

Effects of some environmental parameters on Mycelia Growth of Finnish truffle *Tuber Maculatum*

Matab Nadim, Neila Saidi, Ibrahim W. Hasani, YY El Banna, Omar Samir,
M. El Haj Assad, Salem Shamekh

Abstract— The effects of some environmental parameters such as, carbon source, temperature, pH, salinity and growth media on the mycelial growth of the Finish truffle *Tuber maculatum* were presented for the first time in this study. An experimental procedure was carried out to study these parameters effects and the results of this procedure were analyzed statistically. Four different growth media were used to investigate their effect on mycelial growth. Eight different sources of carbon were used in this study. Salinity effect on mycelial growth was obtained by using four different salt concentrations. Different temperatures as well as different pH values were used to see their effects on mycelial growth. The results of all of the above mentioned parameters effects on mycelial growth rate were presented graphically.

Index Terms— environmental parameters; growth rate; mycelia ; *Tuber maculatum*.

I. INTRODUCTION

Truffle is a hypogeous fungi that undergoes a complex life cycle during which the mycelium establishes a symbiotic interaction with the roots of trees, such as oak, poplar, willow and hazel. As a final step, hyphae aggregate and develop a fruiting body – the truffle – which is an ascoma bearing asci and the products of meiotic events, the ascospores [1]. The true truffles are a group of several valuable and highly sought-after edible species of underground ascomycetes belonging to the genus *Tuber* [2]. *Tuber melanosporum*, the Perigord black truffle, *Tuber magnatum* Pico, the Italian white truffle and *Tuber aestivum*, the summer truffles are among the renowned edible truffles having high ecological

Matab Nadim, Juva truffle center, 51901, Juva, Finland.

Neila Saidi, CERTE - LTEU Technoparc Borj Cedria Tunis Tunisia, 21655641366.

Ibrahim W. Hasani Department of Biochemistry, Faculty of Science, Cairo University, Cairo, Egypt.

YY El Banna Department of Microbiology, Faculty of pharmacy , Ahram canadian university ,6th october city, Egypt.

Omar Samir Misr University for Science & Technology (MUST).College Of Biotechnology. Adress: Al-Motamayez District, P.O.BOX:77, 6th of October City, Giza, Egypt; Tel: +202 -3824-7455 Fax: +202 -3824-7417.

M. El Haj Assad SREE Department, University of Sharjah, P O Box 27,272, Sharjah, United Arab Emirates ,Tel: +971 6 5050911, Fax: + 971 6 558519. (corresponding author)

Salem Shamekh Department of Biotechnology and Chemical Technology, Aalto University, School of Chemical Technology, 00076, Aalto, Finland. And Director of Juva truffle center, 51901, Juva, Finland.

and commercial value [4-5].*Tuber brochii vittad*, commonly called Bianchetto truffle the only truffle species with commercial value found naturally in Finland [6]. *Tuber maculatum* are found throughout Europe [7]. In Autumn 2006, tuffle ascocarps were collected from a natural forest located in Lahti, Finland (100 km north to Helsinki) by the help of a trained truffle dog. By morphological identification, the collected truffles were identified as *Tuber maculatum* and *Tuber scruposum* [8].

Although several biotic (fungi, yeasts, bacteria, mesofauna, plant host) and abiotic (soil composition, weather such as rain, sunshine and temperature) factors could influence truffle life and enhance or inhibit ascocarp formation [9]. As the mycelia is the early stage for the formation of the truffle fruit and it is influenced by the both biotic and abiotic stress many questions stimulated researchers to investigate the distribution of the symbiotic phase of selected truffle-ground where the production of fruiting bodies [3]. Many researchers have investigated the growing mycelia in the broth media and on solid substrates [10-11]. Many studies have determined the optimal growth conditions and nutritional requirements of different fungi [12-13]. The present study is the first report by scientific research work on in vitro to assess the effects of some environmental parameters such as pH, carbon source, salinity, growth media and temperature on the Finnish truffle *Tuber maculatum* mycelial growth.

