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Study the Effect of *Choiromyces meandriformis* – Natural Truffle – on *Taxus wallichiana* Zucc and *Pinus caribaea* Development in Vietnam

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Abstract. *Choiromyces meandriformis* is found in Europe countries, but have never seen in Vietnam. This fungus not only is the value product in the food market, but it also improves the plant development. The aim of this research is to find the effect of *Ch.meandriformis* on *Taxus wallichiana* Zucc and *Pinus caribaea* development in greenhouse, and furthermore to produce the fruit of this fungus in the near future.
The results showed that *Ch.meandriformis* had effects to the researched plants growth. Seedlings inoculated with *Ch.meandriformis*, the Hungarian truffle source, gave the good results for *Taxus wallichiana* Zucc development both on the plant diameter, height and buds forming. Inoculation of *Pinus caribaea* with this truffle resulted in diameter and height, respectively, better than without truffle. In some cases, using Vietnamese selected symbiotic fungus and *Trichoderma* also gave a good results.

Keywords: *Choiromyces meandriformis, Taxus wallichiana* Zucc, *Pinus caribaea*, plant development, ectomycorrhizal fungus

1. Introduction

Almost all truffles are ectomycorrhizal. The mycelia of truffles form symbiotic make relationships with the roots of several tree species. The association between the truffle and the host root is the initial step in the symbiotic phase and involves the formation of the mantle and Hartig net characteristic of the ectomycorrhizal association where water and nutrition exchanged (Harley and Smith, 1983).

Choiromyces meandriformis is grown in acid soils with high rainfall. This truffle associates with deciduous and coniferous trees, and prefer clayey soils (Svensk bot. Tidskr, 1909). This fungus improves the plant development. *Ch.meandriformis* is found in Navarra associated with *Quercus robur* and in Hungarian with *Picea abies*. They are often visible above ground, although many other specimens complete their development underground. Due to the aromatic volatiles, truffles have long been prized for their gastronomic characteristics (Hall *et al.* 2001). The selling price of *Ch.meandriformis* is between 75–120 USD/kg.

In Vietnam, the truffle is not found yet in the nature and until now there are no researches about this fungus. Our study addresses the effectiveness of *Ch.meandriformis* in *Taxus wallichiana* Zucc and *Pinus caribaea* development in greenhouse, and furthermore we have expected of finding the host plant of *Ch.meandriformis* to produce the fruit of this fungus in the near future.

2. Materials and Method

Research place was in Lam Dong province. This province has a tropical monsoon climate and is located in the Central Highlands region of Vietnam. Lam Dong has mild weather and cools all year. The annual average temperature is from 13-23°C and the annual rainfall is range from 1600 to 2700mm.

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2.1. Materials

Plants: Six-month old seedling of *Taxus wallichiana* Zucc and *Pinus caribaea* were obtained from a commercial plant centre in Vietnam and transferred to a greenhouse of our Institute in Lam Dong province, in May 2011. Seedlings for the experiment were chosen randomly from a batch ready for commercial inoculation.

The natural *Choiromyces meandriformis* (truffle) for inoculation was transported from Pannonia University of Hungary. The mycelia of this fungus were isolated from the original samples with MMN medium.

The natural symbiotic fungus (AM) was isolated from the natural root of *Taxus wallichiana* Zucc.

Trichoderma spp. was received from the IBF laboratory of Ho Chi Minh university of Industry.

Soil which was used for the experiment was of a 2:1:1 mixture of dried natural soil, perlite and peat (v:v:v). This natural soil was sieved to 4mm on site, steam pasteurized at 80^{o}C twice and air-dried. Ground limestone (<1 mm mesh) was applied and mixed at rates determined by means of a pH of 7.5.

Plastic pots were used for the experiment (25 cm diameter) with a fixed amount of soil, perlite and peat.

2.2. Experimental Design and Analysis

This experiment compared the effects on the plant diameter and height development and the ability of bud forming under the effect of *Ch.meandriformis*.

