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GROWTH, YIELD AND NITRATE ACCUMULATION OF IRRIGATED CARROT AND OKRA IN RESPONSE TO NITROGEN FERTILIZATION

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Abstracts

A randomized block experiment conducted on Sultanpur silt loam evaluated the N effects on growth and nitrate accumulation of okra (*Hibiscus esculents* L. cv. Sabz pari) and carrot (*Daucus carota* L. cv. T29). Treatments consisted of a control, 100, 150 and 200 kg N ha^{-1}. Significantly higher yields of carrot and okra were recorded at 150 kg N ha^{-1}. Nitrate concentration in both increased (> 35 to 200%) with increasing N. An excessive nitrate accumulation in both was recorded at 200 kg N ha^{-1}. Okra had 1.7 times higher nitrate accumulation than carrot. Early season okra had a significantly higher nitrate (~10%) than late season. Gross income and profitable return responded quadratically with increasing N. Results suggest that N fertilization of carrot and okra at 150 kg N ha^{-1} is optimum for economical yields with less accumulation of nitrate in vegetables.

Introduction

Vegetables are important dietary source of nitrate for human nutrition (Santamaria, 2006). About 70 to 80% of total intake of dietary nitrate comes from vegetables (Eichholzer & Gutzwiller, 1998). However, vegetables are considered to be a high source of nitrate accumulation (Yimin *et al*., 1991). High rates of N applied to vegetables are often considered by producers to be insurance against yield loss. When excess N is applied, nitrate absorption by vegetables is often more than what can be utilized (Barker *et al*., 1971; Barker & Tucker, 1971; Peck *et al*., 1971; Cantliffe, 1973; and Tarif *et al*., 1987). Increased nitrate accumulation due to indiscriminate applications of N is becoming a public health concern (Santamaria, 2006).

Nitrate by itself is relatively non-toxic, however, its metabolites may cause a number of public health problems (Santamaria, 2006). The toxic effects of nitrate are related to its endogenous conversion to nitrite, which is related to methaemoglobinaemia, gastric cancer and other diseases (Eichholzer & Gutzwiller, 1998; Santamaria, 2006). Excess nitrate can also block iodide uptake of the Sodium iodide symporter in a competitive manner (Tonacchera *et al*., 2005).

Commercial production of vegetables has increased substantially in Pakistan (www.Pakistan.gov.pk/divisions/food-division). Since N is the most deficient nutrient in soils, l producers apply heavy amounts of N for growing vegetables. Nitrogen fertilizers being used for vegetable production has increased by 21% between 1997 to 2003 (http://engrozarai.com). In addition to N fertilizers, there are widespread applications of high nitrate sewer water used for irrigating vegetables in Pakistan.

Since N fertilization is a critical factor influencing marketable yields and nitrate accumulation in vegetables, a balanced fertilization program can support profitable yields with an associated reduction in nitrate concentration to minimize public health risks (Barker *et al*., 1971; Barker and Tucker 1971; Peck *et al*., 1971; Cantliffe, 1973; and Tarif *et al*., 1987).

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Although limited attention has been paid to drinking and ground water nitrate levels in Pakistan, there is a lack of information about the dietary nitrates (e.g., vegetables) being consumed by humans. The present study was conducted to determine the optimum level of N that needs to be applied for increased plant growth and yield of irrigated carrot and okra. Moreover, nitrate accumulation was evaluated in response to N fertilization.

Materials and Methods

Description of the site: A field production experiment of carrot and okrawas conducted at the research farm of University College of Agriculture, Bahauddin Zakariya University ($71^0$37’79”E and $30^0$16’49”N), Multan, Pakistan in 2004 and 2005 growing seasons. The climate of the region is arid. Mean air temperature is 25^oC with highest temperature (40^oC) recorded in June and lowest temperature (7^oC) recorded in December. Mean relative humidity is 64% with the highest values (~ 90%) recorded in December and lowest values (25%) recorded in May. Average rainfall is 17.5 mm with the highest rainfall in July-August and lowest rainfall in October–November. Maximum daily sunshine (9 hr) was recorded in May-June and minimum of 6-hr was recorded in January with an average daily sunshine of 8 hr.

