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# A Review on the Nematicidal Activity of Volatile Allelochemicals against the Pinewood Nematode †

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**Abstract:** The pinewood nematode (PWN), *Bursaphelenchus xylophilus*, induces shoot yellowing and wilting by injuring pine vascular tissues and resin canals. In Asia, it has devastated wide forests of susceptible pine with drastic ecological, economic and cultural repercussions. In 1999, it reached Europe (Portugal) and despite the actions of the authorities, it rapidly advanced to the border areas of Spain, threatening other European pine forests. Chemical control has been used in Asia with remarkable success, yet most nematicides are dangerous to human health and the environment. Natural volatile allelochemicals (VA) and derivatives are sustainable alternatives, providing many advantages to commercial nematicides. The present work summarizes available bibliographic information on VAs with activity against the PWN and reviews the chemical properties leading to nematoxicity. Published works have reported over 250 VAs in direct contact assays. Compounds highly active against the PWN belong to monoterpenoid, phenylpropanoid and aliphatic alcohols; aldehydes, ketones, carboxylic acids, esters and sulphide groups. The presence of highly electronegative elements in these structures appears to increase activity. Trisulphides, coumarins, medium carbon chain length aliphatic alcohols and derivatives comprise the top five most active allelochemicals. An indication of their mechanisms of action can be obtained from studying the reported structure–activity relationships.

**Keywords:** *Bursaphelenchus xylophilus*; monoterpenoids; nematicide; oxygenated aliphatic compounds; phenylpropanoids; pinewood nematode; sulphides; toxicity

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## 1. Introduction

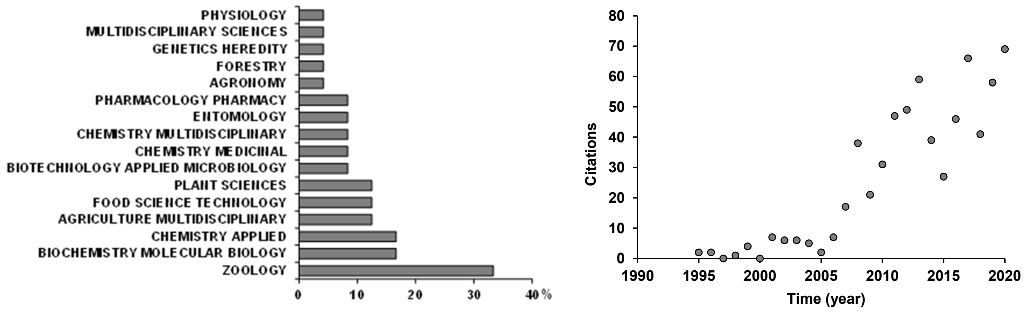
Pine wilt disease (PWD) induces pine shoot yellowing (chlorosis) and wilting (caused by embolism), which can kill a mature tree in less than 50 days. It is caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner & Buhrer 1934), an invasive microscopic plant parasitic nematode with a short life cycle and an impressive spread rate. It was introduced from North America to Japan around 1905 and devastated vast pine forests. The PWN has since spread to neighboring China (1982) and Korea (1988). Susceptible Asian pine forests were heavily affected causing irreversible changes to native forest ecosystems and significant losses to national economies [1]. In 1999, Portugal became its entry point into Europe, most likely through imported goods from Asian countries [2]. It since spread throughout Portugal and reached Spanish border cities after only 10 years [3,4]. Due to the environmental conditions imposed by climate change, there is a very high risk for a Europe-wide epidemic of this forest pathogen [5]. The PWN is now classified as an A2 quarantine pest, and the European Union (EU) has taken steps to ensure its control beyond the present geographic area and to eradicate it from EU territory. PWN containment strategies generally rely on the removal of the insect vector through aerial application of synthetic insecticides, the establishment of pine tree-free buffer zones, the fumigation of infected trees or the use of the insect vector's natural enemies [1]. In the most affected countries, chemical control is considered one of the most effective and reliable containment strategies within integrated management. Directly killing the nematode is performed by trunk injection of synthetic or hemisynthetic nematicides, like avermectins, carbamates or neonicotinoids [6]. Unfortunately, common use nematicides are generally toxic to other beneficial microorganisms, humans and animals and can accumulate in the soil and in food plants. The development of environmentally safer strategies to control invasive PWN populations has become paramount due to the ban imposed on hazardous pesticides and the recent fear of drug resistance on the PWN [6]. Strong pressure on the development of improved ecological biopesticides has prompted researchers to screen for environmentally friendlier natural compounds with increased anti-PWN properties, which are, at the same time, cost effective. Primary breakthroughs have been reached by screening highly active volatile allelochemicals (VAs) that are commonly produced by plants and microbes [7,8]. These compounds have the advantage of not accumulating in the environment and having a broad range of activities, which diminishes the risk of developing resistant pathogenic strains. Mixtures of VAs, such as essential oils (EOs), have shown great promise due to their high nematicidal activities and for being easily obtained [9]. Research has been mainly performed in the most affected countries: Japan, China, Korea and recently Portugal. Faria et al. [10] screened over 80 EOs and identified their chemical composition through gas chromatography coupled with mass spectrometry (GC-MS). Through EO fractionation, the authors were able to attribute anti-PWN activity to the oxygen-containing EO components. Other authors have also identified the chemical composition of EOs and tested the main components of the active EOs by using commercially acquired chemicals with noteworthy results [11,12].

