Biological Studies on Airborne *Cladosporium* Species Isolated from Riyadh City

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Abstract: The biological studies were performed using five different Cladosporium species. The five fungal species were isolated during investigation of the geographical distribution of Cladosporium at Riyadh. The five fungal species were with the codes; Clad#4, Clad#5, Clad#8, Clad#12, and Clad#16). In order to investigate the effect of environmental and physiological factors on the mycelial growth of Cladosporium spp., the effect of temperature, pH, carbon and nitrogen sources, and different culture media on mycelial growth of Cladosporium species were studied in vitro. Analysis of variance (ANOVA) showed very highly significant (P = 0.0001) effects of Temperature, and significant effects of Temperature × isolate interaction on mycelial dry weight of Cladosporium. Analysis of variance of the effect of pH, Cladosporium isolate, and their interaction showed that they were all very highly significant sources of variation in mycelial dry weight of Cladosporium. Also, Carbon source, isolate, and Carbon source × isolate interaction were all very highly significant sources of variation in mycelial dry weight of Cladosporium. ANOVA showed very highly significant effects of Nitrogen source, isolate, and Nitrogen source × isolate interaction on mycelial dry weight of Cladosporium. Out of five solid media tested for their effect on mycelial growth, for most of Cladosporium species the maximum colony diameter was recorded on sabouraud dextrose agar medium followed by potato dextrose agar medium.
[Mohammed S. Alhussaini, M.A. Moslem, Mohammed I. Alghonaim, Abdullah A. Al-Ghanayem and Hamido M. Hefny. **Biological Studies on Airborne *Cladosporium* Species Isolated from Riyadh City.** *Life Sci J* 2015;12(6):83-91]. (ISSN:1097-8135). http://www.lifesciencesite.com. 11

Keywords: *Cladosporium* genus**,** *Cladosporium* spices.

1. Introduction

Although *Cladosporium* genus is among the most common fungi to be isolated from the environment almost anywhere in the world, few biological studies were done with it. The genus *Cladosporium* is one of the most important group of fungi which includes many saprophytic and some pathogenic species. Also, its ability to biodegrade some aromatic compounds in industry has been well established. In spite of the importance of *Cladosporium* in medicine, agriculture and industry, very little documented data have been presented about cellular and molecular aspects of this cosmopolitan fungus (Ghahfarokhi *et al.*, 2004). Qi *et al.* (2002) tested the ability of five fungal species, included, *C. resinae*, *C. sphaerospermum* for their ability to degrade nine volatile organic compounds (VOCs) commonly found in industrial offgas emissions under static conditions. Compounds tested included aromatic hydrocarbons (benzene, ethylbenzene, toluene, and styrene), ketones (methyl ethyl ketone, methyl isobutyl ketone, and methyl propyl ketone), and organic acids (n-butyl acetate, ethyl 3-ethoxypropionate). Experiments were conducted using three pH values, 3.0, 5.0 and 7.0 to determine the optimum pH for fungal growth on the compounds tested. Results indicate that *C. sphaerospermum* can readily utilize each of the nine VOCs as a sole carbon and energy source. *C. resinae* was able to degrade both organic acids, all of the ketones, and some of the aromatic compounds (ethylbenzene and toluene); however, it was not able to grow utilizing benzene or styrene. Maximum growth for most fungi was observed at a pH of approximately 5.0.

Ohkawa *et al.* (2000) investigated the degradation of two kinds of polyion complex (PIC) fibers, chitosan-gellan (CGF), and poly(Llysine)-gellan (LGF) fibers, by *Cladosporium* sp. and found that the fungus was able to grow on the two fiber materials. Microscopic observation of the biodegradation processes revealed that *Cladosporium* sp. Partly digested the fiber matrices (Ohkawa, *et al.*, 2000). The biodegradation of electrospun nano-fibers of poly (e-caprolactone) (PCL) was initially investigated with respect to the environmental application of PCL non-woven fabrics, using pure-cultured *Cladosporium* sp. All of the pure-line *Cladosporium* sp. tested grew on the fiber materials. *Cladosporium* sp. were well grown on the PCL non-woven fabric, but the degradation of the PCL nano-

fibers were not clearly observed (Ohkawa *et al.*, 2004). Cellobiose dehydrogenase (CDH; EC.1.1.5.1) is an extracellular enzyme that mainly produced by wood-degrading fungi. It oxidizes cellobiose to cellobionolactone using a wide spectrum of electron acceptors. The genus *Cladosporium* is a well-known cellulolytic fungus. The early screening of *Cladosporium* revealed the presence of CDH as an extracellular form in all of the examined isolates. Submerged cultivation of the best producer of CDH (selected from initial screening) on a specific medium showed the maximum amounts of enzyme produced in shaking cultures with pH 4.5 at 28°C for a 14-day period (Ghahfarokhi *et al.*, 2004). Gross and Robbins (2000) regarded *Cladosporium herbarum* (Pers.) Link ex Gray as acid-tolerant fungi and able to grow under pH ranges from 3.5 to 6.7. The main goal of this research was studying the main eco-physiological factors that affect mycelial growth rate including temperature, pH, nitrogen sources and carbon sources.

