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ESTIMATION OF NITRATE AND NITRITE CONTENTS IN THE LOCALLY GROWN VEGETABLES

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Local leafy, stem and root tuberous vegetables grown with different forms of nitrogen application were analysed for nitrate by Londmann's method and nitrite by diazotization. The contents of nitrate in spinach were found higher in comparison to carrots, radish, turnip, cabbage and potatoes. Carrots grown using nitrogenous fertilizers contained nitrate contents 59.4 per cent more than those grown only with well water.

The nitrite contents of these vegetables ranged between (0–0.2 parts/10^6) and the contents of nitrites in spinach were significantly higher. It was also observed that nitrite contents of the analysed vegetables were dependent upon the nature of irrigation system and a significant difference was noted between the conditions in respect of accumulation of nitrites.

INTRODUCTION

The fast increasing nitrogen input due to excessive use of nitrogenous fertilizers may become a health hazard through nitrate pollution of water and food resources in the coming decades.

The present investigations were therefore conducted to analyse some of the local leafy stem and root tuberous vegetables grown with different forms of nitrogen applications for nitrate and nitrite contents which are persumed to be health hazard.

Bock and Schuphan (1959) estimated the nitrate and nitrite contents of 17 varieties of white, red and savoy cabbages given normal fertilizer treatment. The nitrate contents ranged from 27 to 87 average 50 mg% fresh matter. Increasing $Ca(NO_3)2$ from 45–180 KgN/ha raised nitrate values in fresh matter from 100 to 170 mg%.

Simon (1966) determined that fresh spinach and lettuce, 12 samples of each contained from 70 to 2100 and from 860 to 2100 mg nitrate/Kg; nitrite occurred only in one withered sample of spinach. Endwes, khlrabi, green beans,

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cress and stinging nettles contained over 1000 mg nitrate/Kg. Values were lower in leeks, cauliflower and brussels, Sprout, sugar peas, field beans, comfrey and gorrel contained none. In 32 samples of imported canned spinach for infants nearly all with potato added there was from 40–1900 mg nitrate/Kg, but no nitrite or bacteria. Storage at 20°C of tightly packed fresh spinach or of deep frozen spinach before and after cooking, led to formation of nitrite after 3 or more days.

Richardson (1970) found high nitrite concentration (30–75 parts/10^6) in celery, lettuce, beet root and radish. He found that increased use of nitrogenous fertilizers resulted in higher nitrate concentrations in plants.

Cantliffe (1973) proved that in a wide range of plant species, application of nitrogenous fertilizers led to accumulation of nitrate and supplementation with potassium also favoured such accumulation. The rate of reduction of nitrate by endogenous nitrate reductase depends on genetic factors, substrate induction and the supply of electrons from NADPH.

MATERIALS AND METHODS

1. Samples Collection

½ Kg of carrot (*Dacus carota*), radish (*Raphanus sativa*), turnip (*Brassica napus*), potato (*Solanum tuberosum*), cabbage (*Brassica oleraceae*) and spinach (*Spinach oleraceae*) vegetables samples were collected from various vegetable fields, grown under four different agronomic conditions of Tehsil Chiniot, Distt: Jhang, Punjab during Nov-Dec., 1976. The detail of the agricultural conditions were as follows:

I. Tube well water
II. Severage water
III. Tube well water in combination with farm yard manure (25 cart loads/hac.)
IV. Tube well water in combination with nitrogenous fertilizers (100 Kg/hac.) and farm yard manure (25 cart loads/hac.)

The freshly harvested samples of vegetables were brought to the Chemistry laboratory, University of Agriculture, Lyallpur. Before subjecting them to analysis they were thoroughly washed with distilled water.

