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Propagation Principles in Using Indole-3-Butyric Acid for Rooting Rosemary Stem Cuttings

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Abstract

Indol-3-butyric acid (IBA) was applied in 0-100 ppm concentrations as a soaking medium of rosemary cuttings for one, three and six hrs or in low (0-20 ppm) concentrations continuously for cuttings rooting. Cuttings number of induced roots and root length and rosmarinic acid, phenolic and alkaloids contents, giving rosemary plant its edible and pharmaceutical importance, were studied. Results revealed that rooting ability of cuttings was improved as a number of induced roots andby time of cutting soaking in the different concentrations of IBA. Optimal root number, which was five times the control value was achieved by soaking cuttings for three hours in 60 ppm IBA. Length and weight of induced roots were significantly varied by both time of soaking and concentration of IBA. Rosemary cuttings length slightly increased (4%) by six hours of soaking in 40 ppm IBA while their leaves areas increased (20%) by one hour of cuttings soaking in 60 ppm IBA. Leaves chlorophyll a, b and carotenoids were not affected significantly by IBA concentrations, while time of soaking was effective in increasing chlorophyll a. Rosmarinic acid was increased (5.6%) by 60 ppm IBA, but soaking in 80 ppm for one and three hours decreased it (32% and 33%). Cutting mortality during transplantation into soil was improved by low concentration of IBA or by short time of Soaking in high IBA concentration. Study preferred soaking cuttings in low concentrations of IBA especially 5 ppm continuously as it was found effective in cuttings rooting and vigor that will insure success of plant growth by such cuttings schema.

Keywords: Propagation; IBA; *Rosmarinus officinalis* rosemary; Pigments; Rosmarinic acid

Introduction

Rosemary "*Rosmarinus officinalis*" belongs to the family lamiaceae (labiatae) and is native to southern Europe and the Mediterranean area, and northwestern Spain. Traditionally, the Romans used rosemary to strength memory functions and scholars take rosemary during examinations to improve memory and concentration and people have regarded rosemary as the herb of remembrance because of the plant richness in volatile oils [1]. Also, rosemary oil is used in medicine for improving bad memory, reducing headache, tension, insomnia, fever and respiratory system diseases. It is used as a natural antimicrobial and antifungal against *E. coli*, *Pseudomonas*, *Aspergillus*, *Staphylococcus* and an insecticide and as a fragrant repellant [2]. Rosmarinic acid has potential in the treatment of toxic shock syndrome, whilst the flavonoid diosmin is reputedly more effective than rutin in reducing capillary fragility. Rosmarol, a compound from the leaves, has shown remarkably high antioxidant activity [3].

The whole rosemary plant is antiseptic, antispasmodic, aromatic, astringent, cardiac, carminative, cholagogue, diaphoretic, emmenagogue, nervine, stimulant, stomachic and tonic [4-6]. Rosemary is used as a cardiac stimulant, and for the cure of indigestion, flatulence, common cold, rheumatism and dandruff [7]. An infusion of the flowering stems made in a closed container to prevent the steam from escaping is effective in treating headaches, colic, colds and nervous diseases [5].

Cutting is a well-known common and relatively cheap method used in the propagation of many ornamental plant species. It overcomes the difficulties of propagation by plant seeds. Induction of adventitious roots on cuttings is governed by the complex interaction of several factors that could be classified under two major sections. The first would involve the stock mother plants' physiological status and their environmental conditions in addition to treatments applied to the mother plants' themselves (e.g. etiolation, girdling and spraying with chemicals) [8]. The second includes factors concerned with the post harvested cuttings which basically include both chemical and mechanical treatments imposed on cuttings [e.g. wounding, centrifugation and growth regulator application] [8]. Physical factors such as cutting length, cutting type, stem diameter, presence or absence of a leaf and stock plant from which the cuttings are taken, play an important role in rooting ability of cuttings [9].

It has been widely documented that auxins promote adventitious root development of stem cuttings through their ability to promote the initiation of lateral roots premordia and to enhance transport of carbohydrates to the cutting base needed for root growth [8,9]. The purpose of treating cuttings with auxins is to increase the percentage of rooting, root initiation, number and uniformity of rooting [10]. It also accelerates the translocation of nutrients from upper part of the cuttings to their basal ends by increasing the activity of enzymes. This increases hydrolysis of carbohydrates for providing enough energy in rooting respond of the cells [11]. As reported by Al-Barazi and Schwabe [10], occasionally IBA treatment seems to stimulate cell division in the ray cells between the primary bundles which improved root initiation and increased uniformity of rooting.

Stem cuttings of rosemary (*Rosmarinus officinalis*) were treated with IAA, IBA and NAA. Soaking and quick dipping are the best treatments for initiation of early rooting, root length and survival percentage under field conditions. The quick dip method was found most effective and time saving [12]. Rosemary have a good rooting ability, and the effect of the root-forming solution induces better results, both in terms of the rooting percentage and the qualitative features of the new root system [13]. Deen and Mahmoud [14] stated that auxins stimulates root formation of rosemary if applied in a dilute concentration but higher dosage (0.2%) vigorously caused inhibitory effect in rooting of rosemary cuttings. Rosemary branches were collected at the end of each season (spring, summer, autumn and winter) and the end of winter was the best season for rooting cuttings [15]. IBA is a synthetic root-promoting chemical compound that has been found most reliable in stimulating rooting of cuttings in a large number of plant species and is non-toxic to plants over a wide concentration range [8].

The present study aims at studying the effect of applying different high concentrations of IBA for different time or low concentration for long time on the characteristics of the induced roots and morphology, growth and chemical constituents of rooted cuttings.

Materials and Methods

The present work was carried out in laboratory of Physiology, Botany department, Faculty of science Minufia University. It was carried out on rosemary (*Rosmarinus officinalis* L.). Cuttings of rosemary were collected from shrubs which planted in the faculty garden in clay soil.

Effect of IBA concentration and soaking time

Different concentrations of IBA (Merck) (0, 20, 40, 60, 80 and 100 ppm) were prepared. IBA was firstly dissolved in few drops of 1N KOH before diluting by distilled water to the needed solution concentration. Cuttings of rosemary, fixed at about 15 cm in length by cutting under water, were sterilization by washing with $HgCl_2$ (0.1%) and washing several times with sterilized distilled water. The lower leaves (at the lowest 3 cm) of the rosemary cuttings were removed and then two cuttings were immersed in 50 ml of each IBA concentration in beakers (50 ml) for three fixed times (one, three or six hours). The lowest three cm were totally immersed in the hormone solution. After that, cuttings were transferred into another beakers containing 50 ml sterilized distilled water after washing with distilled water. Three beakers were used as replicates for each concentration. The beakers were covered with aluminum foil to keep lower part of cuttings in continuous darkness to enhance root initiation as auxin works efficiently in darkness. The water in the beakers was kept at specific level by adding distilled water and the beakers were agitated from time to time for gently enriching the water with air. The experimental beakers were kept under laboratory conditions, air temperature 30 ± 2°C, relative humidity 80%, with 12 hours natural light and 12 hours dark. Died cuttings were recorded and excloded while others were harvested for measurements and analysis at the end of experiment.

Effect of continuous soaking in low concentrations of IBA

Low concentrations of IBA (0, 1, 2, 3, 5, 10, 15 and 20 ppm) were prepared and in 50 ml of each concentration two prepared rosemary cuttings were soaked, three beakers replicates for each concentration. The beakers were agitated from time to time and their solutions were completed to the specific level by distilled water or the same concentration. The beakers were covered with aluminum foil for darkness and kept under the previously mentioned laboratory conditions. Then died cuttings were recorded and excluded while others were harvested for measurements and analysis at the end of experiment.

