

**PHYSIOLOGICAL RESPONSE OF ROSE GERANIUM (*Pelargonium graveolens*, L.) TO PHENYLALANINE AND NICOTINIC ACID  
BY**

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**ABSTRACT**

A pot experiment was conducted in the screen of the National Research Centre to study the effect of foliar application of phenylalanine and nicotinic acid on the growth and chemical constituents of geranium plants (*Pelargonium graveolens*). The results indicated that foliar application of phenylalanine, nicotinic acid or their combinations influenced the vegetative growth of geranium plants, especially when plants were sprayed with phenylalanine (100 mg/l) combined with nicotinic acid (50 mg/l). Data also show that foliar application of phenylalanine (100 mg/l) combined with nicotinic acid (50 mg/l) significantly increased total protein contents, essential oil% and essential oil yield. On the other hand, phenylalanine (100 mg/l) combined with nicotinic acid (100 mg/l) resulted in the highest content of linalool and citronellol contents in geranium oil.

The hormonal picture indicated that the bioregulators alone or in combinations caused an increase in IAA, GA-like substances and markedly decreased ABA. Plants sprayed with 50 mg/l nicotinic acid combined with 100 mg/l phenylalanine gave the highest biological activity of IAA and gibberellin-like substances in comparison with the other treatments.

**INTRODUCTION**

*Pelargonium graveolens*, Family Geraniaceae is one of the many fragrant species of *Pelargonium* used as a source of geranium oil. As a medicinal plant, geranium has traditionally been considered an astringent and as a folk remedy in the treatment of ulcers (Grieve, 1994; Bown, 1995). A terpine hydrate synthesized from geraniol is known to be, an effective expectorant. Geranium leaves are reported to have antifungal activity. Scented geranium and its oil are reported to cause contact dermatitis. Geranium is also reported to repel insects because of its citronellol content. *Pelargonium graveolens* is an aromatic, rose-scented herb, the whole herb has relaxant, anti-depressant and antiseptic effects, reduces inflammation and controls bleeding (Bown, 1995). All parts of the plant are astringent. It is used internally in the treatment of pre-menstrual and menopausal problems, nausea, tonsillitis and poor circulation. Externally, it is used to treat acne, haemorrhoids, eczema, bruises, ringworm and lice (Bown, 1995).

The leaves can be used at any time of the year. The essential oil from the leaves is used in aromatherapy and is also applied locally to cervical cancer (Grieve, 1994).

Hathout *et al.* (1993a, b) found that the application of 10, 40 and 80 mg/l nicotineamide as foliar spray on tomato plants caused stimulatory effects on growth, yield and endogenous promoters (auxins and gibberellins). The stimulatory effects were found to be correlated with the increase in content and activity levels of endogenous promoters particularly GA<sub>3</sub> and IAA which are known to promote linear growth of plant organs: (Stoddart, 1986 and Wilkins, 1989). In addition, the stimulatory effects of nicotinic acid may be due to its role in increasing pigments, carbohydrates, nitrogen, RNA and DNA contents in plants (Sana and Ota, 1977; Bearder, 1980; Sharaf El-Din *et al.*, 1987 and Hathout *et al.*, 1993a, b).

Aberg (1961) indicated that amino acids can act as growth factors of higher plants since they are the build blocks of protein synthesis, which could be enzymes important for metabolic activities. Moursy *et al.* (1988) working on *Datura stramonium* L. indicated that phenylalanine or ornithine increased the fresh and dry weights of callus explants. Gamal El-Din *et al.* (1997) reported an increase in vegetative growth of lemongrass as a result of ornithine and phenylalanine treatments. In addition, phenylalanine application significantly increased fresh and dry weights of *Datura* during vegetative and flowering stages (Youssef *et al.*, 2004).

## MATERIAL AND METHODS

### Plant material and growth conditions.

This work had been conducted at the Experimental Farm of National Research Centre, Dokki, Cairo. Uniform cuttings (two cuttings/pot) were planted in clay pots, 30 cm diameter, at 10<sup>th</sup> and 15<sup>th</sup> November during two successive seasons (2000-2001 and 2001-2002), respectively.

Plant material used in this work consisted of terminal cuttings of geranium, uniform in size and shape (5-6 buds), kindly supplied from the Experimental Station of Pharm. Sci. Dept., Giza, Egypt.