2. Material and Methods

1. Fungal isolates

The biological studies were performed using five different *Cladosporium* species. The five fungal species were isolated during investigation of the geographical distribution of *Cladosporium* at Riyadh. The five fungal species were with the codes; Clad#4, Clad#5, Clad#8, Clad#12, and Clad#16 (Table 1).

Table 1. Isolate Code, Isolate date, AUMC number and Geographic origin of Cladosporium spp.

Isolate Code	Isolate date	*Cladosporium* spp.	AUMC No.	Geographic origin
Clad#1	27/9/2006	*Cladosporium cladosporioides* (Fresenius) de Vries	4432	South East
Clad#2	12/9/2006	*Cladosporium sphaerospermum* Penzig	4433	South East
Clad#3	12/9/2006	*Cladosporium herbarum* (Persoon) Link	4434	North West
Clad#4	4/10/2006	*Cladosporium sphaerospermum* Penzig	4435	Middle
Clad#5	12/9/2006	*Cladosporium herbarum* (Persoon) Link	4436	South West
Clad#6	4/10/2006	*Cladosporium herbarum* (Persoon) Link	4437	North East
Clad#7	4/10/2006	*Cladosporium herbarum (Persoon) Link*	4438	North East
Clad#8	4/10/2006	*Cladosporium cladosporioides* (Fresenius) de Vries	4439	Middle
Clad#9	27/9/2006	*Cladosporium sphaerospermum* Penzig	4440	South East
Clad#10	4/10/2006	*Cladosporium sphaerospermum* Penzig	4441	North West
Clad#11	4/10/2006	*Cladosporium herbarum (Persoon) Link*	4442	Middle
Clad#12	4/10/2006	*Cladosporium macrocarpum* Preuss	4443	North East
Clad#13	4/10/2006	*Cladosporium sphaerospermum* Penzig	4444	North East
Clad#14	4/10/2006	*Cladosporium cladosporioides* (Fresenius) de Vries	4445	North East
Clad#15	12/9/2006	*Cladosporium macrocarpum* Preuss	4446	South East
Clad#16	4/10/2006	*Cladosporium chlamydosporis* Matsushima	4447	Middle
Clad#17	4/10/2006	*Cladosporium sphaerospermum* Penzig	4448	Middle
Clad#18	12/9/2006	*Cladosporium sphaerospermum* Penzig	4449	South West
Clad#19	27/9/2006	*Cladosporium cladosporioides* (Fresenius) de Vries	4450	South West

(AUMC)* Assiut University, Mycological Centre

2. Preparation of spore suspension

Ten days old cultures of *Cladosporium* were used to investigate the effect of physical and chemical factors on *Cladosporium* growth. Cultures were grown on MEA at 25°C, each plate was then flooded with 20ml sterile distilled water and by using sterile glass rod dislodged the conidia. The suspension was then transferred to 100 ml sterile Erlenmeyer (Conical) flasks. Serial dilution was carried out when needed to be able to reach appropriate spore's concentration/ml. Spore suspensions concentrations ml^{-1} was estimated with a haemocytometer. Spore suspension at concentration (1×10^6 spores ml^{-1}) were used (Pascual, *et al.,* 1997).

3. Dry weight determination

Biomass (growth) were obtained from the investigations of the effect of temperature, pH, carbon sources and nitrogen sources on mycelial growth were filtrated through Whatman No.1 filter papers which were pre-dried in an oven for 24 h or overnight at 70°C and weighed prior to the filtration. While the sample is being filtered it was washed with 20 ml acidified water (pH4) in order to remove insoluble salt. The filter paper containing the fungal biomass was transferred to a dry glass petri dish in an oven for 24 h. or overnight at 70°C. After cooling inside desiccators for 20 min they were weighed again (Vonshak, 1997).

4. Effect of temperature on mycelial growth rate

The effect of temperature on mycelial growth rate was assessed in 100 ml Erlenmeyer flasks containing Czapek's Dox liquid medium which contained; Sucrose 30.0 gm, Sodium nitrate 3.0 gm, Magnesium sulfate 0.5 gm, Potassium chloride 0.5 gm, Ferrous sulphate 0.01 gm, and di-Potassium hydrogen phosphate 1.0 gm, distilled water, 1 liter.

