Results and Discussion

4.1 Starch gel electrophoresis:-

Four enzymatic patterns were investigated including (MDH, EST, GOT and G6PD) in which both GOT and G6PD were investigated before and during flowering. MDH was investigated just before flowering and EST during flowering.

Horizontal starch gel electrophoresis was accomplished as mentioned in (3.2).

4.1.1 G6PD:-

Further characterization of this isozyme was detected using horizontal gel electrophoresis , accordingly three spots were detected for male while two were detected for female before flowering time (Fig. 4.1). During flowering two, extra samples from flowers were characterized in addition to the leaf samples (Fig.4-2). The same results shown for the leaves .Three spots were detected for male while two were detected for female). Samples taken from the flowers showed one clear spot with more extended smear for the male compared with flower samples taken from the female .It showed one clear spot with a less extended smear, *RF* factor was 64 % for both.

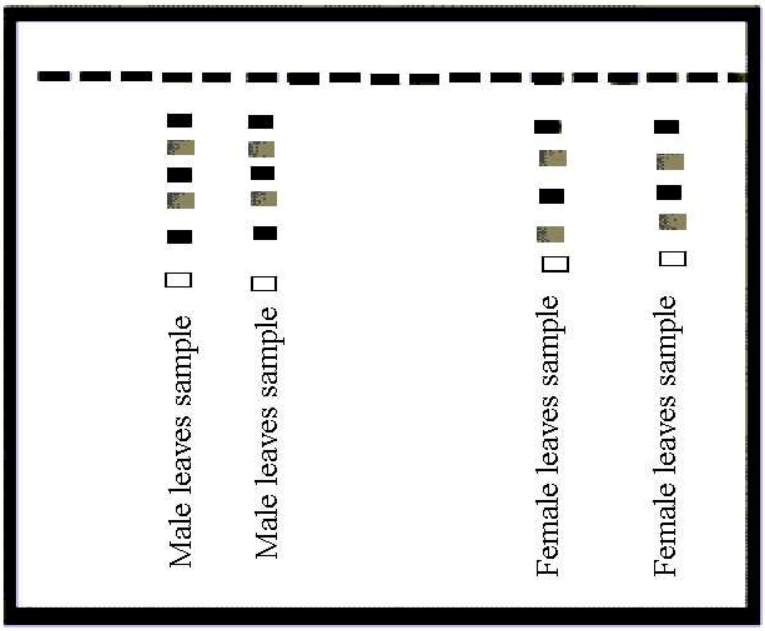


Figure (4.1) G6PD electrophoresis using horizontal gel technique for male and female leaf samples before flowering.

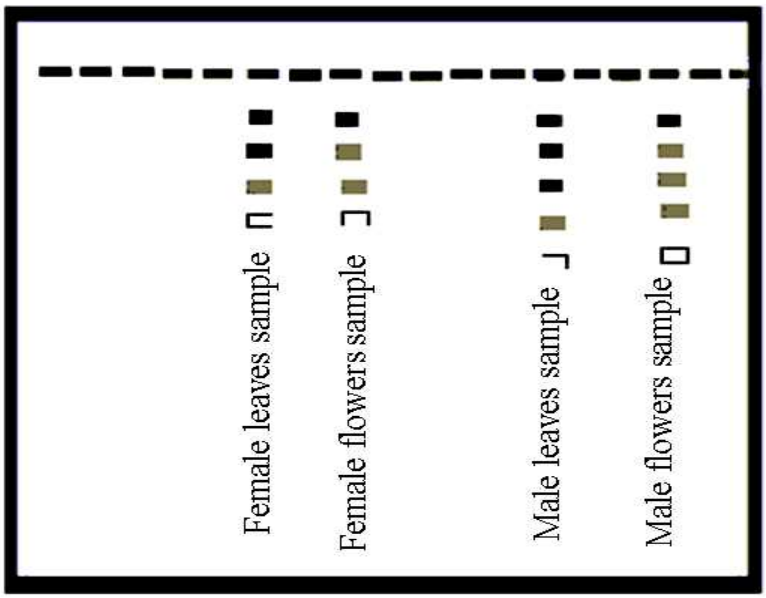


Figure (4.2) G6PD electrophoresis using horizontal gel technique for male and female leaf and flower samples during flowering time .

The energy requirement during this period will be different for the male than the female. The male needs the energy to start the pollination.
The female needs the energy later after fertilization.

G6PD is found to be a trait related with the sex in human, animals and insects (Matizikin, 2004) and shows a relation to oxidation, toxin and oxidant . So the results may suggest a difference in the healthy features as a mark for the certain infection or that the oxidation reaction differs between male and female. Although the G6PD deficiency was shown to female sex – linked gene in the human , horses and drosophila (Rain, 1996). The differences between male and female were recorded before that of the two sexes of the papaya in which a dimmer pattern *(G6PD 1 and G6PD 2)* in male while in female a monomer pattern (*G6PD 1*) (Akah, 1992).

G6PD was investigated to be sex-linked trait, it is related to humans animals and insects. The early behavior of both male and female will be detected according to the enzymatic behavior and there for is no need for the flowering time to detect the sex . The results shows a variation between the two sexes of the mature plant .

G6PD is mentioned to be the variant between the two sexes according to the rainfall gradient (Pastorino, 2001).

4.2.1 GOT :-

The characterization of this isozyme is carried out using the same horizontal gel electrophoresis technique (3.2). GOT enzymatic system showed a variation in the level of the path with RF factor reaching 76 % , as follows :-

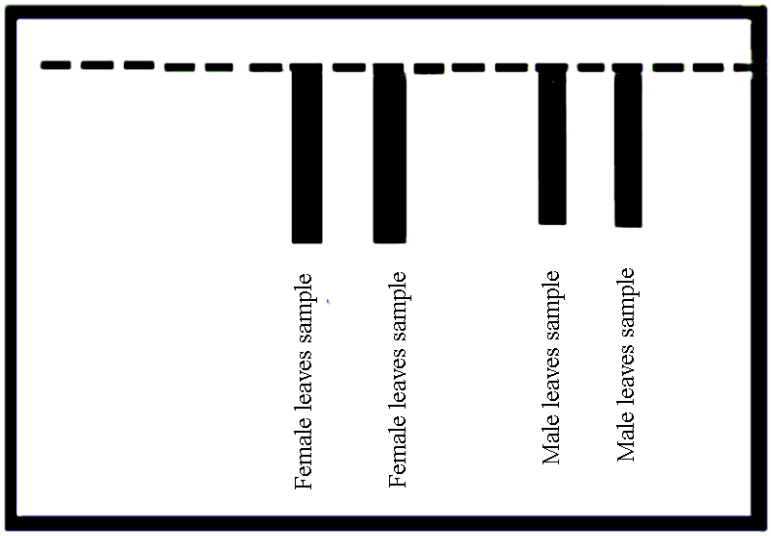


Figure (4.3) GOT electrophoresis using horizontal gel technique for male and female leaf samples before flowering.

The variation increases during the flowering time during February, with three bands for the female and two bands in the male:-

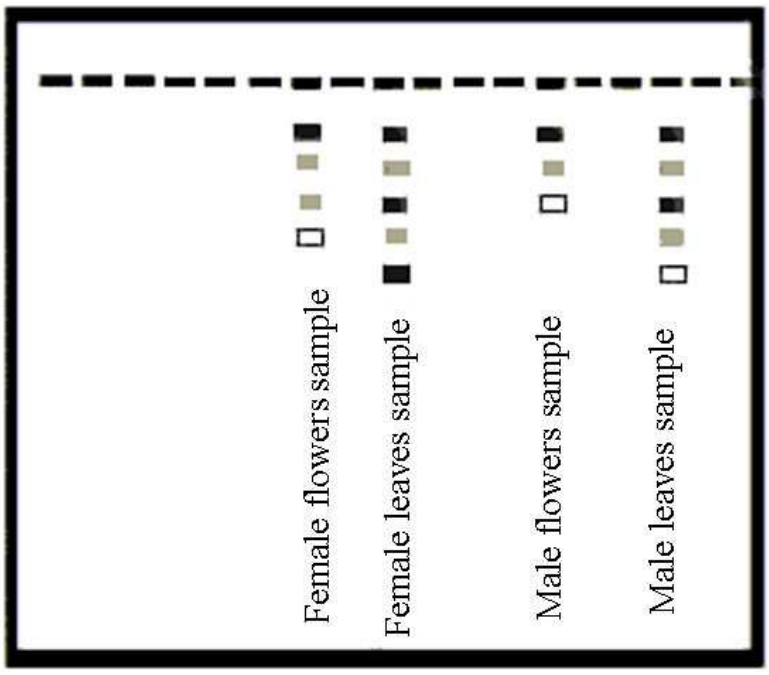


Figure (4.4) GOT electrophoresis using horizontal gel technique for male and female leaf and flower samples during flowering time .

GOT enzymatic system is related to the aerobic mechanism in which the energy is required through. The different energy requirement has shown clearly during flowering because of the fertilization time and the different energy requirement between male and female which is signed to be different in morphology and in the reproduction stage. The differences between the several strains of maize and the Isozyme variation is recorded as a paths level (James, 1994). The difference between the results after flowering and before the flowering time reflect changes between the gene expression during the this period (from September 2004 – February 2005) , which is related with the weather conditions and the age of the plant . All the metabolic pathways that are related with energy shouldn't be equal for both male and female all the times. This would give us a sign for some differentiations between the two sexes.

Certain pathways linked with sex and the differentiation here is shown clearly after the flowering , when the energy is needed for the spring flowering after cold winter. Isozymes study showed that GOT appeared as three loci's for female and two for male. The rainfall gradients may affect the expression for GOT enzymatic system according to (Pastorino, 2001).

4.2.3 Esterase:-

EST characterization was done using the same horizontal gel electrophoresis technique mentioned in (3.2). EST enzymatic system showed a variation in the level of the path with *RF* factor reaching 76 % and as follows:-

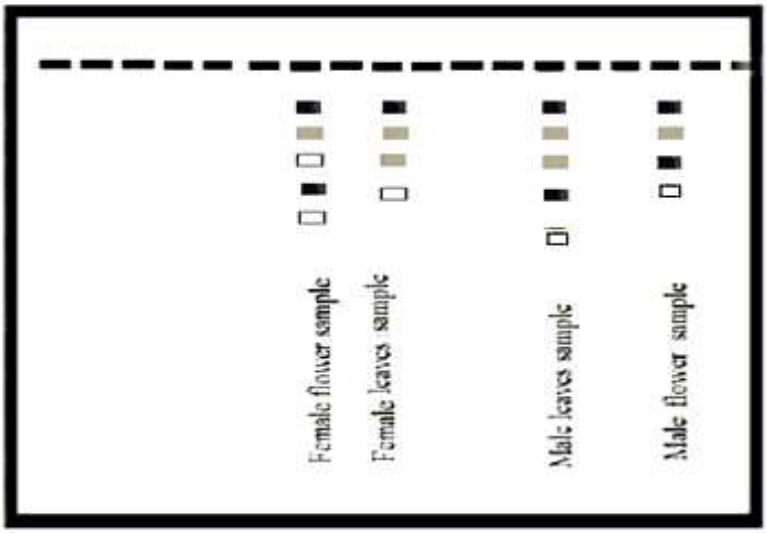


Figure (4.5) EST electrophoresis using horizontal gel technique for male and female leaves and flowering sample before flowering time RF factor reaches 83% .

The seasonal changes of esterase in the various tissues of jojoba were thought to be either related to the annual cycle of cambial activity , or to the types of tissue through cambial dormancy period . Some esterase bands occurred in the epicedium and phloem. These could be related to the protection against frost. EST bands occurred in the cambial region, these could be related to the enlarging of the cambial cells and to the formation of the new xylems and phloem and to be closely related to the differentiation of the phloem and xylem (Somsri, 1998). Papaya showed also a variation in esterase and it was recorded by (Simon, 2003) in which two bands were recorded for male and one for female. This may conferm the results. In addition , esterase used as an enzyme represented as a reversible enzyme its reaction depends upon too many agents like temperature , pH and substrate concentration and which may result in the variation.

4.2.4 MDH :-

MDH electrophoresis shows a variation before flowering time and the samples were taken from leaves , four loci for female and three for male were recorded as shown below:-

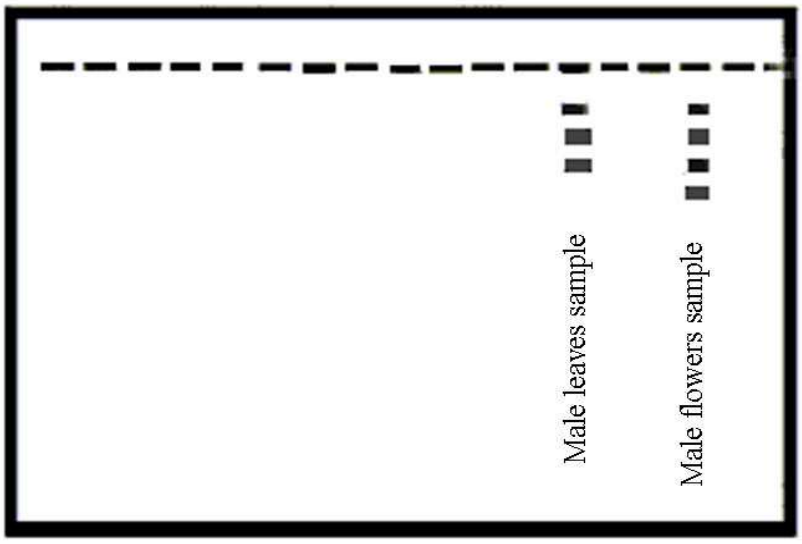


Figure (4.6) MDH electrophoresis using horizontal gel technique for male and female leaves and flowering sample before flowering time RF factor reaches 79 % .

