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RESEARCH ARTICLE

Morpho-agronomic characters of oat growing with humic acid and zinc application in different sowing times

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ABSTRACT

The objectives of the study were to determine the effects of humic acid (HA) and zinc (Zn) applications on winter tolerance and yield performance of oat (cv. Albatros) planted in winter and spring sowing times (ST). We conducted the study in the 2017/2018 growing season. The experimental layout was split-split plots with three replications. Sowing times (winter and spring) comprised of the main plots. Humic acid application (with HA and without HA) was in the sub-plots, and Zn application rates (0, 23 and 46 kg ha^{-1}) were in the sub-sub-plots. Chlorophyll content of plants was measured at the heading stage, while plant height, panicle height, panicle weight, number of grain per panicle, weight of grains per panicle, harvest index and grain yield were determined at the harvest. The effects of HA and Zn applications in both ST increased the chlorophyll content, yield and yield components. The results showed that cold tolerance of oat plants can be increased by the application of HA and 46 kg $ZnSO_4.7H_2O$ ha^{-1}. Overall performance of winter season was better than spring; thus, oat can be cultivated in winter under warm winter climate conditions. In addition, the HA and Zn applications can be used for other cold sensitive plant species to increase the cold tolerance which is a prevalent environmental stress affecting productivity of crops.

Introduction

Oat (*Avena sativa* L.) is cultivated commonly as a forage crop because of the high nutritional value compared to the other cereals. The oat contains useful nutrients for human and animal health (1, 2). Besides the value as forage for animal and food for human, the oat has recently gained a considerable importance for the pharmaceutical and cosmetic industries (1). Despite the increasing demand, the cultivation area of oat is not expanding sufficiently to produce adequate oat for the market (3). The main reasons for the lack of widespread oat cultivation are because of the low grain yield, problems of cold and drought sensitivity, lodging, grain losses and non-simultaneous grain ripening (2). The oat is commonly planted in spring in the Central Anatolia Region of Turkey because of the drought and cold sensitivity of oat. However, irregular and inadequate precipitation in the spring with a short vegetation period affect negatively the yield of oat (1).

Using chemical fertilisers alone will not increase the fertility of soils and improve the resistance of plants to stress conditions and diseases. Therefore, the application of humic acid (HA) recently become common in addition to the use of mineral fertilizers. The amino acid composition of HA increases the population and activity of soil microorganisms and improves plant growth. The molecules in HA bind nutrients and prevents leaching from the root zone, and help plant roots receive water and nutrients (4). The HA stimulate the root development in early plant development stages (5). At the same time, HA control the presence of nutrients and enhance their reception by the plant through increasing the ATPaze activity between soil and atmosphere and transfer between carbondioxide and oxygen (6). In general, metal ion complexes that increase the micronutrients [especially iron (Fe) and zinc (Zn)] availability with low solubility are affected by HA on plant nutrition (7).

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High soil pH in the presence of calcium carbonate decreases the mobility of micronutrients, causing deficiency in many micronutrients including Zn and Fe (8). Zinc deficiency in cereal-grown soils of Central Anatolia is one of the major causes that restrict crop yield. The sensitivity of plants to environmental stress conditions such as drought, high or low temperature etc increases in Zn deficiency. Therefore, the plants are still stunted and the yield is greatly reduced (9). When there is Zn inadequacy in the development stage, photosynthesis rate decreases to 50-70% depending on the plant type and Zn content in the soil (10). In addition, Zn play important roles on water use efficiency of the plant, root development and growth as well as flowering and grain formation. The winter death in seedlings, seeds with low Zn content is more common, especially in the soil which lacks of Zn (8). The positive effects of Zn on photosynthesis, starch accumulation, sucrose synthesis and integrity and durability of biological membranes (9).

Winter cereals survive the winter months with no damage when the seedlings have a minimum of 4-5 leaves and one or two tillers to store enough energy. The application of Zn and HA may have a positive influence on the growth and development of oat plants, and help to increase the cold stress resistance in winter months. Therefore, this study was designed to investigate the effects of HA and Zn applications to the soil on cold stress tolerance and some morpho-agronomic characters of oat grown in a Zn deficient soil of central Anatolia.

