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A preliminary study on domestication of wild-growing medicinal mushrooms collected from Northern Iran

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Abstract

Domestication of wild mushrooms and preserving their mycelia alive is essential to ensure reproducibility of biomedical research and breeding programs. This study was conducted with the aim of domestication of wild-growing mushrooms collected from the northern Iran (Mazandaran province) where is known to be rich in valuable higher fungal reserves. Totally, 40 well-identified wild isolates belonging to Basidiomycota (comprised of 21 different genera) was evaluated in solid media (potato dextrose agar and compost extract agar), spawn (grain- and wood-based spawn), and lignocellulosic substrate (wood chips-based substrate supplemented with 15% wheat bran). Significant differences were found between the isolates and between the media in terms of mycelial growth ($p < 0.05$). While more than half of the isolates showed a growth rate higher than 3 mm per day, few isolates exhibited a growth rate of less than one mm per day in both media. Besides, two isolates failed to grow in grain-based or wood-based spawn, while most of them colonized wheat grains shorter than wood chips ($p < 0.05$). The fruiting tests showed that eight isolates were able to produce fruiting bodies in the substrate, including: *Pholiota aurivella* (GPS 142), *Lenzites tricolor* (GPS 180), *Ganoderma tsugae* (GPS 186), *Cyclocybe* sp. (Darabkola 20), *Trametes* sp. (GPS 130), *Trametes* sp. (Darabkola 13), *Daedaleopsis* sp. (Royan 8), and *Donkia pulcherrima* (Neka 24D). Based on the literature survey, most of these isolates possess antimicrobial and anticancer properties. Therefore, adaptation of these wild species to produce fruiting bodies may facilitate further research on their biological activities.

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Introduction

The local knowledge about production and medicinal properties of non-*Agaricus* wild mushrooms (known as specialty mushrooms) in the East and Southeast Asia dates back to centuries ago (Oyetayo 2011, Wachtel-Galor et al. 2011). However, only less than 10 mushroom species out of 14,000 taxonomically known wild mushroom species around the world have reached large-scale commercial cultivation (Miles & Chang 2004). Among these commercially cultivated species, five main genera of mushrooms, including *Agaricus*, *Lentinula*, *Pleurotus*, *Auricularia*, and *Flammulina* account for 85% of the global mushroom production (Royse et al. 2017).

Despite the global trend in cultivation of non-*Agaricus* specialty mushrooms, the white button mushroom (*Agaricus bisporus*) is still the most cultivated species in countries such as Iran, where is known to be rich in a large number of different species of wild mushrooms (Rahnama & Habibi 2015). A vast number of studies have reported the presence of wild mushrooms in different provinces of the country (Ershad 2009, Asef 2010). Among these regions, forests located in the north of Iran have temperate climate, adequate vegetation, and sufficient moisture, making them suitable to host a wide range of different types of edible, medicinal, industrial, and symbiotic macrofungi. Thus, a significant number of studies performed on Iranian wild-growing macrofungi are related to Mazandaran as one of the Northern provinces. Examples published in English journals include (but not limited to) collection of 100 species of macroscopic fungi from Mazandaran, of which 11 species were recorded as new species in Iran (Borhani et al. 2010). A study on *Ganoderma* spp. also reported collection of several *Ganoderma* spp. from Northern Iran (Keypour et al. 2014). A more recent study collected more than 470 specimens from Kheyroud Educational and Experimental Forest (Mazandaran), of which 200 specimen were identified to belong to 46 agaric species (Asef & Etemad 2016).

Collection and identification of native wild mushrooms may have various applications in taxonomical and ethnomycological studies, documentation related to the traditional use of mushrooms, and local markets (Kamalebo et al. 2018). It may even promote further investigations for the domestication of wild edible mushrooms not yet cultivated in a country (Semwal et al. 2014). However, it does not necessarily address the challenge with production of fruiting bodies and pure culture of wild mushrooms. Adaptation of wild mushrooms in locally available lignocellulosic substrates to produce fruiting bodies is essential to ensure ongoing reliability and reproducibility of research on biomedical and biochemical aspects of wild mushrooms (Lindequist et al. 2005). To the best of our knowledge, limited information is available regarding characterization of growth of Iranian wild-growing macrofungi in solid media, spawn, or locally available substrates, although unpublished efforts might have been made to this end. Following the previous experiences with domestication of Iranian wild-growing Enoki (*Flammulina velutipes*) (Rezaeian & Pourianfar 2017), this study aimed to evaluate adaptation of 40 mushroom isolates indigenous to Mazandaran province (Northern Iran) in solid media, spawn, and locally available lignocellulosic substrate.

Materials & Methods

Mushroom samples

Totally 40 isolates of wild-growing mushrooms were provided as mycelia cultures (in media) as indicated in Table 1. These isolates were selected from a collection of wild mushrooms that have been collected from Mazandaran province (located in Northern Iran that lies along the Caspian Sea) during the year 2015-2017 and have been deposited at Microorganisms Bank of Iranian Biological Resource Center (IBRC). Identification of the collection of wild mushrooms has been performed by experts of IBRC based on morphological and microscopic characteristics. In addition, the strain names assigned to each species utilized in this study were provided by IBRC (indicated in parentheses after the scientific name of each isolate).

Mycelial culture

Specific media for growing mycelia of the samples included Potato Dextrose Agar (PDA) and compost extract agar (CEA). PDA was purchased from Merck (Darmstadt, Germany). CEA was manually prepared as detailed by Masoumi et al. (2015). Three hundred grams of the pasteurized fresh compost of the white button mushroom was boiled in one liter of distilled water for one hour, followed by centrifugation (Hermle Labor Technik GmbH, Germany) at 5000 rpm for five minutes. The upper phase was collected, filtered and used as the compost extract to be mixed with 0.5% agar (Merck, Darmstadt, Germany) v/w to serve as a solid media. In all the experimental steps, deionized water was utilized to prepare solutions and buffers. Both PDA and CEA media were sterilized for 20 minutes at 121℃ and then aseptically poured into gamma-sterilized 8-cm plastic petri dishes. The solid media was inoculated with 1 cm^2 disks of the leading edges of mushroom mycelia. The cultures were then incubated in the dark for two weeks at 25±2℃, after which the daily mycelial growth rate was evaluated (Rezaeian & Pourianfar 2017). Two vertical lines were drawn on each petri dish and four points of mycelia extensions at perpendicular angles were marked. With the aid of a caliper, means of readings of the four points were measured daily. After 14 days, the radial growth rate was calculated based on daily averages and expressed as millimeter per day. Three independent replicates were made for each isolate in each media.

