EFFECT OF SEED RATE AND NITROGEN ON GROWTH AND YIELD OF TEFF GRASS

(*Eragrostis teff* (Zucc.)Trotter

By

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Dedication

To my family

Murtada

Acknowledgements

First of all, I thank and praise my God for supporting me with power, patience and determination to complete my research.

May I take this opportunity to extend my thanks, appreciation and gratitude to Dr. Awad Osman Abusuwar my supervisor whom without his patience, guidence, and assistance during the course of this study, it would have not been possible to complete this thesis.

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Finally, I am thankful and appreciative to all those who support me, and my thankfulness is extend to my dear family and to all those who care.

ABSTRACT

A field experiment was conducted during the season (2003/2004) in the Experimental Farm of the Faculty of Agriculture, University of Khartoum at Shambat in order to study the effects of seeding rate and nitrogen rates on growth and yield of Teff forage *Eragrostis teff* (Zucc.) Trotter. The treatments consisted of three seed rates (2,4, and 6kg/fed) and three nitrogen levels (0, 40, and 80kg N/ha). Urea (46% N) was used as the source of nitrogen. The experiment was laid out in a randomized complete block design with four replications.

The results showed that seed rates significantly increased plant population, while nitrogen significantly increased number of leaves per plant. Both seed rate and nitrogen did not affect plant height and number of tillers per plant significantly. Seed rates and nitrogen affected green and dry forage yield but were not significant.

Seed rates and nitrogen significantly increased crude protein and crude fiber. Minerals (P, K, Mg and Ca) were not significantly affected by seed rates. Nitrogen application caused significant decreases and increases in P and K respectively, while it had no effect on Ca and Mg contents.

خلاصة الاطروحة

أجريت تجربة حقلية لموسم (2004/2003) بالمزرعة التجريبية بكليـة الزراعـة، جامعة الخرطوم شمبات وذلك لدراسة تأثير معدل البذر والنتروجين على نمو وإنتاجية علف ال Teff.

استخدمت ثلاثة معدلات بذر (2، 4، 6، كجم/فدان) وثلاثـة مـستويات نيتـروجين (صفر، 40، 80، كجم نيتروجين للهكتار). استخدم سماد اليوريا (46% نيتروجين) مصدراً للنيتروجين. اتُبع التصميم العشوائي ذو القطاعات الكاملة بأربعة مكررات في تنفيذ التجربة.

أوضحت النتائج أن معدلات البذر ادت لزيادة معنوية في الكثافة النباتيـة بينمـا ادى النيتروجين إلى زيادة معنوية في عدد الأوراق ولم يكن لمعدل البذر والنتـروجين اى تـأثير معنوي على طول النبات وعدد الخلف بالنبات. معدلات البذر والنتروجين اديا لزيـادة غيـر معنوية في انتاجية العلف الاخضر والجاف.

ادت معدلات البذر والنتروجين لزيادة معنوية في محتوى البروتين والالياف. كما لم تؤدي معدلات البذر لاى زيادة في تركيز عناصـر (P, K, Mg, Ca) بينمـا أدت اضـافة النتروجين لنقص وزيادة معنوية في محتوى الفسفور والبوتاسيوم على التوالى، ولم يؤثر على عنصري الكالسيوم والمغنسيوم.

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CHAPTER ONE

INTRODUCTION

For sustainable and stable food production, maintaining forage production to face significant increases in animal population is an appropriate action. Forage production has an important role because it is the basic source of energy for the growth and maintenance of livestock and to increase their products, which they form an essential diets for humans.

In the Sudan forage production is very important due to the fact that Sudan has a huge number of animals which is estimated to about 143 million heads (camels 3 million, cattle 35 million, sheep 42 million, and goats 63 million) in 1998 (Mohamed, 2000).

Over grazing of natural pastures, expanding of rain fed agriculture, and improper conservation measures lead to reduction of rangeland. For these reasons the stocking rate must be adjusted with the potential carrying capacity. Range conservation against seasonal fires must be considered and green fodder production must be encouraged. The expansion of rain fed farming on marginal range-land must be prevented.

Teff*(Eragrostis teff* (Zucc.)) Trotter, which means "lost" in Amharic, is a member of the tribe Eragrosteae, sub- family Eragrostoidae of Poaceae. Ethiopia is considered the site of origin of teff. Teff was domesticated in Ethiopia 4000- 1000 BC. Teff is a warm season, annual C-4 plant (Kebed *et al.,* 1989), which is grown in Australia, India, and South Africa as a forage (Costanza *et al.*, 1979). Teff is characterized by a large crown, many tillers, and shallow diverse root system. The inflorescence is an open panicle and produces small seeds (1000 seed weight from 0.3 to 0.4 g). Teff is self pollinated. Plant height varies depending upon cultivar, type and growing environments.

The great diversity within the species is evident in seed color.There are reports of purple, white, brown and red seeded types (Mengesha 1966; Costanza, 1979). Teff can be planted in late May similar to millets. Seedbed should be firm and prepared similar to planting of alfalfa. Seeding rate is 4.5 to 9.5 kg /ha using implements such as cultipacker or Brillion seeders (Twidwell *et al.,* 1991). Teff should be seeded 12-15 mm deep either broadcast or narrow rows. Teff grain yield in U.S averages from 700 kg /ha dry land to 1400 kg /ha irrigated in Montana (Eckholff *et al.,* 1993; Stallknech *et al.,* 1993).

The major advantages of teff in Ethiopia according to (Ketema, 1987) are as follows: -

- It can be grown under moisture – stress areas and water logged conditions.
- Its suitable and used for relay cropping.
- Its straw is a valuable animal feed during the dry season, it is highly preferred by cattle.
- It can be stored easily under local storage conditions and not attacked by storage pests, thus reducing post-harvest management costs, and it can be stored for a relatively long period of time (3 years minimum) before losing its viability.
- It has less diseases and pests than any other cereals.

- The grain is ground into flour, fermented and made into (Injra) a sour type of flat bread (Ebba, 1975).

Outside Ethiopia teff has been introduced and cultivated as a forage crop in South Africa, and in recent years cultivated as a cereal crop in North Kenya. Teff seed has a good balance of essential amino acids, except lysine (Ebba,1969). Among the cereals, teff straw is the best and comparable to a good natural pasture. The optimal seeding rate and nitrogen levels for teff are very important practices. However, in the Sudan no comprehensive study has been conducted to assess these factors and their effects on yield potential of the crop. The main objectives of this study were:

1- To investigate the effects of different seed rates on growth and yield of teff.

2- To study the effect of different nitrogen levels on growth and yield of the crop.

CHAPTER TWO

LITERATURE REVIEW

2.1 General:

Teff is grown primarily as a cereal crop in Ethiopia. It is also grown for forage. In Ethiopia teff straw from threshed grains is considered to be an excellent forage, superior to straw from other cereal species (Stallknech, 1997). Teff is very adaptable species grown in different climatic and edaphic zones at elevation ranging between 1000-2500m above sea level, (Westphal, 1975). Owing to it is hardly nature, this species has a considerable potential for agriculture in areas un suitable for other cereals.

The increase in production of teff depends on the optimization of crop management and genetic improvement. Unlike other crops of international importance such as rice and wheat, where basic and applied research contributed significantly to their improvement, teff has received little attention.

Teff germinates rapidly when planted at an average depth of 1.2 cm. However, the initial growth is slow until a good root system has been established (Stallknech, 1993) . Harvesting teff for either forage or seed production is easily accomplished, as long as the combine is seed tight (Stallknech, 1993). Late 20th century publications in the United States described teff grain as being marketed as a health food product, or used as a late planted emergency forage for livestock (Gorege, 1991;Weibye, 1991).

