



Phyto-mycological study of agricultural and forestry plants wastes assets on *Pleurotus ostreatus* mycelial growth

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Abstract

This study probes the exploitation of some agricultural wastes and forestry plants for the growth of *Pleurotus ostreatus*. Mycelium of this mushroom species was cultivated on sterile, dried and crushed leaves of wheat straw, *Typha latifolia* L. and *Ampelodesmos mauritanicus* (Poir), olive pomace, and coffee and tea wastes with different particle sizes. Daily elongation of oyster mushroom colony was conducted in Petri dishes. Statistical analyses demonstrated a faster colonization of *A. mauritanicus* and *T. latifolia* media and that substrates particle size between 1-2 mm was the highest granulometric size for oyster elongation. Properties on mineral composition, pH, water retention capacity, lignin, and cellulose were identified for all used media. Principal components analysis, according physicochemical parameters, showed that *T. latifolia* appears very distinguishable and discarded from others substrates. Water capacity retention was appeared the most discriminating and distinctive parameter between all physical and mineral ones for the oyster hyphal elongation.

Keywords: mushroom, mycelium, cattail, coffee, *Ampelodesmos mauritanicus*, wastes

Introduction

Edible mushrooms grow 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]. 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^[2].

Mushroom cultivation is conducted in many countries (Japan, Korea, France, USA, and Italy...) ^[3], and allowed the mushroom production throughout the year to overcome the global demand faces to the naturally limited and seasonal production. This practice generates a fast yielding and nutritious resources of food and a reliable supply of economic incomes ^[4-5]. Mushrooms are also a potential contributor in the world's food supply with their ability to transform nutritionally-worthless wastes into protein-rich food. Since mushroom cultivation is based on bioconversion, it constitutes a recovery and environmental management assets.

Oyster mushroom (*Pleurotus ostreatus*) is one of the most widely cultivated species ^[6]. Many plant wastes were used as substrate for *P. ostreatus* production such as sawdust, cotton waste, leaves of bananas and paddy straw... ^[7].

Oyster cultivation varies with composting process ^[8] and *P. ostreatus* fructification in composted substrates is more significant and repetitively than fructification on non-composted ones since composting appears particularly important to vary the physicochemical natural assets of substrates and reduces infection rates ^[9].

Northern west Tunisia (Khroumirie) forest areas belong generally to humid climate and contain several unexploited plant species. In these areas, economic development is limited with the geographic condition (scarcity of arable plane surfaces and intense drain of water caused by the geographical gradient properties). Cultivation of edible mushrooms like *P. ostreatus* using endemic plant species and wastes may offer an alternative helpful method for socioeconomic development in these lands.

Methodology

Vegetation species used

External parts (stems and leaves) of *Ampelodesmos mauritanicus* (Poir) ^[10], *Phragmites australis*, *Typha latifolia* L. named also cattails, tea wastes, and wheat straw were dried then milled with an electric grinder (Ceramic instruments Srl Sassuolo- Italy HM/5304mm). Sieving was made by an electric strainer (Retsch AS400) at 250 speed rpm/g during 5mn for all substrates. For each substrate, particles size (PS) chosen, location of sampling

and abbreviations were summarized in table 1. Coffee waste and olive pomace were dried at 45 °C/48h and used in their collected physical structure.

Table 1: Sampling location, treatment practice and particles size for studied substrates

Substrates	Used parts	Sampling location	Grinding (+) or not (-)	Tested Particles sizes (mm)	Abbreviation
Coffee wastes	Wastes	Tunis	-	Common size	
<i>Typha latifolia</i>	Stem and leaves	Nefza	+	1-2 > 2	PS 1-2 PS > 2
Wheat straw	Stem and leaves	Tunis	+	1-2 > 4	PS 1-2 PS > 4
<i>Ampelodesmos mauritanicus</i>	Stem and leaves	Nefza	+	1-2 > 2	PS 1-2 PS > 2
<i>Tea wastes</i>	Wastes	Tunis	+	1-2 > 4	PS 1-2 PS > 4
Olive pomace	Wastes	Nabeul	-	Common size	
<i>Phragmites australis</i>	Stem and leaves	Tunis	+	1-2 > 2	PS 1-2 PS > 2

Physicochemical characterization

Three replicates were prepared for each substrate and essay. Analyzed parameters were: water retention capacity, pH (Jenway 3510) and cellulose and lignin estimated with an extraction with the acid detergent permanganate and 72% sulfuric acid after a determination of NDF ^[11]. Mineral analyses for azote (N; Kjeldahl); sodium and potassium (Na, K; flame photometry); carbon (C), phosphorus (P; calorimetric method) and Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn, Cr compositions (mass spectrometry, HGA400-pékinelmer) were conducted in triplicate repetition for all growing medium.

Fungus strain

Strain of *Pleurotus ostreatus* (Jacq.) P. Kumm. Fungus species was provided by KOICA as part of a cooperation project with INRGREF (number14/2012).

Inoculation

Substrate were humidified at 65%, sterilized then filled in glass Petri dishes. Four replicates were prepared for each substrate. Disks of 8 mm of *P. ostreatus* mycelium grown on PDA were inoculated above substrates. Glass Petri dishes were than incubated at 25 °C (incubator type: Binder, BD115 EC).

Evaluation of *P. ostreatus* growth

From the middle of inoculated disk of *P. ostreatus*, 2 diameters at 90° angle were drawn above Petri dishes. Measurements of these diameters' length were executed daily after 24h from inoculation. A graduated scale was used. Measurements were made in mm. Data for *P. ostreatus* colony length is the mean of these 2 diameters and 4 replicates (Petri dishes).

Statistical analyzes

The variance of multiple parameters (diameter colony; substrates type and particle sizes) 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. Principal component analysis (PCA) was applied and the individual factor map was obtained for substrates physicochemical data (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn substrates contents; ratio C/N and water retention capacities) using R software (Ri 3863.1.1).

