chemistry proceedings

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Proceeding Paper

Influence of Carbonyl Position in C9 Ketones Against the Phytoparasitic Pinewood Nematode †

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† Presented at the 29th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-29); Available online: https://sciforum.net/event/ecsoc-29.

Abstract

Medium-chain aliphatic compounds bearing oxygen-containing functional groups—such as alcohols, ketones, or carboxylic acids—have attracted increasing attention due to their potential as bioactive agents in pest management. These compounds have demonstrated diverse biocidal properties, including insecticidal, antimicrobial, fungicidal, and nematicidal activities. In this study, the nematicidal potency of three structurally related C9 aliphatic ketones—2-nonanone, 3-nonanone, and 5-nonanone—was evaluated against *Bursaphelenchus xylophilus*, the pinewood nematode (PWN). These isomeric ketones differ in the position of the carbonyl group, providing a useful model for examining structure–activity relationships (SAR) among positional isomers. The direct-contact bioassays, performed at 1 mg/mL, revealed that 2-nonanone exhibited the highest nematicidal activity, causing 92.3 ± 1.2% mortality on the PWN, followed by 3-nonanone at 80.1 ± 0.8%, while 5-nonanone showed significantly lower activity at 17.1 ± 0.5%. The results suggest a strong dependency of bioactivity on the position of the carbonyl group along the carbon chain. The increasing efficacy from 5- to 2-nonanone suggests that proximity of the carbonyl group to the terminal end may enhance activity, for example by enhancing membrane interaction or disrupting nematode metabolic processes. These findings underscore the importance of molecule structure analysis in designing effective nematicidal agents and support further investigation into terminally positioned oxygenated medium-carbon chain aliphatic compounds as potential leads. This work highlights that subtle structural differences within homologous series can significantly influence bioactivity and provides a foundation for developing targeted, biodegradable nematicides derived from simple aliphatic frameworks.

Keywords: *Bursaphelenchus xylophilus*; isomers; ketones; nematicides; nonanone; pinewood nematode; toxicity

Academic Editor(s): Name

Published: date

Citation: Faria, J.M.S.; Pereira, G. Influence of Carbonyl Position in C9 Ketones Against the Phytoparasitic Pinewood Nematode. *Chem. Proc.* **2025**, *volume number*, x. https://doi.org/10.3390/xxxxx

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1. Introduction

The pinewood nematode (PWN, *Bursaphelenchus xylophilus*) is the causative agent of pine wilt disease (PWD), a destructive condition to coniferous tree forests, that leads to severe ecological and economic damage, particularly in Asia and parts of Europe. Its dispersion is performed by insect vectors of the genus *Monochamus*, that can carry the PWN

to long distances and makes effective pest management very difficult. In susceptible trees, where pine lacks natural defenses, the PWN invades the resin canals, disrupts water transport and leads to rapid desiccation and death. The damage caused by PWD has prompted the urgent need for effective control strategies, especially in areas with limited natural resistance [1].

Chemical nematicides have been employed to control PWD spread in areas with limited numbers of trees, or in city areas. These include broad-spectrum pesticides applied through trunk injections to eliminate nematode populations in affected arboretums [2]. While these compounds can be effective in the short term, their use presents several challenges specific to forest settings. Their non-selective toxicity can negatively impact non-target organisms, including beneficial soil fauna and microbial communities essential for plant health. Also, these chemicals can persist in the environment, raising concerns about contamination of soil and nearby water bodies, especially in sensitive ecological zones [3]. The repeated exposure to the same chemical agents can lead to the development of resistance in nematode populations, ultimately reducing the efficacy of treatment protocols. Furthermore, increasing regulatory restrictions on pesticide use in forestry, particularly within the European Union, have resulted in the withdrawal of many conventional nematicides. This has created a significant gap in effective control measures and highlighted the urgent need for the development of novel, environmentally sustainable nematicidal agents that can be integrated into PWD management programs [4,5].

Among the promising alternatives to traditional nematicides are medium-chain aliphatic compounds, especially those containing oxygen-based functional groups such as alcohols, ketones, and carboxylic acids. These compounds, typically derived from natural sources, have demonstrated a wide range of bioactivities, including insecticidal, antimicrobial, fungicidal, and nematicidal effects [6,7].

C9 ketones are of interest due to their relatively simple chemical structure, ease of synthesis, and biodegradable nature. Structural variations within these molecules can lead to significant differences in bioactivity, offering opportunities for fine-tuning their effectiveness through rational molecular design. For aliphatic oxygen containing compounds, the position of the functional group along the carbon chain appears to play a critical role in determining nematicidal strength [7,8]. Variations in this position can alter the molecule's polarity, hydrophobicity, and capacity for membrane penetration, which in turn affects its interaction with nematode physiology. Previous studies have shown that even minor structural changes—such as shifting the carbonyl group by a single carbon—can lead to drastic differences in bioactivity [7]. Such insights underline the importance of detailed structure activity relationship (SAR) evaluations in the search for next-generation nematicides.