2.2 Nitrogen Fertilization:

The response of crop to nitrogen depends on soil fertility, moisture (Anon, 1972), location and season (Coler and Kroth, 1968). Sharma (1973) reported that addition of nitrogen fertilizer increased plant height, which resulted an increase in leaf number per plant .Increase in growth parameters, is an indication of more herbage yield. The major form of nitrogen fertilizer used is urea. It is efficient , very soluble and moves freely up and down with soil moisture (Samuel *et al.*, 1975).Kidanu (1999)demonstrated that the carryover benefit of applied fertilizer (N) enhanced the yield and (N) content of grain and straw of both wheat and teff , resulting in significant increase in (N) uptake. Application of (N) fertilizer to field crops has been shown to increase post-harvest levels of soil N and /or organic matter on well drained soils (Chaney 1991;Mahli *et al* ,1991; Tanner *et al*,1993)and poorly-drained vertisols (Strong *et al.*, 1996;Tilahun *et al.*, 1998). Gasim (2001) showed that plant height increases as nitrogen levels increased even when the difference was not significant. She also reported that number of leaves per plant was higher when fertilized with nitrogen compared to the control. The effect of nitrogen fertilization on forage fresh yield was significant. With increasing (N) levels forage fresh yield increased progressively, also dry matter yield significantly increased with the increase in nitrogen level (Gasim, 2001). The nutritional value of teff grain is similar to the traditional cereals .Teff is considered to have an excellent amino acid composition. The lysine level is higher than wheat or barley, and slightly less than rice (Stalknecht, 1997). He also reported that teff contains very little gluten, and is also higher in several minerals; particularly iron (Stalknecht, 1997).Teff has a very high calcium, content, and contains high levels of phosphorus, iron, copper, aluminum, barium and thiamine (Mengesha, 1965). The nutritive value of teff for livestock fodder is similar to other grasses utilized as hay or ensiled feed (Boer *et al.*, 1986; Twidwell *et al.*, 1991). Protein and digestibility as forage decreases with increase maturity. Kidanu *et al.*, (1999) reported that the highest grain yield and straw yield were obtained when 60kg N/ha was applied, although, in most cases, there were no significant differences between the yield obtained with 30or 60 kg N. Straw yield differed between the nitrogen rates. They also reported that nitrogen % in teff seed increased with each increment in N fertilizer, while N % in teff straw increased only up to the intermediate level of N fertilizer applied. Grain yield and straw represented the maximum genetic diversity among the observed teff germplasm (Ketema, 1997).

Forage yield varied from 9.0 to 13.5 kg /ha depending upon moisture levels during the growing season (Boe *et al.*, 1986; Eckhoff *et al.*, 1993). In Montana forage yield cut from dry land and irrigated cropping ranged from 2.2 to 15 ton/ha (Stallknecht ,1993).

Nitrogen element plays an important role in quality of forage crops. Increased protein content in maize straw was obtained with increased dose of nitrogen (Rai, 1965). Rajagpal *et al.*, (1974) studied the nutritive value of fodder maize in relation to nitrogen levels, and concluded that protein increase with increased nitrogen dose. Tripathi *et al.*, (1979) reported that the application of nitrogen gave a significant additional increase in crude protein contents of forage oats. In other studies increase in nitrogen rates in forage grasses resulted in an increase in protein content of herbage (Reid *et al.*, 1966; Rhykerd *et al.*, 1966 and Gonzalez & Dodd, 1979). Regarding crude fibre, Primost, (1964) reported that fibre content of fodder maize was slightly affected by nitrogen .Reddy *et al.*, (1985) reported that when maize was fertilized with nitrogen, as urea, at the rate of 40, 60, 80, and 100 kg N/ha ,respectively, crude fibre percentages were 29,31.7,31.3, and 32.4 in dry matter. Gasim, (2001) reported that the increase in nitrogen levels increased crude protein content, but reduced crude fibre content of maize forage . In Montana, teff hay protein content ranged from 13.7 to 9.6 %. Moderate rates of nitrogen and phosphorus are suggested to prevent lodging. Optimum nutrient rate recommendation have been developed for the major cereal crops grown in Ethiopia (ADP/NFIU, 1991). For the high land vertisols of Ethiopia, economic optimum N rates of 55kg /ha for teff (Teckalign *et al.*, 1996). The highest N level approximates the N rates recommended for teff production in Ethiopia vertisols (Workn and Mwangi,1994;Teckalign *et al.*, 1996). Ethiopia high land vertisols tends to exhibit low total N organic matter content, and the application of N fertilizer considered essential to improve cereal production (Teckalign *et* *al.*, 1996). Peasant farmers in Ethiopia commonly apply sub-optimal rate of fertilizer to crop due to limited access to credit and low untimely availability of fertilizer during the planting season (Gezahegn and Teckalign, 1995). Elevated levels of soil N resulting from fertilizer N application can influence the yield and N response of subsequent cereal crops (Shepherd and Sylvester Bradly,1996). In fact, the carry over benefits associated with residual soil N enhance the profitability depleted vertisol in Australia (Strong *et al.*, 1996).

Previous studies indicated that carryover effect on either soil N level or succeeding crop performance were only apparent when high, super-optimal fertilizer level had been applied to preceding crop (Chaney, 1991;Malhi *et al.*, 1991; Shephered and Sylvester Bradley, 1996). The use of mineral fertilizer in modest quantities is necessary. On light soils (Nitisols, Lurisols ,Cambisols)the recommended levels per ha are 25- 40 kg of N and 30- 40 kg 0f P_2O_5all applied at planting (Decker *et al.*, 2001). Systemic studies on the the fertilizer requirement of teff under varying condition and in various regions need further investigation. However, currently the following recommendation are made based on the result obtained from Debre Zeit Agriculture Centre:

On heavy clay soil (vertisols): 60kg N and 26kg P_2O_5 per ha. On sandy clay loam soils (Anasols): 40 kg N and 26 P_2O_5 per ha. Urea is generally recommended to be applied in split application.

Alkamper, (1973) has made the following conclusions regarding the response of teff to fertilizers:

- High rates of fertilizer can be applied at sowing together with the seeds on the bare land without any harm to the germination rate of teff.
- Nitrogen produces more straw while phosphorus encourages good grain production.
- Potassium is of minor importance to teff production.
- The figures concerning the uptake of main elements by teff are relatively low.
- The split application of nitrogen may result in increase in grain yield without influencing the straw yield.

Varities with short growing periods and/or of higher levels of productivity may require lower N and P recommendation.

Tropical grasses are poor in nutritive value (Minson and Mc Leod, 1970) and attempts to enhance quality of the forage (particularly nitrogen component) have been tried through the use of nitrogen fertilization or incorporation with legumes . Appendix (1) shown that yield and chemical composition of various crop residues on dry matter basis (Lulesged and Jamal, 1986).

The crude protein composition of all the forage was higher than 6-7g/100g which is considered as the critical level at which intake could be depressed (Minson,1983) .Grazing livestock in warm climates have to depend largely upon forage to fulfil their animal requirements. Minerals composition of forage plants is affected by soil-plant factors, including pH, drainage, fertilization, forage species**,** forage maturity and interaction among minerals (Gomide, 1978; Reid and Hovarth,1980). Lower mineral and protein concentrations found in the more mature forages grazed during November agrees with Gomide ,(1978), who found that decreases forage nitrogen, Pand K with increasing forage maturity.

Joseph *et al.*, (1998) reported that increasing N application reduced P concentration linearly and concentration of K decreased linearly as N application increased. The concentration of K in vegetative forage were generally higher compared with many forage (Minson, 1990). The content of Ca and Mg was the most positively affected by decreasing N fertilization (Segaard, 1990). Increasing N application increased Ca and Mg concentration linearly (Joseph *et al.*, 1998). A complete study of minerals utilization in tropical forages is therefore very essential.

