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Effect of organic and inorganic fertilizers levels on spinach (*Spinacia oleracea* L.) production and soil properties in Khost Province, Afghanistan

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Abstract

A pot experiment was conducted in a four replicated at the research farm, Shaikh Zayed University, Khost, Afghanistan to examine the production of spinach (Spinacia oleracea L.) and soil properties under varying levels of cow manure, inorganic fertilizer and cow manure + inorganic fertilizers. The treatments included: T1= Control, T2= CM 6 t ha⁻¹, T3= CM 8 t ha⁻¹, T4= CM 12 t ha⁻¹, T5= 100 Kg N ha ⁻¹, T6= 150 Kg N ha ⁻¹, T7= 200 Kg N ha ⁻¹, T8= 3 t CM + 90 Kg N ha ⁻¹, T9= 5 t CM + 80 Kg N ha ⁻¹ and T10= 7 t CM + 70 Kg N ha⁻¹. The results showed that highest spinach yield were obtained in T7 (200 Kg N ha $^{-1}$) and followed by T8 (3 t CM + 90 Kg N ha $^{-1}$), which were 4.2 and 4.1 t ha $^{-1}$ respectively. Control treatment pot had significantly lower yield as compare with others treatments. The crop fertilized with T7 (200 Kg N ha⁻¹), T8 (3 t CM + 90 Kg N ha⁻¹) and T6 (150 Kg N ha⁻¹) produced highest plant heights which were 27.8, 25.8 and 24.3 cm respectively. All treatments except from T2 (CM 6 t ha⁻¹) had significantly higher plant height than control treatment. The highest amount of soil NO₃-N was obtained in T7 (200 Kg N ha⁻¹) and followed by T8 (3 t CM + 90 Kg N ha⁻¹), which were 23.8 and 22.5 mg/L respectively. The lowest amount was obtained in control treatment. In T2 (CM 6 t ha⁻¹), T3 (CM 8 t ha⁻¹) and T4 (CM 12 t ha⁻¹) the cow manure decreased soil NO₃-N and negatively impact in spinach yield. The highest amount of Ws- P2O5 and Ws- K2O in T9 (5 t CM + 80 Kg N ha⁻¹) and T10 (7 t CM + 70 Kg N ha⁻¹) were 20.3, 22.8 and 32.3, 34.8 respectively, and the lowest amount of Ws- P2O5 and Ws- K2O were obtained in control treatment. The results suggested that cow manure with applied N application can be a valuable practice for increasing yield and available nutrients in soil.

Keywords: Spinach production, organic fertilizer, inorganic fertilizer, soil properties

1. Introduction

Afghanistan is an agricultural country where farmers can grow various types of vegetables in different areas. Spinach (*Spinacia oleracea* L.) is leafy green vegetable that originated from South-West Asia and nowadays grown in most parts of the world (Gaikwad *et al.*, 2010)^[9]. In Afghanistan, it is important horticultural species, since its leaves have been used in human nutrition. Plant production is directly related to soil chemical and physical properties. The potential of conventional farming system increased vegetable yield. However, their reliance on deep-inversion of using chemical fertilizers can weaken the agro ecosystem (Ali *et al.*, 2006)^[2].

In Organic farming instead of chemical fertilizers, farmers using compost, green manure, and crop rotation including legumes to maintain soil fertility and high yield. The cost of inorganic fertilizers is increasing enormously, to the extent that they are out of reach for small and marginal farmers (Kühling and Trautz, 2013; Larsen *et al.*, 2014) ^[13, 14]. Organic manures like farmyard manure, compost and cow manures have long been used by farmers for nutrients availability for plant growth especially N and improve soil quality. Organic manures contain most essential plant nutrients especially N in organic which not meeting the plant nutrient demand at critical growth stages, due to low mineralization process and this process results in low plant productivity (Zaman *et al.*, 2004) ^[22]; however, the best practice for long time have the potential to improve and sustain soil quality and productivity (Ahmad *et al.*, 2013) ^[1]. Shaheen *et al* (2014) ^[20] reported that using organic manure increased

spinach yield and nutrients levels of N, P and K in soils. The combination of organic and inorganic fertilizers can increased plant production. Mahmoud *et al.* (2009) ^[15] reported that the fruit and shoot dry weights of cucumber in the 75% mineral nitrogen + 25% plant compost treatment were higher than in the other treatments. These results suggested that organic manure can increase crop yield when practiced together with the use of inorganic fertilizers.