II. MATERIAL AND METHODS

A. Isolation and Culture of the mycelia

The truffle fruiting body was topically sterilized with 70% ethanol. Small pieces of tissue 1-2mm were aseptically excised from the inner part of the fruit bodies and cultivated on malt extract agar (MEA) medium (Sigma Aldrich, Finland). Incubation was carried out in dark at 22°C. Mycelia growth and purity were investigated weekly microscopically. Mycelial discs of 5 mm diameter were transferred to fresh MEA plates and incubated at 4°C for experimental studies. Three replicates were prepared for all experiments and incubated for one week. The radial extension of the mycelium was measured daily as described by [14] with a caliper gauge along two diameters at right angles to one another and the average for each plate calculated.

B. Determination of the effects of various environmental conditions

A. Carbon sources

Fructose, glucose, sucrose, maltose, lactose, xylose, mannose and Dextrose were used as carbon sources and added at a concentration of 1% to Malt extract agar (30 g/l

Effects of some environmental parameters on Mycelia Growth of Finnish truffle Tuber Maculatum

malt extract -5g/l pepton – 15g/l agar) according to [13]. Carbon sources were separately sterilized by filtration and concentrated to prevent possible heat damage.

B. Temperature and pH effects

Truffle mycelia growth on malt extract agar was investigated at different temperatures (4, 15, 25, 30, 35 and 40 °C) were used to determine the optimal temperature for promoting mycelia growth of Tuber maculatum. Regarding pH, three experiments were used to screen an optimal pH for mycelia growth of Tuber maculatum. In first experiment the pH of agar media was adjusted with NaOH or HCl to a pH range of (5.8-6.0-6.6-7.0-7.6-8.0), while the second experiment was adjusted with Potassium and Sodium Phosphate Buffers to the same pH range for comparison between the two. Broth media adjusted with NaOH and HCl was used to determine the change in pH between the initial pH and the final pH (pH after growth) by pH meter. [11-15]

C. Salinity

Malt extract agar amended with different concentrations of NaCl (0.5%, 1.0%, 2.0% and 5.0%) was prepared and autoclaving for 15 min at 121°C for salinity studies experiment [15-16]

D. Different types of growth media

Different types of growth media such as potato dextrose agar (PDA), potato glucose agar (PGA), malt extract agar (MEA), m17, yeast extract agar (YEA), beef extract agar (BEA) were used to investigate the effect of media on the growth of the Tuber maculatum mycelia. The compositions of different types of growth medium are presented in Table 1.

Growth Medium	Composition
Potato dextrose agar	Standard composition (39.0g/l)
Potato glucose agar	Standard composition (39.0g/l)
M 17	Standard composition (50.0g/l) + 5 ml lactose solution (separately sterilized)
Malt extract agar	Malt extract (30g/l); Peptone (5g/l); Agar (15g/l)
Yeast extract agar	Tryptone (6.0g/l); Yeast extract (3.0g/l); Agar (15g/l)
Beef extract agar	Tryptone (6g/l); Beef extract (3.0g/l); Agar (15g/l)

Table 1. Growth media used

E. Statistical Analysis:

Experimental data was analysed by statistical package for the social sciences (SPSS program, version 21.0) and considered statistically significant at a two-sided $P < 0.05$. The growth rate values of mycelia were calculated by the ratio of colonies diameter and time (days). The data was expressed as mean \pm standard deviation and one way

ANOVA was used to compare between means followed by post-hoc test (LSD) for detection of significance. Comparisons between the growth rate of agar media adjusted with NaOH or HCl and buffered agar media were performed using Student's t-test. Figures.

III. RESULTS AND DISCUSSION

A. Effect of carbon sources

In this study, eight different sources of carbon, namely sucrose, fructose, xylose, glucose, dextrose, mannose, lactose and maltose were used to determine the most suitable carbon source for mycelia growth of Tuber maculatum. Statistical analysis unveiled that the mycelia grew equally with no significant differences ($P > 0.05$) in medium containing different carbon sources as shown in Table 2. The present study showed that lactose is the best carbon source. In a recent study it was found that when the citric acid or glucose serve as the carbon source truffle grows well [17]. On the other hand [18] reported that none of the carbon sources except the sucrose supported the growth of *T. melanosporum*. [19] showed that when they used mannose or mannitol in the liquid media in order to obtain an increased fungal biomass for different *T. borchii* mycelial strains, some strains grew in mannose or mannitol equally well as in glucose. In another study, *Morchella* spp. showed that fructose was the best carbon source for *M. rotunda*, glucose for *M. elata* and *M. esculenta*, maltose for *M. costata* and *M. intermedia* and sucrose was the best for *M. hortensis* [20]. A study on a medicinal mushroom showed that the best mycelial growth area and mycelial colony diameter of *Hydnum repandum* were obtained in the media including mannitol and glucose [21].

