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Genetic Diversity Using Random Amplified Polymorphic DNA (RAPD) Analysis for *Aspergillus niger* isolates

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Abstract

This study includes isolation, purification, and identification of *Aspergillus niger* from different sources (soil, Seeds, powdered milk and factory waste water) by traditional methods (macroscopic and microscopically). and study the relationships of genetic among the *A. niger* isolates based PCR (RAPD) technique (find the DNA fingerprint). The results of this study revealed that genetic diversity and relationships among twenty four isolates were determined by using the random amplified polymorphic DNA (RAPD) technique. Genomic DNA of each isolates was extract at final concentration of (115-959) µg /l per (2-3) gm of wet mycelium, and at a purity (1.7-1.9). Each DNA of isolates were amplified with each 24 primers and the products were resolved electrophoretically on 1.2% agarose gel, stained with ethidium bromide and photographed under UV light. Ten primers failed to amplify DNA of *A. niger* isolates but the rest 14 primers produced 367 bands, of these bands 99.4% (365) bands were polymorphic. The size of the amplified bands ranged between 130-2840 bp. The genetic polymorphism value of each primer was determined and ranged between 86-100%. All primer have given unique bands. The primers were fingerprinted 21 of the 24 isolates. Genetic distances ranged from 0.11496 to 0.68088 between *A. niger* isolates. Also, cluster analysis of genetic distances between these isolates have divided into groups according to the source, which isolated them and this indicates the presence of a high level of genetic kinship samples isolated from the same source at the same time which indicates the presence of a high level of genetic distance between samples isolated from different sources**.**

Keywords: DNA, RAPD, genetic distance, *Aspergillus niger*

التغايرات الوراثية باستخدام مؤشرات التضاعف العشوائي لعزلات فطر الرشاشيات الاسود

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الخلاصة

تضمنت الدراسة الحالية عزل وتنقية وتشخيص فطر الرشاشيات الاسود من مصادر مختلفة (التربة، البذور، الحليب المجفف، فضلة مياه صناعية) باستخدام الطرق التقليدية التي تعتمد على المظهر الخارجي للمستعمرة وشكل الفطر تحت المجهر الضوئي و ايجادالعلاقة الوراثية للعزلات باستخدام تقنية مؤشرات التضاعف العشوائي متعدد الاشكال للدن(ايجاد البصمة الوراثية)، وجرى استخلاص الدنا من كل عزلة وبتركيز نهائي قدره (115–959) مايكروغرام/مللتر لكل 2–3 غرام من الغزل الفطري الرطب وبنقاوه (1,7–1,9). تم ايجاد العلاقة الوراثية بين 24 عزلة من فطر الرشاشيلت الاسود بواسطة 24 بادئا وفرزت النتائج بالترحيل

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الكهربائيا خلال هلام الاكاروز ذي تركيز 1,2% والمصبغ بالمادة المتالقة بروميد الاثيديوم والتصوير تحت الاشعة فوق البنفسجية ، وقد اخفق عشرة من البوادئ في دعم عملية التضاعف بينما افلح 14 بادئ اخر في ذلك. كل البواديء اعطت بصمة وراثية، ولقد استطاعت تقنية التضاعف العشوائي بتحديد البصمة الوراثية 21عزلة من العزلات الاربعه وعشرين المعزولة وذلك من خلال ظهور حزم مؤشرات فريدة اثناء مضاعفة الدنا لكل عزلة مع واحد او اكثر من البوادئ الاثنى عشر، البعد الوراثي يتراوح بين (0,68088−0,11469) بين عزلات فطر الرشاشيات الاسود المدروسة وقد تجمعت العزلات الاربعة والعشرين في المخطط الشجري في مجموعتين رئيسيتين. كما أن التحليل العنقودي للمسافات الوراثية بين قد قسم العزلات في مجموعات حسب المصدر التي عزلت منها وهذا يدل على وجود مستوى عال من القرابة الوراثية للعزلات المعزولة من نفس المصدر و في نفس الوقت يدل على وجود مستوى عال من البعد الوراثي بين العزلات المعزولة من مصادر مختلفة.

Introduction

The genus *Aspergillus* comprises about 175 species. *A. niger* is one of the most common *Aspergillus* species that are among the most abundant fungi worldwide. Soil and decaying material are the natural habitats of Aspergilli and they were considered important food spoilage fungi . Although most Aspergilli normally exhibit a saprophytic life style [1]. *A. niger*, like other fungi, secretes a wide variety and large amounts of enzymes. These enzymes degrade dead and living organic material into molecules that can be taken up to serve as nutrients. Protein secretion is thus an essential step in nutrient uptake and might therefore explain the exceptionally high secretion capacity of filamentous fungi in general and *A. niger* in particular. Apart from enzymes, *A. niger* secretes large amounts of organic acids such as citric acid [2] Traditional methods for species identification were mainly based on morphological parameters, including colony diameter, color and texture, size and texture of conidia and conidiophore structure [3-4] However, species identification may be difficult due to extensive divergence of morphological characters produced by a high level of genetic variability both inter- and intraspecific. [5] Despite intense investigation, the identification of this group of fungi is still highly complex and the genetic techniques to detect the level of polymorphism and similarity among species help minimize the problem. Molecular markers provide additional tools for germplasm characterization and assessment of genetic relatedness and diversity in collections. DNA markers are particularly useful for characterizing individual genotypes and selection of the parents for successful hybridization. Among all molecular markers mentioned above, RAPD technique of DNA fingerprinting have been used in the present study, which is widely used in conservation biology because of quick results, cost-effectiveness, easy analyzed results and reproducibility. The PCR-based RAPD approach by using arbitrary primers requires only little quantities of DNA template, no radioactive probes, and is relatively simple compared to other techniques [6-8]The Aim of the study investigation of the genetic diversity, relationships and attempt to find the DNA fingerprint of isolates by PCR-RAPD technique, in order to identify the various isolates and studying genetic distance between *A. niger* isolates.

Materials and Methods

Samples collection

1. Soil samples

Ten soil samples (at depth 4-5cm) were collected from different places of Baghdad University gardens by putting sterile polythene bags.

2. Seeds samples

Forty-five samples of seeds (pistachios10, Ground nut10, walnuts10, Almonds10 and cashew5) were collected from different Baghdad markets at rate (50 g), transferred in sterile packets to the laboratory and kept in a cool place (3 - 5°C).

3. Powdered milk samples

Eighteen samples of instant full cream milk powder (Almudhish, Anchor, Diallaak) were collected from markets (500 g).

