MOLECULAR STUDY ON *CLADOSPORIUM* SPECIES ISOLATED FROM AIR OF CAIRO, USING UNIVERSALLY PRIMED-PCR (UP) AND INTERNAL TRANSCRIBED SPACER (ITS) PCR TECHNIQUES

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ABSTRACT

Universally Primed-PCR (UP-PCR) and Internal Transcribed Spacer-PCR (ITS-PCR) based genomic fingerprinting techniques are considered a good methods that rely on specifically targeted primers. These techniques, which analyse the rDNA, have been shown to be relatively robust and discriminatory. This study was designed to investigate and characterize the molecular variation among *Cladosporium* strains collected at different sites in Cairo by using two different fingerprinting methods, Universally Primed- PCR (UP- PCR) and Internal Transcribed Spacer (UP- PCR) technique. The *Cladosporium* isolates investigated were isolated from air of Cairo by settle plate method. The samples were then purified and identified by using culture based techniques, microscopical methods, and biochemical reactions followed by confirmation in the regional center for mycology and biotechnology (RCMB). Molecular fingerprinting, and genetic similarities among *Cladosporium* species populations depending on microsatellites-polymerase chain reaction (ITS-PCR). Primers used are ITS4, and ITS5. PCR products were digested with 3 restriction enzymes and separated by agarose electrophoresis. Restriction patterns generated by *Cfo*I, *Msp*I and *Rsa*I. In addition, we have applied the Universally Primed PCR (UP-PCR) technique using two primers L21 and Fok1.The current work showed prominent discriminatory power given by amplification of internal transcribes spacers PCR regions followed by restriction with *Cfo*I enzyme than other endonucleases. moreover, Fok1 primer revealed minor variability among *Cladosporium* strains using UP-PCR genotyping technique.

Key words: Molecular study, *Cladosporium*, Universally Primed-PCR (UP-PCR), Internal Transcribed Spacer-PCR (ITS-PCR)

INTRODUCTION

Cladosporium species are well known for their production of substances with antimicrobial activities, several of which have formed the basis for the development of new clinically important antimicrobial agents.

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. The dematiaceous fungi have been recognized as a possible useful source of bioactive secondary metabolites, especially in anticancer application **(Ibrahim *et al.*, 2018).** Universally primed PCR (UP-PCR) is a PCR fingerprinting method similar to the well-known RAPD technique in that it is possible to amplify DNA from any organism without previous knowledge of DNA sequences and to generate multibanding profiles (fingerprints) following gel electrophoresis. Some of the advantages of UP-PCR are the use of relatively high annealing temperatures, fast ramping and relatively long primers, features that seem to enhance the reproducibility which many have found to be problematic with RAPDs **(Lubeck *et al.*, 2005)**. Other advantages are the resulting banding profiles which consist of higher numbers of bands than most RAPDs, facilitating identification of specific markers, and at the same time showing species conservative bands **(Demissie *et al.*, 2019)**. A variant of the UP-PCR technique is UP-PCR product cross hybridization assay that facilitates investigation of sequence similarity (homology) of UP-PCR products. This allows grouping of strains into UP-PCR hybridization groups which we use to separate the strains into genetic entities featured by high genetic similarity (DNA homology) **(Bulat *et al.*, 1998)**. The internal transcribed spacer (ITS) region is well suited for comparison of closely related organisms. This non-coding region is highly polymorphic and provides a useful tool for taxonomic and phylogenetic studies. ITS regions vary between species within the same genus, but show little or no intraspecific variation. Hence, they have been used in many phylogenetic studies of various fungi **(White *et al.*, 1990)**. The internal transcribed spacer (ITS) region is well suited for comparison of closely related organisms. Analysis of the ITS region has been used to distinguish species and higher taxonomic divisions. DNA fingerprint has been used to study several genera. Restriction Fragment Length Polymorphism (RFLP) of the ITS region has been used to distinguish between species of the same or among different genera. Many species among different genera have been studied **(Bernier *et al.*, 1994)**. ITS1 and ITS2 separates the 18S, 5.8S and 28S genes and an external transcribed spacer (ETS) that is located up stream of the 18S gene. The transcribed spacers contain signals for processing rDNA transcript. Nearby copies of the rDNA repeat unit are separated by a nontranscribed spacer (NTS), also called intergenic spacer (IGS) by some workers. This region contains subrepeating elements that serve as enhancers of transcription. The ETS and ITS are removed during rRNA processing **(Kamle and Ali, 2013).** The aim of this study to explore the genetic variation between various *Cladosporium* strains isolated through this investigation using DNA target such as internal transcribed spacer - polymerase chain reaction (ITS-PCR) and Universally primed-PCR (UP-PCR) techniques.

MATERIALS AND METHODS

1. Collection of *Cladosporium* samples

Air samples were collected in Cairo city at 5 distinct geographical regions during all climate seasons from June 2017 to May 2019, using Passive sampling "settle plates" method, the petri dish containing Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA) and supplemented with chloramphenicol (100 mg/L) and gentamicin (40 mg/L) **(Schubert *et al.*, 2007 and Bensch *et al.*, 2012)**.

2. Culture media used in the current study

In the current study, multiple culture media are used for collection, isolation, purification and preservation of *Cladosporium* species including, Dichloran glycerol agar DGA (MERCK) Sabouraud Dextrose Agar (SDA) (Oxoid), Malt Extract Agar (MEA) (MERCK), Potato Dextrose Agar (PDA) (Oxoid-USA) or Czapek Dox Agar (CDA) (MERCK), were prepared according to the supplier's instructions at pH 6.8. Culture media were incubated at 30 ^{O}C for 7-14 days and examined after 4 days **(Asl *et al.*, 2017).**

3. Identification of *Cladosporium* species

Cladosporium isolates were identified based on culture characterization, macroscopic, microscopic properties according to **Crous *et al.* (2007),** and biochemical reactions such as starch hydrolysis test, casein hydrolysis test and gelatin hydrolysis test, according to **Rodarte *et al.* (2011)** the identification was confirmed at the Regional Center of Mycology and Biotechnology (RCMB), Al-Azhar University.