Soil at the experimental site is classified as a Sultanpur silt loam (coarse silty, hyperthermic, Typic Haplocambids) which is calcareous and alkaline in nature. Composite soil samples at 0 to 30-cm depth were randomly collected from the field prior to planting, air-dried for 7days at room temperature, 2-mm sieved, and ground before analysis for selected properties. Soil characteristics were: electrical conductivity (EC) 1.97 dS m^{-1}, pH 8.1, organic matter 5.8 g kg^{-1}, total nitrogen 0.3 g kg^{-1}, C:N 11, nitrate 15 mg kg^{-1}, calcium carbonate 65 g kg^{-1}, and sodium bicarbonate extractable phosphorus 95 mg kg^{-1}.

Experimental treatments and cultural practices: The experiment was established in a chisel plowed field using a randomized complete block design, with three replications. Plot size was 6m x 12m. Treatments were 0, 100, 150, and 200 kg N ha^{-1} (Tables 1 and 2). Urea was used as a source of N. A basal application of phosphorus (75 kg P_2O_5) and potassium (60 kg K_2O ha^{-1}) was applied as triple super phosphate and sulfate of potash, respectively. Okra seed were planted on ridges 20 cm apart in the first week of March, 2004. Plant spacing between rows was 45 cm. Carrot seed were planted on ridges 10 cm apart during first week of September, 2004. Plant spacing between carrot rows was 20 cm. Okra and carrot were irrigated 15 and 9 times, respectively, from a shallow tube-well. The chemical characteristics of the water were: EC 1024 dS m^{-1}, total bi-carbonate 292.8 mg L^{-1}, chloride 61.3 mg L^{-1}, sulfate 356.2 mg L^{-1}, calcium + magnesium 251.2 mg L^{-1}, sodium 55 mg L^{-1}, sodium absorption ratio 1.21 (m mole $L^{-1})^{-1/2}$, and nitrate 8 mg L^{-1}. Standard production practices were followed for growing okra and carrot. Data for plant height, length of green pod, number of pods $plant^{-1}$, individual weight of green pod, and pod yield $plant^{-1}$ of okra were recorded. Okra was harvested by picking green pods four times each growing season. At maximum growth, carrots were harvested manually. After harvest, length and diameter of each carrot root and total yield (Mg ha^{-1}) of were recorded. The experiment was repeated in 2005.

Table 1. Amount of nitrate applied and available for carrot production.

Nitrogen fertilization	Nitrate equivalent	Residual soil nitrate ($kg\ ha^{-1}$)	Nitrate in water	Total nitrate
Control	0	30	55	85
100	414	30	55	499
150	621	30	55	706
200	829	30	55	914

Table 2. Amount of nitrate applied and available for okra production.

Nitrogen fertilization	Nitrate equivalent	Residual soil nitrate ($kg\ ha^{-1}$)	Nitrate in water	Total nitrate
Control	0	30	91	121
100	414	30	91	535
150	621	30	91	742
200	829	30	91	950

Collection, processing and analysis of plant, soil and water samples: Green pods of okra were collected at 56, 66, 76 and 86 days after planting for analysis of nitrate concentration. Carrot plots were analyzed for nitrate concentration after final harvest. The nitrate concentration in both carrot and okra was determined by following standard method (Ryan *et al*., 2001). Okra green pods and carrot was oven dried at 70°C for 48h before analysis. A 0.5g oven- dried sample was taken into 100ml digestion tube with a few pumice boiling granules followed by an addition of 5ml concentrated sulfuric acid. The contents were mixed and heated on an aluminum block digester at 150°C. After digestion, the contents in the tubes were allowed to cool down and mixed with 2ml H_2O_2 followed by heating on a block digester to 280°C for 10-min. After cooling, another 2-mL H_2O_2 was added followed by block digester heating for 10-min to obtain a clear digestate and the nitrate content was determined by steam distillation.