Afghanistan soils are formed under arid and semi-arid climatic conditions, and textural classes are mostly clay loam to sandy loam. Afghanistan soil has high amount of calcium carbonate and high pH, and has low soil organic matter content ranges from 0.2 to 2.5% (Sameen and Zaghard, 2008, Hashimi, 2016) ^[18, 12]. In Khost province, there are many sources of organic fertilizers (animal waste, green manure and chicken waste for compost). These organic fertilizers provide nutritional requirement especially contain macronutrients, essential micronutrient, and increasing yield and beneficial microorganism (Arancon et al., 2004)^[3]. In Khost, province farmers due to insufficient knowledge and experiences of organic manure and very rare using of cow manure and green manure. Lack of attention to crop rotation, organic residue, using compost and extensive tillage has created serious problems in terms of decreasing vields and degradation of lands, such as soil erosion and reduce soil aggregates stability (Favre and Kamal, 2004)^[8]. In Khost province, farmers using inorganic fertilizers to increase vegetables yield but they are not focusing on soil quality, fruits quality and environmental pollution awareness. Some farmers may not apply inorganic fertilizers due to low income and as a result they obtain low yield (Hashimi, 2016)^[12].

Although many studies have examined the effects of organic and inorganic fertilizers on soil quality (e.g. Mahmoud *et al.*, 2009) ^[15] and yield response (e.g. Shaheen *et al.*, 2014; Uddin *et al.*, 2012) ^[20, 21]. In Khost province, there is no information available on the effect of chemical fertilizer along with cow manure on soil quality and the yield of spinach (*Spinacia oleracea* L.), a crop that is widely grown across Asia. In this study, it was hypothesized that organic manure along with additional N would improve soil quality, especially nitrogen concentration, and increase spinach yield. Therefore, this study was undertaken to investigate the effect of organic manure and chemical fertilizer on the agronomic performance of spinach crop under greenhouse condition.

2. Material and Methods

2.1 Site description and field design

The study was performed during autumn season of 2018, to examine the production of spinach and soil properties under varying levels of organic, inorganic and combination of organic and inorganic fertilizers at the research farm, Shaikh Zayed University, Khost, Afghanistan. During experimental period the minimum and maximum air temperature was 5 and 21 °C respectively. This pot experiment was conducted in green house. Organic fertilizer including cow manure (CM) with 3 levels (6 t ha⁻¹, 8 t ha⁻¹ and 12 t ha⁻¹), three levels of (100 Kg N ha⁻¹, 150 Kg N ha⁻¹ and 200 Kg N ha⁻¹) inorganic fertilizer was applied in the form of urea (46% N). The combination of organic and inorganic fertilizers was three levels (3 t CM + 90 Kg N ha⁻¹, 5 t CM + 80 Kg N ha⁻¹, and 7 t CM + 70 Kg ha⁻¹) applied. Pots were filled from air dried soil (sandy loam) and applied cow manure before seed sowing. Spinach seed was direct seeded with 1.2- 2 cm depth in each pot. Chemical fertilizer was applied as top dressing methods in growing stage of spinach. After emergence, spray irrigation using a watering cane was carefully applied at regular interval to maintain soil moisture. There were 10 treatments and each treatment was replicated four times (Table 1). The pots were arranged in a completely randomized design. The pre-planting soil analysis (Table 2) indicates a poor soil fertility status that requires fertilizer application to supplement nutrients to boost yield (Hashimi 2016)^[12].

Table 1: Treatments explanation

Treatment	Description of Treatment
T1	Control
T2	CM 6 t ha ⁻¹
Т3	CM 8 t ha ⁻¹
T4	CM 12 t ha ⁻¹
T5	100 Kg N ha ⁻¹
T6	150 Kg N ha ⁻¹
T7	200 Kg N ha ⁻¹
T8	3 t CM + 90 Kg N ha ⁻¹
T9	5 t CM + 80 Kg N ha ⁻¹
T10	7 t CM + 70 Kg N ha ⁻¹

