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Nitrate accumulation, growth and leaf quality of spinach beet (*Beta vulgaris* Linn.) as affected by NPK fertilization with special reference to potassium

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Abstract: The present study was carried out at the Herbal Garden of Jamia Hamdard, New Delhi on Pusa Bharti variety of Spinach Beet (*Beta vulgaris* Linn.) to understand the effect of potassium with other fertilizers on nitrate accumulation, growth and quality of the leaves. Potassium application along with other fertilizers increased leaf area, per cent dry weight, Chlorophyll Content Index (CCI) and Nitrate Reductase Activity (NRA), but decreased nitrate content. Effect of 2:1 ratio of N and K was found statistically at par with 1:1 ratio of N and K. Fertilizer treatments without potassium had lower level of NRA and higher nitrate content in the leaves as compared to other applications. Application of FYM along with NPK fertilizers increased leaf area, per cent dry weight, CCI and NRA, whereas it decreases nitrate content. As higher NRA and lower nitrate content was recorded in treatments with potassium, application of K is desirable to reduce health hazards related with nitrate toxicity. Based on the results of the study, application of 1:1 ratio of N and K fertilizer and FYM is recommended.

Keywords: Spinach beet, potassium, nitrate toxicity, nitrate reductase, manure; fertilizers, NPK.

Introduction

Due to unplanned use of natural resources and indiscriminate use of chemical fertilizers, pesticides and various other chemicals to increase agricultural productivity, huge pressure has mounted on environment. In modern agricultural systems where plants rely on fertilizers to meet their demand of nitrogen, inadequate practices still cause environmental problems (Bacon, 1995; Lawlor *et al.*, 2001) mainly related to nitrate loss in the environment. When input of nitrogen exceeds the demand, plants are no longer able to absorb it, and nitrogen then builds up in the soil, mostly as nitrates (Nosengo, 2003). Apart from environmental contamination due to leaching volatilization, denitrification, surface run-off, etc. it leads to accumulation of nitrate in leafy vegetables beyond safe limits. Nitrate accumulation in plants is a natural phenomenon resulting from uptake of the nitrate ion in excess of its reduction and subsequent assimilation. Nitrate accumulation in plants varies widely between species, cultivars of same species (Anjana *et al.*, 2006) and is influenced by several factors, especially those factors that tend to limit the growth of the plant while still allowing for the uptake of nitrate. There are several plant species that may accumulate nitrate, including the Brassica plants, green cereal grains (barley, wheat, rye and maize), sorghum and Sudan grasses, corn, beets, rape, docks, sweet clover and nightshades. It is generally known that when plants are provided with excess nitrate, only a small amount of the nitrate taken up by the roots is immediately assimilated in roots and shoots, while the majority is stored in vacuoles of both roots and shoots.

Vegetables eaten by people every day usually contribute about 72-94% of the total daily intake of nitrate (Shen *et al.*, 1982; Dich *et al.*, 1996; Eichholzer & Gutzwiller, 1998). Leafy vegetables occupy a very important place in the human diet, but unfortunately constitute a group of foods which contributes maximally to nitrate consumption by living beings (Anjana *et al.*, 2007). Study done by Anjana *et al.* (2006) on samples of leafy vegetables collected from the local markets of Delhi has revealed that a significant number of Spinach and Chenopodium samples contained nitrate in concentrations higher than the Acceptable Daily Intake (ADI) limit for an average 60 kg person (if consumed at 100 g/d). This causes various health related problems in humans and livestock. Intake of high amounts of N-nitroso compounds by humans may increase the risk of nasopharyngeal and esophageal cancer (Eichholzer & Gutzwiller, 1998). As green vegetables are consumed by many pregnant women as a good source of iron, high nitrate content can cause pregnancy failure. It can also cause various other diseases in humans like stomach cancer Non-Hodgkin's Lymphoma. Thus reducing nitrate content in vegetables can decrease a risk of human illness (Luo *et al.*, 2006). A reduction in nitrate content can add value to vegetable products already very popular for their nutritional and therapeutic properties (Santamaria, 2006).