Spawn preparation

Two types of spawn were evaluated, including grain-based and wood-based substrates. Grain-based substrate was prepared using a mixture of boiled wheat grains supplemented with 1 % w/v $CaCo_3$ and 2 % w/v $CaSO_4$, as described by Ghanbari et al. (2015). Wood based-spawn was made of wheat grains enriched with 25% wood chips, being conditioned with 1 % w/v $CaCO_3$ and 2 % w/v $CaSO_4$ (Erkel 2009). Polypropylene bags of 18 × 32.5 cm were then filled with one kilograms of each substrate and packed tightly followed by sterilization at 15 psi, 121°C for 120 minutes. Inoculation was performed using pure actively growing mycelia. The spawn bags were incubated in 25°±2℃ until they were fully colonized by mushroom mycelia (over a period of two months). Three independent replicates were made for each isolate in each type of spawn.

Fruiting test

Wood chips (obtained from *Populus* alba) were utilized as the main substrate in order to test fructification of the wild strains, based on the assumption that the collected mushroom isolates were wood-decay fungi growing in Mazandaran forests. Wood chips were soaked for 18 hours followed by mixing with 15% wheat bran and 3% $CaCO_3$ in 600g cellophane plastic bags. Then, the bags were sterilized at 15 psi and 121 °C for 120 min. Spawn rate and environmental conditions were adapted from the previous experiments with wild-growing medicinal mushroom *Flammulina velutipes* (Rezaeian & Pourianfar 2017). Thirty grams of the fresh spawn (containing actively growing mycelia) aseptically inoculated one kg of fresh raw substrate, followed by sealing and transferring to the cultivation room. For some isolates, due to the lack of growth in spawn, a couple of 3-cm plug of newly grown mycelium in petri dishes were directly used to inoculate the substrate. All the bags were placed in a dark room at 25℃±2℃ until completion of the mushroom vegetative phase. They were then categorized into two different temperature treatments: (i) pre-cooling in a refrigerator at 7-9°C for 8-10 days before transferring to the cropping room; (ii) transferring to the cropping room directly without the pre-cooling treatment. Three independent replicates with two sub-samples were made for each isolate in each temperature treatment. The relative humidity of 85 to 90% and a room temperature of 18-20°C were maintained in the cropping room for fructification.

Statistical analysis

All the experiment were independently repeated three times. JMP version 8 was utilized to conduct statistical analyses and ANOVA tests. Means were compared using Duncan's multiple range test with a significance level (*p* value) of 0.05.

Results

Adaptation of mycelia of wild mushrooms in solid media

Over a period of 14 days, mycelia of all the wild mushroom isolates grew to some extent in PDA (Figs 1, 2) and CEA media (Figs 3, 4) and were expressed as average daily mycelial growth (mm per day). Overall, there were significant differences in growth rates of mycelia between the media and between the isolates as well ($p < 0.01$). Table 1 illustrates the average daily mycelial growth rates (mm per day) obtained for each isolate in each media.

Among the tested isolates, *Irpex lacteus* (GPS 005) displayed the highest daily mycelial growth rate in both media, including 8.37±0.05 and 8.2±0.07 mm per day in PDA (Fig. 1) and CEA (Fig. 3), respectively (Table 1). The findings also revealed that average daily growth rates of mycelia recorded in PDA for *Donkia pulcherrima* (Neka 24D) (Fig. 1), *Daedaleopsis* sp. (Royan 8) (Fig. 2), *Lenzites tricolor* (GPS 180) (Fig. 2), *Trametes* sp. (GPS 122), *Trametes versicolor* (GPS 107) and *Stereum hirsutum* (Darabkola 1) (Fig. 1) were significantly higher as compared with those in CEA medium ($p < 0.01$) (Figs 3, 4). *Donkia pulcherrima* (Neka 24D) showed a daily growth rate of 4.34±0.22 (mm per day) in PDA, while the records in the same media for *Daedaleopsis* sp. (Royan 8), *Lenzites tricolor* (GPS 180), and *Stereum hirsutum* (Darabkola 1) were 3.23±0.07, 3.81±0.12, and 4.82±0.04 (mm per day), respectively (Table 1) ($p < 0.05$).

Hypholoma fasciculare (GPS 176) (Fig. 4), *Cyclocybe* sp. (Darabkola-20) (Fig. 4), *Hypholoma* sp. (GPS 106) (Fig. 4), *Coprinellus disseminates* (Nur 9) (Fig. 3), and *Psathyrella candolleana* (Nur 10) (Fig. 4) had higher daily radial growth rates in CEA as compared with PDA medium. *Coprinellus disseminates* (Nur 9) displayed an average daily mycelial growth rate of 5.66±0.21 (mm per day) in CEA. The average daily mycelial growth rates in the same media for *Cyclocybe* sp. (Darabkola-20), *Hypholoma* sp. (GPS 177), and *Psathyrella candolleana* (Nur 10) were 3.29±0.07, 1.69±0.58, and 3.45±0.02 (mm per day), respectively ($p < 0.01$) (Table 1).

A high daily growth rate (more than 4 mm per day) was also observed in mycelia of *Trametes* sp. (GPS 130), *Trametes* sp. (Neka 25-2), *Trametes* sp. (GPS 179), *Marasmiellus* sp. (GPS 30), *Hydnopolyporus fimbriatus* (GPS 29), *Trametes* sp. (Darabkola 13), *Trametes versicolor* (GPS 007), *Trametes gibbosa* (Nur 8), *Trametes gibbosa* (GPS22), *Irpex lacteus* (GPS 005), *Trametes hirsuta* (GPS 042), *Ganoderma* sp. (GPS 52), *Trametes gibbosa* (GPS 63) and *Trametes* sp.(GPS 119) .However, there was no significant difference between PDA (Figs 1, 2) and CEA (Figs 3, 4) in terms of mycelial growth rates of these isolates ($p \geq 0.05$) (Table 1). In addition, no significant difference ($p \geq 0.05$) was observed between the media in the average daily mycelial growth of *Macrolepiota konradii* (GPS 101), *Hohenbuehelia auriscalpium* (GPS 196), *Armillaria* sp. (GPS 173), *Ganoderma* sp. (GPS 17), and *Ganoderma* sp. (Bozchaft 2), all of which showed a slow daily growth rate less than 2 mm per day in both media, PDA (Figs 1, 2) and CEA (Figs 3, 4) (Table 1).

