2012 3rd International Conference on Biology, Environment and Chemistry
IPCBEE vol.46 (2012) © (2012) IACSIT Press, Singapore
DOI: 10.7763/IPCBEE. 2012. V46. 14

Compare the Effect of *Terfezia* and *Tuber aestivum* on *Pinus caribaea* Development in Vietnam

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Abstract. *Terfezia* and *Tuber aestivum* are not only the kinds of valuable mushroom in gastronomy; they also can help the tree development with the symbiotic ability with host tree roots where water, mineral nutrients and organic carbon are exchanged. The aim of this research is find the effect of those truffles on *Pinus caribaea* development in the greenhouse and in the natural forest of Vietnam. The results showed that when *Pinus caribaea* was planted in the green house, *Tuber aestivum* had the better effects on the researched plants growth than *Terferzia* both in the diameter and length development and buds forming of plant, while *Trichoderma* spp. reduced this effect. After 4 months when this tree was transfer to the natural forest without human care, all *T.aestivum,* and *Terfezia* had good effect on this tree; most samples which were using *Terfezia* had survival and grown better than others.
So, the results can be seen that *Terfezia* and *Tuber aestivum* gave good effects on *Pinus caribaea* growth in the green house and in the natural forest. But we should be care when using with *Trichoderma*.

Keywords: *Tuber aestivum*, *Terfezia, Pinus caribaea*, symbiotic, ectomycorrhizal fungus, truffle

1. Introduction

Fusarium oxysporum is the fungi which remarkably diverse and adaptable fungi have been found in soils ranging from the Sonoran Desert, to tropical and temperate forests, grasslands and soils of the tundra (Stoner, 1981). From more information on *Fusarium oxysporum* it reacts as a plant pathogen – plant wilt. But when this *Fusarium oxysporum* is associated with endomycorrhizobial organisms, which can enter the cells in the roots and colonize the root system, the plant wilt symptom may be reduced (Gordon and Martyn, 1997)

Truffles are not only known as the valuable in gastronomy, but their effect on the plant development was also studied (Harley, Smith, 1983). Colonisation of the host plant 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, mineral nutrients and organic carbon are exchanged (Hall et al., 2001; Harley and Smith, 1983). The mycelia of truffles form symbiotic relationships with the host plant roots of several tree species. Their fruiting bodies grow underground.

Phenological development (height and stem diameter), photosynthetic activity (SPAD-values) and mycorrhiza level of summer truffle mycorrhized host plants were examined in order to reveal correlation (Csorbainé, 2001). Results were in harmony of previous findings on host plant depending, partial or environment affected vitalizing effect (Bratek, 2008). Therefore plant growth indicates the mycorrhiza level development and vice versa.

In Vietnam, there are many kinds of microorganism using to improve the quality of the soil, but the truffle is not found yet in the nature. The aim of this study was to find the ability of association between

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Tuber aestivum and *Terfezia* with other fungi and the root of *Pinus caribeae* in the greenhouse and in the forest, that improve the tree development both in length, diameter and bud forming.

2. Materials and Method

Research place was in Lam Dong province: the greenhouse in Da Lat and the Forest in Lam Ha village. This place has a tropical monsoon climate at an altitude around 900 – 1200m above sea level. The annual average temperature is from 13-23°C and the annual rainfall is range from 1600 to 2700mm. The soil is clayey and acid.

2.1. Materials

Plants: Six-month old seedling of *Pinus caribaea* were obtained from a commercial plant centre in Vietnam and transferred to a greenhouse in Da Lat, Lam Dong province, in May 2011. From April 2012 all survival trees were planted to the natural forest in Lam Ha village, Lam Dong province.

The natural truffles: *Tuber aestivum*, *Terfezia* material 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 fungus *Fusarium oxysporum* (AM) was isolated from the nature. This fungus was found in most places in the plant parts.

Trichoderma spp. was received from the IBF laboratory of Industrial University of Ho Chi Minh City.

Soil 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

The green house experiment followed a completely randomized design with 4 inoculation treatment (uninoculated, natural truffle, mycelia of truffle and mix form of truffle with natural and mycelia of truffle), 4 kinds of formula (uninoculated, different formula of truffle; different formula of truffle was mixed with *Fusarium oxysporum* (AM); and different formula of truffle was mixed with *Fusarium oxysporum* (AM) and *Trichoderma* (Tr)), and 2 kinds of truffle (*T. aestivum, Terfezia*) with 15 replicates of individual treatments giving total of 360 pots.