2.3 Effect of seed rate:

Seed rate, which is the amount of seed sown per unit area, is an important factor for forage production because it affects plant density. The highest plant density is always obtained at the highest seed rate. The optimum plant density for production is higher than that for grain production (Lucas, 1986). The influence of plant density on growth and yield of crops is through its influence on competition for light, nutrients in soil and available moisture that affects leaf area index and dry matter yield (Karlen and Camp, 1985; Barrere and Traneau, 1986; Giskin and Efron, 1986).

The most important aspect for forage production is plant population. Narrow within –row spacing which results in high density, significantly increased plant height and leaf area index and consequently forage yield in maize (Mohammed, 1998). Early *et al.,* 1967) reported that increasing population increased plant height and reduced stem diameter producing thin palatable forage plants.

Evans (1975) attributed the increase in height with increase in plant density due to the competition for available light. In another study plant height was found to decrease with increase in seed rate (Crossman, 1967). Plant height at maturity was not affected by plant population as reported by Roy and Biswas (1992). This difference in response of plant height to seed rate might be attributed to the levels of seed rates , and condition under which the crop is grown . Since higher densities contain more plants per unit area, dry matter yield per unit area increased with increasing plant density (Esechie, 1992).

Gasim (2001) reported that the effect of seed rate on plant population increased with increase in seed rate .Plant height increased with increase in seed rate, and number of leaves per plant was significantly affected by seed rate. Moreover, he also reported that seed rate significantly influnced forage fresh yield and forage dry matter. As the seed rate increases crude fibre increases.

Increase in plant population results in a decrease in yield per plant due to mutual shading (Prine, 1971) and increase lodging (Kaliappa, 1974) due to thin stem. High forage yields were obtained from the closet spacing (high seed rate) of sorghum compared to the wider one (low seed rate(Hunter *et al*, 1970; Carvetta *et al.*, 1990).

Protein content in forage decreased with increasing seed rate in sorghum (Abu suwar 1981, Hago and Mahmoud, 1996). Plant density and spacing has little or no effect on crude fiber (Powell *et al.*, 1991).This indicates that forage quality can be maintained at higher plant densities (Graybill *et al.*, 1991).

About 15 – 55kg of teff seeds are sown per hectare under different conditions .If a manually or motor driven broadcaster or a drill is available, a lower seed rate (about 15kg/ha) is recommended .If sowing is done by hand-broadcasting, it would be difficult to evenly distribute the 15 kg/ha of seeds because of small seed size. Therefore, 25-30 kg/ha seeds are recommended for broadcast sowing. Farmer traditional practice is to broad cast teff at the rate 40-50 kg/ha (Ketema 1997). Seed rate broadcasted at a rate 25 -30kg/ha and covered lightly with soil by hand-held twigs (Decker *et al.*, 2001). Seeding rate is 4.5 to 9 kg/ha using implements such as cultipackers or Brillion (Twidwell *et al.*, 1991). Seeding rates varies from 2.3 to 9 kg/ha, with 5 to 8kg/ha generally recommended (Stallknech, 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site description:

A field experiment was conducted at the Experimental Farm of the Faculty of Agriculture, University of Khartoum at Shambat, Sudan, from June to September 2003 to study the effect seeding rate and nitrogen levels on growth and yield of teff .The farm is located in semi-desert zone, (latitude 15 40 N and longitude 32 32E , elevation is 380 m above sea level). Monthly mean maximum and minimum temperature, relative humidity, and rain fall during the experimental period were recorded and presented in Appendix, (2). The soil at the farm is alkaline, pH (7.8 to 8) cracking with about 48% clay content with low permeability. It also low in nitrogen content (0.065%), but potassium (K) and aviable phosphorus (P) content are (0.23Meq /1 and 0.1935 Meq l1, respectively.

3.2 Land preparation:

The experimental site was disc ploughed, disc harrowed, leveled and ridged up 70 cm apart. The area of the experiment was divided into 36 plots each 3× 4 m, with four ridges.

3.3 Treatments:

The treatments consisted of three nitrogen levels (0, 40, and 80 kg N/ha) designated as 0N, 1N, and 2N, respectively. Three seeding rates were used 2, 4, and 6kg /ha designated as S1, S2, and S3, respectively. The seed was obtained from the Department of Agronomy U of K. Urea (46% N) was used as the source of nitrogen. The treatments were assigned as:

S1 0N, S1 1N, S1 2N, S2 0N, S2 1N, S2 2N, S3 0N, S3 1N, and S3 2N. The experiment was laid out in randomized complete block design with four replications. The crop was sown on 16th June and seeds were sown at narrow rows in Eastern side of the ridge. Urea was applied three weeks after sowing on one side of the ridge. The crop was irrigated immediately after sowing then irrigation was done at an interval of a week.

Pre-sowing irrigation was done for weed control and to obtain a fine seedbed. One meter sample from the two outer ridges was used for destructive sampling which was taken two month after sowing, whereas the two middle ridges were left for the final yield by using a sickle to clip the plants 2-5 cm above the soil surface .

3.4 Parameters measured (Sampling):

1. Plant density (population): An area of one square meter was permanently marked in each ridge. Plant counts were made after 10, 30, 60 days from sowing and at harvest.
2. Plant height (cm): Ten plants were randomly selected from each plot to measure plant height from the soil to the tip of the flag leaf.
3. Number of leaves per plant: Ten plants were randomly taken from each treatment and the mean number of leaves per plant was calculated.
4. Number of tillers per plant: Ten plants were randomly taken from each plot to calculate this parameter.
5. Fresh yield (ton/ha): The entire plot for each treatment was clipped and the fresh forage was weighed to estimate this parameter.

6. Dry matter yield (ton/ha): The green forage of one square meter in each plot was air dried and weighed to estimate dry matter yield.

7. Chemical analysis: proximate analysis was performed on a composite of 2.0 g of dried leaves and stems include:

Crude protein %: determined by using macro kjeldhal method (Bremner, 1965).

Crude fiber %**:** determined according to the method of AOAC (1975) using fibertic system 1010 heat extraction.

Minerals :

Phosphorus (p) was measured spectro-photometrically according to Pearson (1970).Potassium (K) was determined by coring EEL flame photometer after extract preparation according to procedure described by Chapman and Pratt(1961). Calcium (Ca) and Magnesium (Mg) were determined by EDTA, according to the method described by Chapman and Pratt (1961). See appendices 3,4,5,6,7 and 8.

3.5 Statistical analysis:

The collected data of each parameter were subjected to analysis of variance (ANOVA) according to the method described by Gomez and Gomez (1984). Means were compared using LSD.

CHAPTER FOUR

RESULTS

4.1 Growth attributes:

4.1.1 Effect of seeding rates and nitrogen levels on plant density:

Plant density was significantly affected by seeding rate in three out of four counts. These were counts number one, tow and three (Table 1). Non significant effect was observed in count number four. The treatment (S3) gave a higher number of plant than other treatments (Fig.1).

The highest nitrogen level (2N) produced greater number of shoots over 0N and 1N, but there was no significant difference (Table 1). The nitrogen level 2N increased plant density by 10 % and 14% over 0N and 1N, respectively.

There was a significant interaction of seeding rates and nitrogen level observed in two out of four counts. These were counts number one and two (Table 2), Where the combination of highest levels of both (S3 2N) gave the greatest effect, while the treatment (S2 1N) gave the lowest.

Table (1) Plant density (plant/m^2)of teff. *Eragrostis teff* (Zucc.) Trotter as affected by seeding rates and nitrogen.