Carbon source	Growth rate Cm/Day	ANOVA test
Sucrose	3.46 \pm 0.14	P = 0.457
Fructose	3.43 \pm 0.09	
Xylose	3.47 \pm 0.1	
Glucose	3.24 \pm 0.13	
Dextrose	3.42 \pm 0.21	
Mannose	3.43 \pm 0.5	
Lactose	3.50 \pm 0.06	
Maltose	3.25 \pm 0.34	

Table 2. Effect of carbon source on the mycelial growth rate of (*Tuber maculatum*).

B. Effect of the salinity

Different concentrations of NaCl (0, 0.5, 1, 2 and 5 %) were screened to determine the influence of salinity on the mycelial growth rate of *Tuber maculatum*. Our results showed that the best growth rate value was found in the control (0%) then the growth rate values were decreased dramatically with increase of the NaCl concentrations in the medium as shown in Figure 1. In a previous study, it was found that all strains of six *Morchella* species could tolerate NaCl at different concentrations, the mycelia of the morels grew on all the salt concentrations [20]. In another study, it has been reported that

the mycelial growth of *Pisolithus tinctorius* at 100mM NaCl was significantly higher than other concentrations. They also found the mycelial growth of *Cenococcum geophilum* and *Suillus luteus* decreased with increasing NaCl concentrations [22]. Moreover, [23] reported that *Pleurotus tuberregium* grew best in no salt medium.

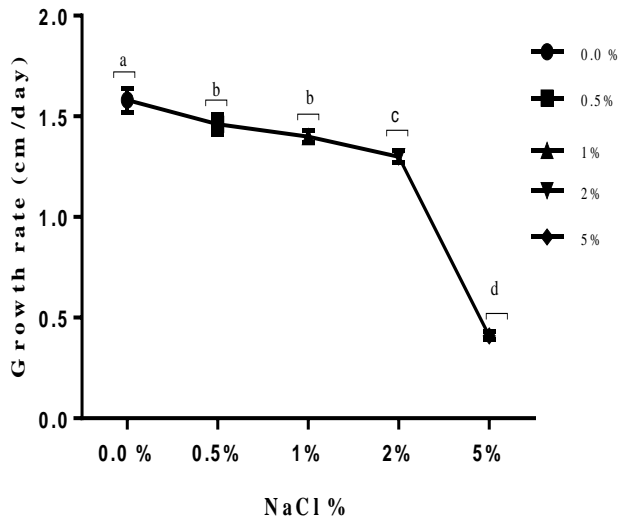


Figure 1. Effect of concentrations of NaCl % on the mycelial growth rate of *Tuber maculatum*. Different letters represent significant difference ($P < 0.05$).

C. Effect of temperature on mycelia growth

Tuber maculatum was screened for suitable temperature for inducing mycelia growth on MEA medium. Temperatures of 4 °C, 15 °C, 25 °C, 30 °C, 35 °C and 40 °C were used, the results of the present study showed that *Tuber maculatum* was able to grow at a temperature range of 4-30°C. The statistical analysis showed that 15°C was the best temperature for mycelial growth of *Tuber maculatum* with the growth rate of $(1.96 \pm 0.06 \text{ cm/day})$ followed by 25 and 30°C with the growth rate of $(1.19 \pm 0.39, 0.97 \pm 0.00 \text{ cm/day})$ respectively and it grew slowly in 4°C with growth rate of $(0.46 \pm 0.02 \text{ cm/day})$, while it did not grow in 35 and 40°C at all as illustrated in Figure 2. The inhibition of *Tuber maculatum* growth at 35 and 40 °C may be due to denaturation of some important enzymes that catalyze mycelial metabolic processes [24]. It was reported the optimum temperature for truffle to grow is 22-24 °C. This result is close to the natural ecological environment temperature conditions, for example, from the the Panzhuhua region survey Truffle township to natural temperature and the ground temperature is consistent with truffle growing area, the average annual temperature is about 20 °C (According to the survey truffle growing area months mean temperature from March to August temperature was gradually rising trend in September, began to decline, approximately 14,16,20,23,25,28,26,25 °C) [17]. Accordingly, it was found that the optimal temperature for growth of *Morchella* spp were 20 and 25°C [20]. In agreement with this study, It was found that the best temperatures for mycelial growth of *H. repandum* were found to be 20 and 25°C, while no mycelial growth was observed at 30°C, moreover, the mycelial growth drastically decreased at 15°C, because of the decrease of metabolic activities of the fungus