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

Kinds of truffle which was associated with *P.caribaea*	Experiments	Form of Truffle			Non truffle (T0)
		Natural (T1)	Mycelia (T2)	Mix (Natural and Mycelia) (T3)	
Non truffle	Non microorganism				P0CT
	Mix with *Fusarium oxysporum* (AM)				P0AM
	Mix with *Trichoderma* (Tr)				P0Tr
T.aestivum	Control (CT)	P1TaCT	P2TaCT	P3TaCT	
	Mix with *Fusarium oxysporum* (AM)	P1TaAM	P2TaAM	P3TaAM	
	Mix with *Fusarium oxysporum* and *Trichoderma* (Mx)	P1TaMx	P2TaMx	P3TaMx	
Terfezia	Control (CT)	P1TeCT	P2TeCT	P3TeCT	
	Mix with *Fusarium oxysporum* (AM)	P1TeAM	P2TeAM	P3TeAM	
	Mix with *Fusarium oxysporum* and *Trichoderma* (Mx)	P1TeMx	P2TeMx	P3TeMx	

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 Truffles to the Rate of the Living of *Pinus caribaea*

Table 2: The rate of living *Pinus caribea* under the effect of *truffle* in green house and in the forest (%)

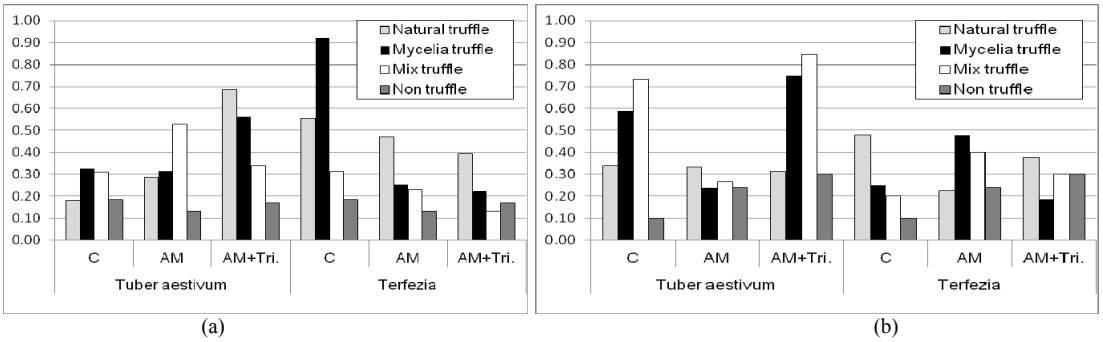
Growing place	Formula	*Tuber aestivum*			*Terfezia*		
		Control (CT)	Mix with (AM)	Mix with AM, *Trichoderma* (Mx)	Control (CT)	Mix with (AM)	Mix with AM, *Trichoderma* (Mx)
After 9 months in the green house	Natural truffle	45.0	60.0	86.7	83.3	56.3	71.4
	Mycelia truffle	70.0	73.7	80.0	60.0	61.5	90.0
	Mix truffle	33.3	60.0	86.7	66.7	37.5	42.9
	Non truffle	54.6	57.1	46.2	54.5	57.1	46.2
After 4 months in the forest	Natural truffle	88.9	66.7	61.5	100.0	88.9	90.0
	Mycelia truffle	64.3	72.7	50.0	100.0	50.0	44.4
	Mix truffle	80.0	66.7	76.9	75.0	66.7	33.3
	Non truffle	50.0	50.0	33.3	50.0	50.0	33.3

After 9 months in the green house, the plants which were treated with *T.eastivum* in the mycellia form were alive more. In most cases, with *T.aestivum* the plants were adapted more quickly than with *Terfezia* (table 2).

After 4 months when *Pinus caribaea* was transferred to the natural forest, both *T.aestivum* and *Terfezia* affected better on this tree than none using truffles. Especially when using only truffle, without other fungi, the tree grew better. In most cases, when the plants were only treated with *Terfezia*, all samples were alive. The results also showed that, *Trichoderma* effected worse on *Pinus caribaea* surviving when the root of this tree was associated with the mycelia or mix form of truffles (table 2).

3.2. The Effect of Truffles to the Diameter of *Pinus caribaea* during Tree Growing

After 09 months in latest treatment in greenhouse, with different kinds of truffle the results were different. In usual the diameter of seedling *P. caribaea* inoculated with the different truffle source had grown faster than those inoculated with the non truffle (Fig.1a). Especially the mixture of mycelia form of *Terfezia* and AM gave the best results.



