Scientia Horticulturae 189 (2015) 86–93

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of microelemental fertilizers on yields, mineral element levels and nutritional compositions of the artificially cultivated *Morchella conica*

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ARTICLE INFO

Article history:
Received 22 November 2014
Received in revised form 4 March 2015
Accepted 18 March 2015

Keywords:
M. conica
Microelement
Mycelia growth
Amino acid
Mineral elements
Heavy metals

ABSTRACT

This study analyzed the effects of four microelemental fertilizers not only on growth rates and sclerotia formation of mycelia, but also the yields, amino acids contents and mineral elements of fruit body from artificially cultivated *Morchella conica*. Results showed that Fe·EDDHA had the strongest promoting effect on the mycelia growth, sclerotia formation and resulted in the highest yield, while $CuSO_4·5H_2O$ resulted in an adverse effect. Fe·EDDHA, Nutriagent and $ZnSO_4·7H_2O$, respectively, increased the yields of morel's fruit bodies by 63.4%, 59.9% and 25.9% when compared with that of the control, but the yield of $CuSO_4·5H_2O$ group was reduced by 19.3%. The total amino acids contents of Fe·EDDHA, Nutriagent and $ZnSO_4·7H_2O$ groups were also significantly increased. Six microelements (Fe, Zn, B, Cu, Mn and Mo) and six heavy metals (Ni, As, Pb, Cr, Cd and Hg) were assayed and the highest Fe content was found in the Fe·EDDHA group, 7.10 times higher than that of the control group.

1. Introduction

Morels has been highly prized for their medicinal and nutritional qualities, widely consumed as a vegetable or a functional food and used as a medicine to cure some diseases, such as hypercholesterolemia, hypertension and cancer for centuries throughout the world (Elmastas et al., 2007; Wong and Chye, 2009; Gençcelep et al., 2009; Kanwal and Reddy, 2012). The morel's fruit body yield is highly limited to temperate regions of the Northern Hemisphere, such as Europe, North America and China (O'Donnell et al., 2011; Du et al., 2012) and the harvesting season is short. Due to increasing popularity and commercial demand, wild morels are cultivated commercially and exported extensively from China, India, Turkey, Mexico and the United States (Pilz et al., 2007; Taşkin et al., 2010). Meanwhile, wild morels are at risk of accumulating heavy metal content such as arsenic (As), cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni) and mercury (Hg) from natural habitats (Vetter, 2004; Gençcelep et al., 2009; Gursoy et al., 2009; Zhu et al., 2011). Therefore, it is very important to study a mature approach to commercially cultivating morels.

Many researchers have attempted to cultivate morels in controlled conditions. Up to now, most reports mainly focus on the development of fruit bodies, modes of reproduction, nutritional sources, life cycle (Pilz et al., 2007), external ultrastructure, sclerotium formation and germination (Masaphy, 2010). Some studies have demonstrated that minerals play an important role in the metabolic processes of mushrooms (Stihi et al., 2011; Cheung, 2008), and more extensive studies have been published (Vetter, 2004; Ouzouni et al., 2009; Ayaz et al., 2011; Falandysz et al., 2013). Gursoy et al. (2009) determined the metallic elements content of *Morchella conica* collected from the Mugla Province in Turkey, and it was found that the morel fruit body was rich in Fe, Zn and Mn. In addition, some nutritional components in mushrooms, such as protein, vitamins, carbohydrate and minerals, could be varied because of categories, anthropogenic factors, soil structure and region (Szefer, 2007). However, all of these reports were about wild morels, not artificially cultivated ones. In the present study, four fertilizers including zinc sulfate ($ZnSO_4·7H_2O$), copper sulfate ($CuSO_4·5H_2O$), Fe·EDDHA and Nutriagent, were chosen for analyzing the efficacy of these microelements on the growth of mycelium, the formation of sclerotia and the nutritional value of

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http://dx.doi.org/10.1016/j.scienta.2015.03.047
0304-4238/

artificially cultivated *M. conica* compared with wild *M. conica* (data of the literature by Liu et al., 2012).

2. Materials and methods

2.1. The strain and chemical agents

The *M. conica* strain Mc-5 has been isolated and preserved in the authors' laboratory for 6 years, whose molecular identification results were reported previously by one of the authors (Li et al., 2013). Zinc sulfate ($ZnSO_4{\cdot}7H_2O$), copper sulfate ($CuSO_4{\cdot}5H_2O$), ethanolamine, disodium edentate dihydrate (EDTA·2Na), manganous sulfate ($MnSO_4{\cdot}4H_2O$), ferrous sulfate ($FeSO_4{\cdot}7H_2O$), ammonium molybdate [$(NH_4)_2MoO_4$], boracic acid, isopropyl alcohol and other chemical agents were analytically pure and purchased from local reagent chemical companies except Fe·EDDHA, which was purchased from Nobel Azol Co, Ltd.

