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Original Research Article

The effect of different levels of amino acid and zinc on the quality and quantity of Berseem (*Trifolium alexandrinum*)

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Abstract

The unavailability of green fodder throughout the year and low quality fodder are some of the main constraints that contribute in low yield of livestock. To overcome the restraint in quality and quantity, an experiment was designed at Agriculture Research Institute, Tarnab - Peshawar to investigate the effect of different levels of amino acid (aspartic acid) and zinc (zinc sulphate) on the quality and quantity of berseem. The experiment was laid out in randomized complete block design with split plot arrangement having three replications. Different levels of amino acid were applied @1000, 2000 and 3000 ml ha^{-1} to main plot while Zinc was applied @14 and 28 kg ha^{-1} to sub plot. Maximum stem height (73.77 cm), branches per stem (20.39), highest percentage of crude protein (17.67 %), crude fiber (33.59 %), dry mater (21.75 %) and green fodder yield (27.84 t ha^{-1}) was recorded in the plots that received the amino acid @ 3000 mlha^{-1}, while the plots that received amino acid at lowest rate i.e. 1000 ml ha^{-1} yielded the lowest stem height (60.22 cm), minimum number of branches per stem (12.72), low percentage of crude protein (15.85 %), crude fiber (31.21 %), dry matter (19.38 %) and lowest green fodder yield (23.72 t ha^{-1}). Similarly, zinc applied at higher rate of 28 kg ha^{-1} boosted the stem height (70.89 cm), number of branches (16.62), crude protein (17.86 %), crude fiber (35.01 %), dry matter (21.53 %) and green fodder yield (28.91 t ha^{-1}) compared to zinc applied @ 14 kg ha^{-1}. It is therefore, recommended that while growing berseem amino acid @ 3000 ml ha^{-1} and Zinc @ 28 kg ha^{-1} should be applied in order to get good yield and a quality crop.

Keywords: Amino acid, Zinc, Berseem, Quality and quantity

Introduction

For maintaining the normal health and reproduction of livestock it is essential to feed them with green fodder and forage of high quality (Roy and Khandaker, 2010). In Pakistan, 90% of the livestock diet consists of poor quality of roughages and yield of these forages are also less than its diet requirement. As a result the livestock is under fed and their health is poorly maintained. Furthermore, this problem is becoming more severe as the population of cattle are increasing and most of the land is diverted to the cereals grain production for human consumption (Roy and Khandaker, 2010). Therefore cultivation of good quality forages with high yield is necessary to alleviate the shortage of fodder and forage for feeding livestock in Pakistan.

Berseem is one of the fast growing and high quality leguminous forage that is fed to the animals as green chopped forage. In Pakistan, the yield and quality of berseem is low due to many environmental factors and certain management constrains. Among these, zinc deficiency is considered as one of the major nutritional constraint (Asif et al., 2013). Zinc plays a vital role in plant growth process and enhance metabolism. It is also considered to be involved in the formation of auxin and chlorophyll pigment which is essential for carbohydrate metabolism, enzyme formation and proper root development (Asif et al., 2013). This micronutrient is also required for nitrogen metabolism and is considered important element in the process of photosynthesis. In soil, deficiency of zinc may lead to poor quality and low yield of fodder crop (Rathore et al., 2015). Zinc is an important nutrient; therefore, its proper application may mitigate zinc deficiency in animals. Zinc interacts with other macro and micronutrients in crops and tends to increase the production. In pearl millet it was reported that application of zinc along with nitrogen increased the dry matter yield of a crop (Kumar et al., 1985). Furthermore, it was also observed that application of zinc enhances the fresh matter production of rice (Malik et al., 2011). Zinc also plays an important role in improving the quality of the crop and in mung bean it is observed that zinc treatment tends to increase the protein content (Krishna, 1995). Similarly, in sweet potatoes application of zinc increases the crude fiber content of a crop (Khairi et al., 2016).

Similarly, crops yield is also improved by the application of amino acids. Amino acid is used to increase the overall production and quality of a crop. It plays fundamental role in the synthesis of photo assimilates and can directly or indirectly influence the physiological activities of a crop (Liu and Lee, 2012). Amino acid applied to the soil is believed to improve the soil micro flora, which further facilitate the availability of nutrients to the crops. It is also not known that application of amino acid along with zinc can bring some changes in the morphological and quality characters of crops. Therefore the present study was designed to study the effect of different level of zinc and amino acid on the yield and nutritive value of berseem. Objective was to investigate the optimum level of amino acid and zinc for improving the growth, yield and quality of berseem.