MDH isozyme is used in clonal identification studies as shown in (Bergmann, 1987). It is used in population structure surveys and molecular taxonomy between plant sexes (Jelinski and Cheliak, 1992).

MDH was described as a single locus with three alleles . Although no indication is given of their interpretation of the banding patterns (Bergmann, 1987). Five patterns were obtained (pentameric) (Cheliak and Pit, 1984) . It shows a variation in *lepidoptera yponomeutidae* which is a small ermine moths between the two sexes one loci for the male and two for the female (Raijmann *et al* .,1996). This would partially agree with our results in which one loci is the difference between the two sexes.

This may lead to conclude that one loci at least is related to the sex, or it might be related to the expression mechanism of the allele during the period of the test. *Austrocedrus chilensis* is one of the most important tree species of the Patagonian Andean forests. Variation between male and female is shows as three spots for female and four for male (Pastorino, 2001).

4.3 Total protein concentrations between the two sexes:-

Results show differences in female are more clear than in male (table. 7) (The time of the calculations includes the period during the last two weeks of July and first week of Augusts):-

Table (7) Total protein values for jojobas male and females:-

Weeks no.	*Jojoba Male mg/ml*	*Jojoba female mg/ml*
1	**7.2**	**7.8**
2	**7.3**	**7.9**
3	**7.2**	**7.9**

Total protein test shows a difference through the three weeks started from the middle of April till the beginning of August. The high value is due to the high protein level in both male and female because jojoba contains about 30% protein of its weight and so the difference in these levels could explained as the follows. Protein is a materials which includes amino acids-enzymes-nucleic acids-general proteins product-toxins and a specific type of secondary metabolites. The male and female should be of similar weight in the nucleic acid but the other product like the secondary metabolites, enzymes and amino acids may vary according to the type of its functions and the role of the plant sex Female and male differ in the sexual activity and so the difference between the sex enzymes concerned showed as an accumulative result that is obtained by

the total protein. Recent studies shows a less values in rice (Totingham, 2000).

4.4 Results for Terpenoids, Eugenol, Flavonoids, Geraniol and Terpens concentrations by HPLC test:-

4.4.1:-Results for terpenoids, eugenol, flavonoids, geraniol and terpens concentrations in jojoba male:-

The concentrations of the terpenoids, eugenol, flavonoids, geraniol and terpens were estimated, as described in (3.3).

Figure (4.7) shows the peaks that representing the different chemical composition:-

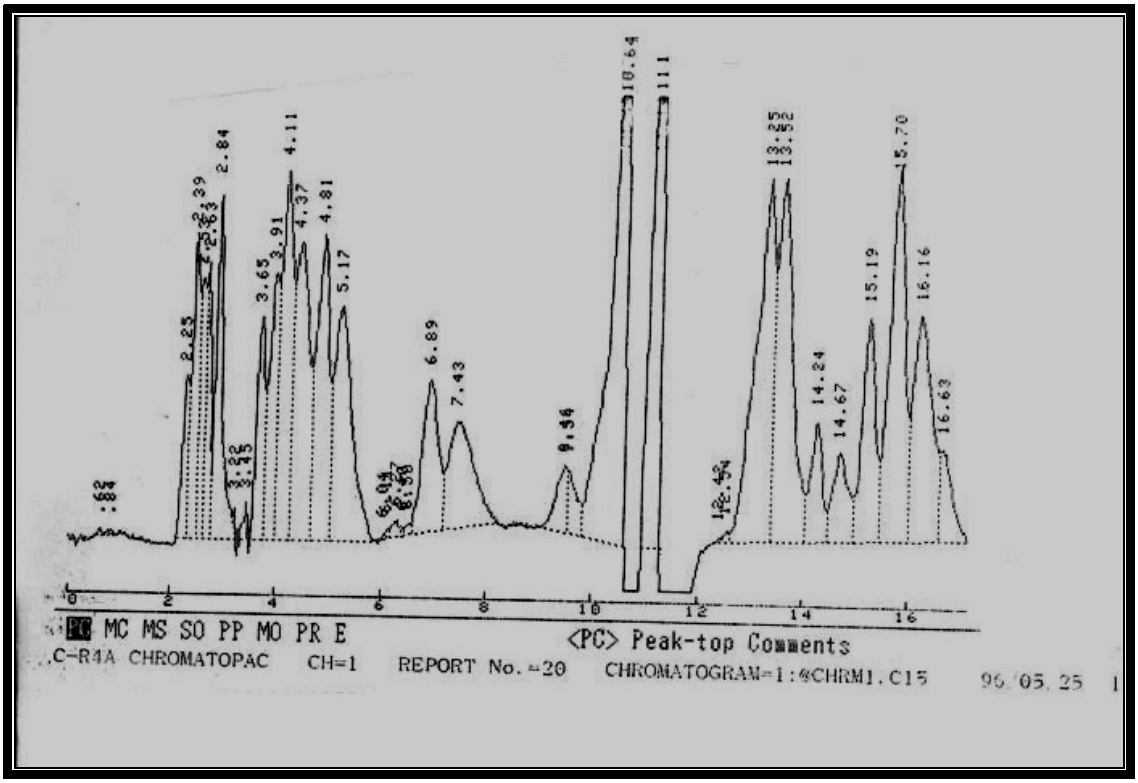


Figure (4.7) Different concentrations of tepenoids, coumarin, terpens, Euginol and penins in jojoba mature male.

4.4.2 Results for terpenoids, eugenol, flavonoids, geraniol and terpens concentrations in jojoba female:-

Fig. (4.8) shows the concentrations of the terpenoids, eugenol, flavonoids, geraniol and terpens concentrations in jojoba female.

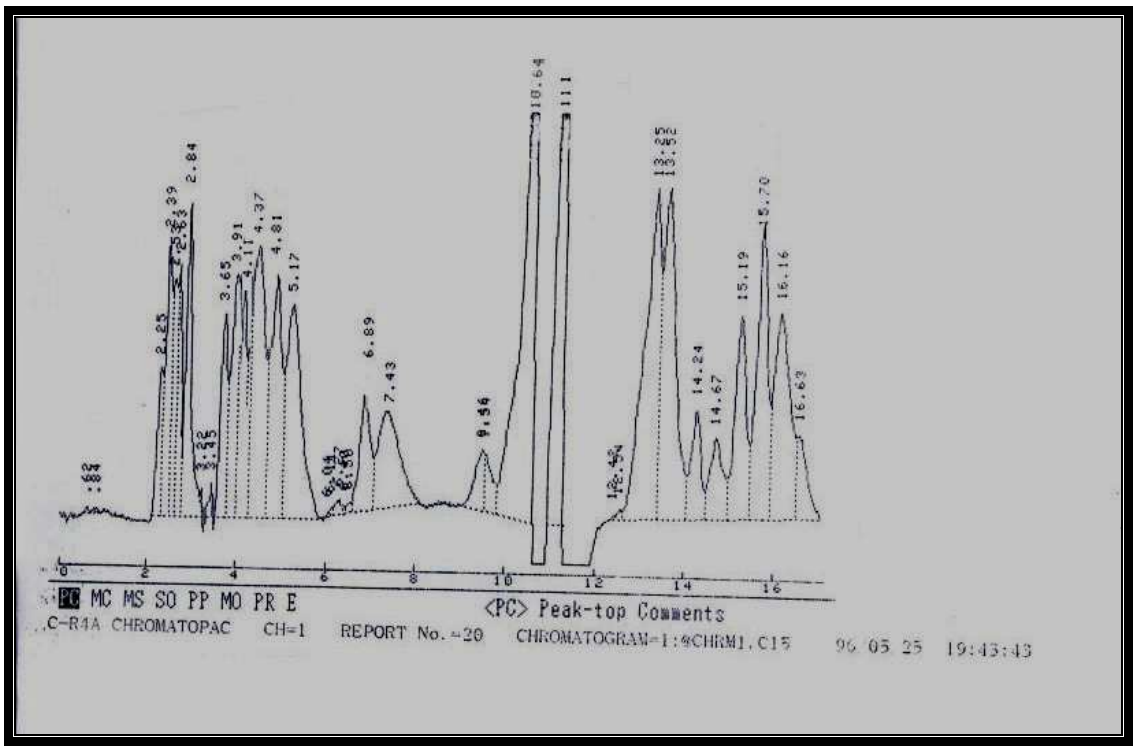


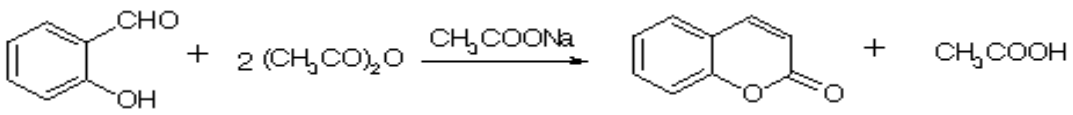
Fig. (4.8) Different concentrations for tepenoids, coumarin, terpens, Euginol and penins in jojoba mature female.

Concentrations of the terpenoids, eugenol, flavonoids, geraniol and terpens in jojoba male and female are shown in table (8):-

Table (8) HPLC results for different chemical materials between the male and the female of jojoba:-

Phenol type	Concentration in male (micro mg/ml)	Concentration in female (micro mg/ml)
Alpha penin	**1.25**	**1.83**
Geraniol	**7.77**	**4.05**
Camphore	**2.30**	**2.52**
Eugenol	**10.32**	**9.52**
Myrecene	**1.75**	**1.53**
Menthol	**3.98**	**2.11**
Rutin	**5.68**	**4.45**
Methyl cinamate	**2.21**	**2.86**
Coumarin	**7.44**	**7.84**
Linalool	**0.4**	**0.69**
Betacaryophiline	**8.32**	**10.89**

The differences between the compounds of the two sexes could be explained by the biosynthesis of coumarin in plants occurred via hydroxylation, glycolysis and cyclisation of cinnamic acid



These operations release energy for which both sexes of jojoba showed difference in their need , depending on the time and whether its related to flowering or not.

Coumarin is found in a variety of plants such as Tonka bean, lavender, sweet clover grass, and licorice, but it also occurs in food plants such as strawberries, apricots, cherries, and cinnamon. It is thought to be serving as a pesticide for the plants that produce it (Scholde, 1994).

Coumarin is higher than (in other plants like *Mikania glomerata*) which is found to be (5.0 µmg/ml) (Sandy, 1999) while no study has been conducted reinvestigate the differences between the two sexes .

The determination of oil in the *A. galanga* leaf oil is camphor (15.6%), pinene 5.0 (µmg/ml) , methyl cinnamate (4.6 µmg/ml), bornyl acetate (4.3 µmg/ml) and guaiol (3.5 µmg/ml). The stem oil contains 1,8-cineole (31.1 µmg/ml), camphor (11.0 µmg/ml), methylcinnamate (7.4 µmg/ml, guaiol (4.9 µmg/ml), pinene (3.3 µmg/ml) (Jirovetz, 2003).

The terpens compounds are investigated during the flowering time ,penine and camphor are two types of terpens that show variation in which camphor is higher in male than in female , while the opposite is in the female . In recent studies, terpens show a lower concentration in *Agrobacterium rhizogenes* , it shows 1.2 and 1.4 (µmg/ml) for camphore and penine consequently (Kovalenko, 2002). No study shows the differentiation between the two plant sexes mentioned before.

Euginol and geraniol are found in *Pelargonium grossularioides* hebra with about 15.9 and 11.2 (µmg/ml) consequently. This result is much higher than our results which are 10.32 and 7.77 (µmg/ml) in the male (

Lisbalchin, 1993), while linalool (0.40 (µmg/ml) for jojoba male and 0.69(µmg/ml) for jojoba female) and geraniol (7.77 µmg/ml for male and 4.05 µmg/ml for female) shows 5.4 µmg/ml and 3.7 µmg/ml consequently in *agrobacterium rhizogenes* (Kovalenko, 2002) .

Euginol is highly related to vitamin E which is the downstream product of euginol. So we suggest a variation in the vitamins between the two sexes.

Linaloo, Eugenol, Carviol and Geraniol are materials related to defensive state against any pathogenesis stage. That is different, according to the plant and whether there is pre infection state in the both sexes or not (Lisbalchin, 1993).

Rutin shows 4.68 µmg/ml in male and 4.45 µmg/ml in female of jojoba. This could be related to the nutrition activity that could be related to the fertilization time and the energy requirement of the male and female that will not be equal for both of them. The energy would be needed more in male during pollination and so later it will be raised in female since the test is done in the flowering time.

A comparative study of essential oils of *Cymbopogon Citratus* and some members of the genus *Citrus* show that myrecene reachs 0.72 µmg/ml, that is lower than our result we have in which indicates 1.25 µmg/ml for male and 1.83 µmg/ml for female of jojoba , while β-caryophiline shows 0.8 µmg/ml in which our results are much higher in concentration.

This is because the oily nature of our plants reflect a much higer concentration in the essential oils in addition to the jojoba oil (Sonam, 2000).

4.5 GOT activity's :-

(Table 7) shows the activity of GOT enzyme for both male and female of jojoba during flowering enzyme according to GOT KIT (Biomerix., 2004) before starting the purifications.

