World Applied Sciences Journal 9 (1): 49-54, 2010
ISSN 1818-4952

Prospective Study in Influences of Using Bio-Organic Farming System on Growth, Nitrate, Oxalate and Ascorbic Acid Contents in Spinach

[1]A.A. Alderfasi, [2]A.E. Moftah and [3]A.M. Aljuaed

[1]Department, Plant Production Faculty of Food and Agricultural Sciences, King Saud University, Saudi Arabia
[2]College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia
[3]Princess Noura Bint Abdurrahman University for Girls, Saudi Arabia

Abstract: The ability of using bio-organic farming system may offer an opportunity to improve soil fertility and crop productivity using less chemical fertilizers. Therefore, the present study was carried out in the green house of Agriculture and Veterinary Medicine Experiment Station, Qassim University. The experiments included 7 treatments, three were main factors viz., chemical fertilizers; organic compost and biofertilizer as well as their combinations. The main objective is to evaluate a sustainable, ecofriendly, economically viable integrated plant nutrient supply system for maximum growth with useful components and minimum leaf contents of nitrate and oxalate of Spinach (*Spinacia oleracea* L.). Data recorded included, growth characters expressed as number of leaves, leaf area, fresh and dry weight, leaf concentration of nitrate and oxalate, carbohydrate; vitamin B and C percentages, water content (W.C), macronutrients (N, P, Ca and K) and Fe uptake. Results indicated that integrated nutrient supply with organic fertilizer in combination with chemical and bio-fertilizer increased the availability and uptake of nutrients which would have favored promoted growth vigor, increased photosynthesis productivity, macro and micro-nutrients as well as vitamin B and C concentration, contrary decreasing accumulation of nitrate and oxalate in leaves. Primary results highlights the benefit use of bio-organic farming system conjunction with chemical fertilizers in reducing the cost of farming, improved eco-system and maintains high quality yield of spinach.Thus, more studies are needed for constructing recommendation can be followed by farmers.

Key words: Vegetable crops · Spinachyield · quality · leaf nitrate and oxalate content

INTRODUCTION

Sustainable agriculture development, in arid and semi-arid is influenced to a great extent by soil supplying with nutrients. In general, under such condition the production of most crops is very low due to low soil fertile and always is not economic too. Farmers and agriculture companies have to apply high rates of chemical fertilizers and large amount of irrigation water to maintain satisfactory yield level [1]. Widespread uses of agro-chemical have virtually become a necessity for the growth of high-yielding varieties of crops which highly consumes large amount of fertilizers, will likely result in resource degradation and environmental pollution with adverse effect on human health. Saudi Arabia consumes nearly about 400 thousand ton/year of chemical fertilizers [2]. Recently,enormous changes as the result of development of innovative technologies including management practices of integrated nutrient application by using beneficial bacteria(biofertilizer),which has the ability to fix nitrogen, solubilize phosphorus, stimulate plant growth and organic manure (contains higher levels of essentially,easily available and slow release nutrients for plant growth), [3, 4]. Leafy vegetables spinach (*Spinacia oleracea* L.) represent good sources of vitamins B (folic acid) and C (ascorbic acid) as well as minerals such as iron (Fe), calcium and magnesium, besides the dietary fibers. Spinach is commonly used as fresh or cooked in local meals. Although, richness with high energy, good nutritional values and mineral inputs, it accumulates high levels of nitrate and oxalate. In this concern, earlier studies indicated that the most important and effective factor affecting in nitrate content is the form of applied nitrogen [5, 6]. Shingo *et al.* [7] found that, nitrate content in the leaves of spinach given organic fertilizers decreased by 45% as compared to that of the standard treatment.

Corresponding author: A.A. Alderfasi, P.O. Box 2460, Riyadh 11451, King Saud University Saudi Arabia

Soil supplied with organic fertilizer showed delayed mineralization and nitrification [8-11]. Renard *et al.* [12] showed that yield quality of spinach (expressed in terms of nitrate concentration) was differed as nitrogen sources were changed. Some investigators reported that inoculated both of chemical and organic fertilizers with bio-fertilizers markedly effect on yield and quality of many crops [13-21]. However, other investigators decided that bio-organic farming systems have been found, while no significant differences has been identified for yield and quality [22-25].

The present study was undertaken based on available scientific literature highlights that organic plant products contain markedly less nitrates than inorganic one. Thus the main objective is to evaluate the use of organic compost associated with biofertilizer singly or in combination with chemical fertilizers on growth and leaf content of nitrate, oxalate and vitamins of spinach have been estimated.