When no new root was produced experiments were ended and at that time cuttings of rosemary were collected and washed with distilled water. Then the induced roots on each cutting were counted and the lengths (cm) of the roots were measured, while cuttings length (cm) at start and end of experiments was measured from the end of terminal node to the end of cutting. Fresh leaf samples from cuttings of each treatment were used for the measurements of leaf area and the photosynthetic pigments and rosmarinic acid. The roots and cuttings were weighted as fresh and dried in an electric oven at 70°C for constant weight for the determination of water content percentage. The dried materials were powdered and reserved in paper bags for further analysis.

Estimation of photosynthetic pigments

Fresh leaf samples (100 mg) were homogenized immediately in 5 ml cold aqueous acetone 85% (v/v) solution and centrifuged for 15 minutes at 3000 rpm. These extracts were diluted to the appropriate volume (10 ml) with the previous cold acetone solution and their color intensities were measured against a blank of the 85% acetone solution at three wavelengths, 663, 644 and 452.5 nm by using a spectrophotometer (Metertek SP-850) for the determination of chlorophyll a, chlorophyll b and carotenoids content according to the method of Metzner et al. [16].

Estimation of rosmarinic acid

The method of rosmarinic acid extraction and determination was reported by Lopez-Arnoldos et al. [17] and Komali and Kalidas [18]. Rosemary fresh leaf samples (200 mg) were blended with porcelain mortar in 10 ml of 50% methanol and placed in water bath at 55°C for 2 hours then were centrifuged for 10 minutes at 3500 rpm. One ml of the extract was diluted with 9 ml of the 50% methanol and the absorbance of the formed colour was measured at 333 nm using spectrophotometer (Metertek SP-850) and the rosmarinic acid concentration (mol^{-1} cm^{-1}) was calculated from the following equation:

$$A = \varepsilon bc$$

Where: (A) is the absorbance at 333 nm, (C) is the concentration of rosmarinic acid, (ε) is the extinction coefficient (ε=19000 L mol^{-1} cm^{-1}) and (b) is the width of cuvette (b=1 cm). The content per mol was calculated as mg/g F.wt. by multiplying with rosmarinic acid molecular weight.

Estimation of flavonoids

The aluminum chloride colorimetric method was used to estimate the flavonoids in rosemary. The rosemary cuttings dry leaves sample (10 mg) was extracted by 80% ethanol then a definite volume of solution (0.5 ml) was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer (Metertek SP-850). The amount of 10% aluminum

chloride was substituted by the same amount of distilled water in blank. Quercetin was used to make the calibration curve, the reacted flavonoids of the extracts with aluminum chloride was used for the determination of flavonoids content as mg/g d.wt. in the plant leaves.

Estimation of total alkaloids

Total alkaloids of rosemary cuttings dry leaves were. The plant leaves dry powder samples (1 g) were extracted several times with 4:1 of 70% (v/v) ethanol and glacial acetic acid. The mixture was left to stand for at least 6 hours extraction time during which it was shacked from time to time, and filtrated. The alkaloids content in the collected supernatants was precipitated by a drop wise addition of concentrated ammonia solution until no precipitation was formed. The precipitated alkaloids were filtered on a pre-weighted filter paper (Whatman 102), washed with diluted ammonia and were dried in an oven at 70°C to a constant weight. Alkaloids content was calculated as mg/g dry weight of the plant samples.

Estimation of saponins

The saponin content in the rosemary cuttings was. A known weight (10 mg) of the plant leaves dry powder was extracted three times with 95% ethanol. The clear supernatants were combined and completed into a definite volume, then 0.5 ml of this ethanol extract was mixed with 0.5 ml of 8% vanilline in ethanol (8 g of vanilline dissolved in 100 ml ethanol). The mixture was placed in an ice bath and mixed with 5 ml of 72% sulfuric acid, then heated in a water bath adjusted at 60°C for 10 minutes followed by cooling in the ice-cold water bath. The absorbance of the formed colour was measured at 544 nm using the previously mentioned spectrophotometer. A standard curve by different concentrations of cholesterol was constructed as in the previous steps and used for the determination of the content of saponins (mg/g d.wt) in the plant leaves.

Estimation of total phenolic compounds

Total phenolic content in rosemary cuttings was estimated quantitatively using a method. A known weight (10 mg) of the dried plant leaves was extracted by 95% ethanol three times and the clear supernatants were combined and completed to a known volume (25 ml) by the 95% ethanol. Then 1 ml from this extract was mixed with 1 ml folin reagent and 1 ml N (20% w/v), then the mixture was completed up to a known volume with distilled water. Thereafter, the absorbance of the produced colour was measured spectrophotometerically (Metertek SP-850) at 650 nm after exactly 30 minutes. A standard curve was prepared by using different concentrations of pyrogallol as the previous procedure and used for the determination of the total phenolic compounds content (mg/g d.wt.) in the plant leaves.

Statistical analysis

The obtained results were statistically analyzed using one and two ways analysis of variance (ANOV) to determine the degree of significance for the obtained variations by the used treatments. Also, correlation coefficients were applied for investigating the significance of the relationships between the studied variables of the study plant. All of the statistical methods were according to Bishop [19], while the analysis was carried out by SPSS statistical package.

Results I- Effect of different indole-3-butyric acid concentrations for different times

a- Root characteristics

Root number: Results of induced root number per cutting of rosemary (Table 1 and Figure 1) showed remarkable and significant variations in the number of the induced roots of cutting by time of Soaking and the concentration of the IBA.

Time of soaking (hour) Concentration (ppm)	One	Three	Six
0	38.8	38.8	38.8
20	33	0	33.3
40	17	0	33.3
60	17	17	33.3
80	0	0	33.3
100	0	50	0
Mean	17.5	17.5	28.6
Number of cutting	36	36	36

Table 1: Mortality percentage (%) of rosemary cuttings which soaked in different concentrations of IBA for different times. Mortality percentage (%) of rosemary cuttings which soaked continuously in different concentrations of IBA.

Soaking in the different concentration of IBA for one, three and six hours increased root number progressively in comparison with the control. The maximum root number after Soaking for one, three and six hours was at 100, 60 and 40 ppm IBA concentration respectively these maxima were 17.8,27.8 and 22.5 roots and they were 223.6%,405 and 309% the control value respectively. This showed that root

number was lowered but not lower than that of the control by cuttings soaking in increased concentrations of IBA also, increasing time of soaking increased root number of cutting at lower IBA concentration.

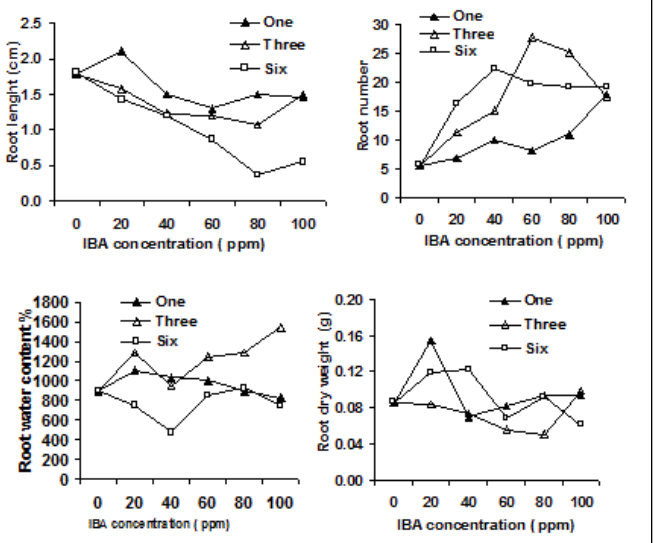

Figure 1: Variations in the induced roots number, length (cm), water content (%) and dry weight (g) on the rosemary cuttings treated with high concentrations of IBA for different times.

