RESULTS AND DISCUSSION

IV. RESULTS AND DISCUSSION

The main topics of these studies were divided into two parts:

1- Isolation and Identification of volatile oils in Genus Citrus.

2- Isolation and Identification of DNA.

I- Isolation and Identifiction of volatile oils:

The data on the concentration of the volatile oil for the three studied genera (***Citrus, Fortunella*** and ***Poncirus*** are found in Table (2). It was revealed that a considerable variation between the three studied genera in the concentration of the volatile oil extracted from leaves. Generally, Kumquat which belongs to genus ***Fortunella*** had the lowest percentage of volatile oil extract (0.12 %) while most of the speces which belong to genus ***Citrus*** had the highest percentage of volatile oil extract (nearly 0.45 %) as compared to the trifoliate orange (genus ***Poncirus*** (0.23 %). On the other hand, varieties which belong to orange group had the highest percentage of volatile oil extract as compared to the other Citrus groups followed by the lemon group. Most of the studied varieties in Pummelos group had the lowest percentage of volatile oil extracts followed by the mandarin group. Within the orange group, the percentage of volatile oil extract ranged from 0.48 % in Jaffa orange to 0.94 % in blood orange. In case of mandarin group, the percentage of volatile oil extract ranged from 0.09 % in Satsuma to 0.45 % in Balady mandarin variety. Within the lemon group, the percentage of volatile oil extract ranged from 0.37 % in rough lemon to 1.03 % in Balady lime. Concerning Pummelos group, the percentage of volatile oil extract ranged from 0.16 % in Kabbad to 0.97 % in Shaddock. These results were

Table (2): Percentage of volatile oil extracted from leaves of the studied Citrus genus and species.

No.	Species	Percentages of extracted volatile oil
1	Sweet Orange (*Citrus sinensis* Var. Gaffa)	0.48 %
2	*C. sinensis* Var.Succari	0.89 %
3	*C. sinensis* Var.Blood o.	0.94 %
4	Sour Orange (*C. grandies* L.)	0.52 %
5	Balady Mandarin (*C. reticulata* L.)	0.45 %
6	Cleopatra Mandarin (*C. rechni*)	0.41 %
7	Santra Mandarin (*C. reticulate* Blanco)	0.17 %
8	Satsuma Mandarin (*C. Unshi* Mar.)	0.09 %
9	Rough Lemon (*C. jambhiri*)	0.37 %
10	Lemon (*C.lemon* L.)	0.47 %
11	Balady Lime (*C. orantifolia* Sw.)	1.03 %
12	Sweet Lime (*C. limetta* Riss.)	0.61 %
13	Grapefruit (*C. paradisi*)	0.21 %
14	Shaddock (*C. grandis*)	0.97 %
15	Citron (*C. medica* Var. Kabbad)	0.16 %
16	(*C.medica* Var. Naffash)	0.21 %
17	Trifoliata orange. (*Poncirus trifoliata*)	0.23 %
18	Kumquat (*Fortunella margarita*)	0.12 %

generally in agreement with the findings of **Agrarwal *et al.*, 1989** and **Rojas** and **Scorza, (1991.) and Mukhtar *et al.*(2001).**

The tables from No.3 to No.21 revealed the components of the volatile oils in the three genera under study (citrus, poncirus and Fortunella

Table (3) revealed the major components of leaves oil extracted from **Jaffa orange**. The volatile oils of Jaffa orange leaves were fractionated into **22 peaks** as revealed by GC/MS. The major components of volatile oil in **Jaffa orange** variety were **Linalool** in a percentage of **25.43**,**α-Pinene** in a percentage of **20.37**, **lemonen** in a percentage of **11.19**. and **β Pinene** in a percentage of **11.03**.Eighteen compounds were found as a minor components as the following; Cis-3hex-1-ol, Trans-2 hex-1-ol, Octanol, Terpinolene, Decanal, Geraniol, Trans Ocimen, β-Cymene, β- Selinene, α–Telhinen, Cis-2-Pent-1-al, Linalool oxide, Geranial, Sabinene, Linalyl acetate, α-Terpineol,Terpinen-4-ol and γ-Terpinene . It was found that these results were in agreement with the findings of **Fischer *et al.*, 2008; Ahmed, 2004; Mukhtar *et al*, 2001; Smith et al., 2000; Blanco *et al.*, 1995; Kekelidze and Attaway *et al.*, 1968.**

Table (4) revealed the major components of leaves oil extracted from **Succari orange**. The volatile oils of Succari orange leaves were fractionated into **21 peaks** as revealed by GC/MS. The major components of volatile oil in Succari orange variety were **β-Pinene** in a percentage of **25.00**, **α- Pinene** in a percentage of **15.59**, **Limonene** in a percentage of **14.55** and **linalool** in a percentage of **10.74**. Seventeen compounds were found as a minor components as : Linalyl acetate, β-Cymene, Caryophyllene, Citronelal, Decanal, β- Myrcene, Octanol,and Terpinen-4

Table (3): Components of volatile oil extracted from leaves of the Jaffa orange.

No.	Components	Retention time	Concentration of the extracted oil
1	**α- Pinene**	3.27	**20.37**
2	β-Pinene	4.00	11.03
3	Sabinene	6.10	00.74
4	Octanol	8.00	00.10
5	α – Telhinen	10.16	00.37
6	**Limonene**	11.95	**11.19**
7	γ – Terpinene	14.06	07.78
8	Trans Ocimen	14.97	00.18
9	β-Cymene	16.11	00.18
10	Terpinolene	17.00	01.10
11	Cis-2-Pent-1-al	17.98	00.49
12	Cis-3 hex-1-ol.	19.07	00.06
13	Trans-2 hex-1-ol.	20.79	00.06
14	**Linlaool**	23.01	**25.43**
15	Linalyl acetate	23.91	01.43
16	Terpinen-4 ol.	25.51	03.31
17	α- Terpineol	27.00	01.88
18	β- Selinene	27.82	00.09
19	Linalool oxide	28.66	00.42
20	Decanal	31.50	00.14
21	Geranial	36.81	01.65
22	Geraniol	39.93	00.16

Table (4): Components of volatile oil extracted from leaves of the Succari orange.