Seeding rates	Date and number of sampling			
	1st	2nd	3rd	4th
	8/7/03	22/7/03	16/8/03	15/9/03
S1	81.33 b	71.5 b	80.33 b	79.66 a
S2	78.08 b	67.08 b	96.66 b	81.83 a
S3	111.25 a	100.08 a	128.75 a	90.41 a
LSD 5%	11.4	11.63	48.13	
Nitrogen	8/7/03	22/7/03	16/8/03	15/9/03
0N	88.08 a	77.91 a	106.08 a	85.08 a
1N	84.33 a	74.25 a	78.50 a	78.16 a
2N	98.25 a	86.5 a	94.16 a	91.41 a
LSD 5%	11.42	11.63	48.13	17.4

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed — 0N = 0 kg N/ha

S2= 4 kg/fed — 1N = 40 kg N/ha

S3= 6 kg/fed — 2N = 80 kg N/ha

Table (2) Plant density (plant/m^2) of teff. *Eragrostis teff* (Zucc.) Trotter as affected by interaction between seeding rates and nitrogen levels in the first sample.

Seeding rates	Nitrogen levels			Mean
	0N	1N	2N	
S1	83.0 a	85 25 a	96.0 a	88.08
S2	87.5 a	52.75 b	112.75 a	84.33
S3	73.5 a	96.25 a	125.0 a	98 25
Mean	81.33 a	78.08	111.25	
LSD 5%	54.72			

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed 0N = 0 kg N/ha

S2= 4 kg/fed 1N = 40 kg N/ha

S3= 6 kg/fed 2N = 80 kg N/ha

Table (3) Plant density (plant/m^2) of teff. *Eragrostis teff* (Zucc.) Trotter as affected by interaction between seeding rates and nitrogen levels in the second sample.

Seeding rates	Nitrogen levels			Mean
	0N	1N	2N	
S1	74 25 b	74.0 b	85.5 b	77.9
S2	77.5 b	43.25 c	102.0 a	74.25
S3	62.75 b	84.0 b	112.75 a	86.5
Mean	71.5	67.o8	100.08	
LSD 5%	11.63			

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed   0N = 0 kg N/ha

S2= 4 kg/fed   1N = 40 kg N/ha

S3= 6 kg/fed   2N = 80 kg N/ha

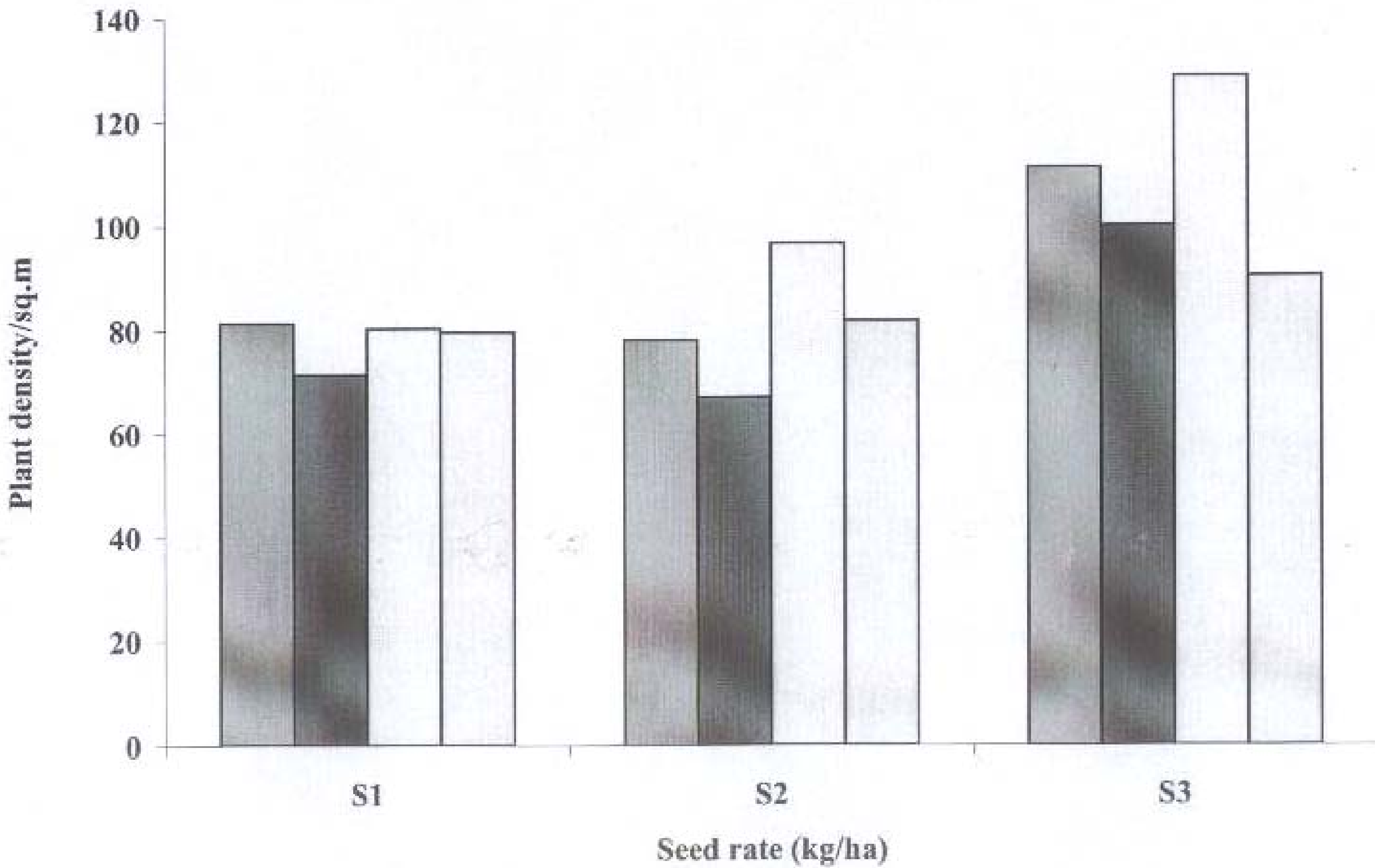

Fig. (1): Plant density/sq.m of the Teff as affected by seeding rate

4.1.2 Effect of seeding rates and nitrogen levels on plant height (cm):

Plant height was not significantly affected by seeding rates (Table 4). Treatment S2 increased plant height more than S1 and S3 in all samplings occasions. S1, S2, and S3 it recorded an average of 76.5, 80.5, and 78.8cm, respectively.

Nitrogen had no significant effect on plant height (Table 4). The combined treatment (S3 1N) was superior with respect to plant height.

4.1.3 Effect of seeding rates and nitrogen levels on number of leaves per plant:

Seeding rates did not significantly affect number of leaves per plant (Table 5) There was no trend of increase or decrease with respect to number of leaves per plant by increasing seeding rates. The treatment S1 gave lower leaves per plant compared to S2 and S1 (Table 5).

Number of leaves per plant was significantly affected by nitrogen levels in all samplings (Table 5). There was a trend of increasing number of leaves with increasing nitrogen levels. The treatment 2N increased number of leaves per plant by 39% and 12.7% over 0N and 1N, respectively in the third reading.

Table (4) Plant height (cm) of teff. *Eragrostis teff* (Zucc.) Trotter as affected by seeding rates and nitrogen.

Seeding rates	Date and number of sampling		
	1st	2nd	3rd
	7/8 03	21/8/03	13/9/03
S1	67.31 a	78.99 a	83.18 a
S2	70.12 a	82.85 a	88.41 a
S3	71.52 a	78.95 a	86.07 a
LSD 5%	16.83	13.37	13.82
Nitrogen	7/8/03	21/8/03	13/9/03
0N	65.6 a	76.15 a	84.09 a
1N	73.09 a	81.71 a	86.41 a
2N	70.26 a	82.91 a	87.16 a
LSD 5%	16.83	13.37	13.82

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed 0N = 0 kg N/ha

S2= 4 kg/fed 1N = 40 kg N/ha

S3= 6 kg/fed 2N = 80 kg N/ha

Table (5) Number of leaves per plant of teff .*Eragrostis teff* (Zucc.) Trotter as affected by seeding rates and nitrogen.