Material and Methods

The present study was conducted at Agriculture Research Institute, Tarnab-Peshawar. The experiment was conducted to know the impact of different levels of amino acid and zinc on the quality and yield of berseem during rabi season 2016. Experiment was arranged in randomized complete block design with split plot arrangement having three replications. Zinc sulphate was used as a source of zinc whereas aspartic acid was used as a source for amino acid. Zinc sulfate was applied as soil application @ 14 kg ha^{-1} and 28 kg ha^{-1} while amino acid was applied as foliar application@ 1000, 2000 and 3000 ml ha^{-1}. All other agronomic practices were kept constant to all the treatments of the experiment.

Crop morphological data

Plant growth parameters including stem height (cm) and number of branches $plant^{-1}$ were collected from five randomly selected plants in each treatment. The stem height was recorded from base of the plant to the top using measuring tape and the average stem height of the plant was calculated using eq. 1. Similarly, numbers of branches per plant were counted on the selected plants and their average was worked out with the help of eq. 2.

∑ Values of stem height ÷ total number of selected stem……………. Eq. 1

∑ Number of branches ÷ total number of selected plants ……………. Eq. 2

Crude protein (%)

Took 30 ml of concentrated sulphuric acid (H_2SO_4), which was added to 1.0 g of finely powdered dried plant material. Then 5g of digested mixture were added to the sample and material was digested in the digestion chamber at 400^{0}C for 2-3 hours. After digestion the material was cooled at the room temperature and the volume of the sample was made 250ml with the addition of distilled water. From this sample 10 ml of aliquot was taken and distillation was done in the kjeldhal apparatus. Nitrogen was evolved from this distillation in the form of ammonia, which was collected in a receiver containing 2% boric acid. This sample was then titrated against 0.1N H_2SO_4until the golden colour appeared. At that point the volume of the acid was recorded. Nitrogen reading was multiplied with conversion factor 06.25 to get crude protein percentage of berseem.

Crude fiber (%)

For determination of crude fiber about 1.0 g of dried plant sample was taken and was wetted with 1.25% H_2SO_4. Distilled water was added to the beaker in order to make up the volume up to 200ml. Material was then boiled on the flame for 30 min and was filtered and washed. To the filtrate 1.25% NaOH and distilled water was added and the volume was made up to 200ml. Again the sample was heated for 30 minute, filtered and the filtrate was washed again. The filtrate was then taken in a pre-weighed crucible and placed in oven for drying at 105^0C for 24 hours. After 24 hours the dry weight of the sample (W1) was recorded. The sample was then placed in the muffle furnace at 600^0C until the grey or white ash was obtained. Sample was then cooled at the room temperature and the weight of the ash (W2) was recorded. From the above recorded data the crude fiber percentage was calculated by using the formula.

Crude fiber (%) = W1 – W2 (g) x 100 ÷ Sample weight

Dry matter (%)

For dry matter estimation, weight of the oven dried aluminum container was recorded. Then 10 g of the green fodder was weighed and was placed in oven at 105^0C for 24 hours. Percentage of dry matter was calculated by using the following formula;

Dry Matter (%) = Weight of dry sample (g) x 100 ÷Weight of green sample (g)

Crude protein, crude fiber and dry matter yield was estimated according to the procedures recommended by AOAC, (1990).

Green fodder yield (t ha^{-1})

Each plot in the experiment was harvested separately. Green fodder of the harvested plots was weighed on the balance so as to determine the total green fodder yield per plot. Data was recorded on kg per plot, which was then converted to tons per hectare.

Statistical analysis

The recorded data was statistically analyzed according to Steel and Torrie (1982) using RCB design with split plot arrangements. To determine treatments mean difference, least significant difference (LSD) was used at 5 % level of significance ($P \leq 0.05$).