MATERIALS AND METHODS

In green house (provided with control system) of the Agriculture and Veterinary Medicine Experiment Station, Qassim University, Saudi Arabia, local variety of leafy vegetables spinach (*Spinacia oleracea* L.) was sown in 35 cm. diameter pots filled with air dried sandy soil (7 kg/pot) and wetted with water after preparation. Chemical and mechanical analyses of the experimental soil are presented in Tables 1 and 2.

Before sowing, numbers of pots were prepared according to the experimental design of complete randomized design as described by Gomez and Gomez [25], in 5 replications. Treatment included 7 fertilization treatments, three were the main factors viz., chemical fertilizers; organic compost and biofertilizer as well as their combinations.Organic matter was applied as the form of organic compost using commercial product produce by El-Bostan Company, chemical analyses of the compost used was presented in Table 3. Compost was mixed with the soil by the rate of 2g/kg soil, whereas biofertilizer treatment was followed using soil inoculation with commercial product of nitro-pen, (containing two specific strains of bacteria, *Azotobacter sp.* and *Azospirillium sp.*) and phosphoren (contain strain of phosphate dissolving bacteria, PDB) and chemical fertilizer was applied as nitrogen fertilizer in the form of potassium nitrate (13% N, 37% K and 44% K_2O) by the rate (150 kg N/ha.). Equal dose of phosphorus fertilizer was applied after one week of pots preparation in the form of super phosphate (15.5% P_2O_5).

Sowing took place by 15 seed per pot on 15 January and December, in the first and second season, respectively. Two weeks after sowing, the plants were thinned and 12 plants per pot were left to grow. During the growth period, three representative samples (30,40 and 50 days from sowing) of four plants from every treatment for three replication were taken for determining, growth parameters expressed as number of leaves, leaf area, fresh and dry weight and water content. Leaves photosynthetic

Table 1: Chemical analyses of the experimental soil used

		Soluble cations ($meq.L^{-1}$)			soluble anions ($meq.L^{-1}$)				
pH*	EC (dSm^{-1})	Na^+	Ca^{2+}	Mg^{2+}	HCO_3^-	SO_4^{2-}	Cl	$CaCO_{3\%}$	O.M%
7.6	2.06	11.0	4.35	2.5	2.99	11.7	7.6	4.0	0.23

* pH of H_2O (soil: water = 2.5: 1)., ** EC = Electric conductivity of the extract soil paste (2:1)

Table 2: Physical properties of the experimental soil used

Fractions (%)			Texture
Sand	Silt	clay	sandy soil
95.30	3.60	1.10	

Table 3: Chemical analysis of organic compost used

Items	value
Organic carbon (%)	13.00
E.C. (dSm^{-1})	2.47
pH	7.76
Total N (%)	0.60
Available P (ppm)	84.50
C/N ratio	21.8

pigments content in the third sample on the basis of blade area (μg/dm^2) i.e., chlorophyll a, chlorophyll b and carotenoids were also determined by the method described by Bruinsma [27] and their concentration were calculated using formula used by Porra *et al* [28]. For measuring leaf water content (WC), plant samples were sealed in plastic bags, placed above ice in cooler and transported to the lab for determination leaf fresh weight, dried at 60°C until constant weight was reached. WC was calculated according to formula described by Barrs [29]. The third sample was taken nearly at the harvest time (end of the season), random sample from all plant organs were taken, washed with 0.001M hydrochloric acid followed by redistilled water to remove contaminations, then dried and ground in stainless steel mill through 0.5 mm. The powdered samples were wet ashed according to the method of Chapman and Pratt [30]. Chemical analyses were determined as follows:-

- Total carbohydrates percentage were determined using spectrophotometer and total soluble sugar using soxhelt apparatus by the method described by Dubois *et al* [31].
- K, Ca and Fe Content were determined using Atomic Absorption Spectrophotometer (AAS, Shimazau, AA6200, Kyoto, Japan).
- N was determined as nitrate using selective specific ion electrode by the method described by Barker *et al* [32]. P was determined in ashed sample according to the method adopted by King [33].
- Determination of vitamin C by calorimetric method according to the method described by Sadasivam and Manickam [34] and vitamin B using Diagnostic Automation according to Pffeifer *et al* [35].
- Determination of oxalate according to the method described by Savage *et al* [36].
- Results were subjected to statistical analysis of variance described by Gomez and Gomez [26]. New LSD test as suggested by Waller and Duncan [37] was used in comprising means.