Materials and Methods

Site description

We established the field experiment on the research fields of Faculty of Agriculture, Eskisehir Osmangazi University, Turkey in the 2017/2018 growing season. The climatic data (monthly total rainfall, average temperature and relative humidity) of the trial area as growing season (2017/2018) and long years (1939 to 2017) are given in Table 1. Total rainfall during the growing season, average temperature and humidity values were higher than the long-term mean values, especially since the winter months were unusually temperature. The experimental soil was slightly alkaline, non-saline and clay-loamy textured. Organic matter, Mn and Zn contents were low, while K, Fe, P, Cu and lime contents were sufficient (Table 2). Fifteen days after the application of HA, Zn and basic fertilisation, soil samples were taken from each application again and analysed (Table 2).

Experimental materials and design

Oat seed (cv. Albatros) was obtained from Ata Seed Company, Turkey. Albatros has plump white grain. It has short vegetation period and is generally spring planted. However, it is also recommended for planting in winter in temperate regions.

Humic acid material used in the study was a commercially available natural organic soil conditioner (TKI-Humas) containing humic and fulvic acids extracted from leonardit. Zinc sulphate ($ZnSO_4.7H_2O$) fertiliser containing 22% Zn was used in Zn application.

The experimental layout was a randomised complete block design with split-split plots in three replications. Main plots (sowing time; winter and spring) were divided into two sub-plots and two HA application (with HA or without HA) were randomly allocated to these. Sub-plots viz. with HA and without HA were then divided into three sub-sub-plots and some of them were not treated with Zn fertilizer, to some were applied 23 kg $ZnSO_4.7H_2O$ ha^{-1} and others 46 kg $ZnSO_4.7H_2O$ ha^{-1} (Fig. 1). Each sub-sub-plots comprised of 1.2 × 4 m size. Zinc and HA were not applied to the control plots.

The HA (20 L ha^{-1}) was applied to the soil surface considering the recommendations of the produce company (TKI Humas) using a mechanical sprayer 24 hours before planting and thoroughly mixed in the sowing deep.

Zinc was applied to soil before planting using a mechanical sprayer at a rate of 23 kg $ZnSO_4.7H_2O$ ha^{-1} as an optimum dose for Central Anatolian (11) and 46 kg $ZnSO_4.7H_2O$ ha^{-1} as high dose.

The 31.3 kg N and 35.2 kg P per ha were applied as di-ammonium phosphate (18-46-0) at the time of sowing and 28.7 kg N ha^{-1} as ammonium sulphate (21-0-0-24) was added to complete 60 kg ha^{-1} of N. Nitrogen topdressing was then applied as 60 kg N per ha as ammonium sulphate (21-0-0-24) during the tillering stage.

Oat seeds (cv. Albatros) as 500 seeds m^{-2} were planted by a small plot seeder (Turan Machine, Turkey) in 24th of October and 20th of March for winter and spring oat cultivations respectively.

The field was irrigated after planting in both ST to help emergence. The plants were irrigated again during stem elongation and flowering periods. Thus, total 300 mm of water were applied.

Measurements

Chlorophyll content was measured in the flag leaves using a chlorophyll meter (Spectrum Field Scout CM 1000, Spectrum Technologies, Inc.) at the heading stage. Yield and quality parameters such as plant height, panicle length, panicle weight, number of grains per panicle, grain weight per panicle, harvest index, grain yield and thousand-grain weight were also determined.

Agronomic zinc efficiency (AE) was calculated using the equation (12);

$$AE = (Yf-Yc)/F$$

where, AE is the agronomic efficiency (%), Yf is the grain yield (t ha^{-1}) with fertilizer treatments, Yc is the grain yield (t ha^{-1}) without fertiliser treatment, and F is the amount of Zn added to the soil (kg ha^{-1}).

Statistical analyses

The data got from the experiment were analyzed using general linear model (GLM) procedure with IBM-SPSS 20 statistical package programme. The means were compared using the Duncan test at

$p \leq 0.05$ and the interactions of applications and genotypes were shown in graphs.

significantly differed for all the traits. The effect of ST × HA interaction on yield and yield parameters such

Table 1. Climatic conditions in growing season and long years at experimental area

	Growing Season (2017–2018)			Long Years (1993–2017)		
	Total rainfall (mm)	**Mean humidity (%)**	**Mean temperature (°C)**	**Total rainfall (mm)**	**Mean humidity (%)**	**Mean temperature (°C)**
October	48.4	72.9	10.7	31.6	64.7	11.8
November	28.6	85.4	5.5	29.8	70.5	5.6
December	41.8	86.3	3.9	42.0	75.9	1.6
January	29.0	86.3	1.6	37.6	75.2	-0.2
February	41.6	82.4	5.8	29.2	70.6	0.9
March	41.1	73.2	9.3	32.5	64.2	4.8
April	9.5	61.6	13.8	41.6	62.7	9.6
May	92.5	74.9	16.7	46.5	59.5	14.6
June	73.8	69.6	19.9	25.6	55.2	19.1
July	60.1	65.7	22.2	13.2	51.9	22
Mean		75.83	10.94		65.0	9.0
Total	466.4			329.7		