2. Analysis of vegetables

(i) *Nitrate Determination*. A 10 grams of vegetable sample was macerated with 40 ml water for 3 minutes in a mechanical blender. It was transferred to

250 ml. beaker with 20 ml water. The slurry was then heated on water bath for one hour. It was cooled, washed into 100 ml volumetric flask with distilled water and the volume was made upto the mark. The macerate was then filtered. 0.1 ml saturated urea solution was added in 1 ml of filterate and the nitrate contents were estimated by the method of Londmann *et al.* (1966).

The standard curve was made by dissolving 1 gm $NaNO_3$ (Merck) in 500 ml distilled water and the volume was made up to 1 litre. It was again diluted to 1:10 to have a final concentration as 100 ug $NaNO_3$/ml. Aliquots of 0-10 ml of diluted stock solution were diluted further up to 10 ml with water. In 1 ml of each solution 1 ml of brucine solution (10% soln of Brucine in 92% ethanol) and 9 ml orthophosphoric acid and sulphuric acid mixture was added with shaking. All these tubes containing the above solutions were kept in boiling water for two minutes and were placed in the refrigerator at once. The maximum obsorbance was read at 420 nm using spectronic-20-spectrophotometer and the standard curve was constructed by plotting optical density against various concentrations of $NaNO_3$.

(ii) *Nitrite determination.* 5 grams of vegetable sample was macerated with 40 ml distilled water. The macerate was heated to 80°C and was transferred to 500 ml volumetric flask adding further 260 ml hot water. The solution was placed in a boiling water bath with occasional shaking for two hours. 5 ml saturated mercuric chloride solution was added. The mixture was cooled, mixed, diluted to mark and filtered. Nitrite contents in various vegetables samples were estimated following the method of Griess & Ilosvay (1889).

RESULTS AND DISCUSSION

The nitrate and nitrite analysis of three root tubers, one stem tuber and two leafy vegetables obtained from various vegetable fields of Tehsil Chiniot, Distt: Jhang was conducted during Nov-Dec., 1976. These vegetables fields were either irrigated with tube well water or city drains (severage) water. Samples collected from fields where they were grown using farm yard manure alone or the combination of farm yard manure and nitrogenous fertilizer have also been analysed and results have been presented in Table 1 & 2.

The nitrate contents listed in Table 1 determined by the method of Londmann's *et al.* (1966) appeared to be very less as compared to the data reported by Richardson (1970).

Londmann's method was based on the oxidation of Brucine. This method has been successfully employed for the determination of nitrates in

Table 1. *Nitrate contents of various vegetables grown under different conditions.*

Irrigation source		Carrot ppm	Radish ppm	Turnip ppm	Cabbage ppm	Potato ppm	Spinach ppm
Well water	(C_1)	2.2	2.2	2.8	2.6	2.1	3.6
Severage water	(C_2)	3.6	3.7	3.6	3.9	3.8	4.3
Farm yard manure	(C_3)	3.7	4.0	4.0	4.1	4.2	4.8
Farm yard manure +Nitrogenous	(C_4)	5.4	5.3	5.8	6.0	6.4	8.3

Table 2. *Nitrite contents of various vegetables grown under different conditions.*

Irrigation source		Carrot ppm	Radish ppm	Turnip ppm	Cabbage ppm	Potato ppm	Spinach ppm
Well water	(C_1)	.066	.08	.07	.052	.06	.092
Severage water	(C_2)	.096	.116	.124	.128	.136	.156
Farm yard manure	(C_3)	.112	.136	.132	.136	.144	.160
Farm yard manure +Nitrogenous	(C_4)	.172	.150	.140	.160	.166	.192

meat and meat products in various analytical laboratories (Schuller and Veen, 1967), it was, therefore attempted to find the feasibility of this method in vegetables. The measurement of such a low contents of nitrates in vegetable by this method proved the specificity of the method to only meat products. The low values of nitrates in vegetable by this method could be due to the interference from nitrites, chlorides and carbohydrates (Boek and Schuphan, 1959).

