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# *Aloe vera* Leaf Extract as a Potential Growth Enhancer for *Populus* Trees Grown Under *in vitro* Conditions

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**Abstract:** Natural plant extracts are a cost-effective and environmental friendly alternative to synthetic plant growth regulators and phytohormones. The present *in vitro* study investigated the promotory activity of various concentrations (0, 10, 20 and 40 mL/L) of *Aloe vera* leaf extract (ALE) on the growth of hybrid aspen (*Populus tremula* L. × *Populus tremuloides* "Michx") clone T89 and aspen (*Populus tremula* L.) clone W52. The extract isolated from *Aloe vera* leaves increased the plant height and weight, number of shoots, leaves and roots, and the root length, as well as mineral concentrations of both *Populus* clones. The rooted plants were acclimatized in the greenhouse with 90% survival. The results showed that ALE is an efficient alternative source to improve the growth of both *Populus* clones under study.

**Keywords:** *Aloe vera* Leaf Extract, Male Trees, Aspen, Hybrid Aspen, *in vitro,* Shoot Tip

## 1. Introduction

Plant hormones and growth regulators have been used in agriculture and tissue culture for many decades. The search for natural compounds to replace the use of hormones and PGRs is becoming popular because of the high cost of hormones and the risk of toxicity in plants, animals, and humans due to overdose [1] Natural plant extracts, which are rich in plant hormones, can be used to improve and stimulate growth of other plant species. Among these is the extract from the leaves of *Aloe vera* plants. *Aloe vera* is an important medicinal plant from the Liliaceae family with African origin. It is a succulent herb which grows in many countries [2]. Its large leaf parenchymatic cells contain liquid of yellow latex and clear gel [3, 4], which is rich in essential amino acids, mono- and polysaccharides, lignin, macronutrients, micronutrients, vitamins, gibberellins and salicylic acid [5, 6, 7, 8, 9, 10]. *Aloe* leaf extract (ALE) has been used to improve the vegetative growth of *Abelmoschuses culentus*, *Oenothera biennis* and *Majorana hortensis* [8, 11, 12, 13] and also as a natural plant growth regulator in *Majorana hortensis* and *Salvia officinalis* [8, 14, 15, 16]. Since the *Aloe vera* extract contains plant hormones such as gibberellin, therefore, we may be able to use it as a source of hormone instead of synthetic growth regulators and purified natural hormones. *In vitro* culture provides controlled environmental conditions for plant culture and shortens experimental time frames.

*Populus* trees is a dioecious plant, native to the Northern hemisphere. It is a rapid-growing tree commonly used for wood and fuel, as well as a model organism for the study of biological functions of trees [17, 18]. Male *Populus* trees tend to have longer internodes, higher dry weight and heavier wood compared with female clones [19, 20].

The present work aimed to study the effect of various concentration of *Aloe* leaf extract (ALE) on vegetative growth of *Populus* clones under *in vitro* conditions.

## 2. Materials and Methods

### *2.1. Establishment of in-vitro Populus Plantlets*

Plant material was obtained from the Experimental Farm of Ismailia Agriculture Research Station, Ismailia Governorate, Egypt. The experiments were conducted from March 1st, 2016 until October 1st, 2016 at the Tissue Culture Lab, Department of Biological Science, Faculty of Science, King Faisal University, Saudi Arabia. The male aspen (*Populus tremula* L) clone W52 and the hybrid aspen (*Populus tremula* L.×*Populus tremuloides* "Michx") clone

T89 were used in this experiment. Shoot tips of 0.5-1.0 cm length, excised from the respective poplar trees (four years old), were sterilized to establish the *in vitro* plantlets [21].

### *2.2. Preparation of Aloe Leaf Extracts (ALE)*

*Aloe vera* leaves cut from the plant (5 years old) which grown in The Agricultural-Veterinarian Training and Research Station, King Faisal University, were cold pressed using a stainless steel drum to obtain the gel solution of *Aloe* leaves. The extract was then filter-sterilized. Determination of mineral and phytohormone contents in the *Aloe vera* leaves extract were performed according to [22] and [10], respectively (Table 1 and 2).

