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Bionematicidal Potential of Undecanoic Acid Against Plant Root Parasitic Nematodes †

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Abstract

The growing demand for sustainable and cost-effective alternatives to synthetic nematicides has driven interest in naturally derived compounds with selective activity against plant-parasitic nematodes. In this study, we evaluated the bionematicidal potential of undecanoic acid, a naturally occurring medium-chain (C11) fatty acid, against two economically important root-knot nematodes: *Meloidogyne ethiopica* and *M. graminicola*. Direct contact bioassays demonstrated 100% mortality of both species within 24 h of exposure to a 1 mg/mL concentration, confirming strong and rapid nematicidal activity. In contrast, exposure of a non-target, the free-living soil nematode *Cephalobus* sp., resulted in only ca. 20% mortality, suggesting a favorable degree of selectivity toward phytoparasites. Additionally, environmental fate modeling indicated a predicted distribution of this compound of ca. 69% in soil, 28% in water, and 3% in air environmental compartments, consistent with its use as a soil-applied agent, while highlighting the need for environmental risk assessment under field conditions. As a naturally sourced compound, undecanoic acid offers advantages over synthetic nematicides, not only due to its biodegradability and potential reduced environmental impact, but also its lower cost compared to commercial pesticide active ingredients. The selective toxicity of undecanoic acid makes it a favorable candidate for integrated pest management programs, particularly in low-input or organic systems. These results underscore the potential of this naturally occurring fatty acid as an effective and sustainable tool for nematode control. Further studies on formulation optimization, persistence, and field efficacy will be needed to fully realize its application in agricultural systems.

Keywords: biopesticide; integrated pest management; *Meloidogyne*; nematicides; toxicity; undecylic acid

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1. Introduction

The escalating demands of a growing global population exert unprecedented pressure on agricultural systems to increase food production efficiently and sustainably. However, this is constantly threatened by countless biotic and abiotic factors, among which plant-parasitic nematodes (PPNs) represent one of the most significant biological constraints on crop yields worldwide. These microscopic roundworms inhabit the rhizosphere, where they directly feed on plant roots, causing extensive damage that compromises nutrient and water uptake, stunts plant growth, and predisposes host crops to secondary infections by pathogenic fungi and bacteria. The collective damage inflicted by these pests results in estimated global crop losses amounting to billions of dollars annually, underscoring their profound economic impact on both large-scale commercial farming and subsistence agriculture [1].

Among PPNs, root-knot nematodes (RKNs, *Meloidogyne* spp.) are considered the most economically damaging genus, with a host range spanning over 3000 plant species, including major food and cash crops like soybean, corn, potato, tomato, and various fruit trees. The characteristic symptoms of RKN infestation include the formation of galls or knots on the root system, which severely disrupt the plant's vascular tissues and lead to visible symptoms of wilting, chlorosis, and overall decline. The RKN life cycle involves a mobile second-stage juvenile (J2) that seeks out a suitable host root, where it penetrates the root tip and migrates into the vascular cylinder. Once inside, the J2s establish a permanent feeding site, a complex process that involves the induction of giant cells and the manipulation of host cell metabolism. This sedentary phase allows the nematode to grow and develop into an adult, which can then produce eggs, leading to a rapid buildup of population densities in the soil and exacerbating damage in subsequent crop cycles [2]. For example, *M. ethiopica* is a particularly aggressive and polyphagous species with a wide geographical distribution, posing a significant threat to perennial crops such as coffee, grapevine, and fruit trees, especially in tropical and subtropical regions [3]. While *M. graminicola*, by contrast, is known as the rice root-knot nematode and is a major constraint on global rice production. This species is highly adapted to both upland and lowland rice ecosystems, causing stunting, chlorosis, and reduced tillering, which directly translates to substantial yield reductions for a crop that is a staple food for half of the world's population [4].

