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RESEARCH ARTICLE

Survey on Wood Decay Fungi *Ganoderma* Species (Ganodermataceae; Polyporales) from Guilan and Mazandaran, Iran

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ABSTRACT

Ganoderma is a genus of fungi belonging to the Polyporales; involving different species that most of them are well known for their medicinal properties. More than 250 *Ganoderma* species are described so far from all over the world. The aim of the study was to identify and classify *Ganoderma* species collected from Iran based on morphological characters. In this research, forests of north Iran were surveyed for *Ganoderma* species. Samples were collected from different sites and hosts. Identification of the species was carried out based on macro/micro morphology of fruiting bodies. Numerical taxonomic studies were performed using morphological characters. Growth Rate (GR) and Growth Coefficient (GC) were also estimated for all purified samples. Isolate GIran 102 identified as *G. resinaceum,* showed the best Growth Rate among 19 collected samples with the GR=9.4 mm/day and GC=14 following by two other samples GIran98 and GIran100 identified as Eurasian *G. lucidum* and *G. resinaceum*. Result revealed that there are some hosts which are new for Iran.

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INTRODUCTION

It is more than thousand years that mushrooms have been used as a source of food and medicine in different civilizations. Mushrooms are considering as a valuable source of functional food and as materials for the development of medicines, pharmaceutical products, dietary supplements and healthy beverages as well as cosmetic products. Ganodermataceae is a unique family among polyporales which possesses double wall basidiospores. The family is also cosmopolitan basidiomycetes that are not just important for the species with medicinal properties but for causing root rot of many hardwoods (Widyastuti 2006). *Ganoderma* P. Karst. is one of the most studied genus among all mushrooms due to the species potential in having different source of bioactive compounds that can be used in different aspects of pharmaceutical industry (El-Mekkawy *et al.,* 1998, Min *et al.,* 1998, Wu *et al.,* 2006, Keypour *et al.,* 2010). The genus *Ganoderma* involves laccate (*Ganoderma lucidum* complex) and non-laccate (*G. applanatum* complex) species (Gottilieb & Wright 1999a, b). Both laccate and non-laccate species were recorded from Iran by different authors (Moradali *et al.,* 2007, Ershad 2009, Keypour *et al.,* in press). More than thirty hosts like *Carpinus betulus* L., *Armenica vulgaris* Lam. and *Ficus benghalensis* L. have been reported for *Ganoderma* in Iran by Ershad (2009), Keypour and her colleagues (2013). This study was conducted according to the potential use of *Ganoderma* species in medicine and their importance in plant pathology researches. The objective of this research was to study and identify *Ganoderma* species of north Iran based on morphological and numerical taxonomy, as well as estimation of the Growth Rate efficiency of the collected species.

MATERIALS AND METHODS

Fungal material and morphological identifications

Fruiting bodies were collected from different hosts and sites of two Northern Provinces of Iran, Guilan and west Mazandaran, in autumn 2012. Most samples were collected from angiosperms and in rare cases from conifers. Both micro- and macro-morphological characters for identification of *Ganoderma* species such as: size, shape and color of fruiting body as well as length

and width of 40 spores and hymenodermis cutis elements, were used according to different identification keys (Strayert 1972, Moradali *et al.,* 2007). All micro-characters were determined by light microscopy. Spores were collected from a block of tube layer and observation of spores were made using Olympus microscope 100X objective with immersion oil. Thin-hand sections were taken from the cutis of each sample for achieving more details. Cutis sections were observed with 40X objective. The measurements were determined by ocular micrometer for spores and club shape elements of cutis. Basidiospores and cutis elements were stained using Melzer's reagent. Amyloid or non-amyloid reactions were also viewed by the former reagent.

