

**EFFECTS OF CHEMICAL AND BIO-FERTILIZATION  
ON THE GROWTH AND THE CHEMICAL  
CONSTITUENTS OF SPINACH PLANTS**

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**ABSTRACT**

Consumption of spinach with high nitrate and oxalate contents may be a health hazard to infants and adults. The crop accumulates  $\text{NO}_3\text{-N}$  and oxalic acid, when grown in a soil with high  $\text{NO}_3\text{-N}$  availability. Therefore, pot experiments were designed during the two successive years of 2000 and 2001 to evaluate the effects of different nitrogenous fertilizers, chemical and bio-fertilizers as well as their combinations on nitrate and oxalate in spinach. One objective of this study was to develop a mean for fertilization for maximum growth with useful components and minimum nitrate and oxalate contents. The trials involved 6 application ratios of the chemical fertilizer "Sangral" and the bio-fertilizer "nitrobin" 0/0, 100/0, 75/25, 50/50, 25/75 and 0/100). Results indicated that growth (monitored by the number of leaves, leaf area, fresh and dry weights) was enhanced when plants were provided with a mixture of chemical and biofertilizer, compared with the control or both fertilizers as single treatments. Nitrate concentration was reduced by 50/50 N-mixture compared with higher ratios of chemical fertilizer. Total and free oxalate in spinach leaves were reduced also by N-mixture of 50/50 compared with no N or either N form alone. The highest vitamin (vit. B1, vit. B2, vit. C) and element (Ca, P, Fe) concentrations were found at 50/50 N-mixture with chemical and bio-fertilizer application. The data suggest that fertilization of N as a mixture of chemical and bio fertilizers (50/50 ratio)

improved plant growth status. Furthermore, nitrobine is a promising factor to minimize hazardous components such as nitrate and oxalate, and to maximize useful components such as vitamins and mineral elements in spinach.

**Key words** *biofertilizers, bio-fertilizer "nitrobine, chemical fertilizer, "Sangral",  $\text{NO}_3\text{-N}$ , oxalic acid, spinach (*Spinacia oleracea* L.).*

## 1. INTRODUCTION

Excessive application of nitrogenous chemical fertilizers, to enhance growth rates and yield of crops, is a common agricultural practice in developing countries. This extreme fertilizer application often leads to the accumulation of high levels of nitrates in plant tissues (Greenwood and Hunt, 1986) and ground water (Viets and Hageman, 1971). High levels of nitrate in the diet can indirectly inhibit oxygen transport by blood, a medical condition known as methemoglobinemia, in infants because of the reduction of nitrate to nitrite (Lyons *et al.*, 1994). Nitrites can react with amines and may be converted to nitrose amines which usually cause cancer (Whitney *et al.*, 1990). Furthermore, oxalate accumulation which may lead to the formation of calcium oxalate calculi in the kidneys (kidney stones) and calcium deficiency (hypocalcaemia) in humans (Schenk *et al.*, 1982) is highly influenced by excessive nitrate fertilization (Goh and Vityakon, 1988).

The leafy vegetable spinach (*Spinacia oleracea* L.) represents a good source of vitamins B and C as well as minerals such as iron, calcium and magnesium, besides the dietary fibers (NRC, 1984). It is commonly used in considerable amounts, either fresh or cooked, in Saudi Arabian meals. Unfortunately, such vegetable accumulates high levels of nitrates (Lyons *et al.*, 1994). In this concern, earlier studies indicated that the most important factor affecting  $\text{NO}_3\text{-N}$  contents in plants appears to be the form of applied nitrogen (Greenwood and Hunt, 1986).

The bio-fertilizer (nitrobine) was found to reduce nitrate accumulation in plant tissues, but may not produce as the much yield as the chemical fertilizer. Furthermore, the possibility of chemical fertilizer toxicity to plants exists when all N fertilizers are ammoniacal (Mengel and Kirkby, 1987a).

The biofertilizer (nitrobine), offers a way to use the chemical fertilizer safely if both types are mixed together in the soil, and it is not harmful to other soil microflora (Mills *et al.*, 1976). Unfortunately, little information is available on the effect of nitrobine and its effect when applied with chemical fertilizers. Therefore, the present study was undertaken to evaluate the interactive effects of the chemical fertilizer (Sangral) and the bio-fertilizer (Nitrobine) on growth and chemical compositions in the leafy vegetable (spinach) under different fertilization regimes. Determining the maximum yield and useful compounds (vitamins and minerals) and the minimum accumulation of hazardous compounds (nitrate and oxalate) in plant leaves was studied.