For decades, the primary strategy for managing RKNs has been the application of synthetic chemical nematicides. These fumigants and non-fumigants agrochemicals have historically offered high efficacy in controlling PPNs and protecting crop yields. However, the widespread and indiscriminate use of these compounds has led to a range of critical drawbacks that have spurred a global reevaluation of their role in modern agriculture [5,6]. Many of the most effective synthetic nematicides are highly toxic to non-target organisms, including beneficial soil microbes, nematodes, insects, and aquatic life, leading to a severe disruption of ecosystem balance. Furthermore, their high mobility and persistence in soil have raised serious concerns about groundwater contamination and the accumulation of toxic residues in agricultural products, posing a significant risk to human health [7]. The most pressing issue, however, is the development of resistance in nematode populations, which diminishes the long-term effectiveness of these chemical controls and necessitates the search for novel, more sustainable management strategies. Thus, there has been a significant shift toward developing and implementing environmentally favorable alternatives for PPN control. The search for a new generation of nematicides has focused heavily on biopesticides derived from natural sources, including plant extracts, microbial agents, and organic compounds. These naturally occurring substances often exhibit high specificity, have a more favorable environmental fate, and are generally considered less harmful to human health and to non-target organisms [8]. The use of botanical

nematicides represents a promising avenue for integrated pest management systems, offering a viable and sustainable alternative that aligns with the principles of ecological agriculture. A wide array of plant-derived compounds, including alkaloids, terpenoids, phenolics, and fatty acids, have been identified as having potential nematicidal properties, but the mechanism of action and practical application of many of these remain to be fully explored [9].

Among the various classes of organic compounds with reported biological activity, saturated fatty acids and their derivatives have attracted considerable attention. Fatty acids are fundamental components of lipids and play a crucial role in cellular metabolism, but at elevated concentrations, some of these compounds can exhibit potent antimicrobial and pesticidal effects [7]. Medium carbon chain saturated fatty acids are naturally occurring compounds found in various plant oils and microbial metabolites. Their relatively simple structure and presence in natural sources make them attractive candidates for development as bionematicides. Although their antimicrobial properties have been studied, the specific efficacy against the most economically significant PPNs, such RKNs, has been scarcely evaluated, leaving a critical knowledge gap in the field of sustainable nematode management [10,11].

The current preliminary study evaluates the nematicidal activity of undecanoic acid, a medium carbon chain unsaturated fatty acid, against *M. ethiopica* and *M. graminicola* under controlled in vitro conditions, in comparison to a free-living nematode of the *Cephalobus* genus, as an ecological indicator. This research seeks to provide data on the potential of undecanoic acid as a natural, low-risk alternative to conventional chemical nematicides. The findings of this study aim to contribute to the growing body of knowledge on natural nematicides and may pave the way for the development of new, more sustainable strategies for RKN management and highlight the broader significance of using natural fatty acids as a viable tool in the future of sustainable agriculture.

2. Material and Methods

2.1. Chemicals

Pure standard undecanoic acid (≥99%) was acquired from Sigma-Aldrich (St. Louis, MO, USA) and diluted in HPLC-grade methanol (Fischer Chemicals, Hampton, NH, USA) to a concentration of 20 mg/mL [12]. This stock solution was stored at −20 °C until used.

2.2. Growth of Root-Knot Nematodes

Meloidogyne ethiopica is regularly maintained in tomato plants, *Solanum lycopersicum* L., cv. Ox heart [3], while *M. graminicola* is maintained in rice, *Oryza sativa* L. var. Ariete [4], the most commonly grown rice variety in Portugal, as reference isolates at the Plant Nematology Lab of the National Institute for Agrarian and Veterinary Research (INIAV, I.P.), in Oeiras, Portugal. For *M. ethiopica*, 1 L black plastic pots were filled with a sterilized mixture of local soil, sand, and substrate (1:1:1), watered to 70% of maximum water holding capacity and maintained in these conditions for 1 week for soil stabilization. Tomato seedlings, obtained by germinating commercially acquired certified seeds on hydrated filter paper, are transferred to the pots and kept at 25 ± 1 °C. For nematode infection of the tomato seedlings, RKN egg masses were added to the soil in the vicinity to the roots of tomato plants and maintained in a growth chamber at 25 ± 1 °C [13]. After ca. 60 days, tomato roots already displayed fully developed root galls with egg masses. For *M. graminicola*, rice seeds were germinated at 26 ± 1 °C on hydrated filter paper and then transplanted into 0.5 L dark plastic pots filled with a sterilized 2:1 mixture of sand and biological substrate (SIRO, Dublin, Ireland). A wet environment was maintained by daily watering, and fertilizer was applied once a week (0.5 g/L; Green House Feeding (Hybrids);