***Table 1.*** *Determination of minerals in Aloe vera extract according to Rawe (1966).*

| Mineral | Result mg/100ml f. w |
|---|---|
| Nitrogen | 81 |
| Phosphorus | 7 |
| Potassium | 61 |
| Iron | 0.3 |
| Zinc | 0.02 |
| Manganese | 0.03 |
| Calcium | 40 |
| Copper | 0.004 |
| Magnesium | 14 |
| Sodium | 51 |

***Table 2.*** *Determination of phytohormons (GA3, IAA and ABA) in Aloe vera extract according to Shyamal et al. (1990).*

| Phytohormone/ Nutrient | Result | Unit |
|---|---|---|
| GA3 | 16 | mg/100gm f. w |
| IAA | 0.6 | mg/100gm f. w |
| ABA | 3.1 | mg/100gm f. w |
| Total carbohydrate | 10 | (%) |
| Glucose | 3 | g/100g |
| Protien | 1.0 | mg/g |
| Cholesterol | 19 | mg/g |

### *2.3. Effect of Different Aloe Leaf Extract (ALE) Concentrations on Growth of Two Populus Clones*

Eight week old *in vitro* shoot tips (0.5-1.0 cm in length) were obtained from established *in vitro* cultures of male aspen *P. tremula* clone W52 and hybrid aspen *P. tremula* × *P. tremuloides* clone T89 from in-vitro grown plantlets. A single shoot tip explant was cultured into a 40 mL capacity jar containing 10 mL of half strength MS basal salts and vitamins medium [23] supplemented with 2.0% (w/v) sucrose and 6.0 $gL^{-1}$ agar. NaOH (0.1 N) or HCl (0.1 N) solution was used to adjust the medium pH to 5.7. The medium was autoclaved for 20 min at 121°C. Various concentrations of filter-sterilized *Aloe vera* extract (0, 10, 20 and 40 mL/L) were added to the medium after the autoclaved medium reached 47°C. Each treatment contained 20 replicate jars. After 8 weeks of culture, growth parameters such as plant height and weight, number of shoots, leaves and roots, as well as the longest root, were recorded. Some of the plantlets were transferred to pots with a moist mixture of sand and perlite (1:1) for acclimatization. Potted plants were grown in a growth chamber and irrigated with a fine mist of water for 4 weeks. The percentage of plants surviving was determined after 4 weeks.

### *2.4. Mineral Composition*

Plantlet samples from both *Populus spp*. after 8 weeks of culturing on MS medium supplemented with *Aloe vera* leaf extracts were dried at 70 ºC for 24 h. The samples were ground and digested with sulphuric acid [24]. Nitrogen determination was carried out using the modified micro-Kjedahl method, as described by [25] The percentage of phosphorus was estimated calorimetrically according to the method of [26]. The Potassium (K) content was determined by using Atomic Absorption Flame Photometry according to [27]

### *2.5. Statistical Design and Analysis*

The experiment was set up in a complete randomized design with 20 replicates per treatment. Data were statistically analyzed using ANOVA\MANOVA of Statistic 6 software [28]. The significance of differences among means was analyses using the Least Significant Test (L. S. D) at p = 0.05.

## 3. Result and Discussion

### *3.1. Effect of (ALE) on Vegetative Growth*

#### *3.1.1. Effect on Plant Height (cm)*

ALE at the concentration of 10 and 20 mL/L increased the plant height of both *Populus* clones as compared with control treatment (Table 3). The ALE concentrations of 10 mL/L produced the highest plant height as (10.0 and 11.86 cm) for hybrid *P. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L. respectively (Figure 1). In contrast, the ALE at 40 mL/L significantly reduced the plant height of both clones as compared with the control treatment. The used of ALE extract in the *in-vitro* culture of *Populus* clones increase the plant height, Data in Table (2) showed that ALE extract contained the GA3 (16 mg/100gm f. w), higher growth observed could be due to GA3 found in ALE. Many reports have described that ALE increases cell membrane permeability, oxygen uptake, respiration and photosynthesis, root and cell elongation and ion transport and effects on different factor of plant growth [12, 13, 16].

#### *3.1.2. Effect on Fresh Weight of Plant (gm)*

ALE at 10 and 20 mL/L increased the fresh weight of both *Populus* clones as compared with control treatment (Table 3). However, the concentration of ALE at 40 ml/L decreased the plant fresh weight. The highest plant fresh weight of 1.32 and 1.02 g were observed in 10 ml/L ALE treatment for hybrid *P. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L respectively. The enhancing effect of ALE on plantlet fresh weight is another indication of the plant growth enhancement effect of ALE on *in vitro* cultured *Populus* plantlets. ALE has been reported to stimulate assimilation of major and minor elements, activates enzyme, and enhances membrane

permeability, protein synthesis and biomass production [29].

### *3.1.3. Shoot Number Per Explant*

ALE concentration at 10 and 20 ml/L produced higher shoot number per explant as compared to control treatment for both *Populus* clones (Table 3). The concentration of 10 ml/L gave the highest shoot/explant as 4.5 and 4.0 shoot/explant for the hybrid *P. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L. respectively (Figure 1). High concentration of ALE (40 ml/L) significantly decreased the shoot number per explants in the case of *P. tremula* as compared to control treatment. The use of ALE as a natural plant growth regulator has been reported by [13, 16].