Table 2: Chemical properties of cow manure and soil using in experiment

Туре	Total C (%)	Total N (%)	C/N	pН	CaO (%)	K2O (%)	MgO (%)	P2O5 (%)
Cow Manure	31.8	1.2	25.9	8.9	0.13	0.75	0.11	0.18
	Total C (%)	Total N (%)	C/N	pН	CaO (mg kg ⁻¹)	K ₂ O (mg kg ⁻¹)	MgO (mg kg ⁻¹)	$P_2O_5 (mg kg^{-1})$
Soil	1.0	0.1	10.3	7.8	4084.0	107.0	55.0	22.0

2.2 Crop growth and yield measurement

The plant height (from the soil surface to the top of the plant) was recorded by using measuring through each replicate pot. For spinach total yield per plant and fresh weight recorded and it achieved from each pot was gathered and on the basis of yield pot⁻¹, the yield ha⁻¹ was calculated. For dry matter and water content the spinach residue was dried at 65°C for 72 h and weighed.

2.3 Soil sampling and measurement

After harvesting, we obtained soil samples from each pot up to 5-cm depth. Soil samples were measured for NO_3 -N, water soluble P_2O_5 , water soluble K_2O using a rapid soil

diagnosis kit "Midori-kun". pH and EC measured by multiparameter tester 35 series.

2.4 Statistical analysis

The statistical analyses were performed using STAT View (STAT View for Windows, version 5; SAS Institute, Cary, NC) ^[19]. Analysis variance was performed within each treatment means were tested using the least significant difference test at a level of 0.05.

3. Result and Discussion

3.1 Plant yield, height and dry matter

Plant yield was significantly influenced (P<0.05) by the

chemical fertilizer, cow manure and cow manure applied with N levels (Table 3). The highest yield was obtained in T7 (200 Kg N ha $^{-1}$) and followed by T8 (3 t CM + 90 Kg N ha⁻¹), which were 4.2 and 4.1 t ha⁻¹ respectively. The yield in T6 and T9 were same and significantly increased from control treatment. All treatments significantly had higher yield than control treatment. These finding are in line with those of (Shaheen et al., 2014) [20], which the highest yield was obtain in organic waste applied treatment. Hashimi et al. (2019) [11] reported that soil nutrient availability particularly soil inorganic nitrogen (NO₃-N + NH₄-N) increased eggplant yield. In the study reported here, spinach yield were significantly higher in those treatments (200 Kg N ha $^{-1}$ + 3 t CM + 90 Kg N ha $^{-1}$ + 5 t CM + 80 Kg N ha $^{-1}$ and 150 Kg N ha ⁻¹) which has higher soil nutrient availability, particularly soil nitrate (Table 4). The yield and soil NO₃-N has significant positive correlation (Figure 1). The addition of chemical fertilizer with organic manure stimulate mineralization process and as a result increase nutrients in soil. Chat et al. (2005) [4] reported that NPK fertilizers in addition to some amounts of cattle manure resulted in higher vegetable yield. Patel et al. (2008) ^[17] also indicated that the farm yard manure in addition to recommended NPK fertilizers resulted in significant increase in spinach leaf yield. Thus, our results also support that in treatments (3 t CM + 90 Kg N ha⁻¹ + 5 t CM + 80 Kg N ha⁻¹) increased plant yield due to increasing mineralization process.

Plant height was also significantly influenced (P<0.05) by the chemical fertilizer, cow manure and cow manure applied with N levels (Table 3). The highest height was obtain in T7 (200 Kg N ha -1) and followed by T8 (3 t CM + 90 Kg N ha⁻¹) and T6 (150 Kg N ha⁻¹), which were 27.8, 25.8 and 24.3 cm respectively. All treatments except from T2 (CM 6 t ha⁻¹) had significantly higher plant height than control treatment. Yield and plant height had significant positive correlation (Figure 2). These results suggest that available soil nutrients especially soil NO₃-N has potential to increase spinach growth as seen in this experiment. Ouda and Mahadeen (2008) ^[16] reported that nitrogen is essential for protein synthesis and chloroplast development and this can increased cell division and enlargement resulting in high and many leaves in Indian spinach.