No.	Components	Retention time	Concentration of the extracted oil
1	Acetaldehyde	2.31	00.75
2	**α- Pinene**	3.27	**15.59**
3	**β-Pinene**	4.00	**25.00**
4	Sabinene	6.10	05.00
5	β- Myrcene	7.65	00.47
6	Octanol	8.00	00.51
7	**Limonene**	11.95	**14.55**
8	Trans-2 hex-1-al	13.09	05.63
9	γ - Terpinene	14.06	07.96
10	β-Cymene	16.11	00.15
11	Cis-3 hex-1-ol.	19.07	01.88
12	Trans-2 hex-1-ol.	20.79	00.08
13	Citronelal	22.60	00.16
14	**Linlaool**	23.01	**10.74**
15	Linalyl acetate	23.91	00.08
16	Terpinen-4 ol.	25.51	00.75
17	Caryophyllene	26.40	00.22
18	α- Terpineol	27.00	01.76
19	β- Selinene	27.82	01.24
20	Linalool oxide	28.66	00.45
21	Decanal	31.50	00.37

ol . It was found that these results were in agreement with the findings of **Fischer *et al.*, 2008**; **Ahmed, 2004**. **Hognadottir and Rouseff, 2003; Mukhtar *et al*, 2001**; **Smith *et al.*, 2000; Blanco *et al.*, 1995;.**

Table (5) revealed the major components of leaves oil extracted from **Blood orange**. The volatile oils of Blood orange leaves were fractionated into 21 peaks as revealed by GC/MS. The major components of volatile oil in Blood orange variety were **Limonen** in a percentage of **19.19**, **α- Pinene** in a percentage of **15.81**, **Linalool** in a percentage of **14.19** and **β – Pinen** in a percentage of **10.48**. Seventeen compounds were found as a minor components as: Linalyl acetate, B myrcene, Cis-2-Pent-1-al,Cis-3hex-1-ol,Octanol, Linalool oxide, Decanal and Caryophyllene . It was found that these results were in agreement with the findings of **Ahmed, 2004**. **Mukhtar *et al*, 2001 Smith *et al.*, 2000; Blanco *et al.*, 1995; Baaliouaamer *et al.*, 1988 and Attaway *et al.*, 1968**; **Fadel,(1991).**

Table (6) revealed the major components of leaves oil extracted from **Sour orange**. The volatile oils of Sour orange leaves were fractionated into **17 peaks** as revealed by GC/MS. The major components of volatile oil in Sour orange variety was **Linalyl acetate** in a percentage of **48.11**, **Linalool** in a percentage of **10.78** and **Myrcene** in a percentage of **9.81**. Fourteen compounds were found as a minor components it was found that these results were in agreement with the findings of **Pasquale *et al.*, 2006; Ahmed, 2004; Mukhtar *et al*, 2001; Lota *et al.*, 2001; Baaliouamer and Meklati, 1986; Dela and Sardi, 1977 and Pieringer *et al.*, 1964.**.

Table (5): Components of volatile oil extracted from leaves of the Blood orange.

No.	Components	Retention time	Concentration of the extracted oil
1	Acetaldehyde	2.31	0.97
2	**α – Pinen**	3.27	**15.81**
3	**β – Pinen**	4.00	**10.48**
4	β– Myrcene	7.65	0.20
5	Octanol	8.00	0.49
6	**Limonen**	11.95	**19.19**
7	Trans-2 hex-1-al	13.09	4.28
8	γ – Terpinen	14.06	10.03
9	β – Cymene	16.11	06.94
10	Cis-2-Pent-1-al	17.98	0.17
11	Cis-3 hex-1-ol.	19.07	0.36
12	Trans-2 hex-1-ol.	20.79	0.46
13	**Linalool**	23.01	**14.19**
14	Linalyl acetate	23.91	0.03
15	Terpinen-4 ol.	25.51	3.65
16	Caryophyllene	26.40	0.80
17	α – Terpineol	27.00	2.85
18	β – Selinene	27.82	3.79
19	Linalool oxide	28.66	0.73
20	Decanal	31.50	0.73
21	Geranial	36.81	1.47

Table (6): Components of volatile oil extracted froms leaves of the Sour orange.

No.	Components	Retention time	Concentration of the extracted oil
1	Nonane	2.90	06.88
2	α- Pinene	3.27	00.07
3	Camphene	3.45	00.06
4	β-Pinene	4.00	02.30
5	Sabinene	6.10	08.90
6	**Myrcene**	7.65	**09.81**
7	α-Phellandrene	7.71	00.51
8	α-Terpinene	7.89	00.09
9	Limonene	11.95	03.40
10	Ocimene	14.97	05.94
11	Terpinolene	17.00	00.89
12	**Linalool**	23.01	**10.78**
13	**Linalyl acetate**	23.91	**48.11**
14	Thymol	24.00	01.78
15	Caryophellene	26.40	00.07
16	α-Terpineol	27.00	03.80
17	Geranyl acetate	27.30	02.60

Table (7) revealed the major components of leaves oil extracted from **Balady mandarine**. The volatile oils of Balady mandarin leaves were fractionated into **24 peaks** as revealed by GC/MS. The major components of volatile oil in Balady mandarine variety were **Linalool** in a percentage of **50.87**, **β- Pinene** in a percentage of **21.11**, It was found that these results were in agreement with the findings of **Ahmed, 2004. Mukhtar *et al*, 2001 Kekelidze *et al*., 1981 a and b and Karawya and Hifnawy, 1977; Fadel,(1991).**

Table (8) revealed the major components of leaves oil extracted from **Cleopatra mandarin**. The volatile oils of Cleopatra leaves were fractionated into **22 peaks** as revealed by GC/MS. The major components of volatile oil in Cleopatra variety were **linalool** in a percentage of **43.58**, **β-pinene** in a percentage of **18.76** and **γ–Terpinene** in a percentage of **5.53** and **α – Pinene** in a percentage of **4.29**.Eighteen compounds were found as a minor components It was found that these results were in agreement with the findings of **Ahmed, 2004. Mukhtar *et al*, 2001., Blanco *et al*., 1995; and Kekelidze *et al*., 1981 a and b**.

Table (9) revealed the major components of leaves oil extracted from **Santra mandarin**. The volatile oils of Santra clement leaves were fractionated into **20 peaks** as revealed by GC/MS. The major components of volatile oil in Santra clement variety were **Linalool** in a percentage of **51.73**, **β- pinene** in a percentage of **16.89** and **α- pinene** in a percentage of **4.17**. Seventeen compounds were found as a minor components it was found that these results were in agreement with the findings of **Ahmed, 2004. Mukhtar *et al*, 2001., Karawya and Hifnawy, 1977 .**

Table (7): Components of volatile oil extracted from leaves of the studied Balady mandarin.

