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Full Length Research Paper

Influence of growth medium on diagnostic characters of *aspergillus* and *penicillium* species

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Three fungal strains; namely, *Aspergillus terreus*, *Penicillium janthinellum* and *Penicillium duclauxii* were cultured on different growth media including yeast extract, malt extract, yeast-malt extract, Czapek's Dox, Sabourod's, Harrlod's, and potato dextrose. The growth and secondary metabolites of the three fungal strains were greatly affected by the growth medium. The colour of the culture and secondary metabolites were noticeably altered and changed according to the growth medium used.

Key words: Growth medium, Aspergillus, Penicillium, Secondary metabolites, Culture characteristics.

INTRODUCTION

The taxonomy of the fungi is in a state of rapid flux at present, especially due to recent papers based on DNA comparisons, which often overturn the assumptions of the older systems of classification (Hibbett, 2007).

The simple morphology, the lack of a useful fossil record, and fungal diversity has been major impediments to progress in this field (Berbee and Taylor, 1992). Classically, studies on fungal evolution have been based on comparative morphology, cell wall composition (Bartnicki; Bartnicki-Garcia, 1970, 1987) cytologic testing (Taylor, 1978), ultrastructure (Fuller, 1976; Heath, 1980; Heath, 1986) cellular metabolism (Vogel, 1964; Le'John, 1974), and the fossil record (Hawksworth et al., 1995). More recently, the advent of cladistic and molecular approaches has changed the existing situation and provided new insights into fungal evolution (Josep et al., 1999).

However, different concepts have been used by mycologists to define the fungal species. The morphological (phenetic or phenotypic) concept is the classic approach used by mycologists; in this approach, units are defined on the basis of morphological characteristics and ideally by the differences among them (Davis, 1995).

The sexual stages are precisely the baseline of fungal taxonomy and nomenclature. It seems evident that in the near future, modern molecular techniques will allow most of the pathogenic and opportunistic fungi to be connected to their corresponding sexual stages and integrated into a more natural taxonomic scheme (Josep et al., 1999). The major divisions (phyla) of fungi are mainly classified based on their sexual reproductive structures. Currently, four divisions are recognized (Alexopolous et al., 1996).

On the other hand, secondary metabolites are compounds neither essential for growth nor key intermediates of the organism's basic metabolism but presumably playing some other role in the life of fungi. They are usually found as a mixture of closely related molecules with a peculiar and rare chemical structure (Hawksworth et al., 1995).

Molds will be affected by all the environmental factors; (chemical and physical). Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Fungal growth (spore germination, vegetative growth and sporulation) has a specific set of conditions that is optimal. Important conditions in this set are nutrient types and concentrations, light, temperature, oxygen and water availability (Kuhn and Ghannoum, 2003). However, the effect of environmental factors on growth of fungi is generally less specific and restricted than the effect on secondary metabolite production (Northolt and Bullerman, 1982).

MATERIALS AND METHODS

Fungal strains

The investigated fungi were isolated from soil and identified at the Regional Center for Mycology and Biotechnology, Cairo, Egypt.

Media

For isolation, culturing, maintenance of stock cultures, and experimental studies the following range of media were used: Czapek-

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Dox Agar (Sucrose, 30 g; Sodium nitrate, 2 g; Potassium hydrogen orthophosphate, 1 g; Potassium chloride, 0.5 g; Magnesium sulphate, 0.5 g; Ferrous sulphate, 0.002 g; Agar, 20 g; Distilled water, 1 L), Malt Extract Agar (Malt extract, 20 g; Peptone, 1 g; Glucose, 20 g; Agar, 20 g; Distilled water, 1 L), Potato Dextrose Agar (Potato extract, 4 g; Glucose, 20 g; Agar, 20 g; Distilled water, 1 L), Yeast Extract Sucrose Agar (Yeast extract, 20 g; Sucrose, 150 g; Agar, 20 g; Distilled water, 1 L), Yeast Malt Agar (Yeast extract, 3 g; Malt extract, 3 g; Peptone, 5 g; Glucose, 10 g; Agar, 20 g; Distilled water, 1 L), Harrold's Agar (Malt extract, 20 g; Yeast extract, 5 g; Sucrose, 400 g; Agar, 20 g; Distilled water, 1 L), and Sabouraud agar (Peptone, 10 g; Glucose, 40 g; Agar, 20 g; Distilled water, 1 L).

