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Agronomic Bio-fortification of Zinc Improves the Yield and Quality of Fodder Oat

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ABSTRACT

Oat is an important winter fodder crop grown in large areas all over the world. Oat is a good source of nutrition and energy, but it is poor in zinc. Presently, livestock is suffering from malnutrition due to a deficiency of zinc, which has a greater impact on livestock and ultimately on human health. An easy and cost-effective approach to adding nutrients to plants without altering their genetic makeup is agronomic biofortification. Application of nutrients to oat via bio-fortification may enhance the overall biomass production and quality of fodder. Hence, a field study was performed to understand the impact of bio-fortification with zinc on quality along with biomass production of oats. Treatment comprised of control (No Zn), Zn at 4 kg soil application, Zn at 6 kg soil application, Zn at 8 kg soil application, Zn at 4 kg soil application + 0.5% zinc sulfate as foliar application, Zn at 6 kg soil application + 0.5% zinc sulfate as foliar application and Zn at 8 kg soil application + 0.5% zinc sulfate as foliar application. The study revealed that Zn at 6 kg soil application + 0.5% zinc sulfate foliar application produced maximum plant height (207.7 cm), leaf area index (15.78), crop growth rate (9.1 $gm^{-2}day^{-1}$), stem diameter (0.073 cm), number of tillers (204), fresh fodder yield (76.6 $t·ha^{-1}$), dry matter yield (32.27 $t·ha^{-1}$), ash contents (10.3%) and plant zinc contents (70.0 ppm). Control treatment produced maximum crude fiber contents (40.70%), acid detergent fiber contents (35.77%), and neutral detergent fiber contents (72.71%). In conclusion, the bio-fortification of zinc not only enhanced the biomass and yield of oat but also increased the availability of zinc in plants.

Keywords: bio-fortification, growth, oat quality, zinc concentration.

INTRODUCTION

Oat is a member of the family Poaceae and is grown as an annual crop having an adventitious root system (Hussain et al., 2013). Oat is grown on more than 35% area under fodder crops in Pakistan. According to world cereal production statistics, oat ranked sixth all over the world followed by wheat, maize, rice, barley, and sorghum (Stevens et al., 2014). Livestock is the backbone of Pakistan's Agriculture. Pakistan ranked the fourth largest milk producer in the world after

China, India, USA. To fulfill the dietary requirements of the livestock industry, it requires good quality fodder (Wu et al., 2014). However, due to the increase in population, the major focus of policymakers is to utilize larger areas in the production of food and cash crops (Ball et al., 2016). Oat is grown in more than 50 countries all around the world for grain purposes, but oat as fodder is limited in the commercial market as a commodity (Ahmad et al., 2014). Oat fodder has become a major crop in a region of the Himalayas, and South American countries such as Argentina, Chile, Uruguay, and North Africa (Suttie and Raymonds, 2014). Oat can grow on a wide range of soil (Siebielec et al., 2017). It performs better in loamy soils and also tolerates acidic as well as low fertility status with pH ranging from 4.5 to 8.5 (Jordan et al., 2021). Oat is a nutritious crop and can lower cholesterol levels in the body (Whitehead et al., 2014). It was found that 100 g of oat contain 17.0 g protein contents, 11.5 g fiber, 66 g of carbohydrates, and almost 7 g of fats. Trace elements present in oats are 2 mg of sodium, 4.1 mg of zinc, and 5 mg of phosphorus (Biesiekieski, 2017). It has the highest digestibility and palatability percentage of other cereal fodder crops (Wadhwa et al., 2010). The main hurdle in obtaining a good yield of fodder is the imbalance use of fertilizer which results in the unavailability of micronutrients like zinc, calcium, iron, and manganese, etc. in grain as well as in straw. This causes harmful effects on animal health and ultimately leads to disease. The implication of micronutrients like zinc is crucial in addition to other macronutrients so that there should be no malfunction in livestock. Uncontrolled use of phosphate fertilizer leads to zinc deficiency in the soil as well as in plants which are an integral part of several metabolic processes of plants, like chlorophyll synthesis, starch, and nitrogen assimilation. It also assists protein production, gene expression, and the Krebs cycle (Mousavi et al., 2013). Oat is mainly cultivated for whole-plant use such as grazing, green fodder, hay, and silage as well as for its grain. Oat is often mixed with legume crops like berseem for livestock feeding. In the Mediterranean region, a mixture of vetch and oat fodder is very popular (Suttie et al., 2014). Oat for fodder purposes is sown without fertilizer application, the nutritional status of oat is dependent upon previously applied fertilizer and crop residues (Kluthcouski et al., 2013). This may cause a decline in the production of fodder, as well as its nutritional status.