4. Purification and preservation of *Cladosporium* species

The purification procedure of the fungal isolate under investigation was carried out by the agar streak plate method. All expected colonies of *Cladosporium* forms on the growth medium were picked up and re-streaked onto the agar surface of plates containing the same medium. Pure colony of *Cladosporium* isolates only were sub-cultured and stored on slants of Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) media at 5 °C and kept for further investigation. Pure cultures were stored in refrigerator at 4 ^{O}C and sub-cultured periodically each 3 months **(Jin *et al*., 2012)**.

5. *Cladosporium* DNA extraction

DNA was extracted using fungal DNA extraction kit (PrepSEQ, Thermo-Scientific, USA) according to developer instructions as follow: Extraction Buffer was warmed to 65 °C at water bath. Fifty mg of the grounded mycelia were transferred to 2 ml microtube. This microtube contains 400µl warmed extraction buffer and 6µl RNAse A. The microtube was incubated at 65°C for 10 minutes. The microtube was gently shaken every 5 minutes intervals. After that, 130µl from sodium acetate solution was added and the microtube was incubated at -20°C for 10 minute. The lysate was centrifuged at 10000 rpm at 4°C for 15 min. The upper aqueous phase was decanted into fresh centrifuge tubes. An equal volume of isopropanol was added, the microtube was

stored at room temperature for 7 minutes and then spun at 6000 rpm for two minutes at 4°C. The DNA pellet was washed two times with 70% Ethanol (700 µl) and centrifuged at 8000 rpm for 1 minute, vacuum dried and dissolved in 100µl of warmed TE buffer . DNA concentration and purity was checked on an 1.5% agarose containing 0.05 µg ml^{-1} ethidium bromide, using (5µl of each sample+3µl gel loading dye + 3µl ultrapure water), 10µl was loaded at each well, 5-6µl of DNA marker 100bp was run. Photograph were taken after 30 minute. Finally, extracted DNA was stored at -20 °C (**Weising *et al.*, 1995; Hajkova *et al.*, 2006)**

6. Universally Primed PCR (UP-PCR) amplification condition

Universally Primed-PCR (UP-PCR) was conducted in a 25-µl reaction volume. Three µl of DNA template (1 ng quantified with a spectrophotometer) was added to a 7µl Master mix of thermostable DNA polymerase, Jena Bioscience GmbH, Cat.-No.PCR-101S. Taq Master/high yield contains all reagents required for PCR (except template and primer) in a premixed 5x concentrated ready-to-use solution for PCR (Thermostable DNA polymerase, dATP, dCTP, dGTP, dTTP, reaction buffer with $(NH_4)_2SO_4$, $MgCl_2$ and Triton X-100, stabilizers), 13µl of PCR grade water (Jena Bioscience GmbH), 2µl of 20 pmol Universally Primed primer. The used Universally Primed primers were; L21 (5`-GGATCCGAGGGTGGCGGTTCT-3`), and FOK-1 (5`-GGATGACCCACCTCCTAC-3`). Primers were brought in from Metabion International AG GmbH. Using a DNA thermal cycler (Techne TC-312, Techne, Stone, UK) thermal cycling parameters were initial denaturation at 94°C for 4 min, followed by 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 2 min. A final extension at 72°C for 7 min followed. PCR product was checked on an 1.5% agarose containing 0.05 µg ml^{-1} ethidium bromide, using (5µl of each sample + 3µl gel loading dye + 3µl ultrapure water), 10µl was loaded at each well, 5- 6µl of DNA marker 100 bp was run. Photographs were taken after 30 minute **(Lubeck *et al.*, 2005).**

7. Internal Transcribed Spacer-PCR (ITS-PCR) amplification condition.

The same as in UP-PCR with exception of using 2µl of 10 pmol of ITS4 primer, and 2 µl of 10 pmol of ITS5 primer . Primers were supplied by GE Healthcare. Using a DNA thermal cycler (Techne TC-312, Techne, Stone, UK) thermal cycling parameters were initial denaturation at 94°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes, then final extension at 72°C for 10 minutes. PCR product was checked on an 1.5% agarose containing 0.05 µg ml^{-1} ethidium bromide, using (5µl of each sample + 3µl gel loading dye + 3µl ultrapure water), 10µl was loaded at each well, 5- 6µl of DNA marker 100 bp was run. Photographs were taken after 30 minutes **(Abd-Elsalam *et al.*, 2007).**

7.1. Digestion of ITS-PCR Product with restriction enzymes.

Internal transcribed spacer fragments were digested with three base cutter restriction enzymes: *Cfo*I, *Msp*I, and *Rsa*I. Fifteen microliters of amplified DNA were digested for 2 hours at 37°C using a DNA thermal cycler (Techne TC-312, Techne, Stone, UK) as follow; 15 µl of ITS-PCR Product, 1 µl of enzyme (U), 2.5 µl of buffers provided

by the manufacturer, and 2.5µl of PCR grade water (Jena Bioscience GmbH). The resulting DNA restriction was electrophoresed on 1.5% agarose containing 0.05 µg ml^{-1} ethidium bromide (Agarose 25). DNA patterns were visualized and photographed under ultraviolet light (UV) **(Troche, 1997).**

8. Statistical analysis and software used in the current investigation.

Levels of genetic similarity between Microsatellite Primed (MP) and Universally Primed (UP) PCR fingerprints were calculated by using Pearson product-moment correlation coefficient. The samples were clustered using the unweighted pair group method of arithmetic average (UPGMA) which resulted in a Dendrogram by website http://genomes.urv.es/UPGMA/, genetic similarity between *Cladosporium* strains calculated using Pearson product-moment correlation coefficient (**Aamir *et al.*, 2015)**.