Table 1 Growth characterization and fructification of wild mushrooms

	Species (strain)	Average daily mycelial growth (mm per day)		Period of spawn development (day)		Fructification
		CEA	PDA	Wheat grains	Wood chips	
1	*Trametes* sp. (Neka 25-2)	3.51±0.04[a]	3.63±0.28[a]	14±1[a]	25±1[b]	No
2	*Trametes* sp. (GPS 179)	4.1±0.11[a]	4.13±0.08[a]	15±1[a]	25±1[b]	No
3	*Hydnopolyporus fimbriatus* (GPS 29)*	5.43±0.05[a]	5.51±0.01[a]	No growth[a]	28±2[b]	No
4	*Trametes hirsuta* (GPS 042)	4.8±0.08[a]	4.64±0.21[a]	No growth[a]	25±1[b]	No
5	*Trametes gibbosa* (GPS 63)	4.76±0.12[a]	4.77±0.15[a]	14±1[a]	26±1[b]	No
6	*Trametes* sp. (GPS 119)	4.91±0.14[a]	5.02±0.13[a]	14±1[a]	25±1[b]	No
7	*Irpex* sp. (GPS 146)*	4.9±0.12[a]	6.67±0.06[b]	18±3[a]	28±2[b]	No
8	*Trametes* sp. (GPS 122)	4.6±0.22[a]	5.13±0.08[a]	15±1[a]	25±3[b]	No
9	*Irpex lacteus* (GPS 005)*	8.2±0.07[a]	8.37±0.05[b]	17±2[a]	27±2[b]	No
10	*Trametes* sp. (GPS 130)	3.72±0.17[a]	4.16±0.06[a]	14±1[a]	27±2	Yes

Table 1 Continued.

	Species (strain)	Average daily mycelial growth (mm per day)		Period of spawn development (day)		Fructification
		CEA	PDA	Wheat grains	Wood chips	
11	*Trametes gibbosa* (GPS 22)	3.22±0.039[a]	4.73±1.19[a]	14±1[a]	25±1[b]	No
12	*Ganoderma* sp. (GPS 37)	3.13±0.04[a]	4.77±0.11[b]	14±1[a]	25±1[b]	No
13	*Ganoderma* sp. (GPS 38)	3.59±0.11[a]	4.25±0.03[b]	No growth[a]	26±2[b]	No
14	*Ganoderma* sp. (GPS 52)	3.91±0.24[a]	4.3±0.48[a]	No growth[a]	26±1[b]	No
15	*Trametes versicolor* (GPS 107)	3.86±0.12[a]	5.07±0.14[b]	14±1[a]	26±1[b]	No
16	*Stereum hirsutum* (Darabkola 1)	2.77±0.12[a]	4.82±0.04[b]	No growth[a]	27±1[b]	No
17	*Donkia pulcherrima* (Neka 24D)	0.66±0.68[a]	4.34±0.22[b]	No growth[a]	No growth[a]	Yes
18	*Coprinellus disseminates* (Nur 9)	5.66±0.209[a]	3.42±0.08[b]	22±3[a]	31±2[b]	No
19	*Marasmiellus* sp. (GPS 30)	3.04±0.021[a]	3.23±0.18[a]	19±2[a]	29±2[b]	No
20	*Trametes* sp. (Darabkola 13)	3.95±0.14[a]	4.08±0.11[a]	14±0[a]	22±1	Yes
21	*Trametes gibbosa* (Nur 8)	3.48±0.023[a]	3.48±0.13[a]	14±2[a]	25±2[b]	No
22	*Trametes versicolor* (GPS 007)	3.29±0.04[a]	3.23±0.05[a]	14±2[a]	28±2[b]	No
23	*Lenzites tricolor* (GPS 180)	2.91±0.09[a]	3.81±0.12[b]	14±2[a]	27±2[b]	Yes
24	*Daedaleopsis* sp. (Royan 8)	2.02±0.13[a]	3.23±0.07[b]	15±3[a]	31±3[b]	Yes
25	*Psathyrella candolleana* (Nur 10)	3.45±0.02[a]	2.23±0.01[b]	18±1[a]	29±2[b]	No
26	*Cyclocybe* sp. (Darabkola 20)	3.29±0.07[a]	2.94±0.09[b]	14±2[a]	25±2[b]	Yes
27	*Ganoderma tsugae* (GPS 186)	2.19±0.54[a]	2.22±0.29[b]	14±0[a]	25±2[b]	Yes
28	*Fomes fomentarius* (GPS 131)	2.79±0.42[a]	3.1±0.38[b]	14±1[a]	25±1[b]	No
29	*Pholiota aurivella* (GPS 142)	2.52±0.39[a]	2.01±0.06[a]	19±1[a]	26±1[b]	Yes
30	*Ganoderma* sp. (Neka 25-1)	2.84±0.03[a]	2.93±0.35[a]	14±2[a]	29±1[b]	No
31	*Lycoperdon pyriforme* (GPS 158)*	1.23±0.11[a]	1.17±0.07[a]	27±2[a]	24±2[a]	No
32	*Leucoagaricus* sp. (Royan 1)	2.08±0.15[a]	1.17 ±0.08[b]	25±2[a]	32±3[b]	No
33	*Ganoderma* sp. (Bozchaft 2)	1.62±0.52[a]	1.69 ±0.19[a]	14±1[a]	30±2[b]	No
34	*Ganoderma* sp. (GPS 17)	1.63±0.61[a]	1.46±0.37[a]	No growth[a]	25±2[b]	No
35	*Hypholoma fasciculare* (GPS 176)	1.23±0.11[a]	0.8 ±0.007[b]	25±2[a]	25±1[b]	No
36	*Hypholoma* sp. (GPS 177)*	1.69±0.58[a]	0.64 ±0.08[b]	17±2[a]	25±2[b]	No
37	*Hypholoma* sp.(GPS 106)	1.81±0.04[a]	0.64 ±0.15[b]	14±1[a]	35±3[b]	No
38	*Hohenbuehelia auriscalpium* (GPS 196)	0.55±0.54[a]	0.26±0.09[b]	No growth[a]	No growth[a]	No
39	*Armillaria* sp. (GPS 173)	0.52±0.10[a]	0.44±0.06[a]	38±2[a]	45±4[b]	No
40	*Macrolepiota konradii* (GPS 101)	1.59±0.02[a]	1.12±0.04[a]	23±3[a]	27±3[a]	No

The wild mushroom species in Table 1 have been identified by Microorganisms Bank of Iranian Biological Resource Center (IBRC), Tehran, Iran. The strain names assigned to these species (given in parentheses) have been provided by the same center. Mycelial growth was measured in Potato Dextrose Agar (PDA) or Compost Extract Agar (CEA). The data of mycelial growth were collected over 14 days and expressed as average daily mycelial growth rate (millimeter per day). The days indicated for spawn development are the period of time required by the wild mushrooms mycelia to fully colonize wheat grains or wood chips. Statistical comparisons have been made between different solid media and between different spawn substrates within each row (for each

mushroom strain). Means followed by the same superscript lower-case letters are not significantly different (Duncan's multiple range test, $p < 0.05$). Asterisks indicate some of the most significant differences among the mushroom strains, determined based on main effects of each strain on mycelial growth averaging across both PDA and CEA media (Duncan's multiple range test, $p < 0.05$).