The green house experiment followed a completely randomized design with 4 inoculation treatments, as following:

- The plant was not inoculated with truffle (control sample);
- The plant was inoculated with natural *Ch.meandriformis*;
- The plant was inoculated with mycelia of *Ch.meandriformis*;
- The plant was inoculated with mix form of truffle with natural and mycelia of *Ch.meandriformis*

There were 4 kinds of formula also were tested, as following:

- The plant was not inoculated with truffle (control sample);
- The plant was inoculated with different formula of *Ch.meandriformis;*
- The plant was inoculated with different formula of *Ch.meandriformis* which was mixed with selected symbiotic fungus of the host plant (AM)*;*
- The plant was inoculated with different formula of *Ch.meandriformis* which was mixed with selected symbiotic fungus of the host plant (AM) and *Trichoderma*,

And 2 kinds of plant (*T.wallichiana* Zucc and *P.caribaea*) with 15 replicates of individual treatments giving total of 360 pots were prepared.

All experiments were using in the research were signed in the following table:

Table 1. The formulas and signs of experiments were using in the research.

Experiments	Formulas of *Ch.meandriformis*	Form of *Ch.meandriformis*			Non truffle (T0)
		Natural (T1)	Mycelia (T2)	Mix (Natural and Mycelia) (T3)	
Pinus caribea without truffle	Non microorganism				P0CT
	With selected symbiotic fungus (AM)				P0AM
	With *Trichoderma* (Tr)				P0Tr
Taxus wallichiana Zucc without truffle	Non microorganism				T0CT
	With selected symbiotic fungus (AM)				T0AM
	With *Trichoderma* (Tr)				T0Tr
Pinus caribea with truffle	Control (CT-with truffle)	P1CmCT	P2CmCT	P3CmCT	
	Mix truffle with selected symbiotic fungus (AM)	P1CmAM	P2CmAM	P3CmAM	
	Mix truffle with AM and *Trichoderma* (Mx)	P1CmMx	P2CmMx	P3CmMx	
Taxus wallichiana Zucc with truffle	Control (CT-with truffle)	T1CmCT	T2CmCT	T3CmCT	
	Mix truffle with selected symbiotic fungus (AM)	T1CmAM	T2CmAM	T3CmAM	
	Mix truffle with AM and *Trichoderma* (Mx)	T1CmMx	T2CmMx	T3CmMx	

The plants were measured a length, a diameter and bud forming by time. The symbiotic between plant and microorganism was tested via microscope.

The design allowed the univariate General linear Model (GLM) ANOVA to be performed on all sets of measured variables. Where significant differences were identified, LSD multiple range tests were performed to identify differences ($P \geq 0.05$).

3. Results and Discussion

3.1. The Effect of *Ch.meandriformis* to *T. wallichiana* Zucc Development

After 09 months treatment, the diameter of seedling *T.wallichiana* Zucc which was inoculated with the truffle (all formula of natural, mycelia and mix truffle) had grown faster than those inoculated without truffle. Especially when the mix form of *Ch.meandriformis* was used, the result was the best (table 2).

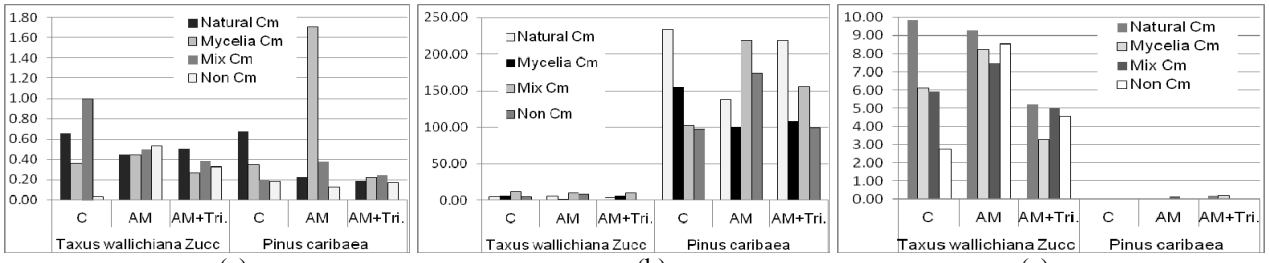
Table 2. The differences between diameter and height of *T. wallichiana* Zucc under the effect of *Ch.meandriformis* after 9 months treatment (mm)

Formula	Differences of diameter (mm)				Differences of height (mm)			
	Natural truffle	Mycelia truffle	Mix truffle	Non truffle	Natural truffle	Mycelia truffle	Mix truffle	Non truffle
Control	**0.65**	**0.36**	**1.00**	**0.03**	4.38	6.00	**12.00**	4.55
Mix with AM	0.45	0.44	0.50	0.53	5.71	1.43	**10.00**	8.18
Mix with AM +*Trichoderma*	0.50	0.27	0.38	0.33	3.79	6.07	**10.00**	0.00

After inoculation with mix form of truffle, the average plant height was 12 mm longer than initial plants (table 2). Plants were inoculated with mix form of truffle and AM or a mixture of AM and *Trichoderma* were also 10 mm taller than the early samples (table 2).