The use of trace minerals in soil fertilization is the key to agriculture in developed countries. Proper nutrition of the plant is one of the most critical factors in yield and quality improvement of fodder. Plant growth is dependent upon the availability of macro and micronutrients in soil and their uptake by the plant. Most importantly, inadequate availability of micro-nutrients like zinc in the soil may reduce the growth of oats (Dhaliwal et al., 2021). Zinc is required in a minute but crucial amount to assist several key plant's physiological metabolisms to function normally (Mousavi, 2013; Yosefi et al., 2011). In zinc-deficient plants, the osmotic potential is low and turgor pressure is higher in leaves, whereas the ability of plants to lower the turgor pressure is low, which is why the stress developed. Zinc deficiency reduced the overall performance of the water used for biomass production and plant efficiency in responding to water stress by osmotic adaptation (Khan et al. 2014).

Most of the world's agricultural soil contains inadequate levels of bio-available form of zinc (Cakmak, 2008). This deficiency is due to increased cropping intensity and preferring high-yielding cultivars. Although some soils contain a sufficient amount of zinc, it is not in the form available for the uptake of plants (Bain et al., 2018). Some of the soil properties like pH, organic matter, soil hardness, available phosphorus, and soil moisture greatly influence the availability of zinc (Chamak and Kuttman, 2018). Some plant-based factor also influences soil-plant zinc availability, such as the presence of mechanisms for zinc uptake (Liu et al., 2017).

Plants take up zinc by root in the form of Zn^{2+} which is a divalent metal ion, through diffusion and as well as mass flow (Gupta et al., 2016). When zinc enters the plant through the root system it is attached to dense ligands like glutathione and phytochelatins due to its low solubility at alkaline and normal pH (Clemen et al., 2013). Further distribution of zinc to the upper part of the plant depends upon the overall potential of the plant to utilize zinc either through symplast or xylem (Olsen and Palmgrem, 2014).

Zinc which was stored in the seed is again distributed from the vegetative part to the reproductive part of the plant (Hegelund et al., 2012). When crop increase zinc uptake under zinc fertilization, this will help to meet the dietary requirements of livestock, especially in South Asian nations where zinc is rare in soil and ultimately in fodder (Ryan et al., 2013). When zinc-enriched fodder is fed to livestock will boost milk production and prevent infectious diseases like mitosis in cattle (Hosnedlova et al., 2017; Gupta et al., 2016).

Deficiencies of vitamin A, iron, zinc, and iodine in the diet of humans and as well as livestock are a great challenge faced by nutritionists. Iron and vitamin-containing supplements are available on the market and zinc has recently now added in mineral supplements (Haider & Bhutta, 2019; Hess and King, 2019). It has recently been noticed that zinc is an important nutritional problem, especially in developed countries. To fulfill the requirement of trace elements, genetic bio-fortification by developing a cultivar that is enriched with zinc is needed. Genetic bio-fortification may be done by a traditional breeding process which may take longer time. Another approach to increase micro-nutrients in the fodder crop is bio-fortification of agronomic mineral (Rengel et al., 2019). Agronomic mineral biofortification involves the addition of fertilizer, seed treatment with minerals, foliar application, or seed priming with synthetic fertilizer (Harris et al., 2018; Erenoglu et al., 2011). The basic requirement of a sustainable and well-organized livestock industry is good quality fodder and surplus for a whole year. Unfortunately, old and conventional methods of fodder production are used by farmers who have not been able to meet the dietary requirements of livestock in terms of quality and quantity. In most cases, livestock is forced to graze in open fields which is a total waste of fodder. Recently, studies showed that soil application or foliar application of trace elements such as zinc, calcium, and iron can boost crop productivity significantly (Wissuwa et al., 2018). Foliar application of zinc is a very reliable method to rectify plant nutrition in cereal grains (Erenoglu et al., 2011).