Fungal mycelium purification and morphological screening of cultures

The collected fungi were isolated on Malt extract agar medium (MEA, Merck, Darmstadt, Germany) and then purified by hyphal tip culture method. The quality of mycelia such as: shape, color and colonial density were recorded. Growth Rate and Growth Coefficient measured using former media, in darkness at 25°C. Growth Rate ($GR=\Delta d/\Delta t$) and Growth Coefficient ($GC=dgh/t$) were estimated for each isolate after the plates were covered with mycelia (Nobles 1965, Stalper 1978). In GR formula, Δd is the average rate of mycelial growth and Δt is the average of growth time. Letter d in the Growth Coefficient formula represents the rate of mycelial growth, g the density of colony and h the height of mycelial growing edge.

Collected samples and strains

All collected samples were deposited in Shahid Beheshti University herbarium. Some purified mycelia were deposited in the Fungi culture collection of the Ministry of Agriculture of Iran (IRAN), (Table 1).

Numerical taxonomy

Twenty five quantitative and qualitative morphological characters were used for numerical analysis such as: Presence of stem, Host, Carpophore shine, Context color, Carpophore length, Spore length, Spore width, Shape of dermal elements and Color of dermal elements. They were scored as multistate. To identify the relationship among all isolates of *Ganoderma* the MVSP (Multi Variate Statistical Package) 3.1 program (Kovach Computing Services, Wales, UK) was utilized. *Trametes versicolor* was chosen as an out group.

RESULTS

The results of morphological identification revealed that the collected samples were composed of three species including; *Ganoderma lucidum* (Curtis) P. Karst., *Ganoderma resinaceum* Boud. and *Ganoderma australe* (Fr.) Pat. Descriptions of the species are available in "Mycelial Growth Rate, Macro-and Micromorphological Characteristics of Species of Genus *Ganoderma* (Higher Basidiomycetes) From Iran" (Keypour *et al.* in press). Among 19 collected samples, six new hosts are identified for *Ganoderma* of Iran (Table 2).

Morphological Screening of mycelia showed that most cultures of *G. lucidum* possess small ellipsoid chlamydospores which gave a dextrinoid reaction in Melzer's reagent but in a sample identified as *G. lucidum,* chlamydospore was not seen. Examining of *G. resinaceum* mycelia lead to observation of innumerable, yellow to brown ovoid shape chlamydospores. In *G. australe* mycelia, no chlamydospore was observed. Most samples showed trimitic system but two samples verified as *G. resinaceum* showed dimitic system. Three shiny samples identified as *G. resinaceum* and Eurasian *G. lucidum* showed the best Growth Rate comparing to others. The isolate morphologically identified as *G. resinaceum* showed the best Growth Rate (GR=9.4 mm/day) but low Growth Coefficient (GC=14 mm/day). Two isolates identified as Eurasian *G. lucidum* and *G. resinaceum* have placed in next places by GR and GC of 9.3, 13.9 mm/day and 9, 27 mm/day, respectively (Table 3). The color of mycelium mat was mostly white and in a rare cases white-yellow (Table 4). None of the isolates produced fruiting body in the artificial culture media.

Table 1: Herbarium codes for collected samples, samples number and IRAN codes are presented in the table. M: Mazandaran province, G: Guilan province.(*Collectors: Keypour & Borhani*)