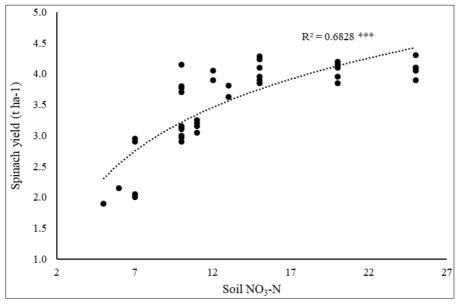


Fig 1: Correlation between spinach yield and soil NO₃-N. *** indicate significance at P <0.001.

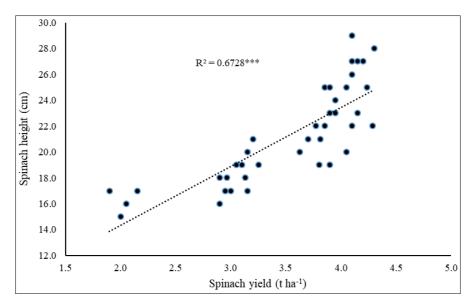


Fig 2: Correlation between spinach yield and spinach height. *** indicate significance at P <0.001.

3.2 Soil chemical properties

The application of chemical fertilizer, cow manure and cow manure applied with N levels significantly (P<0.05) increased soil nutrients levels of NO₃-N, Ws- P₂O₅ and Ws-K₂O (Table 4). The highest amount of Ws- P₂O₅ and Ws-K₂O obtained in T8 (3 t CM + 90 Kg N ha⁻¹), while soil NO₃-N was obtained in T7 (200 Kg N ha⁻¹) and followed by T8 (3 t CM + 90 Kg N ha⁻¹), which were 23.8 and 22.5 mg/L respectively. The lowest amount of Ws- P2O5 and Ws-K₂O were obtained in control treatment. Cow manure exhibited significantly higher NO₃-N, which might be related to their additional inputs through the incorporation of organic materials in soil. After a short period the addition of plant residual into soil, temporary immobilization process occurred and decreased inorganic N pools are often observed (Geisseler et al., 2012)^[10]. Thus in our study, in T2 (CM 6 t ha⁻¹), T3 (CM 8 t ha⁻¹) and T4 (CM 12 t ha⁻¹) the cow manure decreased soil NO3-N and negatively impact in spinach yield. The other reason of low NO3-N is high C:N ratio organic amendment. Hashimi et al. (2019) [11] reported that 23:1 C:N ratio decreased eggplant yield. In the experiment reported here, the average C:N ratio of CM was 25.9, which may explain why the CM treatment without applied N reduced spinach vield. Cheshire et al. (1999)^[6] also reported that straw mulch with a high C:N ratio into the soil, decreased NO₃-N concentrations due to the

immobilization of nitrogen, resulting in no effect on yield. Soil Ws- P₂O₅ and Ws- K₂O was also effected by cow manure applied with N levels. The lowest amount of Ws-P₂O₅ and Ws- K₂O were obtained in control treatment. The amount of Ws- P₂O₅ and Ws- K₂O in T9 (5 t CM + 80 Kg N ha⁻¹) and T10 (7 t CM + 70 Kg N ha⁻¹) were 20.3, 22.8 and 32.3, 34.8 respectively. These treatment showed the most favorable increase in Ws- P2O5 and Ws- K2O availability, which was significantly higher than the other treatments. Similar result was found by David (2015)^[7], who attributed the increased P and K availabilities to the direct addition of P and K in the pig manure. The lowest amount of Ws- P₂O₅ and Ws- K₂O obtained in control treatment, where no fertilizer was added due to removal by crops without addition from external inputs. These amount were Increased under cow manure + inorganic fertilizer treatments. Zsolnay and Görlitz (1994)^[23] reported that incorporation of manure and crop residues has been shown to increase the amount of soluble organic matter and improves the available P content in the soil. Soil pH and EC showed no significant changes in response to the experimental different fertilizer treatments (Table 4), suggesting that changes in pH and EC were not been easily detected in the short term. These results are in line with those of Chen et al. (2010)^[5]. Differences among treatments occurred with respect to available NO₃-N and cow manure concentrations in the pots.