that allow the absorption of essential nutrients needed for growth [21].

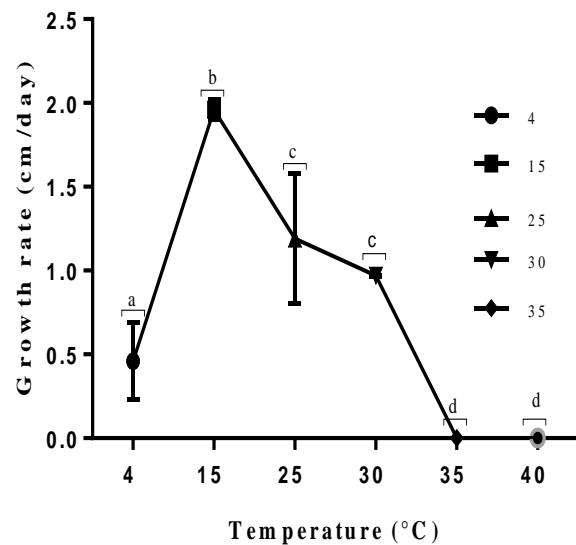


Figure 2. Effect of temperatures on the mycelia growth rate of *Tuber maculatum*. Different letters represent significant difference ($P < 0.05$).

D. Effect of pH on mycelial growth

In the first experiment, the effect of pH on mycelial growth rate of *Tuber maculatum* is shown in Figure 3. The results of the present study showed that *Tuber maculatum* grew well in acidic, neutral and alkaline environments from (pH 5.8 to pH 8) media adjusted with NaOH and HCL. The best mycelia growth rate values were observed in acidic and neutral media of pH (5.8-6.0-6.6-7) followed by alkaline media of pH (7.6-8), while Figure 4 shows the mycelia growth rate in medium adjusted with buffer saline, the acidic media pH (5.8-6.0-6.6) were the best for mycelia growth, while it did not grow at all in the neutral and alkaline environments (pH 7.0-7.6-8.0). In the comparison between the two mentioned experiments, we found that the media adjusted with NaOH or HCl were more suitable and significantly promoted mycelial growth as shown in Figure 5. In order to detect the change in pH of *Tuber maculatum* before and after growth of mycelia, broth media were used to inoculate mycelia and pH meter was used to measure pH values. We observed that the media after growth were turned from alkaline to acidic nearly neutral in range of pH (5.14-6.92) as shown in Figure 6. These results showed that the mycelia have the ability to change the pH environment for its favour when we use the chemical adjustment. In a recent study, it was reported that Truffle grow better in pH between 6.5-7.5, but the optimum pH 6.8-7.0 So the truffles for growth in the ecological environment of the neutral to weak alkaline conditions [17]. Another study indicated that the two strains of *T. melanosporum* differed in their pH requirements. The strain Rey-t grows optimally at the pH 6 whereas the strain Mel-28 prefers pH 6.5 [18]. In addition, it was found that the best pH for growth of *H. repandum* was 5.5 [21]. In a previous study, all *Morchella* species grew over the pH range of 5.5-8.0 with the greatest growth rate at pH 6.0 for *M. intermedia*, pH 6.5 for *M. costata* and *M. hortensis* and pH 7.0 for *M. elata*, *M. esculenta*, and *M. rotunda* [20].

