J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 5 (12): 331 - 356, 2014

GROWTH, ESSENTIAL OIL PRODUCTION AND GENETIC STUDY OF SOME FENNEL VARIETIES UNDER DIFFERENT COMPOST LEVELS IN SANDY SOIL.

Yousef ,R.M.M. and Omneya F. Abu El-Leel

Medicinal and Aromatic Plant Research Department, Horticulture Research Institute (HRI), Agricultural Research Centre (ARC), Cairo, Egypt.

ABSTRACT

The present work was conducted during the two successive seasons of 2011/2012 and 2012/2013 at the experimental farm of El-Quassassin Hort. Res. Station, Ismailia Governorate, and Biotechnology Laboratory, Horticulture Research Institute, Agricultural Research Centre, Egypt. The aim of this study was to investigate the effect of three compost fertilizer levels 4, 6 and 8 ton per Fadden using five varieties of bitter fennel on growth, fruits yield and essential oil production of fennel (*Foeniculum vulgare* Mill). These varieties were Holland, Indian, Azoricum, German and Local fennel. Several trails were studied including growth and yield production, biochemical (the essential oil) and molecular genetic (RAPD- and ISSR-PCR) characteristics under Egyptian sandy soil. The results showed that increasing compost level progressively (form 4 to 8 ton/ Fed) and significantly increased the values of such parameters. Azoricum variety was superior to other varieties under study, as it showed the best growth in terms, fruits yield, fruit essential oil (%) and essential oil production per plant. The main compounds in all fennel essential oils were: Anethole, Estragole, Fenchone and Limonene. The highest percentage of Anethole found in German variety, while the lowest percentage found in Local variety, where the highest percentage of Estragole (= Methyl chavicol) compound undesirable found in Local variety, while the lowest percentage found in Holland and German varieties. Random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) molecular fingerprinting markers were employed as genetic markers for the assay of the genetic relationship of five fennel varieties. In RAPD analysis, 10 selected primers displayed a total of 98 amplified fragments, in which 60 (61.22%) were polymorphic fragments. Thirty-one out of 98 RAPD-PCR fragments were found to be useful as cultivar-specific markers. The largest number of RAPD-PCR markers was scored for Indian variety (68 markers), while the lowest (49 markers) was scored for Holland variety. In the meantime, the highest number of RAPD-PCR cultivar-specific markers was generated by primer OP-C04 (7 markers), while the lowest number of RAPD-PCR specific markers (1 markers) was generated by primers OP-A13 and OP-B04. In ISSR analysis, 4 of the tested ISSR primers generated variable banding patterns. A total of 26 out of 34 ISSR fragments were polymorphic. Eleven DNA amplified fragments were considered as cultivar-specific markers. The varieties distribution on the consensus tree according to the banding patterns of RAPD differed from that based on ISSR. According to the RAPD data, the most two closely related varieties were Indian and Local. While, according to ISSR results, the most two closely related varieties were Indian and German. This may be due to the possibility that each technique of amplified different parts of the genome. Therefore, it would be useful to use a combination of the banding patterns of the two technique in order to use more segments sites of the genome that verify the validity of the consensus tree.

Keywords*:* Fennel varieties, essential oil, DNA fingerprinting and genetic relationship.

INTRODUCTION

Fennel is a plant belonging to the *Umbelliferae* (*Apiaceae*) Family, known and used by humans since antiquity. Because of its flavor, it was cultivated in the countries surrounding the Mediterranean Sea. Fennel is one of the oldest field crop used by the Egyptian for medicinal purposes. Most of the area cultivated with fennel is located in Mid-southern Egypt mainly, El-Fayom, Menia and Assiut Governorates. Only one strain of common or bitter fennel (*Foeniculum vulgare,* Mill.) was cultivated in Egypt for the national and international purposes. Exports of local fennel (*Foeniculum vulgare* Mill.) from Egypt over the past few years have been affected due to the high Estragole but low Anethole content of the oil. Therefore, fennel seeds were imported from different countries to investigate the adaptability of such strains in different locations in Egypt, in comparison with the local one (Shalaby et al., 2011). The essential oil is used as flavoring agent, carminative antispasmodic, stomachic, diuretic, expectorant, aromatic and lactagogue. Fruits are used as spice, in pickles, candies, in liquors and in the preparation of alcoholic beverages. They are also used as remedy for jaundice and menstrual troubles (Kotb, F. T.1985). The cultivar yields big fruits and reasonable percentage of essential oil with particularly high Estragole content, but it is poor in Fenchone which is an important constituent of the fennel essential oil. These, along with some other components, provide the unique aroma and taste. Trans-anethole accounts for the anise taste, Estragole(=Methyl chavicol) compound undesirable, Fenchone the bitterness, and Limonene provides the citrus taste. Commonly, Trans-anethole was used for flavor in the food and liquors industry, which considered non-toxic (Barazani et al., 2002). Moreover, essential oil of fennel has been shown to have antioxidant, antibacterial and antiviral activities (Farag et al, 1989). The fresh leaves and dried fruits of this plant are used as a spice for meat, baked and confectionery products (Davis, 1972). Compost enhances the environmental sustainability of agriculture by decreasing chemical inputs and increasing soil organic matter (Mathur et al., 1993). Many research workers gained best growth, yield, oil percantage and yield and chemical constituents when used compost for several medicinal and aromatic plants, as (Ibrahim, 1999) on *Ocimum sanctum*; (Khalil, 2002) on rosmary (*Rosemarinus officinalis*); (Khalil et al., 2002) on *Tagetes erecta*; (Khalil and El-Sherbeny, 2002) on three *Mentha* species; (Naguib and Aziz, 2004) on *Hyosyamus muticus* and (El-Sherbeny et al., 2005) on *Sidritis montana*. Germplasm is a vital source in generating new plant types having desirable traits that help in increasing crop production with quality and thus improve the level of human nutrition. The genetic diversity is analyzed by using morphological as well as genetic based tools, DNA techniques (Bennici et al., 2003) and advanced molecular methods etc. (Barazani et al., 2002; Shiran et al., 2007). The organic fertilizers is utilized for the change of soil texture, supplying nutrients to the growing plants and organic acids, enhancing nutrients uptake as reported by Lampkin (1990), Mohamed and Matter (2001), Badran (2002) and Yousef (2002) and the organic fertilizers consider save for human health.

Organic fertilization is also one of the methods used to reclaim sandy desert land and to improve the chemical and physical characteristics of the soil (Gomaa1995, Yousef 2002 and Yousef et al. 2008).

Random amplified polymorphic DNA (RAPD) markers are easier and quicker to use and are preferred in application where the relationships between closely related breeding lines are of interest (Hallden et al., 1994). ISSR-PCR is a genotyping technique based on variation found in the regions between microsatellites. It has been used in genetic fingerprinting (Blair et al., 1999), gene tagging (Ammiraju et al., 2001), detection of clonal variation (Leroy and Leon, 2000), cultivar identification (Wang et al., 2009), phylogenetic analysis (Gupta et al., 2008), detection of genomic instability (Anderson et al., 2001), and assessment of hybridization (Wolfe et al., 1998) in many plant and animal species.

The aims of this study were therefore to study the effect of organic compost on growth and essential oil quantity of some varieties of bitter fennel; determine the genetic variability of some fennel varieties by RAPD and ISSR analysis; determine whether secondary metabolites such as essential compounds would be used as taxonomic markers in these varieties and elucidate relationships between genetic and chemical diversity by comparing their hierarchical structures.

MATERIALS AND METHODS

1-Plant materials:

This study was conducted to investigate the effect of different levels of compost fertilizer using five fennel varieties on growth, fruits production, essential oil percentage and essential oil yield. These varieties were Holland, Indian, Azoricum, German, and Local fennel. This investigation was carried out during the two successive seasons of 2011 / 2012 and 2012/2013 at the experimental farm of El-Quassassin Horticultural Research Station, Ismailia Governorate, Agricultural Research Center, Egypt. Table 1 shows the mechanical and chemical analyses of farm soil.

Seeds of bitter fennel obtained from Sekem Company, were sown on 1 November of both years. Seedlings were thinned to single plants and irrigated 15 days after sowing. The experimental unit was 5.4 m^2 ; every unit contained three dripper lines with 3m length. Every experimental unit contained 30 plants (about 22222 plants per Fadden).The experiment was carried out using three replicates in split plot design where the levels of compost were the main plot and varieties of fennel were the subplot. The compost fertilizer was obtained from Arab Organization for Industrialization (A.O.I.); the chemical composition of the compost fertilizer is shown in Table 2.

Table (1): The mechanical and chemical analysis of the experimental soil.