4. Factory waste water

Five samples of water were obtained from batteries factory in Baghdad, the samples were put in dry, sterile polypropylene bottles and stored in the refrigerator (4°C) till the isolation of fungi.

Isolation of fungi from

1. Soil and milk

Series dilutions method wase used for isolation fungi from soil and milk by taken 5g from each soil and milk samples individually, and put into flask (100ml) contain 50 ml sterile distilled water and shaken for making suspensions and dilutions, after that, was taken 1ml from dilution (of each soil and milk) and spread on autoclaved PDA plates, and incubated at 28±2 °C, after 3 days the growing colonies of fungi isolates were recultured on PDA plates individually and incubated at 28±2°C for 5 days for identification.

2. Seeds

Fifty seeds (per each sample) were surface-disinfected with 1% sodium hypochlorite solution for 1 min and then rinsed in sterile distilled water three times. Five seeds were then placed onto the surface of agar plates containing potato dextrose agar (PDA). All plates were incubated for 3 days at 28±2°C, after 3 days the growing colonies of fungi isolates were recultured on PDA plates individually and incubated at 28±2°C for 5 days for identification [9].

3. Factory waste water

Fungi were isolated from factory waste water by a series of dilution. 5ml were taken from collected samples and put into flask (100ml) contain 50 ml sterile distilled water and shaken for making suspensions, after that, 1ml was taken from dilution and spread on autoclaved PDA plates and incubated at 28±2 °C, after 3 days the growing colonies of fungi isolates were recultured on PDA plates individually and incubated at 28±2 °C for 5 days for identification.

Fungal DNA extraction

For DNA isolation cultures were inoculated on the PD broth medium and flasks were put on the shaker at 28± 2°C for 3 days. Culture media were filtered and mycelia were used for genomic DNA extraction by using genomic DNA reagent kit (Geneaid Biotech Ltd., Taiwan, Cat # GR100). Genomic DNA was isolated from 2-3 g mycelium. The purified DNA samples were stored at -20˚C for subsequent downstream studies.

Primer selection and RAPD assay

Twenty four of primers random sequencewere used (Bioneer, operon primers). The primers dissolved in sterile deionizer distilled water to give a final concentration of (10pmol/µl) as recommended by provider the primers were tested in this study show Table-1. The 50 µL RAPD mixture contained PCR buffer (10x), MgCl2 (1.5 mM), dNTPs (0.2 mM), primer (25 pmol/µL), taq polymerase (2.5 U), genomic DNA (1.0 µg). PCR tubes with 50 µL reaction mixture were placed in the PCR thermocycler (Gene Amp Biosysytem-9700, Applied Bio-system) and following temperature cycling conditions were programmed; initial denaturation at 94˚C for 5 min, followed by 40 cycle denaturation at 94oC for 1 min, annealing at (35oC) for 1 min, primer extension at 72˚C for 1 min and final extension temperature 72˚C for 5 min. Twelve micro liter of PCR products were analyzed by electrophoresis in a 1.2% agarose gels at 50 Volt/cm for 2 hour in 0.1xTBE buffer, agarose gels were stained with ethidium bromide 1.2 µg/ml for 2h. The 100bp DNA ladder (100 2,000) bp (Bioneer) was used as a molecular size marker. After electrophoresis, images of gels were captured using Gel Documentation System (Consort - Belgium).

Table 1- Numbers and sequences of the RAPD primers used

No.	Primer name	Sequence	GC content %
1	OPA-09	**3¯**- GGGTAACGCC - **5¯**	**70**
2	OPA-12	**3¯**- TCGGCGATAG - **5¯**	**60**
3	OPA-13	**3¯**- CAGCACCCAC - **5¯**	**70**
4	OPA-18	**3¯**- AGGTGACCGT - **5¯**	**60**
5	OPB-05	**3¯**- TGCGCCCTTC - **5¯**	**70**
6	OPB-10	**3¯**- CTGCTGGGAC - **5¯**	**70**
7	OPB-17	**3¯**- AGGGAACGAG- **5¯**	**60**
8	OPG-04	**3¯**- AGCGTGTCTG - **5¯**	**60**
9	OPG-11	**3¯**- TGCCCGTCGT - **5¯**	**60**
10	OPG-17	**3¯**- ACGACCGACA- **5¯**	**60**
11	OPH-01	**3¯**- GGTCGGAGAA- **5¯**	**60**
12	OPH-03	**3¯**- AGACGTCCAC - **5¯**	**60**
13	OPH-08	**3¯**- GAAACACCCC - **5¯**	**60**
14	OPH-18	**3¯**- GAATCGGCCA - **5¯**	**60**
15	OPM-05	**3¯**- GGGAACGTGT - **5¯**	**60**
16	OPM-18	**3¯**- CACCATCCGT - **5¯**	**60**
17	OPO-18	**3¯**- CTCGCTATCC - **5¯**	**60**
18	OPQ-07	**3¯**- CCCCGATGGT - **5¯**	**70**
19	OPQ-08	**3¯**- CTCCAGCGGA - **5¯**	**70**
20	OPQ-09	**3¯**- GGCTAACCGA - **5¯**	**60**
21	OPQ-15	**3¯**- GGGTAACGTG - **5¯**	**60**
22	OPZ-02	**3¯**- CCTACGGGGA - **5¯**	**70**
23	OPZ-07	**3¯**- CCAGGAGGAC- **5¯**	**70**
24	OPZ-08	**3¯**- GGGTGGGTAA- **5¯**	**60**

Data analysis

Molecular Weight Estimation

In this step we used ladder from Bioneer company 2 kbp (which consist of 13 bands from 100 to 2,000 bp Bioneer).its own molecular weight were known, then curve drawing. The molecular weight of the band of ladder on the Y axis and the distance in the gel on the X axis (from the well to the beginning of each band), measured the distance of the sample on the Agarose gel. Then volume will be strait on the curve from the sample and from the intersection cross volume on the Y axis as [10].

Analysis of RAPD specific primer product

The band that produced from the Amplification has been calculated from the images of the electrophoresis on the Agarose gel since the signal (1) mean found of the band and (0) mean the absence of the band with calculate molecular weight.

Estimation the polymorphism percentage of for RAPD primers

Data generated from the detection of polymorphic fragments were analyzed. The amplification profile of all the used varieties for any given primer was scored as "1" with the presence of each band and was scored as "0" with the absence of the same band of the same size in other isolates [11]. Only major bands consistently amplified were scored. Polymorphism of each primer was calculated based on the following formula:

Polymorphism % = (Np/Nt)×100

(Np)= the number of polymorphic bands of random primer

(Nt)= the total number of bands of the same primer.