No.	Components	Retention time	Concentration of the extracted oil
1	Acetaldehyde	2.31	1.30
2	α – Pinene	3.27	4.56
3	**β – Pinene**	4.00	**21.11**
4	Sabinene	6.10	1.00
5	B– Myrcene	7.65	0.54
6	Octanol	8.00	0.10
7	α – Telhinene	10.16	0.66
8	Limonene	11.95	4.27
9	Trans-2 hex-1-al	13.09	1.76
10	γ – Terpinene	14.06	5.06
11	Trans Ocimene	14.97	0.19
12	B – Cymene	16.11	0.32
13	Terpinolene	17.00	0.27
14	Cis-3 hex-1-ol.	19.07	0.19
15	Trans-2 hex-1-ol.	20.79	0.46
16	Citronelal	22.60	0.73
17	**Linalool**	23.01	**50.87**
18	Thymol Methyl eth.	24.61	1.32
19	Terpinen-4-ol	25.51	0.87
20	Caryophellene	26.40	0.60
21	α –Terpineol	27.00	1.21
22	ß-Salinene	27.82	1.57
23	Decanal	31.50	0.75
24	Thymol	45.73	0.62

Table (8): Components of volatile oil extracted from leaves of the Cleopatra mandarin.

No.	Components	Retention time	Concentration of the extracted oil
1	Acetaldehyde	2.31	0.86
2	**α – Pinene**	3.27	**4.29**
3	**β – Pinene**	4.00	**18.76**
4	B– Myrcene	7.65	0.11
5	α– Telhinene	10.16	0.48
6	Limonene	11.95	4.04
7	Trans-2 hex-1-al	13.09	1.45
8	**γ– Terpinene**	14.06	**5.53**
9	Trans Ocimene	14.97	0.12
10	Terpinolene	17.00	0.20
11	Cis-2-Pent-1-al	17.98	0.17
12	Cis-3 hex-1-ol.	19.07	0.31
13	Trans-2 hex-1-ol.	20.79	0.58
14	Citronelal	22.60	0.53
15	**Linalool**	23.01	**43.58**
16	Thymol Methyl eth.	24.61	1.08
17	Terpinen-4 ol.	25.51	0.73
18	Caryophellene	26.40	0.38
19	α - Terpineol	27.00	1.20
20	Decanal	31.50	0.89
21	Neral	34.84	0.37
22	Thymol	45.73	0.36

Table (9): Components of volatile oil extracted from leaves of the Santra mandarin.

No.	Components	Retention time	Concentration of the extracted oil
1	Acetaldehyde	2.31	0.75
2	**α – Pinene**	3.27	**4.17**
3	**β– Pinene**	4.00	**16.89**
4	β – Myrcene	7.65	0.52
5	α – Telhinene	10.16	0.39
6	Limonene	11.95	2.93
7	Trans-2 hex-1-al	13.09	1.64
8	γ – Terpinene	14.06	3.32
9	β – Cymene	16.11	0.37
10	Cis-2-Pent-1-al	17.98	0.10
11	Cis-3 hex-1-ol.	19.07	0.24
12	Trans-2 hex-1-ol.	20.79	0.59
13	Citronelal	22.60	0.62
14	**Linalool**	23.01	**51.73**
15	Thymol Methyl eth.	24.61	1.01
16	Terpinen-4 ol.	25.51	0.45
17	Caryophellene	26.40	0.72
18	α– Terpineol	27.00	0.86
19	Decanal	31.50	0.94
20	Thymol	45.73	0.83

Table (10) revealed the major components of leaves oil extracted from **Satsuma mandarin**. The volatile oils of Satsuma leaves were fractionated into **22 peaks** as revealed by GC/MS. The major components of volatile oil in Satsuma variety were **Linalool** in a percentage of **47.34**, **β- pinene** in a percentage of **19.06** , **γ – Terpinene** in a percentage of **4.89** and **α- Pinene** in a percentage of **4.02**. Eighteen compounds were found as a minor components it was found that these results were in agreement with the findings of **Ahmed, 2004**. **Mukhtar *et al*, 2001 Blanco *et al*., 1995; Kekelidze *et al*., 1985; Kekelidze *et al*., 1981 a and b and Karawya and Hifnawy, 1977.**

Table (11) revealed the major components of leaves oil extracted from **Rough lemon**. The volatile oils of Rough lemon leaves were fractionated into **17 peaks** as revealed by GC/MS. The major components of volatile oil in Rough lemon variety were **α- Pinene** in a percentage of **26.33**, **phelandren** in a percentage of **16.36** and **limonene** in a percentage of **12.28**. fourteen compounds were found as a minor components it was found that these results were in agreement with the findings of **Ahmed, 2004**; **Mukhtar *et al*, 2001., Blanco *et al*., 1995; and Fadel *et al* 1991.**

Table (12) revealed the major components of leaves oil extracted from Adalia lemon. The volatile oils of Adalia leaves were fractionated into 19 peaks as revealed by GC/MS. The major components of volatile oil in Adalia lemon variety were α- Pinene in a percentage of 29.57, phelandren in a percentage of 15.03 and limonene in a percentage of 14.16. Sexteen compounds were found as a minor components it was found that these results were in agreement with the findings of **Ahmed, 2004**. **Mukhtar *et al*, 2001., Vekiari *et al*., 2002**; **Smith *et al*, 2000; Rodriguez *et al*., 1998; Blanco *et al*., 1995; Rojas and Scorza, 1991.**

Table (10): Components of volatile oil extracted from leaves of the Satsuma mandarin.

No.	Components	Retention time	Concentration of the extracted oil
1	A cetaldehyde	2.31	1.02
2	**α-pinene**	3.27	**4.02**
3	**β – Pinene**	4.00	**19.06**
4	β – Myrcene	7.65	0.72
5	α – Telhinene	10.16	0.29
6	Limonene	11.95	3.38
7	Trans-2hex-1al	13.09	2.38
8	**γ – Terpinene**	14.06	**4.89**
9	β – Cymene	16.11	0.18
10	Terpinolene	17.00	0.22
11	Cis-2-Pent-1-al	17.98	0.10
12	Cis-3 hex-1-ol.	19.07	0.22
13	Trans-2 hex-1-ol.	20.79	0.52
14	Citronelal	22.60	0.71
15	**Linalool**	23.01	**47.34**
16	Thymol Methyl eth.	24.61	0.87
17	Terpinen-4 ol.	25.51	0.98
18	Caryophellene	24.61	0.48
19	α – Terpineol	25.51	1.32
20	β – Selinene	27.82	0.56
21	Decanal	31.50	0.93
22	Thymol	45.73	0.57

Table (11): Components of volatile oil extracted from leaves of the Rough Lemon.