The rate of plant growth depends on the rate of photosynthesis and the rate of respiration. Photosynthesis and respiration, like all biochemical processes depend on the proper functions of enzymes. Enzymes require mineral elements to function effectively. Thus photosynthesis and other metabolic processes depend on an adequate supply of mineral nutrients. Nitrogen is considered to be one of the most important factors limiting plant growth in natural ecosystem and in most agricultural systems. Nitrogen governs plant growth by virtue of being a major constituent of chlorophyll, protein, amino acids and photosynthetic activity. Potash activates plant physiology, improves fruit quality, increases disease resistance, prevents lodging and makes the plants capable of surviving moisture stress. Addition of these

Table 1. Treatment details

Treatment	Concentration of fertilizer applied (Kg/ hectare)	Remark
T_0	$N_0P_0K_0$	Without fertilizer (control)
T_1	$N_{120}P_{60}K_0$	N & P in 2:1 ratio.
T_2	$N_{120}P_{60}K_{60}$	N, P & K in 2:1:1 ratio.
T_3	$N_{120}P_{60}K_{120}$	N & K in 1:1 ratio.
T_4	$N_{240}P_{120}K_0$	N & K in 2:1 ratio without P.
T_5	$N_{240}P_{120}K_{120}$	N, P & K in 2:1:1 ratio.
T_6	$N_{120}P_{60}K_{60}$+ FYM	N, P & K in 2:1:1 ratio along with FYM.

N= Nitrogen, P= Phosphorus, K= Potassium, FYM= Farm Yard Manure

essential nutrients and their uptake by plants has a considerable influence on growth and fruit yield, both qualitatively and quantitatively (Rathore *et al*., 2008). Sustainable nitrogen management should aim at supplying sufficient nitrogen for optimum crop growth and development, while keeping losses to the environment to minimum. Keeping above facts in view, it is of major concern to improve nitrate uptake and reduce its accumulation in leaves of Spinach Beet as well as other leafy vegetables. Proper application of nitrogenous, phosphate, potassium fertilizers, as well as the green and farm yard manure could materially reduce the nitrate accumulation in vegetables (Zhou *et al*., 2000). We should use sufficient amount and proper combination of fertilizers to increase crop productivity so that it does not harm environment. An adequate fertilization program may ensure sufficient plant growth without any risk of plant nitrate levels going too high (Vieira *et al*., 1998). Thus the present study was undertaken to understand the effect of different combinations of NPK fertilizers and FYM on the Nitrate accumulation, growth and leaf quality of Spinach Beet (*Beta vulgaris* Linn.).

Materials and methods

Description of the plant studied

Leaf Beet or Spinach Beet (*Beta vulgaris* Linn.) commonly known as Palak in Hindi is an erect or spreading indigenous annual herb, 30-190 cm in height, cultivated as a leafy vegetable. Spinach beet is a common leafy vegetable cultivated throughout the plains of North India during winter season and is not very popular in South India. It has a wider range of adaptability than Spinach (*Spinacea oleracea* Linn.). Spinach beet is particularly valued for its high ascorbic acid (40.9 mg), calcium, iron (4.8 mg) and riboflavin content. It is regarded as the richest source of vitamin A amongst all the vegetables. The protein of spinach beet is rich in essential amino acids, especially lysine.

Experiment was carried out at the Herbal Garden of Jamia Hamdard, New Delhi. Seeds of Pusa Bharti variety of Spinach Beet (*Beta vulgaris* Linn.) were sown in square beds of size 0.5x 0.5m. Five different treatments of N+P+K fertilizers, one with N+P+K+ Farm yard manure (FYM) and one controlled with no fertilizer or FYM were taken for the study (Table 1). Sources of N, P and K were urea, muriate of potash and single superphosphate. Three replicates of each treatment (7x3=21) were taken. Soil was loamy sand (83.6% sand, 6.8% silt and 9.6% clay, pH 7.1). The available nitrogen (128 mg/kg of soil) and phosphorus (4 mg/kg of soil) were low, whereas the available potassium was 158 mg/kg of soil. Sampling was done at 21, 42 and 63 day stages and all the parameters were studied simultaneously. Total numbers of leaves were counted at each stage. Fresh weight of leaves, stem and root were taken separately. For biomass of the plant first fresh weight (in mg) was taken and then the samples were oven-dried at 60°C for 48 hours and dry weight (in mg) was determined on a digital balance, which was further converted to per cent dry weight per plant. Leaf Area was measured by Potable Leaf Area Meter (model LI-3000, LI-COR, Inc., Lincoln). Net leaf area was expressed in cm^2. Chlorophyll Content Index was measured by CCM-200 Chlorophyll Index Meter (Opti Sciences-USA). Estimation of Nitrate content in leaves was done by the method of Grover *et al*. (1978) and estimation of Nitrate Reductase activity in leaves was done as per Klepper *et al*. (1971).