Results and discussion

Effect of particle size on the growth of *P. ostreatus*

Study of the effect of particles sizes of *A. mauritanicus* leaves on the growth of *P. ostreatus* fungus species (Figure 1) showed that PS >2 mm provided mycelial development of oyster more homogeneously and significantly than PS 1-2 mm. Daily elongation of oyster mycelium curves showed a lag phase of 1 day for both substrates. However, exponential phase was briefer and highest in the growing medium made with *A. mauritanicus* PS >2 mm. Growth curve of *P. ostreatus* on *A. mauritanicus* PS 1-2 mm showed a decrease in the elongation rate of oyster mushroom hyphae at the 13th day of incubation. This instability in the growth of *P. ostreatus* can be caused by an infection which slowed the extension and which forced the fungus to support its resistance metabolism against this infectivity. Stationary phase started in the 15th days after incubation and in 21st day for PS >2 and PS 1-2 mm respectively.

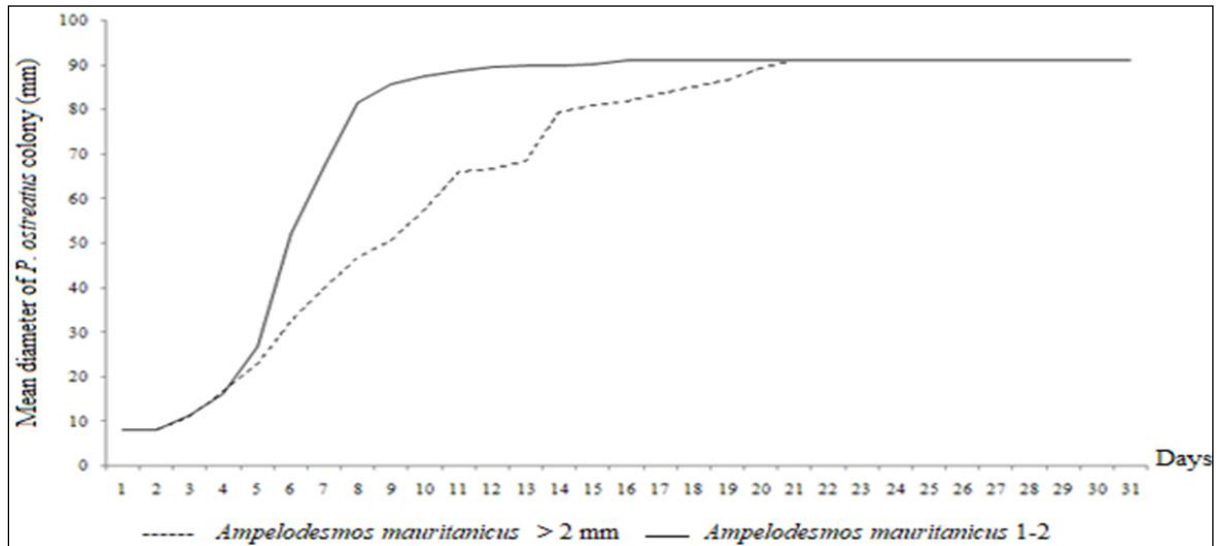


Fig 1: Variation of mean diameter colony (mm), of *P. ostreatus* on *Ampelodesmos mauritanicus* with two particle sizes (>2 mm and between 1-2 mm)

Registered data of the growth of *P. ostreatus* on the same tested particles size of *T. latifolia* (PS>2 and PS 1-2 mm) showed similar curves shape (Figure 2) as for *A. mauritanicus* one. Substrate formed with stems and leaves of cattail species enhanced the development of *P. ostreatus* mycelium. Lag phase lasted one day in the both substrates. Exponential phase spends also an equal period (10 days) and the stationary phase begins in the 11th days in conformity for the two tested particles sizes.

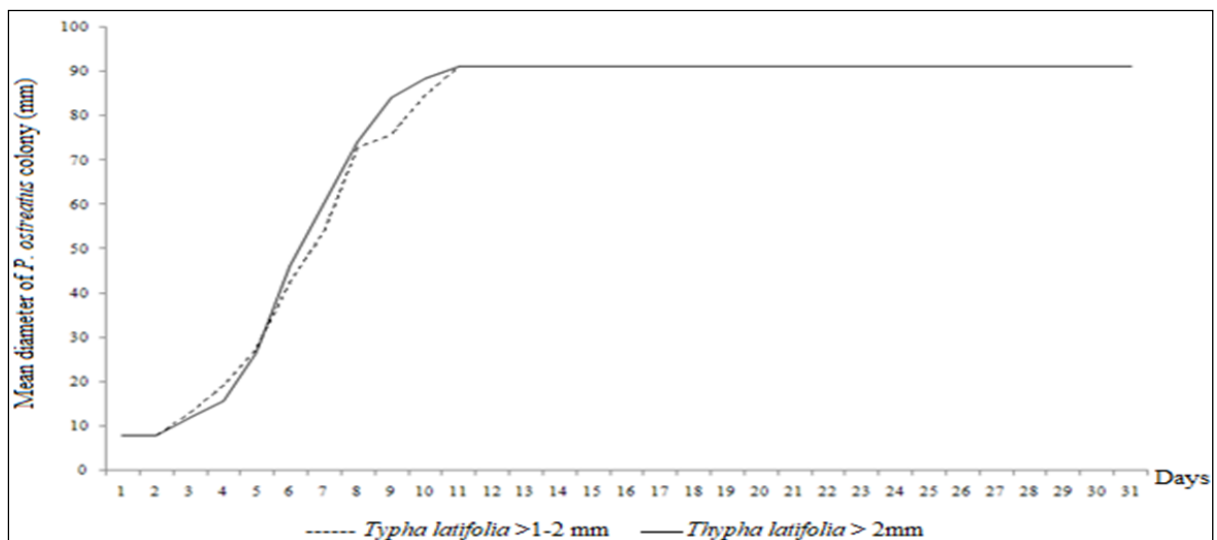


Fig 2: Variation of mean diameter colony (mm), of *P. ostreatus* on *Typha latifolia mauritanicus* with two particle sizes (>2 mm and between 1-2 mm)

In wheat straw, growth of *P. ostreatus* was faster with substrate formed by PS 1-2 mm than PS > 4 mm. PS 1-2 mm of wheat straw (Figure 3) appeared favorable for rapid extension of oyster mycelium. Elongation in the two particles sizes showed a latency period of 2 days, at that point exponential phase promised a faster growth. In bigger wheat straw (PS > 4 mm) exponential phase lasts two days more than the same medium with PS 1-2 mm.