NPK: 15:7:22). After ca. 7 days, seedlings were inoculated with 200 J2 and maintained in a growth chamber at 26 ± 1 °C. After ca. 45 days, host roots already displayed fully developed root galls with visible egg masses. For both species, the plants were harvested and the root systems washed. The eggs were isolated from the roots by mixing with a 0.52% (*v*/*v*) NaOCl solution and were hatched in moist chambers at 25 ± 1 °C [4]. The J2s were counted by sampling 5 aliquots of 100 µL from the 5 mL solution in which the eggs were hatched. The quantification of nematodes and/or assessment of survival rates was performed with an Olympus SZX12 (Tokyo, Japan) stereomicroscope. The free-living *Cephalobus spp.* nematodes were isolated from the vicinity of grown rice roots and identified through morphological assessments and molecular techniques. For molecular identification, genomic DNA was extracted from three separate specimens following the protocol of the DNeasy Blood & Tissue Kit (Ref 69504, Qiagen, USA) as described by the manufacturer. The 18S ribosomal rDNA region was amplified by PCR using the primers 988F/1912R [14] in a total reaction volume of 25 µL with Supreme NZYTaq II 2x Green Master Mix (Ref. MB3600, NZYTech Lisbon, Portugal) in a Biometra Twin 48G thermocycler (Analytik Jena, Germany). The amplicons were sequenced, and the resulting sequences were analyzed by comparison with the GenBank database using BLAST. For in vitro growth, ca. 100 nematodes were surface sterilized with 10% hydrogen peroxide (H_2O_2), according to [3], and transferred to semi-solid Schenk and Hildebrandt [15] medium with 8 g/L agar, 30 g/L sucrose, pH = 5.6. The nematode population increased by feeding on the naturally accompanying bacteria that grew monoxenically in the culture medium. After 4 weeks mixed life stage nematodes were recovered through the modified Baermann tray method [16] and immediately used in direct contact bioassays.

2.3. Direct Contact Bioassays

The direct-contact bioassays were conducted using flat-bottom 96-well microtiter plates (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). Each well was filled with a 95 µL suspension containing approximately 60 RKN J2s, followed by 5 µL of undecanoic acid stock solution prepared in HPLC-grade methanol (at a final concentration of 1 mg/mL). Controls included blank wells containing 5 µL of ultrapure water to measure natural RKN mortality, and wells with 5 µL of methanol to assess mortality caused by the solvent. The microtiter plates were then sealed with plastic film to prevent excessive compound volatilization and shaken on an orbital shaker (IKA labortechnik, Staufen, Germany) at 800 r.p.m. for 1 min. Afterwards, the plates were covered with aluminum foil to maintain darkness and incubated for 24 h in an orbital shaker set to 60 r.p.m. at 25 ± 1 °C [17]. The bioassays were performed in triplicate for each sample, with a total of 9 bioassays conducted.

2.4. Data Treatment and Statistical Analysis

Nematode mortality percentages were calculated using Formula (1):

$$\text{Mortality \%} = (\text{dead RKNs/total no. of RKNs}) \times 100 \quad (1)$$

To obtain corrected mortality percentages Formula (2) was applied:

$$\text{Corrected mortality \%} = [(\text{mortality \% in treatment} - \text{mortality \% in control})/(100 - \text{mortality \% in control})] \times 100 \quad (2)$$

The toxicological strength was classified using previously established criteria, where mortality was deemed complete at 100%, very strong ≥90% strong 60–89%, moderate 37–59%, weak 11–36% and low or inactive <10% [18].