## 2. MATERIALS AND METHODS

In a semi-arid condition, Al Qassim area (Central Saudi Arabia), is a semi-arid region located at 26° 18' N latitude and 43° 58' E longitude and altitude of 725m above sea level. Pot experiments were conducted in a glasshouse during the two successive winter seasons of 2000 and 2001, to test different nitrogen sources and rates in the presence or absence of the biofertilizer nitrobine. The experiments were managed in a randomized complete block design.

In both seasons, spinach (*Spinacia oleracea* L.) var. Balady seeds (local Egyptian variety) were sown on November 10 in pots of 30 cm diameter x 30 cm depth filled with 10 kg sandy-clay soil (pH 7.3). The chemical and physical properties of the soil are shown in Table (1). Fifteen days later, pots were thinned to three uniform plants per pot. Immediately before planting, pots were fertilized with super phosphate (15.5%  $P_2O_5$ ); and soil either mixed or not with the biofertilizer nitrobine (BF) at the rate of 100 mg  $kg^{-1}$  soil (as recommended by the Ministry of Agriculture) as indicated below. The experiment was arranged in a randomized complete block design with 3 replications. Five days after thinning, Sangral chemical fertilizer (CF) was added at the rate of 60 kg/ha, to provide the following treatment combinations: (a) no nitrogen (control), (b) 100% CF, (c) 75% CF + 25% BF, (d) 50% CF + 50% BF, (e) 25% CF + 75% BF, (f) 100 BF.

Table(1). Chemical and Physical Analyses of the pot Soil.

Chemical properties		Physical properties	
pH:	7.30	Fractions (%):	
ECe (ms):	2.06	Sand:	
Soluble cations (meq.L <sup>-1</sup> ):		95.30	
	Na <sup>+</sup> 11.00	Silt:	
	Ca <sup>2+</sup> 4.35	03.60	
	Mg <sup>2+</sup> 2.50	Clay:	
		01.10	
Soluble anions (meq.L <sup>-1</sup> ):		Texture: Sandy Soil	
	CO <sub>3</sub> <sup>2-</sup> + HCO <sub>3</sub> <sup>-</sup> 2.99		
	SO <sub>4</sub> <sup>2-</sup> 11.70		
	Cl <sup>-</sup> 7.60		
	CaCO <sub>3</sub> 4.00%		
	O.M. 0.23%		

Sangral fertilizer consists of N 20%, P (P<sub>2</sub>O<sub>5</sub>) 20%, K (K<sub>2</sub>O) 20%, S 0.4%, Mg (MgO) 0.02%, Fe 70 mg/kg, Zn 14 mg/kg, Cu 16 mg/kg, Mn 42 mg/kg, B 22 mg/kg and Mo 14 mg/kg.

Watering was controlled so that field capacity of the soil was not exceeded and drainage losses of N did not occur. At apparent market maturity, 45 days after planting, spinach plants were harvested with their intact leaves. The following data were recorded at harvest: number of leaves, leaf area, and fresh and dry (at 70°C) weights. Chemical analyses: vitamins B1 (thiamin), B2 (riboflavin) and C (ascorbic acid) were measured spectrophotometrically according to Sadasivan and Manickam (1992). Total N was determined by the modified "Micro-Kjeldahl" apparatus as described by Pregl (1945); P was determined colorimetrically as described by Trough and Mayer (1949); Ca, Mg, K and Na by flame photometry; and Fe by atomic absorption as described by Jones and Steyn (1973). Nitrate concentration was measured spectrophotometry following the method of Cawse (1967).

Total and free oxalates were estimated in spinach leaves by the method of Onayemi and Nwigwe (1987).

All data were statistically analyzed by analysis of variance procedures according to Snedecor and Cochran (1967) with the aid of the SYSTAT computer program. Differences were indicated to be significant at a 5% level and means were separated by the LSD test.

