Research note

The Role of Four Essential Oils on Mycelial Growth and Basidiomatal Formation of *Antrodia cinnamomea*

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【Summary】

Four wood essential oils (*Cinnamomum kanehirai*, *Cin. camphora*, *Cunninghamia konishii*, and *Chamaecyparis formosensis*) were used to test their effects on mycelial growth and basidiomatal formation of *Antrodia cinnamomea*, *A. salmonea*, and *A. heteromorpha*. In addition, these 4 essential oils were also used to test the effects on the mycelial growth of *Penicillium* sp. and *Trichoderma* sp. which are commonly isolated from rotten wood of *Cin. kanehirai*. The growth of *A. cinnamomea* and *A. salmonea* was most promoted with 100~1000 ppm of the 4 test essential oils and even with 5000 ppm of essential oils from *Cun. konishii*. The growth of *A. heteromorpha* and *Penicillium* sp. was most inhibited at all treatments except that *A. heteromorpha* was slightly promoted with 100 ppm of essential oils from *Cin. kanehirai* and *Cin. camphora*. The growth of *Trichoderma* sp. was only inhibiting at a concentration of 5000 ppm of all test essential oils and at a concentration 1000 ppm of essential oils from *Cin. kanehirai* and *Cin. camphora*. Lower concentrations of the 4 test essential oils did not affect the growth of *Trichoderma* sp. There was no basidiomatal formation on PDA medium with any concentration of the 4 test essential oils for the 4 isolates of *Antrodia*. Only isolate, TFRI B191, of *A. cinnamomea* produced basidiomes on PDA medium without the addition of essential oils, while the other isolates of *Antrodia* produced no basidiomes after 3 mo of incubation on the same medium. These results indicated that essential oils provide a favorable environment which promotes the growth of *A. cinnamomea* and *A. salmonea* but inhibits other possible competitive fungi, for better colonization of the fungi on their individual hosts, but they are not essential for basidiomatal formation of the fungi.

Key words: *Antrodia cinnamomea*, basidiomatal formation, mycelial growth, wood essential oils.

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研究簡報

四種木材精油對牛樟芝菌絲生長與子實體形成的影響

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摘　要

以四種木材精油(牛樟、樟樹、香杉、紅檜)測定對牛樟芝(*Antrodia cinnamomea*)、香杉芝(*A. salmonea*)和*A. heteromorpha*菌絲生長和菇體形成的影響。同時，測定分離自牛樟腐木之常見雜菌*Penicillium* sp.和*Trichoderma* sp.菌絲生長的影響。四種精油在添加濃度100~1000 ppm時，對牛樟芝和香杉芝有明顯促進生長，同時，在添加高濃度牛樟精油及香杉精油5000 ppm時分別對牛樟芝TFRI B191及牛樟芝與香杉芝菌絲仍有促進生長。*Antrodia heteromorpha*除在添加100 ppm的牛樟精油及樟樹精油有些微的促進生長作用外，其餘處理濃度均有明顯的抑制生長。四種精油所有處理的濃度均對*Penicillium*的生長有抑制作用。添加高濃度5000 ppm四種精油對*Trichoderma*的生長有抑制作用，且在添加1000 ppm時牛樟精油及樟樹精油就對*Trichoderma*有抑制作用，其餘處理則無抑制與促進生長作用。測定四種精油添加於PDA培養基對牛樟芝、香杉芝及*A. heteromorpha*出菇的影響，這三種菌在所有添加處理均沒有出菇，但在沒有添加的PDA上僅牛樟芝菌種TFRI B191可以出菇。從本研究結果可獲知：牛樟木材精油促進牛樟芝菌絲生長，而抑制其它可能競爭真菌，有助於提供牛樟芝在牛樟木材上建立群落，然而，牛樟芝出菇時，牛樟精油並非必要成份。

