

## PERFORMANCE OF ZINC AND IRON FERTI-FORTIFICATION ON GROWTH AND YIELD OF BERSEEM

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### SUMMARY

A field experiment was conducted during *rabi* 2020 at Research Farm, Agronomy Section, ICAR-National Dairy Research Institute, Karnal, Haryana. The experiment was carried out in Randomized Block Design containing seven treatments which were T<sub>1</sub>: Absolute control, T<sub>2</sub>: 100% RDF, T<sub>3</sub>: 100% RDF + Zn (basal), T<sub>4</sub>: 100% RDF + Fe (basal), T<sub>5</sub>: 100% RDF + 0.5% foliar spray of Zn, T<sub>6</sub>: 100% RDF + 0.5% foliar spray of Fe, T<sub>7</sub>: 75% RDF + 0.5% foliar spray of Zn + 0.5% foliar spray of Fe and these were replicated thrice. Results of the experiment showed that significantly higher value of growth parameters *viz.* plant height, no. of leaves, leaf length, leaf width, no. of regenerated stems etc. at all three cuts were recorded in treatment T<sub>3</sub> which was statistically at par with treatment T<sub>4</sub>. Higher total green fodder as well as dry fodder yield (69.82 and 9.59 t/ha, respectively) was also recorded in treatment T<sub>3</sub> among different treatments in all three cuts while minimum total green as well as dry fodder yield found in treatment T<sub>1</sub> (49.59 and 6.16 t/ha respectively). In nutshell, application of 100% RDF along with Zn as basal could produce better fodder in terms of growth and yield.

**Key words:** Fodder, legume, livestock, nodule, RDF

Livestock, crucial to Indian agriculture, contributes 4% to the national GDP, while agriculture contributes 17%. India's 20<sup>th</sup> livestock census reports a 4.6% increase, totaling 535.78 million animals (Anonymous, 2020). As the livestock population increases, requirement of feed and fodders also increases. But the main limiting factor is the area under cultivated fodder, which is only 8.4 Mha. Therefore, cultivation of crops which give higher yield is most important to combat the scarcity of fodders. According to IGFRI Vision 2050, India has net deficit of 35.6% green fodder, 10.9% dry fodder and 44% feed. As a result, livestock feeding on these roughages have poor health. So, for maintaining the good health of livestock, good quality green fodders are needed. Among legume fodders in India, Berseem (*Trifolium alexandrinum* L.) is an important winter season fodder, popularly known as "King of fodder crops". India occupies the highest area under berseem (2 M ha) followed by Egypt and Pakistan (Muhammad *et al.*, 2014). In India, berseem thrives mainly in North India due to longer winters. As a legume, it enhances soil fertility by fixing atmospheric nitrogen. Berseem is recognized as milk

yield enhancer in lactating cows and buffaloes (Muhammad *et al.*, 2014). It is mostly accepted by livestock because of its nutritious and succulent nature, high digestibility (around 65%) and palatability. It is generally grown as annual crop during *rabi* season which gives 4 to 6 cuts *i.e.* it gave yield in lean period of winters in North India. On dry matter basis, green forage of berseem contains 17-22% crude protein, 42-49% neutral detergent fiber, 35-38% acid detergent fiber, 24-25% cellulose and 7-10% hemi cellulose (feedipedia, 2020). The quality and quantity of berseem fodder is low because of poor management practices and environmental conditions. Also, yield and quality of fodder is largely influenced by the nutrient practices. Intensive farming and improper nutrient use are key causes of widespread micronutrient deficiencies in crops. Despite being needed in small amounts, micronutrients are crucial for crop growth and development. Micronutrients include boron (B), iron (Fe), chlorine (Cl), manganese (Mn), copper (Cu), molybdenum (Mo), zinc (Zn) and nickel (Ni). Indian soils are mostly deficit in micronutrients, on an average Zn-49%, Fe-12%, Cu-4%, Mn-3%. In Haryana, Zn-