Root length: The lengths of the induced roots on the rosemary cuttings by concentration and time of Soaking in IBA treatments were slightly varied (Table 1 and Figure 1). Increasing time of cuttings soaking in IBA decreases root length in rosemary cuttings. Decreases were also, recorded in root length of cuttings with increasing the concentration of IBA when compared with the control, except presoaked in 20 ppm IBA for one hour which increased the cuttings root length by about 17% in comparison with the control. The shortest root lengths were 1.3, 1.1 and 0.4 cm by Soaking in IBA for one, three and six hours in 60, 80 and 80 ppm concentrations respectively. These values were lower by 33.3%, 38.8% and 77.7% than the control value for the previous Soaking treatments respectively.

Root dry weight: The exhausted metabolites in rooting were represented by produced root dry weight under the different IBA treatments (Table 1 and Figure 1). The dry weights of the induced roots on the rosemary cuttings were significantly varied by concentrations of IBA. High dry weight of roots was due to Soaking rosemary cuttings for one hour, followed by six hours in the different concentrations of IBA which progressively increased dry weights of induced roots specially with increasing the concentration of IBA, except 40 and 60 ppm for one hour and 60 and 100 ppm for six hours soaking treatments which decreased the dry weight in comparison with the control. The highest root dry weight was by Soaking for one hour in 20 ppm where the root dry weight was 1.8 that of the control. Soaking for three hours caused decreases in the roots dry weight with increasing IBA concentrations except 100 ppm where the dry weight of roots was higher by 15% than the control value.

Root water content: The percentages of water content in roots of the cuttings (Table 1 and Figure 1) were significantly varied in response to time of cuttings soaking in IBA regardless of the concentration. High percentage of roots water content was induced by soaking rosemary cuttings for three hours in the different concentrations of IBA, followed by one hour and then six hours of soaking. Soaking in most concentrations of IBA increased the percentages of roots water content when compared with the control. The maximum root water content (1544%) was recorded by soaking for three hours in 100 ppm IBA concentration and that was higher by 72% than the control value. On the other side, prolonged Soaking for six hours in all IBA concentrations, except 80 ppm concentration, decreased the percentage of water content of cuttings root compared with the control.

b- Cuttings characteristics

Cuttings length: The length of rosemary cuttings at the end of experiment was not significantly varied by Soaking time of cuttings or by the different high IBA concentrations (Table 1 and Figure 2). Slight increase was found in the length of cuttings by soaking for one, three and six hours in 80 ppm of IBA compared with the control. These increases were only by 3.3%, 2.8% and 4% of the control value.

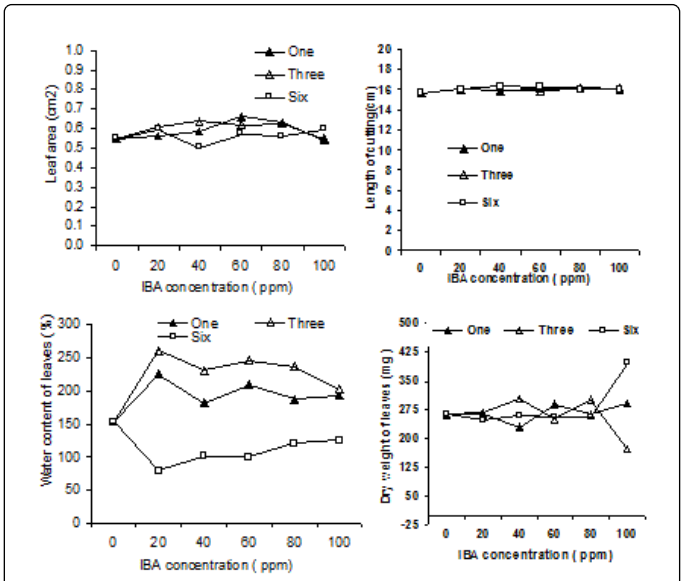

Figure 2: Variations in the cuttings length (cm) and cuttings leaves area (cm^2), water content (%) and dry weight (G) of the rosemary cuttings treated with high concentrations of IBA for different times.

Cuttings leaf area: The leaf area of rosemary cuttings varied significantly by soaking for different times in the different high concentrations of IBA but was not changed significantly by concentrations of IBA (Table 1 and Figure 2). Recorded increase in the cuttings leaf area by concentrations of IBA was slight as compared with the control. The maxima of cuttings leaf area (0.66, 0.64 and 0.60 cm^2) were exhibited by soaking in 60, 40 and 20 ppm concentrations of IBA for one, three and six hours respectively. The maximum values of leaf area of rosemary cuttings were greater by more than 20%, 16% and 9% than the control value by the previous treatments of IBA respectively.

Cuttings leaf dry weight: The dry weight of rosemary leaves (Table 1 and Figure 2) was higher in the presoaked cuttings for six hours than in the presoaked for one and three hours in the different IBA concentrations. The maximum dry weights of leaves (293, 305 and 397

mg/g dry weight) were recorded by soaking cuttings in 100, 40 and 100 ppm IBA for one, three and six hours respectively which increased leaves dry weights by 11%, 16% and 51% respectively in comparison with the control value.

Cuttings leaf water content: Percentages of leaves water content significantly varied as a response to time of cutting Soaking in different concentrations of IBA (Table 1 and Figure 2). Higher water content in the leaves of rosemary cuttings was in presoaked cuttings for three hours than for one or six hours in all IBA concentrations. Six hours as a period of soaking decreased percentage of water content under all IBA concentrations compared with the control value. The highest water content (260%) was recorded in soaked cuttings in 20 ppm IBA for three hours that and was higher by about 70% the control value.

Cuttings leaf photosynthetic pigments: The content of photosynthetic pigments (chlorophyll a, b and carotenoids) in the leaves of rosemary cuttings varied significantly ($p<0.05$) by time of cuttings soaking in IBA but not significantly with IBA concentrations (Table 1 and Figure 3).

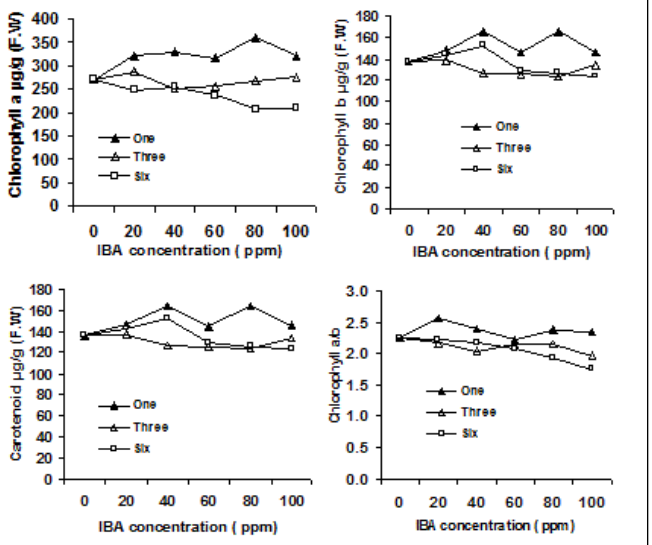

Figure 3: Variations in cuttings leaves chlorophyll a, b and carotenoids content (mg/g f.wt) and the ratio of chlorophyll la/b of the rosemary cuttings treated with high concentrations of IBA for different times.