RESULTS

Data presented in Table 4 showed that, growth parameters of leaf vegetable spinach were greatly influenced by fertilization treatments. In general, a gradual increase in all growth parameters viz., plant height (cm), number of leaves, leaf area (cm^2), fresh weight (g), dry weight (g) and percentage of water content, parallel with the progressing in plant age. Data in the same table also clear that, significant differences among the fertilization treatments were observed with application organic compost in combination with bio- and chemical fertilizers followed by application chemical fertilizer associated with biofertilizer and sole application of chemical fertilizer occupied the third series. The above three treatments recorded the highest values of most growth parameters under investigation and surpassed the other check treatments. In addition, noticeable that fresh and dry matter production was positively influenced by increment in growth parameters. Moreover, obtained data manifested in Table 4, also indicated that the percentage of water content varied (82-85%) as fertilization treatments changed. Although, the extended effect on such traits, no particular trend was observed.

In the respect, macronutrients and Fe concentrations in plant tissues, it is well known that fertilization plants with different fertilizer sources of essential nutrients sustaining the concentrations. Regarding to that, results obtained herein, indicated that, macronutrients and Fe uptake significantly influenced to all experimented treatments (Table 5). In general, organic fertilizer conjunction with chemical fertilizer and bio-fertilizer recorded the highest values of macronutrient and Fe concentrations in spinach tissues, followed by sole application of chemical fertilizer compared with the other check treatments. While, the lowest values of macronutrients and Fe were N (2.13%), P (0.23%), K (3.11%), Ca (0.65%) and Fe (25.5 ppm), registered with sole application of bio-fertilizer. Photosynthetic pigments content of chlorophyll a, chlorophyll b and carotenoids in the leaf tissues of spinach plants (μg/dm^2) recorded maximum values (3.77; 2.18 and 0.88) as application of organic fertilizer in combination with chemical and bio-fertilizer. Whereas, the lowest values for the same parameters with a count of (1.55, 0.76 and 0.56 mg/dm^2) were observed under the treatment of single application of bio-fertilizer (Table 6).

Concerning nitrate, soluble and insoluble oxalate in ppm and carbohydrate percentage, data obtained presented in Table 6, revealed that the highest values (5400, 4.63, 8.42 and 13.3%) were observed as a result of sole application of chemical fertilizers followed by chemical fertilizer conjunction with bio-fertilizer recorded (4500, 3.81, 5.93 and 14.0%). However, the lowest values were obtained as a result of application bio-fertilizer sole. Finally, obtained results herein, noteworthy that oxalate and nitrate concentration in spinach leaves were reduced by sole application of bio-fertilizer or organic fertilizer and their combination as well as their conjunction with chemical fertilizers.

Table 4: Influence of fertilization treatments on some growth parameters of spinach grown in green house. (Average of two growing seasons)

Treatments Character	Chemical fertilizer	Organic (compost)	Biofertilizer	Organic + Chemical	Biofertilizer+ Chemical	Biofertilizer+ Organic	Bio.+ Org + Ch	LSD at 0.05%
First sample (30 days from sowing)								
Plant height, cm.	28.6	19.6	20.2	28.3	30.1	22.1	31.2	2.31
Number of leaves	7.2	4.5	5.1	6.0	6.5	5.8	9.8	1.54
Leaf area,cm^2	150.0	78.0	75.0	118.0	145.0	88.0	184.0	29.12
Fresh weight, g	15.2	4.6	4.2	11.3	14.3	5.7	14.3	2.50
Dry weight, g	2.3	0.80	0.72	1.64	2.53	0.92	2.37	0.22
Water content,%	85.2	82.5	83.0	85.5	82.2	83.9	83.4	--------
Second sample (40 days from sowing)								
Plant height, cm.	44.0	26.9	26.9	39.6	44.3	31.8	45.9	2.41
Number of leaves	10.4	4.9	7.7	9.6	16.8	9.7	16.7	2.12
Leaf area,cm^2	166.5	95.9	97.5	144.0	208.8	104.7	292.6	40.3
Fresh weight, g	30.4	7.9	6.6	18.4	27.4	8.6	26.1	2.64
Dry weight, g	7.7	1.6	1.4	3.1	6.2	1.6	7.0	1.24
Water content,%	74.9	80.4	79.3	83.4	77.5	81.4	73.5	-------
Third sample (50 days from sowing)								
Plant height, cm.	53.7	33.8	34.1	49.5	55.4	42.6	60.6	4.21
Number of leaves	11.7	6.4	10.2	20.8	20.8	11.5	21.8	4.29
Leaf area,cm^2	469.5	115.1	121.9	177.1	396.7	136.1	397.9	61.7
Fresh weight, g	54.2	13.9	14.5	35.8	45.7	13.4	41.7	3.59
Dry weight, g	12.9	3.4	4.2	7.8	11.1	3.1	5.6	1.37
Water content,%	76.3	75.2	70.7	78.2	75.8	76.7	86.5	-------