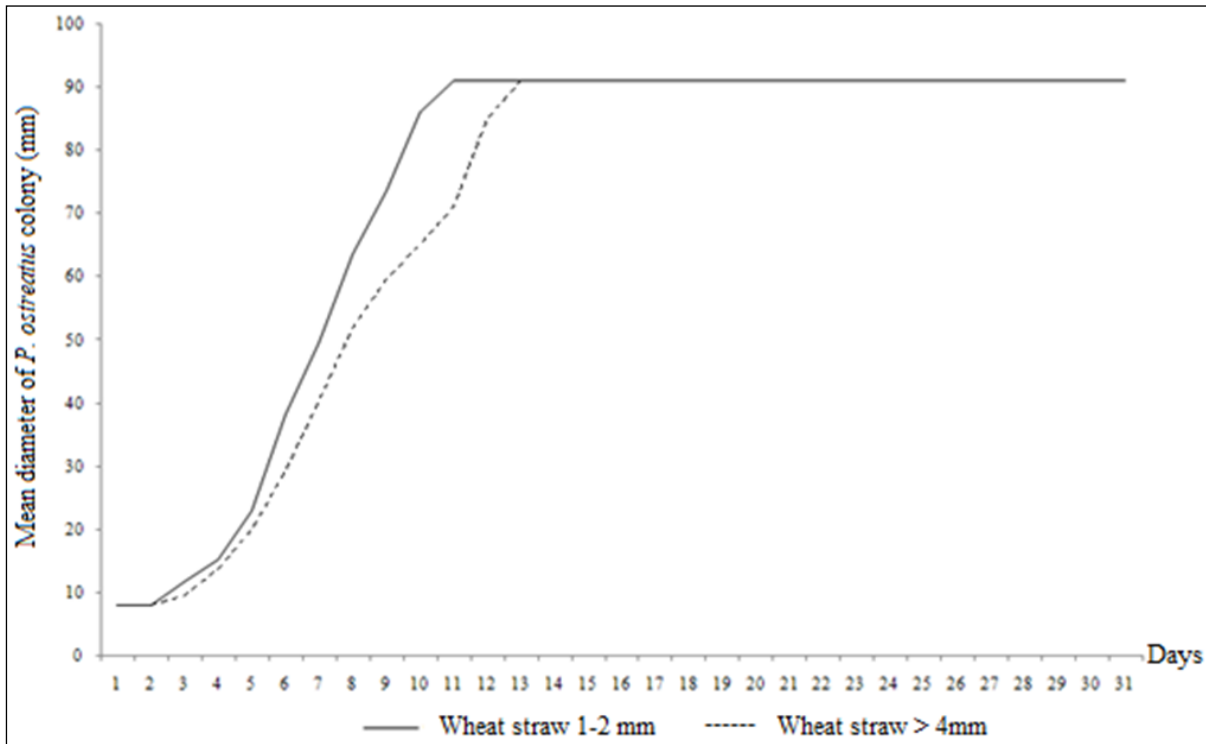


Fig 3: Variation of mean diameter colony (mm), of *P. ostreatus* on wheat straw with two particle sizes (>2 mm and between 4 mm)

Growth of *P. ostreatus* on tea wastes (Figure 4) was less important than on *T. latifolia* and *A. mauritanicus* ones. Growth curve on tea wastes was extended and all phases preserved more duration than those registered for the previous substrates. Tea wastes with PS 1-2 mm showed higher mycelial growth at the exponential phase than PS > 4 mm that begins approximately in the same date after incubation. Compared with tea wastes, growth of *P. ostreatus* on *Phragmites australis* was analogous (Figure 5). Growth curve phases were lengthier than those on cattail and *A. mauritanicus* substrates. *Phragmites australis* substrate with PS > 2 mm provided more hyphal elongation than PS 1-2 mm.

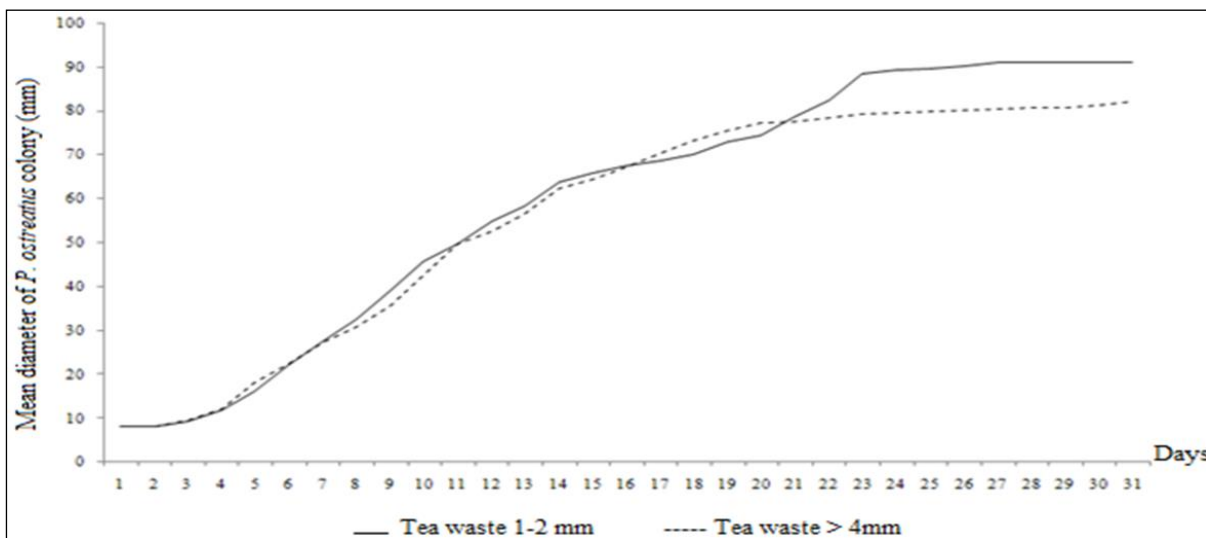


Fig 4: Variation of mean diameter colony (mm), of *P. ostreatus* on Tea wastes with two particle sizes (> 2 mm and > 4 mm)