Estimation the efficiency of primers and the discriminatory power:

Efficiency discriminatory power of each primer calculated according to the formula below:

Efficiency of primer = total number of primer bands / total number of all primer bands according to [12]

Primer efficiency ranged between (0-1).

Discrimination power= (total number of primer bands / total number of all primer bands) ×100% [13].

Results and Discussion

Fungus identification

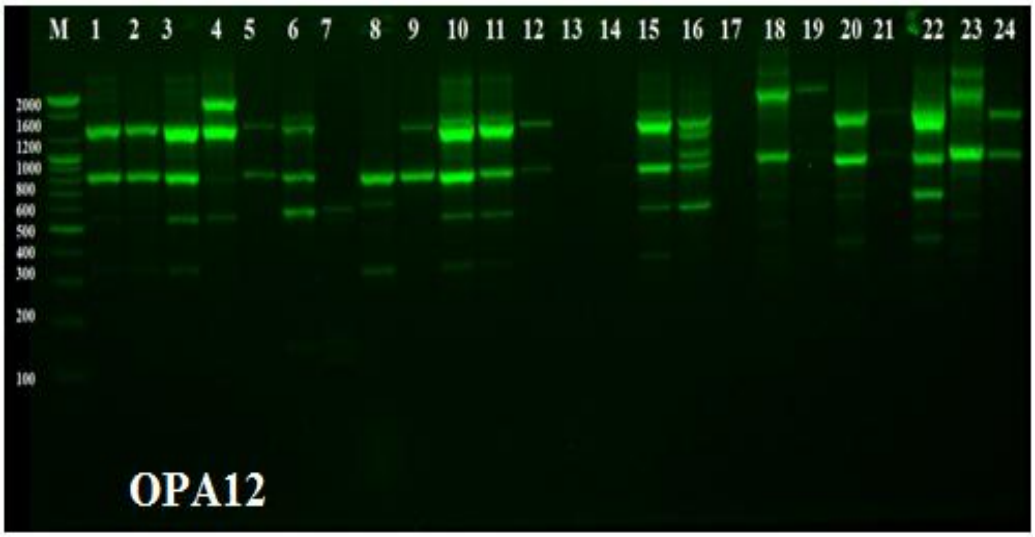
Pure cultures of the isolated fungi were identified on the bases of morphological and microscopically characteristics according to the key of [14, 15].

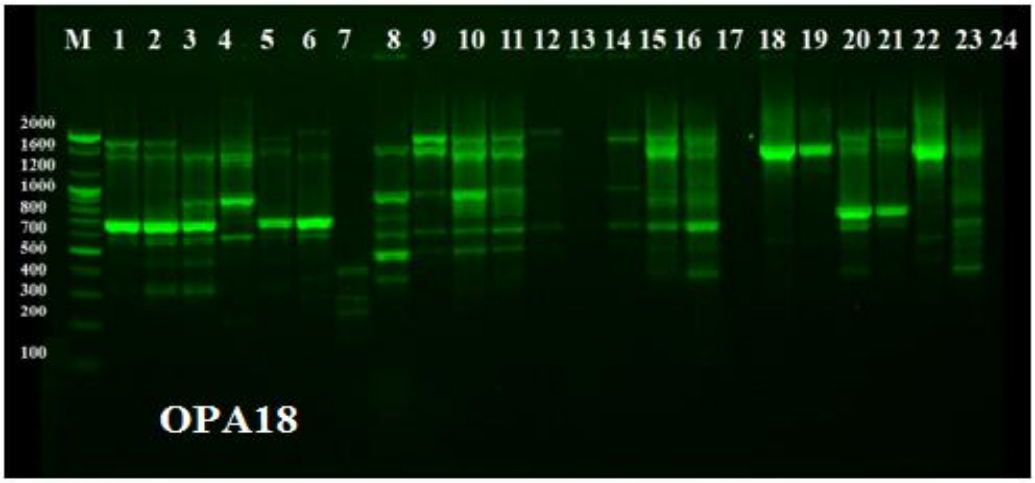
DNA extraction

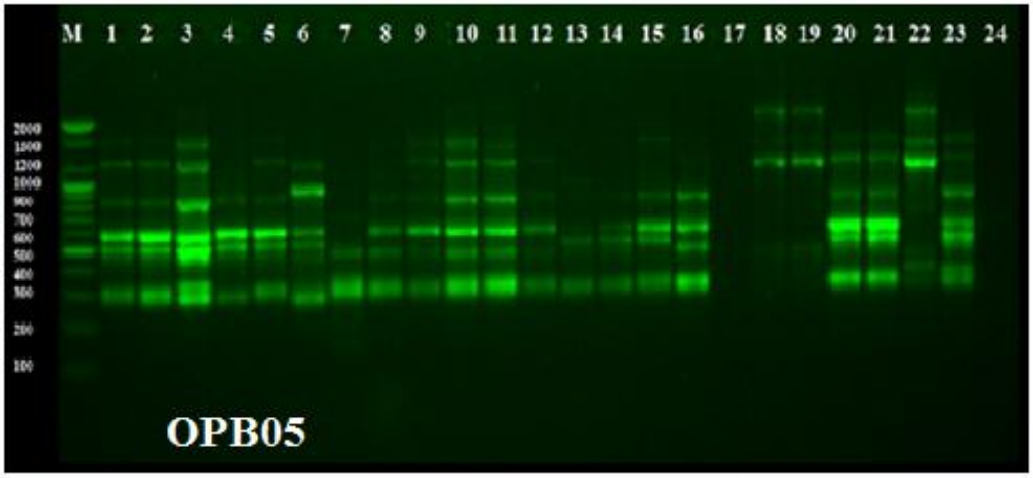
The DNA was extracted efficiently by using commercial kit (Geneaid–Taiwan). Purity and concentration of DNA were measured using the nanodrop. The yield of the DNA extracted from the *A. niger* isolates was in range of (115-959) µg per gram with purity of (1.7-1.9).

RAPD-PCR Analysis

In this study, 24 primers were used; ten primers did not give result (OPA-09, OPA-13, OPG-04, OPG-11, OPH-01, OPH-08, OPM-18, OPQ-07, OPZ-02, and OPZ-08) because there primers do not find the priming site in the genomic DNA of *A. niger* isolates [16] but the rest 14 primers produced bands Figure-1.



