17 *Egypt. J. Bot.,* **Vol**. **56,** No.2 pp. 303-317 (2015)

Effect of Naphthalene Acetic Acid (NAA) on Growth and Yield of Rosemary (*Rosemarinus officinalis* L.) under Salinity Stress.

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THE PRESENT study is an attempt to investigate the effect of saline soil at the levels of 0, 1000, 3000 and 5000 mg/l and foliar application of Naphthalene acetic acid (NAA) at 0.0, 25, 75, 125, 200 and 300 ppm on vegetative growth, chemical composition and oil yield production of Rosemary plant. The results indicated that increasing salinity levels decreased all measured growth parameters, chemical composition and oil production. However, foliar application of NAA reduced the harmful effects of salinity and improved the growth by increasing the concentration of NAA up to 200 ppm then decreased at higher NAA concentration (300 ppm).

Keywords: Rosemary, Salt stress, Synthetic auxins, Naphthalene Acetic Acid (NAA), Oil analysis.

Rosemary, *Rosemarinus officinalis* L. is a flowering perennial herb with evergreen needle like highly aromatic and fragrant leaves, native to the Mediterranean region, Europe and the Near East (Zargari, 1990).

Rosemary is well cultivated in Egypt and available throughout the year. It is one of the important medicinal and aromatic plants used externally as parasiticide, cicatrisant for muscular pain and rheumatism, dermatitis, dandruff and exzema. It promotes hair growth. It is used internally for asthma, bronchitis headache, renal flatulence (Valnet, 1973 and Lawless, 1992) and it is used for its volatile oil which is useful as anti-inflammatory activities, anticancer, muscle contraction, reducing carcinogens and tumors.

Soil salinity is a major abiotic stress in plant agriculture worldwide. Egypt is one of the countries that suffer from sever salinity problems and there are wide saline areas, which cause harmful effects on plant growth and its production. The harmful effects of salinity on medicinal plants and aromatic plants were mentioned by Francois *et al.* (1990), Hamad (1996) and Pathan *et al.* (2000) on guar, El-Shafey *et al.* (1991) and Zidan and Al-Zahrani (1994) on sweet basil, Ali (1996) on hyoscyamus, Dawh *et al.* (1998) on *Taget erecta*, Khalil (1999) on *Nigella sativa*on, Said Al-Ahl (1999) on basil, Ahmed *et al.* (2001) on damisissa and Attia (2003) on cluster bean. Paradia (2002) reported that growth inhibition was a common response to salinity. Shaddad and Heikal (1982) found that salt stress inhibited growth parameters in maize, cotton, pea and wheat.

Salinity induced damage in DNA, Lipids, carbohydrates and proteins and caused aberrant cell signaling (Arora *et al.*, 2002) resulting in delayed germination, poor stand establishment (Almansourit *et al.*, 2001), high seedling motility, stunted growth and lower yields (Allakhverdievet *et al.*, 2006;

Plant growth regulators are synthesized indigenously by plants, however, several studies demonstrated that plants can respond to exogenous application of these chemicals. Exogenous application of plant growth regulators affects the endogenous hormonal pattern of the plant, either by supplementation of sub-optimal levels or by interaction with their synthesis, translocation, activation or inactivation of existing hormone levels (Arshad and Frankenberger, 1993).

Application of growth regulators has good management effect on growth and yield of field crops. Hormones regulate physiological process and synthetic growth regulators may enhance growth and development of field crops thereby increased total dry mass (Das and Das, 1996; Abd-el-Fattah, 1997; Dakua, 2002; Islam, 2007 and Cho *et al*., 2008).

Auxins are phytohormones play an essential role in coordination of many growth and behavioral processes in the plant life cycle. They are major coordinating signals in plant development. Also auxin have bioregulator effects on physiological and biochemical processes in plants such as ion uptake, cell elongation, cell division, cell differentiation, sink/source regulation, enzymatic activities, protein synthesis and photosynthetic activity as well as increase the antioxidant capacity of plants (Blokhina *et al.*, 2003 and El-Tayeb, 2005). Auxins also induce sugar and mineral accumulation at the site of application, induce the formation and organization of xylem and phloem. When the plant is injured, the endogenious auxin can induce the cell differentiation and regeneration of the vascular tissues (Chaparzadeh *et al.*, 2004).