A sample of 10g oven dried equivalent of <2mm sieved air-dried soil was taken in Erlenmeyer flasks and mixed with of 0.1-M 50-ml Copper sulfate. The suspensions were shaken for 15-min and filtered to obtain clear extracts. A 3 ml sample of filtrate was taken into 50-mL conical flasks with drop-wise addition of 1 ml of 0.1% chromotropic acid solution. After mixing, 6 ml concentrated Sulfuric acid was added to the contents and allowed to develop yellow color at room temperature. The absorbance of the colored solution was measured at 430 nm against nitrate standards. The same procedure was followed to measure nitrate concentration in water samples used for irrigation (Ryan *et al*., 2001). Other soil and water properties were determined by following standard methods of analysis (Yadav & Khera, 1993; Ryan *et al*., 2001).

Statistical and economical analyses: Data were analyzed by using Fisher's analysis of variance and Duncan's multiple range tests at $p<0.05$ to compare significant differences among treatment means. Economic analysis for commercial production of carrot and okra was performed by accounting all the expenses related to production and market values of the crops. The carrot and okra price was calculated at \$50 and 133 Mg^{-1}, respectively.

Results and Discussion

Growth and yield of carrot and okra: Nitrogen fertilization treatments were significant on carrot and okra plant growth and marketable yields (Tables 3 and 4). Carrot plant height and diameter have increased quadratically by N fertilization (Table 3). However, the average length of carrot did not vary consistently. Likewise, marketable yield of carrot increased (24 to 42%) quadratically in response to N treatments (Fig. 1). Highest marketable yield of carrot (24.2 Mg ha^{-1}) was recorded when plants were fertilized at 150 kg N ha^{-1} (equivalent to a total input of 706 kg NO3 ha^{-1} from fertilization, residual soil nitrate and irrigation) as compared to 17.1 Mg ha^{-1} in control (equivalent to a total input of 85 kg NO3 ha^{-1} from residual soil nitrate and irrigation).

Okra plant height increased significantly (70 to 90%) with N fertilization (Table 4). Maximum plant height of okra was recorded when fertilized at 200 kg N ha^{-1} (equivalent to a total input of 950 kg NO3 ha^{-1} from fertilization, residual soil nitrate and irrigation) followed by 150 (equivalent to a total input of 742 kg NO3 ha^{-1} from fertilization, residual soil nitrate and irrigation) and 100 (equivalent to a total input of 525 kg NO3 ha^{-1} from fertilization, residual soil nitrate and irrigation) kg N ha^{-1} respectively compared to control treatment (equivalent to a total input of 121 kg NO3 ha^{-1} from fertilization, residual soil nitrate and irrigation). Likewise, maximum length of okra green pod was recorded at 200 kg N ha^{-1}followed by 150 and 100 kg N ha^{-1} as compared to control treatment.

The highest number of okra green pods (17 pods $plant^{-1}$) was recorded at 150 kg N ha^{-1} treatment while a minimum number of pods (14 pod $plant^{-1}$) were observed in control. N fertilization above 200 kg ha^{-1} did not produce any significant increases in number of pods compared to other treatments. On average, maximum individual pod weight and pod yield $plant^{-1}$ were observed when N was applied at 150 kg ha^{-1} while minimum weight was recorded in control (Table 5). The highest green pod yield (19.1 Mg ha^{-1}) of okra was produced with 150 kg N ha-1 where as the lowest yield was recorded in the control treatment (Table 5).

Significant variations in carrot and okra growth and yield may be related to a variation in rates of N. An adequate supply of N (150 kg ha^{-1}) is probably associated with greater photosynthetic activity and optimum vegetative growth which accounted for higher yield of carrot and okra. Havlin *et al*., (1993) reported that N uptake by the plants is assimilated into amino acids that are subsequently incorporated into proteins and nucleic acids to enhance plant growth and yield. Results of this study are in concurrence with other studies (Zanin & Kimoto, 1980; Niak; Srinivas, 1992).

A quadratic increase in carrot and okra yield in response to N fertilization is most probably related to an excessive vegetative growth at higher N level instead of entering into plant's productive phase as marketable yields (Havlin *et al*., 1993). Since plant growth and yield are greatly influenced by N uptake and subsequent biochemical reduction, a greater nitrate reduction to ammonium at 150 kg ha^{-1} may have lead to higher carrot and okra yields, probably by allowing a greater allocation of N to photosynthetic activity and C-metabolism in plants (Djennane *et al*., 2004). It is reported that optimum nitrate supply is related to a high photosynthetic capacity which allowed plants to fix more C and obtain more energy from photosynthesis to enhance plant's C-N metabolism (Behr & Weber, 1992).