For the prediction of the environmental fates (PED, %) of undecanoic acid in comparison to the synthetic nematicides oxamyl and fluopyram [3], the EPISuite freeware [19],

freely available from the US Environmental Protection Agency (EPA) [20], was used with its unified database.

3. Results and Discussion

Natural mortality was assessed for the RKNs after 24 h in water. While for *M. ethiopica* a 2.1 ± 0.1% mortality was recorded, for *M. graminicola* natural mortality was 0.0 ± 0.0%. Control RKN mortality was assessed by exposing the J2 to 5% methanol. For *M. ethiopica* control mortality was 2.3 ± 0.1%, while for *M. graminicola* control mortality was 0.0 ± 0.0%, which suggests that RKNs were mostly unaffected by methanol as a carrier agent for undecanoic acid.

Undecanoic acid tested at 1 mg/mL induced complete mortality in RKN J2s tested, however, free living nematodes (*Cephalobus* sp.) were only weakly influenced (Table 1).

Table 1. Nematicidal activity of undecanoic acid against the plant parasitic nematodes *Meloidogyne ethiopica* and *M. graminicola*, and the free-living nematode *Cephalobus* sp., at 1 mg/mL.

Nematode	PWN Mortality [1]	Nematicidal Strength
M. ethiopica	100.0 ± 0.0	Strong
M. graminicola	100.0 ± 0.0	Strong
Cephalobus sp.	17.1 ± 0.5	Low/inactive

[1] Corrected mortality calculated using formula: Corrected mortality % = [(mortality % in treatment − mortality % in control)/(100 − mortality % in control)] × 100, where control corresponded to the wells with 5 μL of methanol.

RKNs showed the common morphological features of each species. For example, for *M. graminicola*, control J2 were slender, vermiform, and transparent, with an average body length of 400–500 μm (Figure 1a). The body tapered gradually at both ends, with the anterior region bearing a distinct, well-developed stylet that is slender and sharply pointed, adapted for host penetration (Figure 1b). The esophageal region was clearly differentiated, with the median bulb, esophageal glands, and lumen. The intestine extended along the body length and was filled with granular contents, reflecting normal metabolic activity under control conditions. The posterior region narrowed progressively to form a finely pointed tail, which is a key diagnostic feature for this species (Figure 1c).

(**a**) (**b**) (**c**)

Figure 1. Differential interference contrast microscopy (DIC) images of *Meloidogyne graminicola* second stage juveniles under control conditions after 24 h. Whole body showing slender vermiform

morphology with intact internal organization (**a**), anterior region with distinct stylet and esophageal structures (**b**) and posterior region with a gradually tapering, sharply pointed tail and granular intestinal content (**c**). Bar = 30 μm.

However, in contact with undecanoic acid, *M. graminicola* J2s exhibited severe internal structural disruption compared to controls. The entire body appeared degraded, with loss of transparency and distorted internal organization (Figure 2a). In the anterior region, the median bulb and esophageal structures were barely noticeable, indicating degeneration of feeding apparatus integrity (Figure 2b). The mid-body showed marked vacuolization and swelling, suggesting cytoplasmic degradation and organelle breakdown (Figure 2c). The posterior region also presented extensive vacuolization and loss of tissue organization, with the tail region appearing shrunken and irregular (Figure 2d).

