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# Propagation of Portulaca oleracea L. and level of Active Compounds in Callus Culture

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**ABSTRACT**

**This study succeeded in enhancing production of fatty acids in callus cultures of Portulaca oleracea L. with existence Jasmonic acid(JA) in growth medium. Porslane nodal segments were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of Benzyl Adenine (BA) and Naphthalene Acetic Acid (NAA), best initiation callus from these segments after two weeks of culture on MS medium containing 2.0 mg/L BA and NAA from each one (MSt). Then (0.5, 1.0, 1.5, 2.0, 2.5, 3.0) µg/L of JA was added to MSt medium. The results indicated that there is an increase in fresh weights of callus after exposure to JA with low concentration in growth medium, on the other hand a dramatic increase in level of fatty acids was observed after addition (JA) with high concentration in culture medium, Gas chromatography(GC) technique was used for diagnosis of the fatty acid, and found that Oleic acid, Linoleic acid and Palmitic acid were the most common in the callus cultures of Porslane, also found that fatty acids amount were increased with the increase of JA concentration in culture medium, Specially when addition of 3.0 µg/L JA, and that concentration of fatty acids in callus cultures were increased with the increase of callus age.**

**Keywords: Jasmonic acid, NAA, BA, Fatty acids, callus, Portulaca oleracea.**

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## INTRODUCTION

Portulaca oleracea L.an annual succulent in the family Portulacaceae, which may reach 40 centimeters in height[1,2], It is native to the India and grown widely in china, and is used traditionally for alleviating pain and swelling[3]. Extensive studies have been done on P. oleracea regarding it's a pharmaceutically important plant, used for headache, stomach ache, painful urination, enteritis, mastitis, and also eaten as a salad and vegetable all around the world[4,5], leaves may have a protective effect against oxidative stress caused by vitamin A deficiency[6], its used to treat burns, earache, insect stings, inflammations, skin sores, ulcers, itching skin, eczema and abscesses[5,7], P. oleracea has anti-bacterial [8], anti-viral, anti-diabetic, and immuno-modulating activity[3], antifungal, wound healing, anti-inflammatory, uterine stimulant, muscle-relaxant and diuretic properties[9,10]. The whole plant is considered as antiphlogistic bactericide in bacillary dysentery, diarrhea, haemorrhoids, enterorrhaghia, etc. [11]. Portulaca oleracea L. is consider as an important source for unsaturated fatty acids exclusive linolenic acid (Omega-3) [12,13], also Linoleic acid (LA) and α-linolenic acid (α LA) which is important for medicinal properties [14], It possess many antioxidant properties due to the high content of vitamins, minerals, omega-3 essential fatty acids and other healthful compounds [15].

Plant tissue culture is commonly used to describe the in vitro and aseptic growth of any plant part on a nutrient medium. Growth hormone is a natural chemical that exerts strong controlling effects on growth and development. It is used either in low or high concentrations to promote the callus or shoot and root formation [16].

Jasmonic acid(JA) is consider to be one of the growth hormones leading to aging which reduces the level of gene expression [17], It has been reported to influence a wide variety of physiological and developmental responses [18]. The major functions of JA in regulating plant growth include growth inhibition, senescence and leaf abscission. JA has an

important role in response to wounding of plants and systemic resistance in plants inhibiting the insect ability to digest protein, and it has been role in the regulation of root growth and development in response to environmental stresses through interplay with ethylene and auxin [19].

According to the medical importance mention of the plant, the present study demonstrated an efficient in vitro callus induction for this plant, and also detections of the fatty acids in callus cultures with existence Jasmonic acid in growth medium as induced factor.

## MATERIALS AND METHODS

### 1. Explants Sterilization:

Healthy plants of Portulaca oleracea L were identified and collected from the local garden and market place in Mosul city. and washed under tap water for 30 minutes to remove mud, and subsequently submerge in 70% ethanol for(1) minute. Then under sterile condition plant materials were transferred to (1: 2) v/v sodium hypochlorite solution with distilled water respectively and gently agitated, After (10 , 15, 20) minutes plant materials were washed with sterile distilled water four times. The plant materials were placed on sterile filter paper and then cut as explants (nodal segments 2.5 cm long) to be cultured.

### 2. Culture medium and callus initiation:

Nodal segments were cultured on MS [20], basal medium supplemented with BA and NAA at (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5) mg/L for each one together, and(1.0,2.0) mg/L BA and NAA each one alone, Moreover, some nodal segments were cultured on hormone-free MS basal medium as a control, Ten replications were considered for each treatment. The conical containers were incubated at 25 ±2 º C with photoperiod at 16h light / 8h dark/ day. The response of callus induction from explants was recorded after two weeks, and subcultured every 30 days of culture, also shoot regeneration percentage and rooting was recorded in different period after culture.

### 3. Addition of Jasmonic acid in culture medium

Jasmonic acid were provided to MS medium in (0.5, 1.0, 1.5, 2.0, 2.5, 3.0) µg/L with the best level of BA and NAA which where obtained from previous experiment, the response of callus induction from explants was recorded after two weeks, and subcultured every three weeks, also the response of callus induction from callus cultures was recorded as fresh weight of callus after three weeks and six weeks from culture.

### 4. Quantitative and qualitative estimate of fatty acids in callus culture by using Gas Chromatography (GC):

### A -Extraction fatty acids from leaves of plant and callus culture:

Fatty acids were extracted according to Dalgarno and Birt [21], which including eliminate fats from plant tissue by using organic solvent, after that, fatty acids diagnosis by gas chromatography [22].

### A – 1- Prepare of callus extractions:

The purslon callus were taken with 5gm fresh weight, callus which growth on(MSt) medium supplement with different concentration of JA, after (3 and 6) weeks from culture. These sample crushed in 20 ml (chloroforme: ethanol ) (2:1) (v:v )then kept in 25 º C four hours. After that, filtration by using Whatman paper (No.1), then rinsed with 10 ml (chloroforme: ethanol ) (2:1) v/v five times, and evaporated under low pressure with (50-55) º C.

### A – 2 - Prepare of plant leaves extraction:

Plant leaves were identified and collected from the local garden in Mosul city, these leaves were cut down and crushed in 100 ml (chloroforme: ethanol ) (2:1) v/v , then crush completed by using homogenizer from type ( Ultra Turrax blender, Germany ) and ultrasonic with 22 Kilo Hz / minute for 30 second, all these steps were done in ice –bath, then the mixture was kept in 25 º C for 4 hours. Then the mixture was filtered and evaporated by the same steps, mentioned previously.

### A – 3 - Prepare organic salts of fats:

Addition 50 ml of 1.5 normal HCl in methanol to the end product, then heating to the boiling in Reflex with 100 ºC for three hours. Then addition 30 ml from petroleum ether with intensify agitate, then evaporated under low pressure. After that dissolved of fatty acids methyl esters in chloroform, to be ready for analysis by gas chromatography.

### B - Isolation and diagnosis of fatty acids by gas chromatography (GC):

Fatty acids were extracted according to Dalgarno and Birte, 1963[21], to isolation and find the exact amount of fatty acids, gas chromatography system from Hewlett Packard company (Packard Model 438A)was used, with (1/8 Inch – 3meter ) column type E-30, with use of Flame ionization detector (FID) in 325 ºC. Standard fatty acid was obtained from laboratory of Ibn Sina company /Ministry of Industry and Minerals /Baghdad.

Fatty acid was identified according to its retention time, as compared with standard samples. Fatty acids concentration was estimated depending to the area under peak . Fatty acids were separated by using GC column as stated previously after using Helium gas(He) as a carrier gas with speed 30 ml/ minute[23,24].

## RESULTS AND DISCUSSION EXPLANTS STERILIZATION:

The results of sterile nodal explants of Portulaca oleracea L., cultured on media, demonstrated the best treatment when use NaOCl : distilled water (1: 2) v/v at 15 minutes (Table 1). Whereas sterile qualification 90% and growth qualification 95 %, but when increase sterile time at 20 minutes, noted decreased in growth qualification 65 %, (Table 1). Sterile Qualification depend on type of sterile material, concentration its and sterile time with protection on explants vitality and eliminate its from microbes [25,26].

**Table (1): Qualification Sodium Hypochlorite(NaOCl)solution of surface Sterilization on Portulaca oleracea L. nodal explants growth after 10 days.**

| (NaOCl): Distilled Water (1: 2) v/v / Sterile time (minutes) | Sterile qualification % | Growth qualification % |
|---|---|---|
| 10 | 50 | 50 |
| 15 | 90 | 95 |
| 20 | 95 | 65 |

**Response of explants on culture**

Nodal segments, which sterile and cultured, produced callus within 2-4 weeks after culture on MS medium supplied with different combination of growth regulators.

The data in (Table 2) showed that most treatments stimulate callus initiation from nodal segments, but the best callus formation and growth rate was observed in the medium containing 2.0 mg/L BA and NAA from each one , which produced 93.5% callus after two weeks of culture, also observed callus formation in the medium containing 3.0 or 3.5 mg/L BA and NAA for each one, with 83.5% and 88.9% respectively, after three weeks of culture. These results demonstrated that combination of BA with NAA its essential for callus initiation [27]. And this callus was friable and green, (Fig. 1-A ), callus color depending on source of explant and type of plant [28,29].

Similar effect of NAA and BA in callus formation from Portulaca grandiflora leaf explants were observed [30], reported that 10µM NAA combination with 10 or 5 µM BA were found to be suitable for callus production from leaf explants. Again noted decreased in initiation of callus when nodal segments cultured on MS medium supplied with BA or NAA alone, also addition low concentrations from these plant growth regulators in basal medium (MS) caused low rate of response to initiation callus from nodal segments, Table (2).

Initiation of cell by division and subsequent callus production requires cytokinin and auxin in the medium at the proper proportion [27,31].

**Table (2): Assessment of various stimulation media for callus induction from nodal segments of Portulaca oleracea L.**

| Induction medium (mg /L) | No. induction explants | Callus initiation (%) | Period of initiation (wks) |
|---|---|---|---|
| MSO | - | - | - |
| MS+ 1.0 BA | 6 | 56.5 | 3 |
| MS+ 2.0 BA | 7 | 60.7 | 3 |
| MS+ 1.0 NAA | 7 | 60.5 | 3 |
| MS+ 2.0 NAA | 7 | 68.5 | 3 |
| MS+ 0.5 BA + 0.5 NAA | 6 | 58.8 | 4 |
| MS+ 1.0 BA + 1.0 NAA | 7 | 65.5 | 4 |

| | | | |
|---|---|---|---|
| MS+ 1.5 BA + 1.5 NAA | 8 | 75.6 | 3 |
| MS+ 2.0 BA + 2.0 NAA | 9 | 93.5 | 2 |
| MS+ 2.5 BA + 2.5 NAA | 8 | 85.0 | 4 |
| MS+ 3.0 BA + 3.0 NAA | 8 | 83.5 | 3 |
| MS+ 3.5 BA + 3.5 NAA | 9 | 88.9 | 3 |

It is worthy to mention, that the present study growth of shoots from some callus cultures or nodal segments which cultured on MS medium supplemented with BA alone and some concentrations of NAA, after (3 - 4) weeks of culture.

Results in Table (3) demonstrated that best treatments stimulate shoots formation from nodal segments or callus cultures was observed in the medium containing 2.0 mg/L BA alone, with 50% shoots induction after 3 weeks of culture, (Fig. 1- C ) , and the medium containing 2.0 mg/L BA and NAA from each one with 47% after 4 weeks in, this treatment which gave maximum callus induction. The effect of BA combined with NAA on shoots induction percentage was low as compared to the used BA alone, it seems that present of cytokinin necessary to shoot formation, that corresponds with results of Safdari and Kazemitabar [30], whereas, Bhuiyan and Adachi [32], reported that the equivalent concentrations BA is better than kinetin for shoot regeneration from hypocotyl explants of Portulaca species, Safdari and Kazemitabar [33], reported maximum shoot regeneration percentage (78%) in purslane belonging to 8.88 µM BA, while it was low with 4.44 µM of BA (39%).

Also observed roots formation from nodal segments which cultured on MSO medium ( hormone-free), which as a control, after 3 weeks of culture, with 25% roots induction, (Table 3), (Fig. 1- D). also noted 15% growth of roots from some callus cultures which cultured on MS medium supplemented with 1.0 and 2.0 mg/L NAA alone after 3 weeks of culture. Rooting formation of nodal segments or callus cultures probably due to suitability of MSO salts concentration, that where enough to rooting. rooting shoots in MSO probably due to the endogenous auxin that encourage growing zones in shoots that led to roots formation [34,35].

In general, the hormonal balance between auxins and cytokinins is necessary for the organogenesis by using culture, It was found that the high percentage of auxin cytokinins lead to the formation of roots. while, increase in the level of cytokinin / auxins lead to the formation of shoots.the balance levels of plant growth regulators lead to the continued formation of callus tissue [36],

**Table(3):Shoot regeneration and rooting induction of Portulaca oleracea callus culture.**

| Induction medium (mg /L) | Shoots induction (%) | Rooting induction (%) | Period of initiation (wks) |
|---|---|---|---|
| MSO | - | 25 | 3 |
| MS+ 1.0 BA | 42 | - | 4 |
| MS+ 2.0 BA | 50 | - | 3 |
| MS+ 1.0 NAA | - | 15 | 3 |
| MS+ 2.0 NAA | - | 15 | 3 |
| MS+ 0.5 BA + 0.5 NAA | - | - | - |
| MS+ 1.0 BA + 1.0 NAA | - | - | - |
| MS+ 1.5 BA + 1.5 NAA | 35 | - | 4 |
| MS+ 2.0 BA + 2.0 NAA | 47 | - | 4 |
| MS+ 2.5 BA + 2.5 NAA | 45 | - | 4 |
| MS+ 3.0 BA + 3.0 NAA | 40 | - | 3 |
| MS+ 3.5 BA + 3.5 NAA | - | - | - |

**Effect of Jasmonic acid (JA) in callus production:**

**1- Effect of Jasmonic acid in callus initiation from nodal segments:**

Nodal segments which cultured on stander medium (MSt) supplement with 2.0 mg/L BA and NAA for each one, which consider the best medium encourage callus growth in the present study, addition with different concentrations of (JA).These segments produced callus within 2-3 weeks of culture with percentage of response (83.5-98.8) %.

Data in (Table 4) demonstrated that best treatment stimulate callus initiation was observed in the MSt medium containing 1.0 µg /L JA with 98.8% callus initiation after 2 weeks, compare with stander treatment (MSt) which induced callus initiation with 93.4% ( Fig.1-B). also observed other concentrations of (JA), higher or less than 1.0 µg induced callus initiation but with less percentage. these results refer to the great role of JA in stimulate induction and growth of callus. It was found that Jasmonic acid induced high percentage of callus at 1.5 ppm in Vigna mungo [37].

It is worthy to mention, observed growth of roots from nodal segments which cultured on MSt medium supplemented with 1.5 and 2.0 µg/L (JA) with 30.0 and 32.5 % roots induction, after 3 weeks of culture. (Table 4)and (Fig.1-E). which corresponds with the obtained in literature results of [37], that refer to the role of Jasmonic acid in induced high percentage of callus induction at 1.5 ppm and greater rooting response than Salicylic acid on callus initiation and organogenesis in Vigna mungo L. Also observed shoots formation from nodal segments was cultured on (MSt) medium, with 46.8% Shoots induction after 4 weeks of culture (Table 4), that is refer to the role of JA in stimulate rooting and reduced shoots formation.

**Table (4): Effect of addition Jasmonic acid in stimulation of callus initiation from nodal segments of Portulaca oleracea L.**

| Induction medium JA(µg /L) | Callus initiation (%) | Period of initiation (wks) | Rooting induction (%) | Period of initiation (wks) |
|---|---|---|---|---|
| MSt | 93.4 | 2 | - * 46.8 | 4 |
| MSt+ 0.5 JA | 97.0 | 2 | - | - |
| MSt+ 1.0 JA | 98.8 | 2 | - | - |
| MSt+ 1.5 JA | 97.5 | 2 | 30 | 3 |
| MSt+ 2.0 JA | 90.3 | 3 | 32.5 | 3 |
| MSt+ 2.5 JA | 88.5 | 3 | - | - |
| MSt+ 3.0 JA | 83.5 | 3 | - | - |

* **Growth of shoots.**

**2- Effect of Jasmonic acid in growth and fresh weight of callus:**

Callus cultures which production from previous experiments, transfer to MSt medium supplement with different concentrations of (JA), and fresh weight of callus were recorded after three weeks and six weeks from culture.

Results showed a successful growth of callus cultures it is clear, when addition of JA to culture mediums, specially on MSt medium provided with 1.0 µg/L (JA), which fresh weight reached to (2.8 and 6.1) gm after three and six weeks respectively (Table 5), (Figure 1-B). There are several studies indicate the role of (JA) in growth or fresh and dry weights, also in multiplication rate [38,39].

Again low rate of fresh weight noted when concentrations of (JA) was used higher or less than 1.0 µg/L. But addition of JA in different concentration to this medium induced callus with a high percentage compare with callus cultures on MSt , the results in (Figure 2) indicate that. Similar results from Madagascar Periwinkle plant, found that interaction between JA and Glutamine caused the highest significant difference in fresh and dry weight [40].

Also noted fresh weight decrease when use (2.5, 3.0) µg /L JA , perhaps high concentration of JA caused growth inhibition [18]. The results from present study certified the great role of Jasmonic acid to induce callus growth, hence increase in callus fresh weight.

**Table (5) : Effect of addition Jasmonic acid in Fresh weight of Portulaca oleracea callus culture.**

| Induction medium JA(µg /L) | Fresh weight / gm | |
|---|---|---|
| | 3 wks | 6wks |
| MSt | 1.8 | 3.5 |
| MSt+ 0.5 JA | 2.0 | 5.0 |
| MSt+ 1.0 JA | 2.8 | 6.1 |
| MSt+ 1.5 JA | 2.0 | 4.8 |
| MSt+ 2.0 JA | 1.9 | 2.8 |
| MSt+ 2.5 JA | 1.5 | 2.5 |
| MSt+ 3.0 JA | 1.0 | 1.5 |

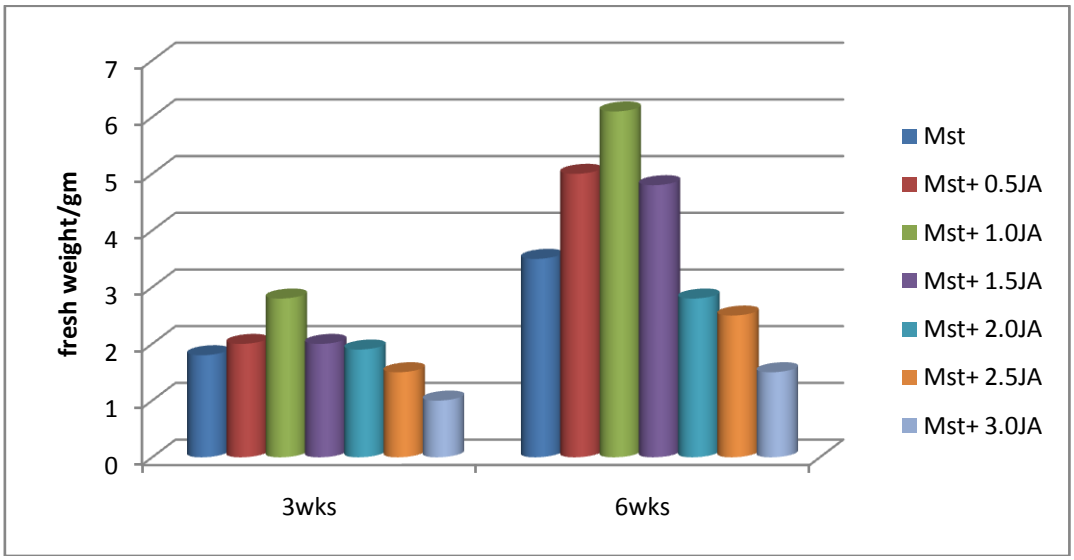



**Figure (2): Effect of Jasmonic acid in fresh weight of Portulaca oleracea L. callus culture.**

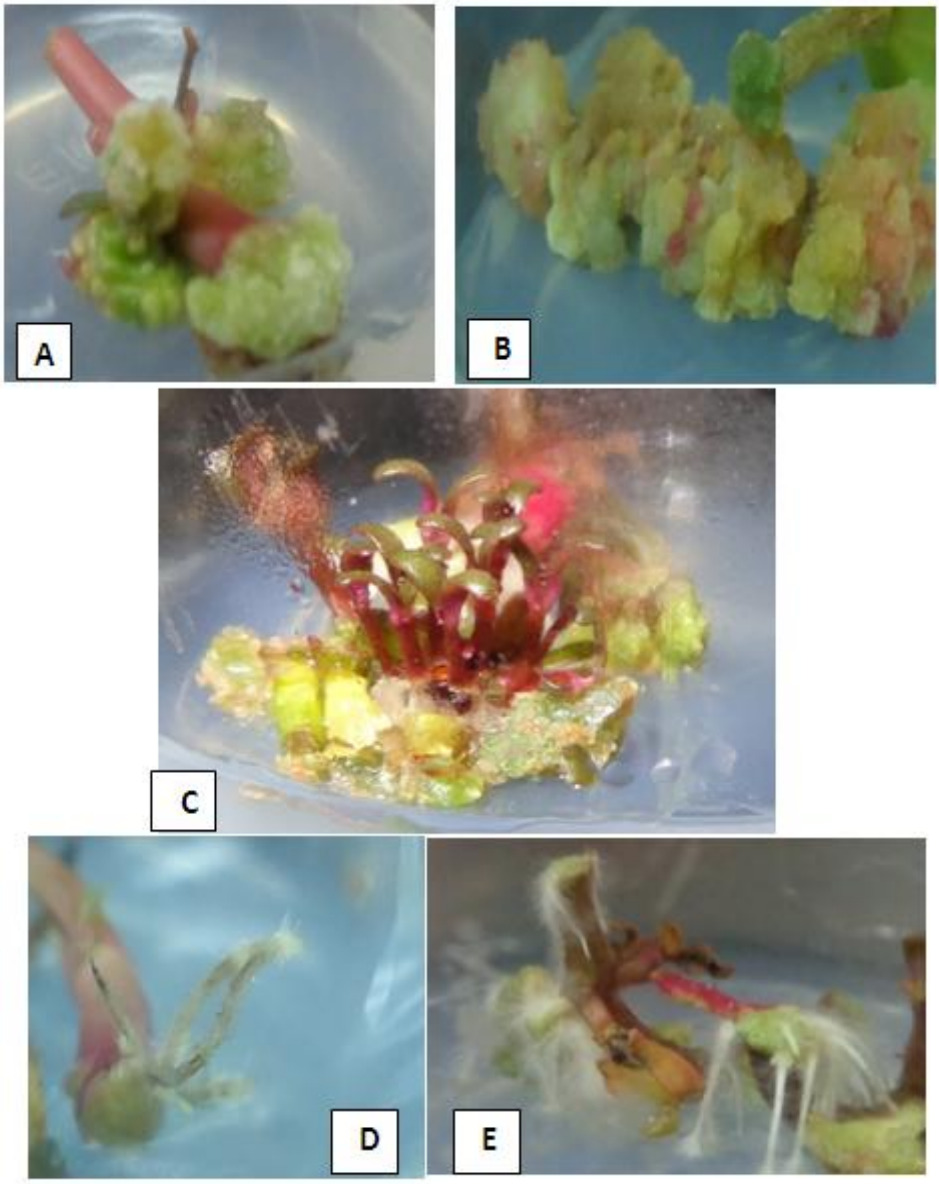



**Fig (1): Different responses of Portulaca oleracea L. nodal explants were cultured on MS medium supplemented with different concentrations of plant growth regulators:**

A: Callus induction in MS+2.0 mg/L BA +2.0 mg/L NAA(MSt) after 2 wks.
B: Induction and growth of callus in MSt + 1.0 µg /L JA after 6 weeks.
C: Induction of shoots in MS+2.0 mg/L BA after 3 wks.
D: Induction of roots in MSO medium ( hormone-free), after 3 weeks.
E: Induction of roots in MSt + 2.0 µg /L JA after 3 weeks.

**Quantitative and qualitative estimate of fatty acids in callus culture by using Gas Chromatography (GC):**

The results of diagnosis and quantitative assessment fatty acids, demonstrated that callus cultures of Portulaca oleracea L. contain several fatty acids ( Oleic, Linoleic, Palmatic, Stearic and Lauric acids, (Table 6).

Diagnosis results of fatty acids callus cultures extractions which growth on MSt medium after 3 weeks, appeared existence absorbent peak of oleic, linoleic, Palmatic acids, with retention time ( 15.52, 14.60, 13.99) minute respectively, also noted increase in concentration of fatty acids after 6 weeks from callus growth, the concentrations was evaluated according the area under peak, (Figure 3).

In (Table 6) generally, illustrated the addition of JA in culture medium caused an increase in fatty acids concentration, compare with stander sample (MSt) or extractions of plant leaves, especially in high concentration of JA in culture medium. Jasmonic acid and its related compounds (all called JA signals) its elicitors could be used as enhancers of plant secondary metabolite synthesis and could play an important role in biosynthetic pathways to enhanced production of commercially important compounds[41,42].

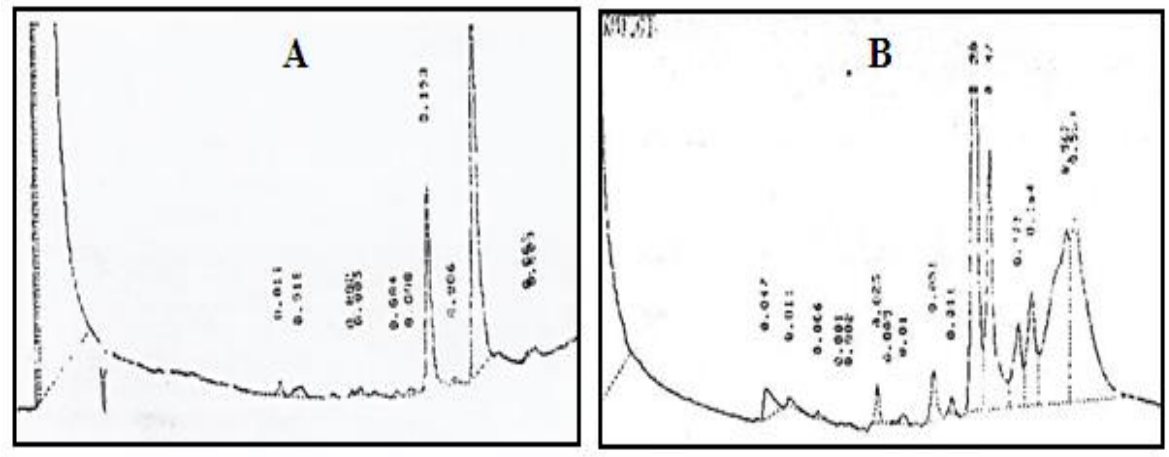
Data in (Table 6) and (Figure 3), also indicated that best treatment stimulate accumulation fatty acids in callus culture, when addition 3.0 µg/L JA in medium , since the concentration of oleic, linoleic and palmitic acid (3.88, 0.99, 1.29) respectively after 3 weeks and ( 5.139, 4.266, 3.53 ) after 6 weeks of culture.

Also noted increase of JA concentration in growth mediums caused increase in fatty acids level in callus cultures, on reverse that, in present study noted low concentration of JA caused increase of fresh weight, However, high concentration of JA caused decrease fresh weight, and growth rate but in the same time, contributed to increasing of level fatty acids.

There are several studies indicate similar role of JA in growth rate and secondary metabolites in different plant, Kee and Paek in 2002, reported ginsenoside content increased significantly by the addition of 10 mg/L JA. However, the root growth was strongly inhibited by increasing JA concentration, also found fresh weight, dry weight, and growth rate of the roots decreased when jasmonic acid concentration increased [38]. Other researchers found Jasmonic acid (JA), and salicylic acid (SA) effected on plant growth and accumulation of hypericins and hyperforin in shoot cultures of Hypericum hirsutum and H. maculatum. These two elicitors, stimulating the accumulation of hypericins[43].

Also Moghadam et al. found that the concentration of methyl Jasmonat is an important factor regarding the increase of producing dopamine secondary metabolite in hairy root cultures of Portulaca oleracea [42].

In the present study, it was found that addition of Jasmonic acid (JA) into the culture medium enhanced the level of callus growth and accumulation of fatty acids in callus cultures of **Portulaca** oleracea L.



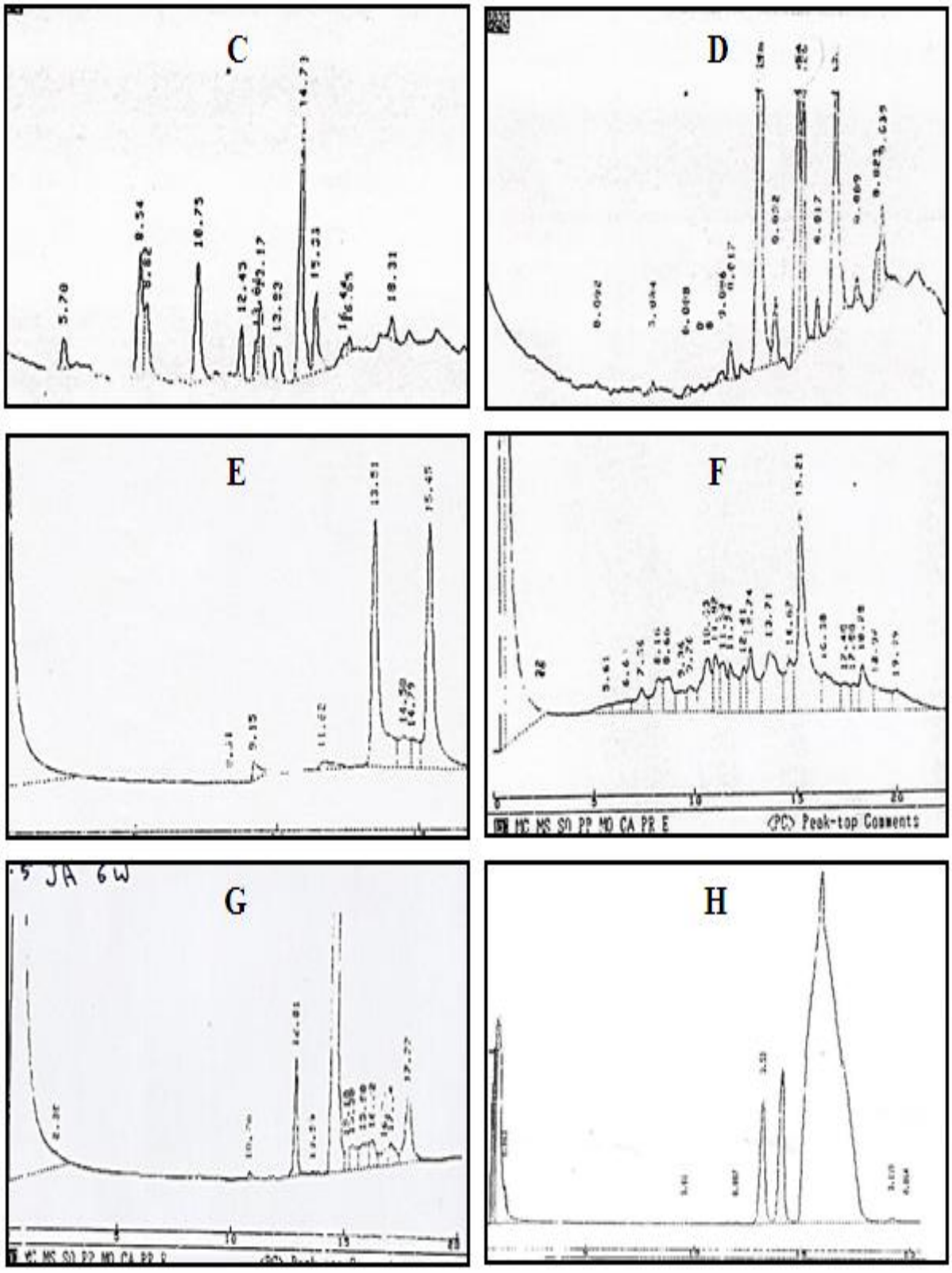



**Figure (3): Retention time(Minute) of fatty acid In Leafs and callus extractions( 6wks ) of Portulaca oleracea L. by Gas Chromatography:**

A: Extract of plant leafs B: Extract of callus growth on MSt

C: Extr. of callus growth on MSt+0.5 JA D: Extr. of callus growth on MSt+1.0 JA

E: Extr. of callus growth on MSt+1.5 JA F: Extr. of callus growth on MSt+2.0 JA

G: Extr. of callus growth on MSt+2.5 JA H: Extr. of callus growth on MSt+3.0 JA

**Table (6): Diagnosis of Fatty Acids in Leafs and callus cultures of Portulaca oleracea L. by Gas Chromatography:**

| Treatments (Callus culture Extractions) | | Stander Fatty acid | Retention time of Stander fatty acid (Minute) | Retention time of fatty acid In callus extractions (Minute) | Fatty acid concentration In sample (calculate from Area under peak ) | |
|---|---|---|---|---|---|---|
| | | | | | 3wks | 6wks |
| MS + (2.0) BA + (2.0) NAA mg/L | MSt | Oleic acid | 15.61 | 15.52 | 0.060 | 0.793 |
| | | Linoleic acid | 14.72 | 14.60 | 0.016 | 0.019 |
| | | Palmitic | 13.63 | 13.99 | 0.037 | 0.059 |
| | + 0.5 JA | Oleic acid | 15.61 | 15.35 | 0.046 | 0.547 |
| | | Linoleic acid | 14.72 | 14.70 | 0.131 | 0.244 |
| | | Palmitic | 13.63 | 13.20 | 0.040 | 0.073 |
| | + 1.0 JA | Oleic acid | 15.61 | 15.30 | 0.013 | 0.125 |
| | | Linoleic acid | 14.72 | 14.69 | 0.027 | 0.158 |
| | | Palmitic | 13.63 | 13.86 | 0.024 | 0.031 |
| | + 1.5 JA | Oleic acid | 15.61 | 15.39 | 1.399 | 3.672 |
| | | Linoleic acid | 14.72 | 14.67 | 0.036 | 0.4131 |
| | | Stearic acid | 14.55 | 14.50 | -- | 0.002 |
| | | Palmitic | 13.63 | 13.70 | 0.601 | 3.9 |
| | + 2.0 JA | Oleic acid | 15.61 | 15.55 | 0.495 | 2.77 |
| | | Linoleic acid | 14.72 | 14.85 | 0.061 | 0.62 |
| | | Palmitic | 13.63 | 14.01 | 0.192 | 1.31 |
| | | Lauric | 9.47 | 9.36 | 0.017 | 0.30 |
| | + 2.5 JA | Oleic acid | 15.61 | 15.69 | 0.255 | 0.38 |
| | | Linoleic acid | 14.72 | 14.81 | 0.677 | 2.97 |
| | | Palmitic | 13.63 | 13.8 | 0.731 | 0.005 |
| | | Lauric | 9.47 | 9.49 | 0.092 | -- |
| | + 3.0 JA | Oleic acid | 15.61 | 16.0 | **3.88** | **5.139** |
| | | Linoleic acid | 14.72 | 14.67 | **0.99** | **4.266** |
| | | Palmitic | 13.63 | 13.18 | 1.29 | 3.53 |
| | | Lauric | 9.47 | 9.057 | -- | 0.01 |
| Leafs of plant | | Oleic acid | 15.61 | 15.596 | 0.495 | |
| | | Linoleic acid | 14.72 | 14.831 | 0.006 | |
| | | Stearic acid | 14.55 | 13.98 | 0.192 | |
| | | Palmitic | 13.63 | 13.35 | 0.007 | |
| | | Lauric | 9.47 | 9.588 | 0.017 | |

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