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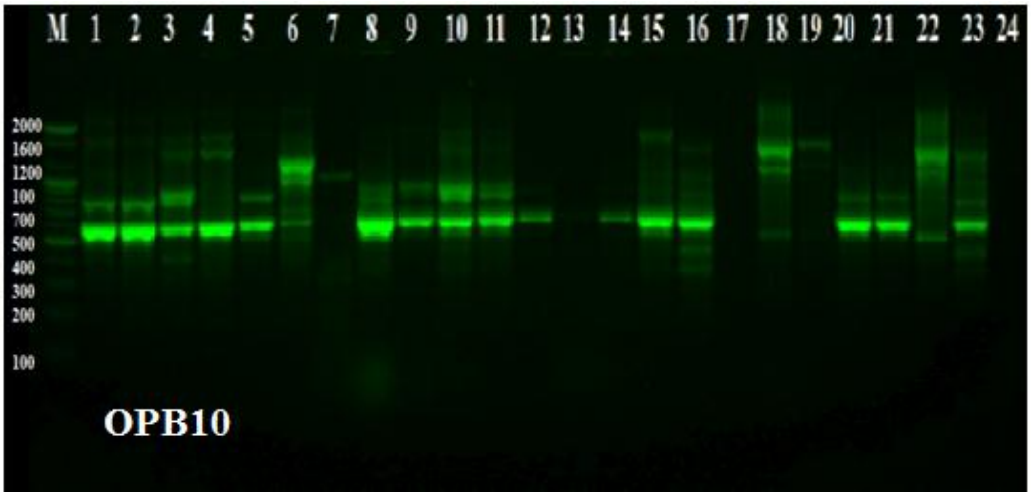
OPB05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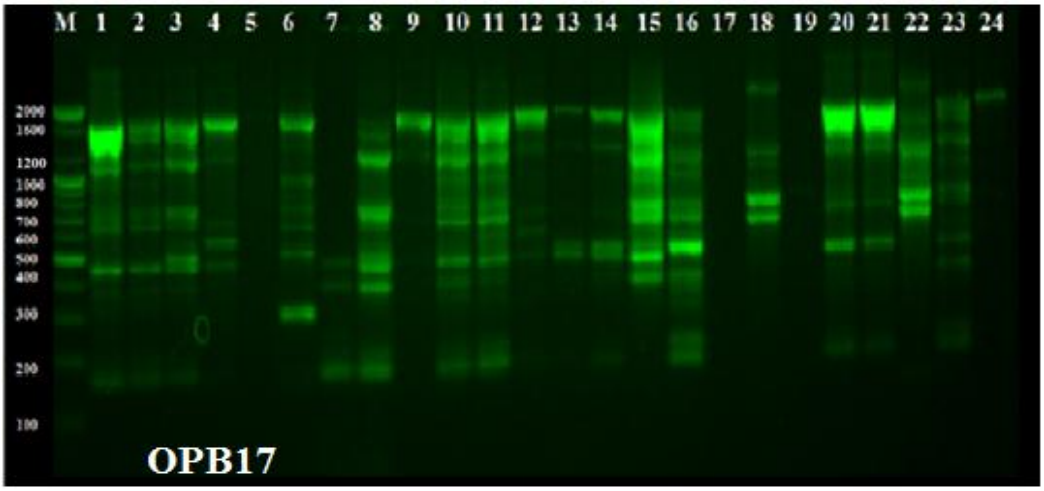
2000
1600
1200
100
700
500
400
300
200
100
OPB10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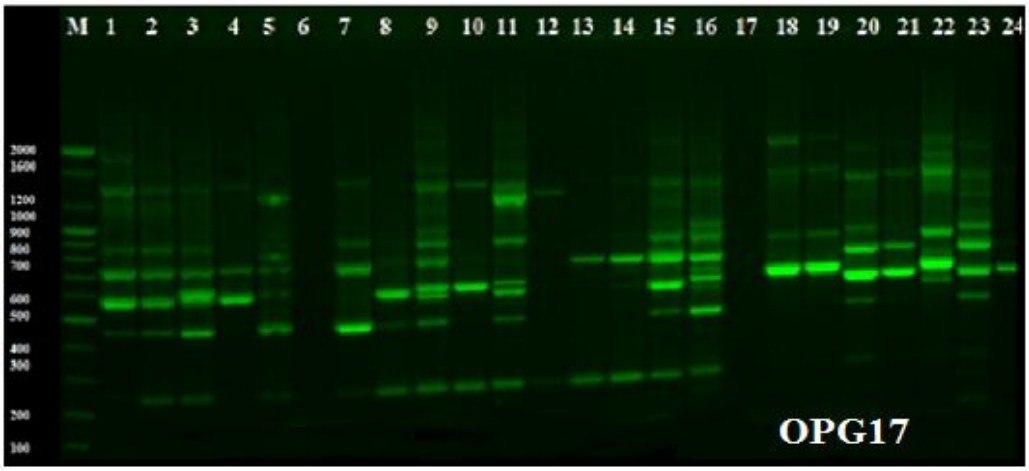
OPB17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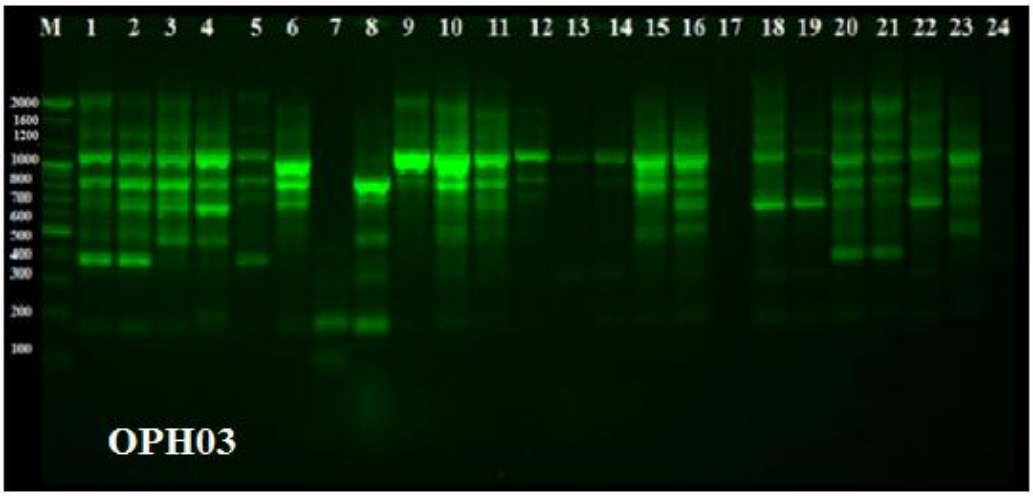
OPG17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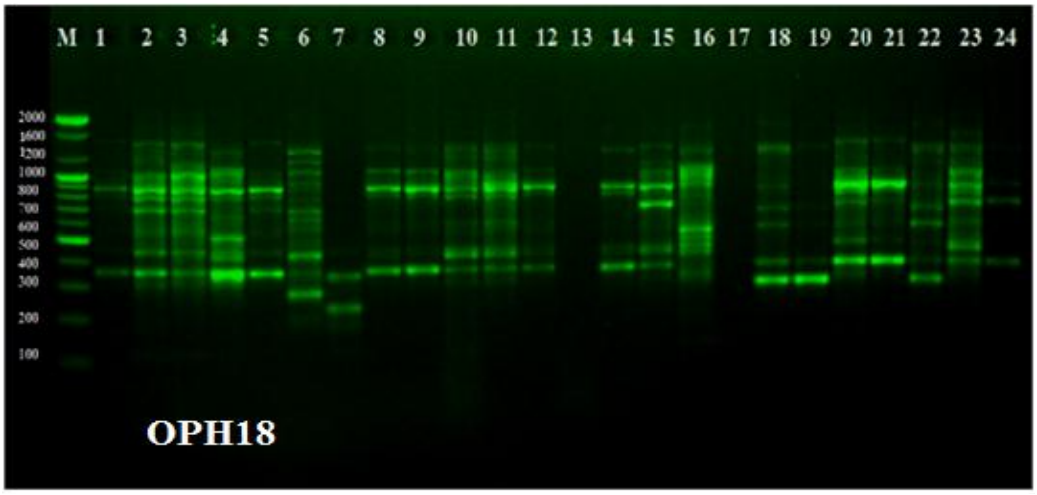