No.	Components	Retention time	Concentration of the extracted oil
1	**α- Pinene**	3.27	**26.33**
2	Myrcene	7.65	07.02
3	Ocimen	9.95	00.78
4	Octararine	10.27	07.90
5	**Phelandren**	11.00	**16.36**
6	**Lemonen**	11.95	**12.28**
7	Cineol	11.24	00.49
8	C8 aldhyd	12.44	04.56
9	C9 aldhyd	12.60	01.25
10	Lenalool	13.29	00.65
11	Cetral b	13.72	00.12
12	α-terpenyol	15.93	04.52
13	α-farmesen	16.39	05.80
14	β-farmesen	16.70	00.97
15	Cariophellen	17.90	01.67
16	Geranil acetate	19.68	01.70
17	Nerolidol	20.40	02.53

Table (12): Components of volatile oil extracted from leaves of the Lemon.

No.	Components	Retention time	Concentration of the extracted oil
1	**α- Pinene**	3.27	**29.57**
2	Myrcene	7.65	06.63
3	Ocimene	9.95	01.93
4	Octacarine	10.27	06.84
5	**Phelandren**	11.00	**15.03**
6	**Limonene**	11.95	**14.16**
7	Cineol	11.24	02.53
8	α-terpinene	11.91	00.34
9	C8 aldhyd	12.44	03.21
10	C9 aldhyd	12.60	01.01
11	C10 aldhyd	13.20	00.89
12	Lenalool	13.29	01.05
13	Lenalyl acetate	13.40	00.53
14	α-terpenyol	15.93	03.12
15	α-farnesene	16.39	04.02
16	β-farnesene	16.70	01.73
17	Caryophellene	17.90	01.22
18	Geranial acetate	19.68	01.30
19	Nerolidol	20.40	02.78

Table (13) revealed the major components of leaves oil extracted from **Balady lime.** The volatile oils of Balady lime leaves were fractionated into **6 peaks** as shown by GC/MS. The major component of volatile oil in **Balady lime** variety were **limonene** in a percentage of **24.79** and **β- Pinene** in a percentage of **21.45**, it was found that these results were in agreement with the findings of **Calvarano *et al.*, 1982, Rojas and Scorza, 1991; Chu *et al.*, 1988; Melendreras *et al.*, 1984; Bukiya *et al.*, 1984;**

Table (13): Components of volatile oil extracted from leaves of the Balady Lime.

No.	Components	Retention time	Concentration of the extracted oil
1	Acetaldehyde	2.31	3.22
2	α- Pinene	3.27	3.40
3	β- Pinene	4.00	**21.45**
4	Myrcene	7.65	2.28
5	A – Telhinen	10.16	0.03
6	**Limonene**	11.95	**24.79**

Table (14) revealed the major components of leaves oil extracted from volatile oil. The volatile oil extracted from leaves of **sweet lime** was determined as shown in Table (14). Results revealed that the chromatographed oil consisted of 22 major and minor components. Both of **camphene** and **limonene** appeared as major components in a concentration of **23.09** and **15.67**, respectively. The obtained results are in accordance with the findings of **Youssef *et al.*,1994 Rojas and Scorza, 1991; Chu *et al.*, 1988; Melendreras *et al.*, 1984; Bukiya *et al.*, 1984; Lund *et al.*, 1982 a and b; Calvarano *et al.*, 1982 and Karawya *et al.*, 1971.**

Table (15) revealed the major components of leaves oil extracted from Grapefruit. The volatile oils of Grapefruit leaves were fractionated into **16 peaks** as revealed by GC/MS. The major components of volatile oil in Grapefruit variety were **Limonene** in a percentage of **29.04**, **α-Pinene** in a percentage of **17.20**, and **Geraniol** in a percentage of **11.48**. Thirteen compounds were found as a minor components it was found that these results were in agreement with the findings of **Ahmed, 2004. Mukhtar *et al*, 2001; Youssef *et al.*, 1994 and Karawya *et al.*, 2000**.

Table (16) revealed the major components of leaves oil extracted from **Shaddock**. The volatile oils of Shaddock leaves were fractionated into **19 peaks** as revealed by GC/MS. The major components of volatile oil in Shaddock variety were **α-terpenyol** in a percentage of **15.46**, **α-Pinene** in a percentage of **10.87**, and **Lenalool** in a percentage of **07.45** . Sexteen compounds were found as a minor components as it was found that these results were in agreement with the findings of **Ahmed, 2004. Mukhtar *et al*, 2001 ;Fadel *et al.*, 1991** and **Zayas *et al.*, 1980**.

Table (14): Components of volatile oil extracted from leaves of the Sweet lime.

No.	Components	Retention time	Concentration of the extracted oil
1	α- Pinene	3.27	3.22
2	**Comphene**	3.50	**23.09**
3	Myrcene	7.65	3.45
4	Ocimene	9.95	7.42
5	Cymene	10.	3.63
6	Octacarine	10.27	9.16
7	Careen		4.84
8	Phellandrene	11.00	1.34
9	**Limonene**	11.95	**15.67**
10	α – Terpinene	11.91	3.35
11	C9 aldhyd	12.60	2.45
12	C10 aldhyd	13.20	1.12
13	Linalool	13.29	3.85
14	Linalyl acetate	13.40	00.73
15	Cetral b	13.72	1.50
16	Cetronellal	14.00	1.39
17	α- Terpineol	15.93	4.02
18	B - Terpineol	15.97	2.76
19	α –Farnesene	16.39	0.32
20	β –Farnesene	16.70	1.12
21	Caryophellene	17.90	0.73
22	Geraniol	19.68	0.93

Table (15): Components of volatile oil extracted from leaves of the Grapefruit.

No.	Components	Retention time	Concentration of the extracted oil
1	**α- Pinene**	3.27	**17.20**
2	β- Pinene	4.00	1.86
3	Myrcene	7.65	3.80
4	Ocimene	9.95	1.31
5	Octacarine	10.27	4.37
6	**Limonene**	11.11	**29.04**
7	α-terpenine	11.91	1.43
8	C9 aldhyd	12.44	2.57
9	C10 aldhyd	12.61	1.85
10	Lenalool	13.29	1.29
11	α-terpenyol	15.93	5.91
12	α-farnesene	16.39	7.38
13	β-farnesene	16.70	5.24
14	α-Caryophellene	17.90	2.52
15	Geranil acetate	19.06	2.75
16	**Geraniol**	19.68	**11.48**

Table (16): Components of volatile oil extracted from leaves of the Shaddock.