Effects of some environmental parameters on Mycelia Growth of Finnish truffle Tuber Maculatum

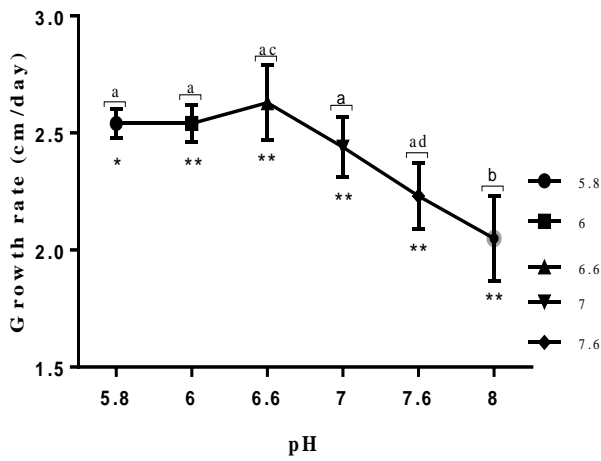


Figure 3. Mycelial growth rate of Tuber maculatum at different pH. Different letters represent significant difference ($P < 0.05$). * The substratum adjusted with HCl and ** The substratum adjusted with NaOH.

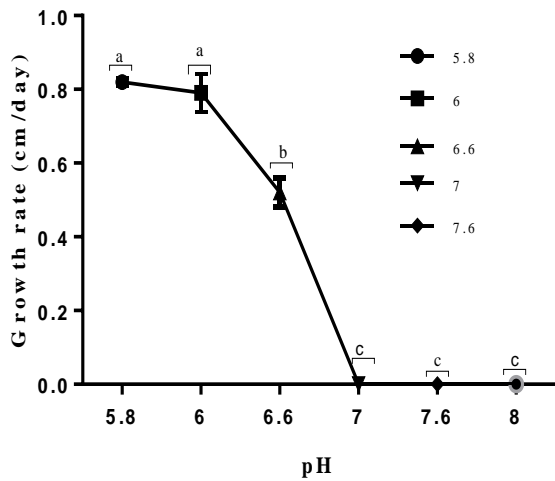


Figure 4. Mycelial growth rate of Tuber maculatum at different pH (pH of media was adjusted with buffer solution). Different letters represent significant difference ($P < 0.05$).

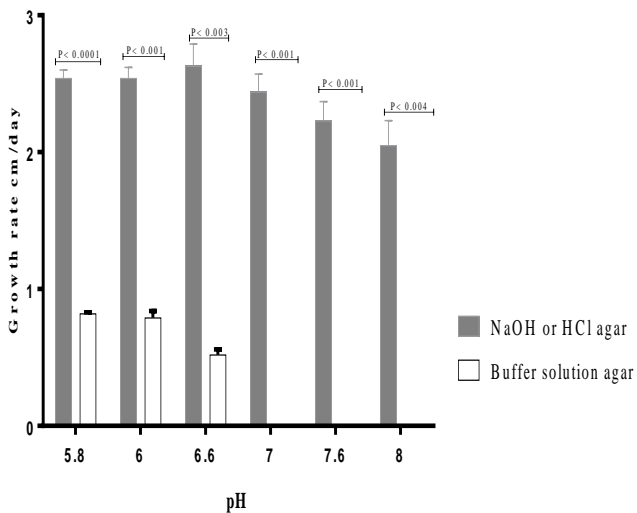


Figure 5. Comparison of mycelial growth rate between NaOH or HCl agar and buffer solution agar media.