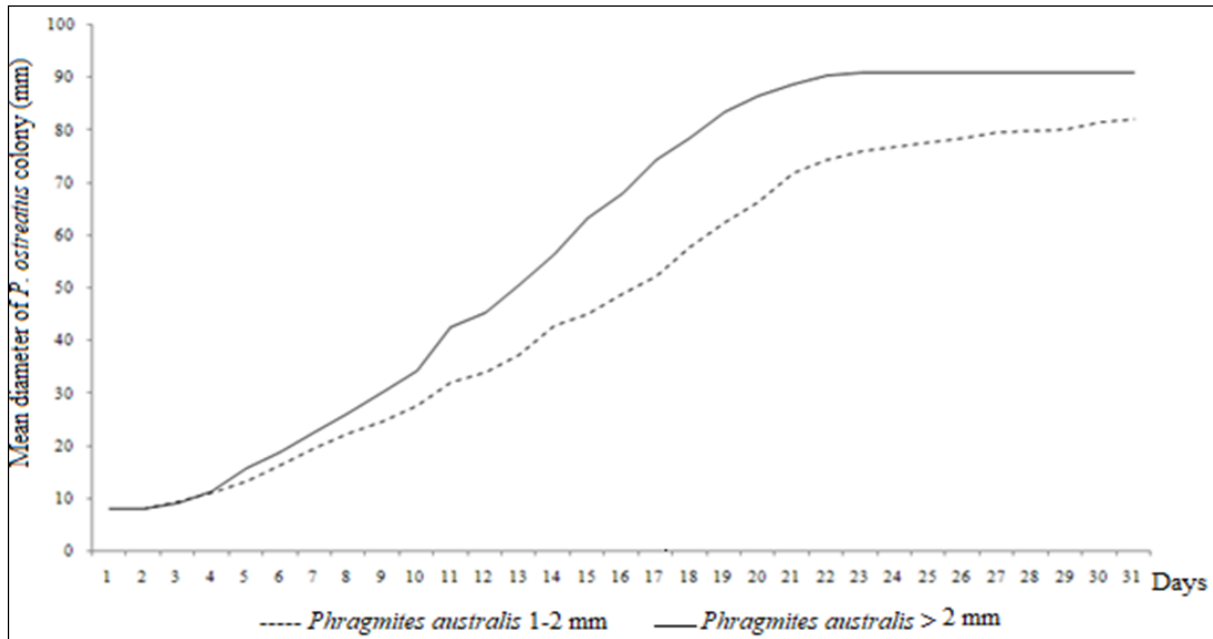


Fig 5: Variation of mean diameter colony (mm), of *P. ostreatus* on *Phragmites australis* wastes with two particle sizes (>2 mm and between 1-2 mm)

Olive pomaces appeared not favorable for oyster hyphal growth; it seems as inadequate substrate. In this medium, latency phase took 5 days (the longest period among all tested substrates). Beyond, a fall in hyphal elongation curve was observed (Figure 6).

Coffee wastes constituted a promising growth medium for *P. ostreatus* mycelium (Figure 6). Lag phase was followed by an acceleration period of 4 days then an exponential phase (7 days) and finally by a stationary stage where mycelium length remained constant. Commercial coffee waste size seems to be favorable for oyster mycelium elongation.

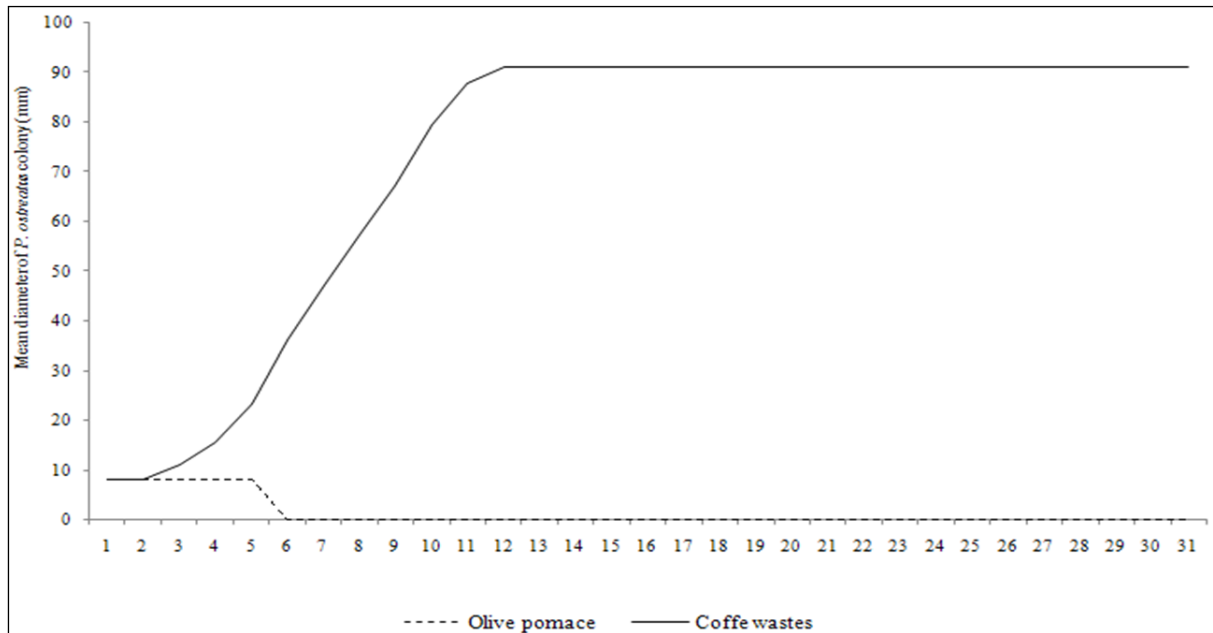


Fig 6: Variation of mean diameter colony (mm), of *P. ostreatus* on coffee wastes and olive pomace

In this study, wheat straw was considered as control according it is used mostly in industry of oyster cultivation. Variation of mean diameter elongation of oyster mushroom according time using only the highest growth for each substrate among tested particles size (Figure 7). Therefore, it is possible to divide tested growth media into 4 groups: the first group including *A. mauritanicus* and *T. latifolia* and characterized by a superior growth than the wheat straw. The second group whose curve growth was similar to wheat straw consists of coffee wastes. *Phragmites australis* and tea wastes substrates allowed lesser grew than wheat straw and assembled in the third group. The last group constituted by olive pomace, was characterized with the whole absence of mycelium development.