No.	Components	Retention time	Concentration of the extracted oil
1	**α- Pinene**	3.27	**10.87**
2	Myrcene	7.65	1.15
3	Ocimen	9.95	00.88
4	Octacarine	10.27	01.64
5	Phelandren	10.54	01.64
6	lemonen	11.95	03.51
7	α-terpenine	11.97	03.91
8	C8 aldhyd	12.44	02.09
9	C9 aldhyd	12.60	03.92
10	C10 aldhyd	12.65	02.18
11	**Lenalool**	13.29	**07.45**
12	Lenalyl acetate	13.62	05.34
13	Cetral b	14.34	03.67
14	**α-terpenyol**	15.93	**15.46**
15	α-farmesen	16.39	04.68
16	β-farmesen	16.43	04.91
17	Caryophellen	17.90	03.67
18	Geranil acetate	19.06	01.89
19	Geranial	19.68	02.24

Table (17) revealed the major components of leaves oil extracted from **Kabbad**. The volatile oils of Kabbad leaves were fractionated into **18 peaks** as revealed by GC/MS. The major components of volatile oil in Kabbad variety were **Octacarine** in a percentage of **18.24**, **α- terpenyol** in a percentage of **17.67** and **leanlyl acetate** in a percentage of **12.66** it was found that these results were in agreement with the findings of **Ahmed, 2004**. **Mukhtar *et al*, 2001** and **Zayas *et al.*, 1980**.

Table (18) revealed the major components of leaves oil extracted from **Naffash.** The volatile oils of Naffash leaves were fractionated into **15 peaks** as revealed by GC/MS. The major components of volatile oil in Naffash variety were **α-terpenyol** in a percentage of **26.59**, **Lenalyl acetate** in a percentage of **24.79** and **α- Pinene** in a percentage of **20.78**, Twelve compounds were found as a minor components for example myrcene, α- farmesen, C8 aldhyd, C9 aldhyd, , C10 aldhyd,, α-terpenine, cetralb, Limonne, β- farmesen, linalool, Phelandren, and α-terpnine. It was found that these results were in agreement with the findings of **Ahmed, 2004**. **Mukhtar *et al*, 2001** and **Zayas *et al.*, 1980**.

Table (19) revealed the major components of leaves oil extracted from **Trifoliata orange**. The volatile oils of Trifoliata orange leaves were fractionated into **15 peaks** as revealed by GC/MS. The major components of volatile oil in Trifoliata orange variety were **α- Terpenine** in a percentage of **3.71**, **Lemonen** in a percentage of **1.90** and **C8aldhyde** in a percentage of **1.01**. Twelve compounds were found as a minor components as the following; octacarine, myrcene, α- pinene, , α-terpenyol, cineol, ocimen, , lenalyl acetate, linalool, , C9 aldhyd, cetral b, and C10 aldhyd,. It was found that these results were in agreement with the findings of **Ahmed, 2004**,and **Mukhtar *et al*, 2001.**

Table (17): Components of volatile oil extracted from leaves of the Kabbad.

No.	Components	Retention time	Concentration of the extracted oil
1	α- Pinene	3.27	00.12
2	Myrcene	7.65	01.49
3	Ocimen	9.95	01.01
4	**Octacarine**	10.27	**18.24**
5	Phelandren	10.54	00.46
6	Lemonen	11.95	02.95
7	α-terpenine	11.98	00.81
8	C8 aldhyd	12.44	05.22
9	C9 aldhyd	12.60	04.03
10	Lenalool	13.29	00.48
11	**Lenalyl acetate**	13.62	**12.66**
12	Cetral b	13.51	06.23
13	**α-terpenyol**	14.26	**17.67**
14	α-farmesen	16.39	03.00
15	β-farmesen	14.34	06.00
16	Caryophellene	17.72	00.48
17	Geranil acetate	19.05	00.18
18	Nerolidol	20.40	00.15

Table (18): Components of volatile oil extracted from leaves of the Naffash.

No.	Components	Retention time	Concentration of the extracted oil
1	**α- Pinene**	5.31	**20.78**
2	Myrcene	10.71	00.9
3	Octacarine	12.65	14.27
4	Phelandren	13.03	01.09
5	Limonene	13.58	03.15
6	α-terpenine	15.26	00.15
7	C8 aldhyd	16.30	07.45
8	C9 aldhyd	18.70	02.28
9	C10 aldhyd	19.19	00.60
10	Lenalool	20.09	00.62
11	**Lenalyl acetate**	23.53	**24.79**
12	Cetral b	23.77	05.39
13	**α-terpenyol**	25.01	**26.59**
14	α-farnesene	28.63	01.63
15	ß-farnesene	29.58	05.82

Table (19): Components of volatile oil extracted from leaves of the Trifoliate orange.

No.	Components	Retention time	Concentration of the extracted oil
1	α- Pinene	4.34	00.06
2	Myrcene	5.31	00.11
3	Ocimen	5.72	00.11
4	Octcarine	6.49	00.22
5	Phelandren	7.75	00.3
6	**limonene**	**8.29**	**01.90**
7	Cineol	8.65	00.03
8	**α-terpenine**	**9.98**	**03.71**
9	**C8 aldhyd**	**10.61**	**01.01**
10	C9 aldhyd	11.08	00.27
11	C10 aldhyd	11.39	00.32
12	Lenalool	12.51	00.50
13	Lenalyl acetate	12.92	00.25
14	Cetral b	13.51	00.37
15	α-terpenyol	14.26	00.56

Table (20) revealed the major components of leaves oil extracted from **Kumquat**. The volatile oils of Kumquat leaves were fractionated into **31 peaks** as revealed by GC/MS. The major components of volatile oil in Kumquat variety were **Isoledene** in a percentage of **17.29**, **Elemol** in a percentage of **12.73** and **Germacrene D** in a percentage of **10.58**. Twelve compounds were found as a minor components as the following; δ-Elemene, β-Elemene, β-Caryophellene, γ- Elemene, β-Chamigrene, α-Cadinene, trans-Nerolidol, Ledol, Globulol, viridiflorol and α-Eudesmol. It was revealed that these results were in agreement with the findings of **Khaleel *et al.***, 2001 who concluded that the volatile oil of the leaf in Kumquate is rich in sesquiterpene hydrocarbons (51.33 %) as well as sesquiterpene alcohols (40.25 %). Isoledene, elemol, β-Eudesmol and germacrene D are the major constituents of the essential oil of the leaves.