Fungal secondary metabolites

The secondary metabolites of the investigated fungal strains were determined according to the method described by Paterson and Bridge (1994). The fungal mycelial mat was removed and the filtrate of metabolized medium was extracted with chloroform in a separation funnel then the extract was evaporated to dryness and then redissolved in a 2 ml volume of chloroform. The extract sample was loaded on thin layer chromatography (TLC) plate (20 × 20cm, Merck aluminum sheet, silica gel G 60, layer thickness 0.2 mm) using volumetric micropipette. Griseofulvin was dissolved in chloroform/methanol (2:1, v/v) and was used as an external standard. The TLC plate was developed in toluene, ethylacetate, 90% formic acid (TEF) (5:4:1, v/v/v). The TLC plates were eluted for 17 cm, removed from jar and left at room temperature till dried. The plates were examined under the normal light, long UV light (365 nm), short UV light (254 nm), and back under long UV light (365 nm). The characteristics of all spots, that is, color and R_f value were recorded. The plates were sprayed with 0.5% (v/v) *p*-anisaldehyde in methanol-acetic acid-concentrated sulphoric acid (17:2:1, v/v/v), heated for 8 min at 105°C and re-examined.

Counting fungal spores

The numbers of fungal spores were estimated by transferring 9 mm disc of fungal cultures into 10 ml test tube containing 5 ml distilled water. The tubes were shaked vigorously using vortex mixer for 5 min. One-tenth ml of fungal spores suspension was transferred into a haemocytometer and then the spores was counted. Accordingly, the number of fungal spores in 5 ml was calculated. The same procedures were repeated three times and the mean number of spores was considered.

Determination of mycelial dry weight

Mycelia were harvested by filtration using Buchner funnel. Then the mycelia were washed thoroughly with distilled water and the excess of water was removed by plotting with filter papers. The mycelia of water was removed by plotting with filter papers. The mycelia were then either directly weighted; mycelial fresh weight, or dried at 80°C till constant weight obtained; mycelial dry weight.

RESULTS

Aspergillus terreus, *Penicillium janthinellum*, *Penicillium duclauxii* were cultured on different kinds of growth media including yeast extract, malt extract, yeast-malt extract, Czapek's Dox, Sabourod's, Harrold's, and potato dextrose media and incubated at 30°C for 7 days; solid media, and 14 days; liquid media.

Results revealed that the culture characteristics and secondary metabolites profiles of *A. terreus*, *P. janthinellum* and *P. duclauxii* were greatly affected by the type of growth medium.

Aspergillus terreus

The surface and reverse culture color, mycelial weight, colony diameter, and number of spores of *A. terreus* were varied and coined to the type of the growth medium (Table 1 and Figure 1). Yeast extract sucrose and Harrold's media showed the best mycelial fresh, dry weight and colony diameter (7.5 g, 2.0 g, 4.5 cm and 7.0 g, 1.6 g, 4.0 cm respectively). However, the Sabourod's medium showed the lowest mycelial fresh and dry weight (1.4 g and 0.5 g respectively) (Table 1).

On the other hand, the highest number of spores per cm2 was obtained on Sabourod's (85 X 10^5 /cm^2), Czapek's- Dox (80 X 10^5 /cm^2), Malt extract (72 X 10^5 /cm^2), and Yeast extract (70 X 10^5 /cm^2) media. The potato dextrose medium showed the lowest number of spores (45 X 10^5 /cm^2).

Interestingly, the surface and reverse culture color of *Aspergillus terreus* were noticeably changed from one medium to another (Figure 1). Surface color was gradually altered from light yellow on yeast extract sucrose to rosy brown on potato dextrose and Sabourod's media. The surface color was old lace on Czapek Dox, tan on malt extract, beige on Harrold's, and antique white on yeast malt extract media (Figure 1).

The numbers of secondary metabolites produced by A. terreus were 11 on yeast extract sucrose, 10 on yeast malt extract and 9 on Harrold's medium. However, only 4 secondary metabolites were detected on potato dextrose medium (Table 2).

The metabolites Aurofusarin, Secalonic acid and unknown (C) were produced on five different growth media; however, they were produced together on only potato dextrose and Sabourod's media (Table 2). While aurofusarin was not produced on malt and Harrold's media, the secalonic acid was not produced on yeast extract sucrose and yeast malt extract media and the unknown (C) wan not produced on malt extract and Czapek's Dox media. However, the terrain was produced on yeast extract sucrose, yeast malt extract, and Harrold's media, the phoenicin was produced only on malt extract and Czapek's Dox media, and unknown was produced on Sabourod's and Czapek's Dox media.