Table 2. Soil properties of research area

	Without HA			With HA		
Characters	**0 Zn**	**23 Zn**	**46 Zn**	**0 Zn**	**23 Zn**	**46 Zn**
pH	8.1	8.0	8.0	8.1	7.9	7.9
Salt (%)	0.01	0.02	0.02	0.02	0.01	0.02
Lime (%)	7.1	7.9	5.5	7.9	8.7	8.3
Organic matter (%)	0.94	1.09	1.59	1.29	1.50	1.68
P_2O_5 (kg ha^{-1})	64.7	130.3	309.4	146	180.9	146.4
K_2O (kg ha^{-1})	5651	4774	6108	3615	4724	2928
Fe (mg kg^{-1})	6.10	6.58	6.78	7.93	7.41	6.44
Mn (mg kg^{-1})	10.5	11.2	8.1	11.2	11.4	9.7
Zn (mg kg^{-1})	0.87	3.03	3.35	1.13	3.31	4.47
Cu (mg kg^{-1})	2.10	1.52	1.65	1.91	1.86	1.80

Main plot 1: Winter sowing					
Sub plot 1: Without HA			Sub plot 2: With HA		
Sub sub plot 1: 0 Zn	Sub sub plot 2: 23 Zn	Sub sub plot 3: 46 Zn	Sub sub plot 1: 0 Zn	Sub sub plot 2: 23 Zn	Sub sub plot 3: 46 Zn
Main plot 2: Spring sowing					
Sub plot 1: Without HA			Sub plot 2: With HA		
Sub sub plot 1: 0 Zn	Sub sub plot 2: 23 Zn	Sub sub plot 3: 46 Zn	Sub sub plot 1: 0 Zn	Sub sub plot 2: 23 Zn	Sub sub plot 3: 46 Zn

Fig. 1. Experimental design of the study.

Results and Discussion

The influences of HA and Zn applications to soil on some physical and chemical properties of the experimental soil are shown in Table 2. Lime and salt contents and pH of soil have not changed with the HA and Zn applications, however organic matter content and nutritional composition of soil significantly changed. The organic matter and Zn contents of soil increased with the increase in Zn application doses, and the increase in organic matter content was higher in HA applications. These findings showed that the addition of HA could increase the available plant nutrient ion content of the soil, especially enhance the content of soil organic matter, which is helpful to the recovery of crop growing soil. Similar results found other researchers (13, 14).

Sowing time had a significant (p<0.01) effect on all traits examined except plant height and panicle length, while the effects of HA and Zn applications as panicle weight was significantly different. The effects of other interactions on the traits determined were not statistically significant (Table 3).

The chlorophyll content and the values of yield and yield parameters determined for winter sowing were higher than those recorded in spring sowing. Application of HA and Zn increased the chlorophyll content and improved the yield parameters. The improvement in properties studied with Zn applications was in good agreement with the increasing doses of Zn to soil (Table 4).

The results of variance analysis indicated that the effects of treatments on chlorophyll content were insignificant however; the chlorophyll content increased with the increasing Zn doses in winter sowing. The increase in chlorophyll content was even higher in HA treatments (Fig. 2). In spring sowing, chlorophyll content in 23 kg of Zn ha^{-1} dose and HA application increased by 26% compared to control. High chlorophyll content of leaves indicates of higher photosynthesis and yield (15). Earlier studies (16, 17) indicated that the chlorophyll content significantly decreased depending on environmental factors such as temperature, precipitation, drought etc. The change of climatic and environmental characteristics depending on the sowing time (season) of the plants affected the chlorophyll content. Since Zn participates in chlorophyll production, the deficiency of Zn negatively affects the chlorophyll content of leaves (18, 19). Therefore, the application of a moderate amount of Zn increases the chlorophyll contents in tissue, promotes the development of aboveground parts, such as leaves and increases the biomass (20, 21). Higher biomass indirectly improves the water absorption of roots and offers a larger surface area for photosynthesis. The positive effects of Zn on photosynthesis cause a significant increase in crop yield (22, 23). Several researchers have reported the improving effect of HA on the chlorophyll content of leaves (24-26).