Each pot was supplied with four g calcium superphosphate (15.5 % P<sub>2</sub>O<sub>5</sub>) and one g potassium sulphate (48 % K<sub>2</sub>O) mixed with the soil before transplanting. Ammonium nitrate (33.5 % N) was applied in two applications (one g for each) with two weeks interval started 30 days after transplanting. Fertilization was repeated after collecting the first cut. Other agricultural processes were performed according to normal practice.

Plants were foliarly sprayed with phenylalanine (99.5%), Sigma, USA (zero, 50 and 100 mg/l) and nicotinic acid (99.5%), ACROS Organics, USA (zero, 50 and 100 mg/l). Untreated plants were sprayed with distilled water. Interaction treatments of the different concentrations of the two factors had been

also carried out. Phenylalanine treatments had been applied at 17<sup>th</sup> March, 2001, 2002 and nicotinic acid at 18<sup>th</sup> March, 2001, 2002. The first cut was collected at 7<sup>th</sup> & 12<sup>th</sup> June 2001 and 2002, respectively. Then, plants of the second cut were sprayed with phenylalanine (18<sup>th</sup> Aug., 2001, 2002) and nicotinic acid (19<sup>th</sup> Aug., 2001, 2002). The second cut was collected at 12<sup>th</sup> & 17<sup>th</sup> Nov. 2001 and 2002, respectively. The experimental design was factorial complete randomized blocks with three replicates, each replicate represented by 3 pots. The volume of the spraying solution was maintained just to cover completely the plant foliage till drip.

**Measurement of growth parameters:**

The plant herbage was harvested, by cutting 10 cm over the soil surface, and plant growth characters in terms of plant height (cm), number of branches per plant, herbage fresh and dry weights were recorded.

**Chemical analysis:**

Total protein was determined using the method of Bradford (1976). A minimum of three representative 100 g samples of the fresh herbage of each treatment were separately subjected to hydro-distillation in order to determine the percentage of essential oil according to the Egyptian Pharmacopoeia (1984). The resulted essential oil from each treatment of the second cut was dehydrated over anhydrous sodium sulfate, then subjected to GLC analysis with Varian VISTA 6000 FID model to determine the essential oil constituents. The separation was carried out with 2 m x 1/8" stainless steel, 3 % OV-101 Column. The flow rate of the carrier gas (nitrogen) was maintained at 50 ml/min. The Column temperature was programmed from 80° to 200° C at the rate of 4°C/min. The injection port temperature was maintained at 180° C and detector at 240° C. The relative percent of the different compounds was determined by Varian 4270 integrator. The identification of these compounds was achieved by matching their retention times with those of authentic samples injected with the same conditions. More conformation was carried out by injection of the authentic samples with the oil samples.

**Extraction, purification and determination of endogenous hormones:**

For the determination of endogenous auxins, abscisic acid (ABA) and gibberellin-like substances the plant samples were weighed and immediately frozen with liquid nitrogen and stored in deep freezer at -20°C (for a period not exceeding 3 weeks) until required for extraction and determination of the hormones. The frozen plants were extracted with ice cold methanol using a blender and the extract was fractionated as described by Badr *et al.*, (1971).

A volume of acidic extracts of IAA, ABA and GA-like substances equivalent to 10 g fresh weight of plant material was loaded onto paper chromatography and developed in an ascending manner in the dark at 25°C ± 2°C using solvent system composed of isopropanol: ammonia: water (10:1:1 v/v) as recommended by Hayashi *et al.*, (1962). The chromatograms were segmented transversely into equal segments (representing the R<sub>f</sub> values) and their activity was estimated biologically using the straight growth wheat coleoptile section bioassay test

developed by Linser (1938, 1940) and modified by Youssef *et al.*, (1970) for IAA and ABA and sorghum first leaf bioassay test developed by Bently-Mowatt (1966) and modified by Abdel-Wahab (1982) for GA-like substances.

#### Statistical analysis:

Data obtained were subjected to standard analysis of variance procedure. The values of LSD were obtained whenever F values were significant at 5% level as reported by Snedecor and Cochran (1980).

