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Lentinus edodes (Berkeley. Pegler.) growth on *Eucalyptus* sp., *Quercus* sp., *Acacia* sp., and carob forestry plants

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Abstract

Lentinula edodes is an edible mushroom best known by its Japanese name, shiitake. Production and consumption of the shiitake mushroom has steadily increased last years. Cultivation of shiitake is dependent on cork logs. Deforestation risk limits the mushroom production interest. This study investigates the effect of some forestry plants on the growth of *Lentinus edodes* mushroom and initiate to this mushroom cultivation essay using novel plant species and combinations**.** The sawdust of four *Eucalyptus* species (*Eucalyptus maidenii, E. bicostata, E. cinerea and E. pauciflora*) and *Acacia salicina* and the addition of carob powder to appropriate substrates were tested in different combinations to evaluate shiitake mycelial growth. Data were compared to control substrate made by *Quercus canariensis* and wheat bran. The best growths of *L. edodes* mushroom were recorded in substrates made with sawdust of *E. pauciflora* and *E. maidenii* supplied to zean oak one. This study made it possible to use combination between Zean oak, 10% *Eucalyptus maidinii*, 20% *Eucalyptus pauciflora* and wheat bran sawdust in shiitake cultivation projects since it has a beneficial effect for the growth of this fungal species. New alternative was here announced to reduce the over- exploitation of *Quercus* species for shiitake cultivation over the world.

1. Introduction

Edible mushrooms appear spontaneously on dead wood and decaying organic matter. Interested mushroom species for human uses, belongs to about 200 genera of macro fungi. At least, twelve species are commonly cultivated for food and/or medicinal purposes. Common edible fungi constitute a source of protein and vitamins including vitamin D and B [1,2]. Mushrooms are also widely used in energy-restricted diets (since they are rich in water 80-90%) and in the treatment of some digestive diseases [3,4]. From these group, shiitake *(Lentinula edodes*) is an edible and medicinal one [5, 6, 7, 8]. It grows naturally on dead wood in oak forests in Asia [4, 9,10,11]. The development of this fungus is inhibited by the considerable lowering of the temperature during the winter. Cultivation in the woods constitutes the only way to produce this mushroom.

Nowadays, the market for mushrooms and shiitake is increasing thanks to their nutritional and dietary characteristics. This mushroom is becoming more and more known for its pleasant taste and its various medicinal properties [5, 12]. These organoleptic qualities have led to renewed interest in its consumption and production around the world in recent years [13-14].

The cultivation of shiitake on wood logs represents a source of income for the inhabitants of the forest areas since it does not require large investments and it makes it possible to intensify cultivation in forest clearings. In 2014, cultivation of shiitake was introduced in Tunisia in the aim to contribute to economic development of underprivileged forestry area. This research establishes the evaluation of local forestry plant species effect on mycelial growth of *L. edodes*.

2. Methodology

Stem sawdusts (**Figure 1a-g)** of *Quercus canariensis*, wheat bran, four *Eucalyptus* species (*Eucalyptus maidenii*, *E. bicostata*, *E. cinerea* and *E. pauciflora*) and *Acacia salicina* plant species were used in different combinations (**Table 1**). Second experiment tested carob (*Ceratonia siliqua*) fruit powder addition and wheat bran with different portions (**Table 2**). All vegetal material was collected from Ain Drahem region (North Western of Tunisia). Chosen particle size was between 2 and 5 mm based on literature information. The mixture was hydrated by adding distilled water. petri dishes were filled with same substrate volume then sterilized. Five dishes corresponding to repetitions were prepared for each substrate.

Table 1. Percentage of plants species sawdust in tested substates.