The mechanical analysis		The chemical analysis	
		Macro elements (ppm)	
		Nitrogen	81
		Phosphorus	23
		Potassium	108
		Micro elements (ppm)	
Sand	89.92 %	Fe	2.0
Silt	4.0 %	Cu	---
Clay	6.08 %	Zn	0.26
The soil was sandy in texture		Mn	0.8
		Anion (mq/100 g soil)	
	11.20 %	Cl^-	0.5
Field capacity (F.C.)	2.20 %	HCO_3	1.0
Welting point (W.P.)	0.42 %	SO_4	0.97
Organic matter	8.1	Cations (mq/100 g soil)	
PH (1 soil : 2.5 d.w.)	0.21	Ca^{++}	1.0
E.C. (mmohs/cm)		Mg^{++}	0.4
		Na^{+}	0.76
		K^{+}	0.31
		$CaCO_3$ (meq/100 g soil	2.6

Table (2): The chemical composition of compost fertilizer

Results	Compost characteristics
The color	Dark brown
The smell	Acceptable
The Strength	Spongy
Wet weight per cubic meter (kg / m^3)	510
Dry weight per cubic meter (kg / m3)	375
The moisture	26.60
PH (1:10)	8.36
otal nitrogen (%)	1.50
Ammonium nitrogen (ppm)	57
Nitrate nitrogen (ppm)	95
Organic matter (%)	35.3
Carbon organic (%)	20.5
The ash (%)	64.7
C:N ratio	1:13.7
Total phosphor (%)	0.38
Total potassium (%)	0.63
Humic acid for organic matter (%)	19.6
Grass seeds	No
Nematode	No
Parasites	No

The organic fertilization treatments as compost fertilizer were applied at the rate of 4, 6 and 8 ton per Fadden. The data of plant height (cm), herb fresh and dry weights (g/plant), number of umbels /plant and number of flowers/umbel were recorded at full flowering stage. The fruits yield (g/plant) was recorded when harvested at fruit maturity stage.

Determination of essential oil content and composition:

Essential oil percentage was determined in fruits according to the method described in the General Medical Council (1963). Essential oil yield per plant was calculated by multiplying essential oil percentage by fennel fruit yield/plant and expressed as ml/plant. Samples taken for the essential oil obtained in the second season were analyzed using DsChrom 6200 Gas Liquid Chromatography equipped with a flame ionization detector for separation of essential oil constituents. The analysis conditions were as follows:-

The chromatograph apparatus was fitted with capillary column BPX-5, 5% phenyle(equiv.) polysillphenylene-siloxane 30m X 0.25 mm ID X 0.25µm film. Temperature program ramp increase with a rate of 10°C/ min from 70º to 200º C. Flow rates of gases were nitrogen at 1 ml / min, hydrogen at 30 ml/ min and 330 ml / min for air. Detector and injector temperatures were 300ºC and 250ºC, respectively. The obtained chromatogram and report of GC analysis for each sample were analyzed to calculate the percentage of main components of essential oil. Essential oil yield/feddan was calculated by multiplying oil (%) by fennel fruit yield.

RAPD -PCR Analysis

Polymerase Chain Reaction (PCR).

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, MgCl2 and Taq polymerase. A total of twenty random DNA oligonucleotide primers were independently used according to Williams et al. (1990) in the PCR reaction. Only ten primers succeeded to generate reproducible polymorphic DNA products. The PCR amplification was performed in a 25 µl reaction volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl2 (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH2O. The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95 ºC for 5 minutes, followed by 35 cycles at 96 ºC for 30 seconds, 37 ºC for 30 seconds, and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 5 minutes. PCR products were run at 100 V for one hour on 1.5 % agarose gels to detect polymorphism between the fennel varieties under study. Only ten primers succeeded to generate reproducible polymorphic DNA products. Table 3 lists the base sequences of these DNA primers that produced informative polymorphic bands. The PCR products were separated on a 1.5 % agarose gels and fragments sizes were estimated with two 100bp ladder markers (1000, 900, 800, 700, 600, 500, 400,300,200 and 100bp) and (3000,2500,2000,1500,1000,750,500,250bp).

ISSR-PCR Analysis

Polymerase Chain Reaction (PCR).

ISSR-PCR reactions were conducted using four primers. Amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl of dNTPs (2.5 mM), 2.5 µl $MgCl_2$ (2.5 mM), and 2.5 µl of 10 x

buffer, 3.0 µl of Primer (10 pmol), 3.0 µl of template DNA (25 ng/ µl), 1 µl of *Taq* polymerase (1U/ µl) and 12.5 µl of sterile dd H_2O. the PCRs were programmed for one cycle at 94º C for 4 min. followed by 45 cycles of 1 min. at 94 ºC, 1 min. at 57 ºC, and 2 min at 72 ºC the reaction was finally stored at 72 ºC for 10 min. The PCR products were separated on a 1.5 % agarose gels and fragments sizes were estimated with the 100bp ladder marker. Only four primers succeeded to generate reproducible polymorphic DNA products. Table 3 lists the base sequences of these DNA primers that produced informative polymorphic bands.

Table(3): List of the used RAPD and ISSR primer names and their nucleotide sequences.

No	RAPD Primer code	Sequence	No	RAPD Primer code	Sequence
1	OP-A02	5´TGCCGAGCTG3´	6	OP-C04	5`CCGCATCTAC 3`
2	OP-A10	5´GTGATCGCAG 3´	7	OP-C05	5`GATGACCGCC 3`
3	OP-A13	5´CAGCACCCAC 3`	8	OP-G14	5`GGATGAGACC3`
4	OP-B04	5´GGACTGGAGT 3`	9	OP-K10	5`GTGCAACGTG3`
5	OP-C02	5´GTGAGGCGTC 3`	10	OP-M15	5`GACCTACCAC3`
No	ISSR Primer code	Sequence	No	ISSR Primer code	Sequence
1	14A	5`CTCTCTCTCTCTCTCTTG 3`	3	HB-09	5` GTGTGTGTGTGTGC 3`
2	44B	5`CTCTCTCTCTCTCTCTGC 3`	4	HB-11	5`GTGTGTGTGTGTTGTCC 3`

Statistical analysis:

The experimental design was factorial experiment between compost fertilizer and the fennel varieties in split plots with three replicates. The compost fertilizers were arranged in the main plots, while the fennel varieties were assigned at random in the sub plots. The data were statistically analyzed according to Steel and Torrie (1960) and L.S.D. at (5% level) for comparison the means of different treatments. The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct dendrogram tree among the fennel varieties under study. Calculation was achieved using Dice similarity coefficients (Dice, 1945) as implemented in the computer program SPSS-10.

RESULTS AND DISCUSSION

1-Effect of different compost levels on growth , fruits and essential oil production of fennel plant:

Data in Table (4) show that organic compost significantly increased fennel growth parameter, fruit production, essential oil % and essential oil yield per plant (ml) of fennel.

Increasing compost level progressively and significantly increased the values of such parameters. Wherever the highest values of increase resulted by the highest level of compost (8 ton/Fed):plant height (105.46, 100.06 cm), herb fresh (180.73, 169.33g) and dry weights (38.74, 31.44g) of plant , number of umbels /plant (20.66,19.40) and number of flowers/umbel(23.87,23.13) in the first and second season respectively. On the other side the lower percentage of increase resulted by the lower level of compost (4 ton/Fed.): (88.80, 82.40 cm) for plant height, (124.51, 113.04g) for fresh weight of herb, (24.34, 21.16g) for dry weights, (11.53, 10.00) for number of umbels /plant and (19.13, 16.13) for number of flowers/umbel in the first and second season respectively. Such results on fennel are in the same line with many researchers on different plants, (Khalil et al., 2002) on *Tagetes erecta*; (Khalil and El-Sherbeny, 2003) on three *Mentha* species, who reported that compost at different levels significantly increased the vegetative growth characters including plant height, number of branches, fresh and dry weight of herb during vegetative growth and flowering stage.

Data in Table 4 revealed that the differences between the various levels of compost were significant in most cases. The highest level of compost (8ton /Fed.) increased yield of fruits per plant, essential oil (%),essential oil yield per plant, by (61.00g, 2.39%, and 1.52ml) respectively in the first season and (54.97g, 2.62% and 1.46ml) successively in the second one, where as the values of increase due to the lower level of compost (4 ton/fed.) were [(50.00,40.78g), (1.62,1.85%) and(0.82,0.76ml)] for the same parameter in the two seasons, consecutively. It is clear that essential oil yield per plant (ml) attained a parallel trend to essential oil (%). The three compost levels significantly raised essential oil yield. Raising compost levels progressively increased fennel essential oil yield. These results agree with those obtained by (Ibrahim, 1999) on *Ocimum sanctum*;; (Khalil, 2002) on *Rosemarinus officinalis*; (Khalil et al., 2002) on *Tagetes erecta*; (Khalil and El-Sherbeny, 2003) on three *Mentha* species and (El-Sherbeny et al., 2005) on *Sidritis montana* L., who mentioned that compost addition markedly improved essential oil %, productivity and essential oil yield.

1-Performances varieties for growth , fruits and Essential oil production of fennel plant over all levels of fertilizers at the two years :

Data in Table 5 show significant differences in plant height (cm/plant) among different varieties. Holland variety was generally the shortest (75.11 and 64.78cm) followed by Local variety (87.56 and 80.33cm) then Indian variety (91.22 and 82.78cm) after that German variety (108.33 and 108.66cm) and the tallest variety was Azoricum (118.22 and 116.11cm) in both seasons.

Concerning the fresh and dry weight, data in Table (5) represent that, in general, the Local variety recorded the lowest value (95.88 and 94.59 g) in fresh weight and (20.71 and 17.12g) in dry weight in both seasons. While German variety recorded the highest value (235.15 and 212.97 g) in fresh weight and (45.80 and 39.91g) in dry weight in the first and second season respectively. Taking number of umbels (umbels/plant) into consideration, data in Table (5) reveal significant differences among all varieties.