### **3. RESULTS AND DISCUSSION**

#### **3.1. Vegetative growth**

Data recorded in Table (2) showed that growth, as represented by the number of leaves, leaf area and fresh and dry weights, of spinach plants was influenced by N application, either in the form of CF or BF compared with the control plants. Furthermore, the plants produced more biomass when N was applied as N mixture than either CF or BF form alone. It is obvious that the maximum growth occurred with the mixture of 50% of each fertilizer form. These findings were true in both seasons. The growth enhancement with N mixture fertilizer on plant growth would be expected since nitrogen is of extreme importance in plants. It is a constituent of amino acids, proteins, cytoplasm, nucleic acids, chlorophyll, and many other important substances within plant cells (Salisbury and Ross, 1992). In addition, plants with high nitrogen contents had high levels of endogenous auxin and high gibberellins activity (Rajagopal and Rao, 1974), which encourage cell division and elongation, increase leaf number and produce a sufficient assimilation area for maximum photosynthesis (Greenwood and Hunt, 1986). Moreover, the beneficial effect of fertilizer mixture on plant growth was also reported by Richer *et al.* (1984) on spinach, Masson *et al.* (1990) on lettuce and Wang and Below (1996) on other field crops such as wheat. The enhanced growth with N mixture rather than either form alone was attributed to the enhancement of plant roots and increased cytokinin levels (Wang and Below, 1996). More efficient utilization of N as mixture than one form alone was found to improve plant growth status (Rickman *et al.* 1985).

#### **3.2. Nitrogen accumulation and nitrogen use efficiency (NUE)**

Concurrent with increasing the number of leaves and biomass of mixed-fertilizer grown plants was an additional N accumulation.

Data displayed in Table (3) show that N concentration (g kg<sup>-1</sup> dwt) and accumulation (mg plant<sup>-1</sup>) were minimum for control plants. Fertilized plants (in CF, BF or mixed form) showed a significant increase in the concentration and accumulation of nitrogen. It was clear that the total N concentration increased with each increment of fertilizer application in the form of CF, but reached the highest level at the point of fertilizer application for the maximum yield with BF form. In this respect, Wang and Below (1996) found that the presence of chemical N fertilizer, either alone or mixed with other fertilizers, increased the proportion of N in wheat shoots. The same pattern of N accumulation was found for radish shoots and roots grown under similar treatments (Mills *et al.*, 1976).

**Table(2): Effects of chemical and bio- fertilizers on the number of leaves, leaf area, and fresh and dry weights of spinach plants during two growing seasons.**

Fertilizer (%)		No. of leaves/plant	Leaf area cm <sup>2</sup> /plant	Fresh weight (g/plant)	Dry weight (g/plant)
CF	BF				
Season 2000					
0	0	4.7	98	18.5	2.2
100	0	9.8	120	32.6	3.0
75	25	10.5	176	40.4	3.9
50	50	14.8	248	58.5	4.7
25	75	12.7	214	46.5	4.1
0	100	9.3	100	30.2	2.4
Mean		10.3	159.3	37.8	3.4
LSD (5%)		2.3	30.5	12.6	1.1
Season 2001					
0	0	3.6	95	17.9	2.3
100	0	8.6	128	30.4	2.9
75	25	10.3	169	39.5	3.4
50	50	15.0	230	42.8	3.9
25	75	11.8	198	32.2	3.4
0	100	9.5	112	26.3	2.5
Mean		9.8	155.3	31.5	3.1
LSD (5%)		1.9	39.6	10.5	1.2

Data in Table (3) also indicate that the increase in bio-fertilizer proportion decreased the concentration of total N when all or 75% of the nitrogen was supplied as BF form. When CF proportion was increased in the fertilizer, N accumulation was significantly higher as compared with the control plants. These findings were true in both seasons.

It seems clear that the production of leaf materials per each nitrogen unit applied to the soil (NUE) as CF/BF mixture (particularly at 50/50 and 25/75 ratios) was much more than that of either form alone, while unmixed CF or BF forms gave the lowest NUE as compared to fertilizer mixture form. These results were true in both seasons. This result emphasizes that the efficiency of N (NUE) applied to the plants was higher in the presence of the biofertilizer, nitroline. On the other side, absorbed N was used most efficiently in producing leaves when CF and BF was applied in mixture rather than separately. In this concern, the highest growth production was observed at 50/50 ratio (Table 2).