***Table 3.*** *Effect of (ALE) on plant height (cm), Plant Friesh weight (g) and number of shoot /explant of two Populus clones, the hybrid aspen clone T89 (Populus. tremula L. x Populus tremuloides Michx.) and clone W52 (Populus tremula L.) after eight weeks from in vitro culture.*

| Aloe leaf extract treatments (ml / L) | Plant height (cm) | | Plant Fresh weight (g) | | No. of shoots (n) | |
|---|---|---|---|---|---|---|
| | Clone T89 | Clone W52 | Clone T89 | Clone W52 | Clone T89 | Clone W52 |
| Control | 7.67 b* | 10.00 ab | 0.51bc | 0.56 bc | 2.56 bcd | 1.86 cd |
| 10 | 10.00 ab | 11.86 a | 1.32 a | 1.02 ab | 4.50 a | 4.00 ab |
| 20 | 9.00 ab | 10.19 ab | 0.73 bc | 0.85 abc | 4.57 a | 3.63 abc |
| 40 | 4.00 c | 4.25 c | 0.44 bc | 0.28 c | 2.75 abcd | 1.50 d |

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L. S. D. test

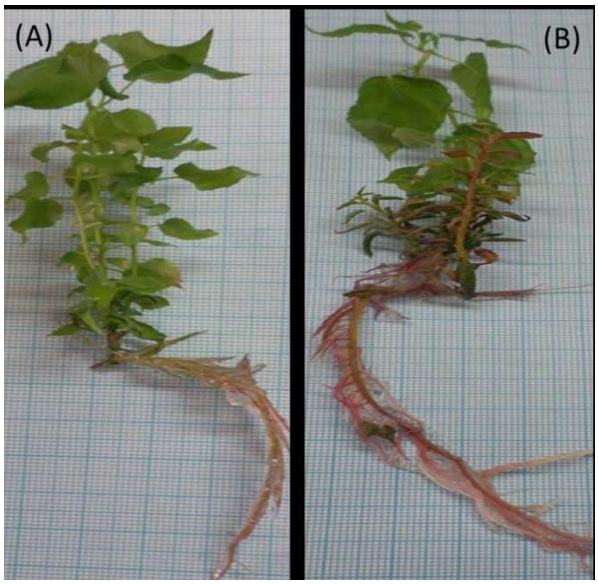


***Figure 1.*** *Effects of ALE extract on growth of Populus hybrid aspen (Populus tremula L. x Populus tremuloides Michx) clones T89 (A) and aspen (Populus tremula L.) clone W52 (B) after eight weeks growth on half MS medium supplemented with 10 ml/l ALE.*

### *3.1.4. Number of Leaves / Explant*

All ALE concentration increased the number of leaves per explant than that of control in both populous clones (Table 4). The highest numbers of leaves/ explant were 34.5and 25.14 for hybrid *P. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L. respectively. These were observed on plantlets grown on media with 10 ml/L of ALE. The obtained results are in line with the findings of [8, 14].

### *3.1.5. Root Number Per Explants and Root Length Per Explant*

ALE at 10 and 20 ml/L increase the number of root per explant for both *Populus* clones (Table 4, Figure 1 a and b). At 40 ml/L ALE concentration, the root number of the two *Populus* clones decreased compared to control treatment. Interestingly, ALE at 10 ml/L only increased the root length of the hybrid *P. tremula* L. x *P. tremuloides* "Michx". As for *P. tremula* L., decrease root length was observed with the increase of ALE concentration. The results showed that ALE at (10 and 20 ml/l) could be considered as an alternative auxin-enriched *in vitro* rooting medium at rooting stage for both clones as ALE containing IAA (0.6 mg/100gm f. w) Table (2).

***Table 4.*** *Effect of (ALE) on number of leaves (n), root length (cm), number of roots and plant height (cm) of two Populus clones, the hybrid aspen clone T89 (Populus. tremula L. x Populus tremuloides Michx.) and clone W52 (Populus tremula L.) after 8 weeks from in vitro culture.*

| Aloe leaf extract treatments (ml / L) | No. of leaves (n) | | No. of roots (n) | | Root length (cm) | |
|---|---|---|---|---|---|---|
| | Clone T89 | Clone W52 | Clone T89 | Clone W52 | Clone T89 | Clone W52 |
| Control | 23.89 bcd* | 14.86 d | 3.22 bc | 4.71 abc | 7.00 ab | 5.29 bc |
| 10 | 34.50 a | 25.14 abc | 6.33 a | 6.14 a | 9.33 a | 5.07 bc |
| 20 | 30.57ab | 21.13 ab | 6.14 a | 5.63 ab | 6.00 bc | 4.56 bc |
| 40 | 27.25 abc | 16.75 cd | 3.00 bc | 2.50 c | 2.75 c | 4.13 bc |

*Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L. S. D. test

### *3.1.6. Ex-vitro Acclimatization*

*In vitro* regenerated plantlets of both *Populus* clones showed 90 % survival when transferred to soil and did not display any phenotypic variation during subsequent vegetative development.