In previous work done by different researchers on cereals and legumes, zinc bio-fortification has been reported in many parts of the world. However, the literature regarding zinc bio-fortification on fodder crops which has economic importance in terms of the overall health of livestock and their consumer products is rare (Capstaff and Miller, 2018). The importance of bio-fortification of nutrients on the quality and yield of fodder crops should be kept in mind. The present study was conducted to check the effect of biofortification of zinc on yield and quality of fodder oat as well as ensure the availability of good quality fodder to animals.

MATERIALS AND METHODS

Experimental site and soil

The experiment was conducted at Agronomic Research Farm, College of Agriculture, University of Sargodha during the winter season 2021–22. Before sowing, the soil was tested for organic matter, NPK, and different physio-chemical characteristics (Table 1).

Experimental design and treatments

The research was designed in a randomized complete block design (RCBD) having seven treatments with four replications. The treatment comprised of control (No Zinc application), soil application of 4, 6, 8 kg Zn ha^{-1}, and in combination with foliar application of 0.5% zinc sulfate $4H_2O$, 45 days after sowing (DAS). The crop was planted with a manual hand drill using 80 kg of seed per ha. N at 85 and P 60 kg/ha was applied. Urea and di-ammonium phosphate were sources of adding nitrogen and phosphorus to the soil. A full dose of phosphorus and half of the nitrogen was applied before sowing, the remaining half of the nitrogen was with 1st irrigation. For the whole experiment, all other agronomic methods such as planting, watering, and gap filling were the same.

Crop husbandry

Pre-sowing irrigation was evenly applied to a depth of four-acre inches as recommended. After attaining optimum soil moisture conditions, a proper seedbed was prepared by applying three cultivations with a cultivator followed by planking with a tractor. Cultivation was done at a depth of 12–14 inches. The crop was sown on 15th November 2021 with the help of a single-row hand drill by using

Table 1. Physio-chemical analysis of experimental soil

Depth (cm)	Texture	E.C (dSm^{-1})	Available N (mgkg^{-1})	Available P (mgkg^{-1})	Available K mgkg^{-1}	Organic matter (%)	pH
0–15	Loam	0.0037	9.2	12.4	160	0.90	7.5

a seed rate of 80 kg per ha to attain the optimum plant population. During sowing, a recommended row-to-row distance of 30 cm was maintained. The fertilizer was applied at a recommended dose of 115 kg of nitrogen and 90 kg of phosphorus per ha. Soil application of different doses of zinc was applied at the time of sowing and foliar application was applied 45 days after sowing (DAS) according to the treatment plan. The source used for zinc application was zinc-sulfate (21% Zn). The field was kept free from weeds by manual hoeing at different stages of the experiment. The crop was harvested with sickle after 110 days when the variety (Super Green) reached at 25% heading stage.

Observations

The data related to oat fodder yield, yield, and quality attributing traits were recorded by using standard protocols as summarized here. Plant height was measured at the time of harvesting. Ten random plants were selected from every treatment. Plant height was measured from base to tip with the help of measuring tape and then calculated the average plant height. The leaf area index was measured at different intervals starting from 45 days after sowing till harvesting. The stem diameter was measured at the time of harvesting. Ten random plants were selected from each subplot and stem diameter was measured from the top, middle, and base of the stem with a digital Vernier caliper, and their average was calculated. Fresh fodder yield was taken by harvesting the whole plot and weighed. Some random plants were selected after harvesting each plot and thoroughly chopped using a chopping machine and weighed the chopped sample. Fresh chopped sample in the amount of 500 g was taken from each subplot and put it in the oven at 70 °C for seven days for dry matter yield calculation. Crop growth rate was determined at 45 days after sowing and after every 15 days till harvesting. The sample was harvested from each sub-plot randomly with sickle. Samples were sun-dried for two to three days to remove the moisture content and then dried in an oven at 70 °C overnight. Then crop growth rate was calculated by using the following formula:

$$\text{Crop growth rate} = (W2 - W1)/(T2 - T1) \quad (1)$$

where: W1 – dry weight of the first sample,
W2 – dry weight of the second sample,
T1 – days when the first sample was taken,
T2 – days when the second sample was taken.