4-5

Generally, it was noticed that Local variety was superior in number of umbels/plant (21.44 and 17.56 umbels/plant). Moreover, it was found that Holland and German varieties were the lowest in number of umbels/plant (14.00 and 10.89 umbels/plant), (13.67 and 12.00umbels/plant) in the first and second season respectively. As for the number of flower (flowers/plant), it was found also that Local variety was superior in number of flowers/plant (25.89 and 24.33 flowers/plant) and Holland variety recorded the lowest value (13.78 and 13.33 flowers /plant) in both seasons.The data on fruits yield/plant as shown in Table 5, show significant differences in these parameters. It was noticed that the German variety showed the lowest value of both parameters (47.55 and 41.24g) for fruits yield/plant in both seasons.Furthermore, Azoricum variety showed the highest value in the same parameters (73.00 and 57.83g) for fruits yield/plant in both seasons. Table 5, demonstrate the essential oil of the varieties.It is generally noticed that, the Azoricum exhibited the highest values in essential oil percentage (2.82 and 3.03%) in the first and second seasons respectively. Moreover, Holland variety exhibited the lowest values (1.61 and 1.88%) in both seasons. Concerning essential oil yield/plant, data in table 5, show that generally speaking, the Azoricum variety was the highest values (2.14 and 1.77 ml) for essential oil yield/plant in the first and second seasons respectively. Also, Holland variety was the lowest values (0.81and 0.81ml) for essential oil yield/plant in the first and second seasons respectively. The results revealed that Azoricum variety was the highest values in essential oil percentage, fruits yield/plant. It is in the same line with Lal (2007), Lopes et al. (2010) and Safaei et al. (2011) which reported that there is a positive and significant correlation between essential oil content and grain yield of fennel. It is clear that, the growth of different varieties of fennel could be arranged in a descending order as follows; Azoricum; German; Local; Indian then Holland from production point of view. Generally, it can be concluded that Azoricum and German varieties were the suitable for sandy soil conditions.

The mean effect interactions at the different compost levels and the varieties of fennel on growth, fruits and essential oil production at the two years:

Tables 6 and Table 7 conclude that, plant height (cm) of the studied varieties. Significant differences were noticed among compost levels and the studied varieties. Also, the Azoricum variety combined with the highest level of compost fertilizer (8 ton/fed) were superior, it recorded (125.33, 122.00cm) in the first and second seasons respectively. While Holland variety was the shortest (66.00, 55.67cm) when applied the compost fertilization level at (4 ton/fed) in the first and second seasons respectively. Generally, increasing compost level led to increase plant height in both seasons. There was a significant interaction between compost levels and varieties of fennel for fresh and dry weight, (Table 6). German variety when applied the highest level of compost (8 ton/fed) appeared the highest value (266.56, 239.10g) for fresh weight and (50.60, 44.93g) for dry weight in first and second seasons respectively. Fresh and dry weight was gradually reduced when compost level was reduced.

6

The results found that Local variety combined with compost fertilizer level (4 ton/fed) exhibited the lowest value (74.13, 66.07g) for fresh weight and (15.40, 11.90g) for dry weight in first and second seasons respectively. As for number of umbels/plant and number of flowers/plant, it was noticed that Local variety was superior in number of umbels/plant (27.00, 23.66) and in number of flowers/plant (29.00, 29.00) in both seasons

At the same time, German variety recorded the lowest value (9.67, 7.00) for number of umbels/plant and Holland variety recorded the lowest value (11.67, 10.33) for number of flowers/plant in both seasons.

Data in Table 7 revealed that Azoricum variety produced the highest value in yield of fruits per plant (79.67, 65.90gm), essential oil (3.50, 3.71%), essential oil yield per plant (2.81, 2.36ml.) in both seasons at rate (8 ton/fed) of compost, While German variety recorded the lowest value in yield of fruits per plant (44.33, 34.86gm) and Holland variety represented the lowest value in essential oil (1.43 , 1.66%), essential oil yield per plant (0.65 , 0.60ml.) in both seasons at rate (4 ton/fed) of compost.

Table (7): The mean interactions at the different compost levels and the fennel varieties for fruits production, essential oil % and essential oil yield per plant (ml.) of fennel, during the two seasons 2011/2012 and 2012/ 2013:

Level of compost	The varieties	yield of fruits per plant (gm.)		Essential oil (%)		Essential Oil yield per plant (ml.)	
		1st	2nd	1st	2nd	1st	2nd
4 ton/fed	Holland	44.50	36.20	1.43	1.66	0.65	0.60
	Indian	54.17	38.03	1.65	1.88	0.89	0.72
	Azoricum	60.00	51.20	1.85	2.04	1.11	1.04
	German	44.33	34.86	1.71	1.91	0.76	0.67
	Local	47.00	43.60	1.50	1.74	0.72	0.76
6 ton/fed	Holland	48.67	42.93	1.66	1.92	0.80	0.83
	Indian	53.67	47.00	1.87	2.10	1.01	0.99
	Azoricum	79.33	56.40	3.12	3.36	2.49	1.90
	German	48.00	41.23	1.92	2.15	0.93	0.89
	Local	54.00	58.53	1.81	2.04	0.99	1.20
8ton/fed	Holland	55.33	48.63	1.74	2.05	0.97	1.00
	Indian	60.00	49.70	2.24	2.44	1.35	1.22
	Azoricum	79.67	65.90	3.50	3.71	2.81	2.36
	German	50.33	47.63	2.34	2.54	1.19	1.22
	Local	59.67	63.00	2.13	2.34	1.29	1.48
	L.S.D at 0.05	3.48	5.72	0.07	0.10	0.06	0.08

It was found that increasing fertilization levels gave the highest values of the studied parameters. These results are in agreement with those

obtained by Haridy et al. (2001) on lemongrass and El-Ghadban et al. (2002) on Origanum majora. The ratios of essential oil from all varieties under this study were between (1.43 and 3.71%); these results are in line with (Miraldi, 1999) who mentioned that essential oil ratio in sweet and bitter fennel samples were found on an average to be 3.26% and 1.47%, respectively. As known, amounts of essential oil in fennel, like other aromatic plants, can be influenced by a lot of factors such as climatic and environmental conditions, season of collection and the stage of ripening of the fruits (Arslan et al., 1989; Miraldi, 1999).

Table 8 showed the essential oil composition (%) for five varieties of fennel at rate of compost (6 ton /fed) in the second season. The results found presence of 11 compounds, five are monoterpenic hydrocarbons comprising between (6.95%) in German variety essential oil and (17.1%) in Local variety essential oil and six oxygenated compounds compromising between (82.89%) in Local variety essential oil and (93.04%) in German variety essential oil.

The major components in fennel fruits essential oil were Trans-anethole, it represented (52.29%, 56.67%, 47.18% and 62.24%) in Holland, Indian, Azoricum and German varieties respectively, the highest values of Anethole found in German variety, while the lowest values found in Local variety.

Estragole (= methyl chavicol) Compound undesirable (24.4%, 27.42%, 39.89% and 26.92%) in the same varieties respectively, the highest values of Estragole found in Local variety, while the lowest values found in Holland and German varieties. Limonene represented (9.81%, 9.05%, 6.19% and 5.12%) in the same varieties respectively, after that Fenchone at percentages (4.77, 4.48, 5.5 and 3.79) in the same varieties respectively. Fenchone has a pungent and camphorate odour; it is present especially in bitter fennel. One of the main components of the fennel is 3-Carene which represents (2.53%, 1.41%, 0.67%, and 0.80%) in the same varieties respectively. While, in Local variety the major components of its essential oil were Estragole, Trans-anethole, Limonene, Fenchone and 3-Carene at percentages (57.96, 18.23, 13.9, 5.99 and 2.15) respectively. Furthermore, the minor compounds were α-Pinene, β-Myrecene, P-Cymene and Camphor at percentages (0.89, 0.21, 0.98, and 0.33) respectively, in Holland variety. While their existed in Indian variety oil at percentages (0.33, 0.9, 0.26 and 0.16) respectively, in Azoricum variety oil at percentages (0.12, 0.4, 0.11 and 0.14) respectively, in German variety oil at percentages (0.03, 0.08, 0.92 and 0.07) respectively, and their percentages in Local variety were (0.57, 0.08, 0.4 and 0.25) respectively. Noteworthy differences were recorded in the precentage of Anisaldehyde an autoxidation product of Trans-anethole, ranged from 0.01% in Indian, Azoricum and Local varieties to the intermediate 0.02% in German variety, up to the highest 3.41% in Holland variety. Percentage of α- Fenchyl acetate ranged from 0.12% in Indian variety,0.15% in Azoricum variety, 0.37% in Holland variety up to the highest 0.45% in Local variety.

8

However, it contents were not available in German variety essential oil. Trans-Anethole, Estragole, Limonene and Fenchone were found to be main constituents in the studied varieties (Table 8). Similar results were recorded by several researchers (Arslan et al., 1989; Charles et al., 1993). It can be seen in table 8 that Local variety essential oil formed the highest percentage of Estragole, while the other varieties essential oil formed the highest percentage of T-anethole. These results are in the line with (Shalaby et al., 2011). It was reported that the chemical composition of bitter fennel essential oil is very variable. The chemo varieties and the environmental conditions cause this variability. The major components from these were found to be Methyl chavicol, Trans-anethole, Limonene, Fenchone, γ-terpinene, and piperitonene oxide (Marotti. et al., 1994). *Foeniculum vulgare* var. presents great composition differences with varying populations with the aim of clarifying the status of var. *vulgare*, the proposed to subdivide it into three chemotypes according to their relative compositions (McDonald, 1999). They called them chemotype Estragole, chemotype Estragole/Anethole and chemotype Anethole. According to that our results divided into two chemotypes.