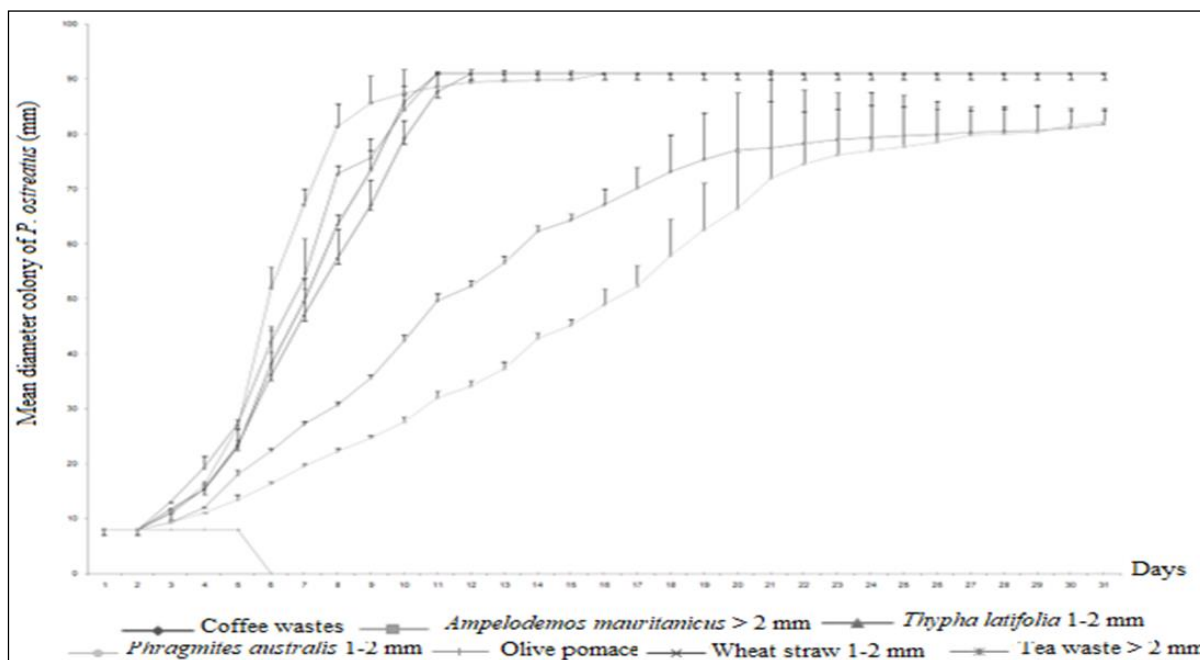


Fig 7: Resume of variation of mean diameter colony (mm), of *P. ostreatus* on higher analyzed substrates

Statistical analysis proved that variation of mean diameter of oyster mushroom by substrates particle sizes was highly significant ($p < 0.0001$). Mostly important particles sizes for hyphal elongation were PS 1-2 mm. Variation of the mean diameter oyster mycelium colony with substrate showed a high significance ($p < 0.0001$). SNK segregate analyzed substrates to 5 groups (table 2) and confirmed that *A. mauritanicus* then *T. latifolia* were the improved substrates allowing fungus growth. However, coffee waste and wheat straw were the lesser with closed means.

Table 2: Student-Newman-Keuls test applied for mean diameter colony variable (*a,b,c,d,e: SNK Groupement)

Substrate nature	Mean value
<i>Ampelodesmos mauritanicus</i>	71.992 ^a ±3.32
<i>Typha latifolia</i>	70.868 ^a ±3.595
<i>Phragmites australis</i>	67.900 ^{ab} ±3.439
Wheat straw	64.176 ^b ±3.317
Tea wastes	46.568 ^c ±2.94
Coffee waste	11.630 ^d ±2.15
Olive pomace	10.036 ^d ±3.29

Substrates physicochemical properties

Water retention capacity

The cattail (*Typha latifolia*) has the greatest water retention capacity (water ret) among all substrates. However, the lowest value was registered for olive pomace. Considering the straw as the control medium, substrates could be divided into 2 groups: the first one with a superior retention capacity value than the straw which were cattail and *A. mauritanicus*. The second group included *Phragmites australis*, tea wastes, coffee and olive pomace. This group was characterized with the inferior RE values. Generally, water retention capacity focused the amount of available water that could contain soluble nutrients. Water retention capacity could allow commensally microorganisms or parasites growth ^[12].

Substrates mineral composition

The results of the cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), plomb (Pb) and zinc (Zn) contents of the tested substrates (table 3) showed that cattail is the most mineral-enriched medium than other substrates, with the exception of the contents of Mn and Pb which were more quantified in tea wastes and *A. mauritanicus* respectively.

Table 3: Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn ($\mu\text{g/g}$ of dried substrate) contents.