For crude protein analysis, the samples were ground in a laboratory grinder. Then, 1 gram of the ground sample was added in the Kjeldahl digestion flask along with 10 grams of digestion mixture and 30 grams of sulfuric acid. The flask mixture was heated to obtain a greenish liquid. The green liquid was shifted into a volumetric flask after sufficient chilling. Then, 10 ml of the liquid was separated, and 15 mL of 40 percent sodium hydroxide was added to each sample. Nitrogen was taken in another apparatus which contained 4% boric acid. The next step was to add methyl red and bromocresol green which act as indicators. The substance from the distillation was then titrated against N/10 sulfuric acid until it turned red. The amount of acid used in the titration was used to calculate nitrogen. Nitrogen percentage was multiplied by 6.25 to obtain crude protein contents. In a volumetric flask, 200 mL of sulfuric acid was added. The acid solution was poured in a conical flask. Afterwards, 2 g of the sample was taken and the sample was placed in a conical flask with an acid solution. For 30 minutes, the conical flask was placed on a heated plate. To ensure proper boiling of the sample, the flask was shaken periodically. The acid solution was drained from the boiled sample. In a volumetric flask, 200 ml of 0.313 M sodium hydro-oxide was measured and added to the acid solution before being placed on a heated plate for another 30 minutes. After 30 minutes of boiling, another conical flask was taken and a funnel with cotton cloth was set on the discard flask. The samples were again filtered to drain sodium hydro-oxide residues and were removed completely with hot water. The filtrate was collected in a dry crucible till no filtrate was left. To evaporate the extra water, the crucible was placed on a hot plate. The crucible was placed in a hot oven at 230 degrees Celsius for two hours. Then, it was cooled in a desiccator for 20 minutes. The crucible containing dry fiber was weighed. It was placed in a muffle furnace and the sample was burned at 540 °C for two minutes and again cooled in a desiccator. The crucible containing fiber was weighed. The crude fiber contents were measured by the following formula:

$$\text{Crude fiber (\%)} = (W2 - W1)/WS \times 100 \quad (2)$$

where: W2 – weight of crucible with fiber,
W1 – weight of crucible without fiber,
WS – weight of sample.

The neutral detergent fiber contents were obtained by weighing one gram of powdered sample of oat plant in a conical flask. Afterwards, 0.5 grams of Sodium Sulfate was added along with 100 ml of reagent for neutral detergent fiber contents. An air condenser was fitted on the conical flask and the flask was heated for 60 minutes. With the help of a suction pump, the sample was cooled and purified. The leftovers placed in a conical flask were rinsed four to five times and then dried. The leftover was shifted in a crucible before placing in the oven for 60 minutes. Then fumes were removed with the help of a desiccator by placing a crucible for 10 minutes. Then, the neutral detergent fiber contents were calculated as follows:

$$NDF\% = (\text{residue} + \text{crucible weight}) - (\text{weight of crucible}) \times 100/\text{weight of sample} \quad (3)$$

The residue present in the crucible from neutral detergent fiber contents was added in 100 ml of reagent for acid detergent fiber contents in a conical flask. Then, the sample was heated to a boiling temperature for 3–4 minutes and suddenly the temperature was lowered. By using a suction pump, the samples were cleaned. Then, the sample was washed with acetone and warm distilled water. The residue was again heated for 24 hrs at 110 °C. Then, the sample was cooled by the condenser and the acid detergent fiber contents were calculated as follows:

$$ADF\% = (\text{ADF residue} + \text{crucible wt.}) \; (\text{weight of crucible}) \times 100/\text{weight of sample} \quad (4)$$

The ash contents were determined by placing 5 grams of ground oat sample in a China dish and placing the sample for six to seven hours at 750 °C in a muffle furnace until grey ash was obtained. After cooling, the samples were weighed and the ash percentage was measured by following the formula.

$$\text{Total ash\%} = (W2 - W1)/\text{weight of sample} \times 100 \quad (5)$$

where: W2 – weight of the sample before placing in the furnace, W1 – weight of ash contents

Plant Zn contents were measured by preparing a Pyrex tube with a volume of 100 ml filled with 1 g of the ground sample. Then, 5 ml of perchloric acid and nitric acid each were added. The sample was then left for 10–12 hours. The Pyrex tube was then placed in the desiccator to chill and raise the temperature to 150 degrees Celsius. A U-shaped glass rod was attached under the funnel to separate the vapor. As the temperature gradually increased the traces of nitric acid were removed. After the appearance of white fumes. temperature was increased up to 235 °C for 30 minutes. The pyrex tube was removed and the samples were cooled in a desiccator. After adding some water, the samples were mixed and left for an hour.