關鍵詞：牛樟芝、菌絲生長、子實體形成、木材精油。

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Antrodia cinnamomea TT Chang & WN Chou is restricted to the host plant, *Cinnamomum kanehirai* Hayata, and causes wood brown rot of the wood. It is believed that the wood essential oils of *Cin. kanehirai* should contribute to its host specificity because the wood extracts, the wood essential oils, and wood dust were able to promote the mycelial growth of *A. cinnamomea* when added to the nutrient agar media (Sheu et al. 2000, Wu et al. 2003). In addition, camphor oils from *Cin. camphora* (L.) Ness et Eberm. also increased the mycelial growth of *A. cinnamomea* (Chian et al. 1997). However, Chang and Wang (2005) reported that 1 isolate of *A. cinnamomea* was able to produce fruiting bodies on PDA (potato dextrose agar) and MEA (malt extract dextrose agar) media that contained no essential oils from *Cin. kanehirai*. This indicated that basidiomatal formation of *A. cinnomomea* was not related to wood components of *Cin. kanehirai*. In this study, we tested the role of different wood essential oils on the mycelial growth and basidiomatal formation of *A. cinnamomea* and some related fungi.

Two isolates (TFRI B86 and TFRI B191) of *A. cinnamomea* isolated from basidiomes were collected from Hsinchu and Taoyuan Counties, respectively. Isolate TFRI B191 is able to produce basidiomes on PDA and MEA media (Chang and Wang 2005), while isolate TFRI B86 cannot form basidiomes on the same media. Two other species of *Antrodia*, *A. salmonea* (isolate TFRI 1012) and *A. heteromorpha* (isolate TFRI 72), were used for the comparison studies on mycelial growth and basidiomatal formation. In addition, 2 fungi imperfecti, *Penicillium* sp. and *Trichoderma* sp., isolated from the rotten wood of *Cin. kanehirai* were used for a comparison study on mycelial growth.

Four isolates of *Antrodia* were used to test the mycelial growth and basidiomatal formation on PDA (Bacto, potato dextrose agar) amended with different concentrations of woody essential oils from *Cin. kanehi-*

rai, *Cin. camphora*, *Cunninghamia konishii* Hayata, and *Chamaecyparis formosensis* Matsum. Those essential oils were bought from markets in Taiwan. Two isolates of fungi imperfecti, *Penicillium* sp. and *Trichoderma* sp., were used to test the mycelial growth on the same media as the 4 *Antrodia* isolates. Culture blocks (3×3×3 mm) of each isolate were placed on Petri dishes (9-cm diameter) containing PDA medium amended with different concentrations of essential oils after autoclaving to test the mycelial growth and basidiomatal formation. One block was placed in each Petri dish at the center of the plates. Cultures were incubated at 24 C in darkness. To measure the mycelial growth, the linear growth of the 4 *Antrodia* isolates, *Penicillium* sp., and *Trichoderma* sp. was determined 3 wk, 2 wk and 5 d after incubation, respectively. To compare the effects of the essential oils on mycelial growth, the growth index (%), defined as (mycelial growth on PDA medium amended with essential oils ÷ mycelial growth on PDA medium without essential oils)× 100, was used. Therefore, a growth index of < 100 indicates that the concentration of essential oils inhibited mycelial growth, while that > 100 indicates that the concentration of essential oils promoted mycelial growth. The 4 *Antrodia* isolates were returned to the same incubator for basidiomatal formation for an additional 2 mo. The methods of Chang and Wang (2005) for observing basidiomatal formation were followed. Five plates were used for each treatment, and the experiment was performed twice.

The growth of isolates of *A. cinnamomea* and *A. salmonea* was promoted with supplementation of 100~1000 ppm of the 4 test essential oils (Tables 1~4). The growth of isolate TFRI B191, and isolates of *A. cinnamomea* and *A. salmonea* were still promoted at 5000 ppm of essential oils from *Cin. kanehirai*, and *Cun. konishii*, respectively. The growth of *A. heteromorpha* was only slightly enhanced at 100 ppm of essential oils from *Cin. kanehirai* and *Cin. camphora*, while growth was inhibited at all other concentra-

Table 1. Effects of different concentrations of essential oils from *Cinnamomum kanehirai* wood on mycelial growth of *Antrodia cinnamomea*, *A. salmonea*, *A. heteromorpha*, *Penicillium* sp. and *Trichoderma* sp.