15.3%, Fe-21.6%, Mn-6.2%, Cu-5.2% and B-3.3% deficiency in soils has been assessed (Shukla *et al.*, 2015). Zinc (Zn) and Iron (Fe) both are essential micronutrients required by both plants and animals for proper functioning and metabolic processes. Zinc and Iron both are transition metals have unpaired electrons, therefore, involved in oxidation-reduction reactions (Zargar *et al.*, 2015). In plants Zn is required for the synthesis of IAA and RNA. Zn is a component of several enzymes like carbonic anhydrase, alcoholic dehydrogenase, superoxide dismutase. Zn is involved in carbohydrate metabolism, maintaining the integrity of cellular membranes, protein synthesis and pollen formation (Hafeez *et al.*, 2013). In plants, Zn deficiency includes symptoms such as stunted growth, chlorosis, smaller leaves, spikelet sterility as well as affect the quality of harvested products. In animals, Zn plays vital role in protein synthesis, carbohydrate metabolism and involved in enzyme systems and reproductive functions. Its deficiency leads to change in taste, limited limb bone growth and sight disorders (Hosnedlova *et al.*, 2007). Another disorder caused from Zn deficiency is *parakeratosis* which is a disorder of skin occurs in calves, sheep, goats and piglets (Sloup *et al.*, 2017). Like zinc, Iron is also an indispensable element of plant which takes part in biosynthesis of chlorophyll, nitrogen fixation, DNA replication and electron transport chain (Zargar *et al.*, 2015). Iron is vital in plants for synthesizing proteins like leghemoglobin and nitrogenase in bacteroids, affecting nodule development and causing leaf chlorosis. In animals, iron is crucial for oxygen transport in hemoglobin and myoglobin, with deficiency leading to anemia in calves. Recent researches revealed that a small amount of nutrients, mainly Zn, Fe and Mn applied by foliar spraying significantly increases the yield of crops (Sarkar *et al.*, 2007; Wissuwa *et al.*, 2008). Application of micronutrient fertilizers to micronutrient-deficient soils

is associated with improved yield and crop quality of cereals, corn, beans, forages, and oil seeds (Malakouti and Tehrani, 2005; Malakouti, 2007). Enhancing micronutrient levels through supplementation, breeding, and genetic engineering is costly and inaccessible to farmers. Under such conditions, agronomic biofortification using fertilizers *i.e.* Ferti-fortification is a complementary solution (White and Broadley, 2009). As Indian soils are deficit in micronutrients therefore there is need of application of micronutrients, so that their deficiency can be cured in the plants as well as in the animals via food chain. Looking to the role of Zn and Fe both in animals and plants, the present study entitled “Performance of zinc and iron ferti-fortification on growth and yield of fodder berseem” was undertaken.

## MATERIALS AND METHODS

### Experimental location

This experiment was conducted at research plot of Agronomy Section, ICAR-National Dairy Research Institute, Karnal in 2020, located at 29°45'N latitude, 76°58'E longitude and at an altitude of 245 m above mean sea level (MSL) under subtropical climate. The soil of the experimental field was near neutral in reaction, medium in organic carbon and low in available nitrogen, high in available phosphorus, medium in available potassium and low in zinc. The texture of the soil was clay loam in nature.

### Experimental details

The details of experiment and treatments are presented in Table 1 and Table 2, respectively.

**Varietal Details:** BL-42 is a quick growing variety which produces more tillers. This variety is

TABLE 1  
Experimental details

S. No.	Particular	
1.	Experimental Design	Randomized Block Design
2.	Variety	BL-42
3.	RDF	20:60:40 kg/ ha, N: P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O
4.	Treatments	7
5.	Replications	3
6.	No. of plots	21
7.	Gross plot size	6 m x 5m = 30 m <sup>2</sup>
8.	Irrigation channel	1.5 m

TABLE 2  
Treatment details

Treatment No.	Treatment details
T <sub>1</sub>	Absolute control
T <sub>2</sub>	100% RDF
T <sub>3</sub>	100% RDF+Zn (basal)
T <sub>4</sub>	100% RDF+Fe (basal)
T <sub>5</sub>	100% RDF+0.5% foliar spray of Zn
T <sub>6</sub>	100% RDF+0.5% foliar spray of Fe
T <sub>7</sub>	75% RDF+0.5% foliar spray of Zn+ 0.5% foliar spray of Fe

stem rot disease tolerant. It gives green fodder yield up to first week of June.