### RESULTS AND DISCUSSION

Data presented in Table (1) show that phenylalanine treatments significantly promoted plant height, number of branches, fresh and dry weights of herb in both cuttings. Application of phenylalanine at 100 mg/l resulted in the tallest plants in most cases. These findings were in agreement with those obtained by Youssef *et al.* (2004) who reported that application of phenylalanine on *Datura* plants significantly promoted vegetative growth of the plant.

The present results emphasized that nicotinic acid significantly increased plant height, number of branches, fresh and dry weights of herb in both cuttings (Table, 1). The highest recorded values were obtained in plants treated with 50 mg/l nicotinic acid. These results are in accordance with those obtained by Tarraf *et al.*, (1999) who reported that foliar application of nicotinic acid to lemongrass plants significantly promoted vegetative growth as well as essential oil percent, oil yield per plant, total carbohydrates and crude proteins.

Data presented in Table (1) show also that foliar spray of pelargonium plants with phenylalanine (100 mg/l) combined with nicotinic acid (50 mg/l) resulted in the tallest plants. It is also clear from the obtained data that foliar spray of geranium plants with 100 mg/l phenylalanine combined with 50 mg/l nicotineamide resulted in the highest pronounced effects on number of branches as well as fresh and dry weights of herb in both cuttings (Table, 1). These results coincided with those obtained by Hathout *et al.* (1993a, b) who found that the application of 10, 40 and 80 mg/l nicotinic acid as foliar spray on tomato plants caused stimulatory effects on growth, yield and endogenous promoters (auxins and gibberellins). Similar findings were also obtained by Tarraf *et al.*, (1999) who reported that foliar application of nicotineamide to lemongrass plants significantly promoted vegetative growth as well as essential oil percentage and yield per plant, total carbohydrates and crude proteins.

The stimulatory effects were found to be correlated with the increase in content and activity levels of endogenous promoters particularly gibberellins and IAA which are known to promote linear growth of plant organs: (Stoddart, 1986 and Wilkins, 1989). In addition, the stimulatory effects of nicotinic acid may be due to its role in increasing pigments, carbohydrates, nitrogen, RNA and DNA contents in plants (Sana and Ota, 1977; Bearder, 1980; Sharaf El-Din *et al.*, 1987).

**Table (1): Effect of phenylalanine and nicotinic acid on the growth of pelargonium plants. (Average of the two seasons)**

Treatment	plant height (cm)		number of branches		Fresh weight of herb (g/plant)		Dry weight of herb (g/plant)	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
<b>Effect of phenylalanine</b>								
Control	41.22	45.56	6.00	7.11	136.82	158.22	25.10	34.96
PA 50	49.22	49.33	6.67	9.22	183.29	181.34	29.81	38.91
PA 100	53.22	52.11	7.89	11.00	219.71	205.20	45.11	40.38
LSD (5%)	1.37	1.75	0.44	0.54	5.40	3.74	1.67	1.01
<b>Effect of nicotinic acid</b>								
Control	46.33	47.78	5.78	7.00	134.85	143.28	27.22	33.35
NA50	49.67	51.56	7.67	11.33	222.29	204.37	38.65	42.89
NA100	47.67	47.67	7.11	9.00	182.68	197.10	34.15	38.01
LSD (5%)	1.37	1.75	0.44	0.54	5.40	3.74	1.67	1.01
<b>Effect of interaction</b>								
Control	41.00	40.67	5.00	5.00	90.54	107.73	15.57	27.78
PA 50	45.33	50.00	5.33	7.33	128.43	153.78	22.58	35.28
PA 100	52.67	52.67	7.00	8.67	185.59	168.33	43.52	36.99
NA50	36.67	50.00	6.33	8.67	177.50	191.73	32.85	40.66
NA100	46.00	46.00	6.67	7.67	142.42	175.19	26.87	36.45
NA50+PA 50	54.67	50.67	7.67	11.67	237.19	195.13	35.15	43.70
NA50+PA 100	57.67	54.00	9.00	13.67	252.19	226.25	47.94	44.31
NA100+PA 50	47.67	47.33	7.00	8.67	184.25	195.13	31.71	37.74
NA100+PA 100	49.33	49.67	7.67	10.67	221.36	221.00	43.89	39.84
LSD (5%)	2.37	3.03	0.75	0.93	9.35	6.48	2.90	1.75

PA = Phenylalanine, NA = Nicotinic acid

Data presented in Table (2) show that foliar application of phenylalanine to geranium plants significantly increased total protein content, especially at 100 mg/l. These increases hold true for both cuttings. These results are in agreement with those obtained by Gamal El-Din and Abd El-Wahed (2005) who reported that application of phenylalanine to chamomile plants significantly increased total amino acids, total nitrogen and crude protein contents in aerial vegetative parts. Tarraf (1999) reported similar results on lupine plants.