OPH03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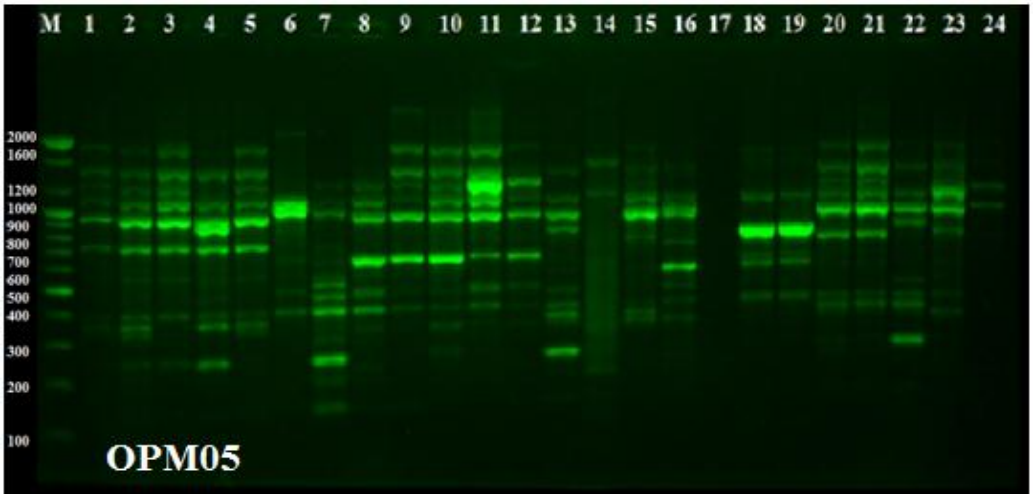
OPH18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1600
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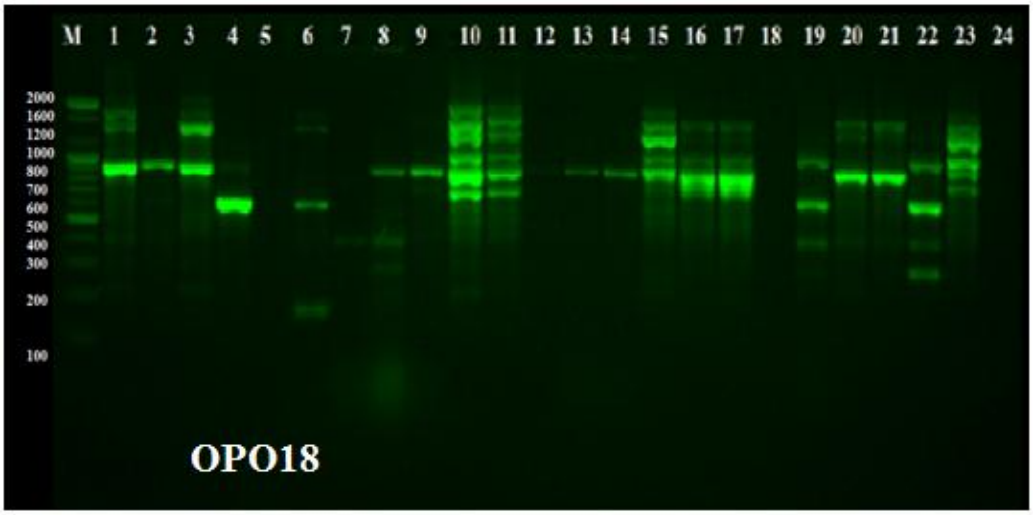
1000
900
800
700
600
500
400
300
200
100
OPM05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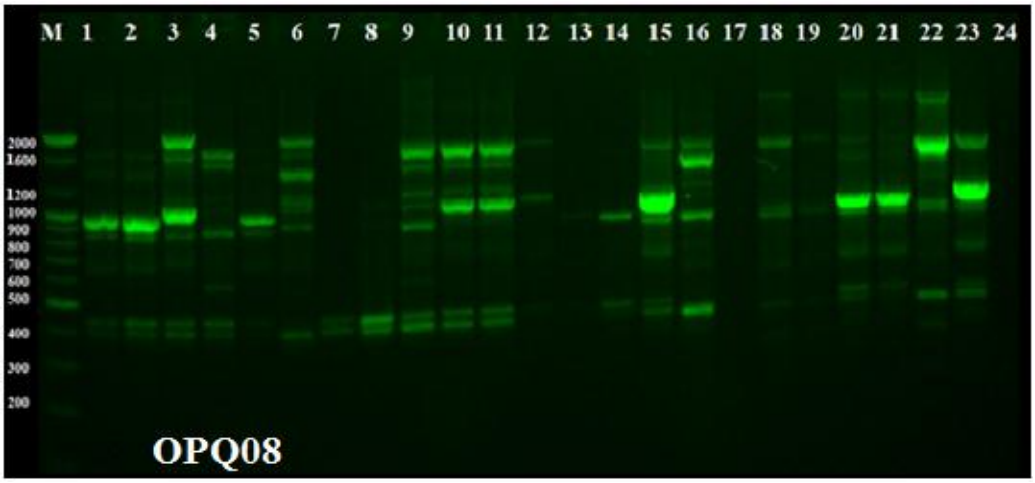
300
200
100
OPO18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OPQ08