Fig. 1 – Mycelial growth of wild mushroom isolates in PDA. Panels a-t illustrate PDA cultures of mycelia of *Trametes* sp. (Neka 25-2), *Hydnopolyporus fimbriatus* (GPS 29), *Trametes gibbosa* (GPS 63), *Irpex* sp. (GPS 146), *Irpex lacteus* (GPS 005), *Trametes* sp. (GPS 179), *Trametes hirsuta* (GPS 042), *Trametes* sp. (GPS 119), *Trametes* sp. (GPS 122), *Trametes* sp. (GPS 130), *Trametes gibbosa* (GPS 22), *Ganoderma* sp. (GPS 38), *Trametes versicolor* (GPS 107), *Donkia pulcherrima* (Neka 24D), *Marasmiellus* sp. (GPS 30), *Ganoderma* sp. (GPS 37), *Ganoderma* sp. (GPS 52), *Stereum hirsutum* (Darabkola 1), *Coprinellus disseminates* (Nur 9) and *Trametes* sp. (Darabkola 13) respectively. Photographs were taken with a digital camera on the fourteenth day of mycelial growth in the first subculture. Each isolate was subjected to three independent replications.

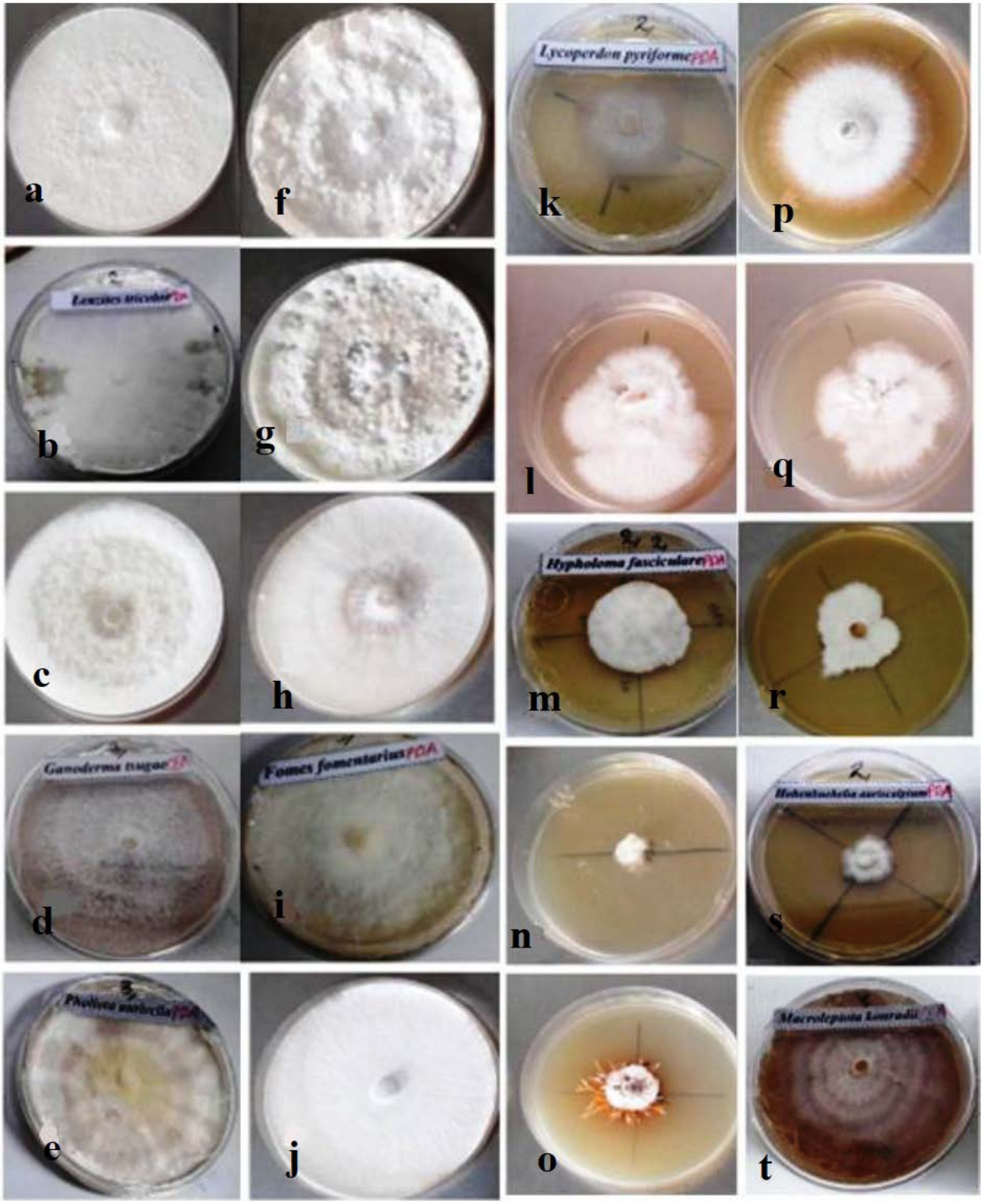

Fig. 2 – Mycelial growth of wild mushroom isolates in PDA. Panels a-t illustrate PDA cultures of mycelia of *Trametes gibbosa* (Nur 8), *Lenzites tricolor* (GPS 180), *Psathyrella candolleana* (Nur 10), *Ganoderma tsugae* (GPS 186), *Pholiota aurivella* (GPS 142), *Trametes versicolor* (GPS 007), *Daedaleopsis* sp. (Royan 8), *Cyclocybe* sp. (Darabkola 20), *Fomes fomentarius* (GPS 131), *Ganoderma* sp. (Neka 25-1), *Lycoperdon pyriforme* (GPS 158), *Ganoderma* sp. (Bozchaft 2), *Hypholoma fasciculare* (GPS 176), *Hypholoma* sp. (GPS 106), *Armillaria* sp. (GPS 173) , *Leucoagaricus* sp. (Royan 1), *Ganoderma* sp. (GPS 17), *Hypholoma* sp. (GPS 177), *Hohenbuehelia auriscalpium* (GPS 196) and *Macrolepiota konradii* (GPS 101) respectively. Photographs were taken with a digital camera on the fourteenth day of mycelial growth in the first subculture. Each isolate was subjected to three independent replications.