It is evident that nitrate contents in spinach (0.83%) when grown under conditions nitrogenous fertilizers combined with farm yard manure are much higher than the nitrate contents in rest of vegetables (.53–.83%) (Table 1). This is true in case of other three conditions also. Statistical analysis has shown that raddish, carrot, potatoes under C_1; Potato, raddish, carrot, turnip under C_2; Potato, turnip, radish under C_3; cabbage and potato under both C_2; and C_3 and raddish, carrot both under C_3 and C_4 do not differ significantly from each other in the nitrate contents while the rest do differ significantly (Table 3) Spinach has shown highly significant difference which possibly means that spinach has the more nitrate accumulating capacity as has already been reported (Richardson, 1907) and (Wilson, 1949).

Table 3. *Statistical analysis of nitrate contents in vegetables.*

ANOVA TABLE

SV	Degree of freedom	S.S.	M.S.	Fcal
C	3	1.3421	.4474	2354.73**
V	5	0.1849	.0370	194.73**
CV	15	0.0876	0.0058	30.52**
Error	48	0.0091	0.000190	
Total	71	1.6237		

** significant at 5% and 1% levels
SE for CV = .00795

	Carrot	Radish	Turnip	Cabbage	Potato	Spinach
C_1	0.23	0.23	0.30	0.28	0.22	0.37
C_2	0.38	0.39	0.38	0.41	0.40	0.45
C_3	0.39	0.41	0.42	0.44	0.43	0.49
C_4	0.57	0.56	0.61	0.63	0.67	0.87

C_1		C_2		C_3		C_4	
Spinach	=0.37	Spinach	=0.45	Spinach	=0.49	Spinach	=0.87
Turnip	=0.30	Cabbage	=0.41	Cabbage	=0.44	Potato	=0.67
Cabbage	=0.28	Potato	=0.40	Potato	=0.43	Cabbage	=0.63
Radish	=0.23	Radish	=0.39	Turnip	=0.42	Turnip	=0.61
Carrot	=0.23	Carrot	=0.38	Radish	=0.41	Carrot	=0.57
Potato	=0.22	Turnip	=0.38	Carrot	=0.39	Radish	=0.56

The four conditions differ significantly among themselves in respect of all the vegetables and has significant effect on nitrate accumulation (Table 3). These findings need confirmation using same rapid, accurate and handy methods for the determination of nitrates in vegetables. The results do indicate some valuable clues and suggest that more work has to be carried out keeping the awareness of increasing level of nitrates, in the environment (Comly, 1955; Crossby, *et al.* 1972, FAO/SIDA, 1972).

The data on nitrite contents of fresh vegetables determined by the method of Griess-illosvay (1889) has been described in Table 2. This method was based on the interaction of nitrite with a primary aromatic amine (Sulphanilic acid) in acid solution to form a diazonium salt. The diazonium salt was then treated with an aromatic compound (1-nepthyl amine) bearing influential amino or hydroxyl substituent to form an azo colour which was finally measured spectrophotometrically. The nitrite values determined in the present investigation were found quite low (0–.2 parts/10^6) and were in agreement with the reported values (0–6 parts/10^6) by Heisler *et al.* (1973) and Kamm *et al.* (1965). On the contrary Richardson (1970) reported a quite high nitrite concentration (30–75 parts/10^6) in celery, lettuce, beet root and radish without mentioning clearly the conditions under which these vegetables were stored prior to analysis. Since post harvest storage of vegetables can lead to the accumulation of high nitrite concentrations, the reported values by Richardson, cannot be easily interpretted.

The permissible values of nitrites range from 5–6 ppm (W.H.O., 1972) and the present survey has revealed that nitrite contents found in fresh vegetables has not crossed the prescribed limits. It is also suggested that fresh vegetables should be used as soon as practicable or processed by sterilization in hermatically sealed container or by deep freezing because vegetables stored at room temperatures or under refrigeration (not frozen) accumulates nitrite (Glaus Simon, 1966).