	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
<i>Phragmites australis</i>	0,57 ± 0.02	1,65 ±0.04	2,78 ±0.06	2,35 ±0.64	471,00 ±0.15	12,22 ±1.01	2,52 ±0.08	4,85 ±0.87	11,42 ±2.03
<i>A. mauritanicus</i>	0,67	1,20	5,78	1,85	386,67	27,37	2,42	6,58	21,90

	± 0.05	±0.03	±0.08	±0.56	±0.18	±1.3	±0.98	±1.03	±2.1
Olive pomace	0,50 ±0.03	1,37 ±0.05	2,88 ±0.07	8,10 ±0.94	249,67 ±0.15	11,05 ±1.09	2,30 ±0.71	4,27 ±0.9	10,27 ±1.3
Wheat straw	0,52 ±0.02	1,48 ±0.04	3,53 ±0.08	2,83 ±0.48	279,33 ±1.2	8,87 ±0.85	2,55 ±0.04	3,67 ±0.5	26,43 ±2.3
Cattail	1,55 ±0.05	6,08 ±0.02	13,62 ±0.08	17,82 ±1.3	890,917 ±4.65	158,83 ±2.04	8,37 ±1.08	5,25 ±0.4	83,85 ±3.2
Tea waste	0,62 ±0.04	2,00 ±0.02	0,00 ±0	9,43 ±0.98	153,00 ±1.1	830,67 ±3.02	3,70 ±0.08	5,67 ±0.4	26,83 ±1.5
Coffee waste	0,37 ±0.03	0,98 ±0.02	0,00 ±0	14,87 ±1.6	155,33 ±0.95	19,75 ±0.9	2,58 ±0.5	3,22 ±0.9	18,02 ±1.7

Substrates composition on cellulose, lignin and carbon

Cellulose was present in higher concentration on cattail (57,549 %). Cellulose minor content was observed in tea wastes (35.2 %). Unlike, among all growth media olive pomace contained the mainly percentage of lignin (55.45 %) and wheat straw enclosed only 18.6 %.

Carbon is the basic element in the structure and the functioning of cells. The coffee has the greatest total carbon content (51.4 %); wheat straw and *T. latifolia* leaves have the same amount (50 %) whereas the lowest value was noted in the rest of tea (42 %).

Nitrogen phosphorus, potassium and sodium levels

Coffee waste, tea waste and olive pomace were the richest substrates in phosphorus. Nitrogen was relatively high on coffee waste (3.6 %) but it was reduced in cattail (0.7 %). However, *Phragmites australis* was the richest substrate on potassium with 1.25 % and 0.12 % of sodium.

Generally, ratio between carbon and nitrogen (C / N) demonstrate the ability of organic matter to be decomposed more or less rapidly. The nutritional balance of microorganisms is located at a C/N ratio of 24. Above, nitrogen is taken from the medium solution to support microorganisms^[13]. Thus, based on C/N ration substrates were divided into 3 groups; first group contain tea and coffee wastes with C / N <15. With this value, there is a nitrogen production and the rate of decomposition increases (with maximum for C / N = 10). The second group including pomace was characterized with 10 < C / N <15. At this level, the nitrogen allows a good decomposition of the carbonaceous material. The last group enclosed straw, cattail and *Phragmites australis* and was characterized by a low nitrogen content (C / N > 20) that reduced or forbid carbon decomposition.

Variable factor map of physicochemical parameters using R software (Figure 8) showed that the points Ni (ni), Na (na), Co (co), Zn (zn) Fe (fe), Cr (cr) and C/N ration (cn) and water retention capacity (water0ret) were very close to the correlation map axis.

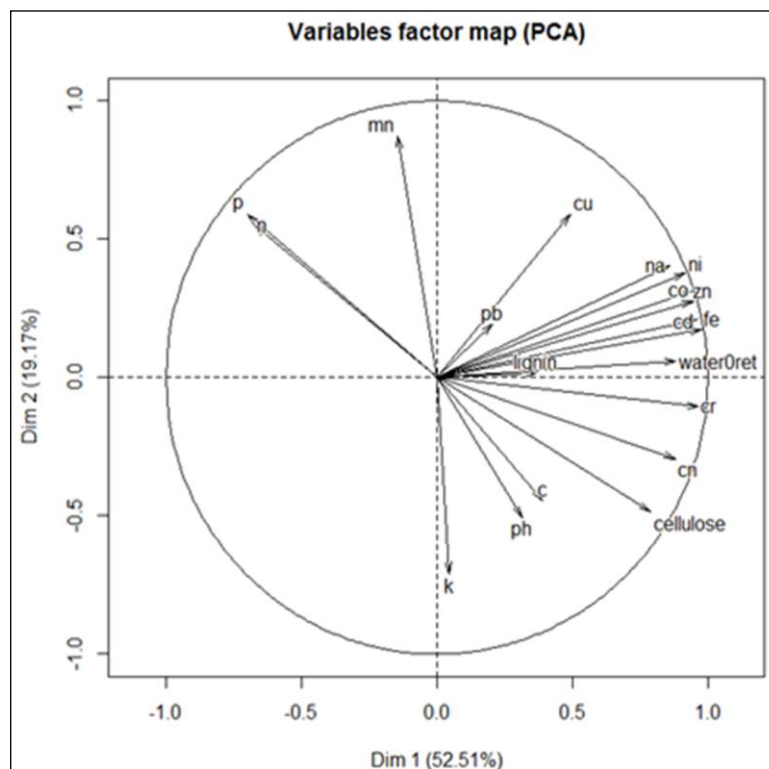


Fig 8: Variable factor map obtained in all individuals by principal component analysis (using R software). Code; (cn) ratio C/N; (water0ret) water retention capacity)

Figure 9 showed the two-dimensional mapping of the principal component analysis based on substrates physico-chemical variability. X-axis accounted for 52.51 %. Y axis explained 19.17 % of total variance. The PCA showed that the cattail was distant from others substrates with the highest correlation degree. *A. mauritanicus*, pomace, *Phragmites australis* and straw were related physico-chemically from each other's, especially by N, P and K contents. Tea and coffee wastes constituted another group. Cattail and tea are quite specific related to their wealth or poverty determined physicochemical parameter. Indeed, *Thypha* showed the higher content of Fe and water retention capacity however, tea is characterized by the lowest rates of Fe and the highest on Cr.