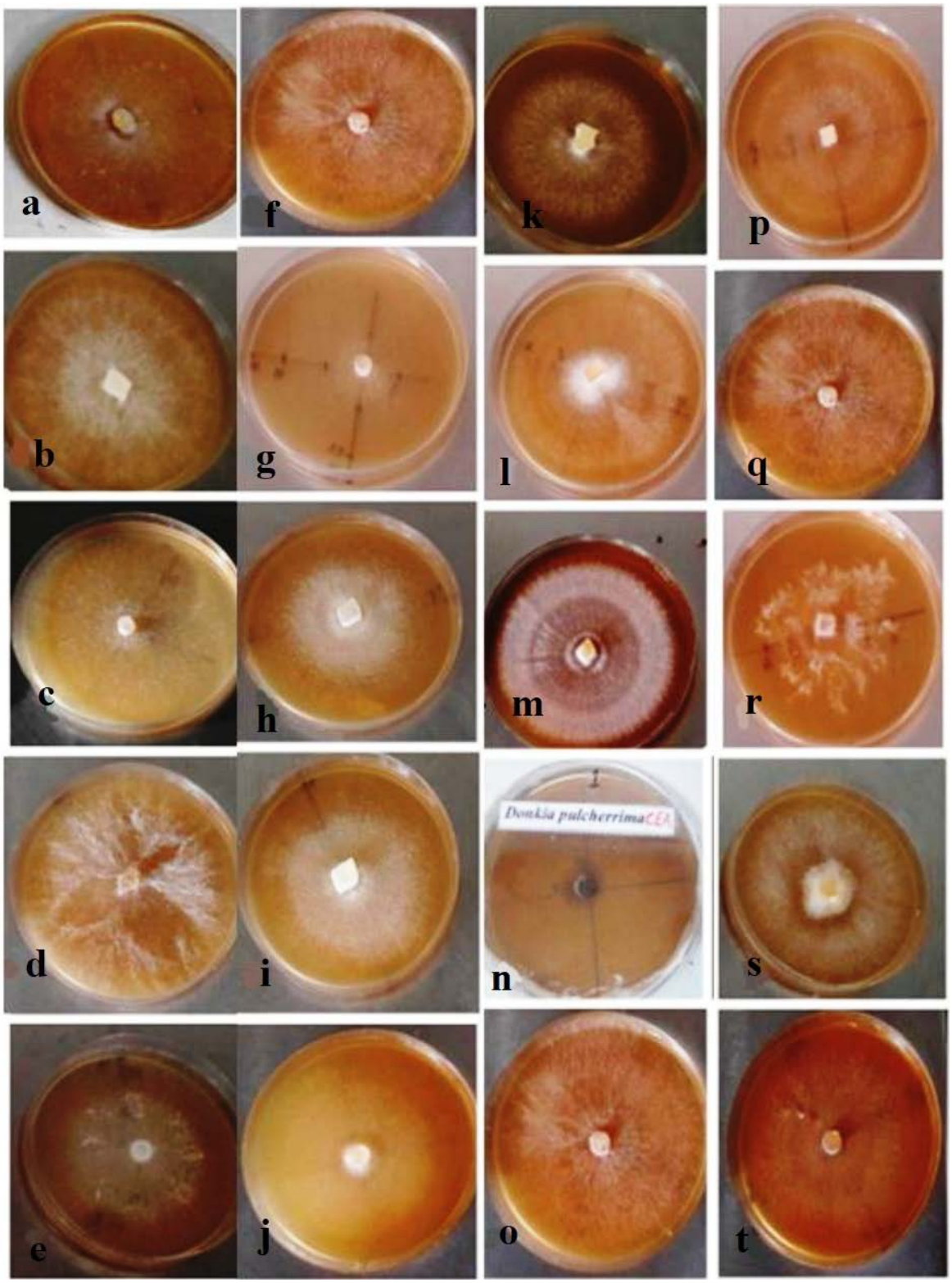

Fig. 3 – Mycelial growth of wild mushroom isolates in CEA. Panels a-t illustrate CEA cultures of mycelia of *Trametes* sp. (Neka 25-2), *Hydnopolyporus fimbriatus* (GPS 29), *Trametes gibbosa* (GPS 63), *Irpex* sp. (GPS 146), *Irpex lacteus* (GPS 005), *Trametes* sp. (GPS 179), *Trametes hirsuta* (GPS 042), *Trametes* sp. (GPS 119), *Trametes* sp. (GPS 122), *Trametes* sp. (GPS 130), *Trametes gibbosa* (GPS 22), *Ganoderma* sp. (GPS 38), *Trametes versicolor* (GPS 107), *Donkia pulcherrima* (Neka 24D), *Marasmiellus* sp. (GPS 30), *Ganoderma* sp. (GPS 37), *Ganoderma* sp. (GPS 52), *Stereum hirsutum* (Darabkola 1), *Coprinellus disseminates* (Nur 9) and *Trametes* sp. (Darabkola 13) respectively. Photographs were taken with a digital camera on the fourteenth day of mycelial growth in the first subculture. Each isolate was subjected to three independent replications.