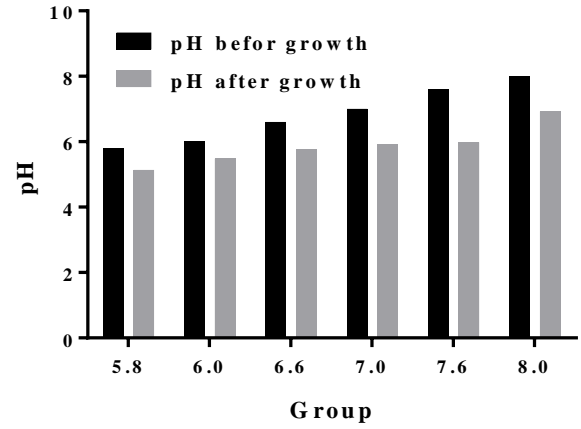


Figure 6. Effect of mycelial growth on the pH of media

E. Effect of culture media on mycelia growth

In our study, five different solid media were tested for the favorable growth of the Tuber maculatum. The best growth rate was observed on MALT and PGA followed by PDA, M17, YEA and BEA respectively. Tuber maculatum had the largest colony diameter on MALT medium (1.42 ± 0.06 cm/days); PGA medium (1.38 ± 0.03 cm/days); PDA medium (1.22 ± 0.02 cm/days); M17 medium (0.81 cm/days); YEA medium (0.71 ± 0.01 cm/days); and BEA medium (0.49 ± 0.01 cm/days), figure (7). In a previous study 10 media were tested for the growth of *T. melanosporum*. Only two media, Malt extract PVP agar and a slightly modified Minimal medium supported the fungal growth. Also MALT extract agar and PDA supported the growth of mycelia with a minimum rate, Malt extract PVP agar supported the maximum growth with a growth rate of 8 mm per week [18]. It has been reported that malt extract agar, wheat agar, potato dextrose agar and complete medium yeast extract agar media containing glucose, sucrose, maltose and starch were the best vegetative mycelial growth of *Morchella conica* [25].

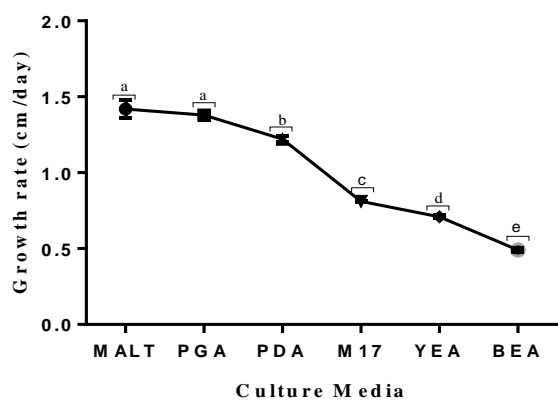


Figure 7. Effect of culture media on the mycelia growth rate of Tuber maculatum. Different letters represent significant difference ($P < 0.05$).

IV. CONCLUSION

Experimental work followed by statistical analysis has been carried out to determine the effects of environmental parameters on the Finnish truffle Tuber maculatum mycelial

growth. The statistical analysis showed that mycelia grew at the same growth rate with no significant differences in medium containing different carbon sources. The best mycelial growth rate was obtained with zero NaCl concentration. The results showed that *Tuber maculatum* could grow at temperature range 4 to 30 °C. Moreover, the analysis showed that the temperature of 15 °C was the best for mycelial growth of *Tuber maculatum*. The results also showed that mycelia growth rate was the best in acidic and neutral media, and the medium adjusted with NaOH or HCl was more suitable for mycelial growth than with buffer solution medium. In this study, it was obtained that the best growth rate of *Tuber maculatum* was for MALT solid medium and the least growth rate was for BEA solid medium.

ACKNOWLEDGEMENTS

This study was conducted at Juva Truffle Center Finland. The financial support of Regional Council of Southern Savo, Finland is appreciated and thanked. Authors are grateful for Mr. Antti kinnunen for administrative service and Mrs. Heli Valtonen for her technical assistant.