Isolate	Growth index (%)[1] for different concentrations of essential oils (ppm)			
	100	500	1000	5000
Antrodia cinnamomea				
TFRI B86	105 (2)[2]	127 (5)	132 (4)	65 (5)
TFRI B191	114 (4)	114 (5)	132 (5)	121 (3)
A. salmonea				
TFRI B1012	137 (6)	180 (5)	183 (4)	74 (5)
A. heteromorpha				
TFRI B72	102 (2)	64 (3)	53 (4)	17 (5)
Penicillium sp.	90 (3)	76 (2)	78 (3)	44 (4)
Trichoderma sp.	100 (2)	100 (2)	58 (4)	9 (3)

[1] Growth index (%) = (mycelial growth on PDA medium amended with essential oils ÷ the mycelial growth on PDA medium without essential oils)×100.

[2] Standard deviation is given in parentheses.

Table 2. Effects of different concentrations of essential oils from *Cinnamomum camphora* wood on the mycelial growth of *Antrodia cinnamomea*, *A. salmonea*, *A. heteromorpha*, *Penicillium* sp. and *Trichoderma* sp.

Isolate	Growth index (%)[1] for different concentrations of essential oils (ppm)			
	100	500	1000	5000
Antrodia cinnamomea				
TFRI B186	114 (3)[2]	114 (4)	129 (3)	41 (4)
TFRI B191	107 (2)	125 (3)	125 (4)	61 (2)
A. salmonea				
TFRI B1012	126 (3)	137 (4)	169 (5)	66 (2)
A. heteromorpha				
TFRI B72	109 (3)	95 (4)	64 (5)	18 (3)
Penicillium sp.	84 (4)	84 (2)	72 (2)	22 (2)
Trichoderma sp.	100 (1)	100 (2)	62 (4)	31 (5)

[1] Growth index (%) = (mycelial growth on PDA medium amended with essential oils ÷ mycelial growth on PDA medium without essential oils) × 100.
[2] Standard deviation is given in parentheses.

Table 3. Effects of different concentrations of essential oils from *Cunninghamia konishii* wood on mycelial growth of *Antrodia cinnamomea*, *A. salmonea*, *A. heteromorpha*, *Penicillium* sp. and *Trichoderma* sp.

Isolate	Growth index (%)[1] for different concentrations of essential oils (ppm)			
	100	500	1000	5000
Antrodia cinnamomea				
TFRI B86	101 (3)[2]	109 (4)	102 (2)	100 (3)
TFRI B191	102 (4)	106 (3)	116 (5)	106 (4)
A. salmonea				
TFRI B1012	162 (5)	135 (6)	159 (4)	159 (3)
A. heteromorpha				
TFRI B72	89 (3)	67 (4)	62 (5)	27 (4)
Penicillium sp.	86 (5)	78 (3)	64 (2)	18 (3)
Trichoderma sp.	100 (2)	100 (2)	100 (3)	10 (3)

[1] Growth index (%) = (mycelial growth on PDA medium amended with essential oils ÷ mycelial growth on PDA medium without essential oils) × 100.
[2] Standard deviation is given in parentheses.

tions (Tables 1~4). The growth of *Penicillium* sp. was retarded at all concentrations, while that of *Trichoderma* sp. was only inhibited at 5000 ppm of all test essential oils and at a concentration of 1000 ppm of essential oils from *Cin. kanehirai* and *Cin. camphora* (Tables 1~4). Lower concentration of the 4 test essential oils had little effect on the growth of *Trichoderma* sp. (Tables 1~4).

The results indicated that the essential oils from woods of the 4 test trees favored the mycelial growth of *A. cinnamomea* and *A.*

Table 4. Effects of different concentrations of essential oils from *Chamaecyparis formosensis* wood on mycelial growth of *Antrodia cinnamomea*, *A. salmonea*, *A. heteromorppha*, *Penicillium* sp. and *Trichoderma* sp.