Plant height and panicle length which are known as significant seconder yield components of crop plants have not been affected by ST. While they are also known to be inherited quantitatively, a lack of water supply can prevent their full expression (27). Since oat plants find the water required for their growth both in spring and winter sowing, there was no significant difference between sowing times as expected. It was reported that the absorption rate of mineral ions on root surfaces and their transfer into the plant tissue cells are enhanced with the supplement of HA in soil which also yields to a raise in the plant height. Therefore, more active metabolism and high respiratory activity and higher plant height are observed (28). The parameters directly affecting the grain yield such as panicle weight, grain number in a panicle, grain weight in a panicle and thousand grain weight significantly increased in 46 kg Zn ha^{-1} application dose especially with HA treatment at winter sowing. Similarly, previous studies also showed that crop yield in Zn deficient soils significantly increased with the Zn application to soil (8, 29, 30).

Table 3. Mean squares obtained from variance analysis of investigated traits

SOV	Df	Chl	PH	PL	PW	GNP	GWP	TKW	HI	GY
ST	1	77469.44**	4.20^{ns}	0.27^{ns}	9.41**	16030.94**	9.43**	185.28**	847.10**	11.94**
HA	1	17689.00*	573.60**	51.60**	6.66**	3678.83**	4.47**	319.75**	278.83*	14.42**
Zn	2	16660.58*	163.97*	11.99*	3.74**	2509.43**	2.06**	172.35**	345.59**	13.28**
ST × HA	1	544.44^{ns}	44.67^{ns}	0.03^{ns}	1.04*	110.67^{ns}	0.46^{ns}	50.10^{ns}	22.36^{ns}	1.10^{ns}
ST×Zn	2	7783.69^{ns}	18.31^{ns}	7.07^{ns}	0.44^{ns}	943.74^{ns}	0.24^{ns}	6.49^{ns}	1.17^{ns}	0.02^{ns}
HA×Zn	2	1535.08^{ns}	13.19^{ns}	0.09^{ns}	0.23^{ns}	117.65^{ns}	0.04^{ns}	15.79^{ns}	25.04^{ns}	0.13^{ns}
ST×HA×Zn	2	1610.53^{ns}	92.93^{ns}	0.54^{ns}	0.22^{ns}	125.28^{ns}	0.18^{ns}	19.47^{ns}	13.20^{ns}	0.07^{ns}
Error	24	3161.92	51.07	2.66	0.23	171.16	0.15	14.26	50.35	0.58

*Chl: chlorophyll content; PH: plant height; PL: panicle length; PW: panicle weight; GNP: grain number per panicle; GWP: grain weight per panicle; HI: harvest index; GY: grain yield; TKW: thousand kernel weight; SOV: source of variance; ST: sowing time; HA: humic acid applications; Zn: zinc fertilization.

Table 4. The mean values of sowing time, humic acid, zinc applications and genotype mean belonging examined traits

	Sowing time		Humic acid		Zinc doses (kg ha^{-1})		
	Winter	Spring	Without HA	With HA	0	23	46
Chl (CM)	494.06±58.8	401.28±72.9	425.50±82.7	469.83±73.9	405.42±76.0	461.75±64.4	475.83±87.5
CV%	11.91	18.18	19.43	15.74	18.75	13.94	18.40
PH (cm)	91.91±7.79	91.22±9.04	87.57±5.99	95.56±8.54	87.64±7.90	92.07±6.75	94.98±9.09
CV%	8.47	9.91	6.85	8.94	9.01	7.33	9.57
PL (cm)	19.39±2.40	19.57±1.83	18.28±1.60	20.68±1.88	18.37±1.68	19.78±2.03	20.30±2.23
CV%	12.36	9.37	8.77	9.07	9.16	10.26	10.99
PW (g)	3.85±1.04	2.83±0.49	2.91±0.69	3.77±1.01	2.81±0.80	3.29±0.72	3.92±1.03
CV%	27.03	17.24	23.57	26.69	28.34	21.85	26.34
GNP	112.18±25.88	69.98±15.15	80.97±26.73	101.19±30.20	75.66±19.76	93.25±25.05	104.33±37.10
CV%	23.07	21.65	33.01	29.84	26.12	26.86	35.52
GWP (g)	3.10±0.79	2.09±0.42	2.24±0.65	2.95±0.82	2.18±0.74	2.59±0.65	3.00±0.86
CV%	25.35	20.08	28.92	27.72	34.01	25.13	28.57
TKW (g)	32.38±7.19	27.84±3.92	27.13±4.34	33.09±6.35	26.34±5.04	30.06±3.66	33.92±7.10
CV%	22.22	14.07	15.99	19.20	19.13	12.16	20.93
HI (%)	41.75±7.86	32.05±8.50	34.11±9.08	39.68±9.22	30.70±9.27	39.81±7.29	40.18±9.08
CV%	18.83	26.53	26.63	23.24	30.18	18.31	22.60
GY (t ha^{-1})	5.81±1.39	4.66±1.18	4.61±1.18	5.87±1.34	4.37±0.95	4.94±1.12	6.41±1.28
CV%	23.86	25.32	25.59	22.76	21.66	22.74	20.01