Penicillium janthinellum

Culture characteristics and secondary metabolites of *Penicillium janthinellum* were significantly affected by the type of the growth medium (Tables 3, 4 and Figure 2). The surface and reverse culture color, mycelial weight, colony diameter, and number of spores of *P. janthinellum* were varied and coined to the type of the growth medium.

Table 1. The culture characteristics of *A. terreus* grown on different growth media.

Growth medium	Mycelial weight (g/100 ml)		Colony Diameter (cm)	Culture color		No. of spores/cm^2 (X 10^5)
	Fresh	Dry		Surface	Reverse	
Yeast extract	7.5	2.0	4.5	Light yellow	Light orange	70
Czapek-Dox	2.7	0.7	3.5	Old Lace	Yellow	80
Malt extract	1.9	0.6	3.0	Tan	Tan	72
Harrold's	1.6	7.0	4.0	Beige	Orange	65
Yeast malt ext.	2.0	0.6	3.0	Antique white	Peach buff	63
Potato dextrose	3.6	0.9	3.5	Rosy brown	Lemon Chiffon	45
Sabourod's	1.4	0.5	3.2	Rosy brown	Saddle brown	85

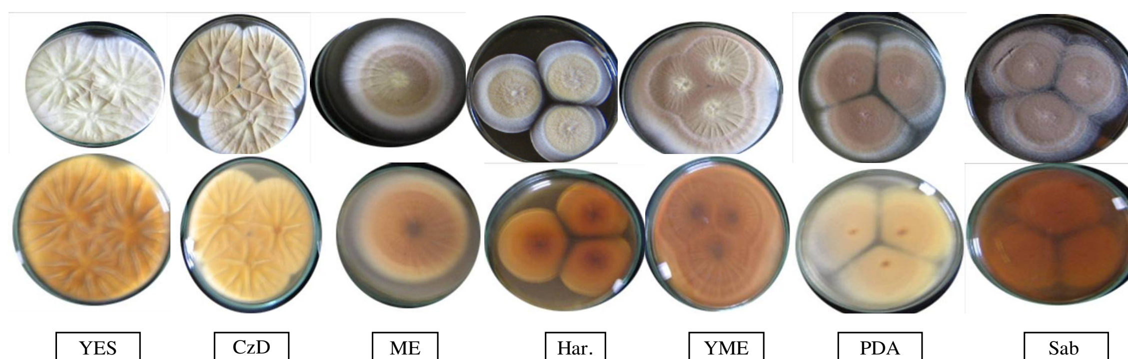

Figure 1. The culture spore mass and reverse color of *A. terreus* grown on different growth media. YES, yeast extract sucrose; CzD, Czapek's Dox; ME, malt extract; YME, yeast malt extract; Har., Harrold's; PD, potato dextrose; and Sab., Sabourod media.

Table 2. Secondary metabolites of *A. terreus* grown on different growth media.

Secondary metabolites	Growth Medium						
	Yeast extract sucrose	Czapek's Dox	Malt extract	Harrold's	Yeast malt extract	Potato dextrose	Sabourod's
Aurofusarin	+	+	-	-	+	+	+
Brevianamin A	+	-	-	-	+	-	-
Equisetin	+	-	-	+	+	+	-
Itaconic acid	+	+	-	+	-	-	+
Lapidosin	+	-	+	+	+	-	-
Phoenicin	-	+	+	-	-	-	-
Secalonic acid	-	+	+	+	-	+	+
Terrain	+	-	-	+	+	-	-
Terrestic acid	+	-	+	+	+	-	-
Wortmannin	+	-	+	+	+	-	-
Unknown A	+	-	-	+	+	-	-
Unknown B	+	-	+	-	+	-	-
Unknown C	+	-	-	+	+	+	+
Unknown D	-	+	-	-	-	-	+

Czapek's Dox medium showed the best mycelial fresh, dry weight and colony diameter (4.6 g, 1.0 g, 4.5 cm respectively) followed by yeast malt extract medium (4.5 g, 1.0 g, 4.0 cm) and malt extract medium (4.5 g, 0.6 g, 4.0 cm). The sabourod medium showed the lowest mycelial fresh, dry weight and colony diameter (2.5 g, 0.3 g, 2.0 cm, respectively) (Table 3).

The highest number of spores per cm^2 was obtained on

Table 3. The culture characteristics of *P. janthinellum* grown on different growth media.