1- chemotype. Estragole (Estragole is the major compound) such as Local variety oil.

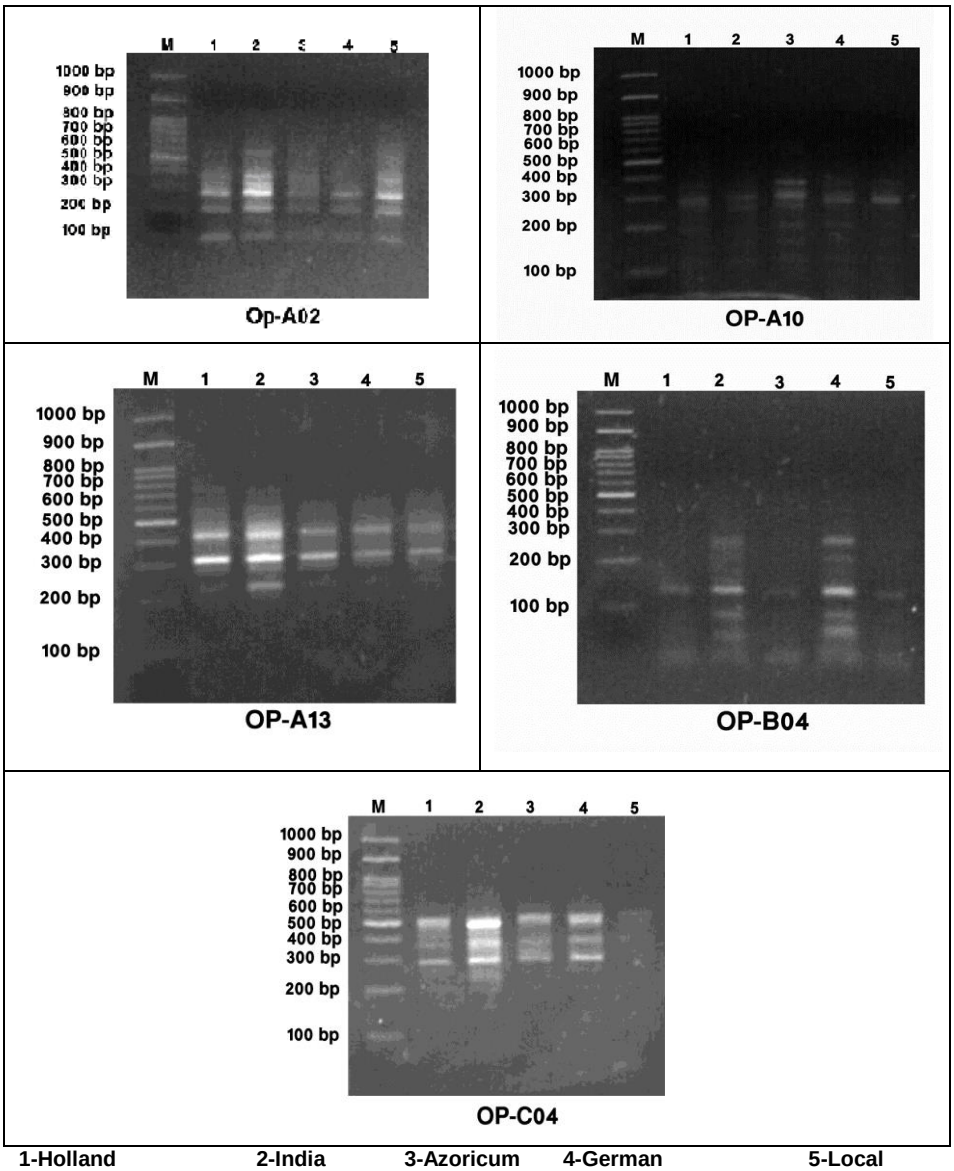
2- chemotype. Anethole(T-anethole is the major compound) such as Holland, Indian, Azoricum and German varieties essential oil.

Molecular genetic identification

Randomly amplified polymorphic DNA (RAPD) markers

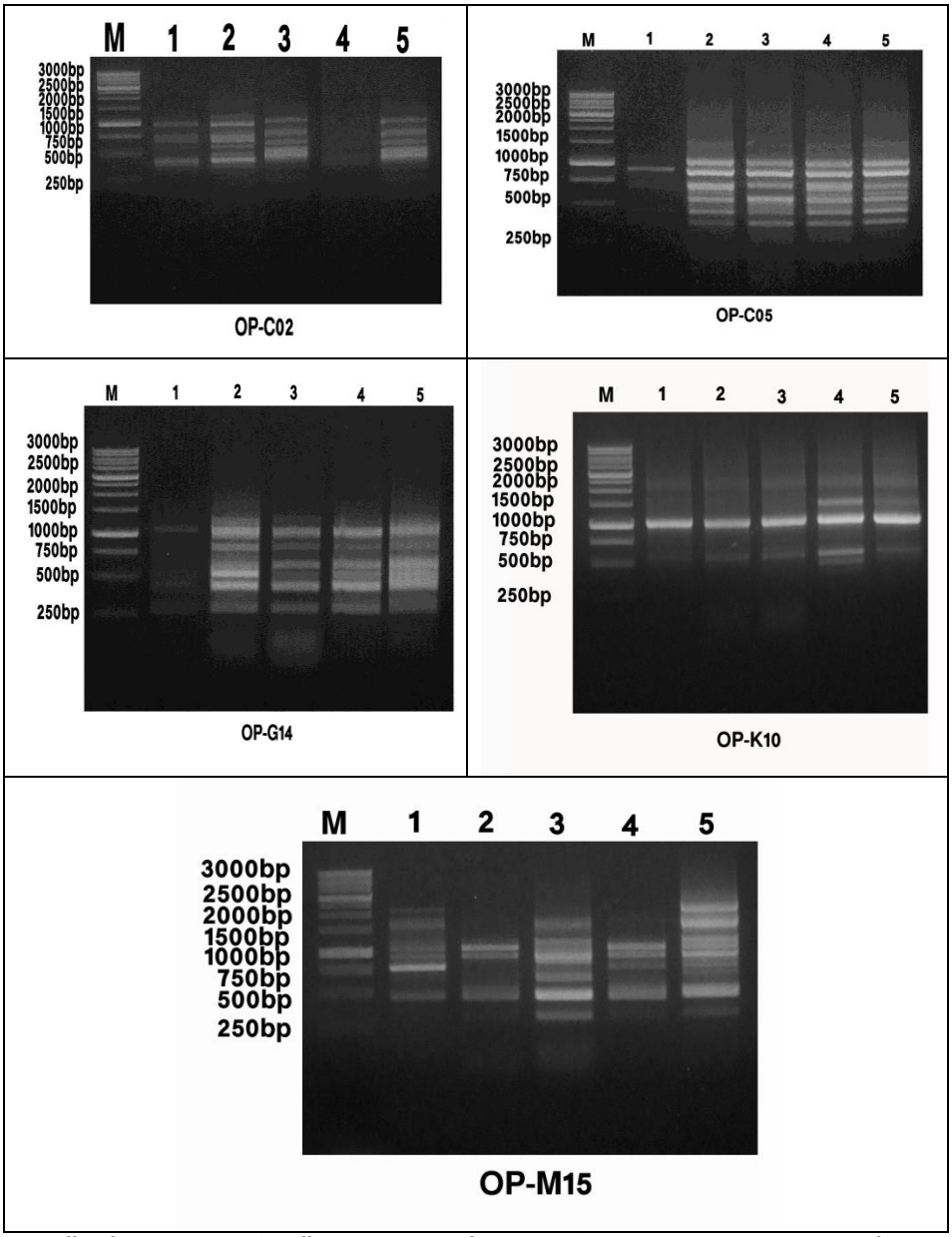
Table (9) and Figures (1 and 2) show the results of total amplified fragments (TAF), amplified fragments (AF) and specific markers (SM) for each variety of Fennel using RAPD-PCR analysis with ten random primers. A total number of 98 DNA fragments were detected, in which 60 (61.22%) were polymorphic fragments. However, 38 bands were common (monomorphic) for all cultivars. Polymorphism levels differed from one primer to another, i.e. The results found that (OP-C02, OP-M15, OP-B04, OP-C04 andOP-A10) primers exhibited high levels of polymorphism (90.91%, 78.57%, 75.00%, 72.73% and 70.00%) respectively. While, (OP-A02, OP-A13, OP-K10 and OP-C05) primers exhibited moderate level of polymorphism (62.50%, 55.56%, 62.50% and 54.55%), and primer OP-G14 represented the lowest level 37.50% as exhibited in Table (9). These results agree with the previously reported for other medicinal and aromatic species like *Lavndaula angustifolia* (Echeverrigaray and Agostini, 2000) and *Ocimom gratissimum* (Vieria et al., 2001). The lowest number of polymorphic fragments was detected for primer OP-M15 (3 out of 14 amplified bands), while the highest number of polymorphic fragments was detected for primer OP-C02 (10 out of 11 amplified bands). Cultivar-specific markers generated from RAPD-PCR analysis are shown in Table (9). Thirty-one out of 98 RAPD-PCR fragments were found to be useful as cultivar-specific markers. The largest number of RAPD-PCR markers was scored for Indian variety (68 markers), while the lowest (49 markers) was scored for Holland variety.