Flasks were inoculated with 1ml of a heavily concentrated spore suspension (1×10^6 spores ml^{-1}) and were incubated in the dark at 5, 10, 15, 20, 25, 30, 35°C for 10 days. Three replicates were prepared for each isolate at each temperature. After 10 days incubation, the mycelia were harvested by filtration through Whatman No. 1 filter paper and the dry weight (mg) was recorded as described before (Kim *et al*., 2005).

5. Effect of pH on mycelial growth rate

The effect of pH values between 3 to 8 on mycelial growth rate was assessed in 100 ml Erlenmeyer flasks, each flask was containing 20 ml of malt extract broth, with the following composition (g/liter); Malt extract 30.0; peptone from soymeal 3.0. pH values between 3 to 5 were prepared using Citrate-Phosphate Buffer and pH values between 6 to 8 were prepared using Phosphate Buffer (Appendix 1). Spore suspensions were prepared as described before. Flasks were inoculated with 1ml of a heavily concentrated spore suspension (1×10^6 spores ml^{-1}) followed by incubation in the dark at 25 °C for 10 days. Three replicates were prepared for each isolate. After 10 days incubation, the mycelium was harvested by filtration through Whatmann No. 1 filter paper and the dry weight (mg) was recorded (Kim *et al.*, 2005).

6. Effect of carbon sources on mycelial growth rate

The effect of different carbon sources on mycelial growth rate was assessed in Carbon -free Czapek's Dox liquid medium, which contained (g/l) Magnesium sulfate 0.5 gm, Potassium chloride 0.5 gm, Ferrous sulphate 0.01 gm, Sodium nitrate 3.0 gm and di-Potassium hydrogen phosphate 1.0 gm, pH 6.8 ± 0.2. Different media were prepared from Czapek Dox liquid medium containing various Carbon sources at a level of 12.62 g L^{-1}. 30 g/l of Sucrose, 31.93 g/l of Mannitol, 31.58 g/l of Glucose, 31.58 g/l of Xylose, 31.58 g/l of Maltose-1-water, and 31.93 g/l of Sorbitol were used. Flasks were inoculated with 1ml of a heavily concentrated spore suspension (1×10^6 spores ml^{-1}) and were incubated in the dark at 25 °C for 12 days. Three of 100 ml flasks were inoculated for each treatment. After 12 days incubation, the mycelium was harvested by filtration through Whatmann No. 1 filter paper and the dry weight (mg) was recorded as described before (Indra and Subbaiah, 2003).

7. Effect of nitrogen sources on mycelial growth rate

The effect of nitrogen sources on mycelial growth rate was assessed in nitrogen-free Czapek Dox liquid medium. Czapek Dox liquid medium containing; Sucrose 30.0 gram, Magnesium sulfate 0.5 gram, Potassium chloride 0.5 gram, Ferrous sulphate 0.01 gram, and di-Potassium hydrogen phosphate 1.0 gram, pH 6.4 ± 0.2. Different media were prepared from nitrogen-free Czapek Dox liquid medium containing various nitrogen sources at a level of 0.33 gL^{-1}. The nitrogen sources used in this study were sodium nitrate, ammonium nitrate, ammonium sulphate and Urea. 2 g/l of Sodium nitrate, 0.943 g/l of Ammonium nitrate, 3.115 g/l of Ammonium sulphate, and 1.42 g/l of Urea were used. These were added to the media individually, and then media were dispensed in 20 ml aliquots into 100 ml Erlenmeyer flasks. The pH of all media was adjusted to 6.4 ± 0.2 using Prepared Phosphate Buffer; which were prepared as described at appendix 1. Flasks were inoculated with 1ml of a heavily concentrated spore suspension (1×10^6 spores ml^{-1}) and were incubated in the dark at 25 °C for 12 days. Three replicates were inoculated for each treatment. After 12 days incubation, the mycelium was harvested by filtration through Whatmann No. 1 filter paper and the dry weight (mg) was recorded (Indra and Subbaiah, 2003; Kanamori *et al.*, 1982; Cohen and Schiffmann-nadel, 1973).