Data presented in Table (2) show that foliar application of nicotinic acid to geranium plants significantly increased total protein content, especially in plants treated with 50 mg/l nicotinic acid. These results could be explained by the findings obtained by Youssef and Talaat (2003) who reported that nicotineamide treatments to rosemary plants significantly increased total nitrogen contents.

These results are also in agreement with those obtained by and Deyab (1989) on wheat plants, and Hathout *et al.* (1993a,b) on tomato plants. The stimulatory effects were found to be correlated with the increase in content and activity levels of endogenous promoters particularly GA<sub>3</sub> and IAA which are known to promote linear growth of plant organs: (Stoddart, 1986 and Wilkins, 1989).

Nicotineamide is considered as one of growth regulating substances which in minute quantities can alter some physiological aspects of plants (Bearder, 1980). Robinson (1973) reported that nicotineamide acts as coenzyme in the enzymatic reactions by which carbohydrates, fats and proteins are metabolized and involved in photosynthesis and respiration.

In addition, the stimulatory effects of nicotineamide may be due to its role in increasing pigments, carbohydrates, nitrogen RNA and DNA contents in plants (Sana and Ota, 1977; Bearder, 1980; Sharaf El-Din *et al.*, 1987 and Hathout *et al.*, 1993a, b).

Data presented in Table (2) show that oil % and total oil yield/plant were significantly increased as a result of foliar spray of phenylalanine, nicotinic acid or their combinations. It is clear from the obtained data that the highest recorded value of essential oil was obtained in the herb of plants treated with 100 mg/l phenylalanine combined with 50 mg/l nicotinic acid treatment. These results hold true for essential oil % and total oil yield/plant in both cuttings.

These changes in the oil percentage could be attributed to nicotinic acid effect on metabolism and enzyme levels responsible for mono or sesquiterpene biosynthesis as reported by Lawrence (1978). Furthermore, it was found that the enzymes responsible for the biosynthesis of higher terpenes arised in plastids as reported by Amelunxen and Arbeiter (1967), and these enzymes could cause an increase in the essential oil. Gamal El-Din and Abd-El-Wahed (2005) also reported that application of phenylalanine to chamomile plants significantly increased essential oil percentage and yield. Tarraf (1999) reported similar results on lupine plants.

The oil of geranium plant from different treatments in addition to that of the untreated control were subjected to fractionation using gas liquid chromatography (GLC) and the data are represented in Table (3). The non-identified compounds ranged from 3.24 to 9.84 %. Twelve hydrocarbon and oxygenated terpenes were markedly identified which are grouped into three classes, i.e., major constituents (more than 10%), minor constituents (less than 10 %) and traces (less than 1 %). Accordingly it is clear from the obtained data that citronellol is the major component of the essential oil and ranged from (30.87% - 35.52%).

Hydrocarbon terpenes ranged from 5.94%-8.80%, while total oxygen compounds ranged from 82.44%-89.20%.

It is also clear from data presented in Table (3) that plants treated with nicotinic acid (50 mg/l) combined with phenylalanine (100 mg/l) recorded the highest level of total oxidized compounds (89.20%). Plants treated with phenylalanine at 100 mg/l recorded the lowest content of total oxygenated compounds (82.44%) and the highest level of total hydrocarbons (8.80%).

Table (2): Effect of phenylalanine and nicotinic acid on some chemical constituents of pelargonium plants.