9



1-Holland 2-India 3-Azoricum 4-German 5-Local

Fig. 1: RAPD-PCR analysis of five fennel varieties cultivated under Egyptian sandy soil condition. (Second season)(Using ladder markers from 100bpto 1000bp)



1-Holland 2-India 3-Azoricum 4-German 5-Local

Fig. 2: RAPD-PCR analysis of five fennel varieties cultivated under Egyptian sandy soil condition. (Second season)(Using ladder markers from 250bp to 3000bp)

In the meantime, the highest number of RAPD-PCR cultivar-specific markers was generated by primer OP-C04 (7 markers), while the lowest number (1 markers) was generated by primers OP-A13 and OP-B04. Moreover, OP-G14 was generated no RAPD-PCR cultivar-specific markers.

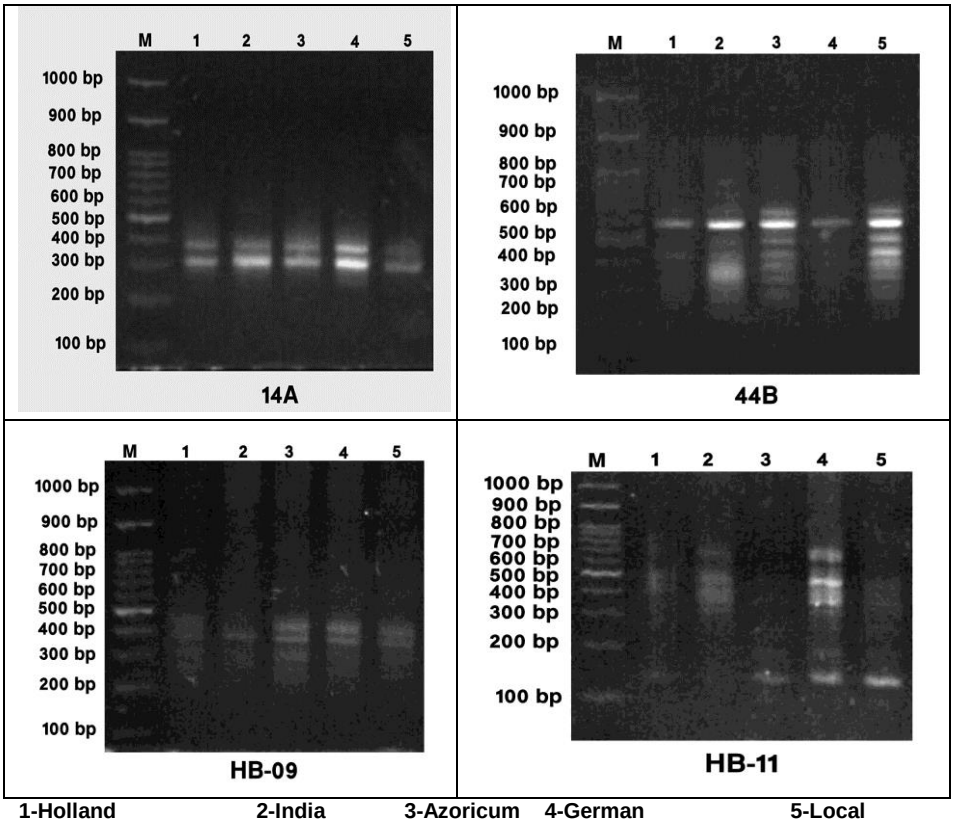
In conclusion, all of the ten primers used allowed enough distinction among the cultivars under study. These cultivar-specific markers can be used in subsequent experiments to detect molecular markers for polymorphic genes with economic importance among these and other cultivars. Similar finding were obtained in mints by Hassan (2005) and Momeni et al., (2006) and in other genera (Choi et al., 1999 and Benedetti et al., 2000).

Genetic similarity and cluster analysis based on RAPD markers:

Genetic similarities among the five fennel varieties were estimated according to the RAPD data by using UPGMA computer analysis (Table 10 and Fig. 1 and Fig. 2). Table 10 showed that the most two closely related varieties were Indian and Local with the highest similarity index (1.000). On the other hand, the results indicated that the two most distantly related varieties were Holland and Local with low similarity index (0.411). The results showed that there was no similarity between Azoricum variety and Local variety. A dendrogram for the genetic relationship among the five genotypes of fennel varieties genotypes is exhibited in Fig. 4, which separated them into two major groups. The first group included Indian variety only, while the second group included two subgroups, the first subgroup involved German variety only and the other subgroup included Local, Azoricum and Holland genotypes.

Inter Simple Sequence Repeats (ISSR) markers:

The four ISSR primers succeeded in amplifying DNA fragments for the five fennel varieties genotypes (Fig.3). Polymorphism levels differed from one primer to another, i.e. HB-11and 44A primers exhibited high levels of polymorphism (93.33% and 77.78%) respectively, while, HB-09 primer exhibited low level of polymorphism (62.5%) as exhibited in Table 9. The number of total amplified fragments (TAF), polymorphic fragments (PF), monomorphic fragments (MF) and specific markers (SM) for each primer of the four primers are shown in Table 9. 14A Primer showed two DNA fragments with molecular size ranging from 279 to 332bp (Fig.3 and Table 9), those two fragments were monomorphic, and there was not any polymorphic fragments or specific markers.



1-Holland 2-India 3-Azoricum 4-German 5-Local

Fig. 3: ISSR-PCR analysis of five fennel varieties cultivated under Egyptian sandy soil condition. (Second season)(Using ladder markers from 100bp to 1000bp)

44B primer showed nine DNA fragments with molecular sizes ranging from 238 to 515bp, seven fragments were polymorphic (77.78 %), and two of them were positive species- specific markers at 429bp for Azoricum genotype and 238bp for Local genotype. HB-09 primer showed eight DNA fragments with molecular size ranging from 207 to 518bp, five fragments were polymorphic (62.50 %), and two of them were positive species- specific markers at (399bp) for Indian genotype and at (207bp) for Azoricum genotype. HB-11 primer showed fifteen DNA fragments with molecular size ranging from 102 to 631bp, fourteen fragments of them were polymorphic (93.33 %), and seven of them were positive species- specific markers at (631 and 357bp) for Holland genotype, (107bp) for Azoricum genotype,(596, 294 and ,114bp) for German genotype and at 102bp for Local genotype.

Genetic similarity and cluster analysis based on ISSR markers:

According to ISSR results, the most two closely related varieties were Indian and German (Table 11) with the highest similarity index (1.000).On the other hand, the most two distantly related varieties were German and Local with low similarity index (0.550) and the two varieties located very far were Azoricum and Local variety with similarity index (0.000).

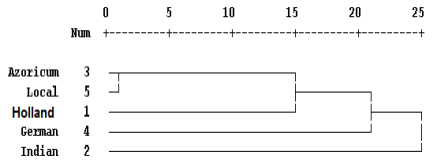


Fig. (4): A dendrogram illustrates the genetic distance for five fennel varieties genotypes based on RAPD data.

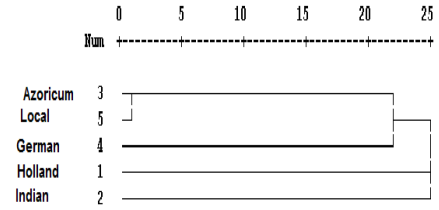


Fig. (5): A dendrogram illustrates the genetic distance for five fennel varieties genotypes based on ISSR data.

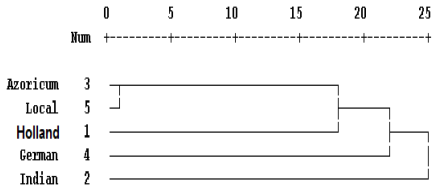


Fig. (6): A dendrogram illustrates the genetic distance for five fennel varieties genotypes based on over-combination of RAPD and ISSR analysis.

Table (10) Similarity value (Pairwise comparison) of five fennel varieties genotypes based on RAPD data.

	Holland	Indian	Azoricum	German
Holland				
Indian	0.763			
Azoricum	0.552	0.936		
German	0.768	0.732	0.746	
Local	0.411	1.000	0.000	0.604

Table (11) Similarity value (Pairwise comparison) of five fennel varieties genotypes based onISSR data.

	Holland	Indian	Azoricum	German
Holland				
Indian	0.835			
Azoricum	0.753	0.835		
German	0.920	1.000	0.908	
Local	0.835	0.679	0.000	0.550

Table (12) Similarity value (Pairwise comparison) of five fennel varieties genotypes based on over-combination of RAPD and ISSR analysis.

	Holland	Indian	Azoricum	German
Holland				
Indian	0.860			.
Azoricum	0.681	1.000		
German	0.904	0.893	0.888	
Local	0.604	1.000	0.000	0.650

Figure 5:indicated that the dendrogram revealed one main group of three varieties including two subgroups. Subgroup 1 included both Azoricum and Local and subgroup 2 included German variety only. The remaining varieties (Indian and Holland) represented distant sequences.