Substrate	**Zean oak**	***Eucalyptus maidenii***	***Eucalyptus pauciflora***	***E. bicostata***	***E. cinerea***	***Acacia salicina***
S 1	100%	-	-	-	-	-
S 2	70%	30%	_	-		
S 3	70%	-	30%	-	-	-
S 4	70%	-	-	30%	-	-
S 5	50%	-	50%	-	-	-
S 6	70%	-	-	-	30%	-
S 7	70%	10%	20%	-	-	-
S8	50%	30%	20%	-	-	-
S 9	50%	20%	30%	-	-	-
S 10	70%	-	-	-	-	30%

Table 2. Combination portions in tested substates

Enriched medium	**Composition**	**Carob powder**	**Wheat bran**
M 1	8 v. S1	2 v	_
M 2	8 v. S1	_	2v
M 3	8 v. S1	1v	1v
M 4	8 v. S7	2v	-
M 5	8 v. S7	-	2v
M 6	8 v. S7	1v	1v
M 7	8 v. S8	2v	_
M 8	8 v. S8	-	2v
M 9	8 v. S8	1v	1v

Figure 1a. *Quercus canariensis* **[9]**

Figure 1b. *Eucalyptus cinerea* **[9]**

Figure 1c. *Ceratonia siliqua* **[9]**

Figure 1d. *Eucalyptus maidenii* **[10]**

Figure 1e. *Eucalyptus bicostata***[11]**

Figure 1f. *Acacia salicina***[12]**

Figure 1g. *Eucalyptus pauciflora* **[13]**

2.1 Inoculation

Disks of 4 mm diameter was isolated from pure culture of *L. edodes* and inoculated in each Petri dishes. Inoculated substrates were incubated at 25 °C in obscurity.

2.2 Evaluation of Lentinus edodes mycelium growth

Mycelial growth is the elongation of mycelium grown on a solid surface. This apical elongation was followed regularly using a graduated ruler and it is expressed in millimeters. For each Petri dish, daily measurements were carried out during ten days of incubation. Value was the average of two perpendicular diameters of the mycelial colony in each Petri dishes and the mean of 5 repetitions.

2.3 Measurement of mycelium growth rate

Mycelium growth rate was the mean of 5 repetitions. It was calculated according [20] equation:

$$\mathbf{Vmoy = [(Dmax - C)/2]/T}$$

With Vmoy: Average apical growth rate (mm/day); Dmax: Diameter of *L. edodes* colony (mm); C: Diameter of inoculum used initially (mm) and T = Time

2.4 Statistical analysis

The variance of multiple parameters (diameter colony; substrates type) was analyzed with the generalized linear model (GLM) using the SAS statistical software (version 9.0). Multiple comparisons of means were performed using the SNK test with a threshold *p* value of 0.05.

3. Results and Discussion

The growth curve of the shiitake mycelium on the culture medium based on Quercus *canariensis* sawdust and wheat bran was established (**Figure 2.**). The shape of this curve is almost linear, this proves a uniform increase (in diameter) over the entire incubation period. The elongation rate of shiitake mycelium on this substrate (**Figure 2.**) was almost uniform. This curve shows that the speed decreases around the 6th day of incubation then it rises from the 7th day. The average speed of mycelium development was about 7mm /day. Data demonstrated that substrate made with zean oak sawdust and wheat bran promoted the growth of shiitake mycelium. The size of its particles as well as the chemical composition of this substrate seem to be adequate for the cu^{lt}ivation of shiitake mycelium.

On *Eucalyptus bicostata* and wheat bran substrate, the fungal colony diameter variation curve with time (**Figure 3.**) showed that the development of *L. edodes* increased with time. Shape curve was generally similar to *Q. canariensis*/ wheat bran one but it spends more time to reach same stationary phase and value. This suggests lesser favor for *Eucalyptus bicostata* and wheat bran substrate than *Q. canariensis*/wheat bran. According to [21], *Eucalyptus bicostata* is rich in lignin and poor in cellulose, whereas zean oak contains less lignin and more cellulose [22, 23, 24]. Lignin composite is hard to be degraded and digested by microorganisms. Its degradation requires the presence of oxidative enzymes. Knowing that the mycelium of saprophytic fungi degrade cellulose more easily than lignin [25, 26, 27], this justifies the more remarkable elongation of shiitake mycelium on the oak-based substrate than *Eucalyptus bicostata* one. The average rate of mycelium development is 5.7 ± 0.2 mm per day.