8. Growth of *Cladosporium* isolates on different media

All isolates of *Cladosporium* were grown on Sabouraud Dextrose Agar (SDA) (Amersham), Malt Extract Agar (MEA) (MERCK), Potato Dextrose Agar (PDA) (MERCK-Germany), Czapek Dox Agar (DOX) (MERCK) or Corn Meal agar (CMA) (WINLAB) plates. Media were prepared according to the supplier's instructions. Sterile media were dispensed in 20 ml into 90 mm Petri-dish. After media solidified three replicates were maintained for each treatment. Each plate was inoculated with the test isolate by using 4 mm diameter agar plugs taken from leads edges of 10 days old culture. Replicate for each treatment were then incubated at 25°C for 14 days (Dean *et al.*, 2005).

9. Statistical analysis

The experimental design of Seasonal variation, the effect of temperature, pH, Carbon sources and nitrogen sources on mycelial growth rate was a randomized complete block design with three replicates. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package (A Microcomputer Program for the Design, Management and Analysis of Agronomic Research Experiments, Michigan State Univ., USA). Duncan's multiple range test or least significant difference (LSD) were used to compare treatment means. Some percentage data were subjected to the appropriate transformation before carrying out ANOVA to produce approximately constant variance.

3. Results

1. The effect of temperature on mycelial dry weight of *Cladosporium*

ANOVA (Table 2) showed very highly significant (P = 0.0001) effects of temperature, and significant effects of temperature × isolate interaction on mycelial dry weight of *Cladosporium*.

Table 2. Analysis of variance of temperature, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* grown on Czapek's Dox liquid medium for 10 days at different temperature.

Source of variation [a]	D.F	M.S	F.value	P>F
Replication	2	0.001	0.1334	
Temperature(T)	6	0.035	5.7897	0.0001
Isolate (S)	4	0.004	0.7179	
T×S	24	0.012	2.0425	0.0115
Error	68	0.006		

[a] Replication is random, while each of temperature and isolate is fixed.

Relative contribution of each source of variation to variation in temperature, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* is shown in Table 3. Temperature × isolate was the most important factor in determining the variation in variation in temperature, *Cladosporium* isolate, and their interaction on mycelial dry weight of *Cladosporium*. It accounted for 56.35% of the explained (model), temperature was the second in importance as a source of variation in temperature, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium*. It accounted for 40%.

Table 3. Relative Contribution of temperature, *Cladosporium* isolate, and their interaction on mycelial dry weight of *Cladosporium* grown on Czapek's Dox liquid medium for 10 days at different temperature.

Source of variation [a]	Relative Contribution [a] to variation in mycelia (Dry weight)
Temperature(T)	40.00
Isolate (S)	3.67
T×S	56.35

[a] Calculated as percentage of sum squares of the explained (model) variation.

Due to the significant interaction between temperature and *Cladosporium* isolate in the case of the mycelial dry weight of *Cladosporium*, a least significant difference (LSD) was used to compare between temperatures within different *Cladosporium* isolates. Comparisons showed that the magnitude of the difference between the mycelial dry weight of *Cladosporium* was affected by different temperatures (Table 4). For example, the rise in temperature from 5˚C to 10˚C caused highly significant variation in the *Cladosporium* dry weight with *C. macrocarpum* Preuss; Similarly, the variation of *Cladosporium* dry weight from *Cladosporium sphaerospermum* Penzig to *C. cladosporioides* (Fresenius) de Vries caused highly significant increase in *Cladosporium* dry weight under 10 ˚C, however, almost no increase in *Cladosporium* dry weight was observed under some treatments, such as temperature 35˚C or isolate *C. sphaerospermum* Penzig.

Table 4. Effect of temperature, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* grown on Czapek's Dox liquid medium for 10 days at different temperature.

Temperature	Isolate					
	Clad#4	Clad#5	Clad#8	Clad#12	Clad#16	Mean
	C. sphaerospermum	*C. herbarum*	*C. cladosporioides*	*C. macrocarpum*	*C. chlamydosporis*	
5˚C	0.015	0.015	0.025	0.027	0.022	0.021
10˚C	0.037	0.029	0.291	0.174	0.023	0.111
15˚C	0.092	0.041	0.003	0.011	0.085	0.046
20˚C	0.127	0.101	0.007	0.028	0.115	0.076
25˚C	0.056	0.117	0.056	0.106	0.054	0.078
30˚C	0.048	0.159	0.257	0.088	0.137	0.138
35˚C	0.006	0.001	0.003	0.00	0.00	0.002
Mean	0.054	0.066	0.092	0.062	0.062	0.067

Least significant difference (LSD) for Temperature×Isolate interaction is 0.126 (P<0.05) or 0.168 (P<0.01).

2. The effect of pH on mycelial dry weight of *Cladosporium*

ANOVA (Table 5) showed that pH, isolate, and pH × isolate interaction were all very highly significant sources of variation in mycelial dry weight of *Cladosporium*.