In this proceedings paper, we present for the first time the results of a comparative evaluation of three C9 aliphatic ketones—2-nonanone, 3-nonanone, and 5-nonanone—against the PWN. These positional isomers differ in the location of their carbonyl group, providing an ideal model for investigating SARs in nematicidal compounds.

2. Material and Methods

2.1. Chemicals

The C9 ketones [2-nonanone (99%), 3-nonanone (99%), and 5-nonanone (98%)] were 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 [9]. These stock solutions were stored at −20 °C until used.

2.2. Pinewood Nematode In Vitro Culture

The PWN population used in the direct-contact bioassays was grown in vitro in aseptic conditions by feeding on axenic cultures of a non-sporulating strain of *Botrytis cinerea* (de Bary) Whetzel. Firstly, certified organic barley grains (*Hordeum vulgare* L.) were hydrated and steam-sterilized (approximately 15 g of barley with 15 mL of ultrapure water in 250 mL Erlenmeyer flasks), inoculated with a fungal culture plug, and maintained for 7 to 10 days at 25 ± 1 °C. The reference isolate Bx0.13.003, kept at the Plant Nematology Lab of the National Institute for Agrarian and Veterinary Research (INIAV, I.P) in Oeiras, Portugal, was used for the experiments [10]. After the cereal was covered by fungal culture, 1 mL of a mixed life stage suspension of PWNs, containing about 1000 nematodes per mL, was added to the culture and kept in darkness at 25 ± 1 °C for 7 to 10 days, or until the fungus was completely consumed. To avoid microbial contamination, the nematodes were surface sterilized with a hydrogen peroxide (H_2O_2) solution (20%, *v*/*v*) for 20 min, and washed 3× with sterilized water, in the flow hood, before being introduced into the axenic mycelial cultures [11]. The modified Baermann funnel technique was used to isolate PWNs [12]. The PWN suspensions were used immediately for the direct-contact bioassays or stored at 11 °C for up to 1 week. PWN numbers and mortality were assessed using an Olympus SX12 stereomicroscope (40×) (Olympus, Tokyo, Japan).

2.3. Direct Contact Bioassays

The determination of compound mortality was performed in flat-bottom 96-well microtiter plates (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). A suspension of mixed life stages of the PWN was prepared so that a 95 µL aliquot contained approximately 60 PWNs. This volume was added to each well with 5 µL of each of the C9 ketones stock solution aiming at a final concentration of 1 mg/mL. Control treatments consisted of wells supplemented with 5 µL of ultrapure water to determine baseline (natural) mortality, and wells containing 5 µL of methanol to evaluate solvent-related effects. To prevent volatilization of the test compounds, plates were sealed with plastic film and briefly agitated on an orbital shaker (IKA Labortechnik, Staufen, Germany) at 800 rpm for 1 min. The plates were then wrapped in aluminum foil to ensure dark conditions and incubated for 24 h at 25 ± 1 °C on an orbital shaker operating at 60 rpm [13]. All treatments were conducted in triplicate, resulting in a total of nine independent bioassays.

2.4. Data Treatment and Statistical Analysis

Mortality percentages for the PWN were calculated using Formula (1):

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

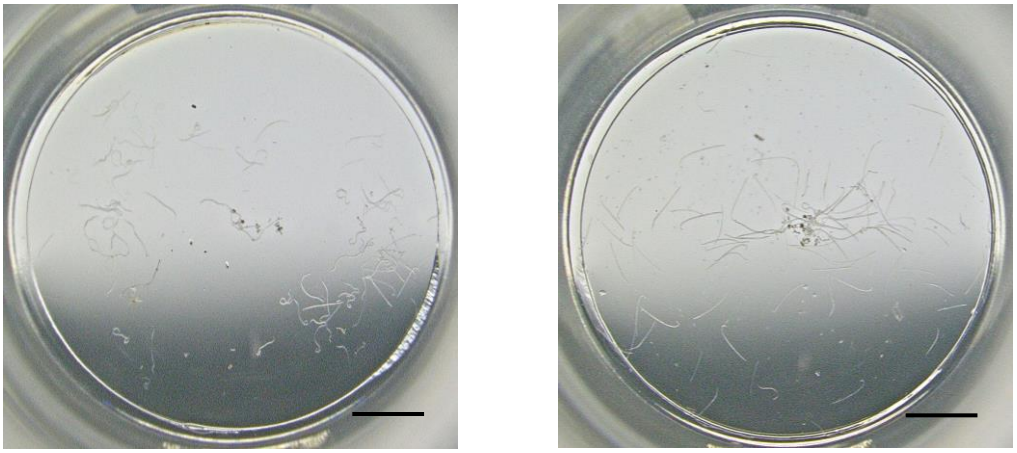
The corrected mortality percentages were obtained using the methanol assays as control through Formula (2):

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

The toxicological strength was classified using previously established criteria, where mortality was deemed complete at 100%, strong above 80%, moderate between 80 and 61%, weak between 60 and 40%, and low or inactive below 40% [14].