The content of chlorophyll a in the leaves of rosemary cuttings decreased in case of elongation of the soaking time of in the different concentrations of IBA. The soaked cuttings for one hour in most concentrations of IBA showed high chlorophyll a content compared to the control. Prolonged soaking time to three and six hours, on the other hand, led to a fluctuation of chlorophyll a contents around a decreasing trend by increasing the concentrations of IBA. The maximum chlorophyll a content (360 µg/g fresh weight) was due to soaking cuttings for one hour in 80 ppm IBA concentration and was higher than the control by 33%.

On the contrary the content of chlorophyll b in rosemary cuttings leaves which was greatly lower than chlorophyll a content decreased due to increasing time of cuttings soaking in the different concentrations of IBA (Table 1 and Figure 3). Chlorophyll b content increased in response to the different concentrations of IBA except for six hours soaking in most of the used concentrations. Soaking in 80 ppm IBA for one hour led to the highest chlorophyll b content (151 µg/g fresh weight) which was higher by 26% than the control value.

The carotenoids content in the leaves of rosemary cuttings was greater by short time of soaking (one hour) than by the long ones (three and six hours) in IBA concentrations (Table 1 and Figure 3). Also, progressive increases in carotenoids content was exhibited by increasing the concentration of IBA up to 80 ppm especially for one hour soaking time. All concentrations of IBA led to higher carotenoids than compared to the control, especially when cuttings were presoaked for one hour, while increasing time of Soaking for three and six hours mostly decreased carotenoids content than that of the control. The highest carotenoids content (165 µg/g fresh weight) was by 80 ppm soaking for one hour and was higher by 20% the control value.

Chlorophyll a/b: Chlorophyll a/b of rosemary leaves was slightly higher by soaking for one hour than for three and six hours (Table 1 and Figure 3). Chlorophyll a/b ratios ranged between 1.74 and 2.56. The highest ratio was due to one hour soaking in 20 ppm that was higher by 13% than the control value. Soaking for three and six hours decreased the ratio especially with increasing the concentrations of IBA. The decrease in chlorophyll a/b ratios was due to more decrease in chlorophyll a than in chlorophyll b by the time of Soaking.

Cuttings leaf rosmarinic acid: The rosmarinic acid contents in the leaves of rosemary cuttings varied significantly ($P<0.01$) by IBA concentrations, time of soaking and their interaction (Table 1 and Figure 4). Soaking for one, three and six hours decreased rosmarinic acid content of cuttings specially with increasing the concentration of IBA when compared with the control value, except the slightly higher content than that of the control by Soaking for six hours in 20 and 60 ppm IBA concentrations. The recorded increases in rosmarinic acid content (by 20 and 60 pmm) were only greater by about 1.2% and 5.6% than the control value. The marked decreases (32% and 33% of the control value) were recorded by soaking for one and three hours respectively in 80 ppm.

Cuttings leaf Flavonoids: The results of flavonoids content in the leaves of rosemary cuttings (Table 1 and Figure 4) varied significantly ($P<0.01$) by concentrations of IBA, time of Soaking and their interactions. The flavonoids content in the leaves of rosemary cuttings was decreased by increasing time of cuttings soaking in IBA. But, increasing the concentration of IBA led to slight decreases in flavonoids content of cuttings when compared with the control. Decreases in flavonoids content ranged between a minimum decrease by 0.32% to a maximum decrease by 42.3% in comparison with the control value and after Soaking for one hour in 100 ppm and six hours in 100 ppm of IBA.

Cuttings leaf Alkaloids: Alkaloids content in leaves of rosemary cuttings (Table 1 Figure 4) showed significant decrease ($P<0.01$) by both IBA concentration and time of Soaking. Soaking for one hour increased alkaloid content of cuttings by increasing the concentration of IBA when compared with the control. But higher than 40 ppm, IBA attenuated alkaloids content gradually. The maximum alkaloids content was 21.4 mg/g dry weight at 40 ppm and that was higher by 27.8% than the control. Also soaking for three hours increased alkaloids with increasing the concentration of IBA in comparison with the control value and its maximum (higher by 29%) was recorded by 100 ppm. Alkaloids content was increased after six hours of soaking in

80 ppm of IBA concentration (17.85 mg/g), while it decreased with the other used concentrations compared to control value.

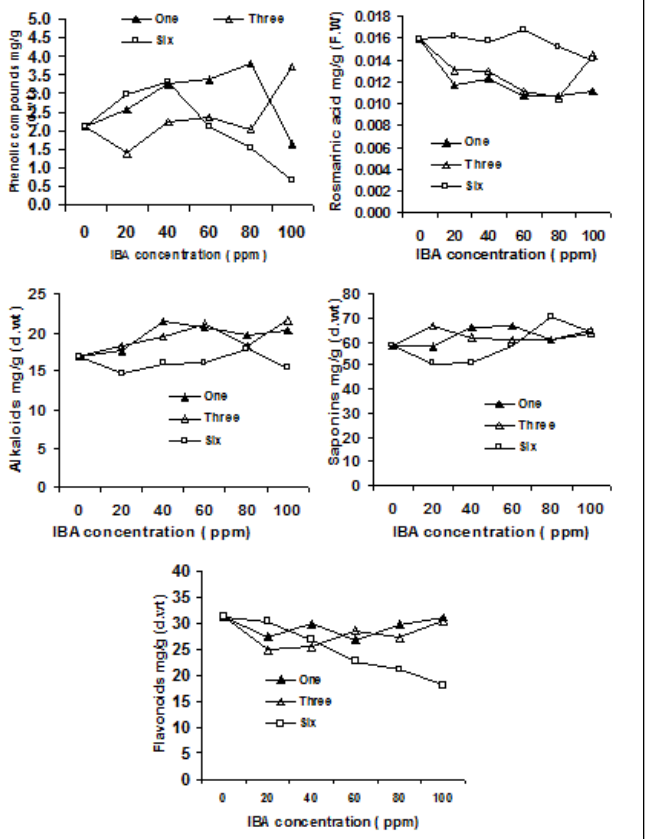

Figure 4: Variations in cuttings leaves phenolic compounds, rosemarinic acid, alkaloids, saponin and flavonoids contents (mg/g d.wt) of the rosemary cuttings treated with high concentrations of IBA for different times.

Cuttings leaf Saponins: Saponin content in leaves of rosemary cuttings did not vary significantly by Soaking in different concentrations of IBA and the different times of Soaking (one, three and six hours) or their interaction (Table 1 and Figure 4). But there were general decreases in mean saponins content by soaking time from one to six hours. Soaking for one hour increased saponins content of cuttings with increasing the concentration of IBA up to 60 ppm then the content was decreased gradually but still higher than the control value. The maximum saponins content was 66.5 mg/g at 60 ppm and was higher by 14.4% than the control value. Three hours Soaking increased also saponins content with increasing the concentrations of IBA in comparison with the control and the maximum content was higher by 15% and was caused by 20 ppm concentration of IBA. Soaking for six hours in IBA concentrations more than 60 ppm increased saponins content with a maximum at 80 ppm and was higher by 20.7%, then the content was decreased but still higher than the control value.

Cuttings leaf Phenolic compounds: The phenolic compounds content in the leaves of rosemary cuttings significantly varied with the Soaking in the different concentrations of IBA ($P<0.01$), the time of soaking ($P<0.05$) and the interaction of the two applied treatments ($P<0.01$) (Table 1 and Figure 4). A general decrease of phenolic compound content in the leaves of rosemary cuttings was exhibited by elongating time of soaking in IBA. Phenolic compounds contents ranged between a maximum of 3.83 and a minimum of 0.64 mg/g dry weight by Soaking in 80 and 100 ppm of IBA for one and six hours respectively. Soaking for one hour, in increasing the concentration of IBA increased phenolic compound content compared with the control value up to the 80 ppm then decreased the content to lower than the control value by 100 ppm IBA concentration. Soaking for three hours led to fluctuation of the content of phenolic compound around an increasing trend with increasing the concentration of IBA. The maximum content (3.72 mg/g dry weight) was by 100 ppm and was higher by 75.4% compared to the control. Soaking for six hours was accompanied with increase in the phenolic compounds content with increasing IBA concentrations up to 40 ppm, which increased the content by 57%, and followed with a sharp decrease with higher IBA concentrations.

