Trends in Horticulture 2026, 9(1), 11718.
https://doi.org/10.24294/th11718

Commentary

Anti-bacterial effect of methyl trans-cinnamate in the mycorrhizosphere of *Tricholoma matsutake*

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CITATION

Kim H, Zhang C, Choe M, et al. Anti-bacterial Effect of Methyl Trans-cinnamate in the Mycorrhizosphere of *Tricholoma matsutake*. Trends in Horticulture. 2026; 9(1): 11718. https://doi.org/10.24294/th11718

ARTICLE INFO

Received: 30 April 2025
Accepted: 02 April 2026
Available online: 24 April 2026

Abstract: In this study, we examined the effect of methyl trans-cinnamate against the growth of the indigenous microbes in the mycorrhizosphere of *Tricholoma matsutake* that forms an ectomycorrhizal association with pines. Methyl trans-cinnamate inhibited the growth of most indigenous bacterial strains isolated from the mycorrhizosphere, but with no negative effect on the proliferation of *Tricholoma matsutake*. We might make use of this material in a successful cultivation of matsutake in the future.

Keywords: ectomycorrhizal fungi; symbiosis; *Tricholoma matsutake*; inhibition zone

1. Introduction

Tricholoma matsutake (matsutake) exists in the mycorrhizal association (often called fairy ring or shiro) consisting of soil, roots, mycorrhizal fungi and soil microbes as an ectomycorrhizal (ECM) symbiotic fungus of Japanese red pine (*Pinus densiflora*) [1]. The infection of pine rootlets by matsutake and the development of shiro are exceedingly important in the cultivation of yet uncultivable matsutake. The inhibiting effects of harmful microbes in soil may be one of the main reasons responsible for the low infection rate of the host by matsutake and for the low survival rate of the host.

Methyl trans-cinnamate is the major volatile component in matsutake mushroom [2,3], and shows the antimicrobial effect against bacteria or/and fungi [4–9]. On the other hand, the cinnamic acid and its derivatives have been shown to promote the growth of plant-seedlings in low concentrations [10,11]. Recently, it has been shown that materials obtained from the fairy ring-forming fungi affect the growth and ectomycorrhizal colonization of herbaceous plants [12,13]. In this study, we assume as follows: the main aromatic component of matsutake, methyl trans-cinnamate, inhibits harmful microbes in shiro soil, while not having any inhibiting effect on matsutake, because it is one of the characteristic odor of the matsutake mushroom. To the best of our knowledge, this is the first report on the antimicrobial effect of methyl trans-cinnamate against microbes inhabiting the mycorrhizosphere of matsutake, though previous studies have shown that it has antibacterial and antifungal potential [14–17]. We investigated the potential availability of methyl trans-cinnamate as an inhibitor against harmful microbes in shiro for the cultivation of matsutake.

2. Materials and methods

Methyl trans-cinnamate was purchased from Sigma-Aldrich (Germany). The fungal strain of *Tricholoma matsutake* used in the present work was AB 188535. To examine the antimicrobial activity of methyl trans- cinnamate, we used the different indigenous microbes (65 of bacteria and 24 of fungi) (**Table 1**) isolated from the soil of the Yangdok region (126°50′24″N, 39°12′36″E, altitude, 560 m), DPR Korea, where matsutake had ever occurred before. Sampling was conducted directly from underneath a stand of P. densiflora. Isolations of the bacterial strains were carried out after the method of Vaario et al. [18]. Fungi were isolated following the method of Oh et al. [19]. The effect of methyl trans-cinnamate against the growth of different isolates was examined using the disc diffusion method. In both studies concerning the antimicrobial effect on the indigenous microbes and promoting effect on the matsutake, methyl trans-cinnamate was in a concentration range from 200 to 1000 ppm. The antimicrobial activities of methyl trans-cinnamate against the growth of different isolates were determined by measuring the zone of inhibition using a digital planimeter. Streptomycin and ethanol were used as positive and negative controls, respectively. *Bacillus cereus* served as a test bacterium. The effect of methyl trans-cinnamate on the growth of matsutake was recorded after the method of Rincon et al. [20]. A plate inoculated with only matsutake without methyl trans-cinnamate served as a control. Data are expressed as Mean ± SD of five independent analyses. One-way ANOVA was used to assess the significance of differences in data obtained from growth cultures ($p < 0.05$).

Table 1. The typical fungi and bacteria isolated from the shiro soil in Yangdok region, DPR Korea.

No	Fungi	No	Bacteria
1	*Penicillium* sp.	1	*Bacillus cereus*
2	*Trichoderma* sp.	2	*Bacillus pumilus*
3	*Aspergillus* sp.	3	*Bacillus mycoides*
4	*Mucor* sp.	4	*Micrococcus* sp.
5	*Laccaria* sp.	5	*Pseudomonas aeruginosa*
6	*Tomentella* sp.	6	*Pseudomonas tolaasii*
7	*Tylopilus* sp.	7	*Pseudomonas fluorescens*
8	*Lactarius* sp.	8	*Pseudomonas* sp.
9	*Rhizopogon* sp.	9	*Agrobacterium* sp.
10	*Russula* sp.	10	*Burkholderia* sp.
11	*Cenococcum* sp.	11	*Acetobacter* sp.
12	*Suillus luteus*		
13	*Amanita* sp.		
14	*Mortierella* sp.		

3. Results and discussion

We evaluated the effect of methyl trans-cinnamate against the growth of 65 bacteria and 24 fungi originating from shiro soil of matsutake in slant culture (**Table 2**). It was found that methyl trans-cinnamate can inhibit the growth of different kinds of microbes from the soil in this study. In the presence of 1000 ppm, methyl trans-cinnamate was effective against all 65 bacteria, with strong inhibition against 42 strains, whereas it was entirely ineffective against any fungi in this concentration.