Table 3: Effect of organic and inorganic fertilizers levels on spinach yield, dry matter and height

Symbol	Treatment	Spinach yield (t ha ⁻¹ of FM)	Spinach dry matter (t ha ⁻¹)	Spinach plant height (cm)
T1	Control	2.1 e	0.33 cd	16.3 g
T2	CM 6 t ha ⁻¹	3.0 d	0.45 c	17.0 fg
T3	CM 8 t ha ⁻¹	3.1 cd	0.48 b	18.0 f
T4	CM 12 t ha ⁻¹	3.2 c	0.45 b	19.8 e
T5	100 Kg N ha ⁻¹	3.9 b	0.60 ab	19.8 e
T6	150 Kg N ha ⁻¹	4.0 ab	0.63 a	24.3 с
T7	200 Kg N ha ⁻¹	4.2 a	0.62 a	27.8 a
T8	3 t CM + 90 Kg N ha ⁻¹	4.1 ab	0.63 a	25.8 b
Т9	5 t CM + 80 Kg N ha ⁻¹	4.0 ab	0.60 ab	22.5 d
T10	7 t CM + 70 Kg N ha ⁻¹	3.9 b	0.58 abc	21.3 d

Note: Values, in columns within treatments, followed by different letters indicate significant differences between treatments at 5% using the Tukey-Kramer test

 Table 4: Effect of organic and inorganic fertilizers levels on soil properties

Symbol	Treatment	pH (H ₂ O)	EC (dS/m)	NO ₃ -N (mg/L)	Ws- P2O5 (mg/L)	Ws- K ₂ O (mg/L)
T1	Control	8.0	0.6	6.3 e	11.0 c	23.0 с
T2	CM 6 t ha ⁻¹	8.0	0.6	8.5 de	11.5 c	23.5 с
T3	CM 8 t ha ⁻¹	7.8	0.5	10.0 cd	13.0 c	25.0 с
T4	CM 12 t ha ⁻¹	7.8	0.5	11.0 cd	17.0 bc	29.0 bc
T5	100 Kg N ha ⁻¹	8.0	0.6	11.0 cd	14.0 c	26.0 c
T6	150 Kg N ha ⁻¹	7.8	0.5	17.5 b	20.5 b	32.5 b
T7	200 Kg N ha ⁻¹	7.8	0.6	23.8 a	17.0 bc	29.0 bc
T8	3 t CM + 90 Kg N ha ⁻¹	8.0	0.5	22.5 a	25.5 a	37.5 a
T9	5 t CM + 80 Kg N ha ⁻¹	7.9	0.6	13.8 c	20.3 b	32.3 b
T10	7 t CM + 70 Kg N ha ⁻¹	7.9	0.6	12.8 c	22.8 ab	34.8 ab

Note: Values, in columns within treatments, followed by different letters indicate significant differences between treatments at 5% using the Tukey-Kramer test

4. Conclusion

It was concluded that the spinach growth and yield was substantially higher under optimum N sources and combined application of cow manure + inorganic nitrogen (As urea); and 4.2 and 4.1 t ha⁻¹ resulted in optimum crop performance; while decrease in control and cow manure application without applied N showed adverse impact on spinach yields. The same trend was obtained in soil properties especially soil NO₃-N, were obtained in T7 (200 Kg N ha⁻¹) and followed by T8 (3 t CM + 90 Kg N ha⁻¹), which were 23.8 and 22.5 mg/L respectively.

5. References

1. Ahmad W, Shah Z, Khan F, Ali S, Malik W *et al.* Maize yield and soil properties as influenced by integrated use of organic, inorganic and bio-fertilizers in a low fertility soil. Soil & Environment. 2013; 32(2).

