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# Acaricidal Activity of Pine Essential Oils and Their Main Components against *Tyrophagus putrescentiae*, a Stored Food Mite

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Some essential oils obtained from the branches of four *Pinus* species (*P. pinea* L., *P. halepensis* Mill., *P. pinaster* Soil in Ait., and *P. nigra* Arnold) have been evaluated for their acaricidal activity by aerial diffusion against the stored food mite *Tyrophagus putrescentiae* (L.). All the essential oils showed a good efficacy, but *P. pinea* oil and its two constituents 1,8-cineole and limonene were the most effective compounds, showing 100% acaricidal activity at 8 $\mu$L; 1,8-cineole showed the same activity at 6 $\mu$L.

**KEYWORDS: Essential oil; 1,8-cineole; limonene; acaricidal activity; *Tyrophagus putrescentiae*; stored food mite; *Pinus pinea***

## INTRODUCTION

The toxicity and the environmental impact of many synthetic drugs and the resistance evidenced by many acari and insect pests (*1*, *2*) justify the study of alternative drugs to control acari and insect pests, such as new and more degradable compounds of natural origin. In light of these problems, we are evaluating the activity of essential oils and other plant extracts against mange infestation in animals.

Essential oils are secondary products of the metabolism of lower (Cryptogamae) and higher plants (Gymnospermae and Angiospermae). Their biological activity against several organisms, mainly bacteria and fungi and arthropods, was confirmed in many reports and it is mainly due to mono and sesquiterpenoids that represent their main components (*3*−*5*).

The acaricidal activity of many essential oils has been previously reported: Charmil gel, containing essences of *Cedrus deodara* Loud. (Pinaceae) and *Pongamia glabra* Vent. (Fabaceae), was used against *Sarcoptes scabiei* (L.) (Sarcoptidae) in dogs (*6*) and pigs (*7*); a phyto-aromatic gel (Canidor), composed of more than 15 volatile oils from plants, was used against the rabbit mite *Psoroptes cuniculi* (Delafond) (Psoroptidae) (*8*); linalool and the essential oils from *Lavandula angustifolia* Mill. (Lamiaceae) and *Artemisia verlotorum* Lamotte (Asteraceae) were also evaluated against *P. cuniculi* in rabbits (*9*−*11*).

Preliminary screening of new compounds against mange mites requires preliminary in vitro tests for the evaluation of their potential effectiveness. Unfortunately, it is not possible to cultivate in vitro the mites responsible for mange infestation, so experimental animals, as donors of mites, must be used for preliminary screenings. On the contrary, stored food mites such as *Tyrophagus longior* (Gervais), *T. palmarum* (Oudemans), and *T. putrescentiae* (Schrank) (Tyroglyphidae) can be successfully used in vitro as target organisms (*12*). These free-living mites are present on the external surface of seasoned hams, sausages, and cheeses.

The present study deals with the acaricidal activity against *T. putrescentiae* of the essential oils obtained from the branches of *P. pinea* L., *P. halepensis* Mill., *P. pinaster* Soil in Ait., and *P. nigra* Arnold (Pinaceae) and of some of their main constituents.

## MATERIALS AND METHODS

Branches (2−5 cm; without leaves) of *P. pinea*, *P. halepensis*, *P. pinaster*, and *P. nigra* were collected during spring 1998 in Pisa and Massa provinces (Tuscany, Italy). All the samples were dried in the air and in the shadow till constant weight was achieved, then ground and submitted to hydrodistillation in a Clevenger-like device for extraction of the essential oils.

Samples were analyzed using a HP-5890 gas chromatograph equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm film thickness), working with the following temperature program: 60° C for 10 min, ramp of 5 °C/min up to 220 °C. The injector and detector temperatures were 250 °C; carrier gas was nitrogen (2 mL/min); detector was set at dual FID; split ratio was 1:30 with injection of 0.5 mL. The identification of the components was performed, for

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**Table 1.** Main Constituents of Essential Oil from *Pinus* spp. (branches)

| constituent | lri[a] | *P. pinea* | *P. halep* | *P. pinaster* | *P. nigra* |
|---|---|---|---|---|---|
| α-pinene | 941 | 3.9 | 61.8 | 61.6 | 70.0 |
| camphene | 955 | 0.1 | 0.6 | 0.7 | 1.3 |
| β-pinene | 981 | 1.1 | 0.7 | 23.5 | 2.2 |
| myrcene | 993 | 2.5 | 20.1 | 5.2 | 0.6 |
| 3-carene | 1013 | - | 2.0 | - | - |
| *p*-cymene | 1027 | 0.1 | 0.1 | - | 0.6 |
| limonene | 1032 | 75.3 | 0.8 | 1.4 | 1.8 |
| 1,8-cineole | 1034 | 4.0 | - | - | 0.4 |
| γ-terpinene | 1064 | 0.1 | 0.1 | - | 1.8 |
| α-terpineol | 1190 | 0.8 | 0.2 | 0.2 | - |
| dihydrocarvone | 1202 | 0.1 | - | - | 4.6 |
| carvone | 1244 | 0.2 | - | - | 2.5 |
| carvacrol | 1299 | 0.4 | - | - | 0.2 |
| α-cedrene | 1411 | 1.5 | - | 3.1 | - |
| β-caryophyllene | 1420 | 3.7 | 8.5 | 1.0 | 0.2 |
| α-humulene | 1459 | 0.8 | 1.9 | 0.2 | - |
| germacrene D | 1482 | 0.2 | 0.6 | 0.9 | 1.1 |
| δ-cadinene | 1524 | 0.1 | - | 0.2 | - |
| caryophyllene oxide | 1583 | 0.3 | - | - | 0.1 |
| total identified | | 95.2 | 97.4 | 98.0 | 87.4 |

[a] lri, Linear retention indices (HP-5 column).

both columns, by comparison of their retention times with those of pure authentic samples, by means of their relative retention times with those of pure authentic samples, and by means of their linear retention indices (lri) relative to the series of *n*-hydrocarbons.