Combined identification based on RAPD and ISSR analysis:

Varieties distribution on the consensus tree according to the banding patterns of RAPD differed from that based on ISSR banding patterns, which may be due to that each technique, amplified different parts of the genome. So, it is better to use the combination of the banding patterns of the two

techniques to use more segments of the genome that will increase the validity of the consensus tree.Results of the combined data as shown in Fig. 6 and Table12 exhibited that the most closely related varieties were Indian and both of Local and Azoricum with the highest similarity index (1.000). On the other hand, the two most distantly related cultivars were Holland and Local with low similarity index (0.604) and also the two varieties located very far were Azoricum and Local variety with similarity index (0.000).

The results of the consensus tree indicated that the tree divided the cultivars into two main clusters, the first included varieties Indian and German. The second one divided into two subgroups, the first one included Local and Azoricum varieties and the other included Holland. This study provides evidence that RAPD and ISSR polymorphisms could be used as efficient tools for the detection of similarities and phylogenetic relationships of the studied genotypes. The same conclusion was obtained by several authors (Alexander, 2002: Abdel-Tawab, et al., 2001 and Heikal, et al 2007). RAPD technique also is an effective technique in studying inter and intra specific variation in fennel. These results are in accordance with (Fu et al., 2003) who reported that out of 92 RAPD primers, 64 gave polymorphism which indicated that 51.2% of total diversity was among populations and 48.8 % within populations of *Changium smyrnioides* Wolff (Apiaceae).

General Discussion:

- Increasing compost fertilizer level progressively and significantly increased the values of such studied vegetative and yield trials. Wherever the highest values of increase resulted by the highest level of compost (8 ton/ Fed.) in sandy soils gave the highest values of growth, fruits and essential oil yield in fennel plant. These results agree with those obtained by (Kandil, 2002) on fennel (*Foeniculum vulgare* Mill.); (Ibrahim and Ezz El-Din, 1999) on catnip (*Nepta cataria* L.)
- In this respect, it is possible that the favorable effect of compost on growth characteristics may be due to their ability to enhance the physical, chemical and biological properties of the soil. A similar suggestion was made by Hanafy et al. (2002) on rocket plants.
- The results revealed that Azoricum variety was the highest values in essential oil percentage, fruits yield/plant, while in another study on fennel varieties (Shalaby et al., 2011) found that the German strain had the higher oil content followed by the Holland and Azoricum strains.
- According to the dendrogram of RAPD, ISSR and the combination between them the results revealed that Holland existed alone in group or sub group of each dendrogram. This would explain the growth results of this study where Holland showed the lowest value in most growth parameter (plant height, No of umbels/plant, No of flowers/umbel, essential oil (%), essential oil yield per plant, essential oil yield per). Also, the results indicated that Holland recorded the highest percentage of Anisaldehyde oil (3.41%). Such results are found for the German variety. From the dendrogram, it would be observed that German variety existed alone in a group or in a subgroup. This would explain the results indicated that German variety recorded the highest value of fresh and dry weight. Also α- Fenchyl acetate oil existed in all studied variety except Geman variety.

CONCLUSION

From previous results it can be concluded that, Azoricum variety gave the highest values of vegetative growth, fruit yield and essential oil content, while German variety gave the highest values of Anethole were main constituents in essential oil, Estragole (= methyl chavicol) Compound undesirable found in Local variety, while the lowest percentage found in Holland and German varieties.

It can expand the cultivation of varieties fennel of German and Azoricum and breeding operations with a Local variety to produce a distinctive variety between the previous varieties.

It can be observed from similarity tables that Local variety and Azoricum variety represented distant sequences. It may explain that Azoricum variety scored higher trans-anethole percentage than Local variety (47.18% and 18.23%) respectively. Also, Local variety scored the highest value of estragole oil (57.96%) while, Azoricum variety recorded (39.89%) and so that, they could be induced into a breeding program in the future for commercial production.

REFERENCES

Abdel-Tawab, F.M., A. Abo-Doma, A.I. Allam and H.A. El-Rashedy,(2001). Assessment of genetic diversity for eight sweet sorghum cultivars (Sorghum bicolar L.) using RAPD analysis. Egypt. J. Genet. Cytol., 30: 41-50.

Ahmed S. Shalaby, Saber F. Hendawy &Mona Y.Khalil, (2011). Evaluation of Some Types of Fennel (Foeniculum vulgare Mill.) Newly Introduced and Adapted in Egypt. J. of Essential Oil Research, 32: 35-42

Alexander, A.J., (2002). Genetic diversity of populations of Astragalus oniciformis using Intersimple sequence repeat (ISSR) markers. M.Sc. Thesis in Botany and Plant Pathology, Oregon State Univ., USA.

Ammiraju J.S.S., Dholakia B.B., Santra D.K. (2001). Identification of inter simple sequence repeat (ISSR) markers associated with seed size in wheat. *Theor Appl* Genet 102:726–732.

Anderson G.R., Brenner B.M., Swede H. (2001). Intrachromosomal genomic instability in human sporadic colorectal cancer measured by genome-wide allelotyping and inter-

(simple sequence repeat) PCR. Cancer Res 61:8274–8283.

Arslan, N., Bayrak, A., & Akgu¨ l, A. (1989). The yield and components of essential oil in fennels of different origin (Foeniculum vulgare Mill.) grown in Ankara conditions. Herba Hungarica, 3, 27–30.

Badran, M. S. S. (2002): Organic US. mineral fertilization on yield and components of some carley varieties under sandy soil conditions. Proc. Minia 1st Conf. for Agric. & Environ. Sci., Minia, Egypt, March 25 – 28 : 917 – 934.

Barazani, O, Cohen, Y., Fait,A., Diminshtein, S., Dudai, N., Ravid, U., Putievsky, E., and Friedman, J.(2002). Chemotypic differentiation in indigenous populations of Foeniculum vulgare var. vulgare in Israel. Biochemical Systematics and Ecology, 30, 721-731.

Benedetti, L., G. Burchi, A. Mercuri and T. Fchida, (2000). Use of RAPD analysis for genotype identification in alstroemeria. Acta Horticulture, 508: 277-279.

Bennici, A., A. Maria and G.V. Giovanni. (2003). Genetic stability and uniformity of *Foeniculum vulgare Mill.*, regenerated plants through organogenesis and somatic embryogenesis. Plant Sci., 161(1): 221-227.

Blair M.W., Panaud O, McCouch S.R. (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theor Appl* Genet 98:780–792.

Charles, D.J., Morales, M.R., & Simon, J.E. (1993). New crops. In J. Janick, & J.E. Simon (Eds.), Essential oil content and chemical composition of finocchio fennel (pp. 570–573). New York: Wiley.

Choi, H.S., K.S. Kim, J.K. Choi, K.K. Lee, D.K. Hong, W.H. Kang and Y.S. Lee, 1999.

Classification of *Lilium* using random amplified polymorphic DNA (RAPD). Korean J. of Hort. Sci. & Technol., 17: 144-147.

Davis, P. H. (1972). Flora of Turkey and the East Aegean Islands, vol.4 University Press, Edinburgh.

Dice, L. R. (1945). Measures of the amount of ecologic association between species. Ecology, 26: 297-302.

Echeverrigaray, S. and G. Agostini.(2000). Avaliacao da variabilidade genetica em lavandas atraves de marcadores de RAPD. Horticult. Bras., 185-186.

El-Sherbeny, S.E., M.Y. Khalil and N.Y. Naguib, (2005). Influence of compost levels and suitable spacing on the productivity of *Sideritis montana* L. plants recently cultivated under Egyptian conditions. Bull. Fac. Agric., Cairo Univ., 56(2): 373-392.

El-Ghadban, E. A. E., A. M. Ghallab, A. F. Abdelwahab, (2002). Effect of organic fertilizer (Biogreen) and biofertilization on growth, yield and chemical composition of Marjoram plants growth under newly reclaimed soil conditions, 2nd Congress of Recent Technologies in Agriculture, 2, 334-361.

Farag RS, Daw ZY, Hewedi FM, El-Baroty GSA (1989): Antimicrobial activity of some Egyptian spice essential oils.J Food Protect 1989;52:665–667.

Fu, C., Q.I.U Yingxiong and H. Kong. (2003). RAPD analysis for genetic diversity in *Changium smyrnioides* (Apiaceae), an endangered plant. Bot. Bull. Acad. Sci., 44: 13-18.

General Medical council British (1963). Pharmacopoeia 1963; 1st Edn., The pharmaceutical press,London, Uk.,Pages;1210.

Gomaa, A.M. (1995): Response of certain vegetable crops to biofertilization. Ph.D. Thesis, Fac. Agric., Cairo Univ.

Gupta S.K., Souframanien J., Gopalakrishna T. (2008). Construction of a genetic linkage map of black gram, *Vigna mungo* (L.) Hepper, based on molecular markers and comparative studies. Genome 51:628–637.

Hallden C., Nilsson N. O., Ebding L. M. and Sdl T., (1994). Evalution of RFLP and RAPD markers in a comparision of *Brassica napus* breeding lines, Theor. Appl. Genet., 123-128.