Naphthalene Acetic Acid (NAA) belongs to synthetic forms of auxins which play a key role in cell elongation, cell division, vascular tissue, differentiation, root initiation, apical dominance, leaf senescence, leaf and fruit abscission, fruit setting and flowering (Davies, 1987). Growth and yield parameters of rice are significantly promoted in response to various auxin levels (Zahir, *et al*., 1998). Naphthalene acetic acid had a significant effect on plant height, number of fruiting branches, volume of boll and yield in cotton (Abro *et al*., 2004). Naphthalene acetic acid 20 ppm showed better performance in enhancing the straw and grain yields of wheat cultivars (Alam, *et al*., 2002). Naphthalene acetic acid has been used for the enhancement of growth and yield of cereals (Lilani, *et al.*, 1991). It produces significant effects in promoting development of pointed ends for the root system, resulting in more, straighter and thicker roots. NAA can increase fruit setting ratio, prevent fruit dropping, promote flower sex ratio. Foliar application of NAA has also found to increase plant height, number of leaves per plant, fruit size with consequent enhancement in seed yield in different crops (Lee, 1990). Favourable influence of auxins such as NAA has been reported on invertase content of sugarcane (Sacher and Glasziou, 1962; Sacher *et al.*, 1963). Govindan *et al.*, (2000) indicated that soybean plants sprayed with NAA at 40 ppm after 35 days of sowing had significant increases in growth characters, yield and its attributes including number of pods and seeds, plant, seeds/pod and 100 seed weight. Senthil *et al.*, (2003) investigated the effects of NAA at 40 ppm and IAA at 100 ppm supplied as foliar spray at 35 and 60 days after sowing on some biochemical and physiological aspects including total chlorophyll and soluble protein of soybean plant. They reported that all treatments increased the biochemical parameters of soybean and IAA treatment had the highest effects on the plant Naphthalene acetic acid is a synthetic growth regulator has proved its potentiality that in appropriate concentration NAA affects the growth and yield of a number of plants *viz.* tomato (Chhonkerar and Singh, 1959), bitter gourd (Jahan and Fattah, 1991) and cowpea (Ullah *et al.,* 2007).

Material and Methods

The pot experiment was carried out on 2013/2014 to examine the physiological responses of *Rosmarinus officinalis* L. to salinity and foliar spray of naphthalene acetic acid (NAA) treatments. Also their combined treatments were investigated. Trials were conducted in triplicate using a randomized complete block design. Terminal cuttings of rosemary plant (15-20 cm long) were transplanted in 40 cm diameter plastic pots filled with 15 kg dry clay. Salinity stress was applied a month after transplantation. Four saline water samples were used as 0.0, 1000, 3000 and 5000 mg/l (NaCl). Pot irrigation was carried out three times a week. Weeds were removed mechanically and no pesticides were applied during the growing season. Plants were thinned after rooting, three plants / pot were left. Two cuttings (10 cm) above the soil surface were taken from rosemary plant; the first one was seven months after transplanting, while the second was carried out after three months from the first cut.

A plot design with three replicates was used; the main plot was the different concentrations of Naphthalene acetic acid (0.0, 25, 75, 125, 200 and 300 ppm), while the sub-plots were the different concentrations of salinity (0.0, 1000, 3000 and 5000 mg/l). Twice foliar spray of NAA was done. The first spray was applied on two months after transplanting, while the second spray was applied one week later after the first one. Spraying with NAA was repeated again after two months from the first cut. Control plants were irrigated and sprayed with H2O. Urea was applied twice at the rate of 1 g per pot. At full blooming stage, the aerial parts were harvested by cutting 10 cm above the soil surface and plant growth parameter were recorded *i.e.* plant height (cm), number of branches per plant as well as fresh and dry weights (g). Chemical analyses were done including photosynthetic pigments, carbohydrates and oil contents. Chlorophyll a, b and carotenoids contents in the fresh leaves (mg/g.F.W) were determined in samples according to Saric *et al.* (1967). Total and soluble carbohydrates contents were determined according to the method described by Dubois *et al.* (1956). Insoluble carbohydrates were estimated by subtraction of soluble carbohydrates from total carbohydrates. Representative fresh samples were taken from each treatment for determination of essential oil percentage and essential oil yield/plant using British Pharmacopoeia (1963) method. All obtained data were statistically analyzed by the method of Snedecore and Cochran (1981).