Herbarium code	Samples no.	IRAN code	Place of collection	Date of collection	Host
HSBU-200884	GIran84	-	Dohezar forest, M	30-10-2012	*Carpinus Betulus*
HSBU-200885	GIran85	-	Dohezar forest, M	30-10-2012	*Dead Carpinus Betulus*
HSBU-200886	GIran86	-	Dohezar forest, M	30-10-2012	*Dead Carpinus Betulus*
HSBU-200887	GIran87	-	Dohezar forest, M	30-10-2012	*Gleditschia caspica*
HSBU-200888	GIran88	-	Separdan village, G	31-10-2012	*Dead Albizzia julibrissin*
HSBU-200889	GIran89	-	Siahkal, G	31-10-2012	*Gleditschia caspica*
HSBU-200890	GIran90	-	Siahkal, G	31-10-2012	*Dead Pinus taede*
HSBU-200891	GIran91	-	Siahkal, G	31-10-2012	*Dead Pinus taede*
HSBU-200892	GIran92	IRAN 2218 C	Siahkal, G	31-10-2012	*Dead Pinus taede*
HSBU-200893	GIran93	-	Siahkal, G	31-10-2012	*Dead Pinus taede*
HSBU-200894	GIran94	-	Siahkal, G	31-10-2012	*Dead Pinus taede*
HSBU-200895	GIran95	IRAN 2222 C	Lounak water fall, G	31-10-2012	*Dead Fagus sp.*
HSBU-200896	GIran96	-	Lounak water fall, G	31-10-2012	*Dead Carpinus Betulus*
HSBU-200897	GIran97	IRAN 2219C	Amlash, G	31-10-2012	*Gleditschia caspica*
HSBU-200898	GIran98	-	Amlash, G	31-10-2012	*Gleditschia caspica*
HSBU-200899	GIran99	-	Amlash, G	31-10-2012	*Gleditschia caspica*
HSBU-2008100	GIran100	-	Amlash, G	31-10-2012	*Gleditschia caspica*
HSBU-2008101	GIran101	-	Amlash, G	31-10-2012	*Gleditschia caspica*
HSBU-2008102	GIran102	IRAN 2217 C	Amlash, G	31-10-2012	*Gleditschia caspica*

Table 2: New hosts for *Ganoderma* species collected from Guilan province.

Species	Hosts
G. austral	*Gleditschia caspica, Pinus taede, Albizzia julibrissin*
Eurasian *G. lucidum*	*Gleditschia caspica*
G. resinaceum	*Gleditschia caspica*
G. lucidum	*Pinus taede*

Table 3: Growth Rate and Growth coefficient for purified samples belonged to *Ganoderma* species.

Samples code	GR=Δd/Δt (mm/day)	GC=dgh/t	Identification
GIran84	3.5	71	*G. lucidum*
GIran85	3.3	29.3	*G. australe*
GIran86	4.2	9.4	*G. australe*
GIran87	3.3	16.6	*G. australe*
GIran88	4.2	15.6	*G. australe*
GIran89	3.5	10.4	*G. australe*
GIran91	5.5	21.8	*G. australe*
GIran92	3.3	10	*G. lucidum*
GIran94	6.5	19.4	*G. australe*
GIran95	4.4	8.9	*G. lucidum*
GIran96	3.1	18.9	Eurasian *G. lucidum*
GIran97	4.4	32.8	*G. australe*
GIran98	9.3	13.9	Eurasian *G. lucidum*
GIran99	3.3	14.6	*G. australe*
GIran100	9	27.0	*G. resinaceum*
GIran101	3.3	14.9	*G. australe*
GIran102	9.4	14.0	*G. resinaceum*

Table 4: Color of mycelia in different species of *Ganoderma*

Species	Color range for the mycelia (top/bottom of the plate)
G. lucidum	White /White, cream, yellow, orange
G. resinaceum	White /White, cream-yellow, orange
G. australe	White, white-yellow/White, white-yellow, cream to orange

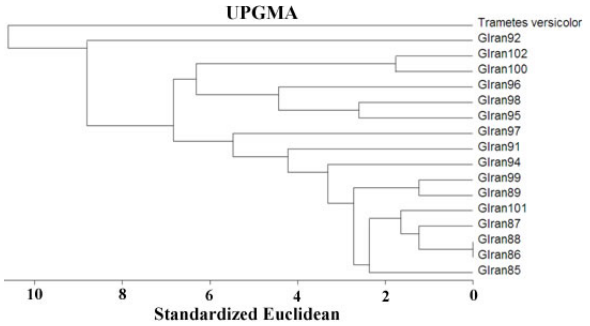

Fig. 1: UPGMA dendrogram showing relationships between *Ganoderma* species.

