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In Silico Screening of Agonist and Antagonist Natural Compounds from Reported Essential Oils against *Bursaphelenchus xylophilus* †

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Abstract: Chemical control has been the most effective and reliable containment strategy in integrated pest management of pine wilt disease (PWD), caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus*. Yet, large spectrum nematicides can be dangerous to human health and the environment. Essential oils (EOs) are safer sustainable alternatives, being composed of highly active natural compounds. A survey of bibliographic data on the detailed chemical composition and activity of the EOs used against the PWN allowed pinpointing monoterpenes as the main source of structures with agonist or antagonist properties. Transversal EO data treatment can identify potential highly active anti-PWN compounds.

Keywords: monoterpenes; nematicides; pine wilt disease; pinewood nematode; toxicity; volatiles

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1. Introduction

Plant parasitic nematodes are still a major threat to the sustainability of world agriculture, forestry, and related industries. Their impact on global crop yield is an estimated $157 billion, twice as much as that of invasive insects [1]. The increased spread of these plant pathogens takes advantage of the increased use of global trade transportation brought about by a reduction in shipping costs and the increased reliance of the commerce industry. The pine wilt disease (PWD), caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner and Buhrer 1934) Nickle 1970, is an example of an extremely hazardous forest disease with a high spread rate. In its native range, in North America, it causes damage only to introduced exotic pines, while autochthonous pines show tolerance. Yet, after its introduction to Japan in the 1900s, vast susceptible pine stands were devastated with grave environmental and economic repercussions [2]. Unsuccessful containment strategies lead to its spread to neighboring China (1982) and Korea (1988) and, in 1999, to Europe (Portugal) [3].

PWN containment generally relies on the removal of the insect vector through aerial application of synthetic insecticides, establishment of pine tree-free buffer zones, fumigation of infected trees or on the use of vector natural enemies [4]. Additionally, the application of synthetic or hemisynthetic chemical nematicides is currently the most effective and reliable containment practice in integrated management, and is widely used in the most affected countries [5]. These contact nematicides act by killing the nematode through direct exposure, after trunk injection of lethal concentrations of the compound.

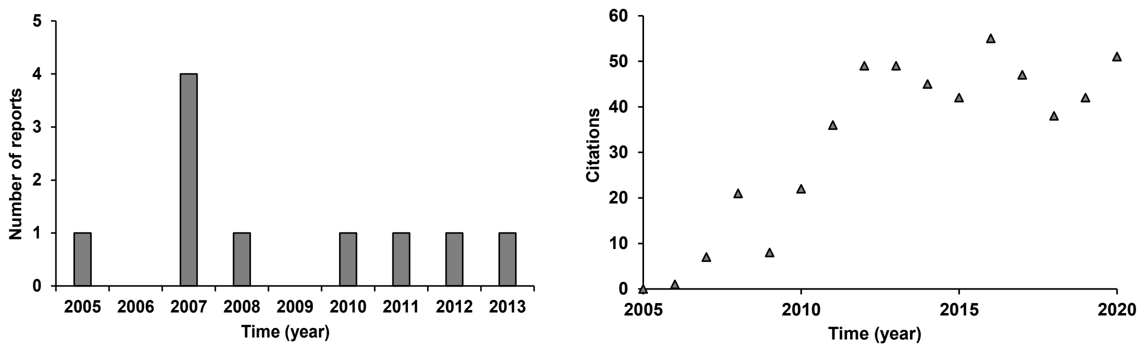
Nevertheless, common use broad spectrum nematicides show several disadvantages. Besides being toxic to other beneficial microorganisms, some have been banned due to hazardous effects to humans and animals, and most can accumulate in the soil and in food plants. Moreover, due to the recent development of drug resistance to these pesticides in insect pests, the fear of lack of efficiency on the PWN has arisen [5]. Worldwide research has directed its efforts towards the screening of environmentally friendlier natural compounds with increased anti-PWN properties. Plant extracts and essential oils (EOs) have proven to be effective alternatives given that, besides being easily obtained and highly active, they do not accumulate in the environment and have a broad range of biological activities, which diminishes the risk of developing resistant pathogenic strains [6]. EOs are composed of highly active chemical classes of compounds, generally terpenoids (mono-, sesquiterpenes and a few diterpenes), phenolic compounds, such as phenylpropanoids, but other groups of volatile compounds can occur in high relative amounts [6]. The biological activities displayed by these complex mixtures often result from the combined effect of volatiles that show direct activity with those that show no direct activity on the biological system, but that are capable of influencing resorption, rate of reactions and bioavailability of the first. Interactions can be additive, synergistic or antagonistic, if the combined effect is equal, exceeds or is less than to the sum of the individual effects, respectively [7]. Most studies neglect to disclose the full EO chemical composition even though this is a valuable information towards understanding anti-PWN activities. In the present work, the available bibliography on detailed chemical composition and biological activity of the EOs used against the PWN was thoroughly screened. The correlation between each EO component abundance and the anti-PWN activity of the respective EO was used to pinpoint compounds with a high probability of showing agonist or antagonist responses to anti-PWN activity. Lastly, volatiles with these potential interactions were investigated in data from reported EOs of pine species with recognized variation in susceptibility to the PWD. The information gathered in the present work may provide the groundwork to understand the complex biochemical mechanisms responsible for volatiles' nematotoxic activity and role in host tolerance to the PWD.