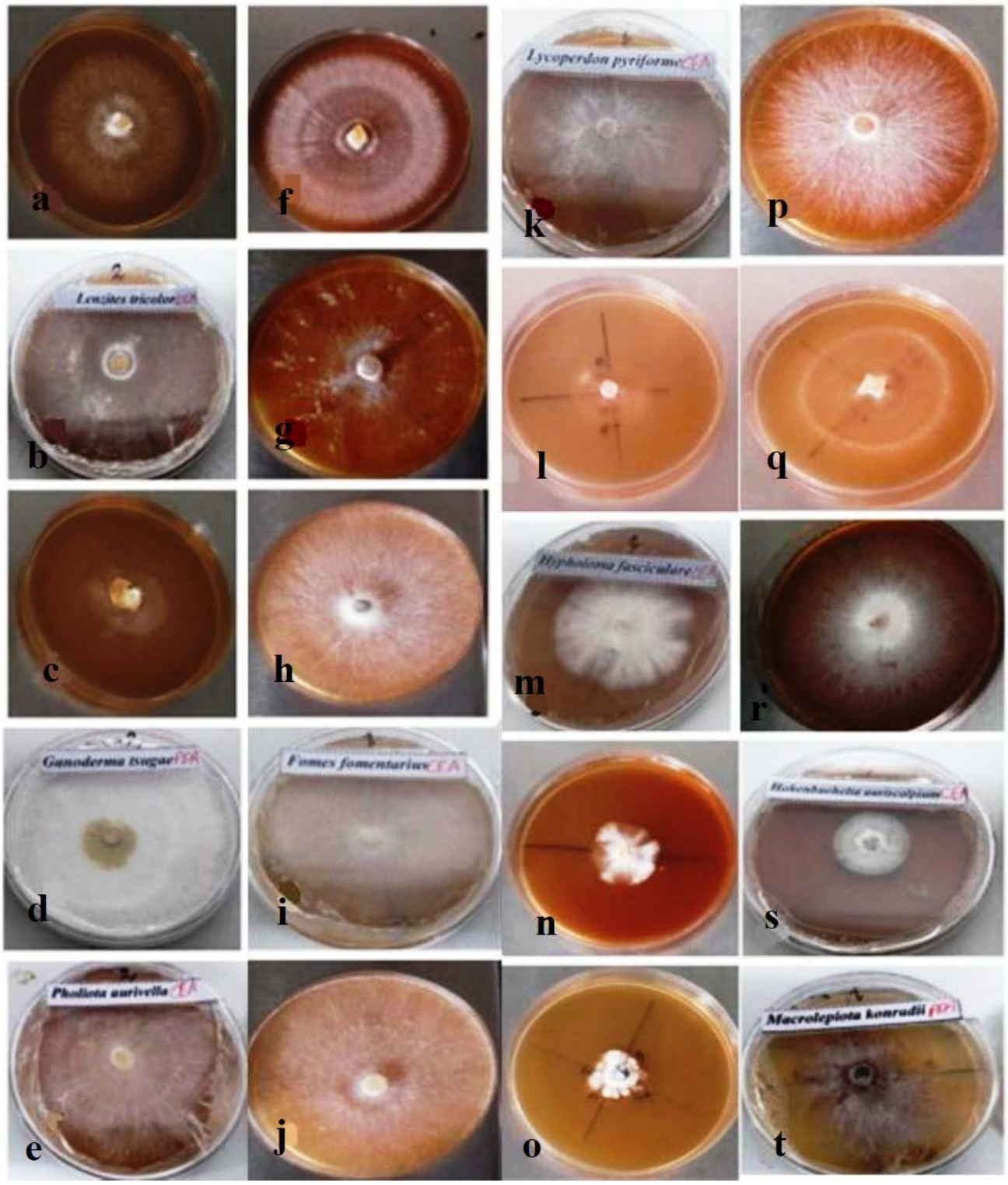

Fig. 4 – Mycelial growth of wild mushroom isolates in CEA. Panels a-t illustrate CEA cultures of mycelia of *Trametes gibbosa* (Nur8), *Lenzites tricolor* (GPS 180), *Psathyrella candolleana* (Nur 10), *Ganoderma tsugae* (GPS 186), *Pholiota aurivella* (GPS 142), *Trametes versicolor* (GPS 007), *Daedaleopsis* sp. (Royan 8), *Cyclocybe* sp. (Darabkola 20), *Fomes fomentarius* (GPS 131), *Ganoderma* sp. (Neka 25-1), *Lycoperdon pyriforme* (GPS 158), *Ganoderma* sp. (Bozchaft 2), *Hypholoma fasciculare* (GPS 176), *Hypholoma* sp. (GPS 106), *Armillaria* sp. (GPS 173) , *Leucoagaricus* sp. (Royan 1), *Ganoderma* sp. (GPS 17), *Hypholoma* sp. (GPS 177), *Hohenbuehelia auriscalpium* (GPS 196) and *Macrolepiota konradii* (GPS 101) respectively. Photographs were taken with a digital camera on the fourteenth day of mycelial growth in the first subculture. Each isolate was subjected to three independent replications.

Colonization of spawn by wild mushrooms

Overall, most of the wild mushrooms filled wood spawn for 24-45 days after inoculation, which was significantly longer than the period observed in grain spawn ($p < 0.01$) (Table 1). Mycelia of *Lenzites tricolor* (GPS 180) (Fig. 5a), *Trametes* sp. (GPS 119), *Daedaleopsis* sp. (Royan 8) (Fig. 5b), *Trametes versicolor* (GPS 107), *Trametes* sp. (GPS 122), *Trametes* sp. (GPS 130) (Fig. 5e), *Ganoderma tsugae* (GPS 186) (Fig. 5g), *Trametes* sp. (Darabkola 13), *Trametes gibbosa* (Nur 8), *Ganoderma* sp. (Neka 25-1), *Ganoderma* sp. (GPS 37), *Ganoderma* sp. (Bozchaft 2), *Hypholoma* sp. (GPS 106), *Trametes gibbosa* (GPS 63), *Trametes gibbosa* (GPS 22), *Cyclocybe* sp. (Darabkola 20) (Fig. 5f), *Trametes* sp. (GPS 179), *Trametes versicolor* (GPS 007), *Trametes* sp. (Neka 25-2), and *Fomes fomentarius* (GPS 131) completely colonized wheat grains for 14-15 days after inoculation. The spawn-growing periods for *Pholiota aurivella* (GPS 142) (Fig. 5c), *Macrolepoita konradii* (GPS 101), *Hypholma fasciculare* (GPS 176) and *Lycopedron pyriforme* (GPS 158) were 19, 23, 25, and 27 days, respectively ($p < 0.05$). No growth of mycelia in wheat grains or wood chips ($p > 0.05$) was observed for *Donkia pulcherrima* (Neka 24D) (Fig. 5d) and *Hohenbuehelia auriscapium* (GPS 196) (Fig. 5h) over one month of incubation (Table 1). Thus, mycelia plugs of these mushrooms grown in the solid media were utilized to inoculate substrate directly.

Observations also showed that mycelium of *Hydnopolyporus fimbriatus* (GPS 29), *Ganoderma* sp. (GPS 17), *Ganoderma* sp. (GPS 38), *Ganoderma* sp. (GPS 52), *Stereum hirsutum* (Darabkola 1), and *Trametes hirsuta* (GPS 042) did not grow in grain spawn, while they colonized wood chips 25-28 days after inoculation ($p < 0.01$) (Table 1).