In the present review, a comprehensive bibliographic research was performed to catalogue the tested VAs and identify groups with the highest activities against the PWN. A critical overview of their chemical structures permits the highlighting of the most successful anti-PWN chemical properties in an effort to uncover novel, highly nematicidal environmentally friendly volatiles and develop eco-friendly anti-PWD strategies.

## 2. Bibliographic Data

Research was performed using the Web of Science® search engine on published works in all available databases using the topics "*Bursaphelenchus xylophilus*" and "nematicide," "nematocide" or "nematodicide". To ensure a direct causality between allelochemicals contact and PWN mortality, only works reporting a direct contact bioassays were selected. Works were identified and catalogued, and information on a compound's chemical nature and half-maximal effective concentration ($EC_{50}$) against the PWN was collected when available. A total of 24 publications were identified reporting the activity of anti-PWN VAs and derivatives. The first identified report dated to 1993 and the latest to 2020 (with 2 reports by September 10). The highest number of reports was published during 2007 (5 publications). Regarding the scientific fields covered by the journals that published these reports, 33% were in zoology, 17% in biochemistry and molecular biology and 17 % in applied chemistry (Figure 1a). The identified publications were cited 650 times (611 when excluding self-citations) by a total of 478 articles (462, excluding self-citations), with an average of 27 citations per work. From 1995 to 2005 this field showed consistent low interest, with an average 3 citations per year, yet after 2005 the study of VAs and derivatives as anti-PWN agents gained momentum and showed linear growth, tripling citations each year (slope = 3) (Figure 1b). The pattern of increased citation since the mid-2000s may be due to a similar increase in reports on the nematicidal activity of essential oils (EOs)

[13]. These complex mixtures of volatiles are mainly comprised of mono- and sesquiterpenes (and a few diterpenes), phenylpropanoids and other groups of volatile compounds, in relevant amounts. Their biological activity can generally result from the combined effect (synergistic or antagonistic) of compounds with direct activity and those with no direct activity towards the biological system that can influence resorption, rate of reaction and bioavailability of the active compounds [10]. In the 24 publications identified, EOs were tested and profiled through gas chromatography coupled with mass spectrometry (GC-MS), to identify the component(s) responsible for the nematicidal activity against the PWN.

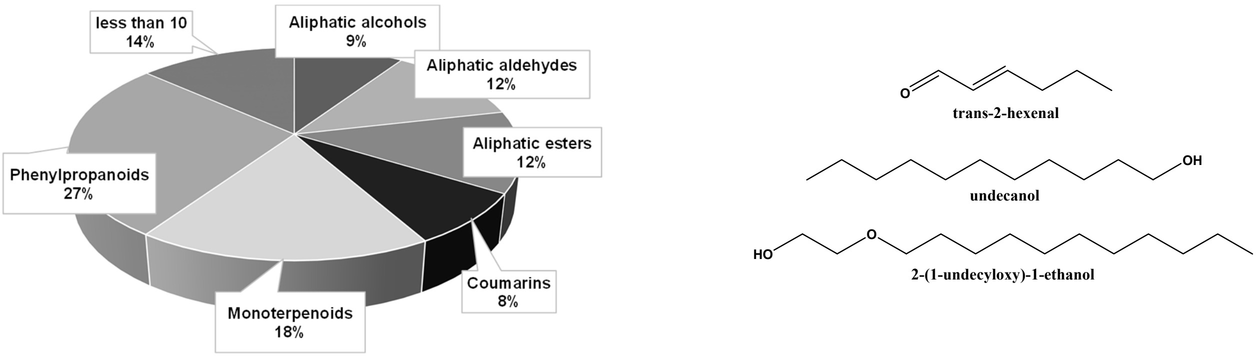



(a) (b)

**Figure 1.** (**a**) Percentage of reports on the activity of volatile allelochemicals and derivatives against *Bursphalenchus xylophilus* distributed by scientific journal areas and (**b**) yearly citation evolution of these reports.