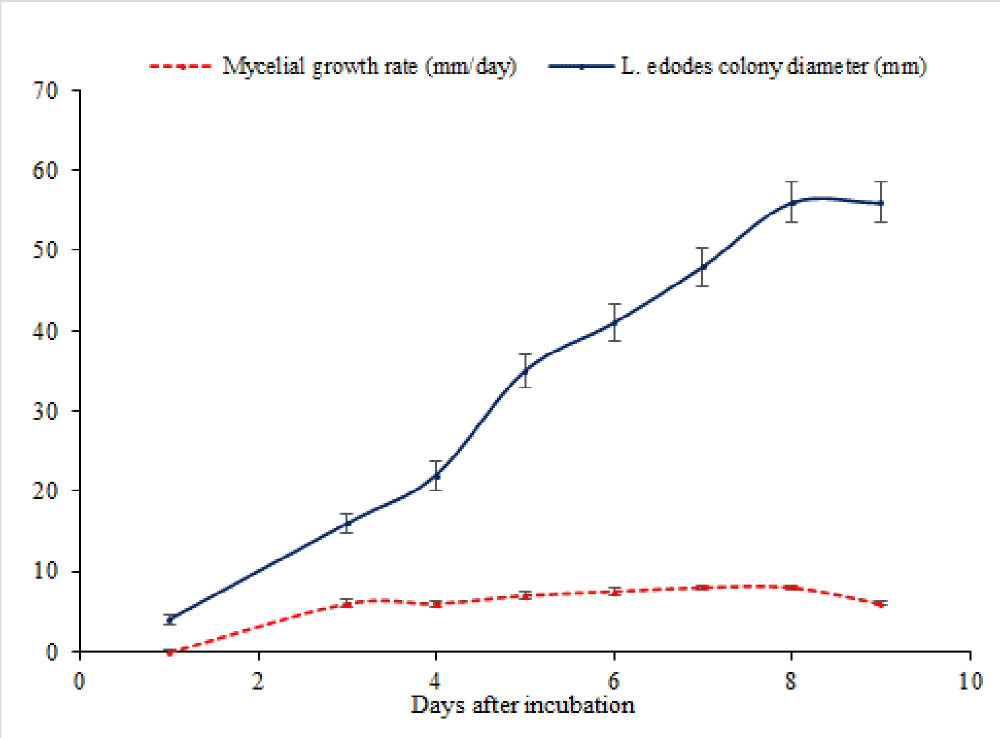

Figure 2. *L. edodes* diameter colony growth (mm) and mycelial growth rate (mm/ day) on a substrate based zean oak sawdust and wheat bran sawdust

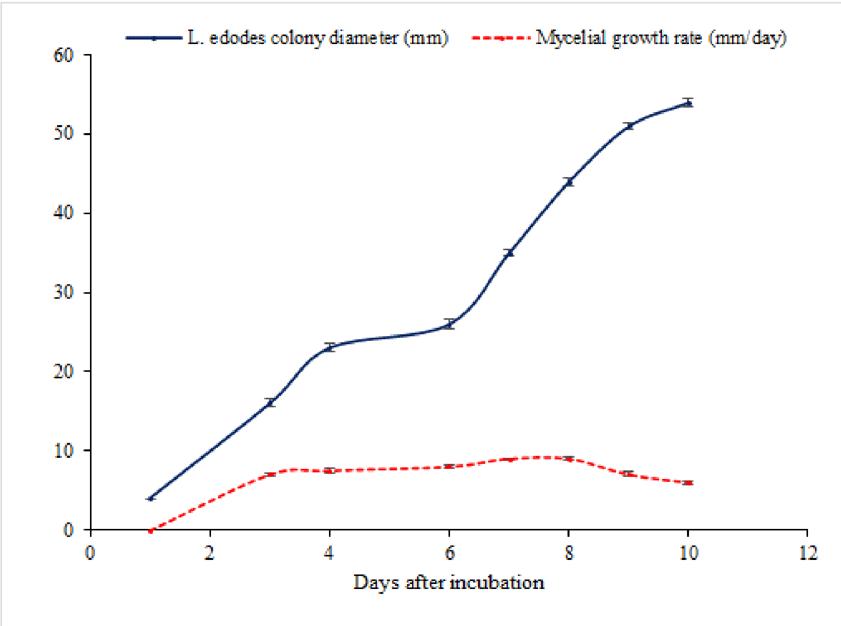

Figure 3. *L. edodes* diameter colony growth (mm) and mycelial growth rate (mm/ day) on a substrate based *on Eucalyptus bicostata* sawdust