Hanafy Ahmed, A. H., M. K. Kahlil, A. M. Farrag, (2002). Nitrate accumulation, growth, yield and chemical composition of Rocket (Eruca vesicaria Sub sp. sativa) plant as affected by NPK fertilization, kinetin and salicylic acid, Annal. Agric. Sci. Ain Shams Univ., Egypt, 47, 1-26.

Harridy, I. M. A., S. G. I. Soliman, T. A. Mervat,(2001). Physiological, chemical and biological studies on Lemongrass Cymbopogon citratus (DC) stapf in response to diazotrophic bacteria, Agric. J. Sci. Mansoura Univ., 26, 6131-6152.

Hassan, A.H.M., 2005. Identification of molecular markers for some morphological and

biochemical characters in some medicinal plants. M.Sc. Thesis. Ain Shams Univ., Fac. Agric.

Heikal, A. Hadia, Y. Mabrouk, O.M. Badawy, A. El-Shehawy and Effa t A. Badr, (2007).

Fingerprinting Egyptian Gramineae Species Using Random Amplified Polymorphic DNA

(RAPD) and Inter-simple Sequence Repeat (ISSR) Markers. J. Cell and Molecular Biology (RJCMB). 1(1): 15-22.

Ibrahim, M.E., (1999). Physiological and chemical studies on Tusli plant (*Ocimum sanctum*). Egypt. J. Hort., 26(2): 147-165.

Ibrahim, M.E. and A.A. Ezz El-Din, (1999). Cultivation of *Nepta cataria* L. in Egypt: its growth yield and essential oil content as influenced by some agronomic practices. Egypt. J. Hort., 26(3): 281-302.

Kandil, M.A.M., (2002). The effect of fertilizers for conventioal and organic farming on yield and oil

quality of fennel (Foeniculum vulgare Mill.) in Egypt. Ph.D.Thesis. Fakultat der Techischen Univ.,

Carlowilhelmina Univ.

Khalil, M.Y., (2002). Influence of compost and foliar fertilization on growth, chemical composition of *Rosemarinus officinalis* L. Egypt. J. Appl. Sci., 17(10): 684-699.

Khalil, M.Y., N.Y. Naguib, and S.E. El-Sherbeny, (2002). Response of *Tagtes erecta* L. to compost and foliar application of some microelements. Arab Univ. J. Agric. Sci., Ain shams Univ., Cairo, 10(3): 939-964.

Khalil, M.Y. and S.E. El-Sherbeny, (2003). Improving the productivity of three *Mentha* species recently cultivated under Egyptian conditions. Egypt. J. Appl. Sci., 18(1): 285-300.

Lal RK., (2007). Association among agronomic traits and path analysis in fennel. Journal of Sustainable Agriculture 30, 21-29.

Lampkin, N.(1990):Organic Farming. Farming Press Book.United Kingdom. P. 63.

Leroy X.J., Leon K. (2000) A rapid method for detection of plant genomic instability using unanchored-microsatellite primers. *Plant Mol Biol* Rep 18 (2000) 283a–283g.

Lopes VR, Barata AM, Farias R, Mendes MD, Lima AS, Pedro LG, Barroso JG, Figueiredo AC.,(2010). Morphological and essential oil variability from nine Portuguese fennel (*Foeniculum vulgare* Mille.) accessions. Acta Horticulturae, 860, 33-49.

Marotti M, Piccaglia R, Giovanelli E, Deans SG, Eaglesham E; (19945): Effects of variety and ontogenic stage on the essential oil composition and biological activity of fennel (*Foeniculum vulgare* Mill.). J Essent Oil Res 1994;6:57–62.

Mathur, G., Owen, G., Dinel, H. & Schnitzer, M. (1993). Determination of compost biomaturity.Biological Agriculture & Horticulture, 10, 65-85.

McDonald, T. A., (1999). Evidence on the carcinogencity of estragole, Ph.D. staff Toxicologist Reproductive and cancer Hazard Assessment Section office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Miraldi, E. (1999). Comparison of the essential oils from ten *Foeniculum vulgare* Miller samples of fruits of different origin. Flavour and Fragrance Journal, 14, 379–382.

Mohamed, S. A. and F. M. A. Matter (2001): Effect of ammonium nitrate and organic fertilizers on growth, essential oil yield and chemical constituents of marigold (*Tagetesminuta L*.) plant. Fayoum J. Agric. Res., & Dec. 15 (1): 95 – 107.

Momeni, S., B. Shiran and K. Razmjoo, 2006. Genetic variation in Iranian mints on the bases of RAPD analysis. Pakistan J. of Biological Sciences, 1898-1904. URL http://www.ansinet.org/pjbs.

Naguib, N.Y. and A.A. Aziz, (2004). Yield and Quality of *Hyoscyamus muticus* L. in relation to some fertilizer treatments. Egypt. J.Hort.,29(4):115-126.

Safaei L, Zeinali H, Afiuni D., (2011). Study of genetic variation of agronomic characteristics in Foeniculum vulgare Mill. Genotypes Iranian Journal of Rangelands and Forests Plant Breeding and Genetic Research 19, 167-180. [with English abstract]

Shiran, B., N. Amirbakhtiar, S. Kiani, Sh. Mohammadi, B.E. Sayed-Tabatabaei and H. Moradi.

(2007). Molecular characterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. Scientia Horticulturae, 111: 280-290.

Steel, R. R. D. and J. H. Torrie (1960). Principles and Procedures of Statistics. MC. Graw-Hill International Book Company, 3rd, Ed. London, pp. 633.

Vieria, R.F., R.J. Grayer, A. Paton and J.E. Simon. 2001. Genetic diversity of *Ocimum gratissimum* L. based on essential oil constituents, flavonoids and RAPD markers. Cab. Int., 7(4): 23-24.

Wang H.Z., Wu Z.X., Lu J.J. (2009). Molecular diversity and relationships among *Cymbidium goeringii* cultivars based on inter-simple sequence repeat (ISSR) markers. Genetica 136(3):391–399.

Williams, J.G.K., A.R.K. Kubelik., T. Livak., J.A. Rafalski, S.V. Tingey. (1990). Nucleic Acids Research. 18: 6531-6539.

Wolfe A.D., Xiang Q-Y, Kephart S.R. (1998). Assessing hybridization in natural populations of Penstemon (Scrophulariaceae) using hypervariable inter simple sequence repeat markers.Mol Ecol 71:1107–1125.

Yousef, R. M. M. (2002): Effect of irrigation and fertilization on *Matricariachamomilla* L. growth and productivity in sandy soil. Ph. D. Thesis, Fac. Agric., Zagazig Univ.

Yousef, R. M. M. , A.M. A. Hamouda and G. G Nawal. (2008): Effect of irrigation and organic fertilization on growth and productivity of *Majoranahortensis* in sandy soil. Mansoura University Journal of Agricultural Sciences 33(11):8039-8056.

النموواِنتاج الزيت الطيار والتركيب الوراثى لبعض أصناف الشمر تحت مستويات مختلفة من الكومبست فى الأراضى الرملية

ربيع محمد مصطفى يوسف , أمنية فاروق أبو الليل

قسم بحوث النباتات الطبية والعطرية-معهد بحوث البساتين – مركز البحوث الزراعية-القاهرة-مصر

اجرى هذا البحث خلال موسمين متتالين هما: (2011 – 2012) و(2012-2013) فى مزرعة التجارب بمحطة بحوث البساتين بالقصاصين - محافظة الاسماعيلية- مصر وذلك لدراسة تأثير معدلات من الكومبست (4-6-8طن /فدان) لخمسة أصناف مختلفة من الشمرهم: (الهولندى , الهندى , الأزوريكم , الألمانى , البلدى) لدراسة تأثيره على النمو وإنتاج المحصول ونسبة الزيت الطيار للشمر وكذلك دراسة الفروق الوراثية والكيميائية لأصناف الشمر لمعرفة مدى العلاقة بينهم وهل من الممكن إستخدام الخصائص الجزيئية لتمييزهم.

وقد وجد أنه كلما زاد معدل التسميد بالكومبست (من 4-8طن/فدان) كلما زادت قيم الصفات الخضرية. و قد وجد أن اعلى القيم كانت لصنف الأزوريكم حيث سجل اعلى قيم للنمو والأنتاج والنسبة المئوية للزيت الطيارو إنتاجية الزيت/ نبات فى كلا الموسمين. كما تبين ان اهم المركبات الرئيسية لزيت الشمرهى : الأنيثول و الأستراجول و الفنشون و الليمونين. وقد سجل الصنف الشمر الألمانى اعلى نسبة من الأنيثول بينما أقل قيمه له كانت فى صنف الشمر البلدى واعلى نسبة من الأستراجول (ميثايل شافيكول) كانت في صنف الشمر البلدى وهومركب غير مقبول واقل قيمه له كانت فى صنفى الهولندى والألمانى.

وللدراسة الجزيئية فقد تم أستخدام طريقة ال RAPD وهى طريقة فعالة فى الكشف عن التنوع أو الأختلاف الوراثى داخل وبين أصناف الشمر بأستخدام 10بوادىء جينية أعطت مجموعها 98حزمة جزيئية منها 60حزمة متشابهة بنسبة61.22%. وتم العثور على 31حزمة جزيئية تعتبر علامات للصنف.