Results

Effect of salinity and NAA treatments on vegetative growth (plant height, number of branches, fresh and dry weights) of rosemary

Table 1.a, 1.b, 1.c and 1-d clarify that, plant height, number of branches/plant, fresh and dry weights of rosemary plant significantly increased by low level of salinity 1000 mg/l, while declined with increasing salinity concentration from 3000 mg/l to 5000 mg/l in both seasons. NAA application showed a significant positive effect on the mentioned growth parameters up to 200 ppm, whereas higher NAA concentration (300 ppm) showed a negative effect on vegetative growth. The highest lengths, the maximum branch numbers, the highest fresh and dry weights were detected at salinity concentration of 1000 mg/l and 200 ppm NAA.

TABLE 1.a. Effect of salinity and NAA treatments on plant height (cm) of *Rosmarinus officinalis* L. plant.

Salinity (mg/l)	NAA (ppm)					
	0.0	25	75	125	200	300
0.0	33.2	36.7	38.1	41.6	45.8	23.1
1000	37.2	39.5	42.4	45.5	53.3	28.4
3000	24.9	30	35.5	39.2	42.4	20.0
5000	15.4	19.2	24.8	29.7	38.7	10.2
L.S.D.at 5%	1.01	0.62	0.63	1.63	1.07	1.03
L.S.D at 1%	1.98	1.11	0.83	1.91	1.75	1.71

TABLE 1.b. Effect of salinity and NAA treatments on number of branches/plant of *Rosmarinus officinalis* L. plant .

Salinity (mg/l)	NAA (ppm)					
	0.0	25	75	125	200	300
0.0	8.7	12.7	14.2	16.4	18.8	7.2
1000	10.2	13.8	15.5	18.9	20.8	8.5
3000	7.1	10.7	12.4	14.3	16.2	4.2
5000	5.7	8.1	10.0	11.9	13.8	2.7
L.S.D at 5%	0. 84	1.0	0.96	1.11	1.02	0.31
L.S.D at 1%	0.98	1.7	1.22	1.76	1.42	0.92

TABLE 1.c. Effect of salinity and NAA treatments on fresh weight (g) of *Rosmarinus officinalis* L. plant.

Salinity (mg/l)	NAA (ppm)					
	0.0	**25**	**75**	**125**	**200**	**300**
0.0	150.3	159	164.5	172.6	182.7	120.4
1000	156.6	161.4	168.2	177.3	195.3	124.1
3000	140.3	144.4	148.7	153.9	160.3	111.2
5000	84.2	95.7	124.4	130.7	136.4	60.4
L.S.D at 5%	0.41	0.83	0.99	0.88	1.05	1.10
L.S.D at 1%	1.2	0.98	1.80	1.76	1.41	1.83

TABLE 1.d. Effect of salinity and NAA treatments on dry weight (g) of *Rosmarinus officinalis L.* plant.

Salinity (mg/l)	NAA (ppm)					
	0.0	**25**	**75**	**125**	**200**	**300**
0.0	26.4	31.2	35.1	42.3	46.1	18.3
1000	32.2	35.7	39.1	44.1	49.4	21.4
3000	20.1	24.1	26.3	28.2	34.2	16.9
5000	12.4	19.8	21.1	24.1	29.9	9.9
L.S.D at 5%	0.88	0.98	0.83	0.79	0.89	0.52
L.S.D at 1%	1.10	1.32	1.72	1.42	1.22	0.83

Effect of salinity and NAA treatments on photosynthetic pigment content

Data recorded in Table 2. a, b and c shows that low salinity concentration (1000 mg/l) increased chlorophyll a, b and carotenoids (mg/g. Fresh weight of leaves), whereas higher salinity concentrations reduced their contents. NAA treatments up to 200 ppm reduced the harmful effect of salinity, but higher NAA treatment (300ppm) had an adverse effect on photosynthetic pigments concentration.