RESALTS

1. Isolation and identification of *Cladosporium* species

A total number of 886 fungal colonies were collected, of 212 *Cladosporium* isolates of 10 different species with isolation rate 23.9%. depending on culture properties, microscopical examination and biochemical properties, a total 10 species of *Cladosporium* were identified which are: *C. asterinae, C. uredinicola, C. acaciicola, C. macrocarpum, C. herbarum, C. nigrillum, C. oxysporum, C. cladosporioides, C. sphaerospermum* and *C. chlamydosporis*. In the current work, the most predominant species was *C. herbarum*, with 38 isolates represented by 17.9% of total collected isolates, followed by *C. cladosporioides* (28 isolates) 13.2% and *C. oxysporum* (27 isolates) 12.7 %, followed by followed by *C. acaciicola* and *C. sphaerospermum* that were identified with rate 10.4 % (22 isolate) for each, while *C. chlamydosporis*, *C. nigrillum* and *C. uredinicola* were isolated in lowest isolates rates 5.66 %, 6.6 % and 7.8 % (12, 14 and 15 isolates) respectively, at collection sites as showed in **table (1)**.

Table 1: Frequency of *Cladosporium* species collected in the current study

Serial	*Cladosporium* Species	Number of isolates	Frequency (%)*
1	*C. herbarum*	38	17.9
2	*C. cladosporioides*	28	13.2
3	*C. oxysporum*	27	12.7
4	*C. acaciicola*	22	10.4
5	*C. sphaerospermum*	22	10.4
6	*C. macrocarpum*	18	8.49
7	*C. asterinae*	16	7.55
8	*C. uredinicola*	15	7.08
9	*C. nigrillum*	14	6.6
10	*C. chlamydosporis*	12	5.66
Total		212	100%

***Percentages were correlated to the total number of *Cladosporium* isolates (212)**

2. Molecular characterization of *Cladosporium* strains

Thirty *Cladosporium* strains selected on the basis of biodiversity in which they selected from different geographical, climate and biological conditions, as shown in **table (2)**.

Table 2: *Cladosporium* strains used in the molecular study.

No.	*Cladosporium* Species	Region of collection	Site of collection	Time of collation
1	*C. chlamydosporis* HMA-13	East Cairo	El Salam city	December
2	*C. herbarum* HMA-36	East Cairo	El Salam city	March
3	*C. chlamydosporis* HMA-M_4	Downtown	Mokattam	December
4	*C. sphaerospermum* HMA-265	West Cairo	El Zamalek	November
5	*C. macrocarpum* HMA-109	East Cairo	Ain shams	June
6	*C. asterinae* HMA-188	East Cairo	Nasr City	March
7	*C. oxysporum* HMA-10	East Cairo	El Salam city	March
8	*C. asterinae* HMA-300	North Cairo	El Matarya	April
9	*C. herbarum* HMA-N_9	Downtown	Mokattam	March
10	*C. oxysporum* HMA-M_2	Downtown	Mokattam	October
11	*C. cladosporioides* HMA-731	Southern Cairo	Dar el salam	January
12	*C. asterinae* HMA-566	North Cairo	Shobra	October
13	*C. cladosporioides* HMA-132	East Cairo	Ain shams	November
14	*C. uredinicola* HMA-216	West Cairo	El Zamalek	October
15	*C. acaciicola* HMA-235	West Cairo	Bolaq	March
16	*C. nigrillum* HMA-225	North Cairo	Shobra	December
17	*C. nigrillum* HMA-221	West Cairo	El Zamalek	January
18	*C. uredinicola* HMA-59	East Cairo	El Nozha	July
19	*C. oxysporum* HMA-680	Downtown	Mokattam	September
20	*C. herbarum* HMA-348	North Cairo	Shobra	April
21	*C. cladosporioides* HMA-232	West Cairo	Kasr El Nile	February
22	*C. macrocarpum* HMA-144	West Cairo	Bolaq	February
23	*C. chlamydosporis* HMA-870	Downtown	Mokattam	November
24	*C. sphaerospermum* HMA-62	East Cairo	El Nozha	September
25	*C. macrocarpum* HMA-410	Southern Cairo	El Maadi	December
26	*C. sphaerospermum* HMA-268	West Cairo	El Zamalek	July
27	*C. acaciicola* HMA-238	West Cairo	Kasr El Nile	April
28	*C. cladosporioides* HMA-285	West Cairo	El Zamalek	December
29	*C. sphaerospermum* HMA-182	West Cairo	Bolaq	October
30	*C. cladosporioides* HMA-407	Southern Cairo	El Maadi	January

2.1. Molecular variation of *Cladosporium* species using Universal primed PCR (UP-PCR) technique.

2.1.1. UP-PCR patterns using L21 primer

The dendrogram revealed 8 well-separated clusters, each one corresponding to a different species. The genetic similarity between *Cladosporium* strains calculated using Pearson product-moment correlation coefficient, genetic similarities oscillated between 42% to 71% for inter-specific and 71%to 100 %for intra-specific comparisons. UP-PCR markers detected a very high level of polymorphism between and among *Cladosporium* strains as shown in **figure (1)**.