Growth medium	Mycelial Weight (g/100ml)		Colony Diameter (cm)	Culture Color		No. of spores/cm^2 ($X 10^5$)
	Fresh	Dry		Surface	Reverse	
Yeast extract	3.8	0.7	3.5	Blue	White with red peripheral	88
Czapek-Dox	4.6	0.1	4.5	Blue	Deep red	94
Malt extract	4.5	0.6	4.0	Greenish blue	Red	91
Harrold's	3.5	0.6	2.5	Greenish blue	Pale yellow	56
Yeast malt ext.	4.5	1.0	4.0	Green	Deep red	95
Potato dextrose	3.5	0.6	2.5	Green	Pale yellow	53
Sabourod's	2.5	0.3	0.2	Greenish	Light red	44

Table 4. Secondary metabolites of *Penicillium janthinellum* grown on different growth media.

Secondary metabolites	Growth Medium						
	Yeast extract sucrose	Czapek's Dox	Malt extract	Harrold's	Yeast malt extract	Potato dextrose	Sabourod's
Carlosic acid	+	-	+	+	+	-	-
Cyclopenin	+	+	-	+	+	+	-
Erythroskyrin	+	-	+	+	+	-	-
Kojic acid	+	+	+	-	-	+	+
Patulin	+	+	+	-	-	+	+
Unknown A	+	+	+	+	+	+	+
Unknown B	-	-	-	+	+	-	-
Unknown C	+	-	-	-	-	-	-

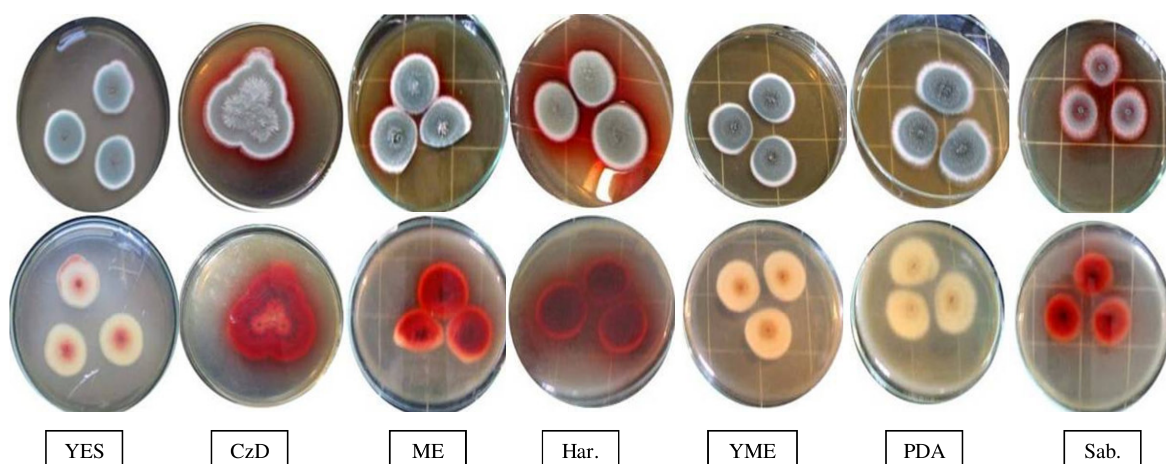

Figure 2. The culture spore mass and reverse color of *P. janthinellum* grown on different growth media. YES, yeast extract sucrose; CzD, Czapek's Dox; ME, malt extract; YME, yeast malt extract; Harr., Harrold's; PD, potato dextrose; and Sab.

yeast malt extract (95 X 10^5/cm^2), Czapek's-Dox (94 X 10^5/cm^2), malt extract (91 X 10^5/cm^2), and yeast extract (88 X 10^5/cm^2) media. The Sabourod's medium showed the lowest number of spores (44 X 10^5/cm^2).

The surface of *P. janthinellum* was noticeably affected by the type of the growth medium and has been changed from one medium to another (Figure 2). The color was steel blue on yeast extract sucrose, malt extract; potato dextrose media, slate grey on Czapek's Dox and Harrlod's media, and olive green on yeast malt extract medium (Figure 2).

Secondary metabolite produced by P. janthinellum was affected by the type of growth medium (Table 4). Secondary metabolites produced by *P. janthinellum* were 7 on

yeast extract sucrose, 5 on malt extract, yeast malt extract and Harrlod's media. However, only 4 secondary metabolites were detected on Czapek's and potato dextrose media and 3 on Sabourod's medium.