**Table (3) : Effects of chemical and bio-fertilizers on N accumulation and vitamin concentrations of spinach leaves during two growing seasons.**

Concentrations of spinach leaves during two growing seasons.					
Fertilizer (%)		N accumul. mg/plant	Vit. (B1) (mg/kg Fwt)	Vit. (B2) (mg/kg Fwt)	Vit. (C) (mg/kg Fwt)
CF	BF				
Season 2000					
0	0	46.2	10	12	120
100	0	145.5	13	14	210
75	25	126.9	16	17	340
50	50	98.6	22	28	460
25	75	80.5	14	17	320
0	100	68.4	12	13	200
Mean		94.4	14.5	16.8	275
LSD (5%)		28.2	3.3	6.7	95
Season 2001					
0	0	54.2	11	14	145
100	0	137.2	14	16	268
75	25	120.4	18	19	425
50	50	90.6	25	29	500
25	75	85.2	12	14	422
0	100	75.4	13	13	214
Mean		93.8	15.5	17.5	329
LSD (5%)		35.5	4.5	5.9	118

Although it is distinctive why this leafy-crop was more efficient in utilizing the nitrogenous fertilizer for leaves and leaf material production, particularly in the presence of biofertilizer. It is well known that nitrogen source with nitrification suppressor and/or biological fertilizer source requires less energy for assimilation than does chemical fertilizer alone (Peuke and Jeschke, 1993). Moreover, mixed fertilizers were found to be assimilated immediately following uptake in the roots, which results in a shift in the distribution of photoassimilates between shoots and roots and greater quantities of nitrogenous compounds in the roots (Lewis *et al.*, 1987; Peuke and Jeschke, 1993).

### 3.3. Vitamin concentrations

Regardless of the chemical and bio-fertilizer treatments, the data recorded in Table (3) indicate that vit. B1 (thiamine), vit. B2 (riboflavin), and vit. C (ascorbic acid) concentrations were generally higher in the first than in the second season. That may be related to climatic differences during the course of the two experiments. Data reported in the same table showed also that nitrogen in all forms increased significantly the concentration of vitamins in spinach leaves. Moreover, the biofertilizer application enhanced the formation of vitamins particularly under 50% CF + 50% BF treatment; under which vit. B1, vit. B2 and C increased by about 120%, 133%, 280% and 127%, 107%, 240%; in the first and the second season, respectively, as compared with the control plants. In this concern, Borowski and Michalek (1994) found that vitamins were increased significantly in the leaves of many leafy crops when N fertilizer was added in a mixed rather than single fertilizer form. Moreover, Takebe *et al.* (1995) showed that ascorbic acid (vit. C) increased with increasing mixed nitrogen sources in the growth media.

The positive effect of nitrogen on vitamin concentrations was expected since vit. B1 and vit. B2 are synthesized *via* the pathway of amino acids, glycine and asparagine (Metzler, 1977); while the enhancement of vit. C, which is formed *via* the pathway of glucose, was ascribed to the stimulative effect of mixed N on the photosynthetic processes (Yamaguchi and Wu, 1978).

**Table (4): Effects of chemical and bio- fertilizers on the elemental concentrations in spinach leaves during two growing seasons.**

Fertilizer (%)		Elemental concentration (g/kg dwt)					
CF	BF	Ca	Mg	K	P	Na	Fe
<b>Season 2000</b>							
0	0	12.5	10.3	65	8.6	11.5	0.32
100	0	14.4	12.6	98	10.3	10.5	0.33
75	25	15.9	14.2	102	11.5	10.2	0.34
50	50	17.5	15.8	108	13.8	9.6	0.37
25	75	13.7	13.2	83	11.6	9.5	0.35
0	100	14.2	12.8	75	9.8	8.4	0.30
Mean		14.7	13.2	88.5	10.9	10.0	0.35
LSD (5%)		2.1	3.6	22.5	2.2	0.5	0.11
<b>Season 2001</b>							
0	0	11.5	11.3	59	7.5	10.3	0.36
100	0	12.5	14.2	88	9.5	11.4	0.42
75	25	14.9	15.2	113	10.4	10.8	0.45
50	50	16.5	17.6	121	11.4	9.8	0.50
25	75	13.6	12.8	87	10.9	8.8	0.32
0	100	11.8	11.5	68	9.5	8.0	0.30
Mean		13.5	13.8	89.3	9.9	9.9	0.39
LSD(5%)		2.6	1.5	20.6	1.8	1.1	0.13