It was noted that the variation in shiitake colony diameter on *Eucalyptus maidinii*/ wheat bran (**Figure 4.**), demonstrated that the addition of *Eucalyptus maidinii* sawdust appears favorable to fungal growth. The average growth rate of shiitake mycelium on *Eucalyptus maidinii* sawdust is 7 mm± 0.1 /day. This result reminds that found in the Zean oak. These two substrates allowed good mycelial development

According to [21] and [26], the comparison between the two species of *Eucalyptus* (*E. maidinii* and *E. bicostata*) showed that the cellulose content is higher in the first species while the second contains more lignin. Thus, shiitake mycelium growth was easier and noticeable on *E. maidinii*. The fungal hyphae found, in fact, easy way to degrade nutrient content (cellulose). This is reflected by an increasing elongation rate. Whereas, on *Eucalyptus bicostata*, the growth of shiitake was less important, the fungus requires enzymatic synthesize (oxidative enzymes) to degrade the lignin present in large quantities.

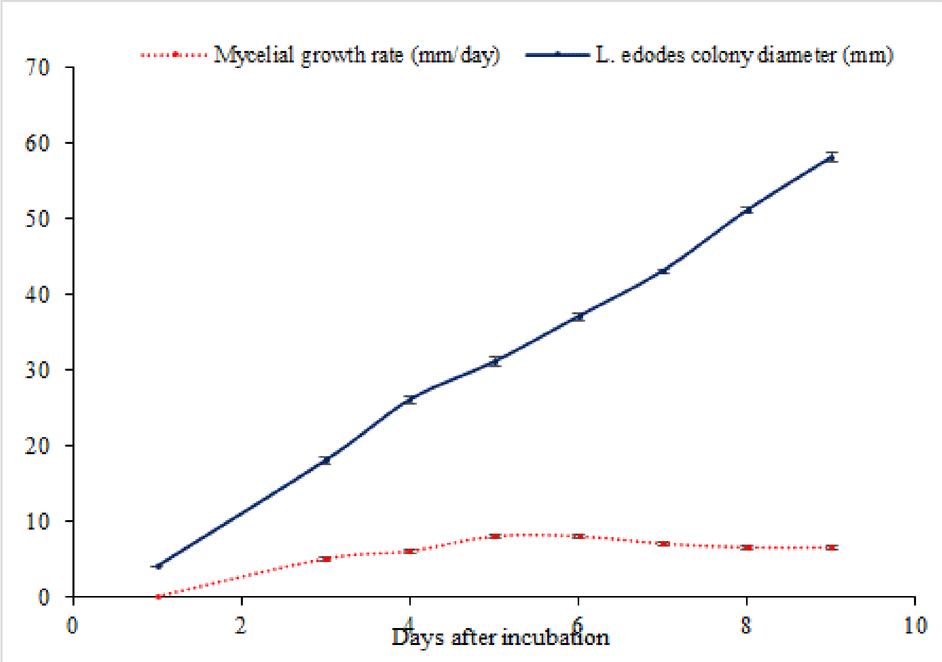

Figure 4. *L. edodes* diameter colony growth (mm) and mycelial growth rate (mm/ day) on a substrate based on *Eucalyptus maidenii* sawdust

A daily increase in mycelium was recorded throughout the incubation period on substrate containing *Eucalyptus pauciflora* (**Figure 5.**). It was found that after 10 days, the diameter of the shiitake colony reached 60 mm (the largest value recorded in all tree sawdust plant species tested). The growth rate on the substrate based on Eucalyptus pauciflora is variable during the incubation period (**Figure 5.**). The maximum speed is recorded on the 7th day beyond which the speed drops considerably then decreased. This decrease in the result of limitation in the volume of culture substrate and nutrients contents for the growth of shiitake hyphae growth.