Relative contribution of each source of variation in pH, *Cladosporium* isolate, and their interaction on mycelial dry weight of *Cladosporium* is shown in Table 6. pH was the most important factor in determining the variation in variation in pH, *Cladosporium* isolate, and their interaction on mycelial dry weight of *Cladosporium*. It accounted for 50.00% of the explained (model), pH x isolate was the second importance as a source of variation in in pH, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium*. It accounted for 36.27%.

Table 5. Analysis of variance of pH, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* grown on Malt Extract broth for 10 days at 25°C with different pH values.

Source of variation [a]	D.F	M.S	F. value	P>F
Replication	2	0.003	0.5729	
pH (T)	4	0.097	21.4953	0.0000
Isolate (S)	5	0.020	4.4486	0.0017
T×S	20	0.014	3.1220	0.0004
Error	58	0.004		

[a] Replication is random, while each of pH and isolate is fixed.

Due to the significant interaction between pH and *Cladosporium* isolate in the case of the mycelial dry weight of *Cladosporium*, a least significant difference (LSD) was used to compare between pH within different *Cladosporium* isolates. These comparisons showed that the magnitude of the difference between the mycelial dry weight of *Cladosporium* was affected by different pH (Table 7). For example, the increase of *Cladosporium* mycelial dry weight from pH 3 to 6 caused highly significant variation in the *Cladosporium* mycelial dry weight with *C. sphaerospermum*; Similarly, the increase of *Cladosporium* mycelial dry weight from *C. herbarum* to *C. cladosporioides* caused highly significant variation in *Cladosporium* dry weight under the pH 4.

Table 6. Relative Contribution of pH, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* grown on Malt Extract broth for 10 days at 25°C with different pH values.

Source of variation [a]	Relative Contribution [a] to variation in mycelia (Dry weight)
pH(T)	50.00
Isolate (S)	12.95
T×S	36.27

[a] Calculated as percentage of sum squares of the explained (model) variation.

Table 7. Effect of pH, *Cladosporium* isolate, and their interaction on dry weight of *Cladosporium* grown on Malt Extract broth for 10 days at 25°C with different pH values.

pH	Isolate					
	Clad#4	Clad#5	Clad#8	Clad#12	Clad#16	Mean
	C.sphaerospermum	*C.herbarum*	*C.cladosporioides*	*C.macrocarpum*	*C.chlamydosporis*	
3	0.138	0.202	0.407	0.035	0.137	0.184
4	0.258	0.141	0.406	0.321	0.152	0.256
5	0.258	0.092	0.386	0.308	0.307	0.270
6	0.282	0.326	0.366	0.292	0.200	0.293
7	0.245	0.262	0.346	0.195	0.177	0.245
8	0.244	0.213	0.347	0.241	0.194	0.248
Mean	0.238	0.206	0.376	0.232	0.195	0.249

Least significant difference (LSD) for pH×Isolate interaction is = 0.103 (P<0.05) or 0.138 (P<0.01).

3. The effect of Carbon sources on mycelial growth rate

Carbon source, isolates, and Carbon source × isolates interaction were all very highly significant sources of variation in mycelial dry weight of *Cladosporium* (Table 8).

Table 8. Analysis of variance (ANOVA) of effect of Carbon source, *Cladosporium* isolate, and their interaction on mycelial dry weight of *Cladosporium* grown in carbon-free Czapek's Dox liquid medium with various Carbon sources in the dark at 25 °C for 12 days.

Source of variation [a]	D.F	M.S	F.value	P>F
Replication	2	0.000	0.2687	
Isolate (S)	4	0.007	14.5397	0.0000
Carbon source (C)	5	0.015	30.0867	0.0000
S×C	20	0.002	4.6292	0.0000
Error	28	0.001		

[a] Replication is random, while each of Carbon source and isolate is fixed.

Table 9. Relative contribution of carbon source, *Cladosporium* isolate, and their interaction on mycelial dry weight of *Cladosporium* grown in carbon-free Czapek's Dox liquid medium with various Carbon sources in the dark at 25 °C for 12 days.

Source of variation [a]	Relative Contribution [a] to variation in mycelia (Dry weight)
Isolate (S)	18.95
Carbon source (C)	49.67
S×C	30.72

[a] Calculated as percentage of sum squares of the explained (model) variation.

Relative contribution of each source of variation in Carbon source, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* is shown in Table 9. Carbon source was the most important factor in determining the variation in Carbon source, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium*. It accounted for 49.67% of the explained (model). Carbon source x isolate was the second in importance as a source of variation in in carbon source, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium*, it accounted for 30.72%.