### Cultural practices

The field was ploughed twice using cultivators to obtain a fine tilth for better crop germination and weed control. After ploughing, planking was done to level the land uniformly, preventing water stagnation. A bund former was used to create bunds and irrigation channels as required for the experiment. According to the recommended package of practices, the prescribed dose of fertilizers for berseem, including urea, SSP, and MOP, was applied. Basal applications of zinc and iron was applied at 25 kg/ha each, in the form of zinc sulphate heptahydrate and ferrous sulphate. Foliar sprays of 0.5% zinc sulphate and 0.5% ferrous sulphate were applied to respective treatments at 30 days after sowing (DAS), and 15 days after the first, second, and third cuts. Before sowing, berseem seeds were treated with liquid rhizobium culture at a rate of 50 ml/acre. Hand weeding was performed to control post-emergent weeds. The crop was irrigated whenever rainfall was insufficient, typically at 10-15-days interval. Harvesting with the first cut at 50 DAS and subsequent cuts every 40 days.

### Biometric observations

Five plants were selected randomly from the net plot for biometric observations by leaving 2 rows from border as a means to avoid border effect. Plant height measured from ground level to the base of peduncle at time of each harvest. Number of leaves was counted in berseem by counting fully opened trifoliate leaves from same five random plants. The leaf length was measured from leaf base to tip of middle leaflet of third leaf from top on main branch

and same leaf for measuring leaf width. Leaf stem ratio in berseem was measured at each harvest by separating leaves and stem and then weighed separately. Ratio was calculated using formula.

$$L: S = \frac{\text{Weight of leaves from five randomly selected plant}}{\text{Weight of five randomly selected plant}}$$

Dry matter accumulation assessed after harvesting by gathering known quantity of sample *i.e.* 500 g followed by drying under shade for 2-3 days and kept in hot air oven at 65°C until constant weight arrived. After that, dry matter was multiplied with green fodder yield to estimate dry fodder yield and combined total dry matter accumulation expressed in g/m<sup>2</sup>. No. of regenerated stems were counted after 15 days of each cut. For nodule count, five plants were selected randomly and uprooted using garden trowel and roots was soaked in bucket of water to remove soil particles. The nodules were then separated and counted per plant.

### Statistical data analysis

The data collected during field experiment was analyzed using analysis of variance (ANOVA) as described by Gomez and Gomez (1983) in MS EXCEL. Statistical significance of the experimental data was determined at 5% level of significance by using “F test” and wherever F value was found significant, critical difference (p=0.05) value was calculated.

## RESULTS AND DISCUSSIONS

### Plant height (cm)

Plant height is a parameter by which one can conjecture the growth of a plant. The data pertaining to height of berseem as affected by zinc and iron nutrients noted at each harvest and are presented in Table 3 and Fig. 1. Among different treatments, maximum plant height was found in treatment T<sub>3</sub> *i.e.* 100% RDF + ZnSO<sub>4</sub> (basal) in all three cuts, I (84.60 cm), II (86.93 cm) and III (88.40 cm) which is statistically at par with treatment T<sub>4</sub>, T<sub>5</sub>, T<sub>6</sub> and T<sub>7</sub> while minimum plant height was observed in T<sub>1</sub>, absolute control *i.e.* in I (55.70 cm), II (59.95 cm) and III (61.53 cm). Application of 100% RDF + Zn (basal) significantly increased the plant height by 21.7%, 20.9% and 21.9% in I, II and III cut

respectively over the 100% RDF alone. The higher plant height of berseem may be because of Zn crucial role in cell differentiation, cell elongation, auxin synthesis as well as enhancement in the metabolic activity which lead to vigorous plant growth. Similar result was also reported by Usman *et al.* (2014).

### Leaf length (cm)

Leaves are the main organs of plant where chlorophyll is present, that captures the sunlight and hence photosynthates are formed by a process called photosynthesis. With the help of leaf length, leaf area can be determined which help us to know how much light has intercepted by a leaf and how much photosynthates were formed which directly related to the yield and quality of a crop. The effect of Zn and Fe ferti-fortification on leaf length of berseem at each harvest is represented through a tabular form in Table 3. The data related to leaf length of berseem shows significant difference among different treatments at each harvest. The highest leaf length is found in treatment, T<sub>3</sub> in all three cuts I (5.85 cm), II (5.94 cm) and III (6.00cm) which is at par with treatment T<sub>4</sub> and minimum leaf length was found in absolute control *i.e.* I (4.57 cm), II (4.49 cm) and III (4.59 cm). Treatment T<sub>3</sub> showed increment by 20.1, 19.5 and 20.5% in I, II and III cut, respectively over the RDF alone. The highest leaf length may be due to zinc role in activating the cell division and elongation. Similar results were also reported by Kumar *et al.* (2016) in maize.