Treatment (mg/l)	Total proteins ( $\mu\text{g/g}$ FW)		Total Oil %		Oil yield (ml/plant)	
	First cut	Second cut	First cut	Second cut	First cut	Second cut
<b>Effect of phenylalanine</b>						
Control	1609.79	1640.49	0.16	0.18	23.13	29.04
PA 50	1652.92	1677.04	0.18	0.19	32.93	34.73
PA 100	1742.83	1765.49	0.19	0.22	42.59	44.99
LSD (5%)	22.51	20.04	0.01	0.01	1.69	1.01
<b>Effect of nicotinic acid</b>						
Control	1563.01	1650.73	0.17	0.17	23.13	25.12
NA50	1745.02	1736.98	0.19	0.22	41.89	45.30
NA100	1697.51	1695.32	0.18	0.19	33.62	38.34
LSD (5%)	22.51	20.04	0.01	0.01	1.69	1.01
<b>Effect of interaction</b>						
Control	1535.23	1614.18	0.15	0.16	13.58	17.24
PA 50	1565.93	1644.88	0.16	0.17	20.55	26.14
PA100	1587.86	1693.13	0.19	0.19	35.26	31.98
NA50	1653.65	1666.81	0.17	0.20	30.18	38.35
NA100	1640.49	1640.49	0.18	0.18	25.64	31.53
NA50+PA50	1699.70	1732.60	0.19	0.21	45.07	40.98
NA50+PA100	1881.72	1811.54	0.20	0.25	50.44	56.56
NA100+PA50	1693.12	1653.65	0.18	0.19	33.17	37.07
NA100+PA100	1758.91	1791.81	0.19	0.21	42.06	46.41
LSD (5%)	38.99	34.70	0.01	0.01	2.92	1.74

PA = Phenylalanine, NA = Nicotinic acid

In this respect several investigators studied the effect of growth regulators on the different constituents of the essential oil (Youssef and Talaat, 1998; Tarraf *et al.*, 1999; Refaat and Balbaa, 2001 and Talaat and Youssef, 2002). However, our findings reveal that application of phenylalanine and/or nicotinic acid may favour the conversion among the terpenic constituents through some enzymatic systems. In this connection El-Keltawi & Croteau (1986 a, b) reported that the influence of growth regulators on essential oil composition and oil yield are most readily explained by alterations in the levels or the activities of the relevant enzymes. Farooqi *et al.*, (1999) determined the biosynthesis and catabolism of menthol stereoisomers using peppermint as model system. The metabolic turnover of monoterpenes in mints represents a mechanism for recycling carbon and energy from terpenes into other metabolites of the rhizome.

These results are in agreement with those obtained by Trehasne *et al.* (1970), who found that plants treated with growth regulators showed a high carboxylating activity due apparently to enzyme activation, and Mohamed *et al.* (1992) discuss the effect of vitamin B6 on bornyl acetate and borneol the main constituents of essential oil of *Alpinia nutans* and concluded that the low

concentration 50 ppm resulted in maximum value of bornyl acetate and the minimum value of borneol. On the other hand, the high concentration showed the positive effect. This result may be attributed to the positive effect of vitamin B6 on the enzymatic systems responsible for acetylation.

**Table (3): Effect of phenylalanine and nicotinic acid on the essential oil constituents of geranium plants.**

Treatments (mg/l) Constituent (%)	Control	PA 50	PA100	NA50	NA100	NA50+PA50	NA50+PA100	NA100+PA50	NA100+ PA100
A-pinene	2.16	1.88	1.75	1.75	1.92	2.72	2.29	1.67	2.10
B-pinene	5.06	4.80	7.05	4.19	4.68	5.56	5.27	4.59	5.44
1,8-cineol	2.76	2.58	2.72	2.32	2.79	3.29	2.59	2.66	2.74
Linalool	10.03	10.13	9.97	10.48	10.13	11.37	10.84	9.21	13.24
Isomenthone	2.48	2.48	2.89	2.47	2.70	2.64	2.50	2.52	2.66
Citronellol	31.91	31.98	34.32	32.15	31.83	35.27	35.42	30.87	35.52
Geraniol	10.21	9.91	10.19	9.93	11.37	9.90	11.03	11.77	9.87
Carvon	2.67	3.06	0.00	2.77	2.77	2.70	2.70	2.90	2.25
Citronellylformate	14.07	14.66	14.64	15.40	14.02	13.71	15.11	14.07	13.47
Geranylbutyrate	7.47	7.53	6.15	8.02	7.09	6.87	7.97	7.73	6.17
geranyltiglate	3.58	1.90	1.31	2.06	0.85	0.80	1.05	1.30	0.00
Eugenol	1.10	0.48	0.24	0.65	0.00	0.00	0.00	1.92	0.00
Hydrocarbons	7.23	6.68	8.80	5.94	6.61	8.28	7.56	6.27	7.55
Oxidized compounds	86.27	84.70	82.44	86.25	83.55	86.57	89.20	84.94	85.93
Total Identified	93.50	91.38	91.24	92.19	90.16	94.85	96.76	91.21	93.48