Chlorophylls and carotenoids concentrations were markedly improved in rosemary leaves as a result of foliar spray of low concentrations of NAA. The lower level of salinity 1000 mg/l combined with 200 ppm NAA produced the highest pigments concentration, whereas the highest NAA concentration (300 ppm) reduced significantly pigments concentration compared with control.

TABLE 2.a. Effect of salinity and NAA treatments on chlorophyll "a" content (mg/g fresh weight) in leaves of *Rosmarinus officinalis* L. plant .

Salinity mg/l	NAA (ppm)					
	0.0	**25**	**75**	**125**	**200**	**300**
0.0	1.17	2.06	3.06	4.60	7.68	0.72
1000	2.12	3.01	4.13	5.96	9.11	1.21
3000	1.01	1.93	2.62	3.71	6.55	0.51
5000	0.61	0.71	0.94	1.27	2.92	0.35
L.S.D at 5%	0.25	.17	0.52	0.22	0.52	0.49
L.S.D at 1%	0.44	0.29	0.74	0.37	0.72	0.61

TABLE 2.b. Effect of salinity and NAA treatments on chlorophyll "b" content (mg/g fresh weight) in leaves of *Rosmarinus officinalis* L. plant.

Salinity mg/l	NAA (ppm)					
	0.0	**25**	**75**	**125**	**200**	**300**
0.0	0.39	0.46	0.53	0.79	1.97	0.22
1000	0.71	0.80	1.01	1.98	3.76	0.37
3000	0.27	0.36	0.45	0.62	0.94	0.19
5000	0.17	0.24	0.38	0.43	0.24	0.11
L.S.D.at 5%	0.044	0.071	0.013	0.032	0.022	0.031
L.S.D.at 1%	0.082	0.090	0.051	0.052	0.076	0.092

TABLE 2.c. Effect of salinity and NAA treatments on carotenoids contents (mg/g fresh weight) in leaves of *Rosmarinus officinalis* L. plant.

Salinity mg/l	NAA (ppm)					
	0.0	**25**	**75**	**125**	**200**	**300**
0.0	0.130	0.182	0.191	0.242	0.403	0.075
1000	0.155	0.191	0.261	0.390	0.621	0.081
3000	0.046	0.063	0.088	0.130	0.255	0.031
5000	0.034	0.048	0.090	0.098	0.119	0.019
L.S.D.at 5%	0.009	0.004	0.005	0.003	0.008	0.004
L.S.D.at 1%	0.015	0.008	0.009	0.004	0.003	0.090

Effect of salinity and NAA treatments on content of total, soluble and insoluble carbohydrates:

The increases of the photosynthetic pigments in the treated rosemary leaves were concomitant with a gradual increase in total, soluble and insoluble carbohydrates as shown in Table 3.a, 3.b and 3c.

TABLE 3.a. Effect of salinity and NAA treatments on total carbohydrates content (mg/g fresh weight) in leaves of *Rosmarinus officinalis* L. plant.

Salinity mg/l	NAA (ppm)					
	0.0	25	75	125	200	300
0.0	313.9	371.5	442.3	500.1	612.4	218.4
1000	435.8	504.4	592.6	638.2	701.3	294.1
3000	251.5	270.5	339.8	391.6	458.5	158.7
5000	140.1	200.4	291.6	320.3	432.9	71.4
L.S.D.at 5%	3.20	3.83	4.78	2.93	4.66	3.65
L.S.D.at 1%	4.57	5.14	5.43	4.21	6.21	4.06

TABLE 3.b. Effect of salinity and NAA treatments on soluble carbohydrates content (mg/g fresh weight) in leaves of *Rosmarinus officinalis* L. plant.

Salinity mg/l	**NAA (ppm)**					
	0.0	**25**	**75**	**125**	**200**	**300**
0.0	79.2	88.4	103.7	113.5	128.7	61.3
1000	99.3	110.4	121.7	131.4	152.1	72.6
3000	59.4	63.6	70.6	80.5	88.44	52.8
5000	43.9	50.4	55.7	59.4	71.3	32.6
L.S.D.at 5%	1.04	3.73	3.12	2.60	3.56	3.19
L.S.D.at 1%	2.51	4.97	5.84	3.63	4.90	5.22

TABLE 3.c. Effect of salinity and NAA treatments on insoluble carbohydrates content (mg/g fresh weight) in leaves of *Rosmarinus officinalis* L. plant.