(a) (b)

Fig. 1: Comparison of *P.caribaea* diameter differences (mm) under the effect of truffle a) the tree planted in the green house and b) the tree grew in the forest

When the samples were mixed with *T. aestivum*, the development of the diameter of *P. caribaea* was most steady in every formula. The results showed that the diameter of seedling *P. caribaea* inoculated with the different *T. aestivum* source always had grown faster than those inoculated without *T. aestivum*. AM or the mixture of AM and *Trichoderma* also improve the diameter of researched samples (Fig 1a).

Compare with control sample (non using truffle), *Terfezia* also affected much on *P.caribaea.* Especially when using natural and mycelia form of this truffle or when mixing with AM, the results were the best on the diameter development. But *Trichoderma* reduced this effect.

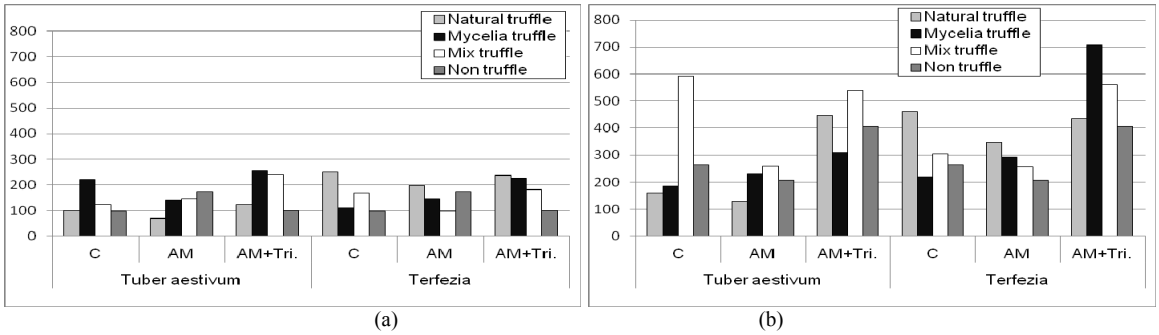
After 4 months *Pinus caribaea* was transferred to the forest, the diameter of those plants was growing similar than it was in the green house (Fig.1b). But in most cases, *T.aestivum* was affected on *Pinus caribaea* better then *Terfezia* when it was in the green house, especial in mycelia and mix form.

3.3. The Effect of Truffles to the Height of *Pinus caribaea* during Tree Growing

Compare the effect of two kinds of truffle, from the results it can be seen that both *T.eastivum* and *Terfezia* affected much on the height development of *Pinus caribaea* than the others none using truffles when it was in the green house, except with AM (Fig. 2a). After inoculation with mycelia form of *T.eastivum*, the average of plants lengths was 221.63 mm longer than initial plants. *Trichoderma* also increased the effect of AM on the plant development. After treatment with mix form and *Trichoderma* the plant was 254.7 mm longer than the beginning (Fig.2a). The mix formula of treatment with *T.eastivum* also gave the similar effect on the plant growing. But the natural formula gave the effect not as much as mycelia form.

The effect of *Terfezia* also can be seen as much as previous truffles. When mixing with AM, the plant height development was slower than other formula. Maybe this effect was from AM on the tree development, not sure from *TerfeziaI*.

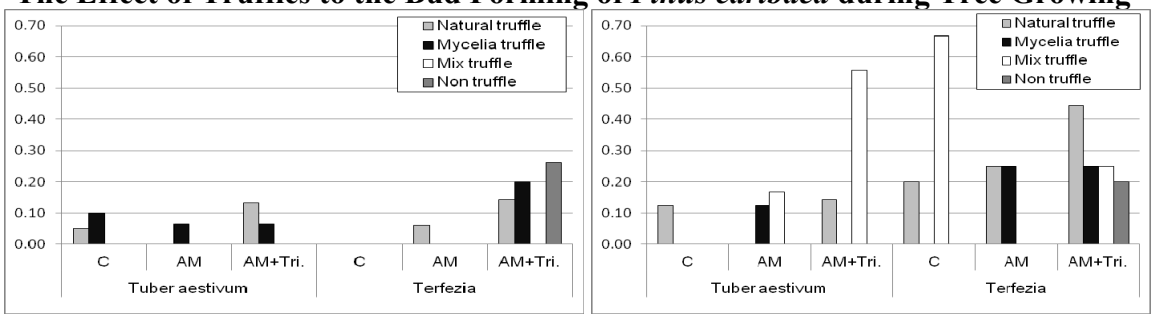
Pinus caribaea grew faster when it was moved to the natural forest (Fig. 2b) in the most formulas. Both *T.eastivum* and *Terfezia* affected much on the height development of *Pinus caribaea*. In some cases, *Terfezia* affected better on the height development of this tree and better than in the green house. With *Terfezia*, *Trichoderma* effected better on the plant height development.