Table 3. Effect of N fertilization on plant height, root length diameter and yield of carrot.

Nitrogen fertilization (kg ha^{-1})	Plant height	Root length (cm)	Root diameter	Yield (g plant^{-1})
Control	22.1c	11.5c	2.7b	82.1c
100	24.2b	12.6b	3.2a	101.8b
150	25.1a	13a	3.4a	116.2a
200	25.5a	12.8ab	3.4a	113.2a

Means followed by same letter were not significantly different at p<0.05.

Table 4.Effect of N fertilization on plant height, pod length, number, weight and yield of okra.

Nitrogen fertilization (kg ha^{-1})	Plant height (cm)	Pod length (cm)	Pod number (plant^{-1})	Pod weight (g)	Pod yield (g/plant^{-1})
Control	40.9d	11.1b	14b	12.9c	180.6d
100	70.2c	12.1a	16a	14.3b	228.6c
150	76b	12.3a	17a	14.9a	258.2a
200	78.6a	12.7a	17a	14.3b	238.7b

Means followed by same letter were not significantly different at (p<0.05)

Table 5.Effect of N fertilization on nitrate accumulation in okra at various times of harvesting.

Nitrogen fertilization (kg ha^{-1})	------------------ Picking days after planting ------------------			
	56	66	76	86
	------------------ Nitrate conc. (mg kg^{-1}) ------------------			
Control	913.3ns	890	839.3	790.6
100	1235.6	1194.3	1130.6	1120.6
150	1384	1371.3	1290.6	1277
200	1842.3	1826.3	1761	1720
Average 1343.7a	1343.7a	1320.5b	1255.3c	1227.1d

Means followed by same letter were not significantly different at p<0.05.
ns=Non-significant interaction between N fertilization and picking days.

The significant quadratic response of carrot and okra yield at higher N level (200 kg ha^{-1}) is most probably associated with the toxic effects of nitrate metabolites on plant's C-N metabolism. Several studies have reported that a higher nitrate accumulation by vegetables may be responsible for rapid biochemical conversion to nitrite followed by nitric oxide (Durner & Klessig, 1999; Lamattina *et al*., 2003). The nitric oxide and O2 - could be rapidly catalyzed by nitrate reductase into peroxynitrites, and subsequently exert toxic effects on plant growth and yield (Reddy & Menary, 1990; Durner & Klessig, 1999; and Lamattina *et al*., 2003).

Therefore, a higher rate of N (200 kg N ha^{-1}) was found detrimental to both carrot (3% yield reduction) and okra (8% yield reduction) as compared to 150 kg N ha-1 treatment. In other words, excess available N in soil may lead to a significant decrease of yield of vegetables.

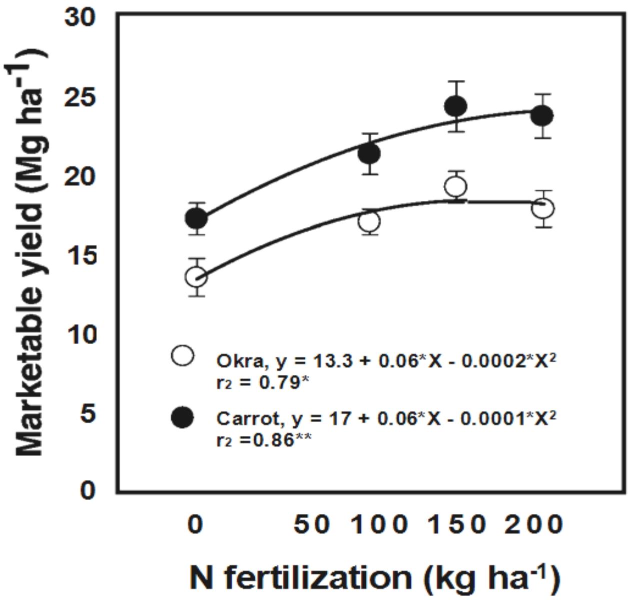

Fig. 1. Carrot and okra marketable yields response to nitrogen (N) fertilization.