Data analysis

Statistical analysis was done by using Fisher's analysis of variance technique and means of treatments were compared using Tukey's honest significant difference (HSD) test at 5% probability level (Steel et al., 1997).

RESULTS AND DISCUSSION

Crop growth rate and leaf area index

Crop growth rate and leaf area index are important physiological determinants of crop yield, especially in fodder crops, where a high biomass of plants is required. The effect of different Zn application combinations is summarized in Figure 1. The Figure shows a uniform trend in CGR from 20 DAS to 110 DAS in all treatments but all differ in their CGR values. CGR in all treatments increased with increasing days after emergence till 90 DAS. After 90 days, a decreasing trend in CGR was observed and lowest at 110 DAS. The maximum value of CGR was observed at 90 DAS in all treatments, whereas among the treatments maximum values of CGR 2.28, 4.57, 9.13, and 4.56 gm^{-2} day^{-1} at 30, 60, 90, and 110 DAS were observed, respectively, in the treatment where 6 kg Zn was applied in soil + 0.5% zinc sulfate as a foliar application. In turn, the least CGR was observed in control, where no Zn was applied, which produced CGR of 1.38, 2.77, 4.56, and 2.28 gm^{-2} day^{-1} at 20, 60, 90, and 110 DAS respectively. Similar results were observed by Dhaliwal et al., 2020 and Rengel et al. (2019) that an appropriate dose of zinc improves the crop growth rate of oat fodder which also helps to reduce the weed in the field due to their shadow effect and improves the photosynthetic process of plants which improve in crop growth rate also. Foliar application of zinc assists cell elongation and cell division, ultimately increasing the photosynthesis rate (Afzal et al., 2017). The leaf area index in all treatments increased with increasing days after emergence till 90 DAS. After 90 days, a decreasing trend in leaf area index was observed and lowest at 110 DAS. Maximum LAI (15.3) was observed when the treatment combination was Zn at 6 kg·ha^{-1} soil application + 0.5% zinc sulfate as a foliar application. The lowest leaf area index (0.38) was observed under control conditions. The same results were observed by Dhaliwal et al. (2020) who also found a mark in LAI with Zn application.

Plant height (cm)

The data regarding plant height at maturity (Table 2) shows significant variation in plant height due to the influence of different treatments which varies from 96.52 to 207.65 cm. Treatment combination of Zn at 6 kg ha^{-1} soil application + 0.5% zinc sulfate foliar application produced the tallest plants (207.65 cm) overall treatments whereas the shortest plants were observed under control conditions (96.52 cm). Similar findings were observed by Rizwan et al., 2019 who presented that the use of an adequate amount of zinc improves plant height, because it reduces oxidative stress and activates enzymatic activities. Results similar to the study were also noticed by Adhikari et al., 2015. Zinc performs an essential role in plant metabolism by neutralizing the component of the ribosome and integral part of the production of cytochrome (Hafeez et al., 2013). These results also correlated with the findings of Erenoglu et al. (2011) who revealed that zinc improved the height of plants significantly because photosynthesis and nutrient uptake was increased by zinc application.

Stem diameter (cm)

The largest stem diameter (0.070 cm) was observed from a combination of Zn at 6 kg ha^{-1} soil application + 0.5% zinc sulfate foliar application. The lowest plant diameter was observed in the control where no Zn was applied (0.047 cm). The results obtained were quite similar to the findings of Tahir et al., 2019. They reported that foliar application of Zn along with soil application significantly improves leaf area index and stem diameter which ultimately improves fodder yield. A similar observation was noticed by Pawar et al. (2016). The benefit of the addition of micronutrients like zinc in soil is that trace elements remain available for longer periods, and the slow-release nutrients remain available throughout the growth period of the crop (Meena et al., 2016).