PA = Phenylalanine, NA = Nicotinic acid

Foliar application of phenylalanine (100 mg/l) combined with nicotinic acid (100 mg/l) possessed the best quality of the essential oil because of its low content of 1,8-cineol and a high content of linalool and citronellol which is a desirable character.

#### Endogenous hormones

##### Effect on endogenous IAA and ABA

The biological activities of endogenous IAA and ABA in wheat coleoptile section bioassay test of purified acidic extracts of treated and untreated pelargonium plants are illustrated in Figure (1).

Two promotion zones were detected with all extracts, first at  $R_f$  0.0-0.5 and second at  $R_f$  0.9-1.0. The promotion activity was higher with all treatments compared with control plants.





The first promotion zone of activity was correlated with authentic IAA. Moreover, this zone in all cases gave purple colour with Erlich reagent, pink colour with Salkowski reagent and fluorescent colour when exposed to UV. On the basis of  $R_f$  value, colour reactions and ultraviolet fluorescence, confirmed that this growth promoter is IAA.

An inhibition zone was evident at  $R_f$  0.6-0.8 which is corresponded with  $R_f$  of ABA authentic standard. In addition, this zone of inhibition gave the colour of fluorescence of ABA on TLC plates after spraying with 5%  $H_2SO_4$  and examined by UV. The inhibitory level was lower in all treatments compared with untreated plants. The magnitude of inhibition in treated plants declined to reach about quarter the value of that shown by control plants. It is worthy to mention that the highest activities of IAA and disappearance of ABA was given with foliar spraying of pelargonium plants with 50 mg/l nicotinic acid combined with 100 mg/l phenylalanine, followed by 100 mg/l nicotinic acid combined with 100 mg/l phenylalanine.

#### **Effect on endogenous gibberellin-like substances**

The biological activities of endogenous gibberellins in sorghum first leaf bioassay test of purified acidic extracts of treated and untreated pelargonium plants are illustrated in Figure (2).

All used treatments increased promotion activities over control plants. Plants sprayed with 50 mg/l phenylalanine showed an increase in promotion activities of gibberellin with maximum activity at  $R_f$  0.4-0.5. In case of 100 mg/l phenylalanine showed also maximum activities of gibberellin at  $R_f$  0.1-0.2.

Extracts of plants treated with 50 mg/l nicotinic acid show one zone of gibberellin activities located at  $R_f$  0.0-1.0. On the other hand, plants sprayed with 50 mg/l nicotinic acid combined with 50 mg/l phenylalanine showed that three zones of gibberellin activities located at  $R_f$  0.0-0.3, at  $R_f$  0.4-0.5 and 0.6-1.0 and two zones of growth inhibitor at  $R_f$  0.3-0.4 and at  $R_f$  0.5-0.6.

Plants sprayed with 50 mg/l nicotinic acid combined with 100 mg/l phenylalanine gave the highest biological activity of gibberellin-like substances in comparison with the other treatments.

Regarding plants treated with 100 mg/l nicotinic acid, one zone of gibberellin inhibitor at  $R_f$  0.3-0.4 was obtained and this treatment gave a lower amount of gibberellin activities in comparison with other treatments.

In case of plants sprayed with 100 mg/l nicotinic acid combined with 50 mg/l phenylalanine, the regions of gibberellin activities were clear at  $R_f$  0.0-0.3 and at  $R_f$  0.4-1.0 and region of growth inhibitor located at  $R_f$  0.3-0.4.

In case of 100 mg/l nicotinic acid combined with 100 mg/l phenylalanine it showed also one zone of gibberellin activities located at  $R_f$  0.0-1.0.