Jojoba male	***Jojoba female***
7 international unite (i.e)	***19 international unite (i.e)***

GOT activity is accomplished according to biomerix kit which is a colorimetric method depending on the enzyme action against the substrate. The method suggests a fully control of the temperature to avoid denaturation of the enzyme which leads to less activity. A variation in the enzyme activity between the two sexes was observed, the difference in the isozymes pattern could be related to this. Many purification tests done for GOT isozyme and the activity have values more than this.

In purification of GOT from pig heart (24 *i.e*) recorded (William, 1998). This is approximate to the value in the jojoba female which partially agrees with the result of this work. No significant differences between the two sexes were noticed.

4.6.1 GOT Purification:-

4.6.1.1 GOT purification in jojoba male:-

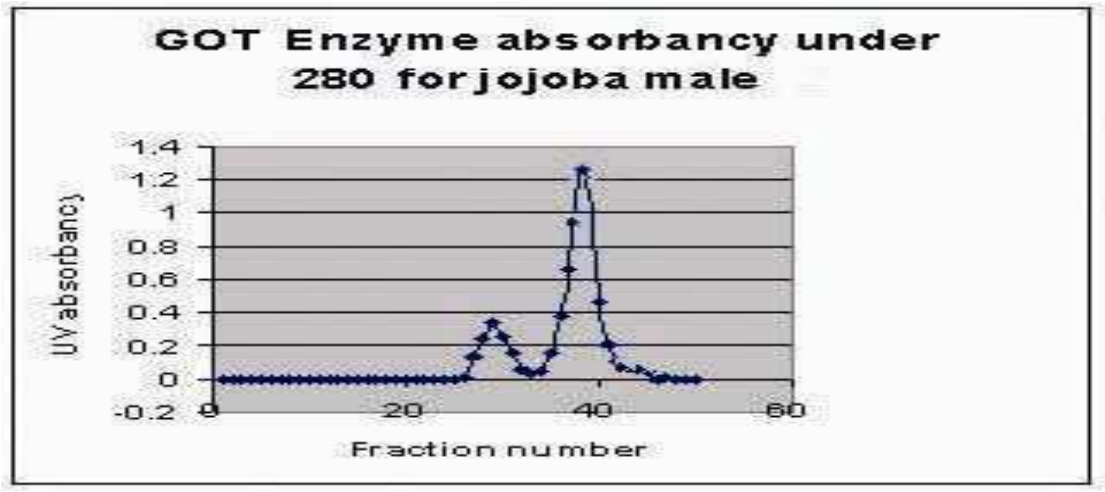


Figure (4.9) GOT purification in jojoba male flow rate is 1 ml / minute, 2 fractions/ ml.

4.6.1.2 GOT purification in jojoba male:-

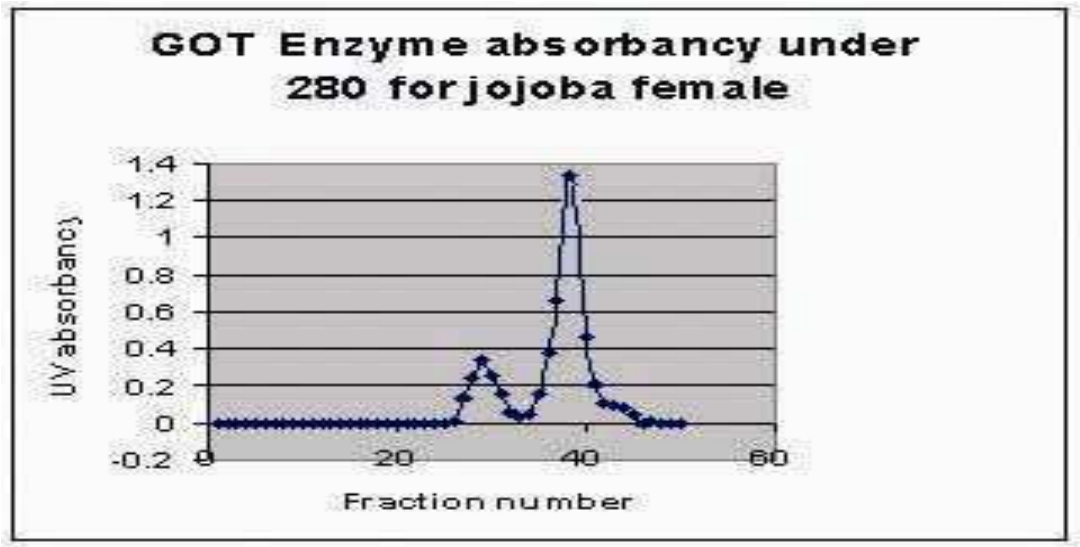


Figure (4.10) GOT purification in jojoba male flow rate is 1 ml / minute, 2 fractions/ ml.

Purification of GOT is carried out by ion exchange chromatography using the methods described previously in (3.5). The result shows that washing with (50 ml) of o.o2M Tris-Hcl buffer (pH 8.0), allows the appearance of small peak representing the fractions (28-50), plooting (200 ml) gradient of (0.02) M Tris-HCL buffer with 0.3 M NaCl solution. One peak is obtained and represented by fractions (37 - 50), each fraction is tested for protein concentration and specific activity. Protein concentration after ammonium sulphate precipitations is 5.4 and 5.7 mg / ml for male and female consequently . After purification it reached 0.42 and 0.46 mg / ml , which is higher than other results . In this respect, no study has been found to be associated with this conclusion.

4.7 Ags – Abs reactions:-

The result below represent the absorbency at 280 nm for the reaction of Abs against crude solution from mature plant (Ag) from both male and female of mature jojoba :-

Serum Absorbency at 280 nm.	Abs reactions against – (GOT) Ags derived from jojoba male Absorbency at 280 nm .	Abs reactions against – (GOT) Ags derived from jojoba female Absorbency at 280 nm.
0.2	0.32	0.34
0.2	0.29	0.37
0.2	0.30	0.33
0.2	0.24	0.31
0.2	0.33	0.36
0.2	0.31	0.29
0.2	0.29	0.34
0.2	0.33	0.31
0.2	0.24	0.30
0.2	0.29	0.27

While Abs reaction against crude solution derived from newly formed plant (absorbency at 280 nm) gave the following results:-

Abs reactions against – (GOT) Ags derived from jojoba male	Abs reactions against – (GOT) Ags derived from jojoba female
0.24	0.25

The statistical differences depending on T-test showed no differences between the two values that represent the Abs –Ags reaction between the Abs derived from each host and the Ag of the host. The mortality rate is 14.28% and 21.42 % for male and female consequently. The lowest values of the newly formed plant reflect a less level of the enzyme expression which was more in the mature plant.

Similarly, the values of the rabbit antibody's reactions may be due to the following reasons:-

1- The similarity between the immune system reactions toward Ags since Ags has similar characters.

2- The immunogenicity of the two enzymes is the same because of the similar distribution of the active site upon the two isozyme molecules or the differences in the molecule shape which affect the immune response.

The active three dimensional structure types and number of isozymes and the concentration of the dozes show no differences.

Conclusions

1- Jojoba two sexes are differ in the isozymes patterns under investigation. They showed bands variation except the GOT which showed variation in the level of the enzyme path was before flowering .

2- Jojoba sexes differ in the protein concentration of the crude solutions and in the purified solutions after ion exchange purification.

3- Both sexes differ widely in several chemical compounds like geraniol, caryophilin and menthol with more than 2 micrograms between the two sexes.

4- The anti-GOT Abs doses not act as a possible way for detecting the differences between the two sexes.

Recommendations

1- Investigating the MDH enzymatic system during the flowering time.
2- Purification of enzymes (EST, MDH and G6PD) to find Abs-Ags reactions .
3- Study the features of the new plant since early shoot formation ending with a conclusion whether the differences in these enzymes related to the sex or not.
4- Attempt to find differences in DNA between male and female using multiple steps technique like the PCR.

Introduction

Jojoba is considered as one of the most important shrubs in the present time. Many products have been successfully isolated, manufactured and used in many applications.This shrub was first mentioned by the Mexican historian Francisco J. Clavijero in 1789 (Hartwell, 1971).

Simmondisin (the protein) and Jojoba oil are the major products obtained from jojoba .

Simmondisin, the protein has been reported as epitate suppression natural drug. It forms more than 30% of the total seed, so it will be very good for investments.

Jojoba oil represents another source of investment, it is considered as an important source of wax which has a wide range of industrial uses mainly in cosmetics. It is used especially in the formulations and preparations of skin care products such as lotions, moisturizers, massage oils and soothing creams.

In general, there are two sex types in Jojoba male and female. However, they cannot be distinguished at the seedling and vegetative stages of growth. The selection of the appropriate sex type of the progeny for commercial planting would be beneficial, since only the female plants are grown for fruit. The identification of sex types prior to propagation would result in higher fruit production and increased profitability.

The flowering of the shrub starts after three years. The early diagnosis of the female will be of a great importance for the economic side. As long as

the females will form the higher percentage of the product population, as good is the product will be more.

The aims of this project :-

The aim of the project is to :-

1- Find a simple profile to distinguish between the males and females from two sides; the first is to find the differences between different chemical compounds like terpens, coumarin, terpinoids and alcoholic derivatives by using HPLC techniques.

2- Detect the total proteins levels in the two sexes, monitoring the enzymes expression by using horizontal electrophoresis technique that showed the variations in the expressions between them.

3- Purify one of Isozymes which shows variation between the two sexes (Glutamate Oxaloacetate Transaminase (GOT)) from the two sexes using ion exchange chromatography. Peaks that shows the highest activities was dialyzed and injected in to (lab rabbits) trying to find a different level in the immune response between the two sexes.

4- Abs reaction against extraction solutions from newly formed plant (Ags) and mature plants were estimated and finding whether the sex in the newly plant is male or females. This depends on the similarity between the two levels of the Abs-Ags reactions.

Materials and Methods

3.1 Instruments and Chemicals:

Table (2) Instruments used in the experimental work.

Instrument	Source
Autoclave	Harayma (Japan)
Oven	Galenkamp (England)
HPLC	Schmidate (Germany)
Electrophoresis	Fisher (Germany)
Balance	Sartorius (Germany)
Sensitive Balance	Sartorius (Germany)
pH meter	Metler Toledo (England)
Vacuum Pump	Schu Co. Inc. (England)
UV – Visible spectrophotometer	Aurora Instruments Ltd (England
Centrifuge	Heraeus (England)
Chromatography Column	Pharmaci Fine Chemicals (Sweden)
Water Bath	Atom (England)

Table (3) Chemicals used in the experimental work.

Materials	Origin
Boric acid	BDH
Tris-base	BDH
Absolute ethanol	BDH
Laboratory starch	BDH
Commassi brilliant blue stain	BDH
Acetic acid	BDH
EDTA	BDH
2-Mercaptoethanol	BDH
Sodium tri sulphate	BDH
Ammonium sulphate	BDH
KCl	BDH
G6PD Quantitive KIT	Biomrix
GOT KIT Quantitive	Biomrix
MDH Colorimetric Reagent	Globel USA
Estarase Colorometric reagent	Globel USA
G6PD Colorometric reagent	Globel USA
GOT Colorometric reagent	Globel USA
NaCl	BDH
Tris-HCL	BDH

3.2 Preparations of Solutions:-

3.2.1 Phosphate buffer:

This buffer was prepared according to the instructions of the manufacturer company.

3.2.2 Electrophoresis buffers:-

Solution (A) boric acid (64%) was prepared by adding 64 g to 100 ml distilled water and the pH was adjusted to (8.3) .

Solution (B) Tris base (53%) was prepared by adding 53 g to 100 ml distilled water and pH was adjusted to (8.3) (May.,1998).

3.2.3 Plant extraction method:-

Extraction solution was prepared by mixing 8 grams of plant material (leaves or flowers) with (30) ml of tris base and (50) µml mercaptoethanol pH was adjusted to 8.3 (May, 1998).

3.2.4 Loading buffer:-

Loading buffer consisted of Solution A which is prepared as in 3.2.2 (May, 1998).

3.2.5 Ion exchange buffer (Maison , 1997):-

Buffer A: 50 mM Tris, pH 7. 5.

Buffer B: 50 mM Tris, 0.30 M NaCl, pH 7.5.

3.3 Starch Electrophoresis:-

3.3.1 Samples collection and preparation

A total of 4 samples (two samples for leaves and two for flowers) were collected from Jojoba tree located in Baghdad (The University of Baghdad campus) from one single tree . Samples were homogenized with extraction solutions and centrifuged for 10 min at 20,000 g . The supernatant was collected and stored in the refrigerator until use (May, 1998).

3.3.2 Electrophoresis gel preparation:-

Electrophoresis gel was prepared by mixing (9 parts of solution B with one part of solution A (pH adjusted to 8.3)) with 14 gm of laboratory starch of the hole quantity was 150 ml (Andrews, 1993).

3.3.3 Emigrations by electrophoresis:-

Gel electrophoresis was carried out in which 30 µl of sample solutions were placed in the gel wells and 10 µl of emersion oil were added to avoid sample floating, gels buffer was added till it coverd the wells and electrophoresis started.

Voltage was adjusted to 170 volt through the first half of an hour and raised to 300 volt for the next one and half hour , marker was placed in another well to indicate *Rf* value, Commassie Brilliant Blue was used as a marker (Andrews, 1993).