Due to the significant interaction between carbon source and *Cladosporium* isolate in the case of the mycelial dry weight of *Cladosporium*, a least significant difference (LSD) was used to compare between carbon source within different *Cladosporium* isolates. These comparisons showed that the magnitude of the difference between the dry weight of *Cladosporium* was affected by different carbon source (Table 10). For example, the increase of *Cladosporium* mycelial dry weight from Sorbitol to Sucrose caused highly significant variation in the *Cladosporium* mycelial dry weight with *C. herbarum*; Similarly, the increase of *Cladosporium* mycelial dry weight from *C. sphaerospermum* to *C. cladosporioides* caused highly significant variation in *Cladosporium* mycelial dry weight under the Xylose.

Table 10. Effect of Carbon source, *Cladosporium* isolate, and their interaction on mycelial dry weight of *Cladosporium* grown in carbon-free Czapek's Dox liquid medium with various Carbon sources in the dark at 25 °C for 12 days.

Carbon source	*Cladosporium* Isolates					
	Clad4	Clad5	Clad8	Clad12	Clad16	Mean
	C.sphaerospermum	*C.herbarum*	*C.cladosporioides*	*C.macrocarpum*	*C.chlamydosporis*	
Sorbitol	0.020	0.016	0.045	0.018	0.015	0.023
Maltose-1-water	0.063	0.059	0.082	0.153	0.064	0.084
Sucrose	0.056	0.134	0.125	0.134	0.041	0.098
Glucose	0.071	0.076	0.086	0.060	0.044	0.067
Xylose	0.045	0.117	0.149	0.046	0.061	0.084
Mannitol	0.019	0.027	0.043	0.027	0.016	0.026
Mean	0.046	0.072	0.088	0.073	0.040	0.064

Least significant difference (LSD) for Carbon source × Isolate interaction = 0.052 (P<0.05) or 0.069 (P<0.01).

4. The effect of nitrogen sources on mycelial dry weight of *Cladosporium*

ANOVA in Table 11 showed very highly significant effects of Nitrogen source, isolates, and Nitrogen source × isolates interaction on mycelial dry weight of *Cladosporium*.

Table 11. Analysis of variance of effect of Nitrogen source, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* grown in Nitrogen -free Czapek's Dox liquid medium with various Nitrogen sources in the dark at 25 °C for 12 days.

Source of variation [a]	D.F	M.S	F.value	P>F
Replication	2	0.003	0.2115	
Isolate (S)	4	11.603	968.6713	0.0000
Nitrogen (N)	4	2.746	229.2467	0.0000
S×N	16	0.334	27.8530	0.0000
Error	48	0.012		

[a] Replication is random, while each of isolate and Nitrogen source is fixed.

Relative contribution of each source of variation to variation in Nitrogen source, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* is shown in Table 12. Isolate was the most important factor in determining the variation in variation in Nitrogen source, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium*. It accounted for 73.98% of the explained variation. Nitrogen source was the second in importance as a source of variation in Nitrogen source, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium*. It accounted for 17.51%.

Due to the significant interaction between Nitrogen source and *Cladosporium* isolate in the case of the dry weight of *Cladosporium*, a least significant difference (LSD) was used to compare between Nitrogen source within different *Cladosporium* isolates. These comparisons showed that the magnitude of the difference between the dry weight of *Cladosporium* was affected by different *Cladosporium* isolate (Table 13). For example, the increase of *Cladosporium* mycelial dry weight from *C. cladosporioides* to *C. macrocarpum* caused highly significant increase in *Cladosporium* mycelial dry weight under the Ammonium nitrate.

Table 12. Relative contribution of nitrogen source, *Cladosporium* isolate, and their interaction on mycelial dry weight of *Cladosporium* grown in Nitrogen -free Czapek's Dox liquid medium with various Nitrogen sources in the dark at 25 °C for 12 days.

Source of variation [a]	Relative Contribution [a] to variation on mycelia (Dry weight)
Isolate (S)	73.98
Nitrogen source (N)	17.51
S×N	8.51

[a] Calculated as percentage of sum squares of the explained (model) variation.

Table 13. Effect of Nitrogen source, *Cladosporium* isolates, and their interaction on mycelial dry weight of *Cladosporium* grown in Nitrogen -free Czapek's Dox liquid medium with various Nitrogen sources in the dark at 25 °C for 12 days.