Results and Discussion

Stem height (cm)

Data pertaining to the stem height treated with different levels of $ZnSO_4$ and amino acids is presented in Table 01. The data revealed that stem height increased with increasing the level of amino acid and $ZnSO_4$. Maximum plant height was obtained with the application of highest level of amino acid and $ZnSO_4$and vice versa. Similar results were reported in mung bean by Samreen et al. (2017) who found out that application of $ZnSO_4$ increased the stem length of mung bean as compared to control. Furthermore, our results are also in agreement with the findings of Alam and Shereen (2002) who studied the effect of various levels of zinc and phosphorous on wheat and observed that the stem length of wheat was increased vs control in all the treatments. On the other hand application of amino acid in the form of aspartic acid increased the plant height of rice seedling, when applied in foliar form (Rizwan et al., 2017).The gain in stem height due to zinc and amino acid might be due to the zinc appetizing effects on crop enabling it to absorbed maximum available plants nutrients while the amino acid helped the crop in making photosynthates rapidly during photosynthesis.

Branches per stem

The results as reflected in table 02 revealed that both amino acid and zinc significantly affected number of branches per stem while the interaction of amino acid and zinc on branches per stem were found non-significant. The results of increased branches per stem with increased levels of amino acid and zinc are aligned with the findings of Sahito et al. (2014) who reported the increase in number of branches per plant of mustard with increase in level of zinc. The increased in number of branches per stem is due to the foliar application of amino acids which specifically useful in providing the readymade building blocks for protein synthesis without going through the cycle of amino acids synthesis within the plant.

Crude protein (%)

Table 03 shows the mean crude protein content of berseem treated with zinc sulphate and amino acid. The data exposed that crude protein content was positively correlated with zinc and amino acid treatment.

These results are in agreement with the findings of Hisamitsu et al. (2001). Similarly, Krishna also found increase in the protein content of mung bean treated with zinc. This increase might be due to the fact that zinc is involved in a catalytic and structural component of protein and enzyme which is essential in normal growth and development of a crop (Broadley et al., 2007). Similarly, Mohan also reported significant increment in the protein content of maize crop treated with zinc sulphate at the rate of 30kg ha^{-1}.Similarly the results of amino acid on increasing the protein content of berseem clover are supported with the findings of Abd Allah et al. (2015)who reported increment in protein content when amino acid was applied in the form of glutamic acid.

Crude fiber (%)

Data regarding crude fiber (Table 04) discovered that application of different levels of $ZnSO_4$ and amino acid enhanced the percentage of crude fiber. Maximum crude fiber (33.59%) of berseem was recorded when amino acid was applied at highest rate of 3000 ml ha^{-1} while minimum crude fiber (31.21%) was observed with the application of amino acid @ 1000 ml ha^{-1}.The same pattern results were observed for zinc application as increase in zinc levels has increased the crude fiber content of berseem clover. All these outcome are in line with the findings of Khairi et al. (2016) who find out that increased the dose of zinc increased the crude fiber content of sweet potatoes. The increase in crude fiber of berseem with the increase in amino acid and zinc application might be due to the appetizing effect of zinc in crop and the foliar application of amino acid resulted in the increase in chlorophyll content which expedite the rate of photosynthesis, boosted up the carbohydrates formation which ultimately contributed in the increase crude fiber content of berseem clover.

Dry matter (%)

It is evident (Table 05) that application of zinc and amino acid increases the dry matter production of berseem. Increasing the levels of zinc and amino acids has positive effect on the dry matter yield. Maximum dry matter yield was observed when zinc was applied at the rate of 28 kg ha^{-1} and amino acids at the rate of 3000 ml ha^{-1}. Malik et al. (2011) reported that application of zinc increases the average root and shoot dry matter yield of rice. Our results are also supported by the findings of Kumar et al. (1985) who also observed increase in the dry matter production of pearl millet fertilized with high dose of zinc. Similar results were also discussed by Kumar et al. (2016) where application of zinc sulphate enhances the dry matter content of maize over the control. Abd Allah et al. (2015) reported increase in the dry matter content of rice when treated with high level of amino acid.

Table 1: Stem height (cm) of berseem as affected by different levels of amino acids and zinc sulphate.

Amino Acid Levels	Zinc Levels		Mean
	14 (kg ha^{-1})	28 (kg ha^{-1})	
1000 (ml ha^{-1})	56.10	64.33	60.22b
2000 (ml ha^{-1})	57.11	70.11	63.61b
3000 (ml ha^{-1})	71.44	76.11	73.77a
Mean	61.55b	70.89a	

LSD value for amino acid and zinc sulphate levels at ($P \leq 0.05$) = 10.96 & 5.17

Table 2: Number of branches $stem^{-1}$of berseem as affected by different levels of amino acids and zinc sulphate.