NAA (ppm)						**Salinity mg/l**
300	**200**	**125**	**75**	**25**	**0.0**	
157.1	483.7	386.6	338.6	283.1	234.5	**0.0**
221.5	549.2	506.8	470.9	394	336.5	**1000**
105.9	370.1	311.2	269.2	206.9	192.1	**3000**
38.8	361.6	260.9	235.9	150	96.2	**5000**
4.04	3.91	4.61	4.07	4.55	2.11	L.S.D.at 5%
6.11	5.11	5.32	5.11	5.29	3.74	L.S.D.at 1%

Effect of salinity and NAA treatments on oil percentage and oil yield of rosemary plant:

The results in Table 4 a-b indicated that there was a significant increase in oil percentage and oil yield due to the lowest concentration of salinity (1000 mg/l), whereas higher salinity levels drastically affected oil percentage and yield.

Concerning NAA treatments, the data indicated that there was a significant increase in oil percentage and oil yield by increasing the NAA concentration up to 200 ppm, whereas 300 ppm NAA considerably decreased both parameters. Moreover, the combination between the highest salinity concentration (5000 mg/l) and the highest NAA treatment (300 ppm) caused the least oil percentage and oil yield and the maximum levels of both parameters were obtained by using 1000 mg/l salinity and 200 ppm NAA.

TABLE 4.a. Effect of salinity and NAA treatments on volatile oil percentage of *Rosmarinus officinalis* L. plant.

NAA (ppm)						Salinity mg/l
300	**200**	**125**	**75**	**25**	**0.0**	
0.17	0.92	0.66	0.54	0.43	0.32	**0.0**
0.23	1.85	0.88	0.71	0.52	0.43	**1000**
0.11	0.76	0.61	0.44	0.35	0.25	**3000**
0.077	0.58	0.46	0.34	0.25	0.13	**5000**
0.009	0.031	0.023	0.021	0.011	0.009	L.S.D.at 5%
0.011	0.044	0.057	0.033	0.017	0.011	L.S.D.at 1%

TABLE 4.b. Effect of salinity and NAA treatments on volatile oil yield (ml/plant) of *Rosmarinus officinalis* L. plant.

NAA (ppm)						Salinity mg/l
300	**200**	**125**	**75**	**25**	**0.0**	
0.241	0.952	0.762	0.653	0.532	0.402	**0.0**
0.303	1.711	0.871	0.721	0.666	0.533	**1000**
0.123	0.823	0.610	0.501	0.332	0.211	**3000**
0.083	0.605	0.541	0.454	0.334	0.145	**5000**
0.010	0.012	0.074	0.009	0.053	0.009	L.S.D.at 5%
0.014	0.019	0.098	0.012	0.088	0.019	L.S.D.at 1%

Discussion

The stimulative effect of lower level of salinity stress shown in our data may be attributed to the production of great amount of secondary plant products such as phenols, terpenes, N as well as essential oils by plants under salt stress (Mosaleeyanonet *et al.*, 2005). Also plants commonly react to salt stress by accumulation of solutes in cells, or osmotic adjustment, which has resulted in improved environmental stress tolerance (Mathews *et al.*, 1984). Solutes involved in osmotic adjustment are typically sugars, amino acids, inorganic ions and organic acids (Morgan, 1984). Glycine betaine (GB) and proline are two major organic osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals. Although their actual roles in plant osmotolerance remain controversial, both compounds are thought to have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions.

The harmful effect of excess soluble salts in the soil may be attributed to osmotic stress, specific ion toxicity and ionic imbalances and consequently plant death or yield loses in crop species and medicinal plants (Munns, 2003 and Le Rudulich, 2005).

According to the results shown in our work, NAA has both stimulatory and inhibitory effect on the different measured growth and yield parameters, this depends on its concentration. NAA at low concentrations showed a progressive increase in plant height, number of branches/plant, fresh and dry weights up to 200 ppm at which the highest length of plants, maximum number of branches and the highest fresh and dry weights of plants were recorded. Whereas 300 ppm NAA led to reduction in these growth parameters. These results are corroborated with those of El-Tayeb (2005) on barley, Gunes *et al.* (2005) on maize and Dawood *et al.* (2012) on sunflower.