GC/EIMS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures, 220 and 240 °C, respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 mL/min; injection of 0.2 μm (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (*13*−*18*). Moreover, the molecular weights of all the identified substances were confirmed by GS/CIMS, using MeOH as CI ionizing gas.

Mites were isolated from samples of seasoned Parma ham and identified according to the key and descriptions given by Robertson (*19*).

The procedure carried out to test the activity of the essential oils and pure principles against mites was planned in order to ascertain the acaricidal activity of their volatile fraction without direct contact with the mites. For this purpose a piece of Parma ham harboring at least 20 adult mites was put in a 6-cm Petri dish covered with a filter-paper disk that allowed gas exchanges; each dish was placed in a 9-cm Petri dish containing the test substance. The entire system was maintained at 25 °C in a humidified atmosphere (80−85% relative humidity). Each compound was tested at 8 μL; 6 μL was also used when 100% efficacy was observed at 8 μL. A 6-cm Petri dish containing the mites placed in a 9-cm empty Petri dish served as controls. All the trials were repeated in triplicate.

The acaricidal efficacy was evaluated by considering the motility of the mites. Mites mortality was evaluated after 72 h by stimulating them with a needle; lack of any reaction and the persistence of immobility was taken to indicate death.

Percentage data were transformed arcsin $\sqrt{\%}$ before ANOVA. The means were separated on the basis of the LSD test only when the *F* test of the ANOVA per treatment was significant at the 0.05 or 0.01 probability level (*20*).

## RESULTS

**Table 1** shows the compositions of the four essential oils used in the acaricidal tests. The acaricidal effectiveness of the essential oils and of some pure constituents of *P. pinea* oil are summarized in **Table 2** and **Table 3**, respectively.

**Table 2.** In vitro Activity of Essential Oils from *Pinus pinea, P. halepensis, P. pinaster*, and *P. nigra* against *Tyrophagus putrescentiae*

| | death (%) | |
|---|---|---|
| essential oil | 8 μL | 6 μL |
| *P. pinea* | 100a[a] | 20d |
| *P. halepensis* | 60b | 10e |
| *P. pinaster* | 53c | 10e |
| *P. nigra* | 10e | 10e |
| control | 10e | 10e |

[a] Means followed by the same letters are not significantly different at the 0.01 probability level according to the LSD test.

**Table 3.** Acaricidal Activity against *Tyrophagus putrescentiae* of Some Constituents of *Pinus pinea* Branches Essential Oil at 8 μL and 6 μL

| | death (%) | |
|---|---|---|
| pure principle | 8 μL | 6 μL |
| α-pinene | 10c | 10c |
| β-caryophyllene | 10c | 10c |
| myrcene | 10c | 10c |
| limonene | 100a | 32b |
| 1,8-cineole | 100a | 100a |
| control | 10c | 10c |

[a] Means followed by the same letters are not significantly different at the 0.01 probability level according to the LSD test.

The oils obtained from *P. pinea, P. halepensis,* and *P. pinaster* branches showed a good acaricidal efficacy. Among them, the oil from *P. pinea* showed the best activity (100% deaths, while those from *P. halepensis* and *P. pinaster* were only partially effective only at the higher dose); and the dose of 8 μL showed a percentage of dead mites statistically higher than that of the lower dose (**Table 2**).

The main constituents of the essential oil of *P. pinea* branches were α-pinene, β-caryophyllene, myrcene, 1,8-cineole, and limonene (**Table 1**); for this reason we evaluated also the acaricidal efficacy of these terpenoids. Among them α-pinene, β-caryophyllene, and myrcene were ineffective, whereas 1,8-cineole and limonene showed 100% acaricidal activity at 8 μL. Only 1,8-cineole maintained 100% acaricidal activity also at the lower concentration of 6 μL (**Table 3**).

## DISCUSSION

In the present research the essential oil obtained from the branches of *Pinus pinea* evidenced a strong acaricidal activity in vitro against *Tyrophagus putrescentiae* by aerial diffusion. Among its principal components, only 1,8-cineole and limonene mantained acaricidal activity.

The high acaricidal activity of 1,8-cineole has been confirmed in other reports (*8*, *12*, *21*). Limonene is known for its insecticidal activity, and it is the active principle of an insecticidal spray commercialized in the United States to eliminate fleas from dogs and cats (*22*).

This study indicates the usefulness of storage mites as target species to evaluate the acaricidal activity of essential oils and their constituents because of their easy availability in stored food and of the possibility of their cultivation in vitro. Moreover, it is important to point out that these acari can give rise to allergic skin reactions (*23*, *24*) in animals and man, and are responsible for fall in the commercial value of stored food products (*25*).

For all these reasons, *P. pinea* essential oil, as well as 1,8-cineole and limonene, could represent new effective compounds to control *Tyrophagus* spp. mites.

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