Statistical analysis have shown that nitrite contents of the vegetables under all the conditions differ significantly from each other except carrot and turnip under C_1 condition. Spinach has shown highly significant difference in comparison to other vegetables indicating that it has also accumulating capacity for nitrite as reported by (Book and Schuphan 1959 and Cantliffe, 1973).

It may also be seen from Table 4 that nitrite contents of vegetables were dependent upon the nature of irrigation water and there was a significant difference between the conditions in respect to the accumulation of nitrite.

It may therefore be concluded that the nitrite contents of vegetables grown under four different irrigation systems do not exceed the permissible level although it has ten-fold greater toxicity that nitrate and also presents a greater health hazard (Phillips, 1968; Magee and Barnas, 1967). It is suggested that more quantitative work on all preharvest and post-harvest environmental conditions is needed in order to exactly know the extent and magnitude of these food pollutants.

Table 4. *Statistical analysis of nitrite contents in vegetables.*

ANOVA

SV	Degree of freedom	S.S.	M.S.	FR Cal
C	3	1.6632	0.554400	4017.39**
V	5	0.2023	0.040460	293.1884**
CV	15	0.1332	0.008880	64.32**
Error	48	0.0066	0.000138	
Total	71	2.0053		

**Significant at 5% and 1% level
SE for CV = 0.0067

	Carrot	Radish	Turnip	Cabbage	Potato	Spinach
C_1	0.30	0.35	0.31	0.24	0.28	1.25
C_2	0.44	0.51	0.57	0.59	0.61	2.07
C_3	0.52	0.57	0.59	0.62	0.64	2.15
C_4	0.78	0.68	0.62	0.71	0.75	2.59

C_1		C_2		C_3		C_4	
Spinach	=0.41	Spinach	=0.69	Spinach	=0.71	Spinach	=0.86
Radish	=0.35	Potato	=0.61	Potato	=0.64	Carrot	=0.78
Turnip	=0.31	Cabbage	=0.59	Cabbage	=0.62	Potato	=0.75
Carrot	=0.30	Turnip	=0.57	Turnip	=0.59	Cabbage	=0.71
Potato	=0.28	Radish	=0.51	Radish	=0.57	Radish	=0.68
Cabbage	=0.24	Carrot	=0.44	Carrot	=0.52	Turnip	=0.62

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The study was limited to the response of 300 randomly selected farmers from 30 randomly selected villages of District Faisalabad. The validity of the study was further limited to the extent to which the respondents were able to provide accurate information.

METHODOLOGY

The random sample of 300 respondents was drawn from 30 randomly selected villages -9 villages each from Faisalabad and Jaranwala Tehsils, 6 villages each from Samundari and Toba Tek Singh Tehsils. The 300 respondents were interviewed with the help of interview schedule. The data collected were statistically analysed and tabulated. The degree answers of the farmers were given numerical scores i.e. 1, ½ and zero for great extent, some extent and not at all, respectively.