Results and discussion

Results of leaf count in various treatments are given in Table 2. Leaf count (leaves/ plant) ranged between 5 (T_0 and T_1) to 8 (T_6) at 21 days stage, 12 (T_0

Table 2. Results of the leaf parameters at the different growth stages

Treatments	21 days	42 days	63 days	21 days	42 days	63 days
	Leaf Count (Leaves/ Plant)			Leaf Area (cm^2)		
T_0	5	12	16	15.1	100.8	121.4
T_1	5	31	32	19.5	321.6	389.4
T_2	6	24	48	12.0	212.1	440.3
T_3	6	12	34	30.8	281.1	848.2
T_4	6	23	30	21.3	209.8	633.8
T_5	7	27	36	19.7	340.9	1179.4
T_6	8	25	35	33.7	248.5	696.5
Treatments	Chlorophyll Content Index			Total Fresh Weight per plant (g)		
T_0	11.2	9.8	9.2	0.7	4.4	7.6
T_1	10.9	18.4	11.8	0.9	4.9	30.6
T_2	12.2	13.2	12.0	0.9	11.4	31.7
T_3	14.9	20.6	16.4	1.5	19.1	58.3
T_4	13.7	15.7	9.5	0.9	11.8	39.4
T_5	10.5	20.9	19.0	1.7	21.4	84.9
T_6	13.3	19.1	17.1	1.0	17.3	37.6

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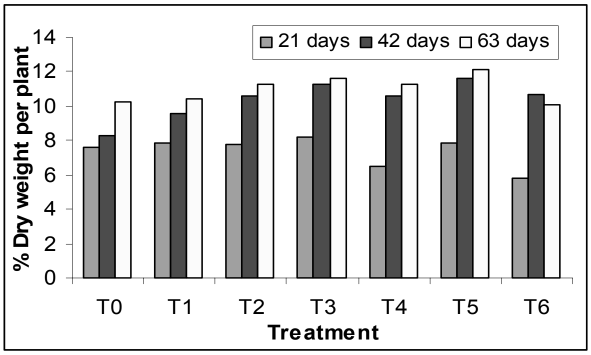
Fig. 1. Per cent dry weight per plant in different treatments at different growth stages



and T_3) to 31 (T_1) at 42 days stage and 16 (T_0) to 48 (T_2) at 63 days stage. There was not much variation as far as leaf count is concerned. Each treatment of fertilizer has increased number of leafs in comparison to controlled treatments where no fertilizer was applied. There is only marginal increase in treatments where potassium was applied as compared to treatments where no potassium was applied. This may be because of faster vegetative growth plants in those treatments. Leaf area is one of the most important growth parameter. Results of leaf area in various treatments are shown in Table 2. Leaf area oscillated between 12.0 cm^2 (T_2) to 33.7 cm^2 (T_6) at 21 days stage, 100.8 cm^2 (T_0) to 340.9 cm^2 (T_5) at 42 days stage and 121.4 cm^2 (T_0) to 1179.4 cm^2 (T_5) at 63 days stage. The increase in leaf area is plausible. This may automatically augment solar energy harvesting ability. As a result there is enhancement in the production of photosynthates and their subsequent translocation vis-à-vis total dry matter production (Blanchet *et al.*, 1962; Linser & Herwig, 1968; Beringer, 1982; Saxena, 1985). Whereas strong reduction of leaf area may be associated with accumulation of chloroplasts but mainly disturbs the integration of chlorophyll molecules into stable complexes (Horvath *et al.*, 1996). Controlled treatment showed that without external supply of N, P and K, leaf area was very small as compared to other treatments which had been given external supply of fertilizers. It has been found that in treatments where K was applied along with N and P had more leaf area then treatments where no K was applied. It may be because K has been reported to augment cell division and cell expansion (Marschner & Possingham, 1975; Mengel, 1977, 1978), which may lead to increase in leaf area. By comparing T_5 i.e., $N_{240}P_{120}K_{120}$ having N and K in 2:1 ratio with T_4 i.e., $N_{240}P_{120}K_0$ where no K was applied we found that T_5 had almost double leaf area at 63 days stage as compared to T_4. At 21 days stage leaf area was almost similar in all the treatments except T_3 i.e., $N_{120}P_{60}K_{120}$ and T_6 i.e., $N_{120}P_{60}K_{60}$+FYM showing that at early stage supply of nutrients was adequate for growth of plants. High leaf area at 21 days stage in T_3 may be because of 1:1 ratio of K and N as N is one of the more mobile elements and is known to be absorbed and accumulated at a fast rate in the presence of K. This is achieved by K activity as counter ion of nitrate in which form N is normally absorbed and translocated from root to shoot (Dijkshroon, 1958; Ben-Zioni *et al.*, 1971). Highest leaf area in T_7 at 21 days stage may be because of FYM, which could have provided adequate amount of other nutrients as Cu, Ca, Mg, etc. From 42 days stage to 63 days stage leaf area of the treatments having K almost doubled whereas of treatments having no K increased marginally. This shows that K has marked effect in increasing leaf area at this growth stage.