Table 5: Mean of macro and micronutrients concentrations in Spanish leaves as affected by different fertilizer sources (average of two growing seasons)

	Percentage of macro- nutrients content and Fe in (ppm)				
Treatments	N	P	K	Ca	Fe
Chemical fertilizer (Ch.)	3.50	0.43	6.80	1.53	54.12
Organic (compost)	2.35	0.25	3.58	0.85	37.60
Biofertilizer	2.13	0.23	3.11	0.65	25.43
Organic + Chemical	3.80	0.90	7.32	2.22	65.51
Biofertilizer+ Chemical	3.70	0.71	7.05	1.70	57.54
Biofertilizer+ Organic	2.75	0.34	4.15	0.95	42.21
Bio.+ Org. + Ch.	5.11	1.12	8.11	2.65	81.11
LSD at 0.05%	0.22	0.65	0.88	0.34	2.92

Table 6: Leaf pigment contents and nitrate, oxalate as well as vitamins concentration spinach leaves as affected by different fertilization treatments (Average of two growing seasons)

	Leaf pigment contents (µg /dm^2)			chemical composition of plant tissues				Leaf vitamin content	
Treatments	Chl. a	Chl. b	Carotenoids	Nitrate ppm	Soluble oxalate, ppm	Insoluble oxalate, ppm	Carbohydrate %	V c mg/100g (Ascorbic acid)	VB9µg/100g (Folic acid)
Chemical fertilizer (Ch.)	2.23	1.57	0.82	5400	4.63	8.42	13.3	42.7	85.0
Organic (compost)	1.75	1.11	0.67	1200	1.13	2.62	12.0	28.4	55.0
Biofertilizer	1.55	0.76	0.56	0942	0.63	2.12	11.6	29.0	40.0
Organic + Chemical	2.88	1.77	0.91	4250	3.12	5.25	15.0	44.7	125.0
Biofertilizer+ Chemical	2.55	1.67	0.74	4500	3.81	5.93	14.0	41.1	100.0
Biofertilizer+ Organic	2.04	1.14	0.81	1150	0.93	2.82	13.5	38.0	65.0
Bio.+ Org. + Ch.	3.77	2.18	0.88	2825	2.11	4.75	21.0	52.2	155.0
LSD at 0.05%	0.34	0.52	0.26	8.25	0.12	1.02	1.53	9.25	35.2

Regarding to the activity ceases of spinach plant to accumulate useful component, such as vitamin C (ascorbic acid) and vitamin B(folic acid) are presented in Table 6, it can be realized that the highest values of both component (52.2, 44.7 mg/100g) and (155.0, 125.0 µg/100g) were attained by fertilized spinach with the combination of three main fertilizer sources (chemical + organic + biofertilizer) and (organic +chemical fertilizers), respectively compared with the other tested check treatments.