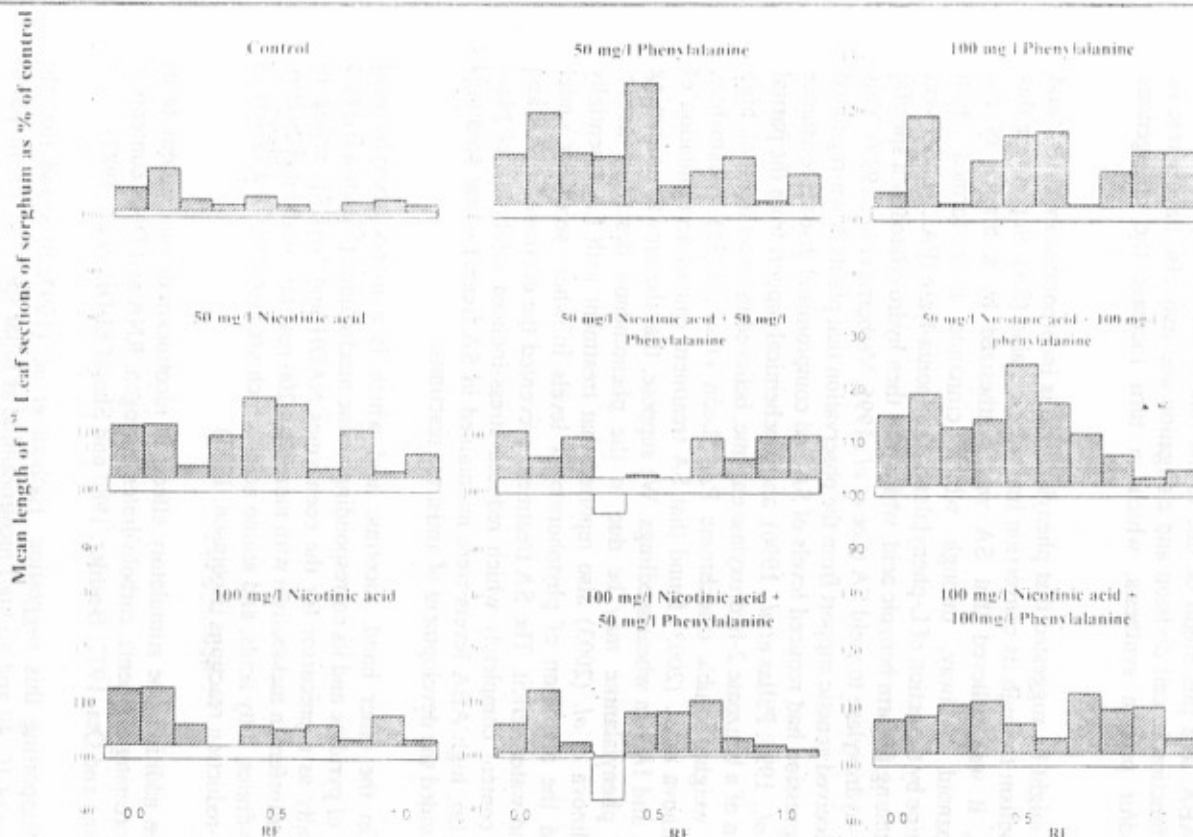


Fig (2) : The biological activities of endogenous gibberellins like substances in sorghum first leaf bioassay test of purified extracts of treated and untreated *Pelargonium* plant.

-Load of each chromatogram equivalent to 10 gms of fresh weight.

-Shaded zones indicate promotion significance.

Regarding untreated plants, very low gibberellin activities were observed at  $R_f$  0.2-0.6 and 0.7-1.0 and one inhibition zone at  $R_f$  0.6-0.7.

It is clear from Figure (2) that gibberellin activities were higher due to all treatments studied than control plants.

The hormonal picture indicated that the bioregulators alone or in combinations caused an increase in IAA, GA-like substances and markedly decreased ABA, and this might be due to the role of these compounds in the regulatory function in cell division and elongation and may also have a role in control and/or protein synthesis, which in turn increase the endogenous hormones.