2. Reports with Detailed Essential Oil Composition

Research was performed with Web of Science® search engine on published works reporting direct contact bioassays, in all available databases, using the topics "*Bursaphelenchus xylophilus*" and "Essential oil". Information on EO qualitative and quantitative chemical composition and PWN mortality, at the various applied EO concentrations, was collected when available. Information was compiled in a single table and dose-response curves were fitted to each EO chemical component, transversally to all EOs, using Origin 2019 statistical software [8]. Information on positive or negative correlation and goodness of fit (coefficient of determination [R^2] values) was obtained.

Full or partial identification of anti-PWN EO composition was retrieved from ten reports, dating from 2005 to 2013 (Figure 1a) [8–17]. These were published in journals covering mainly areas of zoology (70%), biochemistry and molecular biology (20%) and chemistry (20%).

The listed publications were cited 513 times (483, excluding self-citations) by a total of 378 reports (370, excluding self-citations), with an average of 51 citations per work. Citing articles were published by journals publishing on plant sciences, chemistry, agriculture, environmental sciences and ecology research areas. Research interest increased from 2005 to 2012 but has since become stable (Figure 1b).

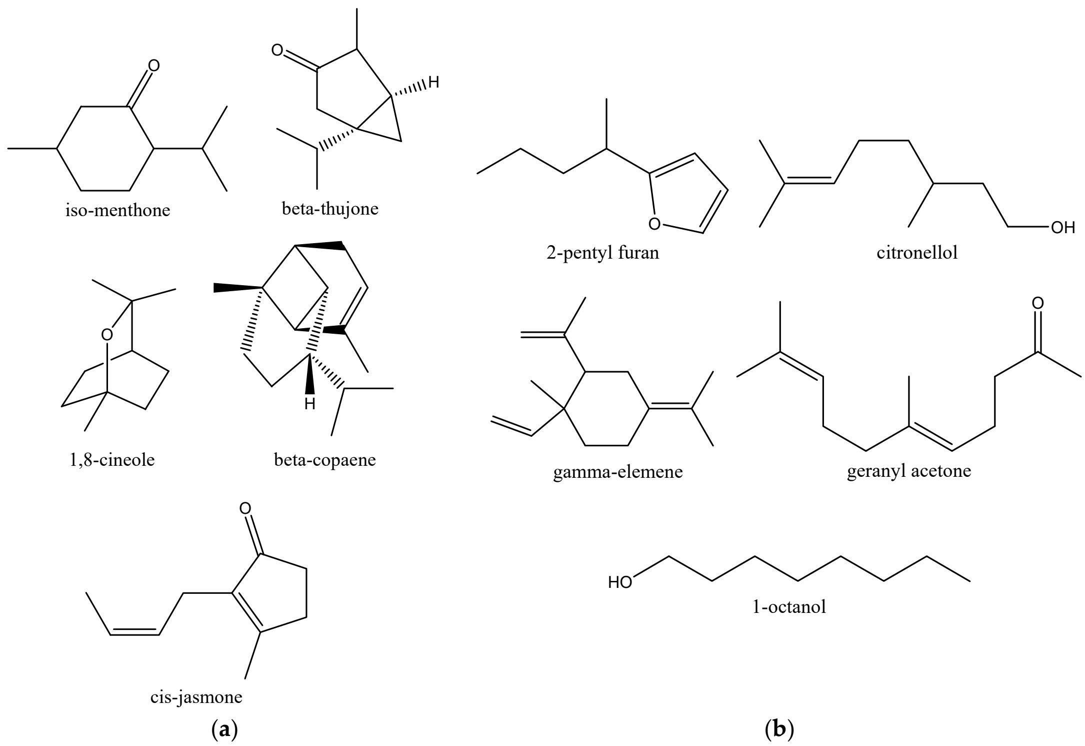


(**a**) (**b**)

Figure 1. (**a**) Yearly number of reports on the activity of essential oils, with detailed chemical composition, against *Bursphalenchus xylophilus* and (**b**) yearly citation evolution of these reports.

3. Potentially Agonist and Antagonist Essential Oil Components

The identification of reported EOs composition was performed using gas chromatography coupled to mass spectrometry (GC-MS) in every publication. A total of 109 EOs were used, summing a collective 325 volatiles, from eight chemical classes; namely, alcohols, aldehydes, carboxylic acids, esters, ethers, hydrocarbons, ketones and sulphides. From these, negative and positive correlations with anti-PWN activity were identified for 36 compounds, mainly monoterpenoids. Selected compounds displayed datasets with more than three different entries obtained from three different EOs and a goodness of fit value above 0.2. Ten compounds showed negative correlations and 26 showed positive correlations. The top five compounds with negative correlations (potential antagonists) were 1,8-cineole, β-thujone, β-copaene, cis-jasmone and iso-menthone, with R^2 values between 0.43 and 0.99, and datasets that ranged from 3 to 55 entries (Figure 2a). The top five compounds with positive correlations (potential agonists) were citronellol, 1-octanol, δ-elemene, geranyl acetone and 2-pentyl furan, with R^2 values that ranged from 0.79 to 0.86, and datasets between 4 and 25 entries (Figure 2b).