Adaptation of wild strains in lignocellulosic agro-wastes

The vegetative phase of all the tested wild mushrooms in the substrate (mycelial running) was completed 17-22 days after inoculation, indicating insignificant variations among the mushroom isolates ($p \geq 0.05$). However, fructification was observed only among fully colonized substrates pre-conditioned in the refrigerator, while the substrates that did not pass this period produced no fruiting bodies. Of the substrates pre-conditioned in the refrigerator, eight isolates belonging to seven different genera successfully fructified, including *Pholiota aurivella* (GPS 142), *Lenzites tricolor* (GPS 180), *Ganoderma tsugae* (GPS 186), *Cyclocybe* sp. (Darabkola 20), *Trametes* sp. (GPS 130), *Trametes* sp. (Darabkola 13), *Daedaleopsis* sp. (Royan 8), and *Donkia pulcherrima* (Neka 24D) (Figs 6, 7).

It took about three months (after transferring the fully colonized substrates to the cropping room) for *Ganoderma tsugae* (GPS 186) to develop first pinheads, lasted for more than 35 days. During the fruiting bodies development, the cap color changed from white to yellow and brown (Fig. 6a, b). Besides, the first pinheads of *Daedaleopsis* sp. (Royan 8) emerged 42 days after transferring the fully colonized substrates to the cropping room, lasted for up to 35 days during which the mature cap showed a brown cap (Fig. 6c, d). Observations also showed that the first primordia of *Donkia pulcherrima* (Neka 24D) appeared 90 days after transferring bags to the cropping room, followed by maturation of hairy caps during one week that lasted for three days (Fig. 6e, f).

Daily observations also showed that *Trametes* sp. (strains of GPS 130 and Darabkola 13) and *Cyclocybe* sp. (Darabkola 20) developed pinheads 28 days after transferring to the cropping room. It took 25-30 days (after appearance of pinheads) for fruiting bodies of *Trametes* sp. isolates (strains of GPS 130 and Darabkola 13) to mature. Reproductive structure of both isolates of *Trametes* sp. lasted 25 days during which fruiting bodies showed color changes from a bright cream to orange brown (like turkey tail) (Fig. 7a, b). *Cyclocybe* sp. (Darabkola 20) fruiting bodies showed caps similar to those of the button mushroom (*Agaricus bisporus*), appeared one week after the development of pinheads and lasted about 3 days (Fig. 7c, d).

Pholiota aurivella (GPS 142) and *Lenzites tricolor* (GPS 180) produced early reproductive structures 50 days after transferring the fully colonized substrates to the cropping room. The reproductive structures of *Pholiota aurivella* (GPS 142) emerged in the form of orange-colored

caps as clusters on the substrate (Fig. 7 e, f), while those of *Lenzites tricolor* (GPS 180), appeared as separate multiple fruiting bodies on the substrate (Fig. 7g, h).

Fig. 5 – Differences in the growth of spawn between selected wild mushrooms in wheat grains. Panels a-h illustrate grain spawn of *Lenzites tricolor* (GPS 180), *Daedaleopsis* sp. (Royan 8), *Pholiota aurivella* (GPS 142), *Donkia pulcherrima* (Neka 24D), *Trametes* sp. (GPS 130), *Cyclocybe* sp. (Darabkola 20), *Ganoderma tsugae* (GPS 186), and *Hohenbuehelia auriscapium* (GPS 196), respectively. *Hohenbuehelia auriscalpium* (GPS 196) and *Donkia pulcherrima* (Neka 24D) did not colonize grains over one month of incubation, while wild isolates such as *Trametes* sp. (GPS 130) and *Ganoderma tsugae* (GPS 186) filled spawn less than 15 days. Photographs were taken with a digital camera on the 30th day of mycelial growth in the spawn. Each isolate was subjected to three independent replications. One of the replicates is shown in this figure.

Discussion

In this study, 40 Iranian wild-growing mushroom isolates (belonging to 21 different genera) were successfully adapted under solid media condition, including PDA and CEA media. However, few isolates failed to colonize grain-based or wood-based spawn. Forty isolates of mushrooms, eight wild mushroom isolates belonging to seven genera successfully produced fruiting bodies in wood-based lignocellulosic substrate in the small-scale cultivation tests. In general, domestication of several species of wild mushrooms have been reported globally, including *Pleurotus giganteus* (Klomklung et al. 2012), a range of wild tropical mushrooms (Thawthong et al. 2014), *Panaeolus*

spp. (Bustillos et al. 2014), *Agaricus flocculosipes* and *Agaricus subrufescens* (Thongklang et al. 2014), *Schizopyllum commune* (Herawati et al. 2016), *Flammulina velutipes* (Rezaeian & Pourianfar 2017), and *Agaricus bisporus* (Salmones et al. 2018).

Fig. 6 – Successful fructification of wild mushroom isolates in wood chips-based substrate supplemented with wheat bran. Different developmental stages of *Ganoderma tsugae* (GPS 186), *Daedaleopsis* sp. (Royan 8), and *Donkia pulcherrima* (Neka 24D) are illustrated in panels a–b, c–d and e–f, respectively. Mature fruiting bodies of *Ganoderma tsugae*, *Daedaleopsis* sp., and *Donkia pulcherrima* appeared 90, 42, and 90 days, respectively, after transferring the fully colonized substrates to the cropping room. Each isolate was subjected to three independent replications. One of the replicates is shown in this figure.

Fig. 7 – Successful fructification of wild mushroom isolates in wood chips-based substrate supplemented with wheat bran. Different developmental stages of *Trametes* sp. (strains of GPS 130 and Darabkola 13), *Cyclocybe* sp. (Darabkola 20), *Pholiota aurivella* (GPS 142), and *Lenzites tricolor* (GPS 180) are illustrated in panels a–b, c–d, e–f and g–h, respectively. Mature fruiting bodies of *Trametes* sp., *Cyclocybe* sp., *Pholiota aurivella*, and *Lenzites tricolor* appeared 58, 35, 50, and 50 days, respectively, after transferring the fully colonized substrates to the cropping room. Photographs were taken with a digital camera during fructification. Each isolate was subjected to three independent replications. One of the replicates is shown in this figure.