Seeding rate	Date and number of sampling		
	1st	2nd	3rd
	7/8/03	21/8/03	13/9/03
S1	92.56 a	133.7 a	168.31 a
S2	100.8 a	139.27 a	206.72 a
S3	96.34 a	127.63 a	182.1 a
LSD 5 %	21.32	30.63	43.07
Nitrogen	7/8/03	21/8/03	13/9/03
0N	80.31 a	111.23 a	136.11 a
1N	105.28 b	139.19 b	196.24 b
2N	104.1 b	150.18 b	224.79 b
LSD 5 %	21.32	30.63	43.07

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed 0N = 0 kg N/ha

S2= 4 kg/fed 1N = 40 kg N/ha

S3= 6 kg/fed 2N = 80 kg N/ha

4.1.4 Effect of seeding rates and nitrogen levels on number of tillers per plant:

Number of tillers was not significantly affected by seeding rates Table (6), but the treatment S2 recorded a higher mean number of tillers than S1 and S3 in all samplings. It increased this parameter by 7.2 % and 8.5 % over S1 and S3, respectively.

Nitrogen levels did not affect number of tillers Table (6). However, there was a trend of increasing number of tillers per plant by increasing nitrogen levels.

4.1.5 Effect of seeding rates and nitrogen levels on days to 50 % flowering:

Flowering was not significantly affected by seeding rates Table (7). Nitrogen had no significant effect on flowering Table (7). The treatment (S2 2N) flowered in less days than other treatments.

Table (6) Number of tillers per plant of teff. *Eragrostis teff* (Zucc.) Trotter as affected by seeding and nitrogen.

Seeding rates	Date and number of sampling		
	1st	2nd	3rd
	7/8/03	21/8/03	13/9/03
S1	18.28 a	20.69 a	26.7 a
S2	20.9 a	22.43 a	27.22 a
S3	19.6 a	19.74 a	25.07 a
LSD 5 %	6.09	4.87	7.35
Nitrogen	7/8/03	21/8/03	13/8/03
0N	18.0 a	18.25 a	24.2 a
1N	20.7 a	21.97 a	26 22 a
2N	20.08 a	22.64 a	28.02 a
LSD 5 %	6.08	4.87	7.35

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed — 0N = 0 kg N/ha

S2= 4 kg/fed — 1N = 40 kg N/ha

S3= 6 kg/fed — 2N = 80 kg N/ha

Table (7) Days to 50 % flowering of teff. *Eragrostis teff* (Zucc.)

Trotter as affected by seeding rates and nitrogen

Seeding rates	
S1	62.08 a
S2	66.33 a
S3	62 08 a
LSD 5 %	11.7
Nitrogen	
0N	65.08 a
1N	63.83 a
2N	61.6 a
LSD 5 %	11.7

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed 0N = 0 kg N/ha

S2= 4 kg/fed 1N = 40 kg N/ha

S3= 6 kg/fed 2N = 80 kg N/ha

4.2 Effect of seeding rates and nitrogen levels on fodder production:

4.2.1 Destructive samples:

Seeding rates had no significant effect on fresh weight of teff (Table 8). Treatment S1 gave more yield than S2 and S3. Maximum forage fresh yield (11.88 ton/ha) was recorded by treatment S1. The result show significant difference among nitrogen levels (Table 8). Treatment 2N gave a higher fresh yield over 0N and 1N. It increased forage fresh yield by 43.5 % and 27.5 % over 0N and 1N, respectively. There was a trend of increase in yield with increasing nitrogen levels.

There were no significant differences observed among seeding rate with respect to forage dry yield (Table 8). Dry yield increased with increasing seeding rates. Table (8) showed that there were no significant differences among nitrogen levels with respect to forage dry yield. The highest nitrogen level 2N gave the maximum forage dry yield. It increased dry yield by 31.5 % and 20.5 % over 0N and 1N, respectively.

Table (8) Fresh and dry yield (ton/ha) of Teff. *Eragrostis teff* (Zucc.) Trotter as affected by seeding rate and nitrogen after 60 days.

Treatment	Fresh yield	Dry yield
Seed rate		
S1	11.88 a	3.62 a
S2	9.99 a	3.27 a
S3	11.32 a	3.65 a
LSD 5 %	5.53	1.73
Nitrogen		
0N	8.18	2.91 a
1N	10.50	3.38 a
2N	14.49	4.25 a
LSD 5%	ns	1.73

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed 0N = 0 kg N/ha

S2= 4 kg/fed 1N = 40 kg N/ha

S3= 6 kg/fed 2N = 80 kg N/ha

4.2.2 Effect of treatments on final forage fresh yield (ton/ha):

The seeding rates had no significant effect on fresh yield (Table 9). Treatment S2 gave more fodder yield than S1 and S3 (Fig. 2). S1 gave lower fodder yield compared to S2 and S3. Maximum forage fresh yield (8.6 ton/ha) was recorded by treatment S2.

There were no significant differences observed among nitrogen levels (Table 9). Treatment 2N gave a maximum fresh yield over 0N and 1N. It increased forage fresh yield by 31% and 10% over 0N and 1N, respectively.

4.2.2 Effect of treatment on final forage dry yield (ton/ha):-

There were no significant differences observed among seeding rates with respect to forage dry yield (Table 9). Dry yield increased with increasing seeding rates but it decreased when the seeding rate was S3. Table (9) showed that there were no significant differences observed among nitrogen levels with respect to forage dry yield. The highest nitrogen level (2N) gave a maximum forage dry yield. It increased forage dry yield by 24 % and 12 % over 0N and 1N, respectively (Fig. 3).

Table (9) Final fresh and dry yield of teff. *Eragrostis teff* (Zucc.) Trotter as affected by seeding and nitrogen at harvest.

Treatment	Fresh yield	Dry yield
Seed rate		
S1	7.35 a	5.50 a
S2	8.60 a	6.16 a
S3	8.52 a	5.54 a
LSD 5 %	3.73	1.97
Nitrogen		
0N	6.51 a	4.95 a
1N	8.50 a	5.75 a
2N	9.45 a	6.50 a
LSD 5%	3.73	1.97