3.3.4 Staining methods:-

Four enzymatic systems were detected by using staining kit for MDH, G6PD, GOT and EST and as follows (Conkle *et al*., 1982):-

3.3.4.1 Glucose 6 Phosphate Dehydrogenase Stain :-

The stain contained:-

25ml 0.2M Tris-HCl, pH 8.0

250 mg glucose

1.5ml NAD

0.5ml PMS

These are Incubated in the dark at 37 °C until bands appeared.

3.3.4.2 Malate Dehydrogenase stain:-

The stain contained:-

12.5ml 0.2M Tris-HCl, pH 8.0

12.5ml 0.5M malic acid, pH 7.0

0.5ml NAD

0.5ml NBT

0.5ml PM.8

These are incubated in the dark at 37 ºC until blue bands appeared in two hours.

3.3.4.3 Glutamate Oxaloacetate Transaminase stain :-

The stain contained:-

7.5 ml 1M Tris-HCl, pH 8.0

100 mI dH2C

300 mg Aspartic acid

150 mg a-ketoglutaric acid

7.5 ml Pyridoxal-5-phosphate

Add 225mg Fast Blue BB salt just before pouring

These are incubated in the dark for 30-40 minutes (protect from light).

3.3.4.4 Estarase Stain :-

The stain contained:-

Esterase buffer 25 ml 50 ml

Alpha-Napthyl acetate 0.025 g

Beta-Napthyl acetate 0.025 g

Acetone 2.5 ml 5 ml

Fast Blue RR Salt 0.05 g.

3.4 Samples Preparation for HPLC:-

Extracts were analyzed by following the method described by (Lege, 1995). Leaves were picked from the upper part of the plant and dried in liquid nitrogen. 200 mg leaf powder was extracted in 12 ml of 2% (v/v) glacial acetic acid was placed in a boiling water bath for 10 minutes and centrifuged at 10,000 rpm for 15 minutes. The supernatant was filtered using 0.22µm filters, and then hydrolyzed with 12 ml of 1N HCl in a boiling water bath for 1 hr. After cooling the reaction mixture, 20 ml diethyl ether was added. Ether layer was separated and dried in freeze temperature. Residue was dissolved in 0.5 ml of the solvent consisted of 2.5% butanol: 12.5% methanol: 2% glacial acetic acid: 10% ammonium acetate: 73% water. The dissolved samples were filtered using 0.2 µm filters.

The concentration should be calculated according to the internal standard time, rutin retension time at 6.89 min with area 3260 for 5 microgram /ml Rutin standards.

Concentration Area (B.T.N)

5 3260

x area of sample

$$x = \frac{5 \times \text{area of sample}}{3260} = \text{concentration in micro gm/ml}$$

Table (4) Standard time of different compounds lockage at HPLC.

Sequence	Time
Alpha penin	2.39
Camphore	2.63
Myrecene	2.84
Menthol	4.11
Carviol	4.81
Rutin	6.86
Methyl cinamate	7.43
Coumarin	9.54
linalool	10.4
Eugenol	14.1
Beta caryophilline	13.5
Geraniol	15.7

3.5 Total Protein Determination:-

3.5.1 Bovine Serum Albumin Stock Solution (Bradford, 1976).

Protein Bovine Serum Albumin (BSA) stock solution was prepared by dissolving 10 mg of BSA in10 ml of 0.05 M phosphate buffer (pH 7).

3.5.2 Commassie Brilliant Blue G- 250 (Bradford, 1976).

About 100 mg of Commassie Brilliant Blue G- 250 was dissolved in 50 ml of 95% absolute ethanol, and then 100 ml of 85% orthophosphoric acid was added. The volume was then completed to 1000 ml with distilled water.

2.5.3 Determination of Protein Concentration:

Protein concentration determination was performed as originally described by Bradford (1976) as follows:-

1. Several dilution of standard protein (BSA) were prepared from BSA stock solution (2.3.6) in the same buffer and according to volumes in table 5:
2. Then 1 ml of commassie brilliant blue G-250 (2.4.2) was added to each dilution and left to stand for 2 min. at room temperature.
3. The absorbance at 595 nm was measured: the blank was prepared from 0.1 ml of the buffer and 1 ml of the dye reagent.
4. A standard curve was plotted between the amounts of protein corresponding absorbance of the standard protein. The protein

concentrations of unknown samples were calculated from the standard curve (fig. 2).

Table (5): Preparation of BSA Standard Curve.

BSA conce. (ml)	Buffer (µl)	Protein conce. (µg/ml)	Total Volume ml
2	98	2	0.1
4	96	4	0.1
6	94	6	0.1
10	90	10	0.1
14	86	14	0.1
16	84	16	0.1
20	80	20	0.1

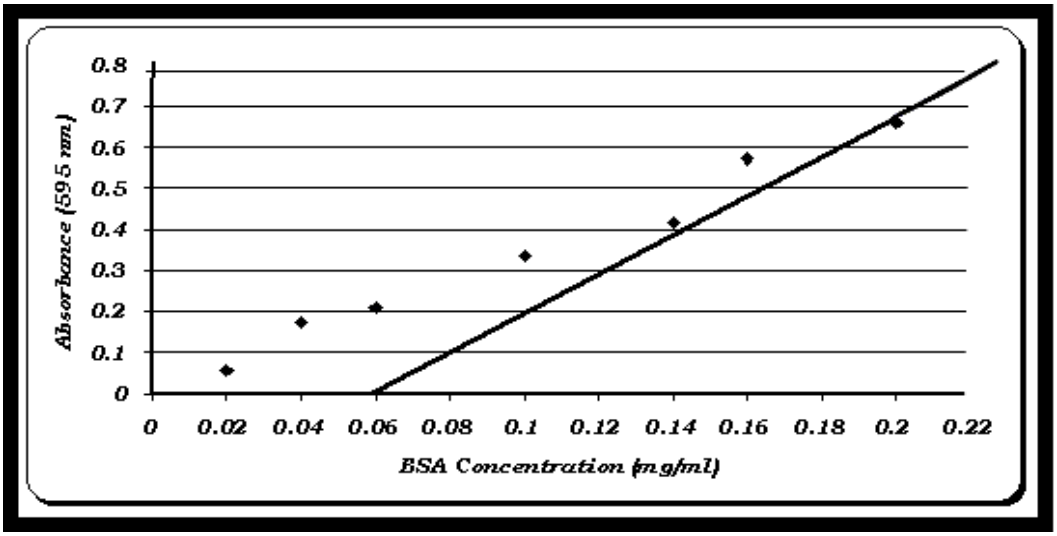


Fig. (2) : Bovine Serum Albumin Standard Curve.

3.6 Purification of Glutamate-Oxaloacetate Transaminase:-

3.6.1 Ammonium sulphate precipitation 70% (Maison, 1997):-

Ammonium sulphate precipitation: a dilution of 70 % was prepared for the precipitation of the GOT, 46 gm of ammonium sulphate was added to the extraction solution and left for 24 houres, the precipitant used for the ion exchange.

3.6.2 Preparation of ion exchange column (Maison, 1997):-

Carboxy methyl cellulose was washed 8 times in water, 8 times in 0.5 M Tris (pH adjusted 7.5), 8 times in 50 mM Tris (pH adjusted to 7.5) and then suspended in 50 mM Tris, pH 7.5 .

We add 50 mM Tris pH 7.5 buffer to the column , the resin beads resuspended uniformly, excess resin was removed by gently stirring the buffer above the resin with a disposable pipet to resuspend beads and remove the excess beads with the disposable pipet , 1 mL of 50 mM Tris pH 7.5 was pipeted onto the packed resin and the column was covered with parafilm until using it, e flow rate adjusted to 1.0 mL/min, 2.0 ml per fraction, the fraction rate was two fractions per minute and I ml for each test tube. 55 small test tubes were collected.

3.6.3 Dialysis (Maison , 1997): -

Supernatant obtained from the above mentioned step was redissolved in distilled water and dialyzed against containing 1 liter of 0.05 M Tris, pH 7.5 and it was place on a stirring motor in cold room and it was slowly stirred for 6 – 8 hr.

3.7 GOT activity test:-

Table (6) GOT Activity's Account.

Instruction	**Sample**	**control**
1- buffer for GOT	0.5 ml	0.5 ml
2- Mix and incubate with glutamate for 30 min at 37 c°	0.5	0.5
3- Add 2,4, dinitro phenylhydrazin	0.5 ml	0.5 ml
4- add extractions solutions	0.1 ml	-
Mix and allow standing 20 min at room temperature.		
5-NaOH	5 ml	5 ml
Mix and stand for 5 minutes then read at 540 nm (Biomerux,2004) and read under the standard values from the kit		

3.8 Rabbit injections:-

The two purified enzymes were injected to the host (rabbit) via foot pad (with 2 ml of each enzyme) and repeated after 2 weeks with the same doze (George, 1996), the number of the hosts was 14 rabbits for both those which injected with male extract and with female extract in repeat to the following equation:-

Mortality rate = (no. of dead rabbits / no. of total rabbits) * 100.

3.9 Ag-Ab reactions:-

It was done by using the precipitation tube in which each tube was placed 0.5 mL of the rabbit serum and the crude extraction solution was added to the tube (0.05 mL), the reaction took about 10-15 minutes the hole solution for each sample(0.55 ml) were readded at 280 nm (George, 1996).

3.10 Statistical analysis:-

T-test was used to compare the means (Kohen, 1984).

Literature review

2.1 Jojoba (*Simmondsia chinensis*):-

Its pronounced (ho-HO-ba) which is an English name , its also called goat nut, deer nut, pignut, wild hazel, quinine nut, coffee berry, gray box bush (a spanish name) (Bianchini and corbetta ,1976; Armstrong, 1998).

2.2 Botanical classification:-

(Table 1 botanical classifications) cited from (Underschold, 1990**).**

Kingdom:	*Plantae*
Division:	*Magnoliophyta*
Class:	*Magnoliopsida*
Order:	*Caryophyllales*
Family:	*Simmondsiaceae*
Genus:	*Simmondsia*
Species:	*Chinensis*

2.3 Historical back ground:-

This shrub is first mentioned in the literature by the Mexican historian Francisco J. Clavijero in 1789, who noted that the Indians of Baja California highly prized the fruit for food and the oil as a medicine for cancer and kidney disorders. Indians in Mexico use the oil as a hair restorer (Hartwell, 1971).

The oil of jojoba was reported to be emetic, jojoba is a folk remedy for colds, obesity, parturition, poison ivy, sores, sorethroat, warts,and wounds. Seri Indians applied jojoba to heal sores aching eyes, colds and to facilitate parturition (Braun and Espericuta, 1979) also for

cooking, hair care, and for treatments of many medical problems such as kidney malfunction. (Benzioni, 1995; Hartwell, 1971).

2.4 Plant origin:-

Jojoba is a desert shrub indigenous to Arizona, California and Northern Mexico. It grows in a number of deserts, including Negev desert **(**Lawrence, 1995**)**, its endemic to the Sonoran desert (South west Arizona and California, Northern Mexico and Baja California) especially suited to the north slopes of granitic hills and upper Bajadas of the Sonoran Desert, it mainly in the Galiuro Volcanics hills and rocky slopes (Shreve ,1964;Dimmit, 2000).

2.5 Morphological description and characterization:-

Jojoba is a woody green shrub ,wind-pollinated, reaching a height of 1-5 meters and having a long life span (100-200 years),jojoba leaves are leathery, thick, grayish green in color, and elliptical in shape (Kohorn, 1990).

These leaves are vertically oriented, an adaptation which lessens sun impact at summer high noon, while exposing each leaf to the less extreme temperatures of morning and evening, their occurrence reflecting the ability of the plant to conduct its leaf functions both in cold months and in dry ones ,the leaves are xerophytic which contain high concentration of phenol. the length of the branch divided by the number of internodes and branching frequency, is 8 branches per plant in the main (Kohorn, 1990).

The plant is drought resistant and to some extent also salt resistant **(**Mills *et al*., 2004).It is adapted to relatively dry climates with annual rain of 500-600 mm and temperatures ranging from (9-50°)C .

Where annual rains are less than 350 mm, supplementary irrigation is required. In the arid areas of occupied Palestine, with less than 200 mm of annual rain, supplementary irrigation of about 300-500 mm is applied. Effluents and saline water may be used to irrigate Jojoba. (Yermanos, 1977; Nicotra,1999).

2.6 Differences between male and female:-

2.6.1 Morphological differences:-

In some populations in xeric sites, males have smaller leaves and more compact canopies than females, female shrubs have larger leaves, more open canopies, and are, on average, smaller plants than males, But while sexes are dimorphic in terms of mean values of these morphological parameters, there is substantial overlap between sexes and considerable within population variation within each sex (Kohorn, 1990).

Female and male flowers occur on separate plants and the male pollens are pale-greenish in color, occurring in a dense cluster. Blossoming times for Jojoba are extremely variable (from December to July), female flowers are also tiny, and greenish yellow in color, eventually forming hard-shelled, acorn-like seeds up to 2.4 cm long, which initially are pale green The seeds eventually ripen to a strong brown color (Dimmit, 2000).

The female flowers are usually solitary, one per two nodes although flowers every node or in clusters are not rare. The male flowers are clustered. Flower buds form in the axiles of leaves on the new vegetative growth occurring during the warm season under favorable temperatures and water regime (Brawn, 1988).

Female plants also have a more open structure which allows more photosynthesis to take place. Together these two strategies permit the female plants to produce the high amounts of energy needed for fruit production. In overall energy expenditures male shrubs only reserve 10-15% of their tissue for reproductive use while female plants utilize 30-40%. Males and females of jojoba can differ in their vegetative structure as well as in floral morphology. Male flowers occur in axillary inflorescences containing from 3 to 20 flowers, while the larger female flowers generally occur singly, also in an axillary position. (Buchmann, 1987).