The diameter of the shiitake colony gradually increased on the *Eucalyptus cineria*-based substrate (**Figure 6.**). This growth, despite its progression, is not exponential. A very large variation in the growth rate of shiitake mycelium was recorded on this medium (**Figure 6.**). Elongation of the hyphae of this fungus was hard and curve showed that all measured value were situated in exponential phase. This suggests that the physical and/or chemical properties of this substrate are not perfect for its cultivation. Since the physical properties of this substrate are close to those of the other *Eucalyptus* species tested. Thus, this result corroborates mainly [28, 29, 30] results. These authors proved that chemical characteristics mostly phenolic products are major compound in Eucalyptus *cinerea*. Phenols are toxic to fungi alive. Mycelium growth rate evolution showed that maximal value was reached after 8 days from incubation date.

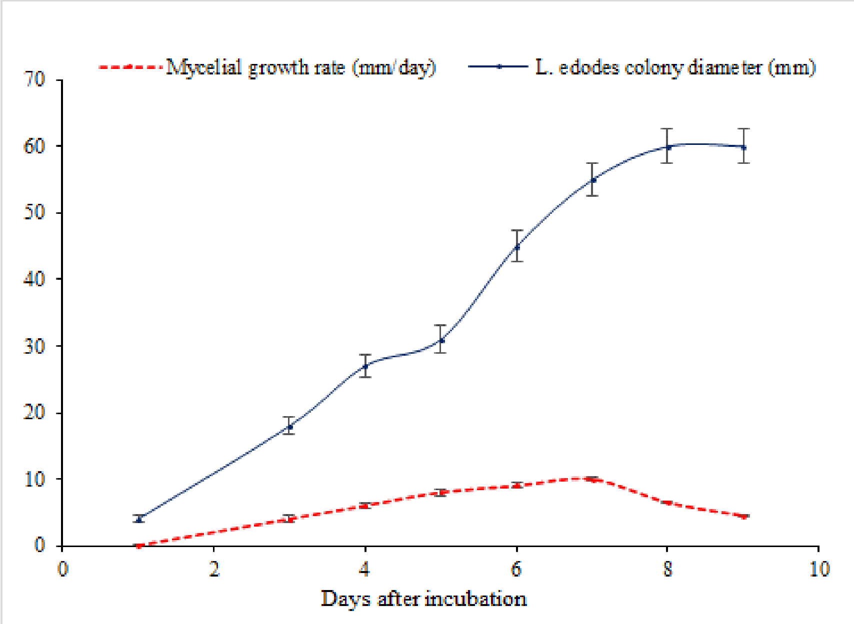

Figure 5. *L. edodes* diameter colony growth (mm) and mycelial growth rate (mm/ day) on a substrate based on *Eucalyptus pauciflora* sawdust.

