***Research article***

**Morphological and ecological screening of different collections of medicinal white-rot bracket fungus *Ganoderma adspersum* (Agaricomycetes, Polyporales) [Times New Roman 18-pt, bold, justified, single-spaced, < 200 char.]**

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**Abstract [Time New Roman 10-pt, bold]**

The screening of morphological and growth characteristics of mycelial collections of medicinal bracket fungus *Ganoderma adspersum* (Agaricomycetes, Polyporales) of different geographical origins (Armenia, Georgia, Iran) on malt-extract agar (MEA) and potato-dextrose agar (PDA) media for 6 days, as well as in submerged culture (malt-extract, ME, 200 rt min-1) for 14 days at different temperature (25, 30, 35, 38 °C) was performed…. [Time New Roman 10-pt, justified, single spaced, < 200 words]

**Key words [Time New Roman 10-pt, bold]:** *Ganoderma adspersum*; mycelium; morphological characteristics; growth rate; chlamydospores; cuticular cells; pellets [Time New Roman 10-pt, justified, single spaced, 5 to 7 key words]

Introduction [Time New Roman 12-pt, bold]

Currently, an interest towards the study of genetic resources of medicinal polyporoid fungi (class Agaricomycetes, order Polyporales), as valuable biological resources with high exploratory potential, including the production of nutraceuticals, nutriceuticals, pharmaceuticals, and cosmeceuticals is increasing (Badalyan and Gharibyan, 2015, 2016; Badalyan et al., 2015; Saltarelli et al., 2015; Taofiq et al., 2016, 2017; Gargano et al., 2017; Badalyan and Zambonelli, 2019). [Time New Roman 12-pt, giustified, single-spaced]

The white-rot cosmopolitan polypores of the genus *Ganoderma* Karst. (family Ganodermataceae) include more than 250 species (Ryvarden, 1991). Among these fungi, *G. applanatum* (Pers.) Pat*.*, *G. lucidum* (Curtis) P. Karst*., G. resinaceum* Boud., and *G. tsugae* Murrill [=*Polyporus tsugae* (Murrill) Overh.] are medicinally important, particularly in the Asian continent and widely used in traditional medicine for the therapy of chronic diseases for over 2000 years (Paterson, 2006)….

The laccate *Ganoderma* species *G. lucidum* and *G. tsugae* grows on maltextract agar (MEA) with the average growth rate of 7.8 mm d-1 at optimum temperature range 30-34 °C and 2.1 mm d-1 at 20-25 °C. *Ganoderma lucidum* formed chlamydospores, while *G. tsugae* did not (Adaskaveg and Gilbertson, 1986)….

Materials and Methods [Time New Roman 12-pt, bold]

*Ganoderma* samples were collected in Armenia during 2002–2015 on *Acacia* sp., *Fraxinus* sp., *M. alba* and *Quercus* sp. trees, in Iran on deciduous tree in 2008 and in Georgia on *Laurus camphora* L. tree in 2013 (Table 1, Fig. 1). Traditional taxonomic keys were used for morphological identification of basidiomata (Phillips, 1981; Ryvarden, 1991). Dikaryotic cultures were isolated from collected basidiomata by tissue method (Bukhalo, 1988) and maintained on MEA or in distilled water at 5 °C in tubes. Mycelial collections were genetically identified and verified using nuclear ITS–rDNA sequence data by previously reported methods (Badalyan et al., 2012b, 2015) (Table 1). [Time New Roman 12-pt, justified, single-spaced]

**Table 1 -** The studied collections of *G. adspersum* [Time New Roman 10-pt, alligned to the table edges, single-spaced]