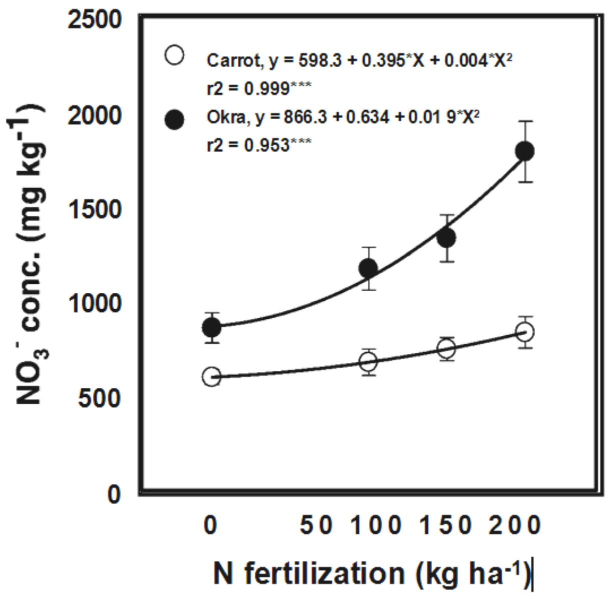

Fig. 2. Nitrate accumulation of carrot and okra in response to nitrogen (N) fertilization.

Nitrate accumulation in carrot and okra in response to N fertilization: Nitrogen fertilization had significant effects on nitrate accumulation in both carrot and okra (Table 5 and Fig. 2). Carrot nitrate concentration increased quadratically (13 to 39%) with a concurrent increase in rate of N fertilization. Maximum concentration of nitrate (834.7 mg kg^{-1}) was recorded in carrot fertilized with 200 kg N ha^{-1} as compared to 598.3 mg nitrate kg^{-1} in the control treatment. However, the nitrate concentration in okra green pods increased exponentially (> 35 to 200%) with an increase in rate of N fertilization (Table 5 and Fig. 2). Averaged across picking days, a minimum nitrate concentration (858 mg kg^{-1}) was found in control plants while the maximum nitrate concentration (1787 mg kg^{-1}) was found in okra pods fertilized with 200 kg N ha^{-1}. Early season picked okra pods had a significantly greater nitrate concentration (~10%) than late season. There was no significant interaction of N fertilization and picking days on nitrate accumulation in okra pods.

A significant difference in nitrate accumulation was found between carrot and okra (Fig. 2). Averaged across N fertilization rates, 714.1 mg NO3 kg^{-1} of carrot was measured as compared to 1236.7 mg NO3 kg^{-1} of okra. In other words, about 1.7 times more nitrate was found in okra than carrot. Even in the control treatment, nitrate accumulation in okra was 1.4 times higher than carrot. Maximum nitrate concentration was 2.1 times higher in okra as compared with carrot.

The significantly higher accumulation of nitrate found in both carrot and okra in response to increased N fertilization is perhaps due to an imbalance between uptake and translocation of nitrate by the xylem and its subsequent biochemical reduction to ammonium by nitrate reductase enzymes for incorporation into amino acids metabolism (Maynard *et al*., 1976; Scaife, 1989). With 200 kg N ha^{-1} supply, a higher nitrate accumulation in both carrot and okra is possibly due to greater uptake of nitrate more than its utilization by the plants (Barker *et al*., 1971; Barker & Tucker, 1971; Peck *et al*., 1971; Cantliffe, 1973; Tarif *et al*., 1987). It has been reported that when plants are provided excess nitrate, only a small portion of the nitrate is taken up by the plants and immediately assimilated in roots and shoots, while the major portion is accumulated in root and shoot vacuoles (Olday *et al*., 1976). This implies that an excess of N fertilization may lead to a significant decrease of yield with an associated accumulation of nitrate in plants. In turn, a greater nitrate reductase activity may also account for the low nitrate accumulation in both carrot and okra at lower rates of N fertilization (Olday *et al*., 1976). Studies have reported that excess N fertilization often increased the nitrate accumulation in root and leafy vegetables (Barker *et al*., 1971, Peck *et al*., 1971, Cantliffe 1973; Yimin *et al*., 1992, John *et al*., 2003).