Number of tillers

The number of tillers varied from 114 to 224. Oat produced maximum tillers (224.0) till maturity where a combination of Zn @ 6kg·ha^{-1} soil application + 0.5% zinc sulfate foliar application was used and the lowest were observed under control conditions (114.75). Similar findings were reported by Castaranga et al. (2012). They reported that zinc application improved the vegetative growth of crops which boosted the photosynthetic rate. Another reason for an increase in the number of tillers is due to an increase in the availability of zinc in the rhizosphere which ultimately increases the uptake of Zn and other nutrients. Shivay et al. (2015) observed that zinc

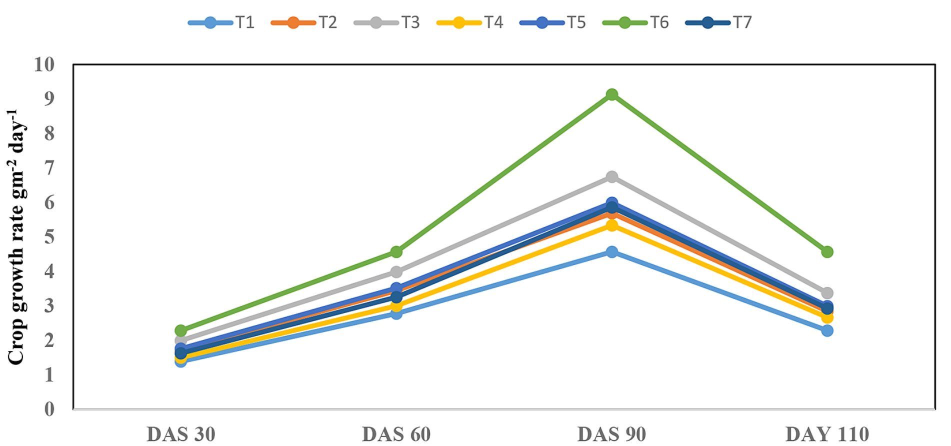

Figure 1. Effect of zinc bio fortification on crop growth rate (gm^{-2} day^{-1}) of oat. T1: Control; T2: 4 kg zinc soil application; T3: 6 kg zinc soil application; T4: 8 kg zinc soil application; T5: 4 kg zinc soil application + 0.5% $ZnSO_4$ foliar application; T6: 6 kg zinc soil application + 0.5% $ZnSO_4$ foliar application; T7: 8 kg zinc soil application + 0.5% $ZnSO_4$ foliar application.

Table 2. Effect of Zn bio fortification on growth and yield parameters of oat fodder

Treatments	Plant height (cm)	Stem diameter (cm)	Number of tillers (m^{-2})	Fresh fodder yield (t ha^{-1})	Dry matter yield (t ha^{-1})
T_0	96.52d	0.047c	114.75f	26.275f	22.57e
T_1	105.41cd	0.056bc	126.75ef	35.77e	24.42d
T_2	125.73b	0.067ab	184.25b	67.250e	29.77b
T_3	198.75a	0.067ab	131.75de	42.80d	25.40cd
T_4	118.75bc	0.059abc	146.00d	43.52d	26.95c
T_5	207.65a	0.070a	224.00a	76.62a	32.27a
T_6	103.50cd	0.061ab	163.50c	52.95c	24.87d
HSD	19.714	0.0128	16.63	4.47	1.67

Note: T_0: control, T_1: 4 kg Zn soil application, T_2: 6 kg Zn soil application, T_3: 8 kg Zn soil application, T_4: 4 kg Zn soil application + 0.5% zinc sulphate foliar application, T_5: 6 kg Zn soil application + 0.5% zinc sulfate foliar application, T_6: 8 kg Zn soil application + 0.5% zinc sulfate foliar application.

improved the initial growth of plants, availability of an adequate amount of zinc improved the initial metabolic process such as the availability of food to the embryo of seed from the endosperm.

Oat fresh fodder yield (t·ha^{-1})

Fresh fodder yield is one of the major outcomes of all the growth and yield parameters like plant height, stem diameter, and crop growth rate. Results varied from 26.2 to 76.6 tons per ha. The highest fodder yield (76.62 tons per ha) was recorded under the combination of Zn at 6 kg·ha^{-1} soil application + 0.5% zinc sulfate foliar application and the lowest fresh fodder was observed under control conditions (26.27 tons per ha). Similar results were presented by Kumawat et al., 2017. They observed that biological yield, grain yield, and fresh biomass production were improved by a combination of soil and foliar application of adequate doses of zinc over control. Zinc is a key component in activating different enzymes such as glutathione and phytochelatins that not only improve the biomass of crops but also boost the nutritional status of fodder which helps to reduce malnutrition in livestock (Amin, 2011; Kumar et al., 2015).