Sex ratios (52 males: 48 females) obtained from seed in horticultural propagation are consistent (Yermanos, 1973). There are no reports of plants changing sex, confirming that environment does not play a role in sex expression. Hermaphroditic plants are only rarely found (Buchmann , 1987). Sex chromosomes have not been identified, nor has their presence been ruled out (Lee and Sherman, 1985).

2.6.2 Genetic variation between dioeciously male and female:-

In contrast to most animals, many plant species generate both male and female organs on the same individual. Only a few plants are unisexual, that is “dioecious”. Male and female plants of dioecious species may differ in many leaf and whole plant traits, including life history, reproductive, allocation, phenology, and growth .

Some of these plants have heteromorphic sex chromosomes by which sex is genetically determined, In the well studied dioecious plant, *Silene latifolia*, the Y chromosome dominantly and positively induces male development by its presence in an XX/XY system similar to the

mammalian pair of sex chromosomes. However, the primary structure and molecular function of the sex chromosomes in plants are largely unknown (Ohima, 2001).

Other plants have the same chromosomes number like the jojoba (the project plant) which had 11, 13 chromosomes (Robin, 1954).

Gene expression variation mechanisms in plant could include many reasons, sex related expression is one of them in which the identification of certain ribosomal protein that is related with male and female was reported ,certain gene from *Mercurialis annua* by mRNA differential display was investigated in which sequence analysis of a cDNA clone of this gene showed that it encodes a protein that belongs to the eukaryotic ribosomal protein family, Northern blott analysis showed a stronger expression of this gene in female flowers as compared with male flowers. Analysis of the genomic DNA sequence showed the presence of one untranslated and three translated exons, the association of habitat (substrate type and light availability), plant size, and inflorescence production included also as gene expression effectent ,the expression of sexually antagonistic alleles generates intersexual genetic conflict during ontogeny because they produce a phenotype that moves one sex toward its optimum, but when expressed in the other sex, moves the phenotype away from that sex's optimum (Rice and Chippindale, 2001).

The Variation could be ruled by the following formula:-

Environmental variation + Genetic variation = Phenotypic variation

Genes may have different effects in different environments in which many genes are expressed depending on temperature variation.

The alleles expression dominance is a good example of this effect (Lewis, 1982).

The different genes that may either be completely absence in one sex and presence in another or either suppressed totally or temporary will result in the differences in these gene products (Lewis, 1982).

Isozymes are one of a number of enzymes that catalyse the same reaction but differ from each other in primary structure and electrophoresis mobility (Duran and Rodriguez, 1987).
Its also could be defined as one of several forms of an enzyme, produced by different loci in an individual organism‘s genome (Crawford, 1983).
Its importatnt in studying the genetic inheratince trait (Biesberg, *et al*., 1987) and it have been exploited to identify markers that could co inherit with sex type like in papaya (Scharpf and Hawksworth, 1999).

G6PD which is the key enzyme that catalyzes first the step of the hexose monophosphate pathway (Ozer *et al* ., 2001).
The principal source of cytoplasmic NADPH in many cells is the hexose monophosphate pathway and is specially catalyzed by the two deydrogenases glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. These reactions appear to be necessary for the maintenance of cellular integrity (Morelli, 1983).

G6PD is one of these enzymes as example it was found to be linked with the chromosome X as inhibitors trait that will not be founded because of the different gene code in human while in plant the most theories that deal with the gene expression mention certain mechanism for gene regulation which depend on the promoter and termination action which direct the action of the gene and so the

switching on and off will be related with the expression of the gene that could be related with it (Ulusu, 1999).

2.7 Seed Preparation and Germination:

Jojoba may be propagated by direct seeding, in which case about half of the seedlings are males which should be roughed as 10% of males are sufficient for pollination. The fact that only 50% of the plants bear seed and the large heterogeneity of the plants those originate from seed lead to low average yields. Hence, vegetative propagation that enables the planting of desired proportion of female plants of superior clones is preferred. Rooted cuttings are used for this purpose (Benzioni, 1995).

Jojoba can be planted by direct seeding or by transplanting seedlings to the field. In the southwestern U.S., many growers prefer direct seeding because it is less expensive, faster and requires less hand labor. Seed can be germinated in vermiculite or sand at about 31 ° C. Emergence occurs in 15 to 20 days, and the seedlings are ready for transplanting when they are 15-30 cm tall (8 to 10 weeks). Propagation from clones or from tissue culture is a more rapid method of varietal improvement (Verbiscar and Banigan, 1978).

2.8 Cultivation :-

Jojoba is cultivated mainly in Arizona, Northern Mexico, Argentina and Palestine. In Australia, Chile and India small scale planting is done. All new plantations are from vegetative propagated plants originating from cuttings of selected clones (Benzioni, 1995).

2.9 Harvesting

The only method for harvesting is hand collecting from the plants, since mature seeds fall from the bush. Under cultivation, hedge-row, or orchard-like plantations, without undergrowth, seeds could be raked from under the bushes and then picked up by suction. Pruning the lower branches might be advantageous if this method be used. A device could be designed to pick the seeds from the bush prior to the time of falling. Cost of harvesting would depend on the method. (Underschold, 1990).

2.10 Yields and Economics

A number near 1,650 kg/ km^2 was recorded for yields of jojoba. Individual plants may yield 5 kg (dry weight) seeds and more, of which 50% (43–56%) by weight is a colorless, odorless liquid wax commonly called "jojoba oil" (Buchanan and Duke, 1981).

(N.A.S., 1975; Yermanos, 1973) suggested that after 5 year the yield which is about 82.5 kg of nuts for km^2 will increase to 412.5 kg / km^2 in the 12th year. Such yields may be optimistic, even for well managed plantations. Estimates of the amount of wild nuts available each year range from one hundred million to one billion pounds, the plants growing over 2 million km^2 in California, Mexico, and Arizona. Usually plants in cultivation yield oil in 6–7 years, the best specimens in Middle East yield 2 or more kg of seed in the fourth year, wild plants yield about 1 kg of nuts per year, and cultivars should yield twice that amount or more. The value of *Simmondsia* oil as a hard wax was estimated at $.55 per kg. Because of the present demand for the wax and oil. Jojoba is being considered as a non-competitive crop that could replace wheat and cotton in Texas and Southern California, with as much as the yield from 70,000 kg is being absorbed by industry.

The Chemical Marketing Report (Dec. 28, 1981) stated that jojoba prices doubled in 6 months to $200 / kg, the cost of establishing a plantation can vary from $3,000/ km^2 on land with irrigation available to $5,600/ km^2 on rough desert terrain, maintenance costs are low only $200/ km^2. One km^2 can yield 1,125–2,250 kg oil per year (Jad, 1997).

2.11 Energy:-

With 6,41 plants per km^2, the aerial phytomass (over 6% of total phytomass) was 157,3 kg/ km^2 and annual productivity only 3,27 kg/ km^2 (Braun and Espericueta, 1979). 500 kg/ km^2 oil for jojoba, can nearly 100/ km^2 for cottonseed, 200 / km^2 for flaxseed, 250 / km^2 for soybean, and nearly 300 / km^2 for sunflower (based on 10 year averages for the conventional oilseeds, speculation for jojoba). (Daugherty *et al*., 1953).

2.12 Ecological requirements :-

Jojoba is found in diverse climatic, geographic and edaphic conditions, at altitudes from sea level to about 1,200 m. Native jojoba populations can be found in areas receiving an annual precipitation of 80-450 mm and having temperatures ranging from 9°C to 50°C**,** When temperatures drop below 0 °C (Gentry,1958), flowers and terminal portions of young branches of most jojoba plants are damaged. During early seedling development, excessive cold may kill an entire plantation. Frost may not damage taller plants to the same degree, but it can reduce yield. Jojoba is very tolerant of high temperatures. Irrigation has produced more luxuriant vegetative growth, this increased growth results in higher seed yield. Jojoba requires the most water during late winter and early spring (Benzioni, 1995)**.**

2.13 Soil:-

Most wild jojoba populations occur on coarse, light or medium textured soils with good drainage and good water infiltration. Planting on heavy soil results in later blooming, slower growth and more problems with fungal diseases (Underschold, 1990).

Little information is available on the response of cultivated jojoba to lime or fertilizer applications. Jojoba grows wild on soils of marginal fertility with soil pH ranging from 5 to 8. The soils that jojoba is adapted to in the semiarid regions of Arizona, southern California and northwestern Mexico are generally slightly alkaline and have native high potassium levels. Based on this, one might assume that for best growth, soils should have a pH of 6 or more and available K levels of at least 100 ppm. Apply enough dolomitic lime according to soil test recommendations to raise soil pH to 6. Approximately 10 to 15 pounds of Potassium fertilizer should be applied if available K levels are less than 100 ppm. Yield trials conducted in California have not shown any improvement in vegetative growth with the addition of nitrogen or phosphorus, therefore no additional N or P_2O_5 fertilizers are recommended (Braun and Espericueta, 1979).

2.14 Seeding Date:-

Jojoba can be seeded or transplanted to the field when the soil temperature reaches 38 Cº, low soil temperature may delay emergence by as much as 2 to 3 months (Underschold, 1990).

2.15 Flowering:-

Flowering begins in early February, when water is often available and temperatures are relatively moderate. Fruit development continues in to the summer, to late June or early July, as water becomes less available and temperatures rise (Dimmit, 2000).

2.16 Methods and rate of seeding:-

Seeds are planted 2.4 cm. deep, and growth usually occurs within 20 days. The soil should be kept moist but not wet through growth. Individual seeds or seedlings are planted 29-45 cm. apart in rows. Spacing between rows depends on the harvester to be used. With hand harvesting and cultivation, rows can be as close as 30 cm.To obtain the proper female: male ratio (6:1), it is advisable to over-plant (12-14 kg/2.4 km^2 of seeds). As male plants flower, they should be thinned out to 1 male every 120 cm on the row. As female plants flower, usually in the third year, any slow growing or unproductive plants are thinned out, leaving 1 female plant every 60-90 feet on the row (Yermanos, 1977).

2.17 Drying and storage:-

Jojoba seed that has been dried to around 10% moisture and protected from pest damage will keep for several years (Lee and Sherman, 1985).

2.18 Yield Potential and Performance Results:-

Jojoba generally does not produce an economically useful yield until the fourth or fifth year after planting. Seed yields in natural stands of jojoba range from a few seeds to as much as 40 kg of clean, dry seed per plant. Production of seed varies greatly from plant to plant in a stand and from year to year for a particular plant , Currently, the average yield of commercial jojoba plantations is less than 2000 kg/ Km^2 (Shreve, 1964).

2.19 Products and chemistry of jojoba :-

2.19.1. Jojoba products:-

2.19.1.1:- jojoba oil:-

Jojoba seed contains a light-gold colored liquid wax ester which is the primary storage lipid of the plant (its own common name, “Jojoba Oil”) and it's unlike conventional oil seed crops, such as soybean, corn, olive, or peanut which produce oils as the primary storage lipid.

Jojoba oil make up 50% of he seeds dry weight ,only the jojoba tree produces commercial quantities of this functional liquid material in its seeds (while for example solid carnauba wax is from the leaves of palm trees),jojoba oil is a complex mixtures of organic compounds that include carboxylic acids, long chain alcohols and to a much lesser extent, alkanes, its also a mixture of longed chained unbranched liquid wax esters that result from the esterification of an Omega-9 (double bond located between the ninth and tenth carbon atom).

Acid value is the most frequently determined property of jojoba oil. Total acid value of jojoba is typically in the range of 0.2-0.5 (mg of KOH to neutralize acid in 1 gram of sample) and it can be reduced to less than 0.2 with refining methods (Johnson, 1992). Jojoba's oil oxidative stability, thermal stability and lack of support for microbial growth can also increase a products safety and decrease its dependence on antioxidants, It has a comparatively low saponification value and contains little to no lecithin. The iodine value is a measure of unsaturation specific gravity that indicates the heavy feel of an oil

Jojoba oil is one of the finest cosmetic ingredients in the world. Its excellent inherent emolliency, moisturization and oxidative stability properties rank it as one of the top cosmetic lipid materials, natural or synthetic, in use today (N.A.S, 1975). Jojoba wax (called oil) makes up 50% of the seed's dry weight, Jojoba oil esters are designed to resist hydrolysis and oxidation for their intended use in non-occlusive moisture control and photo protection on external surfaces of skin, hair, eyes and plant leaves, Preservatives and stabilizers. Extensive use of jojoba as an ingredient in a wide diversity of products by millions of consumers over the past 15 years, as well as widespread use of 100% pure jojoba on skin and hair without adverse effects also verifies its safety. Many of the most effective ingredients for skin care formulations are those with chemical composition and physical properties similar to the skin's own surface layers. Since jojoba is completely miscible with sebum, when it is applied to the skin, a very thin, non-greasy lipoid layer of jojoba and sebum forms. This partially porous layer provides exceptional transepidermal respiration and moisture control. Unlike greasy occlusive materials such as petrolatum, mineral oils and some lanolin products, jojoba provides an absolutely non-tacky and non-greasy, dry emolliency. At the same

time jojoba significantly reduces transepidermal water loss without totally blocking transpiration of gases and water vapor. This function is enhanced by the kinking at jojoba's cis configuration that helps avoid tight packing of hydrocarbon chains. Jojobas oil serves as an excellent moisturizing agent with exceptional spread and lubricity, and leaves a rich velvety non-oily feel on the skin while retarding water loss and enhancing the flexibility and suppleness of the skin.