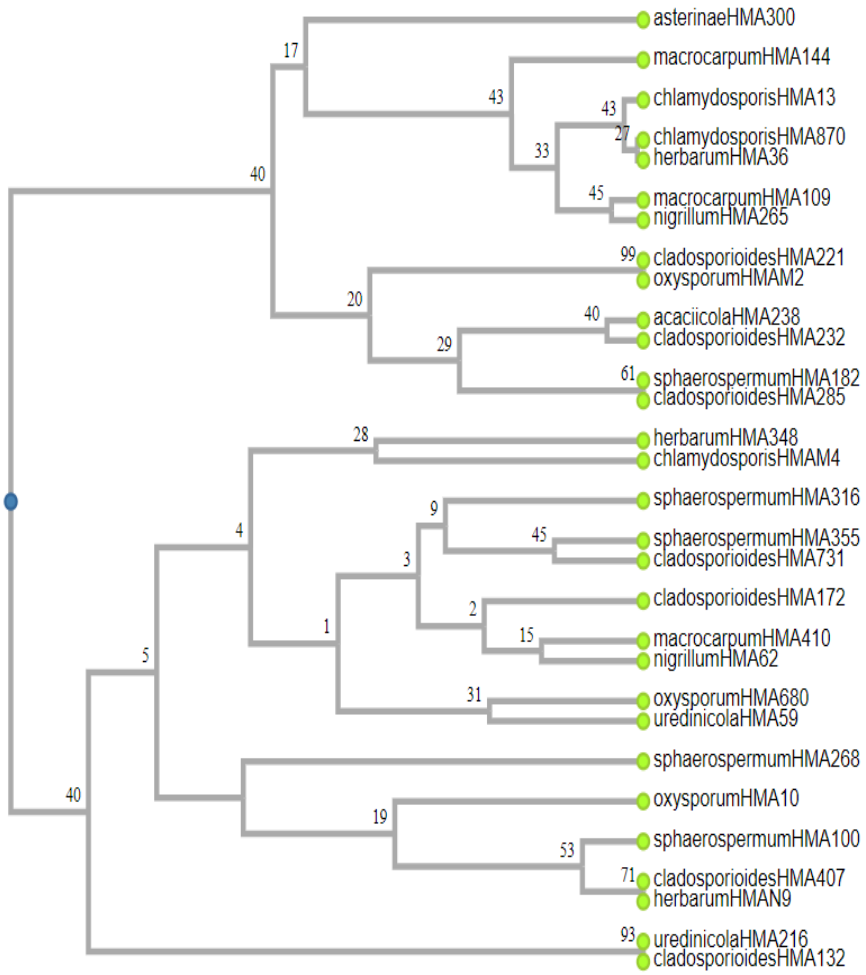

Figure 1: The Dendrogram of *Cladosporium* isolates was constructed after cluster analysis of L21-PCR marker with the UPGMA

2.1.2. UP-PCR patterns using Fok1 primer

The obtained dendrogram depicts that all isolates were separated from each other into 2 major distinct groups and 4 minor groups with genetic similarity between *Cladosporium* species isolates ranged from 26 to 53 % for inter-specific and 53 to 100% for intra-specific comparisons as shown in **figure (2)**.

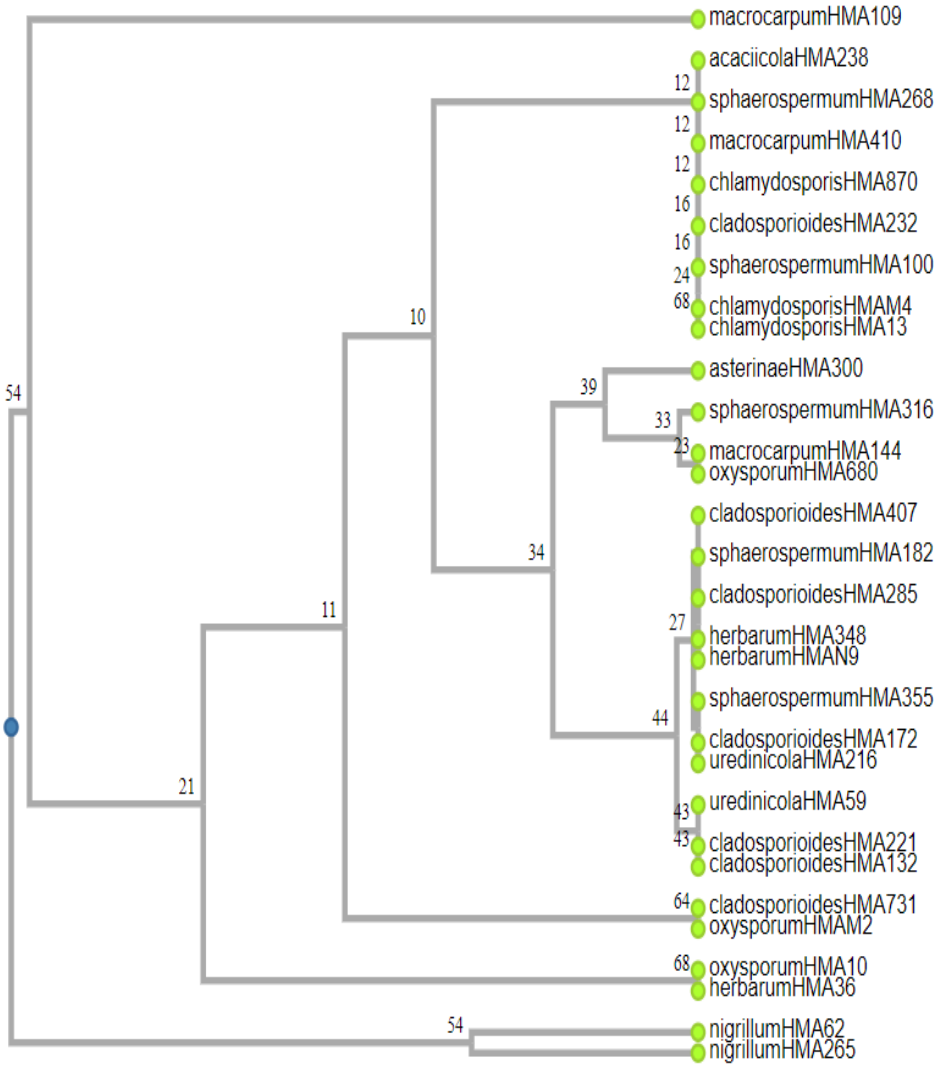

Figure 2: The Dendrogram of *Cladosporium* isolates was constructed after cluster analysis of Fok1-PCR primer using UPGMA