Dry matter yield (t·ha^{-1})

The maximum dry matter yield (32.27 tons ha^{-1}) was observed in the treatment combination of Zn at 6 kg·ha^{-1} soil application + 0.5% zinc sulfate foliar application whereas the lowest (22.57 tons per ha) was observed under control conditions. They observed that the addition of calcium and zinc improved the dry matter yield to at great

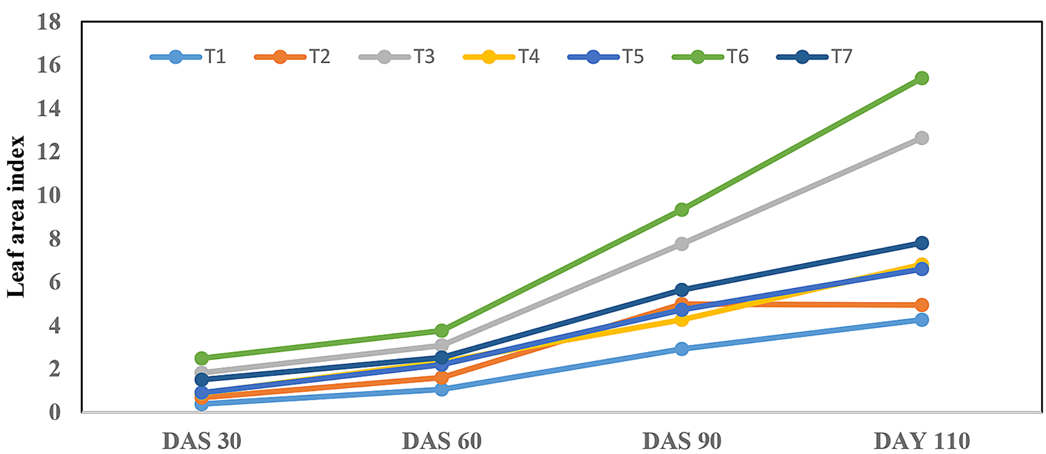

Figure 2. Effect of zinc bio fortification on leaf area index of oat. T1: control; T2: 4 kg zinc soil application; T3: 6 kg zinc soil application; T4: 8 kg zinc soil application; T5: 4 kg zinc soil application + 0.5 % $ZnSO_4$ foliar application; T6: 6 kg zinc soil application + 0.5 % $ZnSO_4$ foliar application; T7: 8 kg zinc soil application + 0.5% $ZnSO_4$ foliar application

extent (Sher et al., 2022). Bio-fortification improved the crop yield mainly due to zinc which plays an integral role in enhancing metabolic processes like photosynthesis, respiration, and other physio-chemical activities (Salih, 2013) (Fig. 2).

Crude protein contents (%)

An increase in crude protein contents improves the fodder quality. Zn treatment combination influenced the crude protein contents and ranged from 5.1% and 20.0%. Maximum crude protein (20.03%) was obtained in a combination of Zn at 6 kg·ha^{-1} soil application + 0.5% zinc sulfate foliar application and the lowest (5.17%) was observed in control conditions. Dhaliwal et al. (2020) reported similar results that soil application of zinc significantly improved the crude protein contents. It is possible because zinc is an integral part of many enzymatic processes. Biel et al. (2014) reported a significant increase in crude protein contents in soybean crops. The increase in protein content is due to a significant increase in the photosynthetic rate of plants.

Crude fiber contents (%)

Crude fiber contents vary from 29.33% to 40.79% (Table 3). Maximum crude fiber contents (40.70%) were observed in control and minimum (29.33%) were obtained where a combination of Zn at 6 kg·ha^{-1} soil application + 0.5% zinc sulfate foliar application. Similar findings were reported by Barszcs et al. (2019). They observed that zinc application reduced the chemical composition of amylose and minerals. It also reduces the availability of anti-nutritional organic factors such as phytates, tannins, and inhibitors of activity such as phytase enzymes.