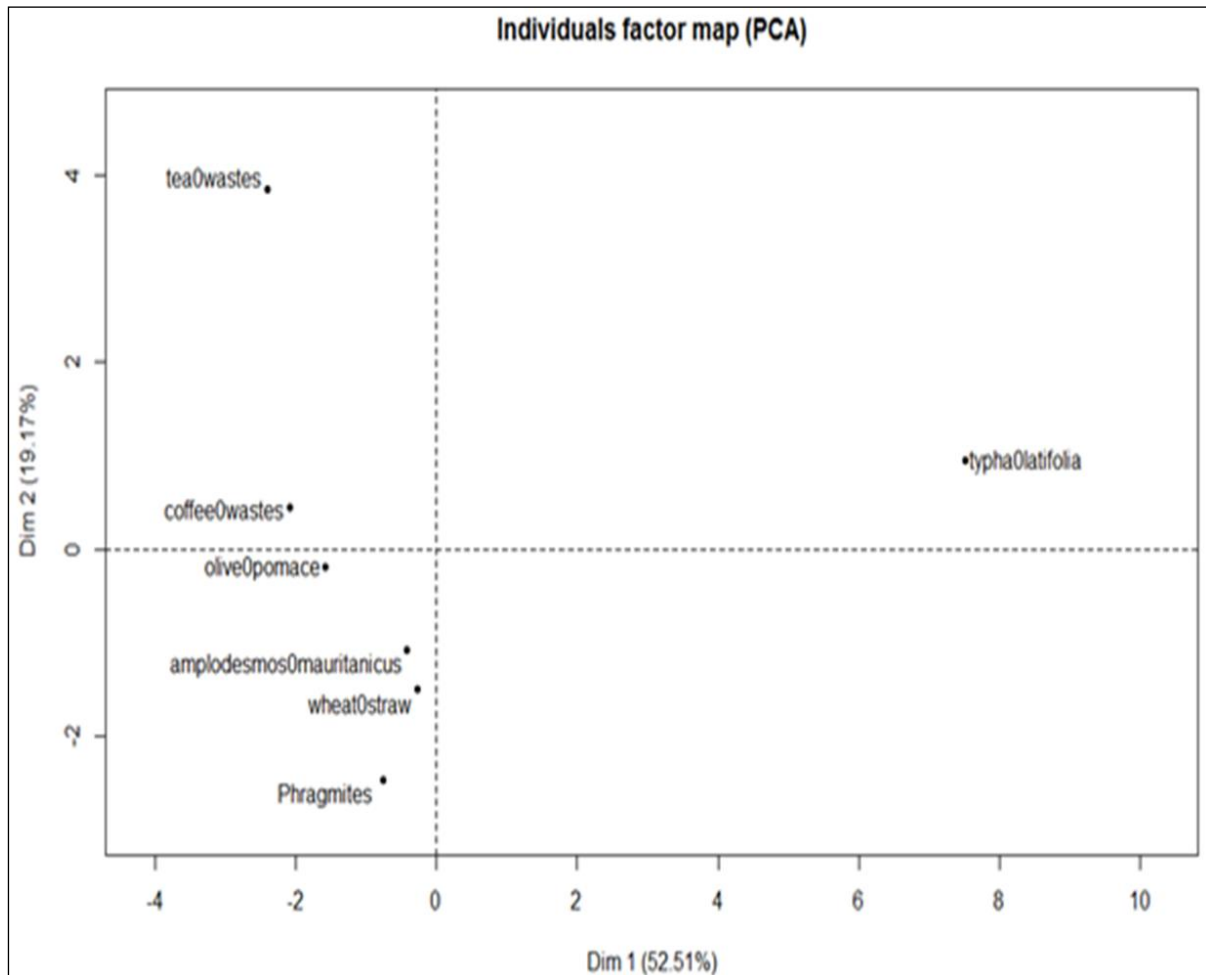


Fig 9: Individual factor map obtained from the PCA of data about physicochemical characterization of analyzed substrates

Code: (tea0wastes) tea wastes; coffee0wastes coffee wastes; (thypha0latifolia) *Thypha latifolia*; (amphedesmos0mauritanicus) *Ampelodesmos mauritanicus*; (olive0pomace) olive pomace; (wheat0straw) wheat straw.

Linking PCA map with the results of *P. ostreatus* mycelium elongation demonstrated that water retention capacity is the most important parameter that regulates fungus elongation. All others analyzed parameters appeared no such discriminatory for differentiation between oyster cultivation substrates.

Kinetics of the variation of organism growth according time follows generally a modeled curve. Commonly this curve is divided into 5 phases and Lag phase is short in the controlled system^[14]. All curves of *P. ostreatus* elongation on analyzed wastes and substrates corroborated with this last proposal.

Stationary phase is a state of absence of net growth. Generally, this phase indicates that either nutrients or at least one important constituent have been exhausted, it is also the stage of excretion and a surcharge of secondary metabolites. In the environment, stationary phase exists for a very short period before entering dormancy and awaiting the next substrate pulse^[15]. According the same authors, the most experimented measures for fungal growth are colony diameter and dry weight. This remark highlighted the methodology used in this research.

Generally, mycelium propagates in the substrate on account of its available nutrients. Many organic substances could be used as energetic sources during the growth of fungi. Fungal species are often extremely selective in their nutritional requirements and grow in preference to certain media over others. This proposal explains difference on oyster growth found in this study. Generally, fungi need important quantities of macronutrients (N, P, K, Mg, S and Ca) during their development^[16]. Inorganic elements could be divided into toxic elements (Cd,

Hg and Pb) and core elements (Fe, Mn, Zn, Ni, Mo, Cu, Cr, Co) and requirement on these elements depends on species and on their living mode ^[17]. According to Paracelse (Swiss doctor and chemist: 15-16th century) "Everything is poisonous, nothing is poisonous. It is the amount that makes the difference". While, mushrooms, heterotrophic organisms, do not require the presence of inorganic elements in their growth media besides they are inhibited by the high quantity of several toxic element. Toxicity may affect osmotic shock; alter thin proteins, enzyme or even destruct the fungal tissues ^[18]. The presence of measured mineral elements in different amount in cattail, *A. mauritanicus*, *Phragmites australis*, wheat straw, olive pomace, tea and coffee wastes suggest that these founded concentrations are tolerable by *P. ostreatus*.