3. Results and Discussion

The activity of the C9 ketones was tested against freshly extracted PWNs, for 24 h. Blank wells consisting of 5 µL of added ultrapure water showed a mortality percentage of 2.0 ± 0.5%, while for control wells, with 5 µL of added methanol, a mortality of 2.6 ± 0.5% was recorded (Figure 1a).

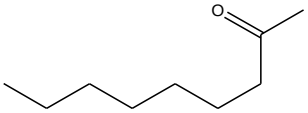
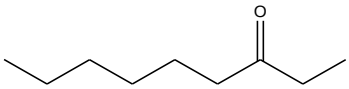
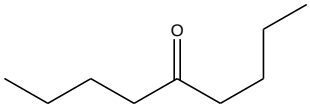


(**a**) (**b**)

Figure 1. Example of direct-contact bioassay wells with pinewood nematodes (PWN) after 24 h of exposure to control conditions (5 µL of added methanol) (**a**) or 2-nonanone (at 1 mg/mL) (**b**). Bar = 1 mm.

For the C9 ketones, mortality values were corrected by accounting for mortality observed in the control wells. The highest corrected mortality was recorded for 2-nonanone, reaching 92.3 ± 1.2% (Figure 1b, Table 1). Shifting the ketone functional group to the C3 position (3-nonanone) resulted in an approximately 12% decrease, yielding 80.1 ± 0.8% of corrected mortality. In contrast, positioning the ketone group at the C5 position reduced corrected mortality to low or inactive levels (17.1 ± 0.5%) (Table 1). These results classify 2- and 3-nonanone as strong nematicidal agents, while in contrast 5-nonanone exhibits negligible activity under the tested conditions.

Table 1. Nematicidal activity of the C9 ketones against the pinewood nematode (PWN).

Ketones	Chemical Structure	PWN Mortality [1]	Nematicidal Strength
2-Nonanone		92.3 ± 1.2	Strong
3-Nonanone		80.1 ± 0.8	Strong
5-Nonanone		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.

The corrected mortality percentages reported for the tested compounds highlight a strong influence of carbonyl position on nematicidal potency (Table 1). As the carbonyl group shifts from C2 to C5, mortality decreases sharply, confirming that subtle changes in molecular structure can drastically impact biological activity. Similarly, Seo et al. reported varying levels of nematicidal activity of oxygen containing aliphatic compounds (C6–C14) against the PWN, with several compounds (e.g., C9–C11 alkanols and alkanonic acids) achieving 100% mortality rates at 0.25 mg/mL [7].

The present series (2-nonanone > 3-nonanone > 5-nonanone) suggests that terminal or near-terminal carbonyl positioning may enhance interaction with nematode membranes or disrupts metabolic pathways, while more central positioning (C5) diminishes activity. Comparable SAR effects have been observed in natural esters and related oxygenated compounds [8,15]. The strong activity observed for 2-nonanone is consistent with previous reports on medium-chain aliphatic volatiles as effective nematicidal agents [3,6]. These molecules are attractive for sustainable pest management due to their relative simplicity, biodegradability, and natural occurrence. The contrasting results between active and inactive isomers, emphasize that even within homologous series, structural changes influence bioactivity (Table 1).

From an application perspective, these results support further evaluation of oxygenated medium-chain aliphatic compounds, particularly ketones with terminally positioned carbonyl groups, as potential leads against the PWN. Given the increasing regulatory restrictions on synthetic nematicides within the European Union and the associated risks of non-selective toxicity, volatile phytochemicals may provide safer alternatives for integration into PWD management programs [2,3,5]. Future work should explore concentration-dependent effects, potential synergisms with other volatiles, and field-scale trials to assess ecological compatibility and long-term efficacy [10,16].

Author Contributions: Conceptualization, J.M.S.F. and G.P.; methodology, J.M.S.F. and G.P.; software, J.M.S.F. and G.P.; formal analysis, J.M.S.F. and G.P.; investigation, J.M.S.F. and G.P.; resources, J.M.S.F.; data curation, J.M.S.F.; writing—original draft preparation, J.M.S.F.; writing—review and editing, J.M.S.F. and G.P. All authors have read and agreed to the published version of the manuscript.

Funding: Partly funded by Fundação para a Ciência e a Tecnologia (FCT/MCTES) through project NemACT (DOI 10.54499/2022.00359.CEECIND/CP1737/CT0002) and structural funding GreenIT (DOIs 10.54499/UIDB/04551/2020 and 10.54499/UIDP/04551/2020).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data is available from the corresponding author (Jorge M. S. Faria) upon reasonable request.

Acknowledgments: The authors wish to thank Cristina Moiteiro at CQE/FCUL, Lisbon, Portugal; and Tomás Cavaco from the Systems Biology Lab at Vrije Universiteit in Amsterdam.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PWN	Pinewood Nematode
PWD	Pine Wilt Disease
SAR	Structure Activity Relationship
HPLC	High-Performance Liquid Chromatography

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