II- Effect of continuous soaking in low concentration of IBA

a- Root characteristics

The experimental results of continuously soaked rosemary cuttings in the low concentration of IBA (Figure 5) significant increase in root number by increasing concentrations of IBA up to 15 ppm where the maximum increase was by 1172% in comparison with control then root number was decreased but still higher than the control value. Root length also showed a general increase with the increase in IBA concentration up to 3 ppm where the maximum increase was recorded and it was by 170% compared to the control. After that the increase was slightly attenuated but root length still higher than that of control.

Fresh weight of the produced roots on the cuttings was not significantly varied. But at low concentrations (1, 2 and 3 ppm IBA) there were decreases, and at the higher concentrations of IBA there were varied increases and its maximum was recorded by the highest IBA concentration (20 ppm) and was by 309% compared to the control. Dry weight of the cuttings root was not significantly varied with the concentration of IBA. Remarkably the root dry weights were lower than that of the control. Water content of root exhibited significant increase with the increase in IBA concentration with a maximum increase at 3 ppm and was by 712% compared to the control.

a- Cuttings characteristics: Length of rosemary cuttings which continuously soaked in low concentration of IBA was not changed significantly by the different concentrations of IBA (Figure 5). The observed increase in comparison with the control cutting length did not exceed 3.2%. Area of leaves of rosemary cuttings also was not significantly by IBA in comparison with the control. There was an increase at 1 ppm and was by 50%, in comparison with the control. Fresh and dry weights of rosemary cuttings leaves (Figure 6) significantly increased with all IBA concentrations and 10 ppm caused the maximum increase (by 149% and 152% compared to control). The variation in water content of rosemary leaves was significant by all IBA concentration. IBA concentration 1 and 3 ppm increased leaf water content, the maximum at 3 ppm was higher by 17% the control value while other concentrations slightly decreased it.

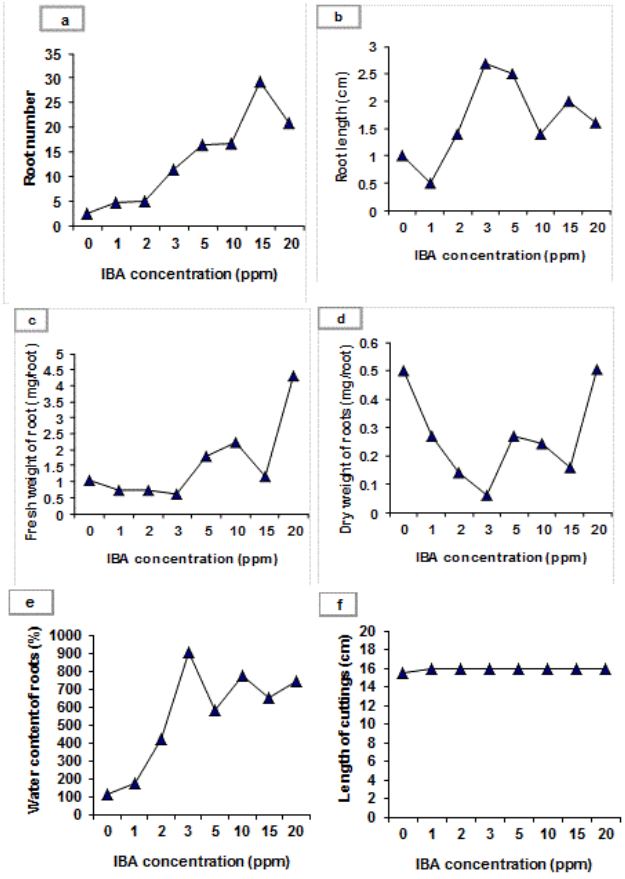

Figure 5: Effect of continuous soaking in different concentrations of IBA on (a) Root number, (b) Root length (cm), (c) Fresh weight of roots (mg/root), (d) Dry weight of roots (mg/root), (e) Water content of roots (%) and (f) Length of cuttings (cm).

Important cutting metabolites: Data of chemical analysis of leaves of rosemary cuttings which soaked continuously in low concentrations of IBA were represented in (Figure 6-8). Chlorophyll a content of rosemary leaves was not significantly varied as a response to IBA concentrations. There were slight increases over the control value by all IBA concentration except 5 ppm and the maximum increase was recorded by 20 ppm and it was by 19.4%. Chlorophyll b content was not also significantly varied but it was decreased than the control value with increasing IBA concentration with a minimum content was at 5 ppm that was lower by 26.8% than control value. Carotenoids in rosemary leaves exhibited slight increase by low concentrations of IBA (1 and 2 ppm) while by high concentrations it decreased. The minimum content was at 5 ppm and was lower by 16.8% than the control. Chlorophyll a/b ratio was higher under in IBA concentrations than under control and acquired its highest at 10 ppm. Also, chlorophyll (a+b)/c ratio slightly increased than the control ratio with the increase in IBA concentration, the highest ratio was at 3 ppm concentration. This ratio decreased compared to that of the control by 15 and 20 ppm concentrations.

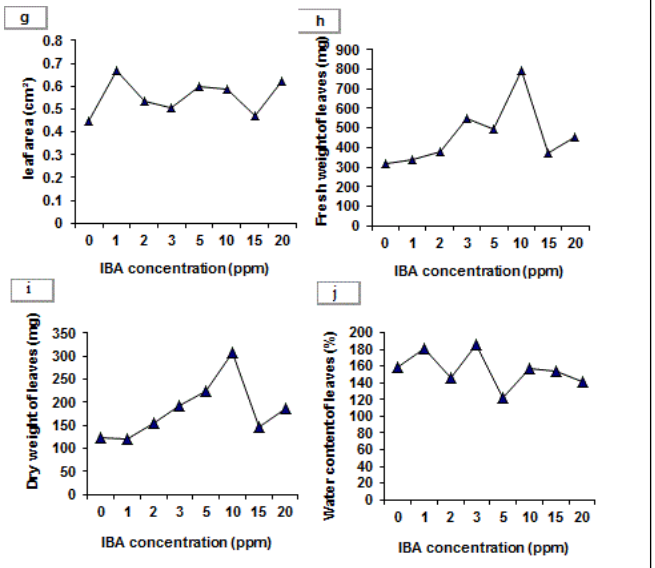

Figure 6: Effect of continuous soaking in different concentrations of IBA on (g) Leaf area (cm^2), (h) Fresh weight of leaves (mg), (i) Dry weight of leaves (mg) and (j) Water content of leaves.