*Chl: Chlorophyll content; PH: plant height; PL: panicle length; PW: panicle weight; GNP: grain number per panicle; GWP: grain weight per panicle; HI: harvest index; GY: grain yield; TKW: thousand kernel weight.

The response of yield characteristics to HA and Zn applications was poor in spring sowing. However, the values obtained were close to those got without HA application in winter sowing. The harvest index values calculated in 23 kg Zn ha^{-1} application treated with and without HA at winter sowing were close to each other. The HA and Zn applications increased the harvest index of oats in spring sowing. Humic acid, a naturally available substance is a cheap source of organic matter that enhances soil fertility, availability of nutrients and water holding capacity

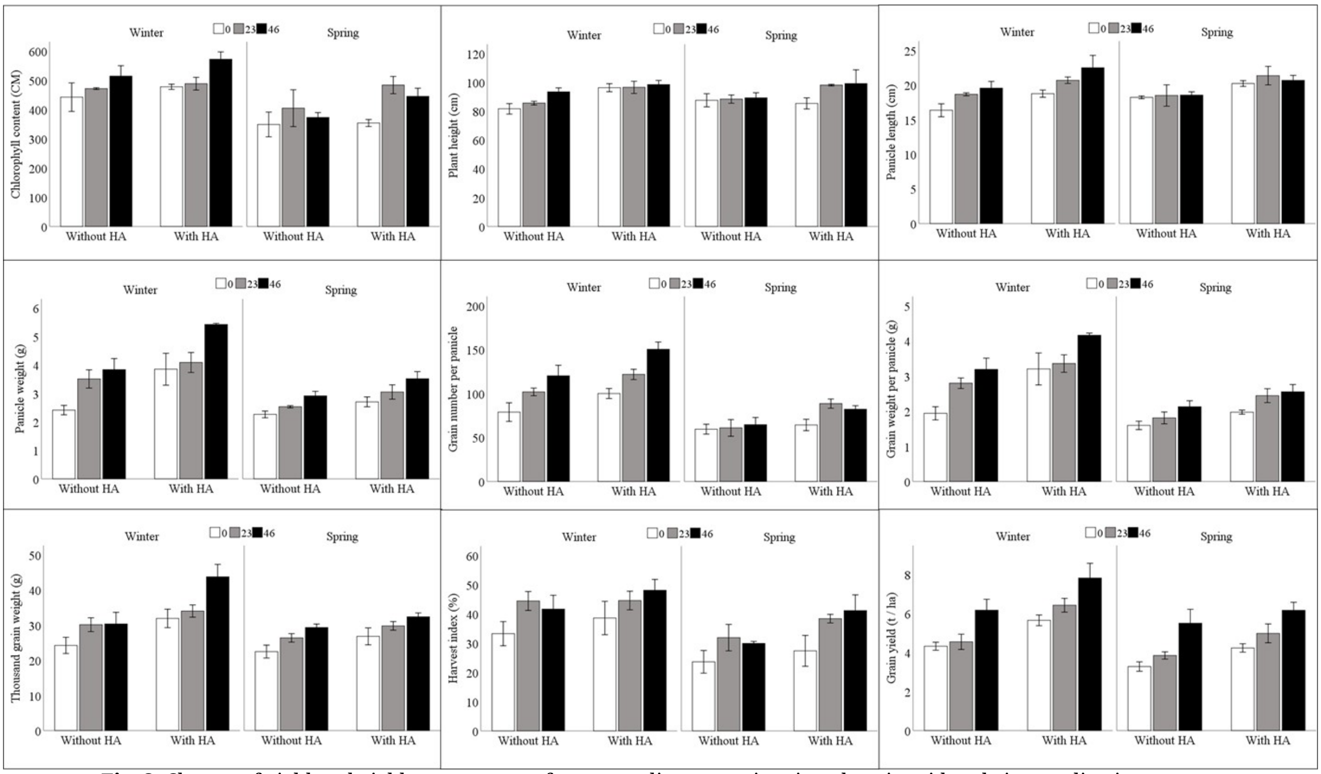

Fig. 2. Change of yield and yield components of oat according to sowing time, humic acid and zinc applications.

of soils. Therefore, the application of HA provides a favourable environment for plant growth, improves the yield parameters and results in higher crop yield (31). In addition, an earlier study (32) reported that liquid humic application increased the winter tolerance of wheat by improving the growth and physiological indices during autumn acclimation in organic and conventional farming systems.