## 3. Anti-PWN Allelochemicals

Worldwide research efforts tested the activity of over 250 allelochemicals against the PWN. Publications reported mainly on compounds identified in plant extracts [14–18] or essential oils [11,12,19–23], microbial extracts [24–26] and compounds synthetized based on highly active analogs [27–35]. The compounds assayed belong mainly to highly active classes of chemical compounds, namely, aliphatic alcohols, aldehydes, ketones, carboxylic acids, esters and sulphides (42%), phenylpropanoids (27%) and monoterpenoids (18%) (Figure 2a). Allelochemicals with direct activity against the PWN showed highly electronegative elements in their composition. The most active were oxygenated compounds with alcohol, aldehyde or carboxylic acid active groups or with sulphur (disulfide or trisulphide functional groups).




(a) (b)

**Figure 2.** (**a**) Percentage of the main chemical groups of the volatile allelochemicals and derivatives reported against the PWN; "less than 10" includes classes with less than 10 instances. (**b**) The top 3 compounds with the lowest reported half-maximal effective concentrations ($EC_{50}$).

The top 3 compounds with the lowest $EC_{50}$ values where trans-2-hexenal, undecanol and 2-(1-undecyloxy)-1-ethanol (Figure 2b). Trans-2-hexenal is an aliphatic aldehyde with a C6-length carbon chain and is very common in fresh green plant EOs [10,17,18,36]. It's used as a flavoring compound in food to induce a leafy fruity note and in perfumes to obtain a green-leaf note. Against the PWN it appears to influence several biologic functions such as reproduction, by reducing rate of reproduction and number of eggs, and development, such as body length, respiration, movement, nutrition and enzymatic activity [17,18]. Undecanol is an aliphatic primary alcohol with a C11-length carbon chain. It's a colorless or pale-yellow, lemon-scented liquid used for some typical aroma fragrances but only at low concentrations. Its activity against the PWN appears to be a part of a mechanism of toxicity imposed by oxygenated aliphatic compounds. Aliphatic compounds with C8 to C11 carbon chain lengths containing alcohol, aldehyde or carboxylic acid functional groups showed increased activity against the PWN in comparison to lower or higher-chain lengths or to hydrocarbons or alkanoic acetates. In this mechanism the presence of double bonds next to the functional group appears to play a role in anti-PWN activity [28]. 2-(1-undecyloxy)-1-ethanol shows similarities to undecanol by having a C11 carbon chain linked to an oxygenated functional group. Interestingly, this molecule is an aggregation pheromone that attracts both conspecific females and males of the *Monochamus* species, which is the PWN insect vector in many countries. This pheromone, known also as monochamol, is commonly used in insect traps against the PWD [31].

*Chemical Characteristics of Successful Anti-PWN Allelochemicals*

The information gathered allowed crossing data on the most nematotoxic compounds and their chemical nature. Some preliminary insights into the chemical basis of anti-PWN efficacy were reported in the publications analyzed [11,23,28,29,31]. Concerning terpenoids, essentially monoterpenoids, compounds with phenol, alcohol or aldehyde functional groups had higher anti-PWN activities than those with ketones or hydrocarbons. Within compounds with the alcohol functional group, primary alcohols showed greater activity than the secondary or tertiary alcohols. Molecule isomerism also appears to contributive to nematicidal activity. The position of the substituent in the geometrical isomer of monoterpenoid molecules influences anti-PWN activity. Regarding phenylpropanoids, in addition to most of the characteristics reported for monoterpenoids, aldehydes, carboxylic acids and alcohols are reported to be more active against the PWN than their corresponding saturated compounds. In the carboxylic acids of these compounds, allyl, ethyl and methyl substituents in the aromatic ring showed higher activity than their isopropyl and vinyl compounds. Phenylpropanoids with hydroxyl or methoxy groups showed higher activity than those with an acetyl group. The reported aliphatic alcohols, aldehydes, ketones, carboxylic acids, esters and sulphides also showed interesting structure or anti-PWN activity relationships. Compounds with alcohol, aldehyde and carboxylic acid functional groups were more active against the PWN than aliphatic hydrocarbons or acetates. The enhancement of anti-PWN activity in aldehydes is linked to unsaturated $\alpha$,β-carbonyl compounds. More interestingly, aliphatic alcohols with specific shape and size showed the most successful activity against the PWN, C9 to C11 aliphatic chain alkanols and trans-2-alkenols showed substantially higher activity than those with lower or higher chain lengths. Finally, sulphides were strongly nematotoxic, showing a positive correlation between anti-PWN activity and sulphur number.

## 4. Conclusions

Research for highly active volatile allelochemicals against the PWN is relatively recent, less than 30 years old, but it has gained momentum in the last 10 years promoted by an increasing search for alternative sustainable strategies to contain this highly damaging forest pest. The works reported so far highlight the success of compounds with oxygenated functional groups. Monoterpenoids, phenylpropanoids and aliphatic oxygenated compounds show the most promising activity. Future research must take advantage of this knowledge to uncover the specific sites and mechanisms of activity employed by these compounds against the PWN. This information will contribute to the development of effective, sustainable strategies to control the spread of PWD.

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