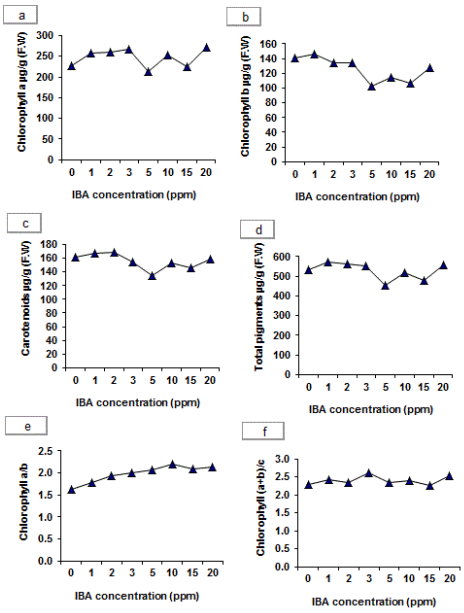

Figure 7: Effect of continuous soaking in different concentrations of IBA on (a) Chlorophyll a µg/g fresh weight, (b) Chlorophyll b µg/g fresh weight, (c), Carotenoids µg/g fresh weight, (d) Total pigment µg/g fresh weight, (e) Chlorophyll a/b, (f) Chlorophyll (a+b)/c.

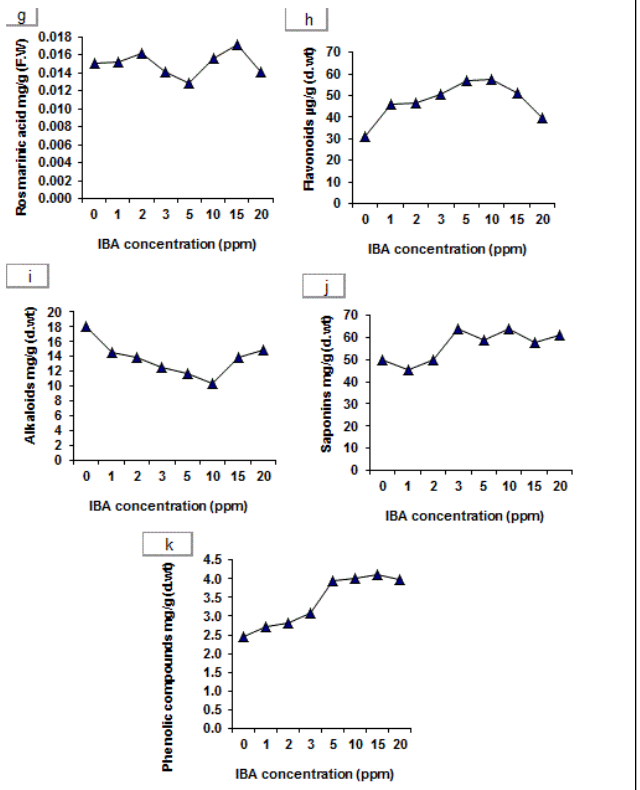

Figure 8: Effect of continuous soaking in different concentrations of IBA on (g) Rosmarinic acid mg/g fresh weight, (h) Flavonoids mg/g dry weight, (i) Alkaloids mg/g dry weight, (j) Saponins mg/g dry weight and (k) Phenolic compounds mg/g dry weight.

The rosmarinic acid content in rosemary cuttings leaves generally, did not significantly increase with the increase in concentrations of IBA. The maximum increase was at 15 ppm and was higher by 14% than the control value. The rosmarinic acid minimum content was at 5 ppm IBA concentration and was lower by 14.6% than the control. On the opposite flavonoids content in rosemary leaves significantly varied by IBA concentrations. Increased in content of flavonoids was recorded with the increase in IBA concentration up to 10 ppm where the maximum content was recorded and it was higher by 83.2% in comparison with the control. Continuous soaking of rosemary cuttings in IBA caused a marked (but not significant) decrease in alkaloids content especially with increasing IBA concentration up to 10 ppm. The minimum alkaloids content was at 10 ppm and was lower by 42.7% of the control value.

Saponins content in rosemary leaves varied significantly by IBA concentrations. At low concentrations of IBA(1 and 2 ppm) saponins content decreased than the control value but at high concentrations it increased. The maximum content of saponins was at 10 ppm IBA concentration and was higher by 27.6% than the control value. Phenolic compounds varied significantly and showed a paralleled increase with the increase in IBA concentration up to 15 ppm at which the increase was by 68.3% as compared with control.

Relationships between the measured parameters in rosemary

Root number significantly and positively correlated with water content of roots (r<0.05), and cut length (r<0.01) (Table 2). Root length significantly and positively correlated with chlorophyll a (r<0.01) and negatively with rosmarinic acid (r<0.05). Dry weight of roots significantly and positively correlated with root length (r<0.01). Water content of roots significantly and positively correlated (r<0.01) with only chlorophyll b. Dry weight of leaves significantly and negatively correlated with carotenoids (r<0.05). Water content of leaves significantly and positively correlated with root length, chlorophyll a, chlorophyll b and with leaf area and carotenoids (r<0.05). Water content of leaves correlated at r<0.01 with rosmarinic acid. Rosmarinic acid acquired significant negative correlations with chlorophyll a and chlorophyll b

Concentration (ppm)	0	1	2	3	5	10	15	20	Number of cutting
IBA continuous	66.6	66.6	50	50	0	16.6	50	50	48

Table 2: Relationships between the measured parameters in rosemary.

The correlation matrix between morphological and chemical characters of rosemary cuttings under the effect of soaking continuously in different concentrations of IBA indicated that root number significantly and positively correlated with root length, phenolic compounds (r<0.01) and water content of roots (r<0.05). Root length correlated significantly and positively (r<0.05) with leaf area and negatively (r<0.05) with chlorophyll a. Fresh weight of roots correlated significantly and positively with dry weight of roots (r<0.01) and leaf area and Saponins (r<0.05). Dry weight of roots significantly and positively correlated with leaf area (r<0.05). Water content of roots significantly and positively correlated with saponins (r<0.01), fresh weight of leaves and phenolic compounds (r<0.05) and negatively with flavonoids and alkaloids (r<0.05). Fresh weight of leaves significantly and positively correlated (r<0.01) with dry weight of leaves and negatively correlated at r<0.05 with flavonoids and alkaloids. Dry weight of leaves significantly and positively correlated (r<0.05) with phenolic compounds and negatively (r<0.05) with alkaloids. Chlorophyll a significantly and positively correlated (r<0.01) with chlorophyll b, carotenoids and total pigments. Chlorophyll b significantly and positively correlated (r<0.01) with total pigments. Carotenoids correlated significantly and positively at r<0.01 with total pigments.

Mortality of cuttings during rooting and their survival after transplantation

Mortality percentage of rosemary cuttings was increased with increasing time of Soaking and decreased with increasing IBA concentrations (Table 3). The mortality of cuttings reached zero by Soaking in the concentrations of IBA (20, 40, 60, 80 and 100 ppm). The greatest mortality was under the control (38.8%). Mortality of cuttings which soaked continuously in IBA decreased with increasing concentration until it reached zero by 5 ppm IBA after it mortality increased again with the increase in IBA concentration, which suggesting this concentration for storage of hormone and could be used for reserving the plant cuttings during manipulation as transportation before cultivation.