It might be suggested that phenylalanine is incorporated into ABA and IAA metabolism through its conversion into salicylic acid (SA). Supporting this suggestion, it was believed that SA was synthesized by a branch of the phenylpropanoid pathway, through which cinnamic acid formed from phenylalanine by the action of L-phenylalanine ammonia-lyase (PAL), underwent chain shortening to form benzoic acid, which was then hydroxylated by a specific benzoate 2-hydroxylase to yield SA (Lee *et al.*, 1995; Verberne *et al.*, 1999). This pathway received genetic support from the observation that plants down-regulated in PAL expression had reduced levels of SA and compromised disease resistance (Maher *et al.*, 1994; Pallas *et al.*, 1996), and biochemical support from the partial purification of a benzoate 2-hydroxylase enzyme, believed to be an unusual, high molecular weight, soluble cytochrome  $P_{450}$  (León *et al.*, 1995). Meanwhile, Sakhabutdinova *et al.* (2003) found that SA treatment caused accumulation of both ABA and IAA in wheat seedlings. We suppose, that the growth promoting effects of phenylalanine may be due to the phenomenon described above. Sakhabutdinova *et al.* (2003) also reported that treatment with SA essentially diminished the alteration of phytohormones levels in wheat seedlings under salinity and water deficit. The SA treatment prevented the decrease in IAA and cytokinin content completely which reduced stress-induced inhibition of plant growth. Also, high ABA levels were maintained in SA treated wheat seedlings which provided the development of antistress reactions.

On the other hand, nicotinic acid which is a monocarboxylic acid derivative of pyridine and its corresponding amine niacinamide (Vitamin  $B_3$ ) acts metabolically as a precursor for the coenzymes NADH and NADPH acting in hydrogen transfers in metabolism with more than 200 reactions in the metabolism of carbohydrates, fatty acids, and amino acids which are essential for a variety of oxidation-reduction reactions (Robinson, 1973).

In addition, the stimulatory effects of nicotineamide may be due to its role in increasing pigments, carbohydrates, nitrogen, RNA and DNA contents in plants (Sana and Ota, 1977; Bearder, 1980 and Sharaf El-Din *et al.*, 1987).

Supporting this suggestion, Hathout *et al.* (1993a,b) found that the application of 10, 40 and 80 mg/l nicotineamide as foliar spray on tomato plants

caused stimulatory effects on growth, yield and endogenous promoters (auxins and gibberellins). The stimulatory effects were found to be correlated with the increase in content and activity levels of endogenous promoters particularly GA<sub>3</sub> and IAA which are known to promote linear growth of plant organs: (Stoddart, 1986 and Wilkins, 1989).

From the above mentioned data, it could be concluded that phenylalanine and nicotinic acid might play a role in plant phytochemical mechanisms through affecting the metabolism of terpenes, essential oil, proteins and endogenous hormones, but further studies are needed to learn more about these mechanisms.

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### الإستجابة الفسيولوجية لنبات العطر للفينيل الانين وحمض النيكوتينيك

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أجريت تجربة أصص فى الصوبة السلكية بالمركز القومى للبحوث و ذلك لدراسة تأثير الرش بالفينيل الانين وحمض النيكوتينيك على النمو الخضرى والمحتوى الكيماوى لنبات العطر. و قد أثبتت الدراسة أن رش النباتات بالفينيل الانين أو حمض النيكوتينيك أدى الى تشجيع النمو الخضرى و زيادة محتوى العشب من الزيت الطيار ، كما أدت المعاملة المشتركة بالفينيل الانين ١٠٠ مجم/لتر + حمض النيكوتينيك ٥٠ مجم/لتر الى أفضل النتائج بالنسبة للنمو الخضرى و محتوى النباتات من البروتين و الزيت الطيار ومحصول الزيت الطيار.

كما أدت معاملة النباتات بالرش بالفينيل الانين (١٠٠ مجم/لتر) + حمض النيكوتينيك (١٠٠ مجم/لتر) إلى أفضل النتائج بالنسبة لصفات الزيت الطيار حيث أدت الى زيادة محتوى الزيت من اللينالول و السيترونيلول.

كل المعاملات تحت الدراسة أدت إلى زيادة فى الأوكسينات وأشباه الجبريلينات بينما إنخفض حمض الأبسيسيك إنخفاضا ملحوظا. خاصة المعاملة المشتركة بالفينيل الانين ١٠٠ مجم/لتر + حمض النيكوتينيك ٥٠ مجم/لتر