Amino Acid Levels	Zinc Levels		Mean
	14 (kg ha^{-1})	28 (kg ha^{-1})	
1000 (ml ha^{-1})	13.11	12.33	12.72c
2000 (ml ha^{-1})	15.22	16.99	16.11b
3000 (ml ha^{-1})	20.22	20.55	20.39a
Mean	16.18a	16.62a	

LSD value for amino acid and zinc sulphate levels at ($P \leq 0.05$) = 1.46 & 1.53

Table 3: Crude Protein (%) of berseem as affected by different levels of amino acids and zinc sulphate.

Amino Acid Levels	Zinc Levels		Mean
	14 (kg ha^{-1})	28 (kg ha^{-1})	
1000 (ml ha^{-1})	14.42	17.28	15.85c
2000 (ml ha^{-1})	15.77	17.75	16.76b
3000 (ml ha^{-1})	16.76	18.57	17.67a
Mean	16.65a	17.86b	

LSD value for amino acid and zinc sulphate levels at ($P \leq 0.05$) = 0.85 & 0.74

Table 4: Crude fiber (%) of berseem as affected by different levels of amino acids and zinc sulphate.

Amino Acid Levels	Zinc Levels		Mean
	14 (kg ha^{-1})	28 (kg ha^{-1})	
1000 (ml ha^{-1})	28.30	34.13	31.21b
2000 (ml ha^{-1})	30.14	34.70	32.42ab
3000 (ml ha^{-1})	30.98	36.20	33.59a
Mean	29.81b	35.01a	

LSD value for amino acid and zinc sulphate levels at (P≤0.05) = 2.12 & 2.08

Table 5: Dry matter (%) of berseem as affected by different levels of amino acids and zinc sulphate.

Amino Acid Levels	Zinc Levels		Mean
	14 (kg ha^{-1})	28 (kg ha^{-1})	
1000 (ml ha^{-1})	18.03	20.74	19.38b
2000 (ml ha^{-1})	19.60	21.14	20.37b
3000 (ml ha^{-1})	20.79	22.71	21.75a
Mean	19.47b	21.53a	

LSD value for amino acid and zinc sulphate levels at (P≤0.05) = 1.09 & 1.44

Table 6.Green Fodder yield (t ha^{-1}) of berseem as affected by different levels of amino acids and zinc sulphate.

Amino Acid Levels	Zinc Levels		Mean
	14 (kg ha^{-})	28 (kg ha^{-1})	
1000 (ml ha^{-1})	20.93	26.52	23.72c
2000 (ml ha^{-1})	22.15	28.89	25.52b
3000 (ml ha^{-1})	24.37	31.32	27.84a
Mean	22.48b	28.91a	

LSD value for amino acid and zinc sulphate levels at (P≤0.05) = 1.21 & 1.44

Green fodder yield (t ha^{-1})

Various levels of amino acid and zinc positively affected the green fodder yield as shown in (table 06). It was observed that increment in application of zinc correspondingly increased the growth and green fodder yield which are supported by the statements of (Kumar and Bohra, 2014) who observed increased yield of corn with increasing the level of zinc. These results are also in line with the findings of Kumar et al. (2016) where zinc soil and foliar application both led to an increase in green fodder yield of the maize crop. Likewise, the linear increase in fodder yield was also noted with the increase of foliar application of aspartic acid. These results are according to the findings of (Abd Allah et al., 2015) who observed the increase in fresh weight of rice tiller and roots with the application of amino acid. The increase in green fodder yield of berseem with increase in amino acid and zinc might be due to the zinc appetizing effects and most of the crop likes to have amino acid as source of nitrogen. Amino acid being a building block of protein, plays a vital role in enhancing the photo synthetic cells division, readily observed and easily converted in to photo assimilates without consuming much energy by the crop.

Conclusion

It is concluded from the study that amino acids and zinc can be applied at the rate of 3000 ml ha^{-1}and 28 kg ha^{-1} respectively to enhance the quality and yield of berseem grown under the agro ecological conditions of Peshawar.

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