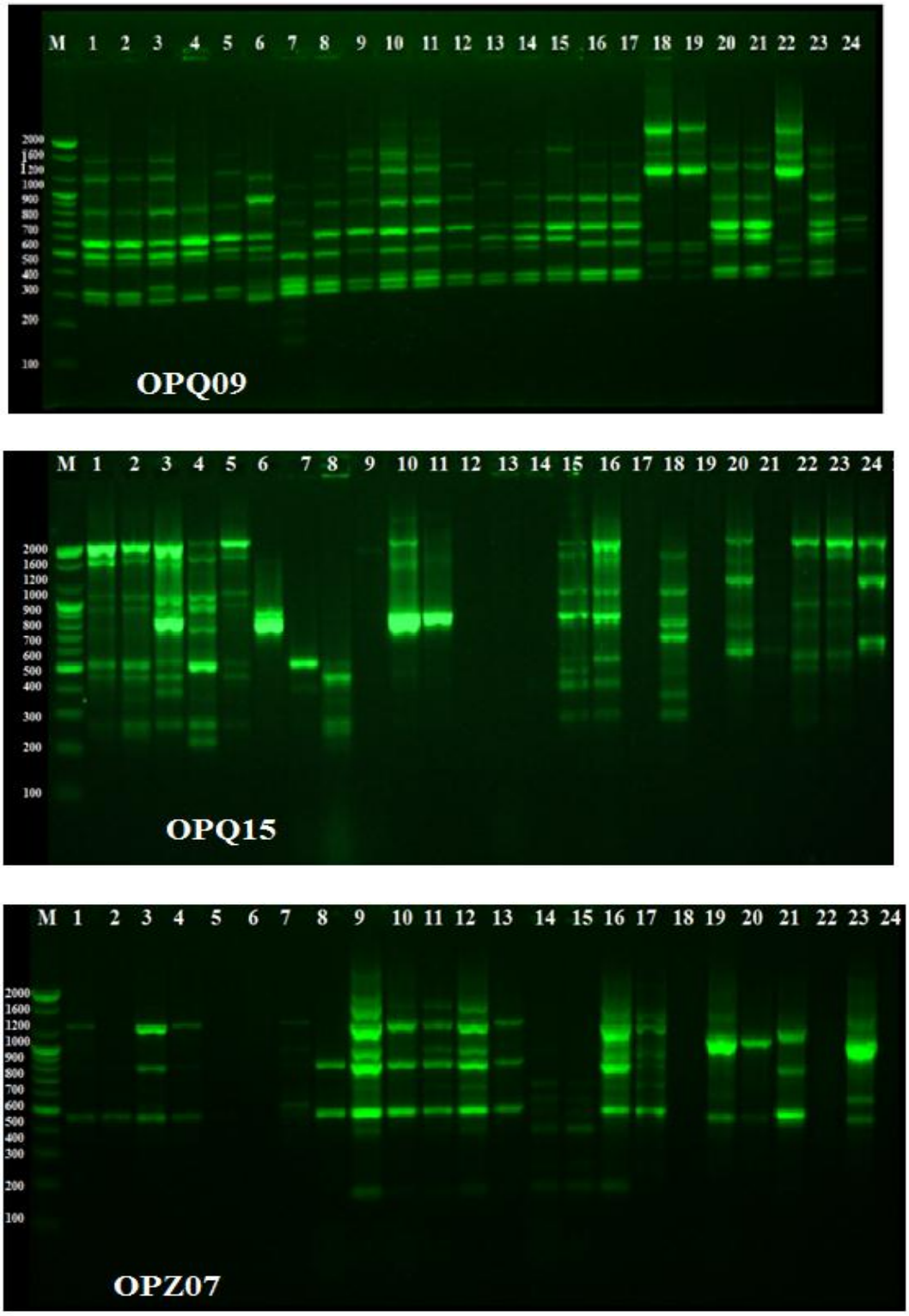

Figure 1- Agarose gel electrophoresis with ethidium bromide of RAPD-PCR reaction 14 primers (OPA12, OPA18, OPB05, OPB10, OPB17, OPG17, OPH03,OPH18, OPM05,OPO18, OPQ08, OPQ09, OPQ15, OPZ07), respectively. DNA samples of *A.niger* isolates (under optimal conditions). Bands were fractionated by electrophoresis on a 1.2% agarose gel (2hr, 50V/cm, 1XTris-borate buffer) and visualized under U.V. light. M: 100 bp DNA ladder. Lanes : *A.niger* isolates from different source : Lanes 1,2,3,4,5 (Soil), Lanes: 6-15 (seeds): 6,7.8.9 (Ground nut), 10,11(Pistachio),12,13,14 (Walnuts), 15,16 (Cashew), Lanes:,17,18,19,20 (Almonds), Lanes: 20,21 (Milk powdery), Lanes:22,23,24 (Factory waste water).

Table-2 revealed the fourteen random primers produced total of (1861) bands across 24 isolates, they were distributed into (367) main bands. Only two main bands were monomorphic, while the rest 365 main bands were polymorphic.The primer OPG17 was generated highest number of main bands (40), while OPB05 was lowest (12) bands. The variation in the number of bands amplified by different primers is influenced by variable factors such as primer structure and less number of annealing sites in the genome [15]. The ratio of polymorphism was ranged between (86-100%), primers (OPA12, OPA18, OPB05, OPB10, OPB17, OPG17, OPH03, OPH18,OPO18, OPQ08, OPQ15 and OPZ07) produced the highest percentage of polymorphism 100%, while primer (OPQ9 and OPM05) produced lowest percentage of polymorphism (86, 87) respectively. As well as noted in the table that the efficiency of primer was ranged between (0.032-0.108), primer OPG17 had achieved a highest efficiency, while the primer OPB05 a lowest efficiency. The primer efficiency represented the ability of primer to appear polymorphic between isolates and not means gave high number of amplified bands [15]. Discriminatory power was ranged between (3.2-10.8), the primer OPG17 had given a highest discriminatory power while the primer OPB05 a lowest discriminatory power. Discrimination power represented the ability to descry and diagnosis of the isolates. From the above results we noted that the proportion of G-C not affected on the discriminatory power, efficiency of primers and polymorphism percentage.