### Leaf Width (cm)

Leaf width is one of a component of leaf area determination as it is related to photosynthesis. Table 3 shows the effect of Zn and Fe ferti-fortification on

leaf width of berseem. At each harvest, it was recorded that all zinc and iron treatments had conspicuously higher leaf width over the RDF alone and absolute control. Among the various treatments, treatment T<sub>3</sub> recorded maximum leaf width in all three cuts I (1.89 cm), II (2.48 cm) and III (2.49 cm) while minimum leaf width observed in absolute control *i.e.* in I (1.37 cm), II (1.87 cm) and III (1.97 cm). It might be due to accelerated metabolism because zinc plays vital role in various enzymes like alcohol dehydrogenase enzyme that is necessary in glycolysis pathway occurred in plant leading to the generation of ATP and hence enhances the photosynthesis. Similar results were also reported by Kumar *et al.* (2016).

### Number of leaves

The data on leaves number of berseem discloses a significant variation among different treatments and are represented in Table 4. With respect to different treatments, treatment T<sub>3</sub> recorded maximum no. of leaves in all three cuts I (29.40), II (31.40) and III (33.13) which is at par with treatment T<sub>4</sub> while minimum no. of leaves observed in absolute control *i.e.* in I (17.73), II (18.90) and III (19.57). The increment by T<sub>3</sub> is 25.5%, 24.4% and 23.9% in I, II and III cut respectively over RDF.

It might be because, zinc increases the translocation of the nutrients to growing points of plant where it increases the auxin build up, that stimulates the division and enlargement of cell, hence enhanced the formation of shoots. Similar findings were reported by Al-Garni (2006) in cowpea and Pandey *et al.* (2019) in cluster bean.

### Leaf: Stem ratio

Leaf: Stem ratio decides the quality of fodder.

TABLE 3  
Effect of Zn and Fe ferti-fortification on the plant height, Leaf length and leaf width of berseem

Treatments	Plant height (cm)			Leaf length (cm)			Leaf width (cm)		
	I Cut	II cut	III cut	I Cut	II cut	III cut	I Cut	II cut	III cut
T <sub>1</sub> -Absolute Control	55.70	59.95	61.53	4.57	4.49	4.59	1.37	1.87	1.97
T <sub>2</sub> -100% RDF	69.53	71.90	72.53	4.87	4.97	4.98	1.70	2.14	2.25
T <sub>3</sub> -100% RDF+Zn (basal)	84.60	86.93	88.40	5.85	5.94	6.00	1.89	2.48	2.49
T <sub>4</sub> -100% RDF+Fe (basal)	82.07	82.27	84.07	5.19	5.20	5.03	1.79	2.17	2.42
T <sub>5</sub> -100% RDF+0.5% foliar spray of Zn	81.33	81.57	83.17	5.40	5.49	5.58	1.82	2.28	2.45
T <sub>6</sub> -100% RDF+0.5% foliar spray of Fe	79.37	79.99	80.33	4.83	5.03	4.79	1.77	2.16	2.33
T <sub>7</sub> -75% RDF+0.5% foliar spray of Zn+0.5% foliar spray of Fe	80.48	80.73	81.00	4.96	5.17	5.24	1.81	2.25	2.39
S. Em±	3.63	4.11	4.03	0.21	0.24	0.23	0.08	0.10	0.07
LSD ( P = 0.05)	11.17	12.65	12.41	0.64	0.73	0.70	0.26	0.32	0.23

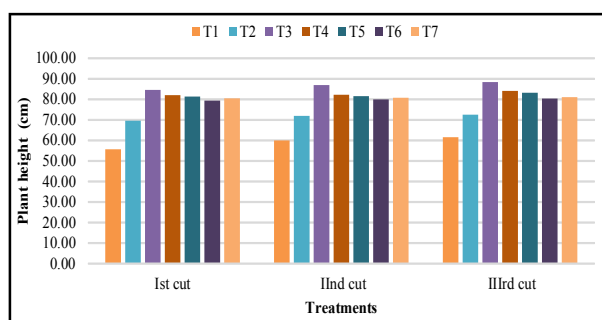


Fig. 1. Plant height of berseem at different cuts.