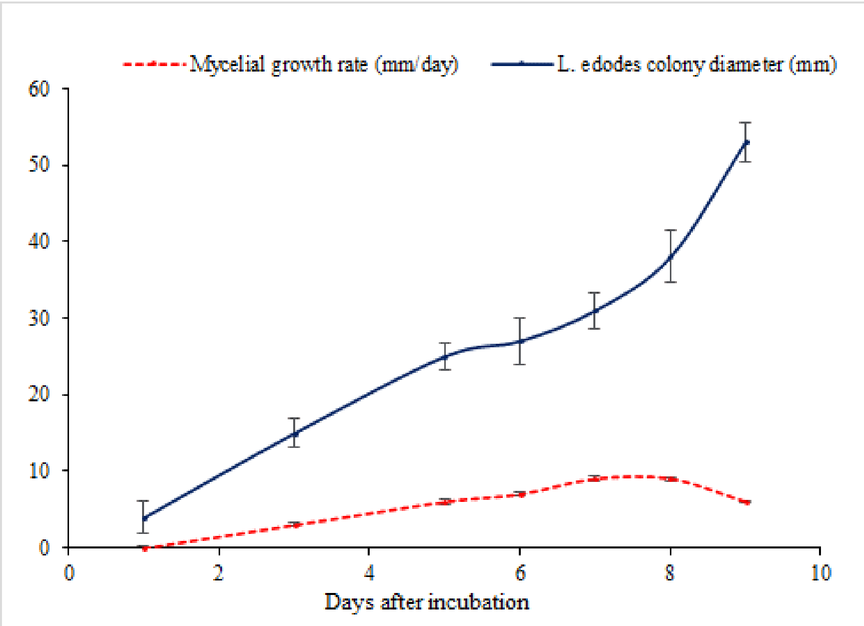

Figure 6. *L. edodes* diameter colony growth (mm) and mycelial growth rate (mm/ day) on a substrate based on *Eucalyptus cinerea* sawdust

An increase in the diameter of the shiitake colony, grown on the substrate based on *Acacia salicina* was recorded throughout the incubation period (**Figure 7.**). This increase starts weakly the first days (from 0 to 5 days). It is the lag phase when microorganism study nutriment composition and synthetize the required enzymes. From the 5th day, mycelium elongation accelerated until reaching its maximum on the 10th day (53 mm). The growth rate on this (**Figure 7.**) is characterized by two phases. The first phase i^{s} stationary where the speed of development is low. The second phase is characterized by an acceleratio^{n} of the elongation rate. Compared to the other substrates used, it can be concluded that growing shiitake on a medium containing *Acacia salicina* allowed heavy growth of *L. edodes* mushroom. This approval was confirmed with the average growth rate data showing of a maximum rate of 5 mm / day far from previous studied substrates.

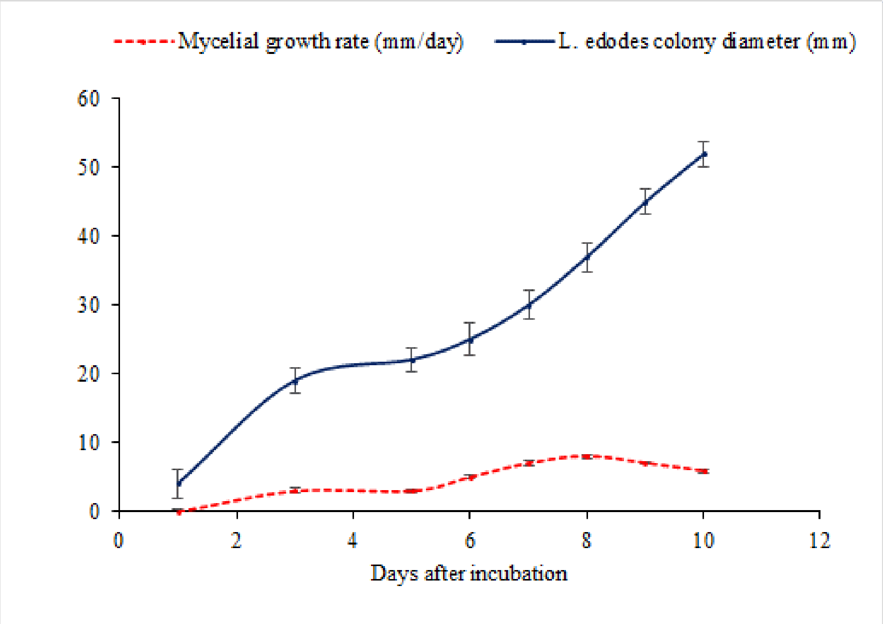

Figure 7. *L. edodes* diameter colony growth (mm) and mycelial growth rate (mm/ day) on a substrate based on *Acacia salicina* sawdust

The results found in the previous part let to conclude that the best growths of *L. edodes* mushroom were recorded in substrates made with sawdests of *E. pauciflora* and *E. maidenii* supplied to Zean oak one.

