides, as limiting the market to the products working through plant resistance improvement. A stand-alone application of these products can be reasonable only in organic crop systems, where few nematode control tools are available, or in short-cycle crops where the short pre-harvest intervals do not allow the use of most synthetic nematicides. However, a combination with other chemical or nonchemical control tools can justify the application of these products also in conventional crop systems. Benefit–cost ratio of treatments with the kind of products analyzed in this work should be always evaluated before their application as nematode suppressants, because of the high market price of these products which limit their use preferably to high value crops.

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