### 3.4. Elemental concentrations:

Data presented in Table (4) show that the concentrations of Ca, Mg, K, P, Na and Fe were generally higher in nitrogen treated than untreated spinach plants. Nitrogen treatments, particularly (50% CF + 50% BF), caused a noticeable increase in the elemental concentration in spinach leaves. It is worthy to mention that the level of cations such as Ca and Mg was low at the high proportion of BF form compared to that under high proportion of CF form. This low level of both elements was ascribed to the competitive depression by the presence of nitrogen source

which is rapidly taken up by plant roots (Mengel and Kirkby, 1987a). Moreover, K and Na increased significantly under CF and BF, with more amounts at CF than BF form; in this regard, Mengel and Kirkby (1987b) indicated that both elements play an important role in plant physiology. They contribute to the osmotic potential of the cell and thus have positive effects on the water regime and hence, on the growth of plants. The extent to which substitution between both elements can occur, however, depends much on the uptake potential for  $\text{Na}^+$ . This differs considerably between plant species. In this concern, spinach was classified as high  $\text{Na}^+$  includer, and other leafy vegetables such as lettuce and cabbage as medium  $\text{Na}^+$  includer crops (Mengel and Kirkby, 1987b).

Data in the same table show also that the enhancing effect of mixture N on the elemental concentrations became more pronounced with the application of biofertilizer. In this regard, the greatest increase in elemental concentrations was often found at the treatment of (50% CF + 50% BF). This positive effect was attributed to the nitrogen efficiency and recovery which are attained when the applied fertilizer is not lost by leaching or denitrification; besides the positive effect of N on mineral absorption and translocation from the soil to plant organs (Mengel and Kirkby, 1987a).

### 3.5. Hazardous compounds

a - *Nitrate concentration.* Data recorded during both seasons indicate that spinach plants accumulated high amounts of nitrate when CF was dominant or in higher proportion than BF form (Table 5). This may be explained as BF is very slowly nitrified so that nitrate accumulation would be low in plant tissues (Mengel and Kirkby, 1987a). However, nitrate concentration ( $\text{g kg}^{-1}$  dry wt) in spinach was much higher at CF than at BF treatments. This difference might be related to the leaf thickness, relatively low water content, high dry matter and, foremost, high N concentration in spinach leaves at CF than at BF treatments.

Data presented in the same Table show that biofertilizer treatments reduced significantly the accumulation of nitrate, particularly when the biofertilizer was used alone or in proportion of 50% and higher with respect to CF. The most reduction in nitrate levels was observed at the combined CF & BF treatment of 50% each, under which nitrate concentration was decreased. These findings are in harmony with those reported earlier by Mills *et al.* (1976) who indicated that the use of mixed

N sources virtually eliminated significant nitrate accumulation in plant leaves; but nitrate increased significantly with single N source.

**Table (5): Effect of chemical and bio- fertilizers on nitrate and oxalate concentrations of spinach leaves during two growth seasons.**

Fertilizer (%)		Total Nitrate	Total Oxalate	Free Oxalate
CF	BF	(g/kg dwt)	(g/kg dwt)	(g/kg dwt)
<b>First season</b>				
0	0	2.4	3.8	1.4
100	0	5.3	9.5	3.2
75	25	4.2	8.5	2.8
50	50	3.0	5.3	1.4
25	75	2.6	4.8	1.2
0	100	2.5	4.5	1.3
Mean		3.3	6.5	1.9
LSD (50%)		1.2	3.1	1.1
<b>Second season</b>				
0	0	2.1	4.1	1.2
100	0	5.4	10.3	3.5
75	25	3.8	8.3	2.6
50	50	3.1	6.1	1.7
25	75	2.8	5.3	1.4
0	100	2.2	4.8	1.3
Mean		3.2	6.5	2.0
LSD (50%)		1.0	3.4	1.3