Table 21 illustrates and concludes all the results shown and discussed in all the previous tables. Accordingly the following are the main points that summaries the major components which differentiate between the species under this investigation

1-Volatile oil component of **Sweet orange** leaves were characterized alone by the presence of **Linalool oxide** component.

2- Volatile oil component of **Sour orange** leaves were characterized alone by the presence of **Nonane** component.

3- Volatile oil component of **Sweet Lime** leaves were characterized alone by the presence of **Carine and β-Terpineol** components.

4- Volatile oil component of **Sweet Lime and Cleopatra mandarin** leaves were characterized alone by the presence of **Neral** component.

5- Volatile oil component of **Santara and Satsuma mandarin** leaves were characterized alone by the presence of **α-3 Dimethyl** component.

6- Volatile oil component of **Rough Lemon, Lemon and Citron (*Citrus medica* var. Kabbad)** leaves were characterized alone by the presence of **Nerolidol** component.

7- Volatile oil component of **Rough Lemon, Lemon and Trifoliata Orange** leaves were characterized alone by the presence of **Cineol** component.

8- Volatile oil component of **Gaffa Orange, Blood Orange and Cleopatra Mandarin** leaves were characterized alone by the presence of **Geranial** component.

9- Volatile oil component of **Gaffa Orange, Blood Orange, Santara Mandarin and Satsuma Mandarin** leaves were characterized alone by the presence of **Cis 2 penta 1 al** component.

10- Volatile oil component of **Gaffa Orange, Blood Orange and Balady Mandarin** leaves were characterized alone by the presence of **Trans Ocimene** component.

11- Volatile oil component of **Gaffa Orange, Succary Orange, Blood Orange and Balady Mandarin** leaves were characterized alone by the presence of **Octanol** component.

12- Volatile oil component of **Gaffa Orange, Succary Orange, Sour Orange and Balady Mandarin** leaves were characterized alone by the presence of **Sabnene** component.

13- Volatile oil component of **Sour Orange, Santara Mandarin, Lemon and Sweet Lemon** leaves were characterized alone by the presence of **Camphene** component

2- Isolation and Identification of DNA.

The banding patterns of ISSR-PCR fragments using the five specific primers with the ten Citrus genotypes (Figs. 1 2, 3, 4, and 5) revealed 62 amplified fragments; 37 of them were polymorphic (59.68%). The total number of amplified and polymorphic fragments obtained with each primer is found in Table (22). ISSR-PCR data revealed 10 positive and 2 negative molecular markers for the ten Citrus species and varieties including their close relatives. Table (23) indicated that the primer (HB09) was specifying to Kabbad, Naffash and Balady lime variety. The primers (HB12 and HB15) were specifying to Balady orange only. The primer (HB13) was specifying to Kumquat only.

ISSR-PCR amplification revealed different degrees of polymorphisms between the Citrus genotypes. The obtained 62 bands were treated with Ntsyspc2 software to release the similarity matrices (Table 24) and the dendrogram of the genetic distances (Fig.6). The highest genetic similarity indices were found between Balady mandarin and Trifoliata orange varieties. The lowest genetic similarity indices were found between Kabbad and Kumquat varieties as they are from different genera.

SSR's have been recognized as good sources of genetic markers including *Citrus* and *Poncirus* **(Akkaya *et al.*, 1992; Wu and Tanksley, 1993 and Kijas *et al.*, 1995).** Standard PCR analysis of micro satellites requires knowledge of genomic sequences flanking the SSR region to design primers that amplify the micro satellite region and reveal polymorphisms resulting from variation in repeat length. Inter simple sequence repeat (ISSRs) amplification is a novel technique which can rapidly differentiate closely related individuals **(Zietkiewicz *et al.*, 1994).** ISSR markers involve PCR amplification of DNA using a single primer

Table (22):The total number of amplified and polymorphic fragments, percentage of polymorphism and specific markers in the ten Citrus genotypes including two close relatives using ISSR-PCR data.

Primer Number	Primer code	TAF	PF	Polymorphism %	SM
ISSR-PCR					
1	HB 09	15	11	73.33 %	+6
2	HB 10	11	05	45.45 %	0
3	HB 12	12	04	33.33 %	+1
4	HB 13	10	08	80.00 %	+2 and -1
5	HB 15	14	09	64.29 %	+1 and -1
		62	**37**	**59.68 %**	**+10 and -2**

TAF= Total amplified fragments, PF = Polymorphic fragments for each primer

SM=Specific markers including either the presence or absence of a fragment.

Table (23): Number of amplified fragments and specific markers of the ten Citrus cultivars based on ISSR-PCR analysis using five primers

Citrus species / Primers		HB 09	HB 10	HB 12	HB 13	HB 15	Total
Naffash	AF	6	9	9	4	7	35
	SM	1	0	0	0	0	1
Kabbad	AF	8	9	11	4	9	41
	SM	1	0	0	0	0	1
Shaddock	AF	7	6	11	7	6	37
	SM	0	0	0	0	0	0
Sour orange	AF	7	10	11	5	12	45
	SM	0	0	0	0	0	0
Grape fruit	AF	5	11	9	5	11	41
	SM	0	0	0	0	0	0
Jaffa Orange	AF	8	7	12	6	13	46
	SM	0	0	1	0	1	2
Balady Mandarin	AF	6	6	9	8	11	40
	SM	0	0	0	0	0	0
Balady Lime	AF	10	11	9	6	9	45
	SM	1	0	0	0	0	1
Trifoliata Orange	AF	8	7	11	8	11	45
	SM	2	0	0	0	0	2
Kemquat	AF	8	11	10	8	9	46
	SM	1	0	0	1	0	2
	TSM	6	0	1	1	1	9

AF = Amplified fragments
SM=Specific markers including either the presence or absence of a fragment,
TSM = Total number of specific markers

Table (24): Similarity index (Pair wise comparison) among ten citrus species and varieties based on ISSR data.