Acid detergent fiber contents (%)

Indigestible cellulose, lignin, and various components are known as acid detergent fiber contents (ADF) and varied between 29.26 to 35.77%. The data regarding acid detergent fiber contents influenced by different treatments are tabulated in Table 3. The maximum value was noted in the control (35.77%) and the minimum (29.26%) was obtained in a treatment combination of Zn at 6 kg·ha^{-1} soil application + 0.5% zinc sulfate foliar application (Sheta et al., 2010).

Neutral detergent fiber contents contents

Maximum neutral detergent fiber contents (72.71%) were observed from the control treatment and minimum (53.70%) from the treatment combination of Zn at 6 kg ha^{-1} soil application + 0.5% zinc sulfate foliar application. The zinc application has extended the growth period of the crop. The decrease in neutral detergent fiber due to zinc application may be attached to the crop receiving zinc application in less advanced growth stages (Chand et al., 2017).

Ash contents (%)

Ash contents give us information about the total amount of minerals available in fodder. The results regarding ash contents (Table 3) varied 10.3% to 13.5%. Maximum ash contents (13.50%) were

Table 3. Effect of Zn bio fortification on quality parameters of oat fodder

Treatments	Crude protein contents (%)	Crude fiber contents (%)	Acid detergent fiber contents (%)	Neutral detergent fiber (%)	Ash contents (%)	Plant zinc contents (ppm)
T_0	5.17e	40.70a	35.77a	72.71a	10.33c	35.20e
T_1	8.39d	38.53b	33.84ab	62.58c	11.20bc	58.50c
T_2	11.58c	31.49d	32.10bc	57.86d	11.72b	66.20b
T_3	15.07b	35.02c	32.33b	62.13c	11.68b	53.56d
T_4	16.33b	37.61b	30.98bc	67.77b	11.51bc	59.26c
T_5	20.03a	29.33e	29.26c	53.70e	13.50a	70.00a
T_6	12.68c	33.64c	32.45b	60.46cd	11.42bc	53.36d
HSD	15.82	1.45	2.96	3.70	1.32	2.75

Note: T_0: control, T_1: 4 kg Zn soil application, T_2: 6 kg Zn soil application, T_3: 8 kg Zn soil application, T_4: 4 kg Zn soil application + 0.5% zinc sulfate foliar application, T_5: 6 kg Zn soil application + 0.5% zinc sulfate foliar application, T_6: 8 kg Zn soil application + 0.5% zinc sulphate foliar application.

observed in the treatment with a combination of Zn at 6 kg ha^{-1} soil application + 0.5% zinc sulfate foliar application and the lowest (10.33%) was observed under control conditions. These results are quite similar to the findings of Dhaliwal et al. (2020). They observed that the foliar application of zinc significantly improves the mineral contents of oat. Zinc is required in a minute but crucial amount to assist several key plant physiological metabolisms to function normally (Yousefi et al., 2011). Zinc deficiency reduced the overall performance of the water used for biomass production and plant efficiency in responding to water stress by osmotic adaptation (Khan et al., 2014).

Zinc contents (ppm)

The zinc contents in oat greatly influenced by Zn application. Zinc contents range between 35.2 ppm to 70.0 ppm on dry weight basis of oat plant (Table 3). Maximum Zn contents (70.0 ppm) were observed when a combination of Zn at 6 kg·ha^{-1} soil application + 0.5% zinc sulfate foliar application was used and the lowest (35.20 ppm) was observed under control conditions. These results are co-related with the findings of Li et al. (2021). They observed that adequate application of zinc significantly improved the zinc content of oat. When zinc concentrations exceed the optimum quantity causes toxicity in plants which results in a decrease of leaf and root growth. As zinc quantity increases in plants, production of NADPH is reduced in chloroplast and free radicals like iron toxicity. Activity of rubilose bi-phosphate carboxylase and photo-system decrease by zinc toxicity. In addition, a large amount of zinc accumulation retarded the uptake of phosphorus and iron (Chang et al., 2017).

CONCLUSIONS

In light of the observed data, it was concluded that the application of 6 kg ha^{-1} Zn as soil application in combination with 0.5% $ZnSO_4$ as foliar application improved the yield and quality of fodder oat. Therefore, it can be an important strategy to overcome malnutrition in livestock. However, the excessive use of zinc can be toxic for plants and the environment, therefore, appropriate measures must be taken to mitigate the hazards of zinc.

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