Jojoba oil is quickly absorbed into the skin. Absorption is apparently via the transappendegeal mechanism and occurs through the pores and hair follicles. Additionally, because jojoba is rapidly absorbed, the pores and hair follicles can remain open and thus maintain their proper functioning ability. From the pores and hair follicles, jojoba diffuses into the corneal layer of the skin probably via a pilosebaceous mechanism (Kadish, 1984).

2.19.1.2 Protien of Jojoba (Simmondisin):-

The other important product we found in jojoba is simmondisin, meal contains up to 30% protein, but toxic compounds (simmondisin) make it currently hazardous as an animal feed (Undersander., 1990).The jojoba meal contain a series of molecules considered to be toxic, with simmondsin [2 - (cyanomethylene) -3-hydroxy- '4,5-dimethoxycyclo-hexylb-D-glucoside] as the most important. Indeed, the extracted and purified simmondsin from jojoba meal caused a food intake reduction in adult rats. Taste is apparently not involved because the same response was seen with intragastric intubation as with oral administration. The food intake reduction is probably due to an inhibition of hunger, rather than to an enhancement of satiation. Simmondsin provokes weight reduction probably by an inhibition of

food intake because the same weight reduction is seen with simmondsin administration as in pairfed animals. The action of simmondsin is observed within the first hour after oral administration and lasts for several hours. Simmondsin treated with beta-glucosidase and taken into the gastrointestinal tract seems to be more active than simmondsin itself with respect to inhibition of food intake. (Miwa, 1984).

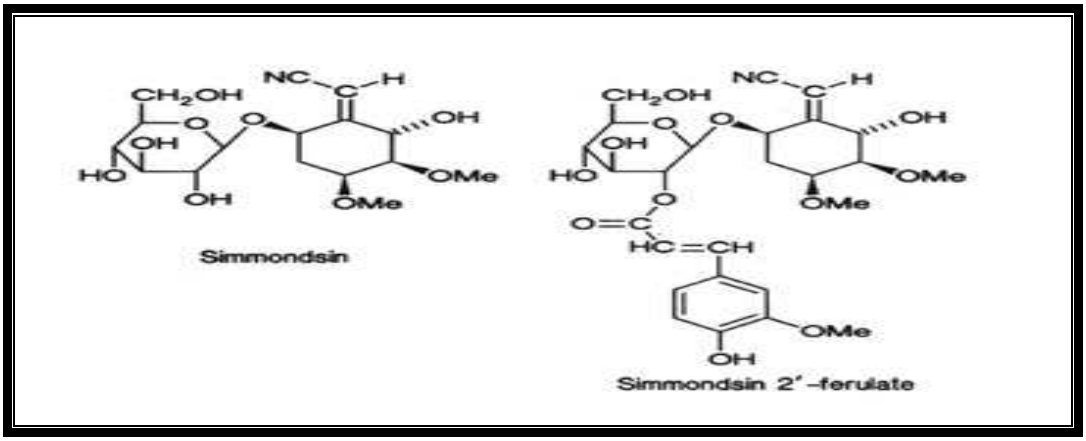


Figure (1-1) Simmondisin structures (Cokelaere, 1985)

2.19.2 Chemistry of jojoba oil:-

Four major carbon numbers are identified in the jojoba oil composition C18:1-C20:1 (CN 38) with C17:0-C19:1 (CN 36) and CN42 of C20:1-C22:1 and C22:1-C20:1 (Rentel, 1999).

The analysis showed some of which follows: per 100 g, the seed is reported to contain 4.3–4.6 % H_2O, 14.9–15.1 % protein, 50.2–53.8 % fat, 24.6–29.1 % total carbohydrate, 3.5–4.2 % fiber, and 1.4–1.6 % ash. Seeds contain 2.25–2.34%, seed hulls, 0.19%. Core wood, 0.45;%, leaves, 0.19–0.23%; twigs, 0.63–0.75%; an inflorescence, 0.22%; simmondsin, a demonstrated appetite depressant, toxicant. Three related cyanomethylenecyclohexyl glucosides have also been isolated from the seed meal. The acute oral LD_{50} for crude jojoba oil

to male albino rats is higher than 21.5 ml/kg body weight.. The amino acid composition of deoiled jojoba seed meal is 1.05–1.11% lysine, 0.49% histidine, 1.6–1.8% arginine, 2.2–3.1% aspartic acid, 1.1–1.2% threonine, 1.0–1.1% serine, 2.4–2.8% glutamic acid, 1.0–1.1% proline, 1.4–1.5% glycine, 0.8–1.0% alanine, 1.1–1.2% valine, 0.2% methionine, 0.8–0.9% isoleucine, 1.5–1.6% leucine, 1.0% tyrosine, 0.9–1.1% phenyalanine, 0.5–0.8% cystine and cysteine, and 0.5–0.6% tryptophane.. Per 100 g jojoba meal, there is 1.4 % lysine, 0.6 % histidine, 1.9 % arginine, 2.6 % aspartic acid, 1.3 % threonine, 1.3 % serine, 3.2 % glutamic acid, 1.5 % proline, 2.4 % glycine, 1.1 % alanine, 0.6 % cystine, 1.5 % valine, 0.1 % methionine, 0.9 % isoleucine, 1.8 % leucine, 1.1 % tyrosine, and 1.2 % phenylalanine (N.A.S., 1975).

2.20 Isozymes :-

Isozyme is a different electrophoretic form of the same multi-subunit enzyme. Unlike allozymes isozymes are due to differing subunit configurations rather than allelic differences, but it has the same activity and specifity to the substrate but it gave differences in physical,chemical,immunological and dynamical properties ,its usually resulted from the fusions of more than one kind as in the five types of lactic acid oxidase,some active groups which found in the enzyme molecule is responsible for the electrical charge that electrophoresis depend emigration technique depend on, the electrophoresis in addition to the chromatography technique is the methods usually used in the separation of isozymes which depend on the nature and the quantity of the proteins charge that form the isozymes molecules in there buffer used and second on the dimensions, volumes and the molecular weight and shapes of the molecules we want to separate , Isozyme analysis has

contributed not only to discrimination of species but also analysis of population biology in species insects and plants are examples, it has an important role in the medical aspects because its effected by the pathogenic cases in which it suffer from changing in types and quantities depending on the pathogenic factor and its intensity (Loxdale and Hollander, 1989) . Many methods used for isozyme analysis like Isoelectric focusing (IEF) is an electrophoretic separation which based on the isoelectric points of proteins. The pI is the point at which the protein has an overall net charge of zero. Differences of only a few hundredths of a pH-unit in isoelectricpoints are sufficient to resolve proteins from each other. IEF is used as an alternative electrophoresis format complementing the widely used SDS-PAGE electrophoresis, which is based on size of proteins (Loxdale and Hollander, 1989).

Enzymes classified to six main groups:-

1- oxidorductase and include oxidation and reduction .
2- Transferase which act as transporting of a certain group from one substance to another.
3- Hydrolases which act on hydrolesis reactions
4- Lyases which act in reactions related with hydrolesis
5- Isomerases which act on isomers which includes photo ismers and geometric isomers.
6- Ligases which act on ligation of between the compounds and help in the product formation

One of these isozymes is Glucose 6 phosphate dehydrogenase (G6PD), the rate limiting enzyme of the oxidative pentose phosphate cycle (OPPC), regulates the NADPH/NADP+ ratio in eukaryotic cells. it is known that decreased G6PD functionality can result in increased susceptibility to ’oxidative stress’, the molecular targets of this stress

are not known., At low levels of oxidative stress, G6PD activity can increase several hundred fold producing NADPH necessary to reduce/repair the oxidized molecules. Severe oxidative stress has been shown to cause inactivation of G6PD which correlated with oxidative damage ,Therefore, NADPH, the major cofactor produced by G6PD/OPPC, is vital to cellular defense against oxidative stress.

Genetic defects in G6PD, an X linked gene, are extremely common occurring in more than 400 million people throughout the world. These G6PD genetic deficiencies result in impaired ability to deal with oxidative stress and can cause various health problems such as cancer, aging related diseases, etc. .

The best known example for the immediate effect is hemolytic anemia caused by treatment of G6PD deficient patients with antimalarial drugs. In addition, several studies using cells from G6PD deficient patients have demonstrated increased sensitivity of these cells to oxidants, toxins and oxidative stress. The effects of these agents are presumed to be due to the interaction of ROS with macromolecular targets such as DNA, lipid and protein but the precise molecular targets are not well defined (Matzkin**,** 2004).

A recent study has demonstrated that the majority of patients with acute non lymphocytic leukemia have decreased G6PD levels ,This study also reported the association of a high percentage of chromosomal abnormalities, lower survival and higher remission rates in patients having decreased G6PD activity vs. normal, respectively. Although some studies have indicated that there was no difference in cancer risk among G6PD deficient Vs G6PD normal subjects, other study showed a significant increase in mortality from non Hodgkin's lymphoma in G6PD deficient subjects (Ulusu, 1999).

In a related animal study,(Wells.,1990)demonstrated that G6PD deficient mice had enhanced embryopathies, indicating a teratological role for endogenous oxidative stress caused by the absence of G6PD In their review, it was suggested that a high prevalence of G6PD deficiency in various ethnic populations may allow the putative role of G6PD deficiency in carcinogenesis and aging to be ascertained ,In addition, to obtain an independent estimate of the level of genetic isolation between the populations, variation at the glucose-6-phosphate dehydrogenase (*G6pd*) gene for the same Baja California and mainland populations of *D. mojavensis* was examined. (Matzkin**,** 2004).

Glutamate, a five-carbon skeleton dicarboxylic amino acid, is known to play a key role in mammalian intermediary metabolism. Glutamate is involved in the synthesis and/or catabolism of many compounds, including amino acids, ketoacids, and peptides. Gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the mammalian central nervous system (CNS), is known to be formed from glutamate by decarboxylation. Glutamate can be reversibly transaminated via glutamate oxaloacetate transaminase (GOT) with oxaloacetate to form α-ketoglutarate and aspartate. Also, it can be oxidatively deaminated to a-ketoglutarate and ammonia via glutamate dehydrogenase (GDH). As such, glutamate is associated directly with aerobic metabolism via the Krebs cycle, glutamate taken up by cells may be oxidatively transaminated by GOT to β-ketoglutarate, which can then enter the Krebs cycle and be oxidized to CO_2 and H_2O, or be recycled, serving as a precursor of transmitter glutamate (Andreas and Shashidharan, 2000).

Esterase is another enzymes that showed a significant variations between the two sexes of the plants, variation in esterase

expression due to the reaction wich is reversible and it could happened forward and back word the esters which the enzyme work on is a non constant and so the esterase will be variable according to its needed.(Maichel, 2002).

Malate dehydrogenize is the another enzyme which was included in this study its founded in many organism and animals and plants, Malate dehydrogenase (MDH) catalyzes the interconversion of L-malate and oxaloacetate using nicotinamide adenine dinucleotide (NAD) as a coenzyme , MDH is found in all eukaryotic cells as two isozymes: mitochondrial (m-MDH) and cytoplasmic (soluble, s-MDH). Prokaryotes contain only a single form. Pig heart MDH has been extensively studied. The two isozymes, both consisting of two very similar subunits of about 35,000 daltons and having similar enzymatic activity appear as different proteins (Bleile *et al*., 1975).

(Noyes *et al*., 1974) report on the structural similarity of mitochondrial MDH to L-3-hydroxyacyl CoA dehydrogenase (the cytoplasmic MDH being somewhat similar to lactate dehydrogenase e). differences in binding sites. have reviewed the enzymes and have compared the amino acid compositions of the two isozymes from pig heart with those of other species. They suggest that the subforms of the isozymes that have been reported may be preparative artifacts. A number of non-mammalian malate dehydrogenases have been included (Banaszak and Bradshaw, 1975).

2.21 Terpenoids, Eugenol, Flavonoids, Geraniol and terpens :-

The terpenoids, sometimes referred to as isoprenoids, are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures which differ from one another not only in functional groups, but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are the largest group of natural products,Plant terpenoids are extensively used for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical effects. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves and ginger and the color of yellow flowers. Well-known terpenoids include citral, menthol, camphor and the cannabinoids found in the Cannabis plant, The steroids and sterols in animals are biologically produced from terpenoid precursors. Sometimes terpenoids are added to proteins, e.g. to enhance their attachment to the cell membrane; this is known as isoprenylation (Hawksworth *et al*., 1983).

Camphor is a white transparent waxy crystalline solid with a strong penetrating pungent aromatic odor. It is a terpenoid with the chemical formula $C_{10}H_{16}O$. It is found in wood of the camphor laurel (*Cinnamonum camphora*), a large evergreen tree found in Asia (particularly in Borneo, hence its alternate name) and some other related trees in the laurel family, notably *Ocotea usambarensis*; it can also be synthetically produced from oil of turpentine. It is used for its

scent, as an embalming fluid and for medicinal purposes (Mann, 1994).

Modern uses include as a plasticizer for cellulose nitrate, as a moth repellent, as an antimicrobial substance, in embalming, and in fireworks. A form of anti-itch gel currently on the market uses camphor as its active ingredient. It is also used in medicine. Camphor is readily absorbed through the skin and produces a feeling of cooling similar to that of menthol and acts as slight local anesthetic and antimicrobal substance. It may also be administered orally in small quantities (50 mg) for minor heart symptoms and fatigue. Camphor is also used as a flavoring in sweets in India and Europe. It is thought that camphor was used as a flavouring in confections resembling ice cream in China during the Tang dynasty (Merc and Co, 1960).