b- *Oxalate concentration.* Data reported in Table (5) show that spinach accumulated potentially hazardous levels of oxalate when plants were treated with all CF application. The total contents of oxalates exceeded the normal limit for oxalates in spinach (Goh and Vityakon, 1986). In this concern, the CF/ BF of (50% : 50%) minimized the level of total oxalate while with 100% BF the total oxalate, was very low compared to other treatments. Free oxalate concentration, is harmful for human health because it reduces the calcium availability and causes urinary bladder stone diseases (Schenk *et al.*, 1982; Whitney *et al.*, 1990), was reduced substantially under the application of 50% CF -- 50% BF. This could be related to the increase in the soil Ca, which enhances Ca-uptake by plants as shown above (Table 4). Thus reduces free oxalates in

plants through the formation of Ca-oxalate complexes (Vityakon and Standal, 1989), a physiologically inactive compound. These findings are in harmony with those reported by Sady *et al.* (1990) and Lyons *et al.* (1994). In this regard, Takebe *et al.* (1995) found that insoluble oxalic acid content increased with increasing Ca/K ratio in spinach leaves, while soluble oxalic acid content decreased.

In conclusion, the combination of CF and BF was much more desirable with respect to growth, nitrate accumulation and total N concentration in leafy vegetables, such as spinach, as opposed to either N source used singly. A fertilizer mixture of 50% CF and 50% BF form was the best combination for maximum growth and total N as well as minimum nitrate and oxalate concentrations in plant tissues.

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## تأثير التسميد الكيميائي والحيوي على النمو والمحتوى الكيميائي لنبات السبانخ

عبد الرحمن بن محمد المشيلح

قسم الإنتاج النباتي و الوقاية - كلية الزراعة والطب البيطري - جامعة الملك سعود  
فرع القصيم - المملكة العربية السعودية

### ملخص

أجريت هذه الدراسة خلال الموسم الخريفي لعامي ٢٠٠٢ ، ٢٠٠٣ وذلك بمزرعة كلية الزراعة والطب البيطري - جامعة الملك سعود فرع القصيم ، بهدف دراسة تأثير مصدرين مختلفين من مصادر التسميد النيتروجيني، أحدهما سماد كيميائي والآخر مخصب حيوي، بالإضافة الى دراسة تأثيرهما المشترك على النمو الخضري ومحتوى نباتات السبانخ من النترات والأكسالات حيث يمثل تراكمها احدى المشكلات الصحية لمستهلكي هذه النباتات. وتهدف هذه الدراسة الى إيجاد أنسب أنواع الأسمدة وأكثر المعدلات المضافة اماناً من حيث تقليل محتوى السبانخ من النترات والأكسالات. وقد تم استخدام السماد الكيميائي " سنجرال" والمخصب الحيوي "نيتروبين" في تجربة أصص بالنسب التالية: صفر/صفر، ١٠٠/صفر، ٢٥/٧٥، ٥٠/٥٠، ٧٥/٢٥ و صفر/١٠٠.

وقد أوضحت النتائج المتحصل عليها أن معدل نمو النباتات ممثلاً في عدد الأوراق للنبات ومساحة الأوراق والوزن الرطب والجاف قد تحسن عند إضافة مخلوط السمادين وذلك بالمقارنة بإضافة كل سماد منفرداً على حدة. وقد وجد أن محتوى النترات في النباتات قد نقص عند استخدام المعدل (٥٠/٥٠) مقارنة بالمعدلات الأخرى أو معاملة المقارنة كما وجد أن محتوى الأكسالات الكلية والحوهة قد تناقص في الأوراق عند استخدام نفس النسبة من الأسمدة بالإضافة إلى زيادة تركيز كل من فيتامين ب١، ب٢ و ج وأيضاً زيادة عناصر الكالسيوم والفسفور والحديد بإضافة نفس النسبة السابقة.

وتوضح النتائج أن نسبة ٥٠/٥٠ % من كلا السمادين (سنجرال ونيتروبين) أدت إلى تحسين نمو النباتات الإقلال من محتوى المواد الضارة مثل الأكسالات والنترات وزيادة تركيز المواد المفيدة مثل الفيتامينات والعناصر المعدنية في نبات السبانخ.

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (٥٥) العدد الثالث  
(يوليو ٢٠٠٤): ٤٤٣-٤٥٨.