The RAPD assay generated isolates specific products in some of the genotypes. These may be used as DNA fingerprints for isolates identification. It would be of immense use for the establishment of proprietary rights and the determination of isolates purity. In this study, there were several unique bands were generated, unique bands means that presence of band in specific isolates and absence of the same band of the same size in all other isolates (Bertout *et al.,* 1999).

Fingerprint determined for twenty one isolates by the presence of a unique band, the isolates (7 and 22) recorded a highest number (8) of unique bands which amounted to, while the isolates (1, 2, 11 and 14) given only one unique band. All primer given unique bands, the primer OPG17 determined highest number of fingerprint, while the primer OPB10 determined lowest number. The isolates (17, 19 and 21) had not given unique bands therefore adopted to determined fingerprint through the absence of a common band that is present in all other isolates amplified by the primer.

In this study, the results of RAPD –PCR analyses of fourteen primers had revealed DNA polymorphism in genomic DNA from *A. niger*. The polymorphism resulted from differences in number of bands or in molecular weight of amplified bands. The variation in the number of amplified bands is influenced by the primer binding sites with genomic DNA. Also there was a difference in the molecular weight of amplified band. Polymorphism in the molecular weight of amplified bands demonstrated the differences in the length between the primer binding sites and the genomic DNA, and resulted in a mobility shift of bands [14

. These results were consistent with [17] they founded sixteen *A. niger* aggregate isolates collected from different crop fields were subjected to RAPD-PCR using 20 operon primers and 8 synthetic primers twenty-two primers led to the amplifi cation of 727. Two bands were monomorphic, while the rest were polymorphic. To study genetic variation between species, RAPD-PCR is a powerful tool [18]. To differentiate different strains of *A. niger* and other strains random primers have been successfully employed worldwide [19-21]

Table 2- Total bands, polymorphic bands and unique bands of each RAPD primers that observed in 24 isolates with efficiency and discriminatory power of RAPD primers

Operon code	No. of bands amplified in all 24 isolates of *Aspergillus niger*				Primer efficiency	Discriminatory power %	Isolates of A niger	Unique bands molecular weight (base pair)
	Total main bands	Total bands	Polymorphic bands	Proporation of polymorphic loci (%)				
OPA12	20	100	20	100	0.054	5.4	4, 8, 10, 16, 18, 23	2840; 560; 1330; 1410, 2425, 460, 290; 790, 260
OPA18	19	109	19	100	0.051	5.1	7,12,13,16, 23	520; 710; 1450; 1170; 500, 400
OPB05	12	127	12	100	0.032	3.2	5,6,7,12,13 ,22	320; 960; 700, 1560; 1280; 1580, 1445, 820, 625, 440
OPB10	13	82	13	100	0.035	3.5	3, 6, 22	880; 710; 580
OPB17	20	135	20	100	0.054	5.4	1, 15, 22, 23, 24	800, 370, 350; 1520; 2030; 1710; 1850
OPG17	40	138	40	100	0.108	10.8	2, 3, 5, 7, 8, 9, 10, 12, 13, 15, 16, 20, 22, 23	1120; 595; 800,250; 260; 265; 2390, 2135; 275; 285; 290; 295; 300; 320; 1780, 550; 1550, 345
OPH03	25	174	25	100	0.068	6.8	4, 7, 24	200; 490, 285, 100; 1510
OPH18	35	218	35	100	0.095	9.5	4, 6, 7, 15, 16, 22, 23, 24	410; 200; 360, 190; 780; 180; 2000; 1100; 1000
OPM05	31	198	30	87.878	0.084	8.4	6, 7, 9, 11, 14, 16, 24	2100, 500; 180, 130; 2200; 2240; 1570; 800; 1450, 1260
OPO18	18	75	18	100	0.049	4.9	6, 8, 10, 22	160; 375; 225; 335
OPQ08	22	125	22	100	0.059	5.9	3, 6, 12, 22	1985; 1945, 1375, 890; 1260; 2215
POQ09	30	173	29	86.127	0.081	8.1	5, 6, 7, 12, 13, 22	320; 960; 700; 1560; 1280; 1580, 1445, 820, 625, 440
OPQ15	19	99	19	100	0.051	5.1	4, 10, 18, 20, 24	200; 1840; 650, 310; 1230; 1410
OPZ07	17	63	17	100	0.046	4.6	7, 12 ,13, 16, 23	520; 710; 1450; 1170; 500,400
Total	367	1861	365					

Genetic Distance

The ratio of genetic distances ranged from (0.11496 to 0.68088) 11%-68% among *A. niger* isolates (Table 4-18). The highest genetic distance 0.68088 (68%) obtained between the isolates number 'C7' and 'C23' and 'C7' and 'C15',in other words, that isolates were similar t0 32%, while the lowest genetic distance 0.11496 (11%) was obtained between the isolate number 'C1' and 'C2', that isolates were similar to 89%

One study reported 12–78% genetic distances among 89 isolates of *A. niger* aggregate using 31 RAPD markers [22]. Another study founded similarity was ranged between (17-79) % among sixteen *A. niger* aggregate isolates collected from different crop fields were subjected to RAPD-PCR [17] Another study showed that the genetic similarity among ten local *A. niger* isolates were subjected to RAPD analysis and out of 20 random oligonucleotide primers was ranged between (22-100)%[23] Genotypic and phenotypic diversity occurred among *A. niger* isolates due to random mutations and environmental variation and stress. Genetic diversity strengthens a population by increasing the likelihood that at least some individuals will be able to survive major disturbances, and by making the group less susceptible to inherited disorders [24].

Table 3-Values of genetic distance between *A. niger* isolates calculated according to [25].