Eugenol ($C_{10}H_{12}O_2$), is an allyl chain-substituted guaiacol, i.e. 2-methoxy-4-(2-propenyl)phenol. It is a clear to pale yellow oily liquid extracted from certain essential oils especially from clove oil and cinnamon. It's slightly soluble in water and soluble in organic solvents. It has a pleasant, spicy, clove-like odor. Eugenol is used in perfumeries, flavorings, essential oils and in medicine as a local antiseptic and anaesthetic. It was used in the production of isoeugenol for the manufacture of vanillin, though most vanillin is now produced from petrochemicals or from by-products of paper manufacture. When mixed with zinc oxide, eugenol forms a cement used in dentistry. Eugenol derivatives or methoxyphenol derivatives in wider classification are used in perfumery and flavoring. They are used in formulating insect attractants and UV absorbers, analgesics, biocides and antiseptics. They are also used in manufacturing stabilizers and antioxidants for plastics and rubbers (McKenzie, 2004).

Rutin, also called rutoside, quercetin-3-rutinoside and sophorin, is a citrus bioflavonoid found in buckwheat and the fruit of the fava D'Anta tree (from Brazil) and other sources, It can combine with cations, supplying nutrients from the soil to the cells in plants. In humans, it attaches to the iron ion Fe^{2+}, preventing it from binding to hydrogen peroxide and creating a highly reactive free radical that may damage cells. It is also an antioxidant, and therefore plays an important role in inhibiting some cancers, Rutin also strengthens the capillaries, and therefore can reduce the symptoms of haemophilia. It also may help to prevent a common unpleasant-looking venous edema of the legs. Rutin, as ferulic acid, can reduce the cytotoxicity of oxidized LDL cholesterol and lower the risk of heart disease (Donald and Crystobal, 2004).

Geraniol, C10H18O, is a primary alcohol, isomeric with linalool, and constitutes the chief part of oil of rose and palmarosa oil; it also occurs in appreciable quantities in geranium, citronella, lemon, and many other oils. It is found both in the free state and in the form of esters, and may be obtained by making use of its property of combining with calcium chloride to form a crystalline compound, thus:—The oil is triturated with an equal weight of anhydrous calcium chloride, and the mixture, which becomes heated to 30° to 40°, is cooled in a desiccator. The solid mass thus obtained is triturated with anhydrous ether or benzene, filtered under pressure, washed with ether or benzene, and decomposed with water, the oily liquid which separates washed with warm water, and finally distilled in a current of steam. Geraniol occurs as a colourless, somewhat oily liquid, having a sweet rose-like odour, and becoming oxidised on exposure to the air. Specific gravity, 0.880 to 0.883; boiling-point, 230°, refractive index, 1.4766, optically inactive. On oxidation, it is converted into its aldehyde geranial or

citral. By heating with acetic anhydride it yields the acetic ester, , which is a fragrant oil of specific gravity 0.917. Geraniol may be changed to linalool by heating with water to 200° under pressure, whilst it may be obtained from linalool by heating the latter for some time with acetic anhydride (McKenzie, 2004).

Terpenes are a large and varied class of hydrocarbons, produced primarily by a wide variety of plants, particularly conifers, but also by some animals. They are the major components of resin, and of turpentine produced from resin. The name "terpene" is derived from the word "turpentine". When terpenes are modified chemically, such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Some authors will use the term terpene to include all terpenoids as well , Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, in aroma therapy, and in traditional and alternative medicines. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives (Red, 1985).

Coumarin is a chemical compound found in many plants, notably in high concentration in the tonka bean, woodruff, and bison grass. It has a sweet scent, readily recognised as the scent of newly-mown grassbiosynthesis of coumarin in plants is via hydroxylation, glycolysis and cyclisation of cinnamic acid. Coumarin can be prepared in a laboratory in a Perkin reaction between salicylaldehyde and acetic anhydride.Some naturally occurring coumarin derivatives include umbelliferone (7-hydroxycoumarin), herniarin (7-methoxycoumarin),

psoralen and imperatorin. Coumarin is found in a variety of plants such as Tonka bean, lavender, sweet clover grass, and licorice, but also occurs in food plants such as strawberries, apricots, cherries, and cinnamon. It is thought to work by serving as a pesticide for the plants that produce it (Scholde, 1994).

Abstract

Four samples were taken from the leaves and the flowers of jojoba plant. Extraction solution were prepared from leaves and flowers of the two plant sexes, Total protein was studied using Bradford procedure during three weeks (the last two weeks of July and the first week of August 2004) for the two sexes and conventional differences were recorded between them, also the Isozymes variation was studied between the sexes for the following enzymatic systems:-

1- Glucose 6 Phosphate Dehydrogenase (G6PD).

2-Glutamate Oxaloacetate Transaminase (GOT).

3- Malate Dehydrogenase (MDH).

4- Esterase(EST).

Isozyme variation was detected between the two sexes of the plant in which leaf samples before and during flowering were tested for the first two enzymatic systems (mentioned above), while the third enzymatic system was tested before flowering (leaf samples only) and the fourth enzymatic systems was studied in the leaves and flowers during the flowering time, the test was done using horizontal gel electrophoresis technique to detect the isozyme variation.

The second enzymatic system was purified from the extraction solutions of the two sexes using the ion exchange technique with cellulose methyl cinamate (CMC) and the protein concentration was studied before the purification in the crude samples , after the precipitation by salts (ammonium sulphate) and after the purification by the ion exchange technique.

The enzymatic extraction solution from the two sexes of jojoba was injected in two groups of hosts (14 rabbits each) .After three weeks, 10 healthy rabbits were selected from each group and serum was taken from each host and tested against Ags from both mature and newly formed plant leaves.

Statistical analysis was conducted using T-test to detect statistical differences between the means. The results show no conventional differences found while differences in the specific activity of enzyme and the protein concentration of the crude solution were recorded (between the two sexes).

Several kinds of terpens, tepenoids, eugenols and coumarin substance were studied in both sexes using high performance liquid chromatography in which differences were indicated as similar and intermediate and acute differences

Acknowledgement

Thanks to Allah ,mercy and prays upon his prophet Mohamed the messenger of Allah.

I should remember my supervisor Dr.Nabeel AL-Ani who gave me time and science to support my work .

I should remember my colleages who supported me during my study , Yarub , Mohannad , Ayat ,Rose and to the Biotechnology department staff Ms.Uruba and Ms.Hiba .

To my family members who supported me during my study.

Sudad

List of abbreviations

HPLC	High performance liquid chromatography
i.e	International unite
IP	Isoelectric point
Abs	Antibody's
Ags	Antigen's
MDH	Malate dehydrogenase
EST	Estarase
GOT	Glutamate oxaloacetate Transaminase
G6PD	Glucose 6 phosphate dehydrogenase
D.W	Distilled water
nm	Nano meter
SKm	Sequer Km

List of Contents

List of figures

References

-A-

Akah, P.A., Oli, A..N., Enwerem, N.M., and Gamaniel, K. (1997). Preliminary studies on purgative effect of *Carica papaya* root extract. Fitoterapia, 68 (4): 327-331.

Andreas, P. and Shashidharan, P. (2000) . Inheritance and linkage relationships of melon (*Cucumis melo* L.) isozymes. J Amer Soc Hort Sci 123:264-272.

Andrews, K. (1993). *Isozymes in Plant and animals, detections and analysis*.53:44-45.

Armstrong, W.P. (1998). "The Wild and Wonderful Family of Gourds." Pacific Horticulture 59 (4): 11-18.

-B-

Bagby, M.O. (1988). Comparison of Properties and Function of Jojoba Oil and Its Substitutes, Proceedingsfrom the Seventh International Conference on Jojoba and Its Uses, Phoenix, AZ.

Baldwin, J. (1989) Chemistry and Properties of Jojoba Oil, Proceedings from the fourteenth International Conference on Jojoba and Its Uses, Phoenix, Az.,.

Banaszak, L., and Bradshaw, R.(1975) : *The Enzymes, 3rd Ed.*,P. Boyer, Academic Press, NY, 369.

Benzioni, A. (1995). Jojoba domestication and commercialization in Israel. Hort. Rev. Janick J (ed) In press.

Bergmann, F. (1987) . electrophoresis technique of Isozymes , a rapid method for genetic detection , Studies in The American Southwest, University of New Mexico Bulletin, Biological Series 7.

Bianchini, F. and Corbetta, F. (1976). The Complete Book of Fruits and Vegetables. Crown Publishers, Inc., New York.

Biesberg, L. H.; Peterson, P. M.;, Soltis, D. E. and Annable,C. R. (1987) *Biology of Dwarf Mistletoes: Proceedings of the*

Symposium (Hawksworth, F. G. and Sharpf, R. F., eds), p. 2. U.S.D.A. For. Ser. Gen. Tech. Rep. RM-111.

Bleile, D. ; Busto, F; and Soler, J.(1975). The Nature of the Substrate Inhibition of Cytoplasmic Malate Dehydrogenase from *Phycomyces blakesleeeanus*, *Biochim Biophys Acta* *784*, 158.

Bradford, M.M. (1976). A rapid and sensitive method for the quantition of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem.,; 72: 248-251.

Braun,W.R.H. and Espericueta, B.M. (1979). Biomesay produccion ecologica de jojoba (*Simmondsia chinensis* Link) en el deserto costero de Sonora. Deserta 557–72.

Brown, J. and Dwyer, K. (1988). The Jojoba Potential: An Overview, roceedings from the SeventhInternational Conference on Jojoba and Its Uses, Phoenix, AZ..

Buchmann, S. L. (1987). Floral biology of jojoba (Simmondsia chinensis), an anemophilous plant. Desert Plants 8(3): 111-124.

Buchanan, R.A.. and Duke, J.A.. (1981). Botanochemical crops. p. 157–179. In: McClure, T.A. and Lipinsky, E.S. (eds.), CRC

handbook of biosolar resources. Vol. II. Resource materials. CRC Press, Inc. Boca Raton, FL.

-C-

Cokelaere, J.N. Jojoba (1985). New Crop for Arid Lands; New Raw Material for Industry, National Academy Press, Washington D.C.,

Conkle, M.T., Hodgskiss, P.D., Nunnally, L.B. and S.C. Hunter. (1982). Starch Gel Electrophoresis of Conifer Seeds: A Laboratory Manual. USDA Forest service General Technical Report PSW-64. Pacific Southwest Forest and Range Experiment Station, Berkeley, California.

Crawford, D. J. (1983) *Isozymes in Plant Genetics and Breeding. Part A* (Tanksley, S. O. and Orton, T. J., eds), p.257. Elsevier, Amsterdam.

-D-

Daugherty**,** E.F. ; Underhill, R.M. ; Lumy, H.V. (1953). Ethnobiological Studies in The American Southwest,University of New Mexico Bulletin, Biological Series 4.

Dimmit, M. (2000). "Simmondsiaceae (jojoba family)," in Steven Phillips and Patricia Comus, eds., , *A Natural History of the Sonoran Desert*, pp. 256-7, Arizona-Sonora Desert Museum Press, Tucson; Epple, Ann, 1995, *A Field Guide to the Plants of Arizona*, Helena, Montana: Fountain Press, p. 137; Shreve, Forrest & Ira Wiggins, 1964, *Vegetation and Flora of the Sonoran Desert, Vol. 1*, Palo Alto: Stanford University Press.

Donald, R. and Cristobal, M.(2004) . Antioxidant activity of flavonoids, department of environmental and molecular toxicology Oregon state university USA) .

Duran, A. and Rodriguez, D. (1987). Changes in serum enzymes of *Saprolegnia-infected* brown trout, *Salmo trutta* L. J. Fish Dis. 10: 505-507.

-E-

Evison, J. and Chester, J.F. (1999). Nature, Soap, Perfume, and Cosmetics , 63 (13),Fieser, Louis and Mary Fieser, Basic Organic Chemistry, D.C. Heath and Company, USA.

-G-

Gentry, W. Jr. (1958). Final Report on the Safety Assessment of Jojoba Oil and Jojoba Wax, J. of the American College of Toxicology, Vol. 11, No.1.

George , S. (1996) . Immunology : practice and methodology ,hand book , Los Angeles, California, USA.

-H-

Hagemann, J.W. and Rothus, J.A..(1979). Journal of the American Oil Chemists' Society, Vol. 56, #629.

Hartwell, J.L. (1971). Plants used against cancer. A survey. Lloydia J. Bacterio., *92*(3): 723-726.

Hawksworth,D. J. (1983). *Isozymes in Plant Genetics and Breeding. Part A* (Tanksley, S. O. and Orton, T. J., eds), p. 257. Elsevier, Amsterdam.

-J-

James, G.T. (1994.). A comparison study between several strains of botanical systems .

Jelinski, G. and Cheliak, H .(1992). Malate dehydrogenize expression in dioeciously behavior plants , U.S.A . Journal Of Botany 37(5): 4-8.

Jirovetz , L. Buchbauri, G. and Leela, N. (2003)Analysis of the essential oils of the leaves, stems, rhizomes and roots of the medicinal plant *Alpinia galanga* from southern India *1 Institute of Pharmaceutical Chemistry University of Vienna A-1090 Vienna, Austria 2 Department of Chemistry Calicut University, Kerala 673 635, India.*

-K-

Kadish, A . (1984). A Search for the Origin of Jojoba, Proceedings from the Sixth International Conference on Jojoba and Its Uses, Beer-Sheva, Israel,.

