Effect of fruit extracts on in vitro growth of *Salvadora persica*

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ABSTRACT

Effect of different fruits and vegetables juices in MS medium was investigated on growth of *Salvadora persica* under in vitro culture. Healthy tissues taken from mature plant and grow on a MS_0 medium for six weeks under 16hr light and 8hr dark condition, after 6 weeks subculture on MS medium [supplement with different juices, MS_1 (Carrot), MS_2 (Coconut water), MS_3 (Orange), MS_4 (Strawberry), MS_5 (Tomato)] and MS_0 use as a control. The growth parameters studied included number of leaves (fresh and pale), number of branches and height of shoots. Result shows that out of 5 medium only MS_2 (Coconut water) shows effective result in all parameters. It is concluded that other natural juices in addition with the natural compounds of *S. persica* have growth inhibition effect instead of enhancing growth.

Keywords :

fruit juices,
Murashige and Skoog (MS),
Salvadora persica

I. INTRODUCTION

Salvadora persica L. is a slow growing, evergreen perennial plant, is a highly salt tolerant tree, capable of growing in dry environment to highly saline soils in coastal region. It is grown in coastal regions and inland saline soils (1). Its seed viability is very low (30%) and has high genetic variability (2). However its seeds exhibit no dormancy, but the fruit pulp contains germination inhibitors, that should be removed before sowing (1).

S. persica is a popular chewing stick commonly known as miswak and it is one of the most common medicinal plants throughout the Muslim world (3). It is traditionally used in the treatment of several diseases such as rheumatism, leprosy, gonorrhea, ulcer, scurvy, tumors, and also several dental disorders (1).

S. persica also contain several biologically active and commercially important compounds such as volatile oils, flavanoids, alkaloids, steroids, terpenoids, saponins (4,5). Almost every part of the plant is pharmaceutically important gradients. The leaves, roots and stem bark contain alkaloid trimethylamine. Benzyl thiocyanate isolated from root contains antiviral activity against Herpes simplex virus-1, which affects oral region. N-benzyl -2- phenylacetamide also contain moderate antibacterial activity against *Escherichia coli.* Other compounds such as benzyl glucosinolate, salvadourea (urea derivative), gammamonoclinic sulfur, sitosterol also found in roots of *S. persica* (6).

Besides its medicinal potentialities, it is also suitable in agroforestry systems as a windbreak and helps in land reclamation (7,8). Its oil has high potential for making soaps, candles and to be used as a substitute for coconut oil (9).

This economically and medicinally viable plant is becoming rare, due to restricted distribution in Pakistan. Furthermore, population is under threat because of deforestation and unsustainable livelihoods.

To safeguard its natural population, in vitro can be used for the commercial production of different medicinal products. Addition of several growth regulators in nutrient media is required for in vitro multiplication to get maximum number of shoots per explants. The same results can also be achieved by including natural organic supplement in nutrient media like fruit juices for that purpose.

Since the plant extracts are a mixture of different organic compounds like hormones, growth regulators, vitamins, complex carbohydrates and several proteins so, the effect of plant extracts in tissue culture has been investigated by a number of workers throughout the world. Effect of coconut water (10,11), carrot juice (12) and orange juice is widely used as growth enhancer (13), some researchers use fruit juices as a complex carbon source for *in vitro* regeneration of plant (14).

II. MATERIALS AND METHODS

Plant material

The samples was collected from mature S. persica tree located at experimental field of "Plant tissue culture laboratory, PCSIR, Karachi". Terminal portion of stems were selected on the basis of healthy physical appearance (vigorous, greenish in color with no sign of infection).

Sterilization of surface of explants

Samples were surface sterilized with a solution contain 20% bleach and few drops of tween 20, on a shaker for 20 minutes, followed by 3-4 rinses with sterile distilled water by shaking. All step done in a laminar flow in front of flame to avoid the contamination. Finally surface sterilized explants were trimmed around 0.5-1 cm pure segment for inoculation.

Media preparation and its condition

The MS (15) medium containing 30gm/L sucrose and 2.4gm/L phytogel to solidify the medium is used for culture along with 5 organic fruit juices of carrot, coconut, orange, strawberry and tomato. Each different medium have different juices as a hormonal supplement (Table-1) and no other growth regulator is added in medium. The MS medium without no growth regulators or juices is used as control.

pH of all medium was adjusted at 5.75 before autoclaving at 121^{O}C, 15 psi for 20 minutes. The culture was incubated at 24^{O}C in 16 hr day and 8 hr dark condition.

Data collection

Visual observations were used to determine the number of leaves (fresh and pale), number of branches and height of explants.

III. STATISTICAL ANALYSIS AND GRAPHICAL PRESENTATION

Table-1: Composition of fruit juices in medium.

Medium code	Juices	Composition (v/v)
MS_0 (Control)	-	-
MS_1	Carrot	100ml/L
MS_2	Coconut	50ml/L
MS_3	Orange	100ml/L
MS_4	Strawberry	100ml/L
MS_5	Tomato	100ml/L

Table-2: Mean height and number of fresh leaves per explants after 6 weeks.