Citrus spp.	Naffash	Kabbad	Shaddock	Sour orange	Grape fruit	Jaffa Orange	Balady Mandarin	Balady Lime	Trifoliata Orange	Kumquat
Naffash	1.0									
Kabbad	0.8	1.0								
Shaddock	0.4	0.6	1.0							
Sour orange	0.4	0.7	0.5	1.0						
Grape fruit	0.4	0.5	0.3	0.8	1.0					
Jaffa Orange	0.1	0.4	0.5	0.9	0.4	1.0				
Balady Mand	0.0	0.0	0.5	0.5	0.6	0.7	1.0			
Balady Lime	0.2	0.3	0.3	0.6	0.7	0.5	0.5	1.0		
Trifoliata Orange	0.1	0.1	0.5	0.6	0.4	0.8	1.0	0.5	1.0	
Kumquat	0.1	0.0	0.3	0.5	0.6	0.3	0.7	0.7	0.7	1.0

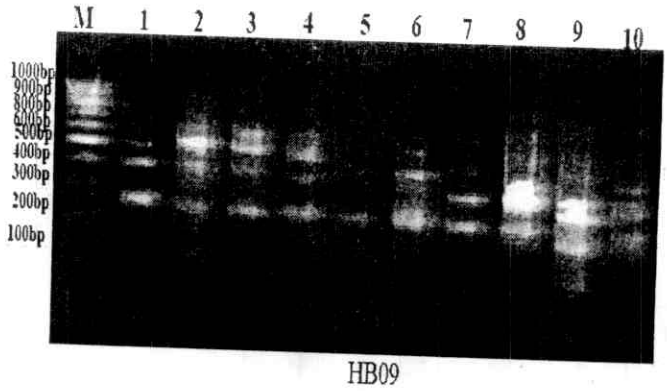

Figure (1): ISSR fingerprints of *10* species and varieties using primer HB 09

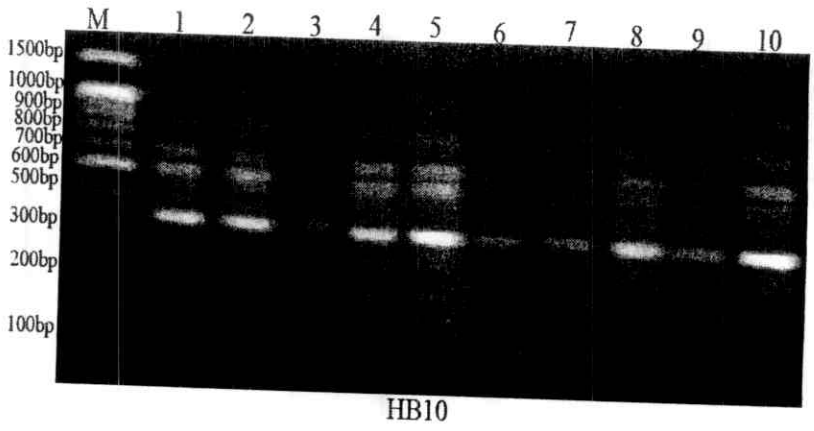

Figure (2): ISSR fingerprints of 10 species and varieties using primer HB 10

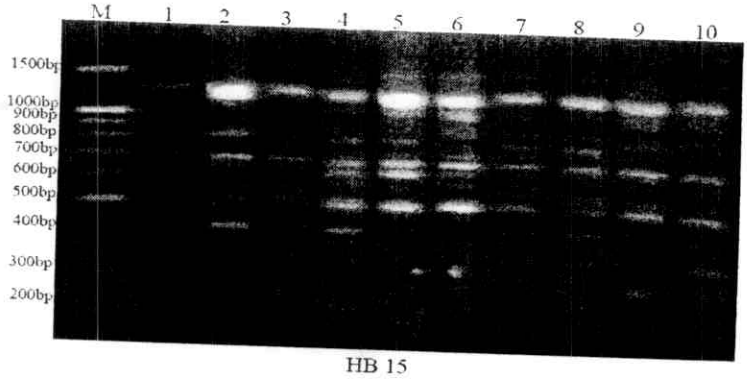

Figure (3): ISSR fingerprints of 10 species and varieties using primer HB 15

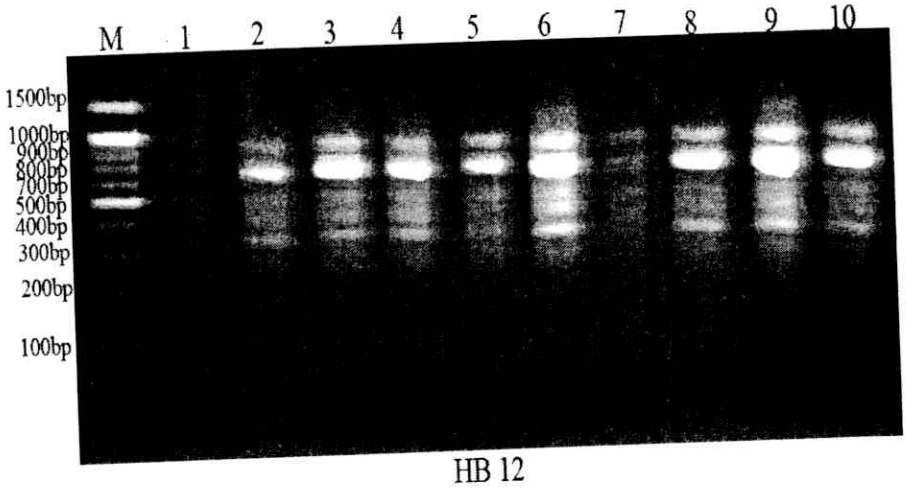

Figure (4): ISSR fingerprints of *10* species and varieties using primer HB 12

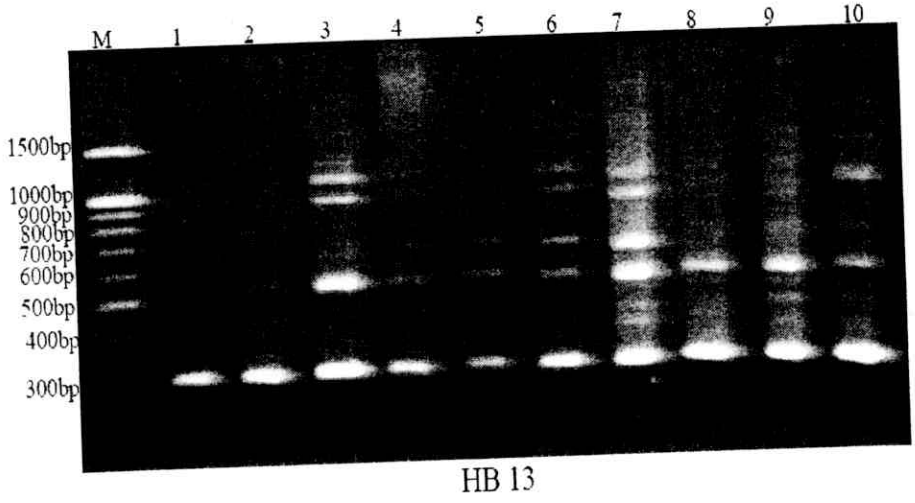

Figure (5): ISSR fingerprints of *10 Citrus* species and varieties using primer HB 13