DISCUSSION

Formulate an efficient, economic and viable integrated system of plant nutrient supply for leaf vegetable spinach is one of the most important issues in improving spinach quality.The salient finding obtained herein that, noteworthy observation was that, the highest increment in growth parameters were detected in early growth stage than the progressing in growth. This is mainly due to the growth habit of spinach plants. Spinach characterizes with rapid growth in early growth stages. Therefore, farmers are used to sowing it two times during the growing season. In the present study, noticeable better performance in all growth parameters accompanying with application of organic compost associated with bio- and chemical fertilizers. Such treatment excelled all other tested treatments and registered higher plant height, more number of leaves and leave area. The increment could be ascribed to add dative effect of both sources of nutrient (organic and inorganic) associated with microbial population through inoculation with Rhizobia bacteria (biofertilizer) helping in mobilizing P and N fixation into soil solution in soluble form, there by higher release of both nutrient forms, this in turn reflected in promoted growth and proliferation of root, increased the rate of absorption, increased photosynthesis productivity and better source-sink relationship. All these put together in higher fresh and dry matter accumulation. These results are in concordance with the findings of Gawish [13], El-Naggar [14], Moftah and Attia [15], Moftah [16], Attia and Saad [17], Al-Humaid [18], Hammad and El-Gamal [19] and Selim *et al.* [21]. Under the present investigation, in nutshell the excellently of integrated nutrient supply may be also due to the state of the experimental soil texture (sandy soil contain, 95.30% sand). According to that ascription, soil have high leaching rate and low nutrient contents (low fertile), thus management nutrient requirements seems useful sates in availability, sustainability and uptake of nutrients which would have favored in increasing photosynthesis productivity (chlorophyll a and b) and better accumulation of micro-nutrients and Fe content and either ascorbic or folic acid concentration, while in the same times contrary decreased accumulation of nitrate and oxalate in leaves and thereby contributed yield quality. Shingo *et al.* [7] reported that, high reduction in nitrate and oxalate leave content resulted by using organic fertilizers. Similar finding was also reported by Okon [8], Goh and Vityakon [9], Mengel and Kirkby [10], El-Gamal and Selim [11], Renard *et al* [12], Selim *et al* [21], Selim *et al* [22], Chaterjee *et al* [23], Singh and Singh [24] and Zaki *et al* [25].

REFERENCES

1. Swaminathan, M.S., 2002. Green revolution is now greedy revolution. The Hindu, dated 02.08.2002, pp: 5.
2. FAO, Food and Agriculture Organization 2004. FAOSTAT Database Results.
3. Sujathamma, P. and D. Srinivasulu Reddy, 2004. Growth, yield and nitrogen uptake of low land rice as influenced by integrate nitrogen management. Indian J. Agric. Res., 38(4): 242-268.
4. Saravanapandian, P., S. Subramanian, P. Paramasivam and K. Kumaraswamy, 2005. Organic farming in sustaining soil health. Areview. Agric. Rev., 26(20): 141-147.
5. Mills, H.A., A.V. Barker and D.N. Naynard, 1976. Effects of nitrapyrine on nitrate accumulation in spinach. J. Amer. Hort. Sci., 101: 202-204.
6. Breimer, T., 1982. Environmental factors and cultural measures affecting the nitrate content in spinach. W.A.U. dissertation no. 909.
7. Shingo, M., A.E. Noriharu and M. Yamagata, 1999. Influence of organic fertilizers on the growth and contents of nitrate, oxalic and ascorbic acid in spinach (*Spinacia oleracea* L.), Japanese J. Soil Science and Plant Nut., 70(1): 31-38.
8. Okon, Y., 1984. Response of cereal and forage grasses to inoculation with N-fixing bacteria. In: Advances in Nitrogen Fixation Research, Veeger, C. and W.E. Newton (Eds.), pp. 303-309, Nijboff/Junk, The Hague.
9. Goh, K.M. and P. Vityakon, 1986. Effects of fertilizers on vegetable production. 2-Effects of nitrogen fertilizer on nitrogen content and nitrate accumulation of spinach and beet root. NZ. J. Agric. Res., 29: 485-494.
10. Mengel, K. and E.A. Kirkby, 1987. Nitrogen Fertilizer Application. In: Principles of Plant Nutrition, P. 347-384. Inter. Potash Inst. Pub. Switzerland.
11. El-Gamal, S. and A.H. Selim, 2005. Response of garlic plants grown in sandy soils to organic and inorganic fertilizers. J. Agric. Sci. Mansoura Univ., 30: 6687-6700.
12. Renard, S., J.P. Goffart and M. Frankinet, 2008. Nitrogen fertilization for spinach-bean rotations and case study on loamy soil in Belgium.ISHS Acta Horticulturae, 817.