Medium code	Height (cm)	No. of leaves
MS_0 (Control)	2.8 ± 0.2	7.57 ± 0.5
MS_1 (Carrot)	2 ± 0.2	0
MS_2 (Coconut)	3.26 ± 0.2	15.6 ± 0.5
MS_3 (Orange)	2 ± 0.2	0.3 ± 0.5
MS_4 (Strawberry)	1.5 ± 0.2	3 ± 0.5
MS_5 (Tomato)	1.8 ± 0.2	0

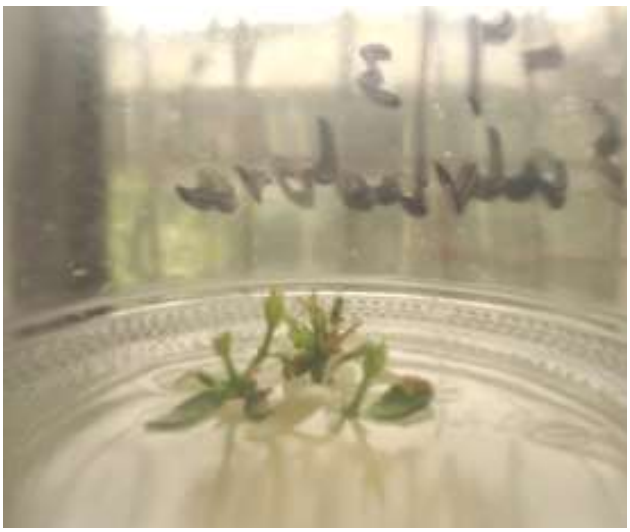
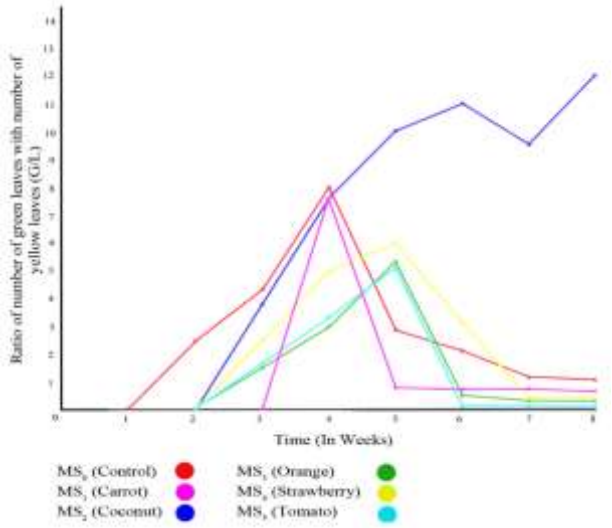
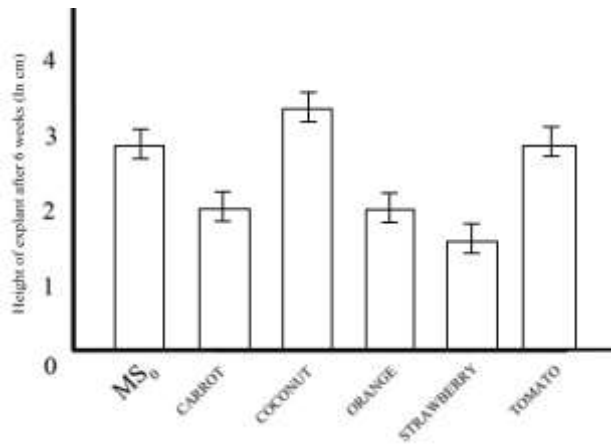


Fig.-1: Explants in coconut water medium.

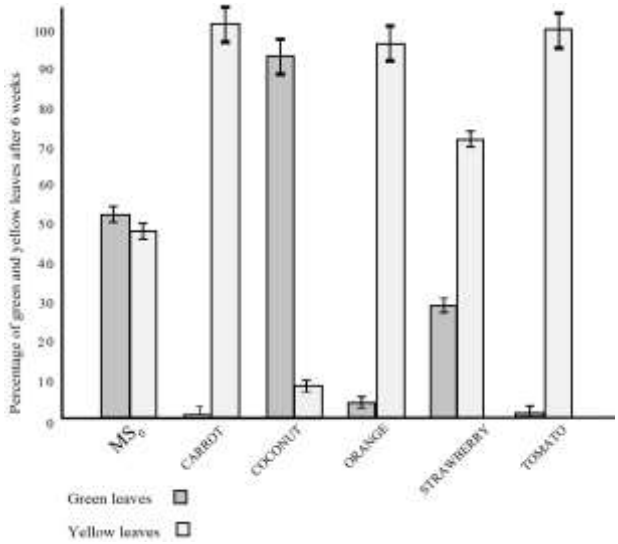
Fig-2: Branches like growth in orange juice medium



Graph-1: Weekly analysis of Growth Pattern of S. persica in different media.