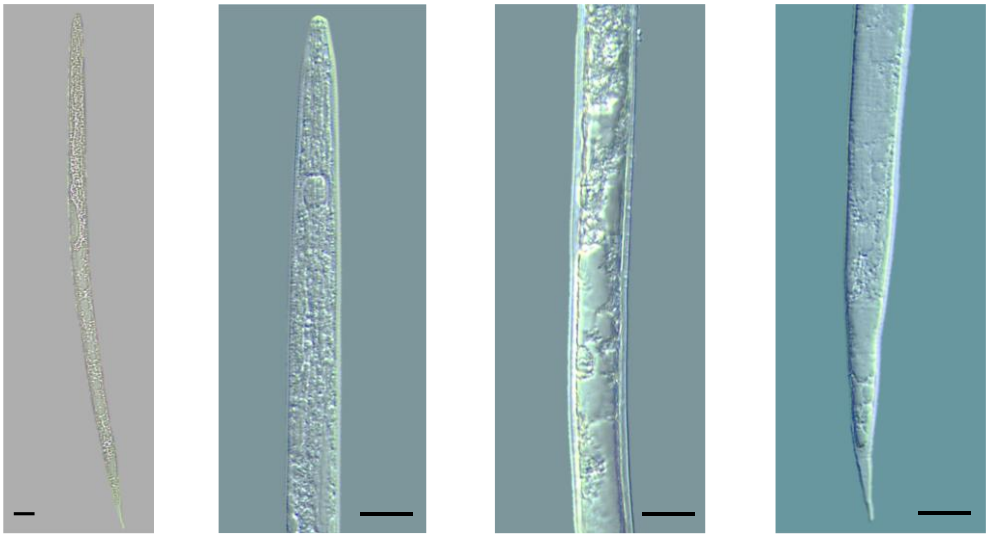

Figure 2. Differential interference contrast microscopy (DIC) images of *Meloidogyne graminicola* second stage juveniles after 24 h in contact with 1 mg/mL of undecanoic acid. Whole body showing internal disruption and loss of transparency (**a**) anterior region with the median bulb and esophageal structures barely distinguishable (**b**) mid-body exhibiting extensive vacuolization and disintegration of internal organs (**c**) and posterior region with disrupted tissues and a shrunken tail (**d**). Bar = 20 μm.

The potential environmental distribution of undecanoic acid was compared to that of the conventional pesticides oxamyl and fluopyram, to understand the possible ecological impacts of using undecanoic acid as a biopesticide. In the analyzed compounds, the soil environmental compartment was favored, with a predicted 80% fluopyram, 69% undecanoic acid, and 65% oxamyl being retained in this compartment (Table 2). For fluopyram, the remaining compound was predicted to be mainly retained in the sediments (17%) and water (3%) environmental compartments. For oxamyl and undecanoic acid, the remaining compounds were predicted to be mainly retained in the water environmental compartment, 35% and 28%, respectively.

Table 2. Predicted environmental distribution (PED) percentages (%) in the air, water, soil, and sediments environmental compartments computed with the EPISuite freeware [19], for undecanoic acid and the conventional nematicides oxamyl and fluopyram.

Compound	Air	Water	Soil	Sediment
Undecanoic acid	2.7	28.2	68.9	0.2

Oxamyl	0.0	35.0	64.9	0.1
Fluopyram	0.0	2.7	80.3	17.0

These preliminary results point towards undecanoic acid as a strong nematicide effective against both *M. ethiopica* and *M. graminicola*, achieving complete mortality of J2 within 24 h at 1 mg/mL. This effect seems specific to root-knot nematodes, as only minor mortality was observed in the free-living nematode *Cephalobus* sp., suggesting a degree of selectivity that could be advantageous in agricultural applications. Morphological observations revealed that activity may be due to marked internal disruption and vacuolization in RKNs treated with undecanoic acid, whereas control juveniles retained intact internal structures. When compared to conventional nematicides, environmental modeling suggested that undecanoic acid may behave similarly to oxamyl and fluopyram, although differences in partitioning suggest that undecanoic acid may behave distinctly in certain environments. Such results highlight both the potential benefits of undecanoic acid as a biopesticide and the need for further research into its environmental persistence, ecological risks, and field-level efficacy.

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Abbreviations

The following abbreviations are used in this manuscript:

PPN	Plant-parasitic nematodes
RKN	Root-knot nematodes
J2	Second stage juveniles

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