2.2.Molecular variation of *Cladosporium* species using ITS-PCR technique

2.2.1. ITS-PCR Patterns of *Cladosporium* species digested with *Msp*I Restriction Enzyme.

In the current investigation, using a universal fungal rRNA primer pair, a 743 bp fragment was successfully amplified from all *Cladosporium* isolates Results of the current investigation showed moderate genetic similarity between *Cladosporium* isolates that calculated using Pearson product-moment correlation coefficient which ranged from 50-70 % for inter-specific and 70-100 % for intra specific comparisons. The dendrogram constructed with ITS-PCR digested with *Msp*I revealed that all isolates of *C. cladosporioides* and almost *C. herbarum* were grouped into a major 9 cluster delimited from other *Cladosporium* species comprising four molecular groups with genetic dissimilarity 30% as represented in **figure (3)**.

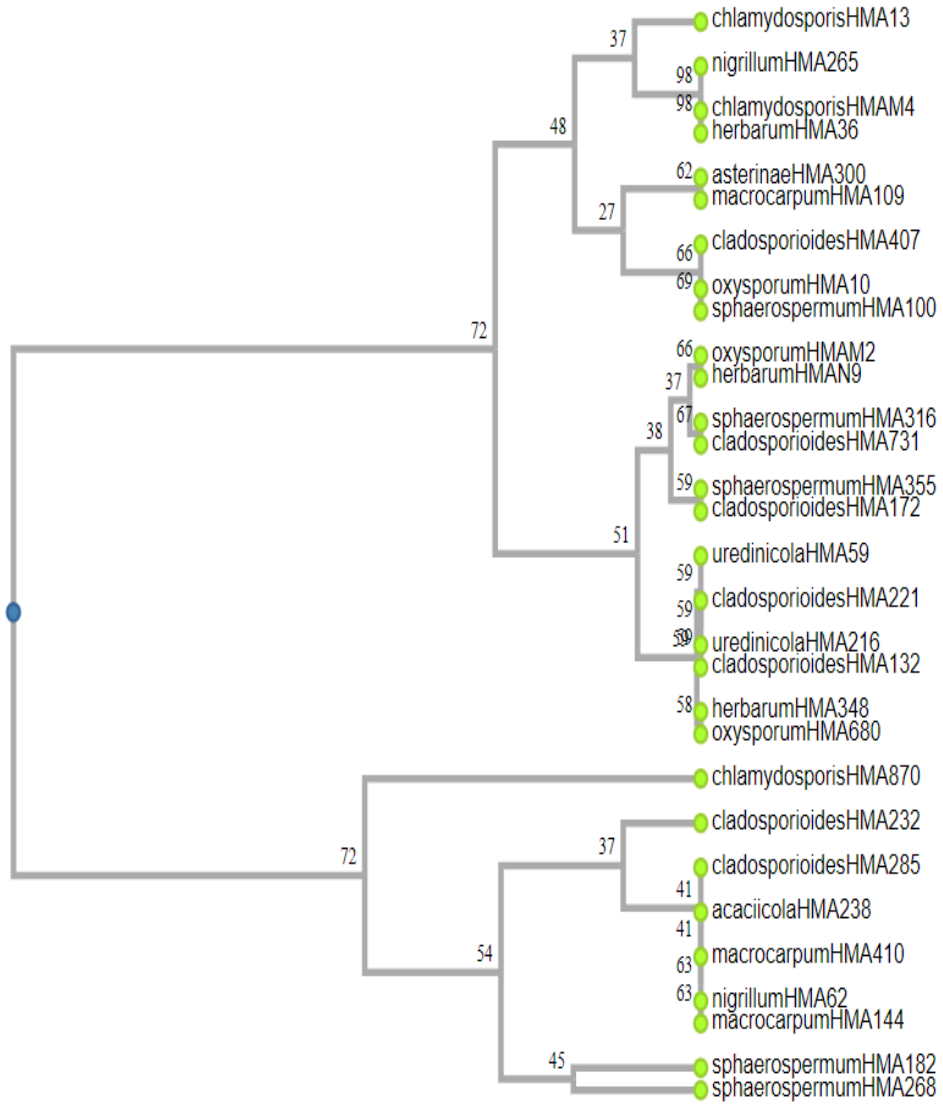

Figure 3: The Dendrogram of *Cladosporium* isolates was constructed after cluster analysis of the digitized ampilicons with *Msp*I using UPGMA

2.2.2. ITS-PCR patterns of *Cladosporium species* digested with *Rsa*I restriction enzyme.

The genetic similarity between *Cladosporium* strains calculated using Pearson product-moment correlation coefficient was ranged from 20 to 42% for inter-specific and 42 to 100 % for intra-specific comparisons. The application of UPGMA clustering produced two large clusters within the population with a branched–off at genetic similarity 20%, each consisting of several subclusters (phenons). Dendrogram of the ITS-PCR patterns digested with *Rsa*I separated the isolates of *Cladosporium* species into 3 main clusters, as represented by **figure (4)**.