Results of fresh weight and dry weight in various treatments are given in Table 1 & Fig. 1 respectively. Total fresh weight per plant oscillated between 0.7g (T_0) to 17g (T_5) at 21 days, 4.4g (T_0) to 21.4 (T_5) at 42 days and 7.6g (T_0) to 84.9g (T_5) at 63 days stage. Applied potassium was found to increase per cent dry weight of plants. At 21 days there was no marked difference in different treatments but in 42 days stage we found treatments applied with K had higher dry weight than other treatments. Maximum dry weight was found in T_5 i.e. $N_{240}P_{120}K_{120}$ having N & K in 2:1 ratio. This can also be associated to increase in leaf area by application of potassium which may automatically augment solar energy harvesting ability. As a result there is enhancement in the production of photosynthates and there subsequent translocation vis-à-vis total dry matter production (Blanchet *et al.*, 1962; Linser & Herwig, 1968; Marschner & Possingham, 1975; Mengel & Kirkby, 1980; Beringer, 1982; Saxena, 1985). The beneficial effect of potassium is of particular importance in tropical crops, since Potassium reduces water loss by reducing transpiration (Brag, 1972; Saxena, 1985). 1:1 ratio of N & K was also found as effective a 2:1 ratio of N & K in increasing dry weight. By comparing treatments of fertilizer of N & P having K with same treatments of fertilizer of N & P or example T_4 i.e., $N_{240}P_{120}K_0$ with T_5 i.e., $N_{240}P_{120}K_{120}$ and T_1 i.e., $N_{120}P_{60}K_0$ with T_3 i.e., $N_{120}P_{60}K_{120}$ we found that there is marked effect of potassium in increasing dry weight. By comparing T_2 i.e. $N_{120}P_{60}K_{60}$ with T_6 i.e., $N_{120}P_{60}K_{60}$+FYM, we found that FYM also increases dry matter by increasing fertilizer use efficiency.

Results of Chlorophyll Content Index (CCI) in various treatments are given in Table 2. CCI ranged between 10.5 (T_5) to 14.9 (T_3) at 21 days stage, 9.8 (T_0) to 20.9 (T_5) at 42 days stage and 9.2 (T_0) to 19.0 (T_5) at 63 days stage. It is well known that the photosynthetic activity is based on the quantity of chloroplast pigment, which is directly proportional to the amount of photosynthetic area of the leaves. Applied potassium was found to increase CCI of leaf which is in accordance with the observation of Bark and Chein (1983), who noted an increase in chlorophyll content by potassium application. Presumably, potassium application promoted the uptake of such nutrients as SO_4^{2-}, Fe^{2+} and Mg^{2+} that are known to be associated with the synthesis of chlorophyll. CCI was minimum in absolute control at all the growth stages. At 21 days stages Chlorophyll content was almost similar in all the treatments except in T_3 i.e., $N_{120}P_{60}K_{120}$ having N & K in 1:1 ratio. Its possible reason may be because 1:1 ratio of N & K increases rapid uptake of Nitrate and K ions both which resulted in increase in chlorophyll content. Chlorophyll content was highest in T_6 i.e., $N_{240}P_{120}K_{120}$ having N & K in 2:1 ratio. In this treatment maximum N could have absorbed and used which could lead to increase in Chlorophyll content. As N is very important for formation of chlorophyll molecule each chlorophyll molecule contains 4N in tetrapyrolle ring. So N can act as limiting factor in formation of chlorophyll. As K enhances N uptake so it effects formation of chlorophyll indirectly as found in our observations.