	RN	FWR	DWR	WCR	FWL	DWL	WCL	CutL	Root L	Leaf A	RA	Ph	Sapo	Flavo	Alka	Chl a	Chl b	Carot	TP
RN	1																		
FWR	-0.011	1																	
DWR	-0.08	.671(**)	1																
WCR	.228(*)	.558(**)	-0.129	1															
FWL	0.048	0.021	-0.145	0.137	1														
DWL	0.107	-0.106	-0.212	0.053	.697(**)	1													
WCL	-0.091	0.146	0.028	0.139	.509(**)	-0.212	1												
Cut L	.471(**)	0.045	0.124	-0.085	0.044	0.18	-0.125	1											
Root L	0.099	.300(**)	.425(**)	0.074	0.175	-0.144	.397(**)	0.048	1										
Leaf A	-0.011	-0.088	-0.161	-0.001	0.073	-0.081	.247(*)	-0.07	0.063	1									
RA	-0.103	0.024	0.171	-0.059	-.531(**)	-0.152	-.574(**)	-0.092	-.329(*)	-0.234	1								
Ph	-0.155	0.134	0.25	-0.132	-.369(**)	-.276(*)	-0.176	-0.043	0.096	0.024	-0.014	1							
Sapo	0.054	-0.234	-0.232	-0.152	-0.067	-0.142	0.125	-0.071	-0.166	.327(*)	-0.164	-0.083	1						
Flavo	0.173	0.021	0.26	-0.156	-.562(**)	0.017	-.776(**)	0.208	-.402(**)	-0.258	.757(**)	0.072	-0.073	1					
Alka	0.078	-0.012	-0.246	0.132	0.191	-0.077	.351(*)	-0.049	0.137	0.158	-.488(**)	0.192	0.209	-.594(**)	1				
Chl a	-0.239	0.088	-0.096	0.23	0.258	-0.159	.553(**)	-0.216	.366(**)	0.256	-.527(**)	0.242	-0.02	-.676(**)	.472(**)	1			
Chl b	-0.072	0.084	-0.251	.358(**)	0.245	-0.131	.517(**)	-0.21	0.194	0.246	-.459(**)	0.117	0.091	-.597(**)	.475(**)	.843(**)	1		
Carot	-0.16	0.112	-0.011	0.193	-0.072	-.304(*)	.286(*)	-0.232	0.189	0.252	-0.183	.375(**)	0.032	-.292(*)	.306(*)	.804(**)	.752(**)	1	
TP	-0.196	0.097	-0.122	0.267	0.204	-0.191	.522(**)	-0.23	.310(*)	0.268	-.472(**)	0.252	0.017	-.619(**)	.467(**)	.980(**)	.912(**)	.878(**)	1

Table 3: Correlation coefficient (r) values of the relationships between rosemary morphological and chemical characters under the effect of presoaking in different concentrations of IBA at different times. Root number (RN), Fresh weight of root (FWR), Dry weight of root (DWR), Water content of root (WCR), Fresh weight of leaves (FWL), Dry weight of leaves (DWL), Water content of leaves (WCL), Cut length (Cut L), Root length (Root L), Leaf area (Leaf A), Rosmarinic acid (RA), Phenolic compounds (Ph), Saponins (Sapo), Flavonoids (Flavo), Alkaloids

(Alka), Chlorophyll a (Chl a), Chlorophyll b (Chl b), Carotenoids (Carot), Total pigments (TP), (*) Correlation is significant at r<0.05 , (**) Correlation is significant at r<0.01.

The transplanted rooted cuttings into peat moss soil survive and continue to growg. All the IBA treatments led to 100% survival of the rooted cutting in the peat moss soil with out any other additions. The continuous observation of the transplanted cuttings for three months indicated more vigor and better growth for IBA treated cuttings in comparison with the control ones.

Discussion

The study was designed to investigate the effect of indole-3-butyric acid (IBA) hormone that might initiate the rooting of rosemary cuttings needed for rosemary plant propagation. Cuttings of rosemary were subjected to high concentrations of indole-3-butyric acid (IBA) for different times or to low concentrations continuously to initiate roots on the cuttings. At the time of no new roots were produced the cutting were harvested and the morphological and metabolical characteristics of the produced roots and of cuttings were studied and the interrelationships between these characters were analyzed.

The number, length, fresh and dry weights, and water content of the induced roots were affected by IBA treatments as also found by Nada et al. [20]. Soaking in different concentrations of IBA for one, three and six hours had various impacts on the morphological characteristics of the rosemary cuttings which are in agreement with the findings of Panwar et al. [21] and Schoellhorn [22]. The number of the induced roots per cutting of rosemary was significantly increased by time of cutting Soaking and concentration of the IBA as also found by Talia et al. [13], Scalon et al. [23] and Nada et al. [20]. The root number was five times the control value by Soaking cuttings in 60 ppm IBA for three hours but greater root number was induced on cutting by increasing time of Soaking in lower IBA concentration as was found by Shah et al. [24] and Silva and Pedras [15]. This increase in root number and length by IBA improve the success and survival of the cuttings after transplanting in the soil. Also, Hartmann et al. [8] appreciated using IBA in stimulating rooting as it is non toxic to plants over a wide concentration range and it improves root initiation and uniformity of rooting. The length of the induced roots on rosemary cuttings was sensitive to both IBA concentration and time of soaking in. however, reduction in root length was exhibited by IBA concentrations and elongation of soaking as was also found by Chauhan et al. [12] while, low concentrations increased the cuttings root length and for example 20 ppm increased root length by about 17%. The marked reduction in root lengths was due to six hours of soaking in the highest IBA concentration (100 ppm), data was in contrast with that of Scalon et al. [23].

The dry weight of induced roots on rosemary cuttings acquired high values the used concentrations of IBA at three hours of soaking but those values accompanied with a decrease in root number. Similar results were also found by Silva and Pedras [15]. This may indicate accumulation in metabolites in the produced root instead of initiation of new ones. Soaking for three hours at 100 ppm of IBA increased dry weight of roots by 15%. Similar effects have been reported by Hosni et al. [25] and Singh et al. [26,27] on Bougainvillea buttiana and by Rowezak [28] on Ficus retusa and F. benjamina. The IBA changes of root fresh and dry weights reduced significantly the water content of the induced root. The response was remarkable by time of cuttings soaking in IBA but slightly by IBA concentrations. This may shortened the time of cuttings tolerance during cultivation as roots will dry earlier.

The growth of rosemary cuttings was slightly during soaking in IBA where the cuttings length was increased by 3.3%, 2.8% and 4.0% compared with the control value by one, three and six hours of Soaking in 80, 80 and 40 ppm IBA concentrations respectively. Similar slight increase was in leaf area of rosemary cuttings by time of soaking in the used concentrations of IBA that reached 20, 16.3 and 9% over the control plant leaf area by one, three and six hours of cuttings soaking in 60, 40 and 20 ppm IBA concentrations respectively. This may indicate that short time of Soaking in high concentration of IBA induces cuttings growth and increases their leaf area which is important for cutting propagation. This also may indicate that metabolism process of cuttings was not only directed by the hormone to root initiation but also to the growth of cuttings. This was also confirmed by the fresh weight of rosemary cuttings leaves which was increased by soaking for one and three hours (33.8% maximum increase), But on the contrary, soaked cuttings for six hours in most IBA concentrations inhibited their growth as was found by Azimi and Bisgrove [29] in Rosa. This inhibition in cuttings growth due to the soaking for six hours caused accumulation of metabolites in the cuttings that increased their dry weight as compare with one and three hours soaking. The effect was magnified by soaking in high concentration of IBA (100 ppm) which led to the high leaves dry weight confirming observed reduction in dry material utilization in root production and or growth in cuttings. These responses were in agreement with the findings of Singh et al. [26] and Sharma et al. [30]. Devlin and Witham [31] attributed the promotion effect of IBA for vegetative growth to the enhancement of rooting and root growth on the treated cuttings that enhances uptake of water and nutrients from the growing medium. The water content percentages in rosemary leaves significantly responded to changes in time of cutting soaking in the different concentrations of IBA [31]. High leaves water content percentages were recorded in the presoaked cuttings for one and three hours in all IBA concentrations and prolonging time of Soaking to six hours remarkably decreased the leaves water content.