	24
24	0.00000 23
23	0.58618 0.00000 22
22	0.62751 0.40236 0.00000 21
21	0.51221 0.30350 0.48810 0.00000 20
20	0.52337 0.32203 0.47148 0.08503 0.00000 19
19	0.55469 0.50972 0.38773 0.39728 0.40373 0.00000 18
18	0.65107 0.51787 0.37534 0.45949 0.45505 0.20129 0.00000 17
17	0.29551 0.57823 0.67767 0.48641 0.55709 0.51589 0.65996 0.00000 16
16	0.62465 0.35469 0.44835 0.39146 0.38521 0.49512 0.41778 0.53823 0.00000 15
15	0.62465 0.35469 0.41366 0.36874 0.36371 0.55484 0.49189 0.57823 0.26028 0.00000 14
14	0.40979 0.47482 0.53798 0.36238 0.41201 0.50249 0.56578 0.38790 0.44583 0.40387 0.00000 13
13	0.46573 0.53613 0.54140 0.40769 0.47331 0.51065 0.61022 0.33486 0.50438 0.52013 0.24464 0.00000 12
12	0.48772 0.48180 0.48876 0.34233 0.41899 0.52434 0.57351 0.42235 0.44153 0.50958 0.29208 0.32258 0.00000 11
11	0.67835 0.40880 0.48586 0.37126 0.35635 0.55327 0.48027 0.57173 0.30138 0.30138 0.45953 0.48731 0.34331 0.00000 10
10	0.67835 0.41916 0.49798 0.37126 0.37740 0.58404 0.51849 0.57173 0.33912 0.29216 0.47392 0.50257 0.36712 0.06274 0.00000 9
9	0.58145 0.49655 0.48311 0.35910 0.38434 0.53106 0.54009 0.49503 0.37736 0.43518 0.37100 0.40056 0.20253 0.27452 0.31534 0.00000 8
8	0.52081 0.48217 0.54247 0.36973 0.36871 0.59640 0.59389 0.51955 0.39516 0.45652 0.36083 0.39229 0.33796 0.31788 0.33986 0.26781 0.00000 7
7	0.53169 0.68088 0.62008 0.60481 0.63609 0.53382 0.63378 0.44527 0.61189 0.68088 0.51872 0.46785 0.53839 0.59280 0.64239 0.54197 0.45152 0.00000 6
6	0.53900 0.52667 0.59095 0.45505 0.49088 0.59623 0.59581 0.56021 0.56749 0.56749 0.45780 0.53027 0.50347 0.46341 0.46341 0.49184 0.46621 0.59159 0.00000 5
5	0.46052 0.47471 0.47488 0.38791 0.37462 0.56052 0.56406 0.58320 0.43744 0.38981 0.39428 0.46007 0.35615 0.42704 0.47583 0.35831 0.38283 0.57324 0.47000 0.00000 4
4	0.52233 0.54311 0.53144 0.43067 0.44208 0.62098 0.59132 0.55852 0.40958 0.43257 0.42178 0.46949 0.41270 0.43471 0.44627 0.38865 0.41234 0.58028 0.44377 0.21797 0.00000 3
3	0.62564 0.40526 0.46876 0.43513 0.41655 0.63876 0.51230 0.66182 0.34583 0.35550 0.48063 0.50928 0.44887 0.25899 0.30344 0.38659 0.36905 0.59951 0.47012 0.25940 0.21598 0.00000 2
2	0.57665 0.43037 0.43160 0.37445 0.34582 0.62311 0.51596 0.63481 0.34611 0.32611 0.44624 0.50755 0.43962 0.32020 0.34976 0.37151 0.35474 0.58331 0.47177 0.17119 0.18551 0.11746 0.00000 1
1	0.59114 0.48982 0.45807 0.35465 0.34962 0.63760 0.54406 0.60582 0.37076 0.34061 0.49059 0.53830 0.45411 0.36426 0.40508 0.38600 0.39305 0.58127 0.47336 0.21495 0.21887 0.16395 0.11496 0.00000

Cluster analysis

Dendrogram was constructed based on [25] Genetic distance using UPGMA cluster analysis and depicted genetic relationships among 24 *A. niger* isolates, showing Cluster analysis Figure 2 divided the A niger isolates into two main clusters distinctly; group I and II. Group I consisted of C24, C17 and C7. While, group II were divided into two groups and each group was divided into subgroups, shown in figure2. cluster analysis of genetic distances among these isolates have divided into groups according to the source, which isolated them and this indicates the presence of a high level of genetic kinship samples isolated from the same source at the same time which indicates the presence of a high level of genetic distance among samples isolated from different sources. Also the dendrogram indicates a clear pattern of division among the *A. niger* isolates based on source isolates.

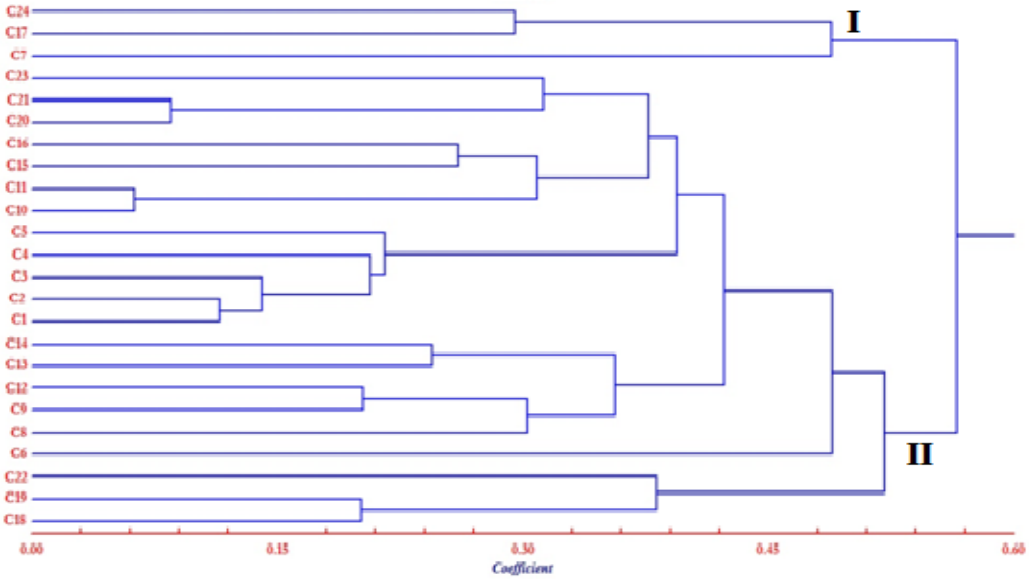

Figure 2- Dendrogram illustrated genetic fingerprint and relationships between *A.niger* isolates developed from RAPD data. *A.niger* isolates from different source: : Lanes C1,C2,C3,C4,C5 (Soil), Lanes:C6-C19 (Seeds): C6,C7,C8,C9 (Ground nut), lanes C10 and C11(Pistachio) , Lanes:,C12,C13,C14 (Walnuts), lanse 15 and 16 (Cashew), Lanes:,17,18,19,(Almonds), Lanes:C20 and C21(Milk), Lanes:C22,C23,C24 (Factory waste water).

Conclusion

A. To study genetic variation between species RAPD-PCR is a powerful tool.

B. RAPD analysis is a truly rapid and reliable tool in DNA fingerprinting.

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