Secondary metabolite unknown (A) was produced on all the investigated growth media. Cyclopenin, Kojic acid, and Patulin were produced on five out of the seven growth media, however, cyclopenin was produced on malt extract and Sabourod's media, while both Kojic acid and Patulin were not produced on yeast malt extract and Harrlod's media (Table 4).

On the other hand, the unknown (C) produced only on yeast extract sucrose and unknown B was produced on yeast malt extract and Harrlod's media.

Penicillium duclauxii

The culture characteristics and secondary metabolites of *Penicillium duclauxii* were affected by the type of the growth medium (Tables 5, 6 and Figure 3). Mycelial weight, colony diameter, culture color, and number of spores of *P. duclauxii* were varied and coined to the type of the growth medium.

Harrlod's growth medium showed the best mycelial fresh, dry weight and colony diameter (7.0 g, 2.0 g, 3.5 cm respectively) followed by potato dextrose medium (6.5 g, 1.9 g, 2.8 cm) and yeast extract medium (5.5 g, 1.8 g, 2.7 cm).

The Czapek's Dox medium showed the lowest mycelial fresh, dry weight and colony diameter (2.0 g, 0.6 g, 2.0 cm respectively) (Table 5).

Interestingly, unlike the vegetative structures, the number of spores produced by *P. duclauxii* was slightly affected by the type of growth media (Table 5). The number of spores per cm^2 was 96 X 10^5 on Harrold's medium, 95 X 10^5 on potato dextrose and yeast malt extract, 94 X 10^5 on yeast extract, Czapek's Dox and Sabourod's media, and 93 X 10^5 on malt extract (Table 5).

The culture spore mass and reverse color of *P. duclauxii* was significantly changed from one medium to another (Figure 3). Spore mass color was steel blue on Harrlod's, malt extract, and potato dextrose media. How-ever, the color was olive green on Czapek's Dox and yeast malt extract media, and light green on yeast ex-tract sucrose and Sabourod's medium (Figure 3). On the other hand, the reverse colour was yellow on malt extract, orange brown on yeast extract and Czapek-dox, light brown on yeast malt extract and Sabourod's media, brown on potato dextrose, and deep brown on Harrold's medium.

Secondary metabolite produced by *P. duclaxii* was affected by the type of growth medium (Table 6). Secondary metabolites produced by *P. duclaxii* were 8 on yeast extract sucrose, 6 on malt extract, Harrlod's and potato dextrose, and 5 on yeast malt extract and Czapek's Dox media. However, only 4 secondary metabolites were detected on Sabourod's medium.

Secondary metabolite Flavoskyrin was produced on all the investigated growth media and unknown (A) was produced on all media except the Czapek's medium. Brevianamide A and Xanthomegin were produced on yeast extract sucrose, Harrlod's, malt extract, yeast malt extract, and potato dextrose media (Table 6).

Citrinin and unknown (B) were produced on 4 different media. They were produced together on yeast extract sucrose, Czapek's Dox, potato dextrose and separately on Harrlod's and Sabourod's, respectively. Patulin and terrestic acid were produced together on yeast extract sucrose and Czapek's media and produced separately on Sabourod's and malt extract, respectively. Duclauxin was produced on Harrlod's, malt extract, and yeast malt extract media (Table 6).

DISCUSSION

The fungal systematic is still based mainly on morphological criteria, observable characteristics. Eventually, fungi are usually recognized and identified basically by their phenotypes. Numerous alternative approaches have been developed, including nutritional and physiological studies, serologic tests, secondary metabolites, ubiquinone systems, and fatty acids. However, some of these are very useful for identifying poorly differentiated fungi such as yeasts and black yeasts; they are only complementary tools of morphological data in most cases (Hawksworth et al., 1995; Alexopoulos et al., 1996; Hawksworth, 2001; Kendrick, 2001; Michael et al., 2001; Bok and Keller, 2004).

Northolt and Bullerman (1982) reported that the growth of fungi depends on the composition of the growth media, water activity (a_w), pH, temperature, light, and the surrounding atmospheric gas mixture. The effect of environmental factors on growth of fungi is generally less specific and restricted than the effect on secondary metabolite production.

However, the results of this study revealed that both the secondary metabolites and culture characteristics including mycelial weight, colony diameter, number of spores, and the spores' colour of *A. terreus*, *P. janthinellum*, and *Penicillium duclauxii* were significantly affected by the type of the growth medium. The obtained results confirmed the effect of environmental conditions on secondary metabolites, in agreement with Northolt and Bullerman (1982), but on the other hand, on contrary of their suggestions a similar effect was obtained on the growth of fungi.