Isolate	Growth index (%)[1] for different concentrations of essential oils (ppm)			
	100	500	1000	5000
Antrodia cinnamomea				
TFRI B86	102 (3)[2]	124 (4)	109 (5)	52 (3)
TFRI B191	100 (3)	101 (2)	72 (4)	56 (5)
A. salmonea				
TFRI B1012	127 (5)	132 (3)	116 (4)	78 (2)
A. heteromorpha				
TFRI B72	53 (4)	27 (3)	18 (4)	18 (3)
Penicillium sp.	64 (3)	46 (2)	44 (2)	28 (3)
Trichoderma sp.	100 (1)	100 (2)	100 (3)	9 (3)

[1] Growth index (%) = (mycelial growth on PDA medium amended with essential oils ÷ mycelial growth on PDA medium without essential oils) × 100.

[2] Standard deviation is given in parentheses.

salmonea, while those essential oils inhibited or did not promote the mycelial growth of *A. heteromorpha*, *Penicillium* sp., and *Trichoderma* sp. *Antrodia cinnamomea* and *A. salmonea* slowly grew on PDA and MEA media, attaining growth rates of 0.8~1.5 and 1.2~1.6 mm/d, respectively (Chang and Chou 1995, 2004). Slow growth of fungal mycelia suggested that they are less competitive than other fungi in terms of colonization of substrates. However, essential oils of *Cin. kanehirai* and *Cun. konishii* favored the growth of *A. cinnamomea* and *A. salmonea* and inhibited the growth of other possible competitive fungi such as *Penicillium* and *Trichoderma*. This phenomenon would support *A. cinnamomea* and *A. salmonea* having better competition with other fungi on their individual host trees. All 4 tested essential oils favored the growth of *A. cinnamomea* and *A. salmonea*, but *A. cinnamomea* and *A. salmonea* are restricted to *Cin. kanehirai* and *Cun. konishii*, respectively. Apparently, the essential oils are not the only factor in *A. cinnamomea* being restricted to *Cin. kanehirai*. This can be explained by host specificity being determined by many factors beyond microbial competition. However, the essential oils do provide a favorable environment for colonization of *A. cinnamonea* and *A. salmonea*. Sheu et al. (2000) reported that the host specificity of *A. cinnamomea* was related to the wood essential oils of *Cin. kanehirai*. *Antrodia cinnamomea* and *A. salmonea* are believed to be close species because both species are morphologically similar but can be separated by the pore surface color of basidiomes and host preferences (Chang and Chou 2004). In addition, both species share most main components of triterpenoids in the basidiomes (Shen et al. 2007). Although essential oils from *Cin. kanehirai* and *Cun. konishii* favored the growth of *A. cinnamomea* and *A. salmonea*, *A. cinnamomea* has not been found on *Cun. konishii* nor *A. salmonea* on *Cin. kanehirai* in nature. Further study is needed to understand factors affecting the host specificity of both fungi with their hosts.

There was no basidiomatal formation on PDA medium amended with any concentration of the 4 test essential oils for isolates of

A. cinnamomea, *A. salmonea*, and *A. heteromorpha*. Only isolate TFRI B191 of *A. cinnamomea* produced basidiomes on PDA medium without amendment with essential oils, while isolates TFRI B86 of *A. cinnamonea*, TFRI 1012 of *A. salmonea*, and TFRI 72 of *A. heteromorpha* produced no basidiomes after 3 mo of incubation. These results indicate that basidiomatal formation of *A. cinnamomea* is not related to the essential oils of *Cin. kanehirai*. In conclusion, the application of essential oils *in vitro* promoted the growth of *A. cinnamonea*, but was not related to basidiomatal formation of the fungus. These results indicate that in nature essential oils provide a favorable environment for colonization of *A. cinnamomea* on *Cin. kanehirai* but are not essential for basidiomatal formation of *A. cinnamomea*.

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