REFERENCES

- [1] Harley, J.L., Smith, S.E. (1983) *Mycorrhizal Symbiosis*, Academic Press, London.
- [2] Murat C. et al. (2008). Edible mycorrhizal fungi: Identification, life cycle and morphogenesis. In *Mycorrhiza* 3rd ed. Ajit Varma Ed. pp. 707-73
- [3] Mello A, Murat C, Bonfante P (2006) Truffles much more than a prized and local fungal delicacy. *FEMS Microbiol. Lett.* 260:1–8.
- [4] Hall I, Brown GT, Zambonelli A (2007) Taming the Truffle: the history, lore, and science of the ultimate mushroom. Timber, Oregon.
- [5] Streiblová E, Gryndlerová H, Valda S, Gryndler M (2010) *Tuber aestivum* – hypogeous fungus neglected in the Czech Republic. *Czech Mycol* 61:163–173.
- [6] Kosonen, L. (2002) Mitä kasvaako Suomessakin tryffeleitä. *Sienilehti*, 54, 105-110.
- [7] Bertault G, Rousset F, Fernandez D, Berthomieu A, Hochberg ME, Callot G & Raymond M (2001) Population genetics and dynamics of the black truffle in a man-made truffle field. *Heredity* 86: 451–458.
- [8] Shamekh S., Donnini D., Zambonelli A. and Leisola M (2009) Wild Finnish Truffles. *Acta Botanica Yunnanica* :69-71.
- [9] Ceruti A, Fontana A & Nosenzo C (2003) Le specie europee del genere *Tuber*, Una revisione storica, Regione Piemonte, Torino.
- [10] Kosaric, N., Miyata, N. Growth of morel mushroom mycelium in cheese whey. *J. Dairy Res.*, 1981; 48: 149-62.
- [11] Volk, T., Leonard, T. Physiological and environmental studies of sclerotium formation and maturation in isolates of *Morchella crassipes*. *Appl. Environ. Microbiol.*, 1989; 55: 3095-100.
- [12] Ohta, A. Fruit-body production of two ectomycorrhizal fungi in the genus *Hebeloma* in pure culture. *Mycoscience*, 1998; 39: 15-9.
- [13] Sanchez, F., Honrubia, M., Torres, P. Effects of pH, water stress and temperature on in vitro cultures of ectomycorrhizal fungi from Mediterranean forests. *Crypto. Mycol.*, 2001; 22: 243-58.
- [14] Weitz, J.H., Ballard, A.L., Campbell, C.D., Killham, K. The effect of culture conditions on the mycelial growth and luminescence of naturally bioluminescent fungi. *FEMS Microbiol. Lett.*, 2001; 202: 165-70.
- [15] Tsai, S.Y., Weng, C.C., Huang, S.J. Nonvolatile taste components of *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. *Food Sci. Technol.*, 2006; 39: 1066–71.
- [16] Kalmýř, E., Kalyoncu, F. Mycelial growth rate of some morels (*Morchella* spp.) in four different microbiological media. *Amer-Euras. J. Agric. Environ. Sci.*, 2008a; 3 (6): 861-4.
- [17] Shang-Qin Hu, Xin Yang, Wei Wei and Xia Guo. Study on the Ecological Environmental Conditions of Truffle with Analysis of Biomaterials. *Advanced Materials Research* Vol 648 (2013) pp 389-393
- [18] Shwet Kamal. Effect of nutrient sources and plant hormones on mycelial morphology of the black perigord truffle tuber *melanosporum*. Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7), 2011
- [19] Ceccaroli, P., Saltarelli, R., Cesari, P., Zambonelli, A. & Stocchi, V. (2001). Effects of different carbohydrate sources on the growth of *Tuber borchii* Vittad Mycelium strains in pure culture. *Molecular and Cellular Biochemistry* 218: 65–70
- [20] Fatih Kalyoncu, Mustafa Oskay and Meryem Kalyoncu. The Effects of Some Environmental Parameters on Mycelial Growth of Six *Morchella* specie. *Journal of Pure and Applied Microbiology*, October 2009. Vol. 3(2), p. 467-472.
- [21] Peksen A, Kibar B, Yakupoglu G. Favourable Culture Conditions for Mycelial Growth of *Hydnum Repandum*, a Medicinal Mushroom. *African Journal of Traditional, Complementary, and Alternative Medicines*. 2013;10(6):431-434.
- [22] Matsuda, Y., Sugiyama, F., Nakanishi, K., Ito, S. Effects of sodium chloride on growth of ectomycorrhizal fungal isolates in culture. *Mycoscience*, 2006;47: 212-7.
- [23] Ayodele, S. M. and Ojogoro, O. J. (2007). Salt stress effects on the vegetative growth of *Pleurotus tuberregium* (FR) sing. *J Biol Sci* 7:1278–1281.
- [24] Jonathan, S.G., Fasidi, I. O. and Ajayi, E. J. Physico-Chemical Studies on *Volvariella esculenta* (Mass) Singer, a Nigerian Edible Fungus. *Food Chem.*, 2004; 85: 339-342.
- [25] Guler P, Özkaya E G. Sclerotial Structures of *Morchella conica* in Agar Media with Different Carbohydrates. *Acta Alimentaria*. 2008;37(3):347–357.