Nitrogen source	Isolate					
	Clad#4	Clad#5	Clad#8	Clad#12	Clad#16	Mean
	C.sphaerospermum	*C.herbarum*	*C.cladosporioides*	*C.macrocarpum*	*C.chlamydosporis*	
Ammonium nitrate	0.058	0.051	0.061	0.105	0.056	0.066
Sodium nitrate	0.056	0.096	0.062	0.066	0.055	0.067
Ammonium sulphate	0.094	0.041	0.120	0.156	0.042	0.090
Urea	0.047	0.040	0.060	0.119	0.031	0.059
Mean	0.064	0.057	0.076	0.112	0.046	0.071

Least significant difference (LSD) for Isolate = 0.025 (P<0.05) or 0.035 (P<0.01). LSD for Nitrogen source is nonsignificant.

5. Growth of *Cladosporium* spp. on different solid media

It is evident from Figures 1, 2, 3, and 4 that out of Five solid media tested for mycelial growth, for all isolated species, the maximum colony diameter was recorded on (SDA) followed by (PDA). The minimum colony diameter was observed in (CMA). Except for *Cladosporium macrocarpum* Preuss (Figure 5) which gave maximum colony diameter on (MEA) followed by (SDA) and (PDA), and the minimum colony diameter was observed in (CDA).

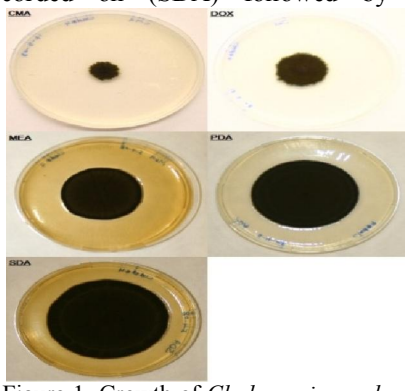

Figure 1. Growth of *Cladosporium sphaerospermum* Penzig on different media in the dark at 25°C for 14 days.

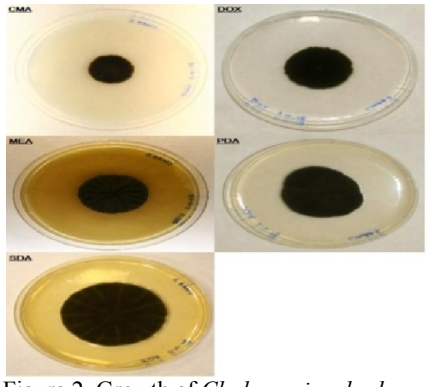

Figure 2. Growth of *Cladosporium herbarum* (Persoon) Link on different media in the dark at 25°C for 14 days.

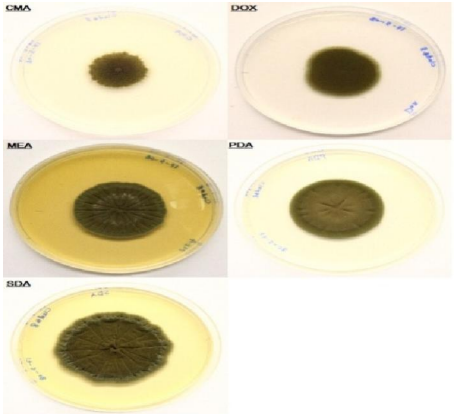

Figure 3. Growth of *Cladosporium cladosporioides* (Fresenius) de Vries on different media in the dark at 25°C for 14 days.

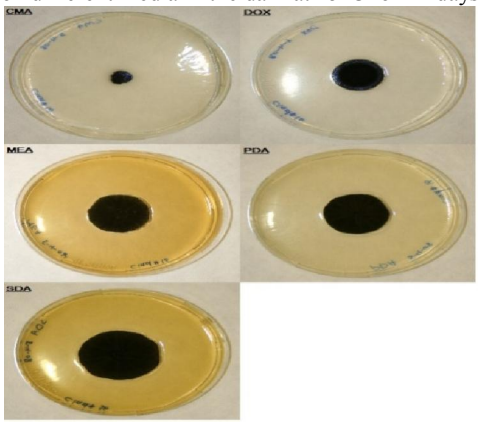

Figure 4. Growth of *Cladosporium chlamydosporis* Matsushima on different media in the dark at 25°C for 14 days.

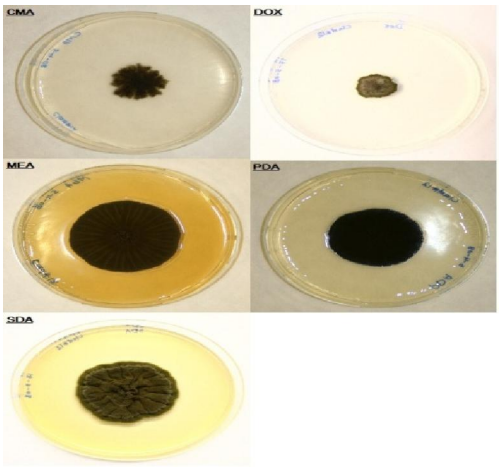

Figure 3-12. Growth of *Cladosporium macrocarpum* Preuss on different media in the dark at 25°C for 14 days.