The data on influence of Zn and Fe ferti-fortification on leaf: stem ratio of fodder berseem presented in Table 4. The data revealed that there is no significant variations occur among different treatments at all three cuts. However, treatment T<sub>3</sub> shows highest leaf stem ratio in all cuts I (0.52), II (0.77) and III (0.75) whereas minimum leaf stem ratio observed in absolute control *i.e.* in I (0.49), II (0.65) and III (0.66).

#### Number of nodules

Nodules are referred to as a seat for atmospheric nitrogen fixation. Table 4 shows the effect of Zn and Fe ferti-fortification on number of nodules of berseem. With respect to different treatments, treatment T<sub>4</sub> recorded maximum number of nodules *i.e.* I cut (73.10), II cut (76.43) and III cut (79.94) which is statistically at par with treatment T<sub>3</sub> while minimum number of nodules found in absolute control *i.e.* in I (52.13), II (55.00) and III (59.47). It may be due to the enhancement in the activity of rhizobium by the ferti-fortification of iron which is required for the nitrogenase activity in biological nitrogen fixation process through nitrogen fixing bacteria. Similar findings were reported by Gupta and Sahu, (2012).

#### No. of regenerated stems/plant at 15 days after each harvest of berseem

The data shows significant variation among different treatments in regard of number of regenerated stems/plant at 15 days of each harvest. The data is presented in Table 4. With respect to different treatments, treatment T<sub>3</sub> recorded maximum no. of regenerated stems in all three cuts I (7.28), II (7.73) and III (8.40) which is statistically at par with treatment T<sub>4</sub> and minimum number of regenerated stems observed in absolute control *i.e.* in I (5.00), II (5.37) and III (5.75) cut. It might be due to zinc role in carbohydrate and nitrogen metabolism as regeneration depends on the mobilization of carbohydrates and protein reserves (Lee *et al.*, 2010). Also, it may be due to better availability of growth resources to the plants (Kumar and Sarlach, 2020).

#### Green fodder yield (t/ha)

Fodder yield of any crop depends on the variety as well as the agronomic practices that are followed, mainly the nutrient management. The data pertaining to green fodder yield shows significant variation among various treatments and are presented in Table 5 and Fig. 2. With respect to different treatments, treatment T<sub>3</sub> showed maximum green fodder yield in all three cuts as well as total (22.50, 22.98, 24.34 and 69.82 t/ha respectively) which was statistically at par with treatment T<sub>4</sub> while the minimum green fodder yield observed in absolute control *i.e.* in I (15.95 t/ha), II (16.14 t/ha), III (17.50 t/ha) and total (49.59 t/ha). The increment in green fodder yield was 28.3, 22.7 and 22.9% in I, II and III cut respectively over RDF alone while in respect of

TABLE 4  
Effect of Zn and Fe ferti-fortification on number of leaves, Leaf: Stem ratio and number of regenerated stems of berseem

Treatments	No. of leaves			Leaf: Stem ratio			Number of nodules			Number of regenerated stems		
	I Cut	II Cut	III Cut	I Cut	II Cut	III Cut	I Cut	II Cut	III Cut	I Cut	II Cut	III Cut
T <sub>1</sub> -Absolute Control	17.73	18.90	19.57	0.49	0.65	0.66	52.13	55.00	59.47	5.00	5.37	5.75
T <sub>2</sub> -100% RDF	23.43	25.23	26.73	0.50	0.66	0.67	59.07	60.20	68.47	5.60	5.80	6.04
T <sub>3</sub> -100% RDF+Zn (basal)	29.40	31.40	33.13	0.52	0.77	0.75	68.43	71.30	75.13	7.28	7.73	8.40
T <sub>4</sub> -100% RDF+Fe (basal)	27.47	30.13	31.20	0.51	0.75	0.71	73.10	76.43	79.94	6.72	7.00	7.60
T <sub>5</sub> -100% RDF+0.5% foliar spray of Zn	26.17	27.63	29.00	0.50	0.73	0.69	62.60	64.47	70.27	6.07	6.57	6.87
T <sub>6</sub> -100% RDF+0.5% foliar spray of Fe	25.80	26.33	28.67	0.49	0.69	0.67	65.67	67.13	74.54	5.63	5.93	6.19
T <sub>7</sub> -75% RDF+0.5% foliar spray of Zn+0.5% foliar spray of Fe	26.00	27.37	28.90	0.50	0.72	0.68	64.10	66.03	74.20	6.02	6.30	6.67
S. Em±	1.02	1.19	1.37	0.02	0.03	0.02	1.89	1.80	1.58	0.19	0.24	0.22
LSD ( P = 0.05)	3.15	3.66	4.21	NS	NS	NS	5.90	5.61	4.92	0.58	0.75	0.67