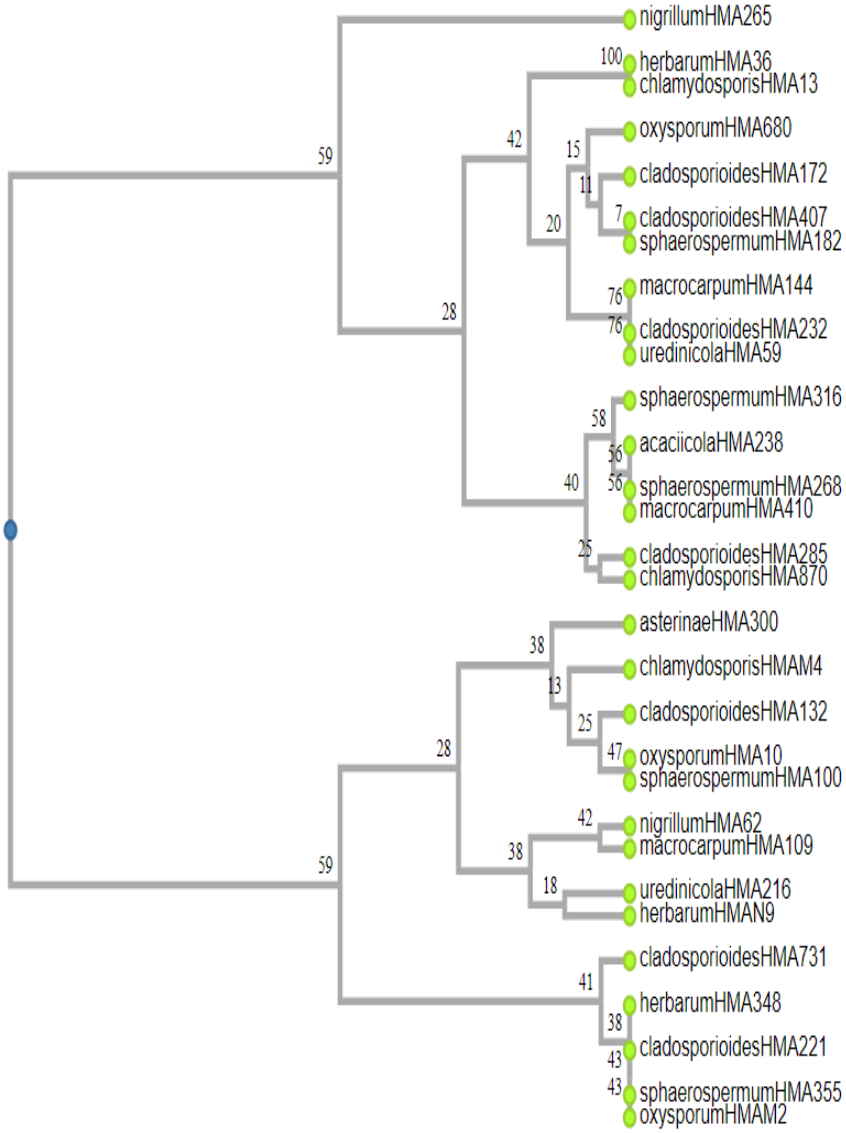

Figure 4: The Dendrogram of *Cladosporium* isolates was constructed after cluster analysis of the digitized ITS4/ITS5 -PCR and digested with *Rsa*I using UPGMA

2.2.3. ITS-PCR Patterns of *Cladosporium* species digested with *Cfo*I restriction enzyme.

The genetic similarity between *Cladosporium* species isolates ranged from 30 to 53% for inter-specific and 53 to 100% for intra-specific comparisons. The application of UPGMA clustering produced two large clusters within the population with a branched–off at genetic similarity of GS=29%, each consisting of several subclusters (phenons). Dendrogram of the ITS-PCR patterns digested with *Cfo*I separated the isolates of *Cladosporium* species into two main clusters as shown in **figures (5)**.