4. Discussion

The present investigation was conducted to study the effect of physiological factors on the mycelial growth of the fungus. In vitro studies were conducted on the effect of culture, media, carbon and nitrogen sources, temperature and pH levels on mycelial growth of *Cladosporium* species. To investigate the effect of different temperature on mycelial growth experiments were carried out at 5 to 35 °C. Temperature x isolate was the most important factor in determining the variation in temperature, It accounted for 56.35% of the explained (model). However, growth of the fungus was drastically reduced below 15ºC and started to decline above 35ºC, as these temperatures did not favor growth of the fungus. No growth was observed at 5 ºC. Gupta *et al*. (1986) reported similar findings regarding temperature.

Due to the significant interaction between pH and *Cladosporium* isolate in the case of the dry weight of *Cladosporium*, a least significant difference (LSD) was used to compare between pH within different *Cladosporium* isolates. These comparisons showed that the magnitude of the difference between the dry weight of *Cladosporium* was affected by different pH. pH was the most important factor in determining the variation in pH, it accounted for 50.00% of the explained (model). Growth of the fungus decreased by increasing or decreasing the pH level from the neutral level. This fungus can tolerate a wide range of pH 3–8. The results of the present study are in agreement with those achieved by Hayes (1978). Great variation in minimum inhibitory concentration is observed between the tested mould species. *C. cladosporioides* was inhibited at much lower concentrations than *C. chlamydosporis*, especially at high pH levels. However, it is not easy to explain the inhibitory effect at high pH values. The optimum pH for mycelial growth of most fungi is 5–6.5 (Ingold, 1973). Fungi develop in optimum pH ranges which are related to enzymatic systems, essential vitamin entry in the cell, surface metabolic reactions and mineral capture. Some fungi, however, are able to adjust to the pH of the medium, optimizing it for their better development (Hung and Trappe, 1983).

To find the most suitable carbon source for mycelial growth and polysaccharide production in *Cladosporium* species, various types of carbon sources including one hexose (glucose), one pentose (xylose), two disaccharides (maltose, sucrose) and one polysaccharide (mannitol) were supplemented instead of glucose as the carbon source in the basal medium. The results of this experiment indicated that all the carbon sources were suitable for the fungus growth. *Cladosporium* was affected by different carbon source. For example, the increase of *Cladosporium* dry weight from Sorbitol to Sucrose caused highly significant variation in the *Cladosporium*. The fungus may convert certain forms of complex carbon compounds into simple form, which may be readily metabolized (Bais *et al*., 1970). The fungus may grow on some carbon sources at a very low rate for maintenance metabolism and therefore may start to sporulate. If this were the case, however, we should have seen a correlation between conidiation and either high or low growth rates. ANOVA calculated for the observed data showed significant variations in the dry weight production of different fungal species grown with different carbon sources ($p < 0.01$). This indicates that different fungal species have different priorities for their carbon source and one carbon source that is less preferred by one *Cladosporium* species could be preferred by another *Cladosporium* species. This might be due to the fact that simple carbon compounds are assimilated directly while complex ones (i.e. polysaccharides) must be converted into simpler forms before their use. Glucose (a monosaccharide) and sucrose (a disaccharide) are well used by the *Cladosporium* species. The number of carbon sources that a species is able to catabolise compared to that of surrounding species gives information about its potential degree of dominance. This approach could also be useful for understanding competitiveness and dominance of fungal species in other ecosystems, especially in relation to impacts of abiotic and biotic factors.

Nitrogen is an important component required for protein synthesis and other vital functions. To investigate the effect of nitrogen source on mycelial growth and polysaccharide production, cells were cultivated in the medium containing various nitrogen sources. Isolate was the most important factor in determining the variation in variation in Nitrogen source. Ammonium sulphate was found to be best

source of nitrogen for *C. sphaerospermum*, *C. cladosporioides* and *C. macrocarpum*, and sodium nitrate was found to be best source of nitrogen for *C. herbarum* and *C. chlamydosporis* on liquid media. Nitrogen is also used both for functional as well as structural purposes by different fungi. The form of nitrogen has a profound effect on metabolism of microorganism. The results obtained imply that the mycelium growth and appearance not only varies with the culture medium, temperature, pH value, carbon source and nitrogen source but also changes with the *Cladosporium* species.

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6/7/2015