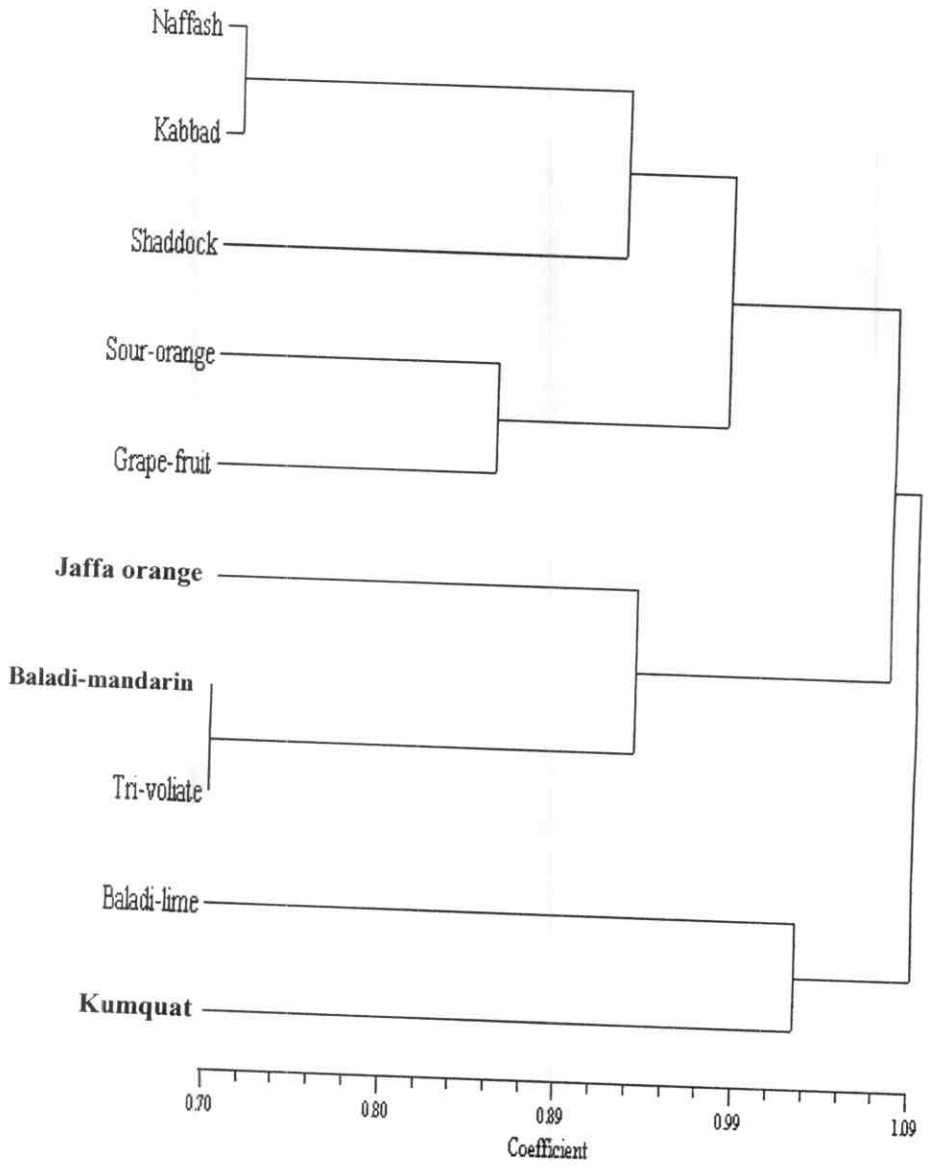

Figure (6):A dendrogram showing the genetic distance among ten species and varieties of Citrus and their close relatives through using ISSR data.

composed of a microsatellite sequence such as (CA)8 anchored at the 3/ or 5/ end by 2 4 arbitrary, often degenerate nucleotides. ISSR has been used to investigate the genomic origins of the genus Eleusine **(Salimath *et al.*, 1995)**, to assess genetic diversity in dent and popcorn **(Kantety *et al.*, 1995)** and in Douglas fir and Sugi **(Tsumura *et al.*, 1996),** and to identify cultivars of chrysanthemum **(Wolff *et al.*, 1995)** and oilseed rape **(Charters *et al.*, 1996).**

The results indicated that it was possible to discriminate between the ten *Citrus* species, since each species banding pattern was not similar to the others. Based on ISSR analysis, the same results was obtained by **Nicolosi *et al.*, (2000)** who used Inter-simple sequence repeat (ISSR) analysis to study the genetic diversity and phylogenetic relationships in 36 accessions belonging to Citrus together with one accession from the related genus *Poncirus*. **Abdel-Tawab *et al.*, 2008** investigated nineteen citrus cultivars for molecular genetic fingerprint and molecular markers were developed to assist selection for these cultivars. PCR reaction was conducted using five ISSR primers. Only two primers HB14 and HA98 showed 100 % polymorphic differences among the cultivars, while three primers exhibited high polymorphism such as HB12 (94 %) and HA99 (93 %).

A dendrogram for the genetic relationships among the ten Citrus genotypes was carried out as shown in Figure (7). The ten Citrus genotypes were separated into two clusters; cluster one included both Balady lime and Kemquat, cluster 2 included; two subclusters; the first subcluster included two groups: group a include Naffash and Kabbad with Shaddock alone. The second group b include sour orange with grape fruit. The second subcluster include Balady mandarin with trifoliata orange and Jaffa orange alone.

This conclusion is in agreement with **Nicolosi *et al.* (2000)** who reported that molecular techniques data are useful for the classification of germplasm and identification of divergent group in Citrus. **Luro *et al.* (1995)** found that DNA amplified fingerprinting could be used in clarifying phylogenetic relationships within a species and also it could be useful in genotype identification in Citrus species.