Interestingly, the color of spores is one of the most common character which is widely used in fungal identifycation and taxonomy, especially among *Aspergillus* and *Penicillium* genera (St-Germain and Summerbell, 1996; Sutton et al., 1998). However, the current results showed that the spores color of *A. terreus*, *P. janthinellum* and *P. duclauxii* was significantly changed according to the type of the growth media. It seems to be that the color variation was mainly attributed to the constituents and/or the ingredients of each medium.

On the other hand, Guarro et al. (1999) reported that the

Table 5. The culture characteristics of *Penicillium duclauxii* grown on different growth media.

Growth medium	Mycelial Weight (g/100ml)		Colony Diameter (cm)	Culture Color		No. of spores/cm^2 ($X 10^5$)
	Fresh	Dry		Surface	Reverse	
Yeast extract	5.5	1.8	2.7	Light green	Orange to brown	94
Czapek-Dox	2.0	0.6	2.0	Olive green	Orange to brown	94
Malt extract	5.0	1.6	2.3	Steel blue	Yellow	93
Harrold's	7.0	2.0	3.5	Steel blue	Deep brown	96
Yeast malt ext.	5.2	1.7	2.5	Olive green	Light brown	95
Potato dextrose	6.5	1.9	2.8	Steel blue	Brown	95
Sabourod's	2.2	0.6	2.0	Light green	Light brown	94

Table 6. Secondary metabolites of *Penicillium janthinellum* grown on different growth media.

Secondary metabolites	Growth Medium						
	Yeast extract sucrose	Czapek's Dox	Malt extract	Harrold's	Yeast malt extract	Potato dextrose	Sabourod's
Brevianamide A	+	-	+	+	+	+	-
Citrinin	+	+	-	+	-	+	-
Duclauxin	-	-	+	+	+	-	-
Flavoskyrin	+	+	+	+	+	+	+
Patulin	+	+	-	-	-	-	+
Terrestic acid	+	+	+	-	-	-	-
Xanthomegin	+	-	+	+	+	+	-
Unknown A	+	-	+	+	+	+	+
Unknown B	+	+	-	-	-	+	+

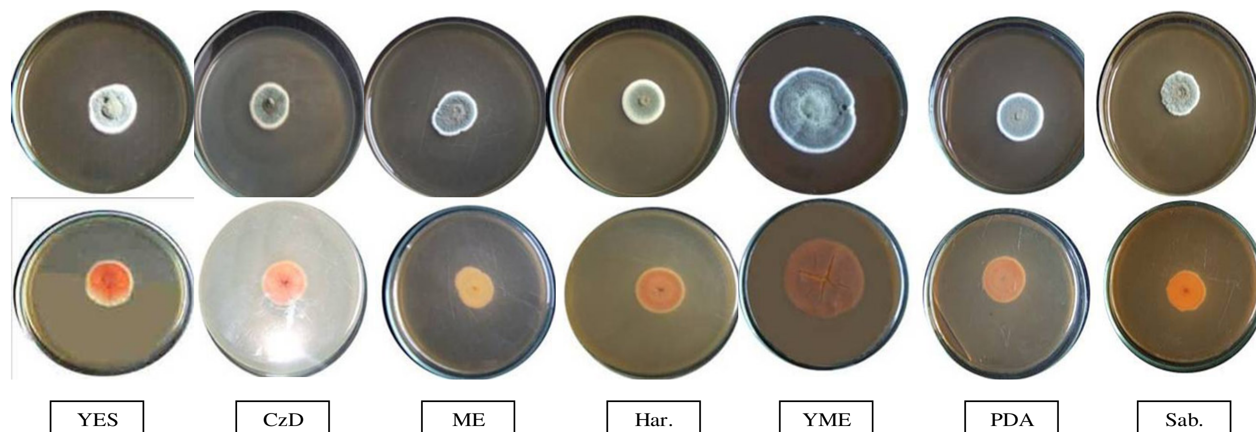

Figure 3. The culture spore mass and reverse color of *P. duclaxii* grown on different growth media. YES, yeast extract sucrose; CzD, Czapek's Dox; ME, malt extract; YME, yeast malt extract; Harr., Harrold's; PD, potato dextrose; and Sab., Sabourod media.

the use of secondary metabolites in fungal taxonomy is not accepted because the production of these compounds can be affected by environmental conditions. The obtained results of the current study revealed that the secondary metabolites are susceptible to the environmental condition and their production is restricted to certain conditions, therefore their use in fungal taxonomy became strongly not recommended.

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