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed 0N = 0 kg N/ha

S2= 4 kg/fed 1N = 40 kg N/ha

S3= 6 kg/fed 2N = 80 kg N/ha

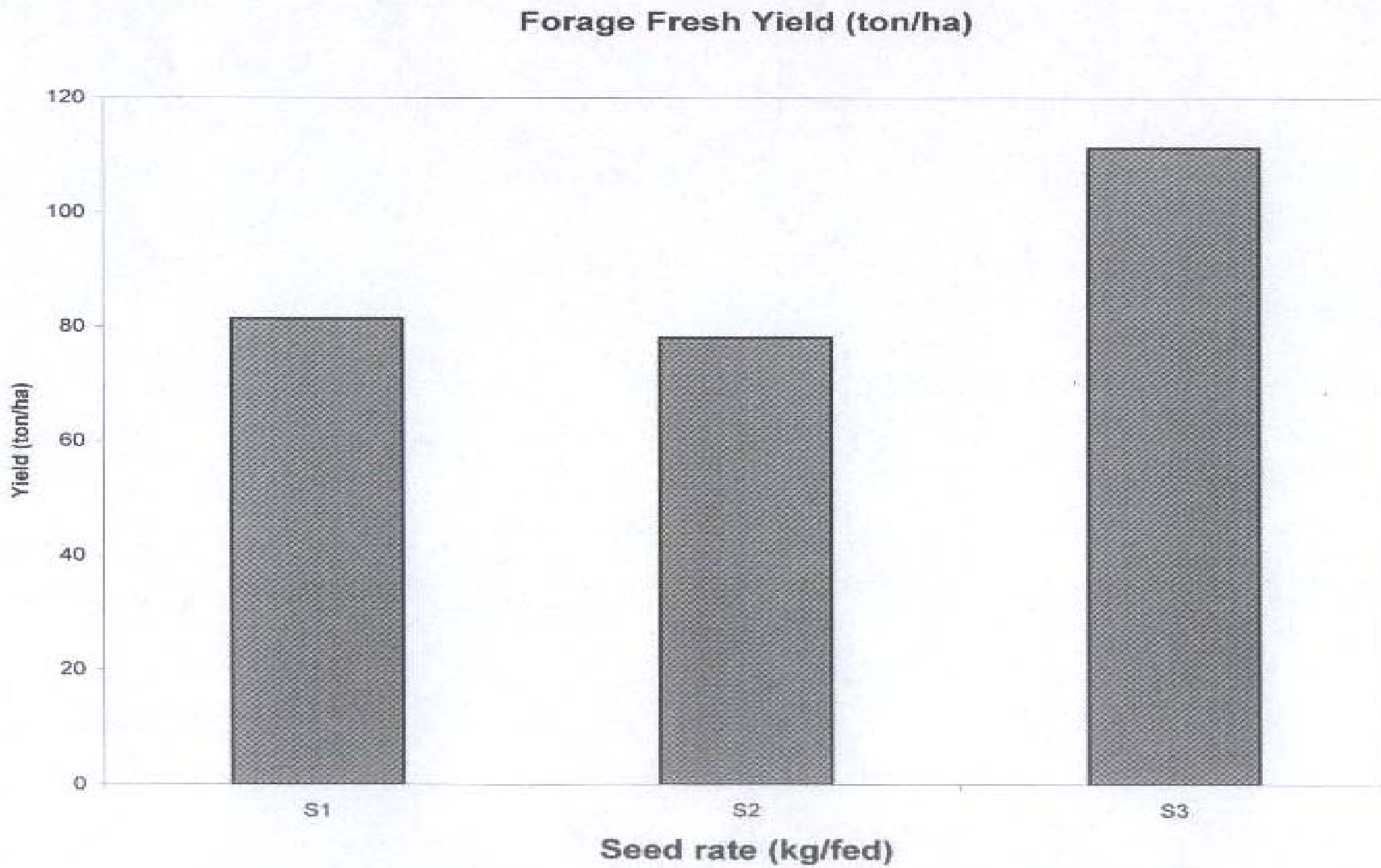

Fig.2. Forage fresh yield of Teff as affected by seed rate

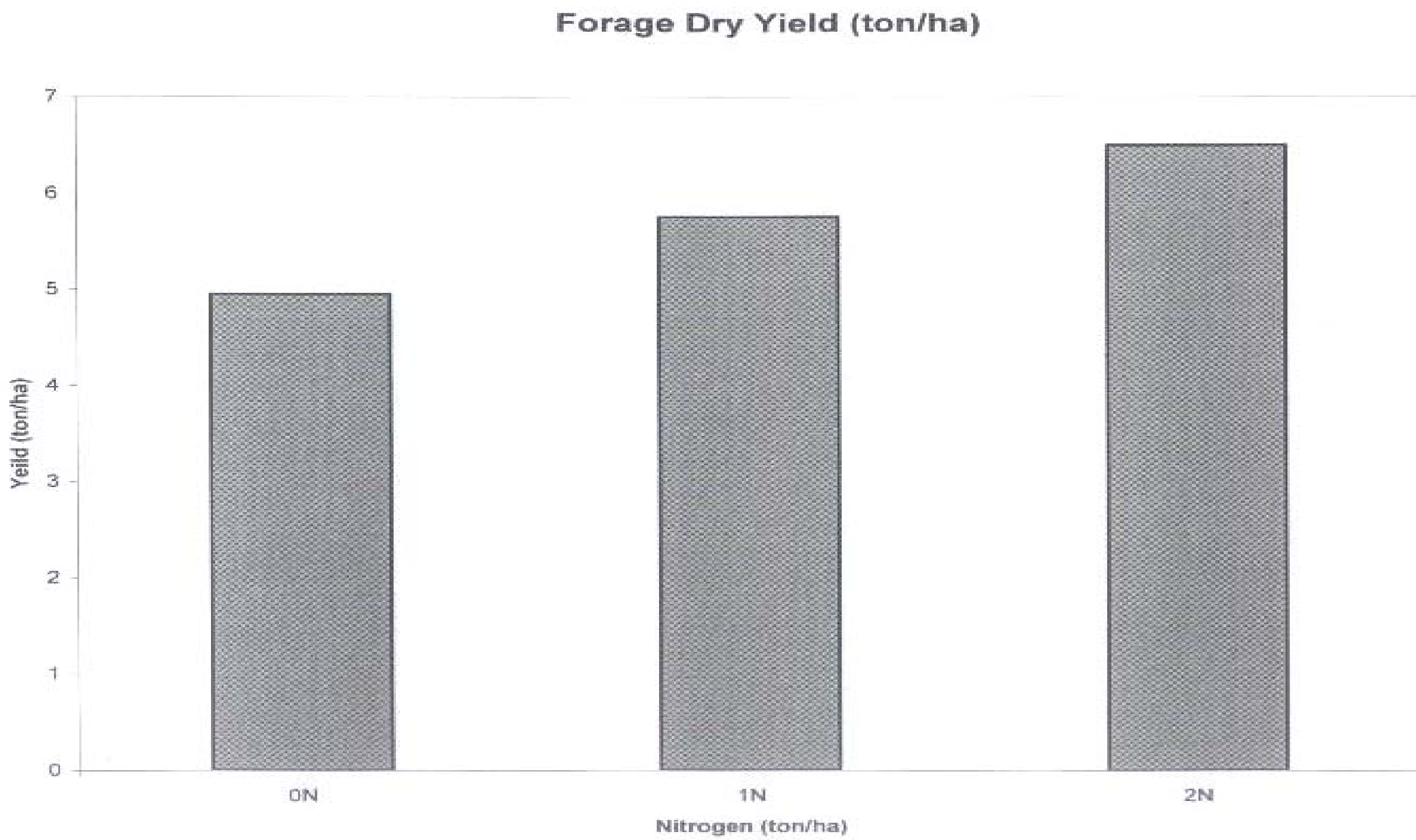

Fig. 3. Forge Dry Yield of Teff as affectec by Nitrogen

4.3 Forage Quality:

Significant difference with respect to forage quality among all seed rates level was observed Table (10). The increament in the protein percentage was observed as seed rates was increased . S3 gave highest percentage than S1 and S2. It increased by 20 .7 and 3.6 over S1 and S2, respectively.

Nitrogen levels significantly affected protein percentage (Table 10). There was a trend of increase protein with increasing nitrogen . 2N scored the highest protein content.

Table (10)revealed that seed rates had significant effect on crude fiber percentage. The increment in the crude fiber was observed as the seed rate increased.

Nitrogen levels significantly affected crude fiber (Table 10). However, the low fertilized treatment (1N) gave higher percentage of crude fiber and 2N gave lower crude fiber.

As shown in (Table 10) these elements P, K, Mg and Ca were not significantly affected by seeding rate. There was no trend of increase or decrease K content with respect to seed rate, but Mg and Ca contents decreased with increased seed rate.

P and K were significantly affected by nitrogen levels (Table 10). Increasing nitrogen application reduced P concentration. The highest nitrogen level (2N) increase K content of teff over 0N and 1N by 25.5 and 22.49 %, respectively. Both Mg and Ca were not significantly affected by nitrogen level (Table 10). But there was a trend of decrease in Mg content with increasing nitrogen level and increased Ca content with increasing nitrogen level.