Fungi assimilate nutriment using hydrolytic enzymes. These enzymes crack the nutrient particles to smaller subunits ^[19]. Inorganic nutrients proportion is critical to the growth, development and survives of fungi. Studies of macronutrients and their behavior approaches are easier than study of trace elements because they are present in relatively high concentrations however the unavailability of micronutrients is difficult to determine.

It's well known that boron, cobalt, vanadium (V) and scandium (Sc) may also be essential to fungi growth but in small quantities ^[20]. Magnesium stabilizes enzymes, ATP, and RNA and may also regulate activities of the fibers of the mitotic spindle ^[21]. Phosphate element is a structural factor of nucleic acids, phospholipids and vitamins and it can also accumulate magnesium and iron ^[22]. Potassium, present on several parts of the cell membrane, maintains electrical and osmotic equilibrium of the cell ^[23]. Sulfur is a constituent of certain amino acids, vitamins and metabolites. Calcium attaches to the cell membrane and also regulates the microtubule and microfilament cytoskeleton ^[24]. These parts clarify fungal requirements to uptake mineral element from their substrates growth to allow hyphal creation and elongation and thus fungus development and survive.

General carbon sources used by the fungi are sugars and polysaccharides, lignin, fatty acids and various types of lipids, amino acids, and carbon dioxide. Fungal growth is also regulated by nitrogen availability in the culture medium; nitrate, nitrite, ammonium, urea and amino acids are the most commonly used sources. Nitrogen origin and dose affect also the timing of mushroom fruiting ^[25].

Cellulose is the main constituent of wood and straw, its decomposition or cellulolysis is favored with the presence of bacterial or fungal cellulase. Contrariwise, lignin covers cellulose fibers of woody and many other plant species (straw). The ligninolysis is slow and it is carrying out by some bacteria species and especially by many basidiomycetes' fungi (Polypores, Agarics).

Substrate thickness or aeration could prevent or reduces mycelium growth ^[26]. If particles' substrates are not close enough, mycelium needs more energy to reach the next piece. Against, a very impenetrable substrate prevents fungal respiration. Excess of water content blocks the flow of air, whereas lack of water could halt mycelium growth. In anaerobic condition, fungal development could be limited with the presence of microbes and toxic substances produced. Each unit of the growth substrate is wrapped in a film of water particularly important because this is where fungal activity is most intense. Accessibility of this water (named "free water") is regulated by osmotic feeds. Generally, quantity of available water in medium (water with many dissolved molecules) is small ^[25] and fine sawdust can hold more water than coarser one. This proves that the water retention capacity (CR) is the major parameter regulated oyster elongation on studied substrates in this research. The optimal value registered on cattail as well as its particles sizes were helpful for the fungus elongation. Approval of [27] elucidates our funding, it seems that in substrate formed from hard organ (leaves and stems) growth of mycelium of oyster mushroom is more important for particles sizes upper than 2mm (such for *A. mauritanicus*, *Phragmites australis* and cattail). For substrate formed of thin texture (such as wheat straw) mycelium growth is optimal with particle size between 1 and 2mm. In friable material, such as for coffee waste fungus could develop in fine particles whereas in non-friable one (tea wastes) it needs greater sizes to grow. Texture and particles sizes support nutriment source, physical material to mycelium collision and permit air circulation. Likewise, the major element considered in the choice of substrates for fungi growth is their availability on O₂ and CO₂ ^[28]. The perfect substrate is that allowing fungal absorption of required concentrations of water and nutrients with favorable granulometry size facilitating hyphal elongation and decomposition.

Marshlands are characterized by low and fluctuated water levels. In these ecosystems grow some macrophytes like cattails (*Typha sp.*). *T. latifolia* species is common in the world. It was found in North America, Mexico, Great Britain, India, Africa, New Zealand, and Australia ^[29]. Cattail is a dominant plant species in southern boreal and temperate marshes and abundant in the low boreal mixed wood eco-region. In Tunisia, the richness of *Typha* leaves on fibres and cellulose was confirmed previously ^[30].

In Canada, cattail leaves are also rich on cellulose as well on gelatine, starch, tannic acid, and lignin as carbon sources (based on calorimetric tests). Many fungi species were able to decompose *T. latifolia* leaves, that's proves a nutriment asset and non-toxicity for fungi ^[31]. According to the same authors, majority of fungi prefers cellulose than gelatine as carbon sources. Tannic acid and lignin are less decomposed. Investigations support our data concluding that cattail leaves permit fungi growth and amount of some toxic elements that is built-in are tolerated by fungi especially by *P. ostreatus*.

Mycelium growth of *P. eryngii* var. *Ferulae* depend on type of used substrates and vary between 9.2 - 13.0 days ^[32]. Generally, mycelium of *Pleurotus sp.* emerges on 10 - 15 days ^[33]. This research corroborates last proof, oyster mushroom spends almost same delay to spread through all tested substrates.

As we know, this study constitutes the first report on the growth of *Pleurotus ostreatus* mycelium on *A. mauritanicus* and *T. latifolia* leaves. Likewise, tea and coffee have a potential to increase the oyster growth when combined with paddy straw and sawdust ^[34]. The same conclusion was established in our study with tea leaves.

Data showed interested growth of *P. ostreatus* mycelium on coffee wastes. This substrate assessed for supporting growth of this edible fungus species^[35].

Conclusion

Mushroom consumption increased last years over the world. Thus, mushroom production projects could allow rural economy, development and employment opportunities in these areas. In developing countries, using of local resources could enhance mushroom productivity assets. This study proves that crushed leaves of *T. latifolia* and *A. mauritanicus*, between 1 and 2mm of size allow hyphae elongation and the growth of *P. ostreatus* edible mushroom species. It also confirms the possibility to grow oyster mushrooms on straw of wheat, coffee wastes, crushed leaves and stems of *Phragmites australis*, and tea wastes. This research offers new prediction to oyster cultivation in forestry areas. It also establishes an idea about compost type and size for studied plant species and wastes to promise faster *P. ostreatus* elongation. Such culture requires more optimization of other growing factors (humidity, temperature, photoperiod...) to ensure the best biological efficiency.

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