Kajikawa, M.;Yamaoka, S.; Yamato, K. T.; Kanamaru, H.; Sakuradani, E.; Shimizu, S.; Fukuzawa, H. and Ohyama, K. (2003) *Biosci. Biotechnol. Biochem.* 67, 605-612.

Kohen, H. S. (1984). Statistical analysis of experimental data. USA, Inc, New York.

Kohorn, L. U. (1990). Sexual dimorphism in Simmondsia chinensis: reproductive effort and physiological function. Dissertation. University of California, Los Angeles, California, USA.

Kovalenko, P.G. (2002). Maliuta Secondary metabolites production from transformed cells of *GLYCYRRHIZA GLABRA* Institute of Molecular biology and genetics, Kyiv, Ukraine.

-L-

Lawrence J. K. (1995). Jojoba, buffalo gourd and Russian thistle: Plant characteristics, products and commercialization potential. Desert Plants 5(3):112-126.

Lee, C. W. and. Sherman, R. A. (1985). Meiosis in jojoba, Simmondsia chinensis. Israel Journal Of Botany 34(1): 1-6.

Lege , O. (1995). HPLC: a new technique for chemical separations Ann Arbor, Michigan. USA.

Lewis, A. (1982). A Study of genes expression in dioeciously patterns of sonaran desert shrebs, LAFR1. EMBO J. *3***:** 3323–3328.

Lisbalchin, M. (1993). The essential oils of *Pelargonium grossularioides* and *Erodium cicutarium* (Geraniaceae). *Journal of Essential Oil Research* **5**: 317-318.

-M-

Maison, F. (1997). purifications of GOT from pig heart K-1 1 151. J. Bacteroil. *176*(12):3584-3588.

Mann, R. S. ;Davidson, J. B.; Hobbs, D. V.; Banthorpe, J. B. and Harborne, C.K (1994). *Natural Products*, pp. 309-311, Addison Wesley Longman Ltd., Harlow, UK,. ISBN 0582060095.

May, D. (1998). Gel Electrophoresis Technique Methods and Applications. Part A 33 (4): 17-18. D.C. , USA.

Merc, H.K and Co, I.D. (1960). *Handbook of Chemistry and Physics*, CRC Press, Ann Arbor, Michigan.

McKenzie, D.H. (2004). Classification of alcohol derivatives Mol. Microbiol. *10:* 233–243.

Matizkin, C.M. (2004). Purification and investigation of some kinetic properties of glucose-6-phosphate dehydrogenase from parsley (petroselinum hortense) leaves. Prep. Biochem., 2002; 32: 173-187.

Mills, D.; Zhou, Y. and Benzioni, A.(2004) Improvement of jojoba shoot multiplication by ventilation in vitro. *In Vitro Cell. Devel. Biol.* 40: 396-402.

Miwa, T.K. (1984). Composition Of Jojoba Oil From Nuts Harvested At Different Geographical Regions, Proceedings from the Second International Conference on Jojoba and Its Uses, Ensenada, Baja California, Mexico.

Morelli, A. and De Flora A.(1983) Biochemical mechanisms of glucose-6-phosphate dehydrogenase deficiency.Proc. Natl. Acad. Sci., 1978; 75.

-N-

N.A.S , (1975). Underexploited Tropical Plants with Promising Economic Value National Academy of Sciences, Washington, DC, USABaldwin.), pp 50–59. AmericanOil Chemists' Society: Champaign Il., USA.

Noyes, B.; Glatthaar, B.; Garavelli,; and Bradshaw(1974). Structural and Functional Similarities Between Mitochondrial Malate Dehydrogenase and L-3-Hydroxyacyl CoA Dehydrogenase, *Proc Natl Acad Sci U S A 71*, 1334.

-O-

Ohima , J.G. and Brown J. H. (2001). Jojoba In Cosmetics, seventeenth International Conference of genetics and featueres inheritance , Sentara, Mexico.

Ozer, N., Bilgi, C., and Ous, H.(2001): Dog liver of glucose-6-phosphate dehydrogenase: Purification and kinetic properties. Int. J.Biochem. Cell B.; 1199: 1-10

-P-

Pastorino, A. G. , Mario D. J., Gallo, U.L. ,Leanardo, K.O. (2001) Isozyme Variation in Austrocedrus chilensis in Relation to Rainfall

Gradients . , Instituto Nacional de tecnología Agropecuaria (I.N.T.A.), CC 277, 8400 San Carlos de Bariloche, Argentina

-R-

Raijmann ,F. Sonima , E. Clous ,S. and Fored , A (1996) . isozyme variations in *lepidoptera yponomeutidae*, Poland .

Rain.D,(1996) characterizations and kinetics of G6PD ,biochemical study , 5: 37- 53.

Red ,R.W.(1985).Biological activity of terpens against *staphylococcus* strains , 4-7 , sec 2.

Rental, C. (1999). The fatty acids of jojoba. In: A.R. Baldwin (ed.), Proc. 7th Int. Conf. on Jojoba and its Uses. Am. Oil Chem. Assoc. Champaign, IL.

Rise, W. R. and Chippindale, A. K.(2001) Sexual recombination and the power of natural selection. Science 294:555-559.

Robin . A.F, (1954). Jojoba: New Crop for Arid Lands; New Raw Material for Industry, National Academy Press, Washington D.C., 1954.

Rohan ,B.T (1987) Isozyme variation in *P. tremuh* and *P. tremuloides* 8(3): 111-124.

-S-

Sandy , J.K .(1999) botanical and chemical study of *Mikania glomerata ,American journal of botany* , U.S.A.

Scharpf, R. F. and Hawksworth F. G.. (1976). Luther Burbank introduced European mistletoe into California. Plant Disease Reporter 60:740-742.

Scholde , J. E. (1994). Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of Ethnopharmacology, 61 (1): 57-65.

Shreve, F. (1964). Vegetation of the Sonoran Desert. In Shreve, F. and I. L.

Shreve, (1964). *Vegetation and Flora of the Sonoran Desert, Vol. 1,* Palo Alto: Stanford University Press.

Simon, S, J.A.(1986). Papaya, p. 256-269. In: Tropical fruits (2nd edition). Tropical Agriculture Series, Longman Inc., New York.

Somsri S.(1998) Improvement of Papaya (*Carica papaya* L.) for South-east Queensland: Investigations of sex-type and fruit quality ,Summary of PhD thesis Submitted June 1998 The University of Queensland Gatton College

Sonam , L.U, (2000) A comparative study of Essential Oils of *Cymbopogon Citratus***,** Wesley Longman Ltd., Harlow, UK

-T-

Totingham , D . (2000) genetic differences and Isozymes variations in different kinds of economical high yield plants .

Tsror , N . P . (1996). fungal pathogenesis Chemistry, Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Reinhardt, J.G.(1990) jojoba Cosmetics, Eighth International Conference on Jojoba and Its Uses, Asuncion, Paraguay.

-U-

Ulusu, N.A(1999). Rapid method for the purification of glucose-6-phosphate dehydrogenase from bovine lens. Int. J. Biochem. Cell. B.,; 31: 787-796.

Underschold (1990). Jojoba: New crop for arid lands, new material for industry. National Academy Press, Washington, DC.

Undersander, D.J. (1990) Sorghum—for Syrup. Alternative Field Crops Manual. University of Minnesota Extension Service, Center for Alternative Plant & Animal Products.

-V-

Verbiscar, A.J. and Banigan, T.F. (1978). Composition of jojoba seeds and foliage. J. Agr. Food Chem. 26(6):1456–1459.

-W-

Wells , D.L.; Cox, M.M.(1990) Glucose-6-phosphate dehydrogenase ktivitesine primaquinin etkisi, Doa - Tr. J. of Med. Sci., 1990; 14: 57- 63.

Wiggins. 1964. Vegetation and Flora of the Sonoran Desert. Stanford University Press. Stanford, California.

Williams, D. A. (1976) *Biometrika* 63, 33.

William , k . (1993) purification of GOT from pig heart , journals of biochemistry , J Amer Soc Hort Sci 123:264-272.

-Y-

Yermanos, D.M. (1973). Jojoba a brief survey of the agronomic potential. California Agriculture (Sept.).

Yermanos, D. M. (1977). Jojoba--genetically controlled botanical traits. Journal of the American Oil Chemists Society 54:545-548.

الاهداء

الى مقام النبي الاعظم
محمد رسول الله

(صلى الله عليه و سلم)..

الى عائلتي الصغيره....

امي العزيزه ...
ابي العزيز...
اخي زياد....

سداد

الخلاصه

اخذت اربعة عينات من اوراق وازهار نبات الهوهوبا وتم تحضير مستخلصات نباتيه من اوراق و ازهار كلا جنسي النبات. تمت دراسة نسبة البروتين بواسطه طريقه برادفورد لكلا المستخلصين النباتيين خلال ثلاثة اسابيع (الاسبوعين الاخيرين لشهر تموز و الاسبوع الاول لشهر اب٢٠٠٥) وتمت تسجيل فروقات معنويه بين النسب كلا الجنسين كما و تمت دراسه الانظمه الانزيميه لكل من :-

١- كلوكوز – ٦- فوسفات ديهايدروجينايز.

٢- كلوتاميت اوكزالو اسيتاتيت ترانس امينايز .

٣- (ماليت ديهايدرو جينايز .

٤- الاسترايس .

وتم تحديد التغاير الانزيمي في النبات بين الجنسين حيث تمت دراسة العينات النباتيه(الماخوذة من الاوراق قبل وقت التزهير و من الاوراق و الازهار خلال وقت التزهير) للنظامين الانزيميين الاول و الثاني اعلاه في حين درس النظام الانزيمي الثالث في عينات من الاوراق قبل فترة التزهير و درس النظام الانزيمي الرابع في عينات من الاوراق و الازهار خلال فترة التزهير فقط(وذلك لندرة وجود الصبغ الخاصه بالانزيمين) . تم استخدام طريقه الهجرة الكهربائيه الافقيه عبر الهلام (النشاء) لتحديد التغاير الانزيمي بين الجنسين النباتيين.
تمت تنقية النظام الانزيمي الثاني (Glutamate oxaloacetate Transaminase) من المستخلص النباتي لكلا الجنسين بواسطة تقنية المبادل الايوني باستخدام مادة السيليلوز ميثايل سينامايت (سي ام سي) و تم قياس نسبة البروتينات في المستخلص الخام و بعد الترسيب باملاح سلفات الامونيوم و بعد التنقيه بالمبادل الايوني.
تم حقن المستخلصين النباتيين من كلا الجنسين في مجموعتين من الارانب المختبريه(مجموعه لكل جنس) وحدد ١٤ ارنب لكل مجموعه وبعد ٣ اسابيع تم اختاير عشرة ارانب صحية المظهر من كل مجموعه وتم عزل السيرم الحاوي على الاجسام المضاده و اختباره على مستخلص

نباتي ماخوذ من الذكر و الانثى البالغين و نبات حديث التكوين ومحاولة أيجاد تشابه بين ردود الفعل المناعية بواسطة اللتحليل الاحصائي (اختبار تي) لغرض التوصل إلى جنس النبات ولم يتم الحصول على فروق معنويه بين الذكر و الانثى في حين تمن الحصول على فروق في فعالية الانزيم و نسبة البروتين بين الجنسين في المستخلص الخام .

كما تمت دراسة بعض أنواع التربينات و التربينويدات و المشتقات الكحوليه و الكومارينات بواسطة تقنيه الفصل بالسائل عالية الدقه و تم الحصول على فروق بسيطه و متوسطه و كبيره بين هذا الانواع.

Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Sciences
Biotechnology Department

Molecular differentiations of Jojoba

A Thesis
Submitted to the College of science
The University of Al-Nahrain
In a Partial Fulfillment of the Requirements for the Degree of
Master in Biotechnology

By

Sudad Tariq Shakir

B. Sc. Biotechnology

(2001)

December, 2005

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانه الاحيائيه

تمييز نبات الهوهوبا بالطرق الجزيئيه

رساله

مقدمه لكليه العلوم جامعه النهرين

وهي جزء من متطلبات نيل درجه الماجستير في التقانه الاحيائيه

من قبل

سداد طارق شاكر البياتي

بكلوريوس تقانه احيائيه ٢٠٠١

جامعه النهرين

شوال ١٤٢٦

كانون الاول ٢٠٠٥

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that, according to our opinion, is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:

Name:

Scientific Degree:

Date:

(Chairman)

Signature:

Name:

Scientific Degree:

Date:

(Member)

Signature:

Name:

Scientific Degree:

Date:

(Member)

Signature:

Name:

Scientific Degree:

Date:

(Member)

I hereby certify upon the decision of the examining committee

Signature:

Name: **Dr. Laith A. Z. Al- Ani**

Scientific Degree: Assistant Professor

Title: Dean of College of Science

Date:

Supervisor Certification

I certify that this thesis was prepared under my supervision in the College of Science, Al-Nahrain University as a partial requirement for the degree of Master of Science in Biotechnology.

Signature:

Supervisor: **Dr. Nabeel K. AL-Ani**

Scientific Degree: Assistant professor

Date:

In review of the available recommendations, I forward this thesis for debate by the examining committee.

Signature:

Name: **Dr. Nabeel K. AL-Ani**

Scientific Degree: Assistant professor.

Title: Head of Biotechnology Department.

Date: