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# EFFECT OF AGEING ON PERMEABILITY PERTURBATION IN MUNG BEAM (*PHASEALUS AUREUS* ROXB.) CUTTINGS AND ITS CONTROL BY SUPPLYING PARSLEY EXTRACT I: MEMBRANE STRUCTURE AND EFFLUX

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## ABSTRACT

Effect of ageing on permeability perturbation in terms of rooting response of mung beam cuttings has been studied. The shortage of protein and phospholipids levels was considered as indicators of permeability perturbation during ageing phenomenon. Meanwhile percentage of electrolytes leakage in terms of electrical conductivity and average of ions leakage (efflux) such as $Ca^{2+}$ and $Mg^{2+}$ in different parts of the cuttings have been measured.

In addition, aqueous extract of parsley (*Petroselinum crispum* Mill.) seeds was used to control meanwhile, the processes that occurs during ageing. The results revealed that ageing caused diminishing of rooting response in aged cuttings (held in $d/H_2O$ during ageing period) compared to fresh cuttings. However, a decline in proteins and phospholipids was coincided with permeability perturbation that represented by increment of ions efflux as mentioned above.

On the other hand, results of ageing control by holding cuttings in aqueous extract of parsley seeds (1%) instead of $d/H_2O$, revealed a complete stopping (overcome) of the processes that occur during ageing. These processes lead to diminish rooting response of subsequent auxin supply. The control of ageing in terms of membrane repair implies maintaining of protein and phospholipids levels in aged cuttings. Thereafter, prevent ions leakage that occurs due to permeability perturbation.

**Key words**: Ageing, Permeability perturbation, Rooting response, Protein, Phospholipids, Electrolytes, Ions leakage, Membrane repair.

## INTRODUCTION

Cuttings were taken from juvenile plants are easy to root compared to cuttings of old plants[1]. Jarvis and Booth[2] confirm the above results in aged cuttings of mung bean

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taken from 10-day-old seedlings. The latter authors attribute these results to a limited supply of IAA from primary leaves of aged cuttings or alternatively from cotyledons in case of excision of primary leave and terminal buds of the same species. It was found that ageing leads to lowering the capability of cuttings for adventitious root formation (ARF)[3]. Ageing was characterized by a progressive or sudden decline in metabolic activities[4] and coincides with changes of component and properties of plasma membrane[5]. Bentalmann and Kende[6] found shortage in phospholipids level of aged tissue in *Ipomoea tricolor*. However, considerable changes were observed in content and properties of phospholipids in *Cassava* roots[7] and protein biosynthesis of potatoes slices[8] during ageing.

The degenerative processes associated with ageing raises the attention of many researchers to offset or stopping these processes by using synthetic or natural substances. For example, Abraham and Reinhold[9] indicate the damage in plasma membrane during ageing of leaf strips of *centranthus ruber*. This damage, that causes increase in uptake (influx), was stopped completely by treatment of these tissues by cerulenin and $CaSO_4$. Thereafter, leads to membrane repair that imply protein and phospholipids biosynthesis.

In terms of Adventitious Root Formation (ARF), Shaheed[10] found that cinnamic acid stop ageing completely in mung beam cuttings, only at high concentration ($10^{-3}$ M). However, some medicinal herb extracts contain secondary metabolites, like phenols, alkaloids, or terpenoids[11] were used to control the processes that occur during ageing of cuttings that leads to diminish rooting response. A successful result were obtained by Shaheed and Al-Alwani[12] about stopping of ageing partially by keeping cuttings during ageing period in aqueous extracts of garden lettuce (*Lactaca sativa L.)* seeds, fenugreek (*Trigonella foenum-graecum L)* shoots, and chamomile *(Authemis nobilis)* flowers at concentrations 10%, 0.1% and 1%, respectively. In addition, the latter authors stop ageing completely by using Anise *(pimpinella anisum* L*.)* seeds, milfoil *(Achillea sentonlina L.)* flowers, Negella *(Nigella sativa L.)* seeds and common Nettle *(Urtica diocia L.)* shoots at concentrations 1%, 1%, 1% and 0.1%, respectively.

It is noteworthy that crude extracts were used in this study without diagnostic test for the specific active compounds of its mechanism of action that involved in ageing phenomenon. The aim of this study is to verify the permeability perturbation hypothesis that caused ageing in terms of ARF of mung bean cuttings via chemical composition of membranes and ions leakage.

# EXPERIMENTAL

## Materials and methods

### Growth of stock plants and preparation of cuttings

Seeds of mung bean (*Phaseolus aureus* Roxb. Var. Local) were germinated in fine granular and sterilized sawdust moistened with tap water. Seedlings were grown in growth chamber at 25 ± 1°C under continuous illumination supplied by warm white fluorescent tubes (3000 - 35000 Lux) and relative humidity of 60-70 %.

Stem cutting were prepared according to Hess[13] from 10 days-old light grown seedlings. The cuttings had apical bud, a pair of expanded primary leaves, epicotyl and 3 cm of hypocotyls under the cotyledonary nodes.

### Preparation of solutions and basal treatment of cuttings

Indolebutyric acid (IBA) was dissolved initially in absolute ethanol to which distilled water was added to prepare the required stock solution ($10^{-3}$M).

Plant extract of parsley (*Petroselinum crispum* Mill) seeds was prepared as aqueous stock solution in concentration of 10% (w/w), by grinding, extraction, filtration through cheese cloth and buchnar funnel with Whatmann No. 1 under vacuum. The other concentrations (1, 0.1, 0.01) % were prepared by dilution with distilled water.

### Extraction and estimation of protein content

Protein was extracted and estimated in mg/g plant tissue of primary leaves, epicotyls and hypocotyls of fresh and aged cuttings according to biuret method[14].

### Extraction and estimation of total phospholipids

Phospholipids was extracted from primary leaves, epicotyls and hypocotyls of fresh and aged cuttings in distilled water as well as in parsley extract (1%) according to Bentelmann and Kende[6]. In addition, phospholipids were estimated by phosphorous determination according to in mg/g plant tissue.

### Efflux analysis

Leakage of ions was estimated as a percentage in different parts of fresh and cuttings aged in distilled water as well as in parsley extract (1%) according to Levitt[15].

### Preliminary test of secondary metabolites

This test has been done according to Harborne[11].

### Basal treatment of cuttings

Fifteen milliliters of test solutions were used for dipping the whole hypocotyls (3 cm depth) in glass vials (7.5 x 2.2) cm. Cuttings C4 (vial) were treated for 24 h in distilled water, auxin (IBA, $10^{-4}$ M) or optimum concentration of test solution (parsley extracted 1%). Then transferred to boric acid (10 µg/mL) for 6 days before estimation of the number of roots per cutting (twelve cuttings were used per treatment for rooting tests).

### Ageing treatment

Cuttings were held immediately after taken from seedlings in distilled water or in appropriate tested solutions of plant extract for 3 days, prior to their treatment with IBA, $10^{-4}$ M (Inductive auxin treatment). During all these treatments, cuttings were held under the same conditions as mentioned above for raising stock seedlings. Completely randomized design (CRD) with 12 replicates was conducted in all experiments for statistical analysis according to Spiegel[16].

## RESULTS AND DISCUSSION

The influence of water extract of parsley seeds on rooting response of mung bean cuttings were raised through Table 1. Data revealed that low concentrations (0.01% and 0.1%) of extract have no significant effect on roots number compared to control. Whereas, cuttings treated with 1% of extract developed approximately 20 roots per cutting. This increment of rooting response over control was counted as 73%. However, the highest concentration (10%) has complete inhibitory effect on rooting response that causes cuttings to wilt and desiccate during treatment with the extract.

On the bases of the above results, extract at 1% concentration was considered as optimum and used for the subsequent experiments that aimed to overcome or stop the metabolic processes that occurs during ageing, which leads to diminish rooting response.

**Table 1: Effect of parsley extract on rooting response of fresh mung bean cuttings**

| Mean roots number/cutting in different concentrations of parsley seed extract | | | | |
|---|---|---|---|---|
| **0.0%** | **0.01%** | **0.1%** | **1** | **1%** |
| 11.5 | 12 | 12.16 | 19.91 * | 0.0 |

**Fresh cutting were treated for 24 h with the above concentrations, and then transferred to boric acid (10 µg/mL) for 6 days.**

**LSP (0.05) = 5.40.**

The controlling of ageing phenomenon by using parsley seed extract in terms of rooting response of mung bean cuttings has been presented in Table 2. Data revealed that fresh, untreated cuttings (general control) and transferred to boric acid for 6 days developed 12.41 roots per cutting. Presumably, this response was attributed to endogenous auxin (IAA). Meantime, boric acid is essential for formation of root primordial, as well as their subsequent growth[17]. However, cuttings treated with ethanol, (2%) developed approximately the same number of roots. The foregoing result confirmed that ethanol used in 2% to dissolve IBA dose not significantly influence rooting response of mung bean cuttings[17]. Whereas, cuttings that were given inductive auxin treatment (IBA, $10^{-4}$ M) for 24 h induces 57.9 roots per cutting. This increment over control was attributed to auxin supplied exogenously and counted approximately as 463%.

Treatment of cuttings (fresh) with parsley extract at optimum concentration (1%) developed 22.75 roots per cutting. This result raises three points. Firstly, parsley extract stimulated rooting response by approximately doubled the roots number compared to control (12.41). Secondly, possibly these extracts may be used as alternative of plant growth regulators particularly, when compared to low concentrations of auxin (e.g. IBA, $10^{-4}$ M, its effect is approximately equal 39.2%). Thirdly, ageing of cuttings in parsley extract may overcome the processe that occur during ageing.

Aged cuttings, that were kept in distilled water or ethanol for 3 days and then transferred to borate without auxin treatment develops approximately the same number 6.33 and 6.66 respectively, with a reduction of ≈50% compared to fresh untreated cuttings. Whereas, aged cuttings in distilled water that were given auxin treatment after 3 days (delaying of auxin treatment for 3 days) develops 22.5 roots/cutting. This means that rooting response was declined in about 61% as compared to fresh cutting treated with

auxin (57.91). The reason is unknown, but could be attributed to these metabolic processes that occurred during ageing, which leads to diminish rooting response in aged cuttings.

As an attempt to control (delay, overcome, offset, or stop), these processes that occur during ageing, cuttings were treated for 3 days with parsley extract (1%) instead of distilled water before giving auxin treatment. Data presented in Table 2 show that water soluble substances in parsley extract were able to delay or stop completely the processes that occur during ageing.

The morphological products in terms of mean roots number/cutting for this extract is 57.58 with no significant difference among them compared to fresh cuttings treated with auxin (57.91). In other words, aged cuttings that were kept in parsley extract respond to auxin treatment after 3 days as was the case in fresh cuttings.

**Table 2: Effect parsley extract on ageing of mung bean cuttings**

| Ageing treatment for 3 days in: | Subsequent treatment for 24 h | Mean roots number per cutting |
|---|---|---|
| None (fresh cuttings) | Distilled water | 12.41 |
| None (fresh cuttings) | Ethanol 2% | 12.58 |
| None (fresh cuttings) | IBA, $10^{-4}$ M | 57.91** |
| None (fresh cuttings) | Parsley 1% | 22.75** |
| Distilled water | Distilled water | 6.33 |
| Ethanol 2% | Ethanol 2% | 6.66 |
| Distilled water | IBA, $10^{-4}$ M | *22.5 |
| Parsley 1% | Distilled water | 27.91 ** |
| Parsley 1% | IBA, $10^{-4}$ M | 57.58** |

* = significant decrease. ** = significant increase.
LSD (0.05) = 8.03

**Cuttings aged in distilled water, ethanol or parsley extract for 3 days, then treated with auxin for 24h and then transferred to boric acid for further 6 days. In addition, fresh cuttings treated with distilled water, ethanol or parsley extract and transferred directly to boric acid for 6 days.**

Table 3 shows a significant decline in protein content of cuttings aged in distilled water for 3 days. This decline approaches its maximum in hypocotyl of cuttings aged in distilled water (1.85 mg/g plant tissue) compared to (3.15 mg/g) of fresh cuttings.

The decline of protein content in cuttings aged in distilled water represented in about 41%. Meanwhile, this decline was reduced to 21% in hypocotyl of cuttings aged in aqueous extract of parsley extract seeds (2.48 mg/g).

As a conclusion, parsley extract retards or prevents the processes that occurs during ageing and leads to decline (hydrolysis) in protein content partially.

**Table 3: Effect of ageing on protein content (mg/g plant tissue) in different parts of mung bean cuttings and its control by using parsley extract**

| Cuttings parts | Fresh cuttings | Aged cuttings in: | |
|---|---|---|---|
| | | Distilled water | Parsley extract |
| Primary leaves | 3.4 | 3.15 ↓ (7%) | 2.80 |
| Epicotyl | 2.47 | 1.95 ↓ ** (21%) | 1.65 |
| Hypocotyl | 3.15 | ** 1.85 ↓ (41%) | 2.48 ** (21%) |

* = significant decrease. ** = significant increase.
LSD (0.05) = 0.51

**Cuttings aged in distilled water or parsley extract for 3 days prior to extraction and estimation of protein content in different parts of the cuttings. Figure between brackets represent the percentage of decline ↓ in protein content.**

The influence of ageing on phospholipids content is shown in Table 4. Data revealed that ageing caused a significant decline in phospholipids content (50%) in the whole cutting and particularly in primary leaves (3.874 mg/g), of cuttings held in distilled water compared to fresh cuttings (7.749 mg/g).

On the other hand, cuttings were kept for 3 days in parsley extract to control ageing, developed a highly significant increase in phospholipids content of epicotyl (4.003 mg/g), which represent 4.4 folds compared to epicotyl of cuttings aged in distilled water (0.903 mg/g). Meanwhile, this increment is not statistically significant compared to fresh cuttings. This means that parsley extract maintaining the same level of phospholipids in epicotyl of aged cuttings as was the case in fresh cuttings.

**Table 4: Effect of ageing on total phospholipids content (mg/g fresh weight plant tissue) in different parts of mung bean cuttings and its control by using parsley extract**

| Cuttings parts | Fresh cuttings | Aged cuttings in: | |
|---|---|---|---|
| | | Distilled water | Parsley extract |
| Primary leaves | 7.749 | ** 3.874 (50%) ↓ | 3.223 |
| Epicotyl | 2.066 | 0.903 (56%) ↓ | 4.0032* |
| Hypocotyl | 5.165 | 2.582 (50%) ↓ | 1.896 |

* = significant decrease. ** = significant increase.
LSD (0.05) = 0.51 LSD (0.1) = 3.07

**Cuttings aged in distilled water or parsley extract for 3 days prior to extraction and estimation of total phospholipids content in different parts of the cuttings. Figure between brackets represent the absolute percentage of decline ↓ in phospholipids content.**

The effect of ageing on percentage of electrolytes leakage in mung bean cuttings has been shown in Table 5. Obviously, there is significant increase on the whole cutting level, and particularly in epicotyl and hypocotyl of cuttings aged in distilled water for 3 days (57.65 and 72.22) compared to fresh cuttings (45 and 62.76), respectively. This increase in electrolytes leakage due to ageing, confirm structurally the perturbation of permeability, which is considered as a fundamental character of cellular membranes.

**Table 5: Effect of ageing on percentage of electrolytes leakage (Efflux) in mung bean cuttings**

| Cuttings parts | Fresh cuttings | Aged cuttings |
|---|---|---|
| Primary leaves | 43.33 | 43.66 |
| Epicotyl | 45.00 | 57.65 ** |
| Hypocotyl | 62.76 | 72.22 ** |

**Stem cuttings aged in distilled water for 3 days prior to their excision into three parts. Percentage of electrolytes leakage was estimated in (1) g of plant tissue.**

The influence of ageing on percentage of $K^+$ ions leakage (efflux) in mung bean cuttings and its control by using parsley aqueous extract is represented in Table 6. The percentage of $K^+$ ions leakage was increased in all parts of cuttings aged in distilled water for 3 days. However, statistically this increase is not significant, but represent in terms of absolute percentage 15%, 21% and 24% in primary leaves, epicotyls and hypocotyls respectively, compared to fresh cuttings.

The results of controlling the processes that occurs during ageing (membrane damage and increase of ion leakage) by using parsley extract (1%) instead of distilled water shows decline in percentage of $K^+$ ions leakage in all parts of cuttings. Statistically, this increase is not significant except the leakage of hypocotyls (58.69), which is significant compared to cuttings aged in distilled water (81.7).

On the other hand, the average percentage of $K^+$ ions leakage in whole cuttings (Fig. 1) decline into (40.90) by holding cuttings in parsley extract during ageing period. The decline was significant compared to cuttings aged in distilled water (54.66), but not significant compared to fresh cuttings (45.14). The foregoing results confirm that parsley extract stop ageing completely in this respect and making aged cuttings respond as was the case in fresh cuttings.

**Table 6: Effect of ageing on percentage of $K^+$ ions leakage (efflux) in mung bean cuttings and its control by using parsley aqueous extract**

| Cuttings parts | Fresh cuttings | Aged cuttings in: | |
|---|---|---|---|
| | | Distilled water | Parsley extract |
| Primary leaves | 26.25 | 3.23 (15%) ↑ | 14.79 (51%) ↓ |
| Epicotyl | 43.18 | 52.05 (21%) ↑ | 49.23 (5.41%) ↓ |
| Hypocotyl | 66.00 | 81.70 (24%) ↑ | 58.69 ** (28%) ↓ |

**Cuttings aged in distilled water or parsley extract for 3 days. Percentage of $K^+$ ions leakage was estimated in (1) g of plant tissues. Figures between brackets represent the absolute percentage for increase ↑ or decrease ↓ of ions leakage.**

**LSD (0.10) = 18.31. LSD (0.05) = 22.19.**

Data presented in Table 7 revealed an increase in percentage of $Mg^{2+}$ ions leakage

in all parts of cuttings aged in distilled water. Although, these increases are not significant but it was equal to 21%, 15% and 4% in primary leaves, epicotyl and hypocotyls, respectively, if estimated as absolute percentages.

Usage parsley extract (1%) to control ageing instead of distilled water for 3 days shows decline in percentage of $Mg^{2+}$ ions leakage in primary leaves, epicotyl and hypocotyls (36.07, 58.63 and 78.02), respectively. This decline is statistically significant in all parts of cuttings aged in parsley extract except hypocotyl. It is noteworthy; the percentage of $Mg^{2+}$ ions leakage of hypocotyls does not differ significantly compared to fresh cuttings. In other words, parsley extract causes cuttings aged in this extract to respond as was the case in fresh cuttings.

On the other hand, parsley extract reduced the increase of percentage of $Mg^{2+}$ ions leakage in a whole cuttings to (57.57) as given in Fig. 1. This reduction was significant compared to cuttings aged in distilled water (71.19) and at the same time was not significant compared to fresh cuttings (63.53). In other words, the extract again completely prevent the processes associated with (permeability perturbation) that leads to increase $Mg^{2+}$ ions leakage and maintaining its percentage in all parts of aged cuttings as was the case in fresh cuttings (membrane repairs).

**Table 7: Effect of ageing on percentage of $Mg^{2+}$ ions leakage (efflux) in mung bean cuttings and its control by using parsley aqueous extract**

| Cuttings parts | Fresh cuttings | Aged cuttings in: | |
|---|---|---|---|
| | | Distilled water | Parsley extract |
| Primary leaves | 42.85 | 51.82 (21%) ↑ | ** 36.07 |
| Epicotyl | 69.74 | 80.41 (15%) ↑ | ** 58.63 |
| Hypocotyl | 78.01 | 81.35 (4%) ↑ | 78.02 |

**Cuttings aged in distilled water or parsley extract for 3 days. Percentage of $Mg^{2+}$ ions leakage was estimated in (1 g) of plant tissues. Figures between brackets represent the absolute percentage for increase ↑ or decrease ↓ of ions leakage.**

*** LSD (0.05) = 15.1.** **** LSD (0.10) = 12.45.**

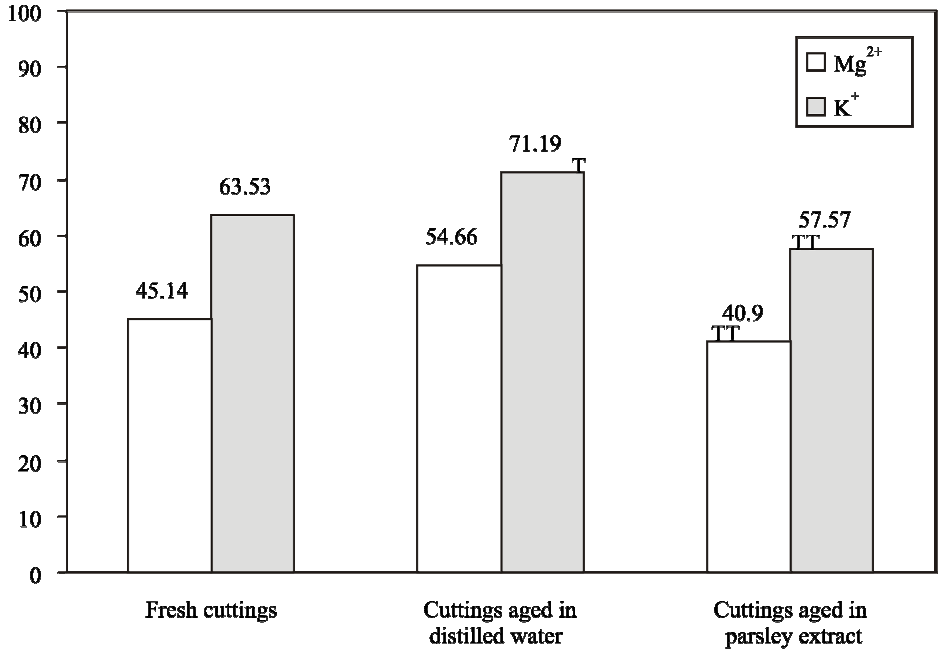


**Fig. 1: Effect of ageing on average percentage of $K^+$ and $Mg^{2+}$ ions leakage in a whole mung bean cuttings and its control by using parsley extract.**

TT LSD (0.05) = (K = 12.81), (Mg = 8.72).

T LSD (0.10) = (K = 10.57), (Mg = 7.19)

T = Significant increase. TT = Significant decrease.

**Effect of ageing on chemical structure of membrane**

It is well known that the chemical compositions of cytoplasmic membranes are proteins and phospholipids. The configuration (architecture) of membranes depend on these materials and its organization in special modal as described by Singer and Nicolson[18], to give the fundamental property of membrane that called "permeability". Changes in the average of fluxes (influx and efflux) considered as indicators for permeability perturbation. The latter caused by two ways; firstly, shortage in protein and/or phospholipd levels. Secondly, alteration or modification of the membrane configuration. Possibly, both ways could happen due to the shortage of proteins and phospholipids.

Ageing caused decline in protein content of cuttings aged in distilled water compared to fresh cuttings (Table 3). There is an agreement with about the relationship between ageing and the decline in protein content in aged Barley and bean seeds[19,20]. This decline of protein level may be attributed to increase of protease activity during ageing[21] or DNase and RNase activity that destroy DNA and is the necessary for protein biosynthesis[22]. In addition, RNA biosynthesis and polyribosomes formation declines during ageing of bean seeds[23]. However, phospholipids are considered important barrier for space (free) diffusion, because it is mostly, if not entirely located in the cytoplasmic membranes[18]. Data presented in Table 4 revealed decline in total phospholipids content in aged cuttings (> 50%) compared to fresh cuttings. These results are in agreement with previous studies by Beutelmann and Kendy[6], Lalaguna and Aguda[7] and Michalczyk *et al.*[20]. The above studies mentioned that the decline in phospholipids level may be attributed to its destruction by increasing the activity of phospholipase or to decline in its biosynthesis or both. Alternatively, the reason may be associated with the activity of free radicals[24] that increases during ageing. The free radical causes lipid peroxidation that implies damage of cytoplasmic membrane. Subsequently, the damage involved decrease in phospholipids content, creating the permeability perturbation.

**The influence of parsley extract in controlling ageing**

Data of Table 2 revealed that parsley extract at the optimum concentration 1% (Table 1) stopped completely the processes that occur during ageing. The mean roots number/cutting that aged in parsley extract was larger compared to cuttings aged in distilled water whether such cuttings supplied, after 3 days, with inductive IBA, $10^{-4}$ M (57.58 roots in case of extract and 22.5 roots in case of distilled water) or not (27.9 and 6.33 roots in extract and distilled water, respectively). Keeping cuttings in parsley extract (1%) for 3 days enhances retention of cuttings sensitivity to subsequent auxin treatment (IBA, $10^{-4}$M). Surprisingly, in terms of adventitious root formation, these cuttings respond (57.58 roots/cuttings) as was the case in fresh cuttings (57.9 roots/cuttings) by reducing the statistical differences between them.

On the other hand, the above extract does not induce more adventitious roots or aged cuttings unless IBA was supplied (27.91 roots in extract compared to 22.75 roots in distilled water). This confirm the solitary role of auxin in stimulation of cell divisions and adventitious root formation without sharing any other substance in initiation phase directly (Eriksen and Mohammed[25], and Blackesly *et al*[26]). Obviously, the dominant role of extract resides in controlling the processes that occurs during ageing phenomenon more than ARF.

The foregoing results confirmed by Tables 3, 4, 5 and 7 and Fig. 1, involving increase in average of ions leakage that caused by ageing and its control by lowering such leakage by parsley extract, Tables 6 and 7 and Fig. 1.The above mentioned increase of ions leakage was correlated with: (a) influence of ageing on decreasing of membrane constitution from proteins (Table 3) and phospholipids (Table 4), as well as (b) physiologically, a decline in rooting response of aged cuttings (Table 2). The foregoing results coincided with effect of extract in maintaining the membrane components such as protein (Table 3) and preventing the decline in phospholipids (Table 4). In other words, parsley extract retard the perturbation that occurred in membrane permeability (Tables 6, 7 and Fig. 1), through maintaining membrane constitution of protein (Table 3) and phospholipids (Table 4). This effect may be attributed to the presence of phenolic compounds in parsley extract (preliminary tests). Harhorre has mentioned that phenolics have the capability of forming complexes with proteins by hydrogen bonds, as mechanism to repair membranes. Shaheed[10] confirmed this idea by stopping ageing completely in terms of ARF in mung bean cuttings by using high concentration ($10^{-3}$ M) of cinnamic acid. However, phenolics (e.g. caffeic acid) has a role in raising IAA level that required for adventitious roots initiation by acting as auxin-protectors via its inhibitory effect for IAA-oxidase[27].

In addition, the influence of parsley extract in promotion of rooting response in aged cuttings may be attributed to the following reasons: (a) Implication of $Ca^{2+}$ in the extract[28] as co-factor in ARF[29] (b) The role of $Ca^{2+}$ in maintaining the physical integrity of membranes or its repairs [30] and (c) The presence of substances acting as anti-oxidant agents through oxidative hypothesis, which is one of the hypotheses that explain ageing causes[24]. These substances (e.g. sucrose or phenolic compounds such as o-coumaric acid, caffeic acid and p-hydroxyquinone) has been found to stop completely the processes that occurs during ageing of mung bean cuttings (its involvement in anti-oxidant defense mechanism) through maintaining IAA levels[31].

Naturally occurring IAA was declined during ageing of mung bean cuttings[12]. Notwithstanding, auxin supplied exogenously doesn't represent the whole prerequisites for processes that occur during ageing. For example, permeability perturbation is one of the above processes.

## REFERENCES


1. F. T. Davies, J. E. Lazarte Jr. and J. N. Joiner, Initiation and Development of Roots in Juvenile and Mature Leaf Bud Cutting of Ficus Pumila L. Aven., J. Bot., **69(5)**, 804-811 (1982).
2. B. C. Jarvis and A. Booth, Influence of Indolebutyric Acid Boron, Myoinsitol, Vitamin and Seedling Age on Adventitious Root Development in Cutting of Phaseolus Aures, Physiol. Plant, **53**, 213-218 (1981).
3. G. Diaz-Sala, K. W. Hutchison, B. Goldfarb and M. S. Greenwood, Maturation Related Loss in Rooting Competence by Loblolly Pine Stem Cutting, the Role of Auxin Transport, Metabolism and Tissue Sensitivity, Physiol. Plant., **97**, 481-490 (1996).
4. S. C. Makrides and J. Goldthwaite, Biochemical Changes During Bean Leaf Growth, Maturity and Senescence. J. Exp. Bot., **32(129)**, 725-735 (1981).
5. Lurie and S. Ben-Yehoshua, Changes in Membrane Properties and Abscissic Acid during Senescence of Harvested Hell Pepper Fruit, J. Amer. Soc. Hort. Sci., **111(6)**, 886-889 (1986).
6. P. Beutelmann and H. Kende, Membrane Lipids in Senescing Flower Tissues of I Pomoeatricolor, Plant Physiol., **59**, 888-893 (1977).
7. F. Lalaguna and M. Agudo, Relationship between Changes in Lipid with Ageing of Cassara Root and Senescence Parameters, Phytochemistry, **28**, 2059-2062, (1989).
8. R. E. Click and D. P. Hakett, The Role of Protein and Nucleic Acid Synthesis in the Development of Respiration in Potato Slices, Proc. Natl. Acad. Sci., **50**, 243-250 (1963). (Cited by Rains (1969).
9. G. Abraham and Reinhold, Mechanism of Effect of Ageing on Membrane Transport in Leaf Strips of Centranthus Ruber. Possible Ethylene Involvement in Cutting Shock, Planta, **150**, 380-384 (1980).
10. A. I. Shaheed, Effect of Secondary Metabolities on the Ageing of Mung Bean Stems Cuttings, Iraq. J. Sci., **38(3)**, 499-509 (1997).
11. J. B. Harborne, Phytochemical Methods, 2nd Edition, Chapman and Hall (1984).
12. A. I. Shaheed and B. A. Al-Alwani, Ageing, Causes and Control in Relation to Adventitious Root Formation in Mung Bean (Phaseolus Aures Roxb. ) Cuttings. I. Decline of Naturally Occurring Auxin (IAA), Iraq. J. Biol., **1(1)**, 161-174 (2001).
13. C. E. Hess, The Mung Bean Bioassay for Detection of Root Promoting Substances. Physiol. Plant., 63, Suppl. 21 (1961).

14. K. Wilson and K. H. Gouldins, A Biologist's Guide to Principles and Techniques of Practical Biochemistry, 3rd Ed., Edward Arnold (1986) p. 38.

15. J. Levitt, Response of Plant to Environmental Stresses. Vol. 11. Water, Radiation, Salt and Other Stresses, Academic Press (1980).

16. M. R. Spiegel, Theory and Problems of Probability Statistic Schaums Outline Series in Mathematic, McGraw Hill Book Company, New York (1975).

17. W. Middelton, B. C. Jarvis and A. Booth, The Effect of Ethanol on Rooting and Carbohydrate Metabolism in Stem Cutting of Phaseolus Aureus, Roxb. New Phytol., **81**, 279-285 (1978).

18. S. J. Singer and C. L. Nicholson, The Fluid Mosaic Model of Structure of Cell Membrane, Science, N. Y., **175**, 720-731 (1972).

19. M. B. Mudgett, J. D. Lewonson and S. Clarke, Protein Repair L. I Soaspartyl Methyltransferase in Plant Phylogenetic Distribution and the Accumulation of Substrate Protein in Aged Barley Seeds, Physiol. Plant., **115**, 1481-89 (1997).

20. D. J. Michalczyk, R. J. Gorecki, K. Kuskka and A. Reiowaki, Change in Phospholipids Fractions of Field Bean (Vicia Faba Var. Minor) Seed Stored At Different Temperature, Plant Breeding and Seed Science, **42(1)**, 37-46 (1998).

21. S. Jana and M. A. Choudhuri, Senescence in Submerged Aquatic Angiospernas Changes in Intact and Isolated Leaves During Ageing, New Phytol., **86**, 191-198 (1980).

22. J. E. Dale, Studies in Biology, The Growth of Leaves. Edward Arnold Ltd., London (1982).

23. K. Zalewski, The Metabolism of Aged Seeds. The Formation of Polyribosomes in Germination Field Bean (Vicia Faba Var Minor) Seeds of Different Ages, Acta. Societatis Botanicorum Poloniae., **61**, 203-210 (1992).

24. R. J. Gorecki, H. Ashino, S. Saton and Y. Esash, Ethylene Production in Pea and Cocklebur Seeds of Different Vigour, J. Exp. Bot., **42(236)**, 407-414 (1991).

25. E. N. Eriksen and S. Mohammed, Root Formation in Pea Cuttings, The Influence of Indole-3-Acetic Acid of Different Developmental Stages, Physiol. Plant., **30**, 158-162 (1974).

26. D. Blakesly, G. D. Weston and J. F. Hall, The Role of Endogenous Auxin in Root Initiation Evidence from Studies on Auxin Application and Analysis of Endogenous Level, Plant Growth Regulation, **10**, 341-353 (1991).

27. B. C. Jarvis, Endogenous Control of Adventitious Rooting in Non Woody Cuttings. in M. B. Jackson (Ed.), New Root Formation in Plant and Cuttings, Martinus Nijhoff Pub., Netherlands, (1986).

28. H. L. Chakravarty, Plant Wealth of Iraq, Vol. 1, Ministry of Agriculture and Agrarian Reform (1976).

29. H. T. Hartmann, D. E. Kester and F. T. Davis Jr., Plant Propagation, Principle and Practices, 5th Ed., Prentice Hall Inc. (1990).

30. E. Epstein, Mineral Mutation of Plant, Principles and Perspectives. John Wiley and Sons, Inc. New York (1972)

31. A. I. Shaheed and N. A. Addulhassan, 2008. Extended Effects of Early Cotyledon Extracts on Rooting Response of Cuttings at Fully Expanded Primary Leaves Stage (Sub. for Pub).

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