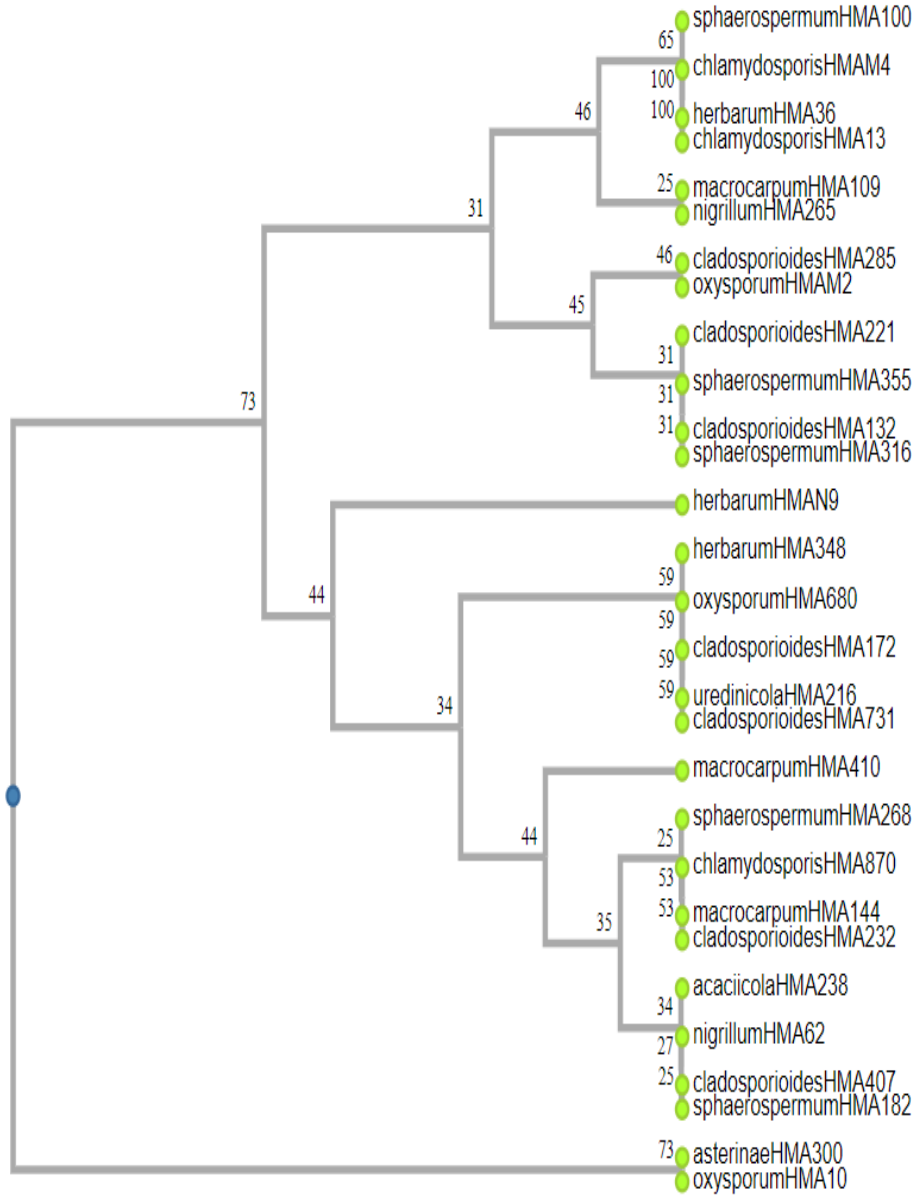

Figure 5: The Dendrogram of *Cladosporium* isolates was constructed after cluster analysis of the digitized ITS4/ITS5 -PCR and digested with *Cfo*I using UPGMA

DISCUSSION

UP-PCR markers were used to estimate the genetic relatedness among *Cladosporium* isolates. Two Universal Primed (UP) primers, individually were tested for the ability to distinguish *Cladosporium* strains. In the present study, molecular variation using Universal Primed PCR (UP-PCR) obtained by L21 primer revealed two main well-separated clusters, each one corresponding to a different species. The genetic similarity between *Cladosporium* strains, similarities oscillated between 42% to 71% for inter-specific and 71%to 100 %for intra-specific comparisons. UP-PCR markers detected a

very high level of polymorphism between and among *Cladosporium* strains. The genetic similarity by UP-PCR using Fok1 primer was more than 55% between *Cladosporium* species isolates ranged from 26 to 53 % for inter-specific and 53 to 100% for intra-specific comparisons. Our observations were comparable with that of **Maymon *et al.*, (2004)**, who reported 3 main different clusters by 76 *Trichoderma* species (genus of dematiaceous fungi closely related to *Cladosporium*) In the same context, **Bulat *et al.* (2000)**, concluded that there is another possible direction for development of gene-specific typing should be noted. In addition **Hafez *et al.* (2013); Chakdar *et al.* (2017)**, reported that universally primed PCR (UP-PCR) has been used to discriminate isolates of *Cladosporium lecanii* and *Fusicladium effusum.* This allows grouping of strains into hybridization groups which can be used to separate the strains into genetic entities with high genetic similarity. Moreover, based on similarity values, UP-PCR is a PCRcharacterization method that it is possible to amplify DNA from any organism without previous knowledge of DNA sequences and to generate multibanding profiles following gel electrophoresis **(Ashfaq *et al.*, 2020)**. Furthermore these results was much comparable with that reported by **Demissie *et al.* (2019)**, who reported that nonspecific primers reveal variability of some isolates, the corresponding PCR technique can be used to fingerprinting *Cladosporium* isolates.

In the current study, we have used the internal transcribed spacer (ITS) to aid compare ITS in length and restriction patterns. The internal transcribed spacer (ITS) was amplified using polymerase chain reaction combining primers ITS4 and ITS5. PCR products were digested with three restriction enzymes and separated by agarose electrophoresis. In the current investigation, using a universal fungal rRNA primer pair, a 743 bp fragment was successfully amplified from all *Cladosporium* isolates. This result was consistent with that of **Asl *et al.* (2017)** by using a universal fungal rRNA primer pair, a 700–800bp fragment was successfully amplified from all the isolates, while no PCR amplification was observed in negative controls. In addition, these observations were in the same line with that of **Dean *et al.* (2005)**, who analyzed the genera *Stachybotrys*, *Penicillium*, *Aspergillus*, and *Cladosporium* in order to identify and characterize by simple ITS method, in which each organism underwent ITS-PCR that amplified ribosomal sequences generating products from 550 to 600 bp followed by enzymatic digestion with *Eco*RI, *Hae*III, *Msp*I, and *Hinf*I, and show that using this combination of restriction enzymes enables the identification of these fungal organisms at the species level. Results of the current investigation showed moderate genetic similarity between *Cladosporium* isolates ranged from 50-70 % for inter-specific and 70-100 % for intra-specific comparisons. The present results indicate that, the dendrogram constructed with ITS-PCR digested with *Msp*I revealed that all isolates of *C. cladosporioides* and almost *C. herbarum* were grouped into a major 9 cluster delimited from other *Cladosporium* species comprising four molecular groups with genetic dissimilarity 30%. These results were similar to that of **Kawasaki *et al.* (1993)** that was conducted on *Cladosporium carrionii* and classified the 38 isolates into 4 mtDNA types (Type I to Type IV) based on the restriction patterns with *Msp*I, *Sau3A*I and *Hae*III. ITS-PCR digested with *Rsa*I revealed that the genetic similarity between *Cladosporium* species isolates ranged from 20 to 42 % for inter-specific and 42 to 100 % for intra-specific comparisons. The application of UPGMA clustering produced 3 large clusters within the population with a branched–off at genetic similarity of GS=20 %, each consisting of several sub clusters (phenons). Dendrogram of the ITS-PCR patterns