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Catalogue number** | **Strain** | **Substrate** | **Origin and date of culture isolation** | **Gene bank accession number\*** |
| 5501 | Ga/1\*\* | *Fraxinus* sp. | Armenia, Yerevan, 2002 | JN588580 |
| 5502 | Ga/2-1 | *Morus alba* | Armenia, Yerevan, 2005 | JN588583 |
| 5503 | Ga/2-2 | *M. alba* | Armenia, Yerevan, 2005 | JN588581 |
| 5504 | Ga/2-3 | *M. alba* | Armenia, Yerevan, 2005  | JN588582 |
| 5506 | Ga/3 | *Acacia* sp. | Armenia, Yerevan, 2010 | JN588585 |
| 5507 | Ga/9 | *Fraxinus* sp. | Armenia, Yerevan, 2011 | not available |
| 5509 | Gad/6 | *Quercus* sp. | Armenia, Tavush province, village Berdavan, 2013 | KP941436 |
| 5517 | Gad/03 | *Laurus camphora* | Georgia, Batumi, 2013 | KP941440 |
| 5518 | Gad/VII | *L. camphora* | Georgia, Batumi, 2013 | KP941441 |
| 5520 | 1016 | Deciduous tree | Iran, Zagemarz-Behshar, 2008 | KP941442 |

\*published by Badalyan et al. (2012b, 2015) [Time New Roman 10-pt, alligned to the table edges, single-spaced]

\*\*The catalogue numbers and accession numbers of strains correspond to strain names with slash published in the catalogue (Badalyan and Gharibyan, 2017a)

The morphology and growth rate of mycelia were studied after inoculation of 5 mm3 inocula (three replicates per strains) into the center of 90-mm Petri dishes at different temperatures (25, 30, 35, and 38 °C) using 1.5% MEA and PDA media (pH 6.0) for 6 days. They were also studied in submerged culture (pH 6.0, 200 rt min-1, 25 °C) using Erlenmeyer flasks (250 ml, 5 inocula each) for 14 days of incubation under dark conditions. Micro- and macro-morphological observation of mycelial colonies and pellets, anamorphs, as well as determination of average growth rates (GRavr) were realized by previously reported methods (Badalyan et al., 2012b, 2015). The preparations were examined under the microscope Omano OM157-T Trinocular (USA) with software program (OC View 7, ver. 7.1) using 15×40 ocular/objective, as well as Axioplan-2 imaging microscope (Zeiss, Germany) using 10×40 ocular/objective….

**Results and discussion** [Time New Roman 12-pt, bold]

*Macro-morphological characteristics* [Time New Roman 12-pt, italic]

The colonies of *G. adspersum* were initially white, cottony-felt, later chamois, leathery, creamy-lemon-yellowish, with suppressed and smooth margin (Fig. 2). The agar is bleached but locally yellowish, in some places light brown. In submerged culture, *G. adspersum* forms small, dense, smooth and leathery pellets (Fig. 3). The development of fruiting bodies with mature basidiospores was only observed in Armenian strains Ga-9 and Ga-2-2. [Time New Roman 12-pt, justified, single-spaced]



**Fig. 1** - Fruiting body of *G. adspersum* collected in 2002 in Yerevan (Armenia) on *Fraxinus* sp. (a); basidiospores (b) [Time New Roman 10-pt, aligned to the figure edges, single spaced]

*Mycelial growth rates*

Depending on media composition and temperature, the variability of mycelial GRavr indicators in tested collections was detected. However, no significant difference was found between tested media for cultivation of *G. adspersum* strains although some strains grow better on PDA (Table 2).

At 25 °C, the cultures grew relatively slower on MEA (GRavr = 3.4-5.1 mm d-1) than PDA (GRavr = 3.5-6.0 mm d-1) (Table 2; Fig. 2a,b,d,e). The GRavr indicators were higher at 30 °C on MEA (4.0-6.8 mm d-1), particularly in Georgian strains Gad-03 (6.3 mm d-1) and Gad-VII (6.8 mm d-1), than PDA (3.0-5.5 mm d-1)….

**Conclusion** [Time New Roman 12-pt, bold]

The revealed taxonomically valuable mycelial characteristics of *G. adspersum* (colony texture, pigmentation, growth rate, presence and form of hyphal clamps, coralloid hyphae, chlamydospore-like swellings and/or cuticular cells, optimal growth temperature) will assist in the proper taxonomic identification of cultures, control their quality and optimal growth conditions during their biotechnological cultivation. [Time New Roman 12-pt, justified, single-spaced]

**Acknowledgements** [Time New Roman 10-pt, bold]

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