13. Gawish, R., 1997. Trials to reduce nitrate and oxalate contents in some leaf vegetables. Zagazig J. Agric. Res., 24: 83-94.
14. El-Naggar, A.I., 1998. Investigation on the response of tuberose plants to biofertilization with nitrogen fertilization under four different soil textures. J. Agric. Sci. Mansoura Univ., 23: 6177-6203.
15. Moftah, A.E. and F.A. Attia, 1999. Compared effects of chemical fertilizer (super phosphate) and biofertilizer (phosphorine) on the growth, chemical constituents and yield of Caraway plants. Zagazig J. Agric. Res., 26: 557-574.
16. Moftah, A.E., 2000. Response of soybean plants grown under saline conditions to the biofertilizers. Ann. Agric. Sci., 38: 869-893.
17. Attia, F.A. and O.A.O. Saad, 2001. Biofertilizer as partial alternative of chemical fertilizer for Catharanthus. J. Agric. Sci. Mansoura Univ., 26: 7193-7208.
18. Al-Humaid, A.I., 2004. Effect of bio and chemical fertilizers on the growth and essential oil yield of fennel (*Foeniculum vulgare* Mill.). Alex. J. Agric. Res., 49: 83-91.
19. Hammad, S. and S. El-Gamal, 2004. Response of pepper plants to biofertilizer and mineral nitrogen. Minufiya J. Agric. Res., 1: 1-27.
20. Alderfasi, A.A., S.S. Alghamdi and A.A. Al-Garawi, 2009. Growth and Yield of Two Soybean Genotypes under Seed Inoculation and Limited Water Conditions of Saudi Arabia. Bull. NRC, 34(2).
21. Selim, M., A.A. Abd EL-Fattah, M.M. Abouel-Magd and M.A. Khalafallah, 2009. Efficiency of Bio-Fertigation on nutrients uptake by broccoli and soil microbial biomass under sandy soil conditions. American-Eurasian J. Agric. and Environ Sci., 6(3): 280-286.
22. Selim, M., A. Bahar and M.O. Kabesh, 2002. Utilization of biofortified system for enhancing lentil (*Lens culinaris* Medik) yield and quality in reclaimed sandy soil. Bull. NAR, Egypt, 27(4): 521-231.
23. Chaterjee, B., P. Ghanti, U. Thapa and P. Tripathy, 2005. Effect of organic nutrition in sprouting broccoli (*Brassica oleracea var.Italica* Plenck).Vegetable Sci., 33(1): 51-54.
24. Singh, V.N. and S.S. Singh, 2005. Effect of inorganic and biofertilizers on production of cauliflower (*Brassica oleracea L.var.Botrytis*). Vegetable Sci., 32(2): 146-149.
25. Zaki, M.F., A.A. Abd EL-Hafez and Y.C. Eldewiny, 2009. Influence of bio-fertilizers and nitrogen sources rates on growth, yield and quality attributes of sprouting broccoli (*Brassica oleracea L.var.Italica*). Egypt. J. Appl. Sci., 24(3): 86-111.
26. Gomez, K.A. and A. Gomez., 1984. Statistical Procedure for Agricultural Research-Hand Book, John WileyandSons, NewYork.
27. Bruinsma, J., 1963. The quantitative analysis of chlorophylls a and b in plant extracts. Photochem. Photobiol., 2: 241-249.
28. Porra, R.J., W.A. Thompson and P.E. Kriedermann, 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta, 975: 384-394.
29. Barrs, H.D., 1968. Determination of Water Deficit in Plant Tissues. pp: 235-368. In T.T. Kozlow-ski (Ed.). Water Deficit and Plant Growth. Academic Press, New York USA.
30. Chapman, H.D. and P.P. Pratt, 1961. Methods of Analysis for Solis, Plant and Water. University of California, Division of Agric. Sci. pp: 209-211.
31. Dubois, M., K.A. Gilles, J. Hamilton, R. Robers and F. Smith, 1956. Colorimetric method for determination of sugar and related substances. Anal. Chem., 28: 350.
33. King, E.J., 1951. Micro-Analysis in Medical Biochemistry 2nd ed. Churchill, London.
32. Barker, A.V., N.H. Peck and G.E. MacDonald, 1971. Nitrate accumulation in vegetables. 1-Spinach grown in upland soils. Agron. J., 63: 126-129.
34. Sadasivam, S. and A. Manickam, 1992. Vitamins. In Biochemical Methods for Agricultural Sciences. Wiley East. Pub. New Delhi, pp: 173-180.
35. Pffeifer, C.M., L.M. Rogers and J.F. Gregory, 1997. Determination of folate in cereal-grain food products using tri-enzyme extraction. J. Agric. Food Chem., 45: 407-413.
36. Savage, G.P., L. Vanhanen, S.L. Mason and A.B. Ross, 2000. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. J. Food Comp. Anal. 13: 200-201.
37. Waller, R.A. and D.B. Duncan, 1969. A boyorule for the symmetric multiple comparison problem.Amer, Stat. Assoc. Jour, Secember, 1485-1508.