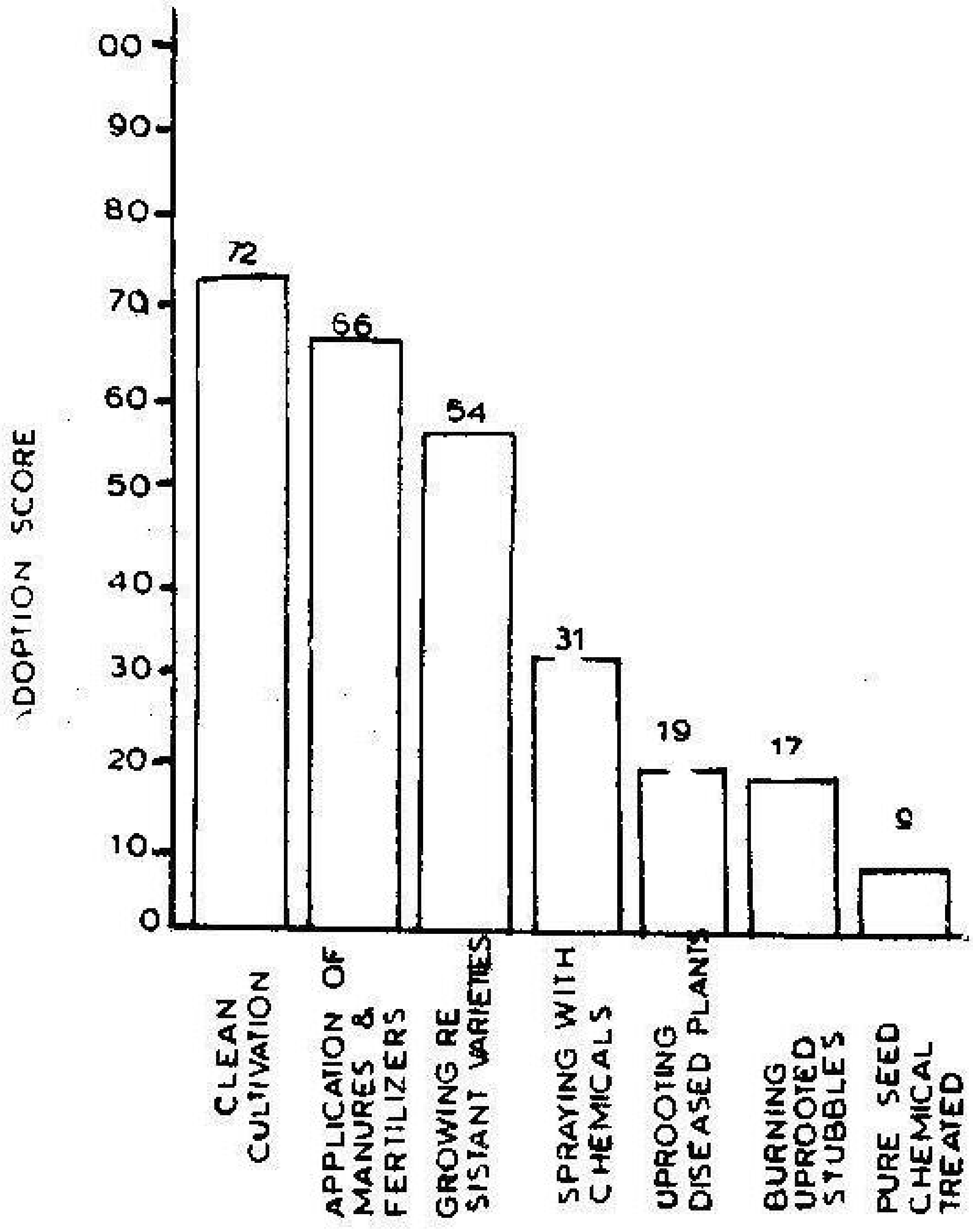
RESULTS AND DISCUSSION

The present study showed that majority of the farmers were aware of various pests and diseases of crops and they were adopting plant protection measures in varying degrees. About 50 per cent of the respondents got help from the extension workers in the form of service facilities such as plant protection, improved implements, seeds of various crops and fertilizers. These findings are not in agreement with those obtained by Gill (1961) and Waraich (1962). However, Razaq (1963) was of the view that majority of his respondents had the knowledge of control measures in cotton and sugarcane crops in Lyallpur Tehsil.

The study indicated that the factors which impeded the adoption of plant protection measures were lack of technical skill, non-availability of insecticides, inadequate help of Agriculture Department and insufficient finance to purchase approved seeds and fertilizers. These findings are similar to those obtained by Ditta (1960).

The study identified a positive significance between size of land holding, type of tenureship, education and distinctive position of farmers, on the one hand, and the adoption of plant protection measures on the other. However, age was not found to have any relationship with adoption. These findings are in agreement with those obtained by Iqbal (1963) and Khan (1965).

ADOPTION OF PLANT PROTECTION MEASURES



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