2.2. Preparation of Nutriagent by three-step cheleration

Nutriagent, one of multi-microelement fertilizers chelated by EDTA, was prepared according to the Chinese Patent No. 200710050488.9 (Long et al., 2009). It consists of six microelements (4.43% Fe, 3.85% Zn, 1.80% Mn, 0.06% Mo, 1.28% B and 0.60% Cu, respectively). First, the ethanolamine chelating boron was synthesized by adding 1.0 kg boracic acid (40 mesh) into the pre-heated distilled water (65 °C) and stirring them for 1 h while 3 kg ethanolamine was mixed with them. Then, the mixture was kept reacting under 80 °C for 2 h followed by another 2 h for evaporation at 110–120 °C. Second, the four-microelement complex for iron, zinc, manganese and molybdenum was prepared according to the following formula: 2 kg $FeSO_4{\cdot}7H_2O$, 1.5 kg $ZnSO_4{\cdot}7H_2O$, 0.6 kg $MnSO_4{\cdot}4H_2O$, 0.1 kg $(NH_4)_2MoO_4$, 3.52 kg EDTA·2Na and 0.5 kg isopropyl alcohol with pure water (w/v = 1:1). These materials were wholly smashed to 40 mesh, and then the mixture was finely mixed with EDTA·2Na until 0.5 kg isopropyl alcohol solution was sprayed onto the mixture. After lying for 24 h under 4 kg/cm^2 to solidify, the complex was finely smashed to 40 mesh and its pH value was adjusted to 5.2 by using sodium hydroxide. Third, the amino acid complex chelated copper was prepared by the following method. Copper sulfate ($CuSO_4{\cdot}5H_2O$, 4.2 kg, 60 mesh in diameter) was evenly mixed with 4.8 kg amino acid complex. The mixture was sprayed by 1.0 kg isopropyl alcohol and then dried under the pressure at 3 kg/cm^2 for 24 h. Finally, all of the complexes were wholly mixed according to the formula (ethanolamine chelated boron 25%, the four-microelement complex 70%, copper complex 5%), dried until the water content was less than 2% by a 90 °C heat treatment and smashed to 40 mesh. The prepared Nutriagent was stored at room temperature before use.

2.3. Experiments on the growth of mycelium of M. conica

The pure culture was inoculated by using the sterile PDA medium (containing 20% potato, 2% glucose and 1.8% agar) in test-tubes and then was sub-cultured in PDA plates. Each microelement group consisted of four final concentration levels (50, 100, 200, 400 mg/L, respectively) in triplicate, and the same volume of distilled water (dH_2O) was added into the medium plates as the control. Mycelia plugs (6 mm in diameter) were prepared from actively cultured mycelia by using PDA medium plate and then were inoculated on Petri dishes (90 mm in diameter) with a PDA medium under 16 °C in dark and natural air conditions for 19 days (Weitz et al., 2001). Sclerotia's color and distribution were observed, and the extension of the mycelia was measured at 24-h intervals with a caliper gauge randomly with three repeats. The mycelia daily growth rate was calculated by the following formula: Mycelia growth rate (mm/day) = (colony diameter − 6)/2 × cultured days.

2.4. Outdoor planting experiment

The outdoor planting experiment was conducted in a wheat field which is located in Longxing Town, Chongzhou, Sichuan, P.R. China, from early November in 2011 to late March in 2012. The content of soil minerals was measured beforehand as the following concentrations (mg/kg dry soil): 0.647 Zn, 3.278 Cu, 70.06 Fe, 0.95 Mo, 0.182 B and 19.66 Mn. Its contents of organic matter, total N, P and K (g/kg) were 46.82, 2.93, 0.89 and 16.83, respectively, while contents of available N, P, K (mg/kg) were 306.42, 25.36, 159.72, respectively. The soil pH value was measured as 6.87. Before the experiment, the spawn for pure *M. conica* was prepared by the following procedure. After the pure culture was activated by a PDA medium in test-tubes for approximately 15 days, the spawn was prepared in large scale by sub-culturing in another new sterilized media (containing wheat 70%, sawdust 10%, chaff 10%, vermiculite 5% and pure soil 5%, natural pH) under 18 °C for over 15 days until morel sclerotia formed well. Approximately 1.5 bottle (500 mL/bottle) spawn were used for experimental plots per square meter (m^2). Four fertilizers, including Fe·EDDHA, $ZnSO_4{\cdot}7H_2O$, $CuSO_4{\cdot}5H_2O$ and Nutriagent were separately sprayed onto the experimental soil plots at the final concentration of 100 mg/m^2 (100 mg solid compound was dissolved into 1 L distilled water), while the same volume of distilled water sprayed as the control. Each group was in triplicate, and every plot was 10 m^2 in effective planting area.

2.5. Analysis of the amino acid composition of fruit bodies

Ten individual fruit bodies (pileus and stipe) of each experimental group were collected to measure the content of amino acids and their compositions. Each fruit body was carefully washed with deionized water and dried at 45 °C until a constant mass was obtained. Then, amino acids in all of the samples were analyzed by the Institute of Quality and Testing Technology for Agro-Products, Sichuan Agricultural Sciences Academy, Chengdu, P.R. China, using a High Speed Amino Acid Analyzer (L-8900, Hitachi, Japan) overnight in a vacuo acid hydrolysis at 115 °C to hydrolyze proteins or peptides into free amino acids (FAA). Each type of amino acid was identified by using the standard amino acid (Sigma) and quantified by the calibration curve of the authentic compound (Heinriksona and Meredith, 1984).

2.6. Minerals determination of morel's fruit bodies

The samples for mineral measurement were prepared by the acid digestion method described in the literature (Liu et al., 2012). First, fruit body samples were dried at 55 °C for 24 h and homogenized using an agate pestle. Then, a 0.5 g dried fruit body sample was placed into a graphite crucible and digested with 10 mL of nitric acid (HNO_3, analytic purity). It was evaporated to dryness in a solution containing 10 mL of concentrated HNO_3 and 2 mL of concentrated hydrogen peroxide (H_2O_2). The residue was dissolved with 5 mL of 0.2% (w/w) HNO_3 and filtered through a filter paper. The filtrate was diluted up to 10 mL with 0.2% (w/w) HNO_3 (Ozturk et al., 2010). Mineral contents were measured by standard procedure using an inductively coupled plasma atomic emission spectrophotometer (ICPQ-1000, Shimadzu, Japan). Six microelements (Fe, Zn, Cu, B, Mn, Mo) and six heavy metals (Ni, Pb, As, Cr, Cd, Hg) were analyzed according to the manufacturer's instruction (Liu et al., 2012).