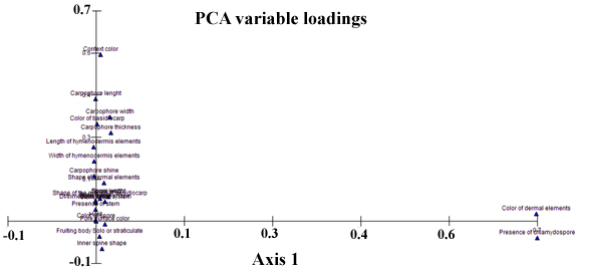

Fig. 2: Principal Component Analysis (PCA) of *Ganoderma* species based on morphological characters.

The numerical analysis resulted in identification of 2 main clusters. The first cluster belongs to laccate species *G. lucidum* complex and the second to the group of *G. applanatum* complex. The first cluster is composed of two subclusters that separate *G. resinaceum* from *G. lucidum.* The second main cluster represents the non-laccate species *G. australe* (Fig. 1). In our study code GIran 92 identified as *G. lucidum* has been separated from the *G. lucidum* group. The GIran 92 has unique aspects comparing to others including:

1) Growing on a Gymnosperm host *Pinus tedae* unlike other collected laccate species.
2) Having smaller cutis elements.

The non-laccate *G. australe* species in the second main cluster were separated by the host relationship. The PCA results showed that three variables including: context color, presence of chlamydospore, color of dermal elements are the main characters for separating the closely related species but the presence of chlamydospore is the most distinguishing character for the species of *Ganoderma* (Fig. 2).

DISCUSSION

Many scientists believe that identification of genus *Ganoderma* is the most difficult among polyporales (Ryvarden 1985, Moncalvo & Ryvarden 1997). The species of *Ganoderma* show a high degree of variation in a single basidiocarp which can lead to misidentification between the species. Therefore, various and accurate characters must be selected for the precise identification. In this study three species of *Ganoderma* were identified based on macro/micro-morphological characters of fruit body and vegetative mycelia. The results of morphological studies showed that the morphological characters are valid for separation of the species but due to the complexity and ambiguity of some morphological characters other analysis like phylogenetic analysis needed to be done for accurate identification of the species.

It has been shown that the mycelial growth rate is a useful character for distinguishing between *Ganoderma* cultures (Moncalvo *et al.,* 1995). It is also an ideal tool for comparing the growth of different fungal species under different environmental conditions (Bilay *et al.,* 2000). In this investigation, linear growth rates were observed for different *Ganoderma* sp. under a certain condition. The species in the first sub clade representing *G. lucidum* complex showed the intermediate growth rate (average of 6.41 mm/day) but the second sub clade representing the non- laccate species *G. australe*, showed the low growth rate(average of 4.1 mm/day). Bilay *et al.* (2000), who compared the growth rate of one *G. lucidum* strain amongst other species, observed a slow growth rate of 2.17 mm /day for *G. lucidum*. Also in 2004 Roberts confirmed that the Australian *G. lucidum* has a slow growth rate while growing on artificial media. In our study three laccate species, including *G. lucidum*, represented the fastest growth rate among all samples which comparing to other studies these samples show a good growth rate which highlights the significance that closely related species or strains from the same or different countries or locations may require different

growth conditions (Roberts, 2004). Thus it is important that these optimal growth conditions be identified for each new isolate. Macrofungi are great sources of secondary metabolites that can be used in medicine or nutraceutical products. They also can convert lignocellulosic biomass waste into human food (Chang, 1999). Thus the first step for achieving the goal would be purification of mycelia and exploring the mycelial growth rate.

Conclusion

In conclusion, this study brings forth the results in which must take into the account that environmental situations, hosts or substrates of saprophytic or parasitic mushrooms like *Ganoderma* plays an important role in mycelial formation.

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