digested with *Rsa*I separated the isolates of *Cladosporium* species into two main clusters. The genetic similarity between *Cladosporium* isolates ranged from 50 to 68% for inter-specific and 68 to 100% for intra-specific comparisons, these results were inconsistent with **Segers *et al.* (2015)** who reported very low genetic similarity (23% for inter-specific comparisons) among *Cladosporium* species using *Rsa*I restriction enzyme after amplification of ITS. The average of genetic similarity based on ITS-PCR patterns digested with *Cfo*I was approximately 50%. Dendrogram of the ITS-PCR patterns digested with *Cfo*I separated the isolates of *Cladosporium* species into 8 main clusters. The first main cluster included all *Cladosporium cladosporioides* isolates at the genetic similarity (GS=76 %). These observations were consistent with **Moslem *et al.* (2010)** study that revealed moderate genetic similarity among *Cladosporium* species using some restriction enzymes included *Cfo*I. On contrary, **Messini *et al.* (2017)** study that showed very low genetic similarity 27% and high molecular heterogeneity among *Cladosporium* species using *Cfo*I for restriction of ITS ampilicons. Our results were consistent with those based on biological characteristics and morphological features. In harmony with our results, ITS molecular marker technique of ITS region represents a possible method for the classification of *Cladosporium* species **(Troche, 1997).** Additionally, the ITS restrictions profiles showed a great genetic similarity between *C. cladosporioides* and almost of *C. sphaerospermum* isolates. In the study carried out by **Park *et al*. (2004)** the sequences of the D1/D2 regions of the LSU rDNA genes and the ITS regions of the rDNA were employed in order to establish molecular standards for the demarcation of the common airborne species *C. herbarum*, *C. cladosporioides* and *C. sphaerospermum*.

CONCLUSION

From the current work, we can conclude that ITS molecular marker technique of ITS region represents a possible method for identification of *Cladosporium* on genus and species level, in addition, subsequent digestion with *Rsa*I was more beneficial than *Cfo*I, *Msp*I, enzymes as a tool for discrimination between *Cladosporium* strains within the same species. Moreover, UP-PCR is a simple method for genotyping of *Cladosporium* species especially when L21 primer used, over than Fok1.

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دراسه ميكروبيولوجيه علي سلالات الكلادوسبوريوم المعزوله من هواء القاهره, بإستخدام تقنيات طرق تفاعل البلمره المتسلسل للوحدات ITS-PCR و UP-PCR

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فطر كلادوسبوريوم هو واحد من أكبر أجناس الفطريات و أكثرها انتشارًا. وذلك بسبب إنتاج أبواغ متفرعة ووفيرة و أيضا بسبب القدرة على النمو على أقل المعطيات الغذائيه تستطيع النمو في أي مكان تقريبًا, ومن اشهر السلالات *C. herbarum, sphaerospermum, C. cladosporioides* و *C. elatum* . في هذه الدراسه, تم عزل عينات الفطريات من مناطق مختلفه وظروف مناخير متباينه من القاهره, وخضعت هذه العينات للعزل والتنقيه والتعرف علي فطر كلادوسبوريوم باستخدام الوسائل الظاهرية والمجهريه وأيضا اعتمادا علي نتائج التفاعلات الكيميائيه, وتم تأكيد تعريف الفطريات في المركز الإقليمي للفطريات والتقنيه الحيويه بجامهة الأزهر. تهدف الدراسه الحاليه لبحث التنوع البولوجي والجزيئي بين سلالات فطر كلادوسبوريوم وذلك باستخدام تقنيه تفاعل البلمره المتسلسلب UP-PCR بإستخدام بادئ L21 و Fok1, تم الاعتماد علي نظام UPGMA لمعرفة مدي التشابه و الإختلاف بين السلالات . اسفرت النتائج. اسفرت النتائج عن وجود تشابه بين سلالات فطر كلادوسبوريوم باستخدام بادءات L21 بنسبة 71%-42 بينما كانت النسبه % 60- 73باستخدام بادءات Fok1 كما يشير العمل الحالي إلى أن طريقة MP-PCR و UP-PCR مناسبة لتوصيف مجموعات كبيرة من سلالات *Cladosporium* نظرًا لبساطتها وكفائتها. تعتبر طريقة (ITS-PCR)مناسبة تمامًا للمقارنة بين الكائنات الحية ذات الصلة الوثيقة. هذه المنطقة غير المشفرة هي متعددة الأشكال للغاية وتوفر أداة مفيدة للدراسات التصنيفية والتاريخية. تم استخدام تحليل منطقة أنظمة النقل الذكية لتمييز الأنواع والأقسام التصنيفية الأعلى. تم استخدام بصمة الحمض النووي لدراسة عدة أجناس. تم استخدام تعدد أشكال طول القطع (RFLP) لمنطقة ITS للتمييز بين الأنواع من نفس الأنواع أو بين الأجناس المختلفة. تمت دراسة العديد من الأنواع من أجناس مختلفة، في العمل الحالي ، استخدمنا (ITS-PCR)للمساعدة في مقارنة ITS في الطول وأنماط الفصل. باستخدام تفاعل البلمرة المتسلسل الذي يجمع بين البادئات ITS4 و .ITS5 تم تجزئة منتجات PCR بثلاثة إنزيمات فصل. كانت أنماط التقييد الناتجة عن *Rsa*I و *Msp*I و *Cfo*I فريدة بالنسبة لمعظم الأنواع. تم الحصول على نتائج واضحة باستخدام ITS-PCR في الدراسة الحالية ، كانت النتائج متسقة مع تلك القائمة على الخصائص البيولوجية والسمات الظاهريه و ITS-PCR هنا أدت إلى تمايز واضح للعزلات على مستوى الأنواع. تم إنشاء ملفات تعريف سمات جينيه التي تم تمييزها بسهولة بين كل من الأنواع العشرة.

الكلمات المفتحيه: دراسه ميكروبيولوجيه, تفاعل البلمره المتسلسل, Internal Transcribed Spacer, Universally Primed