Study of combinations between sawdust plants species on shiitake growth (**Figure 8.**), showed that S8 substrate (50% Zean oak, 30% *Eucalyptus maidinii*, 20% *Eucalyptus pauciflora*) was the best substrate allowing highest mycelial elongation. S7 (70% zean oak, 10% *Eucalyptus maidinii*, 20% *Eucalyptus pauciflora*) substrate was suitable for the cultivation of this mushroom while S1 substrate (100% zean oak) and S9 (50% zean oak, 20% *E. maidinii*, 30% *Eucalyptus pauciflora*) are moderately favorable for such a culture. Among all the substrates analyzed, the S5 medium (50% zea oak, 50% *Eucalyptus pauciflora*) is the least suitable for shiitake growth.

As we Know there no research deals with mushroom species on this plant species sawdust. This promotes data found here and describe new perspectives in *L. edodes* cultivation alternative using substrates described here and to allow protection for Cork oak resources which were usually exploited in shiitake cultivation projects around the world.

The measurement of the diameter of the mycelial colony on each media swith carob powder add (**Figure 9**). As a remind supplied substrates were M1, M4 and M7. Development of L. edodes mycelium was totally absent (0 mm) that why it's not figured in histogram data (**Figure 9.**). During the preparation of these media, it has been found that their appearance has changed, they become firmer and the sawdust particles stick together. This can lead to a reduction in the porosity of the medium and a subsequent reduction in the circulation of the air and available water rate. According to [29, 31], the development of shiitake mycelium requires good aeration in the culture medium.

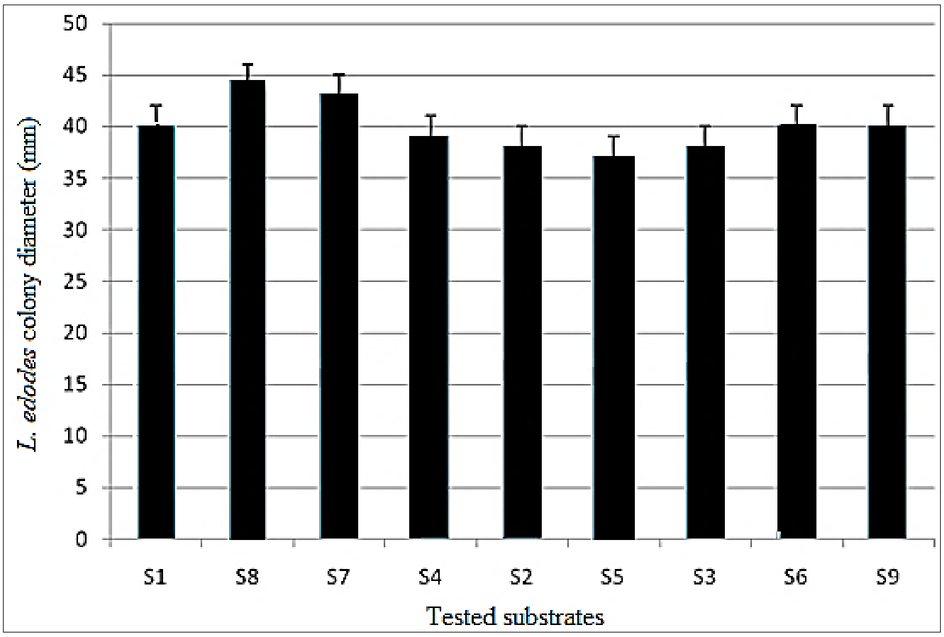

Figure 9. Shiitake colony diameter after 10 days of culture on the analyzed substrates

It was noted that the M5 medium (8 volumes of S7 and 2 volumes of wheat bran) gave the best results (**Figure 10.**); it is the most suitable combination for the culture of shiitake followed by M2 substrate (8 v S1 (100% zean oak)/ 2 v wheat bran) and M6 (8 v S8 (50% zean oak, 30% *Eucalyptus maidinii*, 20% *Eucalyptus pauciflora*) / 1 v of carob tree / 1 v of wheat bran). The lowest values are recorded in the M3 medium (8 v S1, (100% zean oak/ 1 v carob, 1 v wheat bran). It seems that carob powder and zean oak mixture has great effect on shiitake growth. This mixture decreases substrate properties and aspect limiting therefore fungal mycelium growth [32]. As for substrates sawdust combination with carob has never been studied. This valorizes data founded here.