Table 2. The inhibiting effect of methyl trans-cinnamate on the growth of matsutake and the different microbes isolated from shiro soil of matsutake in the Yangdok region, DPR Korea.

	Concentration of methyl trans-cinnamate (ppm)					
	^{0} (CN)	**200**	**400**	**600**	**800**	**^{1} 000**
Bacteria	n	n	n	32/12 [b]	65/31	65/42
Fungi	n	n	n	n	n	n
Colony radius [a]	13.5±1.4	17.0±0.3*	18.2±0.8*	14.8±0.8	14.2±1.2	14.0±1.6

[a] Radial growth of *Tricholoma matsutake* on the 30th day in Petri dish (mm). [b] The total number of strains inhibited/the number of strains strongly inhibited in the presence of methyl *trans*-cinnamate. The term "strong inhibition" represents ones with the diameter of the inhibition zones of over 8 mm. CN: Control (without methyl *trans*-cinnamate), n: not inhibit. * Values with an asterisk represent a significant difference compared to control ($p < 0.05$).

Based on the above results, the inhibition zones of bacteria were measured in a petri dish containing methyl trans-cinnamate (**Table 3**). When the concentration of methyl trans-cinnamate is 1000 ppm, the inhibition zones of methyl trans-cinnamate against bacteria ranged from 8.5 to 9.5 mm. In this study, methyl trans-cinnamate had a positive effect on the proliferation of matsutake in the concentration of 400 ppm, showing no negative effect, even in the 1000 ppm (**Table 2**) and the proliferation of fungi was not inhibited in the concentration of 1000 ppm. Previous studies [14,15] revealed that methyl trans-cinnamate showed an antibacterial and antifungal effect with MIC values ranging between 61 μM and 1.5 mM, and between 50 and 301 μM, respectively. While Ohta [21] reported that the concentrations of methyl cinnamate in the fruiting body of matsutake were about 236 ppm. Compared to these reports, our result may be because the fungi and matsutake have been adapted to their major volatile component, methyl trans-cinnamate, in such an environment for a long time. In general, methyl trans-cinnamate has broad-spectrum antimicrobial activity, that is, it can inhibit not only bacteria but also fungi [9]. Methyl cinnamate also exhibited strong inhibition against collembolans at high concentrations of 1000 ppm [3]. As previously supposed, methyl trans-cinnamate, one of the characteristic odors of matsutake mushrooms, inhibits harmful microbes in shiro soil without any inhibiting effect on matsutake at less than 1000 ppm. We think that most fungi, as well as matsutake, may all be inhibited in above 1000 ppm. But in our case, such a result is insignificant when the proliferation of matsutake is inhibited, and so we have not discussed the effect of this material above 1000 ppm, because the object of this study is to find an inhibitor that can inhibit harmful bacteria to expansion of shiro and has no adverse effect on the growth of matsutake. On the other hand, methyl trans-cinnamate also promoted the generation of new

roots of pine seedlings (our unpublished data). We think methyl trans-cinnamate may be used as a protector of matsutake against harmful bacteria for future cultivation of matsutake in the field, although further analyses are needed to test the inhibiting effect of this material against more microbes originating from the pine forest. If this odor component affects the growth of the beneficial microbes in shiro soil, the ecosystem of the matsutake may be implicated. Therefore, it is necessary to consider and solve such problems in further research.

Table 3. The inhibition zones (diameter, mm) of methyl trans-cinnamate against typical bacteria isolated from shiro soil of the matsutake in the Yangdok region, DPR Korea.

	Bacteria				
	1[c]	**2**	**3**	**4**	**Test bacteria[d]**
Methyl trans-cinnamate (1 000 ppm)	8.9 ± 0.2	9.5 ± 0.2	8.5 ± 0.2	9.2 ± 0.2	10.5 ± 0.5
Positive control [a]	10.2 ± 0.3	16.1 ± 0.5	13.8 ± 0.4	15.1 ± 0.9	17.3 ± 0.6
Negative control [b]	-	-	-	-	

[a] Streptomycin (1000 ppm). [b] Ethanol. [c]Typical strains isolated from shiro soil of matsutake in the Yangdok region (1-*Pseudomonas aeruginosa*, 2- *Bacillus pumilus*, 3- *Agrobacterium* sp., 4- *Acetobacter* sp.). [d] *Bacillus cereus*.

4. Conclusion

The present study reports that methyl trans-cinnamate has an inhibitory effect against the growth of the indigenous microbes in the mycorrhizosphere of *T. matsutake* that forms an ectomycorrhizal association with pines, but with no negative effect on the proliferation of matsutake. For these reasons, Methyl trans-cinnamate can be considered as a useful material in the production of the inoculum of matsutake in the future.

Author contributions: Conceptualization: H-wK, S-iP; Investigation: K-iT; Methodology: H-wK, C-rZ; Formal analysis: C-rZ; Writing—original draft: CK, K-iT; Writing—review & editing: H-wK, S-iP; Supervision: H-wK; Validation: M-hC, S-iP; Resources: M-hC, S-jK; Data contribution: CK, S-jK

Acknowledgement: We thank researchers in the Central Mushroom Institute in Ministry of Agriculture, DPR Korea for their kind help with conservation pine forest. Reviewers are gratefully acknowledged for their kind help in elaborating our manuscript and in encouraging our further study.

Conflict of interest: All authors declare that they have no conflict of interest.

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