Table (10) Chemical composition of teff (*Eragrostis teff* (Zucc).

Trotter as affected by seeding rates and nitrogen.

Seed rate	**C.P**	**C.F**	**P**	**K**	**Mg**	**Ca**
S1	5.93 c	19.74 c	2.43 a	15.91	14.5	14.5
S2	7.21 b	18.05b	2.25 a	16.02	15.16	10.66
S3	7.48 a	24.83 a	2.43 a	13.99	9.66	10.0
LSD5%	0.07	0.31	0.32	ns	ns	ns
Nitrogen						
0N	6.7 c	20.35b	2.68a	13.88b	15.5	10.66 a
1N	6.91 b	21.73 a	2.32 b	13.99 b	13.0	12.66 a
2N	7.01 a	20.45 b	2.1 b	18.05 a	10.83	11.83 a
LSD5%	0.07	0.31	0.32	1.11	ns	2.12

Figures followed by same letter in the same column are not significantly different at 0.05 level of probability.

S1= 2 kg/fed 0N = 0 kg N/ha

S2= 4 kg/fed 1N = 40 kg N/ha

S3= 6 kg/fed 2N = 80 kg N/ha

CHAPTER FIVE

DISCUSSION

5.1 Growth attributes:

The result showed that plant population increased with increased seed rate. High plant density produced at higher seed rate. This result is in agreement with the result reported by Sharma (1969), who noted that highest plant population was obtained from the highest seed rate of maize. Gasim (2001) reported that effect of seed rate on plant population of maize was significant, plant population increased with increased seed rate.

Nitrogen fertilization had no effect on plant population. Plant population is always a function of germination percentage. The non-significant effect of nitrogen may be attributed to the fact that nitrogen has no effect on germination, therefore plant density under different level of nitrogen can be stable. In this study there is a significant interaction of seed rate and nitrogen levels observed in first and second counts. This may be due to the higher levels of seed rate gave a higher number of plants. But in the last two counts there were no significant interaction.

Plant height was not significantly affected by seeding rates. The reduction in plant height with increased seed rate may be due to competition between plants at high densities for moisture and nutrients. Similar results were reported by Crossman (1979), Gasim, (2001). This result is in contrast of that reported by Hozami *et al.*, (1955), Duncan (1980) and Fleming & Wood (1967). This difference in result may be due to the levels of seed rates which used in this study was less than the seed rate which was used in teff forage and causes high competition for light and resulted in taller plants.

Plant height was not significantly affected by nitrogen levels, but plant height increased with increase in nitrogen levels because nitrogen promotes plant growth. This result is in agreement with result reported by Gasim (2001). In contrast Chandler (1969), Turkhende and Jajendra (1978), and Koul (1998) reported opposite results. The explanation of this discrepancy may be refered to the fact that plant height of teff varies depending upon cultivar type and growing environments. Similar results were reported by Stallknech (1993).

Number of leaves per plant were not significantly affected by seeding rates. This may be due to number of leaves is controlled by different factors.

Nitrogen levels cause significant effect on number of leaves per plant in this study. With increasing nitrogen levels increased the average number of leaves per plant. This result is in line with that reported by Gasim (2001) who noted that increasing the level of nitrogen increased the average number of leaves per plant in maize. This may be attributed to the fact that nitrogen increase plant growth and consequently more leaves.

Seeding rates had no significant effect on number of tillers per plant. Similar results were noted by Abu Suwar (1980), who observed that plant density had no significant effect on tillering of fodder sorghum.

Tillering also was not significantly affected by nitrogen. This result is in contrast of that obtained by Abu Suwar (1980) who reported that tillering was affected by fertilization. This may be attributed to high densities which observed in this study resulted in low tillers and this is in line with the fact that more tillers produced at low plant densities.

5.2 Days to 50 % Flowering:

Both seed rate and nitrogen did not significantly affect days to 50 % flowering of teff. This result may be because the teff is sensitive to day length. This is in agreement with that obtained by Stallknecht (1997), who reported that teff is day length sensitive and flowers best during 12 hours of day light. Also this result is in line with the findings of Beech and Basink (1975). They reported that time of occurrence of tesseling, siliking and maturity was not affected by plant population. Ketema (1983) reported that teff is sensitive to day length, the plant performed better in Ethiopia where there is approximately 12 hours of day light. These results are in contrast of that reported by Gasim, (2001) who noted that, both seed rate and nitrogen fertilization affected days to 50% tasseling in maize.

5.3 Forage Yield:

5.3.1 Yield after two month:

Seed rate and nitrogen fertilization had no significant influence on fresh yield. The non significant effect may be attributed to two factors, teff has more tillers producing at low seed rate and this resulted in yield similar to that produced at high seed rate which have high plant population, then they had no differences between them. The other factor was the fact that in low seed rate plants grown with high vigor and good stand establishment.

Nitrogen increased fresh yield but not significantly. This may be due to the nitrogen increase of photosynthetic capacity of plant which enhance growth . This result is similar to that reported by Gasim (2001). Both seed rates and nitrogen fertilizer increased destructive dry weight, but not significantly.

5.3.2 Forage Final Yield:

The results showed that seed rate did not affect fresh and dry forage yield significantly, although there was a higher yield with high seed rate. This may be due to low seed rate resulted in lower fresh and dry yield compared to the higher seed rate. This may be attributed to fact that low seed rate produced low number of plants per unit area. Also at sowing the very small size of teff seed makes it difficult to control population density and it's distribution. This remains true when broadcasting the seed by hand. Similar results were reported by Ketema (1997).

The non-significant effect of nitrogen on fresh and dry weight may be attributed to genetic diversity. Similar result noted by Ketema (1997), who reported that straw yield represented the maximum gentic diversity among the observed teff germplasm.

Generally the destructive sampling gave fresh and dry weight higher than the final yield (fresh-dry). This difference in yield between them may be due to time of harvesting of final yield is more delayed. At harvesting of crop, lodging occurs and this may be due to heavy rain fall often causing lodging associated with crop growth. Similar results were reported by Kidanu (1999), Deaker (2001). Berhane (1985) was found that the loss in yield due to weed competition .

5.3.3 Forage Quality:

Crude protein was significantly affected by seeding rate. It was found that increasing seed rate increases crude protein. But Gasim (2001), disagree with result obtained in this study and reported that increasing seed rate reduced slightly crude protein in leaf and stem.

With regard to the effect of nitrogen on the percentage of crude protein, it was found that increasing the level of nitrogen increased the percentage of crude protein. This result may be attributed to the fact that nitrogen is most important to synthesis of protein. This observation is in agreement with Gasim (2001), who reported that increasing the level of nitrogen increased percentage of crude protein in leaf and stem. Also Singh *et al.*, (1992) reported similar result.

Crude fiber was significantly affected by seed rate . As seeding rate increased crude fiber increased. This result is similar to that obtained by Gasim (2001), and Sanchez *et al.*, (1983). Also crude fiber was affected by nitrogen. Increasing nitrogen level lead to reduced crude fiber. This result is in conformity with finding of Gasim (2001), who reported that the crude fiber content is inversely related to nitrogenous compounds as protein. Several research workers reported similar result (Primmost, 1964; Hago and Mahmoud, 1996 and Powel *et al.*, 1991). This indicates that forage quality can be maintained at higher plant densities (Graybill *et al.*, 1991)

Minerals (P, K, Ca and Mg) were not significantly affected by seeding rate. This may be due to the concentration of these nutrients related to other factors than seeding rate. Similar result was obtained by Gomide (1978), Reid and Horvth (1980). They reported that minerals composition of forage plants is affected by soil-plant factors including pH, drainage, fertilization, forage species, forage maturity and interaction among minerals.