Moreover, our results are in agreement with Akter (2010) who reported an increase in number of cob per plant due to NAA treatment in maize as well as Dasgupta (1975) who obtained reduced pod length with higher concentrations of NAA on groundnut. The results also are in conformity with the findings of Singh *et al.* (1972) on pea and Ullah *et al.*(2007) on cowpea who reported that 1000-grain weight decreased due to higher concentration of NAA application. Moreover, the achieved increase in peppermint yield per plant with low concentrations of NAA might be attributed to increased assimilative area and as a result more photosynthesis throughout the growth period (Balba and Tallat 2007).

The ameliorative effect of NAA could be attributed to its role in coordination of development at all levels in plants from the cellular level to organs and ultimately the whole plant, while the adversely effect of higher concentration of NAA may be attributed to stimulation of ethylene production that can inhibit elongation growth, causing leaf abscission, and even kill the plant (Szepesi et al., 2005).

The enhancing effects of NAA on photosynthetic capacity could be attributed to its stimulatory effects on tryptophan activity and pigment contents (Khodary, 2004) as well as increased CO2 assimilation, photosynthetic rate and increased mineral uptake by the plant (Szepesi *et al.,* 2005). In addition, Arfan *et al.* (2007) pointed that application of auxin improved the photosynthetic capacity and retained pigment content in wheat cultivars under salt stress therefore inhibiting their senescence.

The increase in pigment level by NAA presented in our data could be attributed to the promotion in its synthesis and/or retardation of pigment degradation as recommended by Sharma *et al.* (1995) who found that excised leaves of *Tropaeolum majus* treated with auxin retained more chlorophyll (60% higher at 10^{-3} M) compared to control. Moreover, the potent effects of particularly growth regulator might be ascribed to the reduction in chlorophyll loss due to its ability to increase the antioxidant capacity of the plants or inducing the synthesis of stabilizing substances.

The stimulatory effect of NAA spray on the total carbohydrate content shown in our results is in agreement with those obtained by Balbaa, and Talaat (2007) who reported that, application of NAA increased the total, soluble and insoluble carbohydrate percentage in peppermint leaves. Also Dawood *et al.* (2012) reported a significant increase in total, soluble and insoluble carbohydrate content in leaves of sunflower as a result of NAA application, thus NAA application maintained the carbohydrate pool in the chloroplasts at a high level.

The increment in oil content as a result of NAA treatment might be due to the achieved increase in vegetative growth, metabolism and nutrient uptake. Similar results were reported by Çag *et al.* (2009). In addition, Noreen and Ashraf (2010) mentioned that NAA caused marked increases in sunflower achene oil content.

Thus, our data may lead to conclude that NAA could be a potent growth regulator to improve growth and yield of Rosemary plant cultivated under salinity stress.

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(*Received* 27/7/2015;
accepted 5/10/2015)

تأثير الملوحة والرش الورقى بنفثالين حمض الخليك على النمو والتركيب الكيميائى وانتاج الزيت لنبات حصا البان.

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استهدف هذا البحث دراسة تأثير ملوحة التربه بتركيزات ٠ ، ١٠٠٠، ٣٠٠٠، ٥٠٠٠ ملجم/لتر (كلوريد الصوديوم) وكذا الرش الورقى بنفثالين حمض الخليك بتركيزات (٠، ٢٥، ٧٥ ، ١٢٥، ٢٠٠ ، ٣٠٠ جزء فى المليون) على النمو الخضرى والتركيب الكيميائى و انتاج الزيت لنبات حصا البان.

أظهرت النتائج انخفاض فى كل قياسات النمو الخضرى و التركيب الكيميائى وانتاج الزيت بزيادة تركيز الملوحه ، وأدى الرش الورقى بنفثالين حمض الخليك حتى ٢٠٠ جزء فى المليون الى تقليل الأثر الضار للملوحه و تحسين النمو الخضرى والتركيب الكيميائى ونسبة وإنتاج الزيت ثم انخفض النمو الخضرى والتركيب الكيمياى وانتاج الزيت بزيادة التركيز الى ٣٠٠ جزء فى المليون نفثالين حمض الخليك.