- 2. Ali A, Ayuba SA, Ojeniyi SO *et al.* Effect of tillage and fertilizer on soil chemical properties, leaf nutrient content and yield of soyabean in the guinea savanna zone of Nigeria. Nigerian Journal of Soil Science. 2006; 16(1):126-30.
- Arancon NQ, Edwards CA, Bierman P, Metzger JD, Lee S, Welch C *et al.* Effects of vermicomposts on growth and marketable fruits of field-grown tomatoes, peppers and strawberries: the 7th international symposium on earthworm ecology. Cardiff. Wales. 2002. Pedobiologia. 2003; 47(5-6):731-5.
- 4. Chat TH, Dung NT, Van Binh D, Preston TR *et al.* Effect on yield and composition of water spinach (Ipomoea aquatica), and on soil fertility, of fertilization with worm compost or urea. Livestock Research for Rural Development. 2005; 17 (10): 20-25.
- Chen Y, Zhang X, He H, Xie H, Yan Y, Zhu P *et al.* Carbon and nitrogen pools in different aggregates of a Chinese Mollisol as influenced by long-term fertilization. Journal of Soils and Sediments. 2010; 10(6):1018-26.
- 6. Cheshire MV, Bedrock CN, Williams BL, Chapman SJ, Solntseva I, Thomsen I *et al.* The immobilization of nitrogen by straw decomposing in soil. European Journal of Soil Science. 1999; 50(2):329-41.
- 7. David JM. Biochar and compost increase crop yields but the effect is short term on sand plain soils of Western Australia. Pedosphere. 2015; 25(5):720-8.
- 8. Favre R, Kamal GM. Watershed Atlas of Afghanistan. Kabul: Government of Afghanistan, Ministry of Irrigation. Water Resources and Environment, 2004.
- 9. Gaikwad PS, Shete RV, Otari KV. Spinacia oleracea Linn *et al.* A pharmacognostic and pharmacological overview. International Journal of Research in Ayurveda and Pharmacy (IJRAP). 2010; 1(1):78-84.
- 10. Geisseler D, Joergensen RG, Ludwig B *et al.* Temporal effect of straw addition on amino acid utilization by soil microorganisms. European journal of soil biology. 2012; 1; 53:107-13.
- 11. Hashimi R, Komatsuzaki M, Mineta T, Kaneda S, Kaneko N. Potential for no-tillage and clipped-weed mulching to improve soil quality and yield in organic eggplant production. Biological Agriculture & Horticulture. 2019; 20:1-4.
- Hashimi, R. Potential of no-tillage with clipped weed mulch to improve soil quality and yield responses in organic eggplant production M.Sc. Thesis. Ibaraki University, Japan. 2016; 133-135.
- 13. Kühling IN, Trautz DI. The role of organic farming in providing ecosystem services. International Journal of Environmental and Rural Development. 2013; 4:175-8.
- 14. Larsen E, Grossman J, Edgell J, Hoyt G, Osmond D, Hu S *et al.* Soil biological properties, soil losses and corn yield in long-term organic and conventional farming systems. Soil and Tillage Research. 2014; 1; 139:37-45.
- Mahmoud E, El-Kader NA, Robin P, Akkal-Corfini N, El-Rahman LA. Effects of different organic and inorganic fertilizers on cucumber yield and some soil properties. World Journal of Agricultural Sciences. 2009; 5(4):408-14.

- Ouda BA, Mahadeen AY. Effect of fertilizers on growth, yield, yield components, quality and certain nutrient contents in broccoli (Brassica oleracea). International Journal of Agriculture and biology. 2008; 10(6):627-32.
- 17. Patel KC, Patel KP, Ramani VP, Patel JC *et al.* Effect of Pb and FYM application on spinach yield, Pb uptake and different fractions of Pb in sewage irrigated Fluventic ustochrepts soils of peri urban area of Vadodara. Asian Journal of Soil Science. 2008; 3(2):230-5.
- Sameen AQ, Zaghard MA. Soil Chemistry and plant Nutrients, Kabul: Published by the World Bank. 2008; Pp.58-295.
- SAS Institute. 1998. Stat View for Windows, v. 5.0.1. Cary (NC): SAS Institute, Inc.
- 20. Shaheen S, Khan MJ, Jilani S *et al.* Effect of organic and inorganic fertilizers co-applied with effective microorganism (EM) on growth and yield of spinach (*Spinachia olerace*). Sarhad Journal of Agriculture. 2014; 30(4):411-8.
- Uddin M, Kashem MA, Osman KT *et al.* Effect of organic and inorganic fertilizers on phytoavailability of phosphorus to water spinach (Ipomoea aquatica cv. Kankon). Journal of Agricultural and Biological Science. 2012; 7(3):152-6.
- 22. Zaman M, Chang SX. Substrate type, temperature, and moisture content affect gross and net N mineralization and nitrification rates in agroforestry systems. Biology and Fertility of Soils. 2004; 39(4):269-79.
- 23. Zsolnay A, Görlitz H. Water extractable organic matter in arable soils: effects of drought and long-term fertilization. Soil Biology and Biochemistry. 1994; 26(9):1257-61.