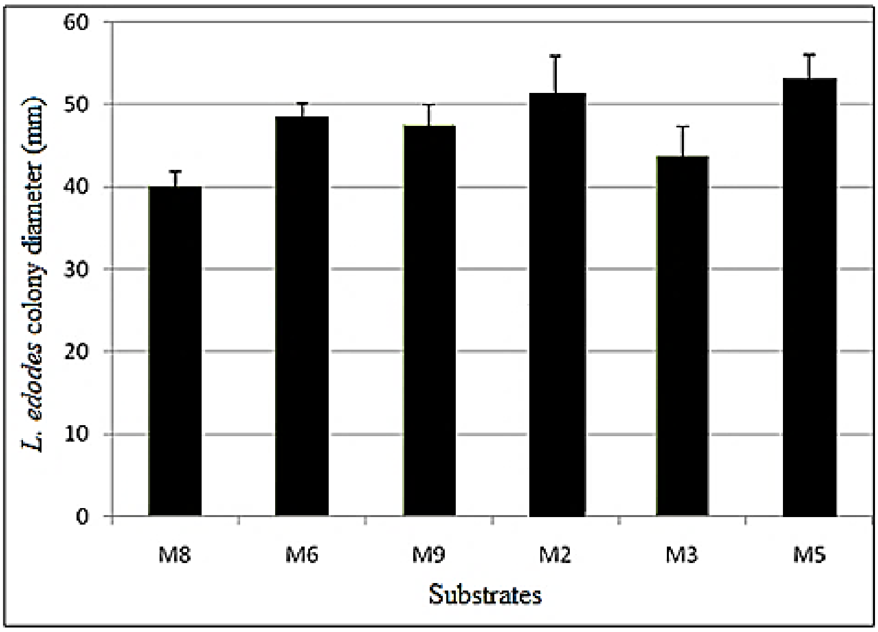

Figure 10. *L. edodes* colony diameter growth (mm) after 10 days in studied media

Statistical analysis showed highly significance variance of shiitake growth with substrate ($p<0.0001$). Thus, substrate nature affected shiitake growth.

From these substrates, statistical analysis distinguished medium M5 (8 v of S7 (70% zean oak, 10% *Eucalyptus maidinii*, 20% *Eucalyptus pauciflora*) / 2 v wheat bran) allowing highest mushroom growth followed by S8 (50% Zee oak, 30% *Eucalyptus maidinii*, 20% *Eucalyptus pauciflora*) and M2 (the control).

This study made it possible to choose the use of combination between zean oak, 10% *Eucalyptus maidinii*, 20% *Eucalyptus pauciflora* and wheat bran sawdust in shiitake cultivation projects since it has a beneficial effect for the growth of shiitake. New alternative was here announced to reduce the over- exploitation of *Quercus* species in shiitake cultivation over the world.

This study deserves to be accomplished by testing the shiitake productivity of different substrates. Study of the Kinetic of enzymatic profiles of shiitake mycelium growing on these substrates could explain its nutritional mode and evolution.

Conclusion

This study made it possible to choose the use of combination between zean oak, 10% *Eucalyptus maidinii*, 20% *Eucalyptus pauciflora* and wheat bran sawdust in shiitake cultivation projects since it has a beneficial effect for the growth of shiitake. New alternative was here announced to reduce the over- exploitation of *Quercus* species in shiitake cultivation over the world and to enhance rural economy, development and employment opportunities in unprivileged regions using local resources vegetation.

This study deserves to be accomplished by testing the shiitake productivity of different substrates. Study of the Kinetic of enzymatic profiles of shiitake mycelium growing on these substrates could explain its nutritional mode and evolution.

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Compliance with Ethical Standards: this article does not contain any studies involving human or animal subjects.

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