The photosynthetic pigments content of rosemary leaves was not affected significantly by IBA concentrations, but time of Soaking in these IBA concentrations was significantly effective as soaking for one hour caused a marked increase in chlorophyll a content which is in agreement with the results of Abu-Grab and Ebrahim [32] and Abdel-Wahed et al. [33]. According to Midan et al. [34], Ludwig- Muller et al. [35] and Ludwig-Muller [36], IBA increases chlorophyll accumulation. Soaking for longer time 3 and 6 hrs) decreased chlorophyll a with progressive values by the increase of IBA concentrations. The minimum content was due to Soaking for six hours in this IBA concentration, results agreed with those of Wiesmann and Lavee [37]. Chlorophyll b metabolism enhancement by one hour of cutting Soaking was also attenuated by the increase in soaking time for three and six hours which decreased chlorophyll b especially with the highest concentration of IBA. Similar effects have been reported by Wiesman and Lavee [37], in the cuttings of olive. Leaves carotenoids markedly accumulated by Soaking for one hour with increasing the concentration of IBA but prolonged soaking to six hours, mostly decreased carotenoids than the control value. The total pigments in the leaves of rosemary cuttings followed the trend of variations in

chlorophyll a however, one hour Soaking in all IBA concentrations led to the highest total pigments while 3 and 6 hours soaking decreased with few exceptions the total pigment content as also obtained before [37]. Chlorophyll (a+b)/c ratio increased by soaking for one and three hours in all concentrations of IBA but prolonged time of Soaking for six hours on the other hand decreased the ratio. This was due to more inhibition in metabolism of chlorophyll a and b than in that of carotenoids.

Rosmarinic acid accumulated in rosemary leaves by about 1.2% and 5.6% due to 20 and 60 ppm IBA concentrations as also found by Munne-Bosch et al. [38] and Ain-Lhout et al. [39] but it was sharply inhibited by higher concentrations soaking for one and three hours (32% and 33%). Frankel et al. [40] and Del Bano et al. [41] due the decrease in rosmarinic acid in the cuttings to the oxidation of caffeic acid by phenolases and peroxidases which is responsible for the decomposition and disappearance of rosmarinic acid, in one hand, and to the limitation of important nutrients such as nitrate and ammonium ions in the medium on the other hand.

However, flavonoid content in the leaves of rosemary cuttings decreased in response to both IBA concentration and time of soaking. Decreases in flavonoids content ranged between a minimum decrease by 0.32% to a maximum decrease by 42.3% due to soaking for one and six hours in 100 ppm of IBA. It is therefore quite possible that the decrease in flavonoids may be due to their transformation into IAA in adventitious root formation as reported by Stenlid [42] and Mosella and Macheix [43].

Both IBA concentration and time of Soaking affect significantly alkaloids of leaves of rosemary cuttings with an exception of one hour soaking that increased alkaloids especially when concentration of IBA increased. The maximum alkaloids content was 21.4 mg/g dry weight. Three hours soaking increased alkaloids with increasing the concentration of. Alkaloids content was reduced after six hours of soaking with most of used IBA concentrations.

Saponin content in leaves of rosemary cuttings exhibited a general inecrease by soaking time and with most of IBA concentrations. The maximum saponin content was 66.5 mg/g dry weight at 60 ppm and was higher by 14.4% than the control value. These results agreed with those of Kim et al. [44]. The later author found in *Panax ginseng* indole-3-butyric acid at 25 μM with methyl jasmonate at 100 μM stimulated ginsenoside saponin accumulation compared with 100 μM methyl jasmonate alone.

Phenolic compounds was found in the plant leaves by small countities compared with the other secondary metabolites and they acquired a general decrease in the leaves of rosemary cuttings by elongating time of soaking in IBA. Phenolic compounds contents ranged between a maximum of 3.83 and a minimum of 0.64 mg/g dry weight. Increase in phenolic compounds by IBA was reported by many authors such as Rowezak [28], Habba [45], Qaddoury and Amssa [46] and Amin et al. [47].

The continuous soaking of rosemary cuttings in low concentrations of IBA increased the produced roots and this increase was a function of concentration of IBA. Maximum rooting was by 15 ppm which increased roots about 10 times compared to the control. These results resembled the results of Shah et al. [24] and Silva and Pedras [15]. The length of produced roots on cuttings increased in response to the increase in IBA concentration. The longest roots were due to 3 ppm concentration of IBA where root length was increased by 170% compared to control. This result is in agreement with those of Scalon et al. [23].

Fresh weight of growing roots on the cuttings increased with high concentrations of IBA. Similar effect had been reported by Hosni et al. [25] and Singh et al. [26,27] on *Bougainvillea buttiana* and Rowezak [28] on *Ficus retusa* and *Ficus benjamina*. Dry weight of the roots decreased with increasing the concentration of IBA in comparison with control. This marked decrease in root dry weight could be a result of utilization of metabolites in the growth of roots length. Water content of root increased with the increase in IBA concentration.

Length of rosemary cuttings and area of their leaves did not show marked effect with continuous application of IBA. This indicated 1- A negligible affect of long application of low concentrations of IBA on the morphology of the cutting 2- Over all metabolites were directed to rooting. These results agreed with those of Hussein [48]. Fresh and dry weight of rosemary cuttings leaves significantly increased with IBA concentrations. The maximum increase was at 10 ppm. The increase in the vegetative growth of rosemary due to IBA treatments was also found by Singh et al. [21] on *Gardenia lucida*. Sharma et al. [30] reported that IBA improved plants vegetative growth.

The photosynthetic pigments chlorophyll a, b and carotenoids of rosemary leaves was affected by continuous soaking of cuttings in IBA. Chlorophyll a metabolism was accelerated by the increase in IBA concentration with a maximum at 20 ppm that was higher by 19.4% compared to control. These results are in agreement with those obtained by Abu-Grab and Ebrahim [32] and Abdel-Wahed et al. [33]. The metabolism of chlorophyll b and carotenoids on the opposite was mostly inhibited and the resulted decreases reached to 26.8% and 16.8% at 5 ppm concentration of IBA. Similar effects have been reported by Wiesman and Lavee [37] and Abu-Grab and Ebrahim [32]. Total pigments were increased with IBA concentrations as a result of the marked increase in chlorophyll a over the decrease of the other pigments. This was also confirmed by the increase in chlorophyll a/b and chlorophyll (a+b)/c ratios. The highest increase in the previous ratios was in chlorophyll a/b especially at 10 ppm where it was by 37.5%.

The prolonged application of the used low concentrations of IBA had no effect or slightly increased rosmarinic acid of rosemary leaves. The maximum increase (14% compared to control) was at 15 ppm. Which agree with results of Reda et al. [49]. Continuous soaking of rosemary cuttings in IBA inhibited the metabolism of alkaloids which decreased by 42.7% at 10 ppm IBA concentration. Creus and Barcklo [50] found slight decrease in the alkaloids content by IBA on *Nicotiana rustica*.

The marked increase in response to prolonged IBA application was in the phenolic compounds of rosemary leaves. The 15 ppm concentration of IBA led to 68.3% increase compared to control. Qaddoury and Amssa [46] reported that phenolic compounds increased immediately after IBA treatment. Saponin in rosemary leaves accumulated only at high concentrations of IBA and Kim et al. [44] found stimulation in saponin accumulation by similar treatments.

The number and character of the induced roots by IBA at specific time correlation coefficients showed significant correlations between cut length, water content, chlorophyll a, total pigments and rosmarinic acid. Also, the content of rosmarinic acid in the rosemary leaves was found to be sensitive to the water content and photosynthetic pigments of the cuttings. Mortality of cuttings which soaked continuously in IBA decreased with increasing concentration until it

reached zero by 5 ppm IBA after it mortality increased again with the increase in IBA concentration, which suggesting this concentration for reserving the plant cuttings during manipulation during transportation.

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