Nitrogen levels significantly affected P concentration .Increasing level of N decreased P content. This observation is in agreement with Joseph *et al.*, (1998) who reported that increasing N application reduced P concentration linearly. Potassium content increased with increasing N levels. This result is in line with that obtained by Bax (1992). But Joseph *et al.*, (1998) disagree with Bax (1992), who reported that K concentration decreased linearly with N application. The different result concerning effects on minerals, may be due to the fact that many parameters affect the concentration of minerals, e.g. temperature and soil type.

Ca and Mg were not significantly affected by nitrogen levels. There was trend of decrease of Ca and Mg content by increasing N levels. This result is in agreement of that reported by Segaard (1990), but in contrast of that obtained by Joseph *et al.*, (1998), who reported that both Ca and Mg concentration increased linearly with increasing N applications.

Summary and Conclusion

A study was conducted to investigate the effect of seed rate and nitrogen fertilization on growth, yield, and quality of the teff. Seed rate of 2, 4 and 6 kg/fed, and nitrogen fertilizer (Urea 46%N) at rates of 0, 40 and 80 kg/ha was used.

The results showed that seed rates significantly increased plant population, crude protein, and crude fiber. While nitrogen fertilization significantly affected number of leaves per plant, crude protein, crude fiber and minerals (P, K) content. The other parameters were not affected by seed rate and nitrogen. From this results we can conclude that:

- High seed rate had more plant population and consequently increased yield.
- Nitrogen increased number of leaves and improved forage quality (protein).

- Forage yield after two month was superior to that at final harvest.

The following recommendations should be taken into consideration when teff is to be planted:

- At sowing it is better to mix teff seeds with sand to avoid drift due to small size of seeds specially when broadcasts the seed by hand.
- A teff field needs plowing (two to five times) more frequently than other cereals.
- The harvesting of the crop as a forage must be performed before lodging occurs.
- Moderate rates of nitrogen fertilizer could be used to prevent lodging and improve teff quality.
- Seeding of teff for rangeland as rehabilitation species in arid zones when rainfall is less than 125mm.

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APPENDICES

Appendix (1) Yield and chemical composition of various crop on dry matter basis.

Crop residue	Yield(ton/ha)	Composition			
		DM	**Ash**	**CP**	**NDF**
Barley straw	10	92.6	8.4	4.7	71.5
Teff straw	5	92.6	8.4	5.2	72.6
Wheat straw	9	93.1	9.0	3.9	79.8
Faba bean	3.8	91.7	10.4	7.2	74.3
Faba pea	5	91.9	6.1	6.7	73.6
Natural pasture	4.1	92.2	9.5	6.6	73.8

DM= dry matter; **CP**= crude protein; **NDF**= neutral detergent fiber

Appendix (2): Mean monthly maximum, minimum temperature(C), Relative humidity% and total rain fall(mm).

Month	Temperature(C)		RH%	Rainfall (mm)
	Max	**Min**		
June	40. 9	27. 0	33	4.8
July	37. 3	25. 2	65	31.2
August	35. 8	25. 3	73	57.6
September	38. 4	25. 2	61	96.o
October	39. 9	22. 9	39	33. 6
November	36. 1	2o. 4	37	-
December	32. 6	15. 9	41	-

Appendix (3) Determination of crude protein:

The procedure**:-**

a) Weight accurately about o.2gm of sample and transfer to kejldhal flask.

b) Add the kejldhal catalyst mixture and about 3.5ml of nitrogen free sulphric acid H2So4.

c) Digest the sample for about 2hours at full heat up to the point that sample solution is clear.

d) Cool the constituents and distillate in micro kejldhal units follows:

I. Take 25cc of 2% acid in conical flask and mixed indicator.

II. Pour the sample gently through the funnel of the distillation unit and add about 20% Na OH 40% slowly and allow the distillation process for 5 minutes, the receiver color change into blue.

III. Take the receiver and titrate against NHCL to the end point which colorless then read the volume HCL used.

Calculation:

Nitrogen content = HCL volume Nitrogen content

Weight of the sample ×1000

Protein content multiply by 6.25

Appendix (4) Determination of crude fiber:

Use the oven dried ether extracted material(Soxhelt Method)

a) Weight out exactly 2gm into 500cc Erlenmey and add 200ml of 1¼% H_2SO_4.
b) Connect up air condenser and boil for exactly 30 min.
c) Watch carefully inclined boil oven especially just on the boil.
d) Filter on a Bucher funnel on a liner cloth and wash with hot water.
e) Scrape off with blunt knife, spatula etc., transfer back to the same Erlenmeyer washing it through a fitter with 200ml 0f 1¼ Na OH.
f) Boil for exactly ¼ hr and filter as before.
g) Transfer to a weighted crucible and transfer to oven.
h) Heat in the oven at 100Cº for 24 hrs (This time being sufficient to remove all moisture .

i) Remove from oven, place in desiccate or until cool(½hr).

j) Weight to constant weight, ash at 600Cº and reweight.

First weight- Ash weight = Fiber weight

Fiber weight /2gm ×100= Fiber on ether extracted material

N.B. Apply ether Extract correction to obtain % fiber on original material:

Wt of Extract/3gm ×100= %Ether Extract.

Appendix (5) Determination of Phosphorus in plant sample:

- Transfer 5ml of HCL to a100 ml volumetric flask.
- Add 10ml ammonia vanadom moybdate reagent and dilute to volume.
- Shake the flask and leave on the bench for 30min.Also prepare a blank containing 10ml ammonia vanadom molydate diluted to 100ml with water.
- Using the blank to zero the spectrophotometer (wave length 400nm), determine the extinction of the sample solutions provided.

Plot the standard curve and calculate the P content of sample.

Appendix (6) Determination of Potassium in sample:

- Weigh out of dried, ground leaf material into a silica or porcelain dish and ash in a muffle furnace at a temperature not to exceed 500°C.
- Weigh out another sample for moisture determination.
- Cool, moisten with 10ml of distilled water, and take up in N nitric acid.
- Filter, wash, and make to volume of 100 ml.
- Take an aliquot containing between 2 and 15 mg of potassium.
- Evaporate to dryness in a 50 ml beaker, add 10 ml of water, 1 ml of N nitric acid, and stiro to dissolve residue.
- Add 5ml of the 20 per cent sodium carbaltinitrite, stiro, and allow to stand 2 hours at 15 to 20°C.
- Filter in a porous-bottom porcelain filtering crucible of tared weight, using th 0.01N nitric acid to wash beaker and precipitate.
- Wash 10 times this dilute acid and 5 times with 95 per cent ethyl alcohol.
- Evaporate until quite dry. Dry for 1 hour at 110°C.Cool in a desiccator and weight.

Appendix (7) Determination of Calcium in sample:

- A 2g sample of ground leaf tissue is ashed in a silica or porcelain dish at a temperature of 550°C until the ash is nearly white. The ash will remain black even after several hours, and a second ashing of the residue may be necessary.
- The ash from the first heating is covered with a watch glass, 20 ml of cold distilled water carefully added after the dish cools, followed by the addition of 2 to 3 ml of 6N hydrochloric acid.
- When the reaction ceases, the spray on the watch glass is washed into the silica dish, the contents of the dish filtered into a 200 ml volumetric flask, and the filtered residue washed.
- If much unburned carbon remains, the paper and residue are again ashed, taken up with water and acid, filtered, and the second filtrate added to the first filtrate in the volumetric flask.
- When the contents of the flask are cool, it is diluted to volume.

Usually, a 100ml aliquot for the determination of calcium.

Appendix (8) Determination of Magnesium in sample:

- Dry ash 2 gm of plant material in a muffle furnace at 550°C.
- Use either silica or porcelain or crucibles.
- Cool, add 20 ml of distilled water, take up in the 3 ml 0f 6N hydrochloric acid, and filter into a 200 ml volumetric flask.

- If much unburned carbon remains, reash residue and combined with first filtrate.