Results of nitrate and Nitrate Reductase activity (NRA) in various treatments are given in table 3. Of the three forms of Nitrogen viz., nitrate, ammonium and dinitrogen, nitrate is usually of greatest importance and is the main form of Nitrogen taken up by the plants. Nitrate is reduced to nitrite by Nitrate Reductase. There is a reverse relationship between nitrate content and NRA (Olday *et al.*, 1976). So accumulation or assimilation of nitrate in cell depends upon activity of Nitrate Reductase (NR). By comparing nitrate and NRA of different treatments at various growth stages we found that increased NR activity decreased nitrate accumulation in leaves of Spinach Beet. Maximum activity of NR was found in T_6 i.e., $N_{240}P_{120}K_{120}$ having N & K in 1:1 ratio. This treatment also showed least amount of Nitrate. When we compared this treatment with T_5 i.e., $N_{240}P_{120}K_0$ having no K we found that K increases NRA and hence decreases nitrate accumulation in leaves. In T_4 i.e., $N_{120}P_{60}K_{60}$ 1:1 ratio of N & K is also shown to have high positive impact on NRA which again showed very low Nitrate in leaves. Maximum nitrate was found in T_2 i.e., $N_{120}P_{60}K_0$ followed by absolute control. It shows that application of N fertilizer without K can increase nitrate accumulation in leaves. The difference in nitrate and NRA in different treatments was apparent at all the growth stages. NRA and nitrate in leaves decreased with plant age. Results of our study suggest that potassium application reduces nitrate accumulation, which is in accordance with the findings of Ahmed *et al.* (2000) and Ruiz and Romero (2002), who reported that increase in the rate of potassium application facilitates uptake and transport of nitrate towards the aerial parts of the plant, promotes the metabolism and utilization of nitrate and ultimately, reduce nitrate accumulation in vegetable crops. Phosphorus, potassium and sulfur have major roles in production of proteins, thereby decrease nitrate within the plant (Brown *et al.*, 1993). Farmyard manure (FYM) is an excellent and balanced source of nutrients, which improves yield and quality of fruits, soil health and increase nutrient uptake (Abusaleha & Shanmugavelu, 1992). When we compare T_3 i.e. $N_{120}P_{60}K_{60}$ with T_7 i.e., $N_{120}P_{60}K_{60}$+FYM we found that addition of FYM is also effective in decreasing nitrate toxicity in leaves by increasing NRA. This may be because incorporation of organic material in the form of FYM enhances the soil organic carbon content and has direct and indirect effects on soil properties and processes (Kundu *et al.*, 2002, Katyal *et al.* 1997). Raupp (1996) also reported that vegetables supplied with organic fertilizers have low nitrate content, compared with minerally fertilized or conventionally grown vegetables. Given above, deficiency of plant nutrients may also favour accumulation on nitrate in plants, whereas integrated use of various fertilizers of potassium, phosphorus and

Table 3. Fresh weight (g) of different plant parts at different growth stages

Treatm-ents	21 days			42 days			63 days		
	Leaves	Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem
T_0	0.56	0.03	0.07	3.56	0.55	0.33	6.59	0.74	0.27
T_1	0.76	0.04	0.09	4.06	0.45	0.42	27.25	2.05	1.30
T_2	0.75	0.04	0.11	10.01	0.70	0.73	27.95	2.65	1.10
T_3	1.24	0.07	0.15	14.52	1.85	2.67	52.65	4.10	1.55
T_4	0.77	0.04	0.11	9.65	1.23	0.92	37.15	1.70	0.55
T_5	1.36	0.08	0.21	19.16	0.82	1.42	77.70	2.50	4.70
T_6	0.87	0.05	0.12	14.25	1.54	1.46	34.30	2.40	0.95

Table 4. Nitrate and Nitrate Reductase Activity in different Treatments

Treatm-ents	Nitrate Content (µ mole NO_3^- g^{-1} fresh wt of leaves)			Nitrate Reductase Activity (µ mole $NO_2^- h^{-1} g^{-1}$ fresh wt of leaves)		
	21 days	42 days	63 days	21 days	42 days	63 days
T_0	5.60±0.17	4.45±0.29	4.22±0.20	1.97±0.22	0.77±0.07	0.37±0.03
T_1	7.83±0.10	6.75±0.22	6.14±0.33	2.34±0.16	1.35±0.09	0.40±0.07
T_2	4.83±0.01	4.07±0.10	3.93±0.17	2.53±0.12	2.23±0.11	0.51±0.13
T_3	1.93±0.15	1.77±0.08	1.70±0.04	3.13±0.26	2.95±0.19	2.85±0.13
T_4	4.18±0.14	3.69±0.15	3.53±0.18	2.71±0.01	2.89±0.08	2.73±0.14
T_5	1.79±0.01	1.70±0.04	1.58±0.03	3.38±0.10	3.64±0.19	3.03±0.09
T_6	3.48±0.11	3.29±0.18	3.06±0.14	2.77±0.26	1.72±0.05	1.36±0.04

nitrogen may improve nitrogen uptake as well as assimilation efficiency. These include application of different types of fertilizers, their mode of application, avoiding runoff, mitigation of losses from soil and plants, use of slow release fertilizers, nitrification inhibitors, use of organic manures, green manuring, use of legumes in cropping systems, correction in their imbalanced use and integrated nutrient management.

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