Graph-2: Height of explants in different medium after 6 weeks.



Graph-3: Percentage of green and yellow leaves.

IV. RESULTS

The tissue of *S. persica* was ~1cm in length and having no leaves at the time of inoculation. It was observed that medium containing coconut water (MS_2) produce maximum number of leaves (15.6 ± 0.5) and length of shoots also appear maximum (3.26 ± 0.2cm) (Fig.-1). After MS_2 only control medium (MS_0) shows promising results. The average shoots length was (2.8 ± 0.2cm) and average number of leaves (7.57 ± 0.5) (Table-2). It was also noted that all growth parameters influence by the addition of juices in the mediums MS_2 shows highest promising result whereas MS_1, MS_3, MS_4, and MS_5 shows negative results (Table-2).

Almost each explant grows with two branches in all medium. In orange juice medium initial growth appear in form of bunches which is quite different from other mediums and then after some days this bunches become pale and growth stops.

Graph-1 shows the growth pattern of S. persica in different organic supplements related to each other in form of ratio between green leaves and pale (yellow) leaves with respect to time.

If we talk about the inhibitory effect of medium then MS_1, MS_3 and MS_5 shows highly negative effect towards the growth of explants (Graph-2). Almost all the leaves become pale after 6 weeks in these mediums. In MS_4 medium around >90% leaves become yellow and in MS_0 medium around 50% leaves become pale after 6 weeks. Almost all leaves remain green in MS_2 medium. Graph-3 shows percentage of green leaves and pale yellow leaves in all medium.

V. DISCUSSION

Result shows that coconut water containing medium shows maximum number of leaves and height. It is due to the presence of Cytokinin, Auxin, Abscisic acid (ABA) and Gibberellin (GAs) in coconut water (16). The ratio of auxin to cytokinin plays an important role in the effect of cytokinin on plant growth, result in coconut medium shows these two hormone must be present in coconut water in appropriate amount which support the optimum growth of S. persica. Cytokinin alone has no effect on parenchyma cells. When cultured with auxin but no cytokinin, they grow large but do not divide. When cytokinin is added, the cells expand and differentiate. When cytokinin and auxin are present in equal

levels, the parenchyma cells form an undifferentiated callus. More cytokinin induces growth of shoot buds, while more auxin induces root formation (17). Since there was no root formation occur in culture medium so we can conclude that the quantity of auxin is quite low in coconut water.

Some leaves become yellow when time passes it is may be due to ABA. ABA was originally believed to be involved in abscission. This is now known to be the case only in a small number of plants. ABA-mediated signaling also plays an important part in plant responses to environmental stress and plant pathogens (18). ABA is also known to slow down the growth and moderate the effect of auxin and cytokinin in in-vitro culture (19).

Initially explants in orange juice medium shows normal growth but the orange juice also contains ABA (20), due to which explant shows negative effect after 4 weeks. Growth inhibitory effect shows that in orange juice ABA level is much high as compare to other 4 juices, and also presence of citric acid in medium cause change in pH during absorption of nutrients and this pH change also reduce the explants growth.

In strawberry medium explants shows exponential growth during first three weeks then growth become decline. Strawberry contain six plant growth regulators (PGRs), 2, 4-dichlorophenoxy acetic acid (2, 4-D), 4-chlorophenoxy-acetic acid (CAP), 4-(3-indolyl) - butyric acid (BBA), forchlorfenuron (CPPU), ABA and trans-zeatin (ZT) (21). All these hormones slightly promote growth except ABA which is reported that it cause growth inhibition during stressful environment and the absence of auxin and cytokinin in strawberry also increase ABA negative growth function environment and the absence of auxin and cytokinin in strawberry also increase ABA negative growth function.

Tomato juice may also contain some growth inhibitory factors while the hormones present in tomato may not report yet. In carrot juice during first week explants grow very rapidly as compare to other explants indifferent medium. Carrot juice contains ABA and indole-3-acetic acid (IAA), IAA is member of auxin family and responsible of inducing cell elongation and cell division with all subsequent results for plant growth and development. On a larger scale, IAA serves as signaling molecule necessary for development of plant organs and coordination of growth. It is reported that presence of ABA and other inhibitory molecule cause growth reduction (12).

Due to the presence of all these hormones and other growth related substances each medium shows distinct result as compare to each other. There are certain other factors related to growth such as concentration of macro, micro nutrient, but our research limited to growth regulators.

VI. CONCLUSION

Coconut water can be used as growth hormone supplement at initial stage of plant growth and then after some time when we transfer the explants from laboratory environment to natural environment it may be capable of grow at higher rate as compare to its normal growth rate due to some physiological changes done by coconut water at initial stage development. In depth study to understand the mode of action and to overcome the inhibitory effect of organic supplements is required.

S. persica is slow growing plant but shows rapid growth in coconut water medium that leads us to the conclusion that coconut water is effective for mass production of *S. persica* could be used for other commercially important plants.

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