TABLE 5  
Effect of Zn and Fe ferti-fortification on green fodder and dry matter yield of berseem fodder

Treatments	Green fodder yield (t/ha)				Dry matter yield (t/ha)			
	I cut	II cut	III cut	Total	I cut	II cut	III cut	Total
T <sub>1</sub> -Absolute Control	15.95	16.14	17.50	49.59	1.43	1.97	2.76	6.16
T <sub>2</sub> -100% RDF	17.54	18.73	19.81	56.08	1.69	2.39	3.15	7.23
T <sub>3</sub> -100% RDF+Zn (basal)	22.50	22.98	24.34	69.82	2.28	3.14	4.17	9.59
T <sub>4</sub> -100% RDF+Fe (basal)	21.12	21.34	22.98	65.44	2.08	2.83	3.84	8.76
T <sub>5</sub> -100% RDF+0.5% foliar spray of Zn	19.69	20.32	21.26	61.27	1.85	2.68	3.44	7.98
T <sub>6</sub> -100% RDF+0.5% foliar spray of Fe	19.43	19.85	20.96	60.24	1.82	2.61	3.37	7.81
T <sub>7</sub> -75% RDF+0.5% foliar spray of Zn+0.5% foliar spray of Fe	19.52	20.03	21.02	60.58	1.84	2.63	3.40	7.86
S. Em±	0.68	0.79	0.86	0.12	0.11	0.11	0.13	0.12
LSD (P = 0.05)	2.10	2.45	2.65	0.37	0.34	0.35	0.40	0.36

absolute control increment was 41.1, 42.3 and 39.1%. The increase in green fodder yield might be due to enhancement of carbohydrate, auxin as well as protein synthesis and their transportation to different organs of plant *viz.* leaves by the ferti-fortification of zinc. Also, green fodder yield is a ramification of other growth-related parameters such as plant height, number of leaves, no. of regenerated stem etc. Similar findings were reported by Kumar *et al.* (2016).

### Dry matter yield (t/ha)

The data regarding the effect of Zn and Fe ferti-fortification on dry matter yield is represented in Table 5 which shows a significant difference in between various treatment. Among different treatments, treatment T<sub>3</sub> in all three cuts shows maximum dry fodder yield I (2.28 t/ha), II (3.14 t/ha) and III (4.17 t/ha) which is statistically at par with treatment T<sub>4</sub> and minimum dry fodder yield was observed in absolute control *i.e.* in I (1.43 t/ha), II (1.97 t/ha) and III (2.76 t/ha). Application of 100% RDF + Zn (basal) leads to the increment of 34.9, 31.4 and 32.3% over the RDF alone and 59.4, 59.3 and 51.1% over the absolute control. It may be due to favorable effect of Zn ferti-fortification on growth parameters like plant height, leaf length, number of leaves etc. which enhanced the area of leaf and finally the photosynthetic efficiency which leads to higher dry matter production. Highest green fodder yield and dry fodder yield attributed in III cut may be because temperature was most favorable for the plant growth while in I and II cut lower temperature affected the growth of plant. Similar findings were reported by Sarangthem *et al.* (2018) and Jha *et al.* (2015).

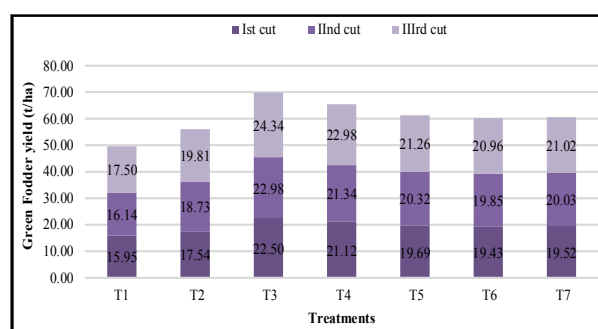


Fig. 2. Green fodder yield of berseem at different cuts.

### CONCLUSION

From the findings of above, it was concluded that application of 100% RDF + Zn (basal) significantly enhanced the growth parameters, green as well as dry fodder yield of berseem in comparison to other treatments.

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