As nitrate accumulation in plants depends on the balance between uptake and biochemical reduction, a higher nitrate accumulation in early picked okra might be due to low nitrate reducing capability in plants (Olday *et al*., 1976). In fully expanded plant leaves with low nitrate reductase activity, the N metabolism was limited as greater nitrate was taken up into the plant due to low mobility of nitrate with the phloem, and therefore nitrate accumulates in the plant tissue (Marschner, 1995). Furthermore, plant growth plays an important role in nitrate uptake and utilization. The efficiency of nitrate uptake substantially increased with the increase of plant relative growth rates (Ter Steege *et al*., 1999). Thus, a low nitrate concentration in okra green pods might be attributable to the decrease in the nitrate uptake owing to the decrease of the plant relative growth rates over time. Nitrate uptake is inversely related to antecedent nitrate concentration in plant. A higher accumulation of nitrates in the shoots than in the roots is possibly related to osmoregulation of the plant cells (Marschner, 1995).

Significantly lower nitrate accumulation in carrot than in okra at all levels of N fertilization may be due to their genetics and low nitrate reductase activity. Several other factors may be related to variations in the absorption, distribution and utilization of nitrate or other element’s needed for nitrate reductase activity are (i) generation of electron donors needed in the assimilative pathway, (ii) photosynthetic activity and (iii) ability to generate and translocate respiratory substrates and reducing equivalents (Cantliffe, 1973; Olday *et al*., 1976; Behr & Weber, 1992).

Economic analysis of carrot and okra production: With variable rates of N fertilization, cost for commercial production of carrot and okra did not vary substantially (Tables 6 and 7). However, the production cost for carrot was less variable than okra. In contrast, the gross income quadratically increased with increasing rate of N fertilization. Both gross income and profitable return were higher (2 and 3 times) for okra than carrot. The cost: benefit ratios were economical when both vegetables were fertilized at 150 kg N ha^{-1}. Increasing rate of N fertilization significantly affected the cost: benefit ratios of both carrot and okra production.

Table 6. Economic analysis for carrot production.

Nitrogen fertilization (kg ha^{-1})	Production cost ($ ha^{-1})	Gross income ($/ha)	Profitable return ($ ha^{-1})	Cost: benefit ratio
Control	519	855d	337d	1: 1.7c
100	557	1060c	503bc	1: 1.9b
150	576	1210a	634a	1: 2.1a
200	607	1180b	573b	1: 1.9b

Means followed by same letter were not significantly different at $p<0.05$

Table 7. Economic analysis for okra production.

Nitrogen fertilization (kg/ha)	Production cost ($/ha)	Gross income ($ /ha)	Profitable return ($/ha)	Cost: benefit ratio
Control	653	1782d	1129c	1: 2.7b
100	798	2248c	1450b	1: 2.8ab
150	875	2540a	1665a	1: 2.9a
200	908	2354b	1446b	1: 2.6b

Means followed by same letter were not significantly different at $p<0.05$.

Significant improvement in profitable return and cost: benefit ratios of commercial production of carrot and okra is perhaps due to higher yield in response to optimum N fertilization. A quadratic effect of increasing N fertilization on return and cost: benefit ratios of both vegetables is most probably related to excessive vegetative growth with an associated decrease in marketable yield. However, the cost: benefit ratios were quite economical for control treatment where residual nitrate in soil and water used for irrigation may have benefited carrot and okra production.

Conclusions

Increasing rates of N fertilization quadratically increased marketable yields, however, accelerated greater accumulation of nitrate in both carrot and okra. Nitrate accumulation in okra was significantly higher than carrot, which increased exponentially in response to N fertilization. Results suggested that N fertilization at 150 kg N ha^{-1} is optimum for producing profitable yields with significantly lower accumulation of nitrate in both carrot and okra. Application of N fertilizer on nitrate accumulating vegetables should be based on crop specific recommendations.

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