Nevertheless, the other formula of treatment with truffle also gave effect on the plants growing but not much.

The numbers of forming buds of *T.wallichiana* Zucc on plants which were treated with truffle were better than without truffle (Fig.1c). But AM also gave the good effect on buds forming. Meanwhile *Trichoderma* reduced this effect.

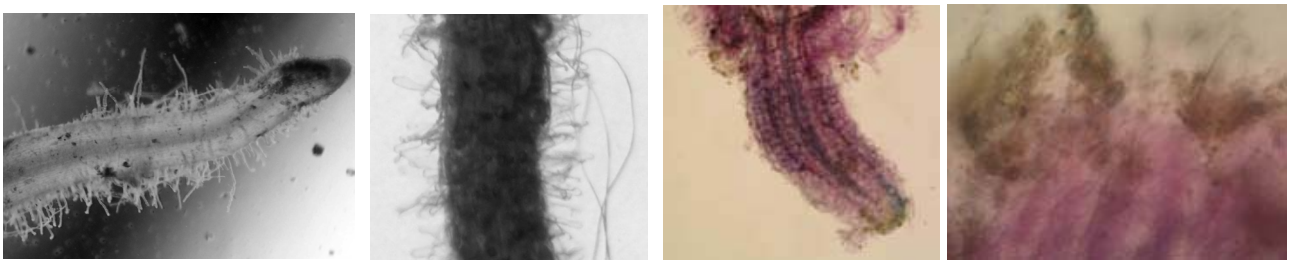


(a) (b) (c)

Fig. 1: Effect of *Ch.meandriformis* on researched trees development: a) comparison of plants diameter differences (mm), b) plants height differences and c) the number of forming buds;

3.2. The Effect of *Ch.meandriformis* to *Pinus caribaea* Development

After 09 months treatment, the diameter of seedling *P.caribaea* inoculated with the different truffle source (natural, mycelia and mix truffle) had grown faster than those inoculated without truffle. Especially when the mixture of AM and mycelia of *Ch.meandriformis* was used the diameter of *P.caribaea* was biggest (1.71mm) (Fig.1a).



(a) (b) (c) (d)

Fig. 2: After infection with *Ch.meandriformis* and a) the root of *T.wallichiana* Zucc, b) the root of *P.caribaea;* c, d) Hartig net between *P.caribea* and *Ch.meandriformis* by SEM

Inoculation with natural or mycelia truffle resulted that the plants height were 233.79 and 155.05 mm taller than the initial samples (Fig.1b). Plants which were inoculated with mix form of truffle also developed 102.48 mm more than the beginning samples (Fig.1b). But, AM and *Trichoderma* also gave a good effects on the plant height development (Fig. 1b).

The truffle, AM and *Trichoderma* didn't give much effect on the buds forming of *P.caribaea*. The amount of buds formed very little (Fig.1c).

The Fif.1b and c also showed that *P.caribaea* developed the plant height faster than *T.wallichiana* Zucc. But *T.wallichiana* Zucc gave more buds than *P.caribaea*

The symbiotic between researched tree and *Ch.meandriformis* was formed via Hartig net (Fig.2). This symbiotic not only provides the nutrition for plant, it also balance the water in the dry season. One of the good characteristic of the truffle is to keep the water for host plant. So, it reduces the using water and keeps the moisture around the root.

4. Conclusion

This study was conceived as a direct response to concerns expressed both to grow planting stock well-infected by *Ch.meandriformis* and to know the importance of other fungus balance for this truffle infection.

It was shown that different state of *Ch.meandriformis* responded differently to addition fungus in symbiotic with host plants. So it can be concluded that *Ch.meandriformis* gave good effects on *T.wallichiana* Zucc and *P. caribaea* growth in the tropical climate. Meanwhile, in some cases, AM and *Trichoderma* also gave a good effects to the researched plants.

The continued research should be considered not only the effect of *Ch.meandriformis* on the other host plants but also the ability of fruit forming from this truffle. So the new valuable product can be produced in the future.

5. Acknowledgements

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