## *3.2. Effect of ALE leaf extract Treatment on Chemical Contents of Leaves*

### *3.2.1. Total Nitrogen (N) Percentage in the Leaves*

The nitrogen percentage increased in parallel with ALE concentration in both *Populus* clones (Figure 2a). At ALE

concentrations of 40ml/L, highest nitrogen percentages were observed, with (3.09 and 3.20 %) for hybrid *P. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L. respectively. Lowest nitrogen percentage was observed in control treatment, with 0.96 and 1.19 % for hybrid *P.. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L. respectively.

### *3.2.2. Total Phosphorus (P) Percentage in the Leaves*

All ALE concentrations increased the phosphorus concentration in the leaves of two *Populus* clones as compared to control (Figure 2b). The higher percentages of phosphorus were (0.37 and 0.43%) for the plants treated with 10 or 40ml/L in the case of *P. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L. respectively.

### *3.2.3. Total Potassium (K) Percentage in the Leaves*

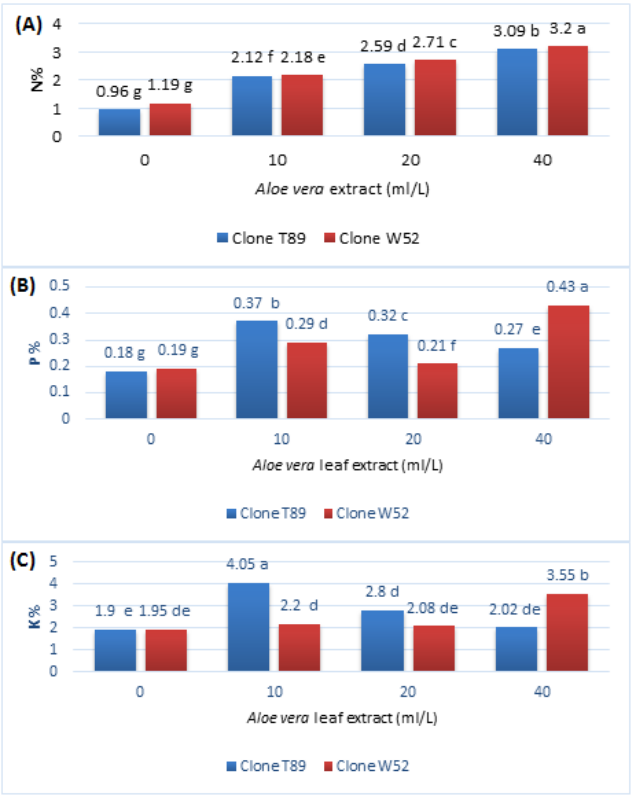


***Figure 2.*** *Effect of (ALE) on the percentage of Nitrogen (N) (a), Phosphorus (P) (b) and Potassium (K) (c) in the leaves of Populus hybrid aspen (Populus tremula L. x Populus tremuloides Michx) clone T89 and aspen (Populus tremula L.) clone W52 after 8 weeks from in vitro culture.*

All concentrations of ALE tended to increase the potassium percentage in relative to the control treatments as for both *Populus* clones (Figure 2c). The potassium percentage were (1.90 and 1.95%) for hybrid *P. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L. in the control treatment. The highest potassium percentage as (4.05 and 3.55%) were recorded by using ALE at concentrations of 10 or 40ml/L in the case of *P. tremula* L. x *P. tremuloides* "Michx" and *P. tremula* L. respectively. Increasing N, P and K in leaves of both *Populus* clones treated with ALE supported the hypothesis that ALE stimulates plant growth by assimilation of major and minor elements. Similar increase in major elements in plant leaves as result of ALE treatments was also reported by [7, 9, 30].

## 4. Conclusion

Results showed that *Aloe vera* leaf extract (ALE) increased the growth (plant height, fresh weight, number of leaves and roots, root length), multiplication (number of shoot per explants), and major nutrients (nitrogen, phosphorus and potassium) contents of leaves in both *Populus* clones. The best ALE concentration was 10 ml/L, which gave the highest recorded value for all the above mentioned parameters. In summary, the results have suggested that ALE is an efficient alternative source to improve the growth of both *Populus* clones under study.

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