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

Figure 2. Identification of the top 5 essential oil (EO) components showing negative (**a**) and positive (**b**) correlations with anti-pinewood nematode EO activity, that show the highest goodness of fit values after dose-response curve fitting.

Occurrence of Selected Compounds on Pinus Hosts with Different PWD Susceptibilities

A previous study reported on the volatile composition of *Pinus* species with different degrees of susceptibility to the PWD. In this study, the EOs of the less susceptible *P. pinea* and *P. halepensis* and the more susceptible *P. pinaster*, and *P. sylvestris* where characterized with GC-MS [18]. The compounds with positive and negative correlations identified in the present study were crossed with the data reported in this work, and several important trends were identified. Shoots of healthy stone pine trees, *P. pinea*, the least susceptible of the four species, revealed EOs with twice as much components with positive anti-PWN correlations as maritime pine, *P. pinaster*, the most affected species in the Mediterranean. In fact, the cumulative relative amount of EO components with negative anti-PWN correlations in *P. pinaster* EO was almost four-fold higher than that in *P. pinea* EO. This is an indication that complex biochemical mechanisms may be implicated in the interspecies variation of host susceptibility to the PWD.

4. Conclusions

EOs can provide powerful alternatives to synthetic nematicides in sustainable PWD containment strategies. They are composed of volatile compounds that can either have a positive, negative or no effect on anti-PWN activity. Furthermore, the total EO activity is a result of compounds' additive, synergistic and competitive interactions. Crossing available data on the composition of anti-PWN EOs reported worldwide allowed the most probable agonist and antagonist EO components to be indicated. Screening these on published pine host EOs revealed that less susceptible *P. pinea* showed a greater number of agonist compounds towards anti-PWN activity while the more susceptible *P. pinaster* showed a higher proportion of antagonists in its EO composition.

Further research is needed to screen the interactions between the components of an active EO to reach definitive EO mechanisms of action on the PWN. Additionally, the connection between biochemical mechanisms of variation in host susceptibility to the PWD and its EO composition should be addressed if a multilayered strategy for PWD sustainable integrated management is to be reached.

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