Cultivation of the Iranian wild mushrooms presented in this study is scarcely reported by researchers from the country. On the contrary, a number of studies from other countries have investigated cultivability of many of these wild species. Although there are taxonomic challenges with the genus *Trametes* and its related genera such as *Lenzites* and *Daedaleopsis* (Ueitele et al. 2018), the scientific knowledge of cultivation of many of the wild mushrooms presented in this study in East and Southeast Asia dates back to several decades ago (Thawthong et al. 2014, Luangharn et al. 2017). Similar to our findings, sawdust-based substrates enriched with wheat bran (or rice bran) have been frequently utilized to domesticate these wild mushrooms. In a relatively recent study, pinhead emergence of – Ganoderma *neojaponicumin* (from Malaysia) was found to be about two months (Tan et al. 2015), which was shorter than that of *Ganoderma tsugae* (3 months) domesticated in the current study. Similar results were also reported with *Ganoderma austral* (from Thailand) where development of pin-heads and maturation of fruiting bodies were completed in a short time (Luangharn et al. 2017). Several strains of wild *G. lucidum* was also collected and domesticated in Philippines with a period of about 17 days for fructification (from primordial formation to fruiting body maturation), even though no data was provided regarding the time required for pinhead emergence in the first harvest (Magday et al. 2014).

In the current study, *Pholiota aurivella* was also successfully cultivated in artificial substrates. Although no similar report is available regarding cultivability of *Pholiota aurivella*, domestication of another species, *Pholiota adiposa,* has been attempted in China (Rong et al. 2016). Similar to our findings, it took around 50 days for initiation of most of strains of *Pholiota adiposa*. However, one of the wild strains displayed better morphological traits and higher chemical contents as compared with the control strain. Cultivation of *Pholiota adiposa* and *Pholiota microspora* has been also reported from Japan (Huang & Ohga 2017).

Fructification of *Trametes versicolor* collected from India was investigated using sawdust enriched with rice bran (Veena & Pandey 2012). Primordial initiation was observed within 14–20 days of opening that was shorter than the period observed in our study. It took 30–45 days for the full development of the fruiting body that was very similar to the findings of the present study.

Cultivation of *Agrocybe cylindracea* in artificial substrate has been reported (Thawthong et al. 2014). In a recent study in Japan, this mushroom was successfully produced using 70% ceramic beads and 30% of the nutrient solution sawdust (Huang & Ohga 2017). However, it appears that no study has been undertaken to evaluate domestication of *Donkia* sp. and thus this study is the first to report successful cultivation of this wild mushroom.

The wild mushrooms adapted for cultivation in the present study have been also reported by other researchers in terms of their medicinal properties. Particularly, studies performed on the genera of *Lenzites, Ganoderma* and *Trametes* have revealed that these mushrooms had significant antimicrobial and anticancer properties (Vamanu & Voica 2017, Yamac & Bilgili 2006). Polysaccharides and sterols present in *Ganoderma applanatum* have shown beneficial effects for the treatment of diseases such as bronchitis, hepatitis, high blood pressure, types of tumors, and immune disorders (Kosanic et al. 2013). It was also found that the compounds extracted from the fruiting bodies of *Ganoderma tsugae* had high levels of phenolic acid, beta-carotene, lycopene, and vitamin C, all of which have high potential for scavenging free radicals (Mshandete 2014).

Species belonging to the genus *Trametes* (Turkey tail mushroom) have been shown to possess significant medicinal and therapeutic properties (Kamiyama et al. 2013). Orally administered preparations from the *Trametes versicolor* showed that up to 9 grams/day of a *T. versicolor* preparation is safe and tolerable in women with breast cancer in the postprimary treatment setting. This preparation may improve immune status in immunocompromised breast cancer patients following standard primary oncologic treatment (Torkelson et al. 2012). However, no final approval has been yet announced by the U.S. Food and Drug Administration (FDA). Phenolic and non-phenolic substances presented in this mushroom have also strong antioxidant properties (Mohd Nor Rasid 2010). Similarly, it has been reported that the compounds extracted from *T. versicolor* had a protective effect against the oxidation of red blood cells (Arteiro et al. 2012, Sun et al. 2014). Protein-polysaccharide complexes of this mushroom have anticancer properties and may boost the

body immune system (Yang & Zhang 2009, Jhan et al. 2016). Furthermore, *T. hirsuta* has been suited for its industrial uses (Vazirian et al. 2014).

The genus *Lenzites* is known as a rich source of antioxidant and antimicrobial compounds (Oyetayo et al. 2009). According to Abubakar et al. (2016), it was determined that the biological compounds presented in *Lenzites quercina* had antimicrobial effects.

Mushrooms of the genus *Daedaleopsis* belonging to Polyloraceae family are usually non-edible due to their hardness and woodiness (Shiono et al. 2005, Yu et al. 2008) *Daedaleopsis confragosa* was found to contain a large amount of phenolic compounds with antioxidant activity (Vidovic et al. 2011). In addition, *Daedaleopsis tricolor* has shown strong antifungal, antibacterial, and antioxidant properties (Zhao et al. 2013).

Pholiota adiposa has been reported to be rich in polysaccharides that have anti-tumor, antioxidant and antimicrobial properties (Dulger 2004, Deng et al. 2011). This mushroom is also rich in protein, essential amino acids, fiber, elements, vitamins, and carbohydrates. It was also determined that *Pholiota aurivella* contains lectin (Kawagishi et al. 1991). Lectins are well-known biologically active compounds that exhibit antimicrobial and anticancer activities (Abubakar et al. 2016).

Methanolic extracts from the fruiting bodies of *Agrocybe* (also known as *Cyclocybe*) *aegerita* contain significant amounts of various substances with high antioxidant properties (Petrovic et al. 2015). In a study performed by Chien et al. (2015), it was determined that *Agrocybe cylindracea* could significantly inhibit the growth of cancer cells.

Conclusion

The present study demonstrated the possibility of adaptation of several Iranian wild-growing medicinally important mushrooms to artificial locally available substrate. At the present, white button mushroom is still the most common cultivated mushroom in Iran (accounting for more than 90% of total mushroom production) followed by cultivated oyster mushrooms (*Pleurotus* spp.). The findings of this study with non-edible specialty mushrooms such as *Ganoderma* sp. or *Trametes* sp. might not be directly implicated in the mushroom industry. However, they may promote further pilot-scale and commercial-scale production of these medicinally important mushrooms. In addition, domestication of *Donkia* sp. was for the first time reported in this study, which may facilitate further investigations on its biological activities, including immune modulation and regulation, anticancer, antimicrobial, antioxidant, and probiotic properties. Further in-depth investigation would be warranted to evaluate different combinations of solid media, submerged media, and lignocellulosic agro-wastes in order to improve mycelial growth, biological efficiency, and other agronomic characteristics of the wild medicinal mushrooms.

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