(a) (b)

Fig. 2: Comparison of *P.caribaea* height differences (mm) under the effect of truffle a) the tree planted in the green house and b) the tree grew in the forest

3.4. The Effect of Truffles to the Bud Forming of *Pinus caribaea* during Tree Growing



(a) (b)

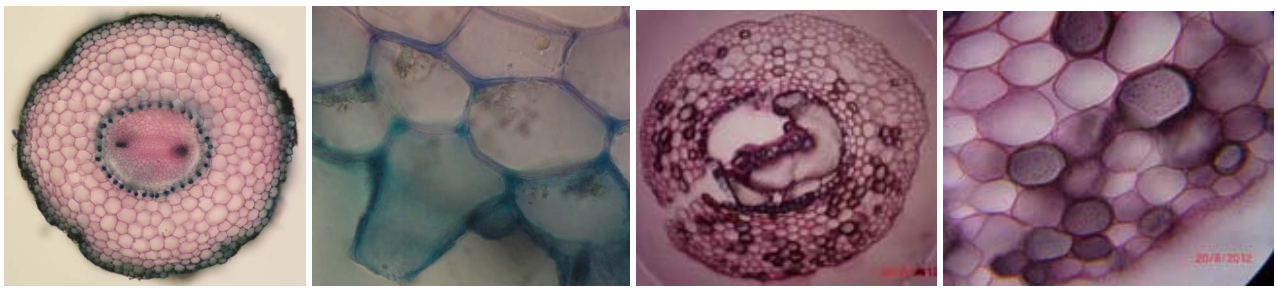
Fig. 3: Comparison of the amount of buds forming of *Pinus caribaea* under the effect of truffle a) the tree planted in the green house and b) the tree grew in the forest

After 9 months growing in the green house, the number of forming buds of *P. caribaea* on all plants which were treated with truffle or without truffle was not significantly different (Fig.3a). Maybe, because *P. caribaea* forms buds less and slowly during its development.

After 4 months in the natural forest each truffle had small effect on buds forming of *Pinus caribaea*. *Terfezia* gave the best effect, while *T.aestivum* gave the least. In most formula *Pinus caribaea* gave only 1 – 3 buds more (Fig.3b).

3.5. Compare the Symbiotic States Between Studied Truffle during *Pinus caribaea* Development

The result showed that all of researched truffle made the infection in the *Pinus caribaea*, but not the same way. In the green house, *Tuber aestivum* affected on the researched plants more than others. But in the natural forest, *Terfezia* had better effects. The Hartig net can be seen inside the cell of the root of the host plant (Fig.4). So the environment could have affection on the plant growing and the fungi infection.



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

Fig. 4: a) and b) The effect of *T.aestivum* on the root of *P.caribaea* during tree development; c) and d) The effect of *Terfezia* on the root of *P.caribaea*

4. Conclusion

This study was considered the effect of two kinds of truffles (*Tuber aestivum* and *Terfezia*) on *Pinus caribaea* in the green house and in the natural forest without human care. It can be understand because of water keeping and nutrient synthesis abilities of truffles. Promotion of absorption of nutrients in complex fungal-vegetation occurs as follows: (1): fungal mitochondria surrounding the roots have helped to increase the surface area of the plant roots, thereby increasing permeability of nutrients around the roots; (2): mycelia systems become a form of " secondary roots" instead of the host plant roots to reach the location where the roots by size too large to reaching; (3): group of symbiotic fungi have the ability to absorb and assimilate the compounds containing N, P, K in the form of difficult indigestion from the soil, then move them into a digestible form to return to the host tree (Bending, 2003). The results showed that different kinds and different state of truffles responded differently to addition fungus in partnership with host plants. Those results also were in harmony of previous other findings (Csorbainé, 2001). So it can be concluded that in the green house *T. aestivum* gave better effects to *Pinus caribaea* growth than the others in the tropical climate. In some cases, the researched truffles also prevented the harmful effects of AM (*Fusarium oxysporum*) on the tree.

After 4 months when *Pinus caribaea* was transferred to the natural forest, all *T.aestivum,* and *Terfezia* had good effects on this tree. Especially the samples which were treated with *Terfezia* had grown better than others.

So, the results can be seen that truffles gave good effects on *Pinus caribaea* growth in the green house and in the natural forest. But it should be care when using with AM or *Trichoderma*.

5. Acknowledgements

Study was funded by project number 02/2011/HD-NDT, title: “Enhancing the potential to improve the fertility of the land around the root with ecto-*mycorrhizal* fungi and other symbiotic microorganisms”

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