وقد سجل الشمر الهندى أكبر عدد من الحزم الكاشفة (68حزم) بينما الشمر الهولندى فقد سجل أقل عدد من الحزم الكاشفة (49حزم). و أوضحت النتائج أن أكبر عدد من الحزم الكاشفة (7 حزم) كان بأستخدام البادىء OP-C04 و أقل عدد (حزمة واحدة) كان بأستخدام كل من البادئين OP-A13 و OP-B04.

فى التحليل بأستخدام طريقة ال ISSR فقد أستخدم 4 بوادىء جينية أعطت نتائج مختلفة. فكان عدد الحزم المتشابهة 26 حزمة من أصل 34و قد تم العثور على 11حزمة جزيئية تعتبر علامات للصنف. وكذلك تم تقدير التشابة الجينى وفقا للنتائج المتحصل عليها من طريقتى ال RAPD وISSR والتى أختلفت فى كلا الطريقتين فقد كان اعلى تشابه بين صنفى الشمر البلدى والشمر الهندى بطريقة ال RAPD بينما بطريقة الISSR فقد كان أعلى تشابه بين صنفى الهندى و الألمانى وقد يرجع ذلك بسبب أختلاف كل طريقة فى درجة تضخمها للجينوم. لذا كان من الأفضل أستخدام مزيج من الطريقتين فى شجرة التوافق.

Table (4): Effect of different compost levels on growth, fruit yield, essential oil % and essential oil yield per plant (ml.) of five varieties of fennel during the two seasons 2011/2012 and 2012/ 2013 over all varieties:

Level of compost	Plant height (cm)		F.W of herb (g)		D.W of herb (g)		No of umbels/plant		No of flowers/umbel		yield of fruits per plant (gm.)		Essential oil (%)		Essential oil yield per plant (ml.)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
4 ton/fed	88.80	82.40	124.51	113.04	24.34	21.16	11.53	10.00	19.13	16.13	50.00	40.78	1.62	1.85	0.82	0.76
6 ton/fed	94.00	89.13	157.25	146.14	30.98	26.44	16.93	13.26	21.47	19.26	56.73	49.22	2.07	2.32	1.25	1.16
8 ton/fed	105.46	100.06	180.73	169.33	38.74	31.44	20.66	19.40	23.87	23.13	61.00	54.97	2.39	2.62	1.52	1.46
L.S.D at 5%	1.80	4.61	3.55	6.23	0.98	1.37	1.17	0.78	1.24	0.30	1.67	1.85	0.06	0.05	0.07	0.05

Table (5): Performances varieties for growth, fruits production, essential oil % and essential oil yield per plant (ml.) of fennel plant during the two seasons 2011/2012 and 2012/ 2013 using the three levels of fertilizers:

The varieties	Plant height (cm)		F.W of herb (g)		D.W of herb (g)		No of umbels/plant		No of flowers/umbel		yield of fruits per plant (gm.)		Essential oil (%)		Essential oil yield per plant (ml.)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Holland	75.11	64.78	118.22	106.71	22.56	19.29	14.00	10.89	13.78	13.33	49.50	42.59	1.61	1.88	0.81	0.81
Indian	91.22	82.78	119.32	110.51	28.71	20.60	15.67	13.89	20.00	17.66	55.94	44.91	1.92	2.14	1.08	0.98
Azoricum	118.22	116.11	202.25	189.42	39.00	34.82	17.11	16.78	24.67	22.55	73.00	57.83	2.82	3.03	2.14	1.77
German	108.33	108.66	235.15	212.97	45.80	39.91	13.67	12.00	23.11	19.66	47.55	41.24	1.99	2.20	0.96	0.93
Local	87.56	80.33	95.88	94.59	20.71	17.12	21.44	17.56	25.89	24.33	53.55	55.04	1.81	2.04	1.00	1.15
L.S.D at 5%	2.01	3.30	3.23	3.48	0.89	0.50	1.01	1.40	1.84	1.06	2.47	1.59	0.04	0.06	0.04	0.05

Table (6): the mean interactions at the different compost levels and the fennel varieties on vegetation growth during the two seasons 2011/2012 and 2012/ 2013:

Level of compost	The varieties	Plant height (cm)		F.W of herb (g)		D.W of herb (g)		No of umbels /plant		No of flowers/umbel	
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
4 ton/fed	Holland	66.00	55.67	93.00	83.37	17.60	15.00	10.67	8.00	11.67	10.33
	Indian	81.67	71.00	102.46	85.80	20.70	16.56	11.67	10.00	17.33	15.00
	Azoricum	116.67	111.33	155.23	144.16	29.03	26.50	11.67	12.00	22.33	18.66
	German	101.33	104.33	197.73	185.83	39.00	35.86	9.67	7.00	21.33	16.00
	Local	78.33	69.67	74.13	66.07	15.40	11.90	14.00	13.00	23.00	20.66
6 ton/fed	Holland	75.00	65.00	124.63	110.16	23.36	19.53	14.00	10.66	14.67	14.00
	Indian	90.67	80.33	114.16	113.40	22.26	20.90	16.00	13.00	20.00	18.00
	Azoricum	112.67	115.00	212.13	197.66	41.33	35.10	17.00	15.66	24.33	22.33
	German	105.33	107.33	241.16	214.00	47.80	38.93	14.33	11.00	22.67	18.66
	Local	86.33	78.00	94.17	95.50	20.13	17.73	23.33	16.00	25.67	23.33
8ton/fed	Holland	84.33	73.67	137.03	126.60	26.70	23.33	17.33	14.00	15.00	15.66
	Indian	101.33	97.00	141.33	132.33	43.16	24.33	19.33	18.66	22.67	20.00
	Azoricum	125.33	122.00	239.40	226.43	46.63	42.86	22.67	22.66	27.33	26.66
	German	118.33	114.33	266.56	239.10	50.60	44.93	17.00	18.00	25.33	24.33
	Local	98.00	93.33	119.33	122.20	26.60	21.73	27.00	23.66	29.00	29.00
	LSD at 0.05	3.48	5.72	5.60	6.03	1.54	0.87	1.79	2.43	3.18	1.83

Table (8): Essential oil composition (%) for five varieties of fennel (*Foeniculum vulgare* Mill.), at rate of compost (6 ton /fed) in the second season.

Peak No.	components	Retention time(min)	Holland	Indian	Azoricum	German	Local
	Monoterpene hydrocarbons						
1	3-Carene	5.882	2.53	1.41	0.67	0.8	2.15
2	α-pinene	6.513	0.89	0.33	0.12	0.08	0.57
3	B-Myrecene	6.58	0.21	0.09	0.04	0.03	0.08
4	P-cymene	7.36	0.98	0.26	0.11	0.92	0.4
5	Limonene	7.418	9.81	9.05	6.19	5.12	13.9
	Total hydrocarbons		14.42	11.14	7.13	6.95	17.1
	Oxygenated monoterpenes						
6	Fenchone	8.45	4.77	4.48	5.5	3.79	5.99
7	Camphor	9.352	0.33	0.16	0.14	0.07	0.25
8	Estragole	10.163	24.4	27.42	39.89	26.92	57.96
9	α- Fenchyl acetate	10.63	0.37	0.12	0.15	---------	0.45
10	Anisaldehyde	11.128	3.41	0.01	0.01	0.02	0.01
11	Trans anethole	11.44	52.29	56.67	47.18	62.24	18.23
	Total oxygenated		85.57	88.86	92.87	93.04	82.89
	Total identified		99.99	100	100	99.99	99.99
	Unknown		0.01	0.00	0.00	0.01	0.01

Values are means of three replicates.

Table (9) : Species-specific RAPD and ISSR markers for five fennel varieties genotypes.

Primers code	Range of M.S.	TAF	MF	PF	SM	Polymorphism (%)
RAPD primers						
OP-A02	128-440	8	3	5	3 (273)-(283)-(278)-(0)-(0)bp	62.5
OP-A10	121-348	10	3	7	3 (0)-(325)-(121)-(117)bp	70.00
OP-A13	210-625	9	4	5	1 (0)-(210)-(0)-(0)-(0)bp	55.56
OP-B04	65-264	8	2	6	1 (0)-(155)-(0)-(0)-(0)bp	75.00
OP-C02	254-1129	11	1	10	5 (574)-(1129,879,553,497)-(0)-(0)-(0)bp	90.91
OP-C04	205-481	11	3	8	7 (344)-(403,251,235,205)-(438,333)-(0)-(0)bp	72.73
OP-C05	325-1311	11	5	6	4 (0)-(558)-(449)-(0)-(1087,583)bp	54.55
OP-G14	174-1097	8	3	5	0	37.50
OP-K10	206-3092	8	3	5	2 (0)-(0)-(0)- (505)-(530) bp	62.50
OP-M15	168-2516	14	11	3	5 (0)-(0)-(599)-(784,339)-(2516,1228) bp	78.57
Total RAPD primers		98	38	60	31	
ISSR primers						
14A	279-332	2	2	0	0	0
44B	238-515	9	2	7	2 (0)-(0)-(429)-(0)-(238)bp	77.78
HB-09	207-518	8	3	5	2 (0)-(399)-(207)(0)-(0) bp	62.50
HB-11	102-631	15	1	14	7 (631,357)-(0)-(107)-(596,294,114)-(102) bp	93.33
Total ISSR primers		34	8	26	11	
Total		132	46	86	59	

TAF = Total Amplified Fragments, MF= Monomporphic Fragments, PF= Polymorphic Fragments, SM= Specific Markers.