The application of 23 kg Zn ha-1 with and without HA in winter sowing slightly increased the oat yield compared to the control. However, oat yield increased in 46 kg Zn ha-1 application dose by 30% compared to the control. In spring sowing, the increase in each Zn dose with HA treatments increased the oat yield approximately by 15% compared to the control (Fig. 2). The results of this study revealed that single or combined applications of HA and Zn improved the yield and yield characteristics of oat. The stimulating effect of both HA and Zn is partly associated with the higher uptake of mineral nutrients (33). Similarly, positive effects of HA and Zn application on the yield of different crops such as pearl millet (34), wheat (30, 31, 35, 36) and sunflower (37) have been reported in other studies. In some studies, it has been shown that 5 or 10 kg Zn ha^{-1} (~25-50 kg $ZnSO_4.7H_2O$) application is sufficient for high yield of cereals (38, 39). Similar results were obtained in this study.

The agronomic efficiency of Zn application in respect to sowing times and HA application indicated that agronomic efficiency positively affected by the HA application in winter sowing. The agronomic efficiency of Zn increased with the application of HA in both sowing times, except for 46 kg Zn ha^{-1} in spring sowing. Considering the Zn doses, the agronomic efficiency of 46 kg Zn ha^{-1} was higher (Fig. 3). Total Zn content of the soil with Zn application in this study is sufficient, because optimum level of available Zn in soil changes between 2 and 20 mg kg^{-1} (40). However, in general, the factors of high calcium carbonate content, high soil pH values, low organic matter content of soils and high phosphorus concentration may prevent the Zn uptake through roots. Humic acid increases the availability of micro and macro nutrients by increasing the organic matter content of soils. Humic acid act as a bridge between nutrients in the soil and the plant roots, as reported in another study (41) that application of HA substances to soil increases the uptake of chelated micronutrients. The agronomic efficiency of Zn values calculated in this study reveals the benefits of HA application in winter sowing.

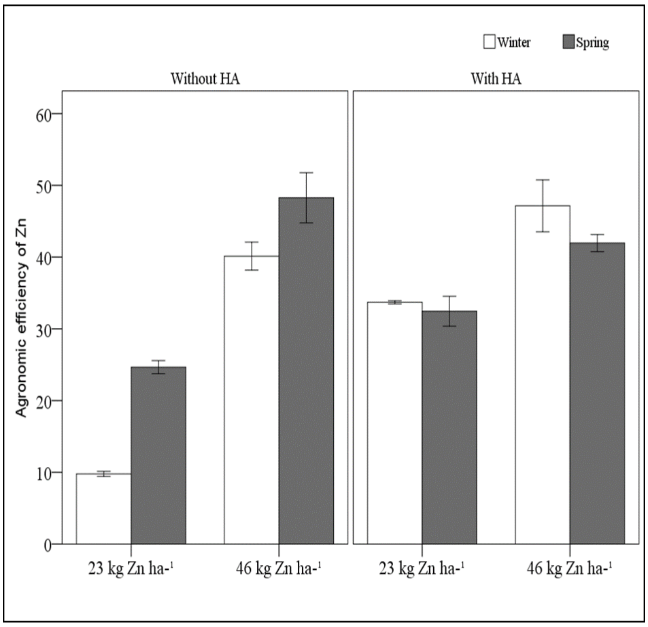

Fig. 3. Agronomic efficiency of Zn ($ZnSO_4.7H_2O$) depending on oat grain yield (kg ha^{-1} / kg ha^{-1}) .

Conclusion

The application of HA with Zn had a positive effect on soil organic matter and nutrient content. The yields of cereals are higher when planted in winter. However, the results revealed that satisfactory oat yield can be obtained in the spring sowing with HA and 46 kg ha^{-1} $ZnSO_4.7H_2O$ applications. In addition, higher yields could also be obtained with the HA and Zn applications in winter sowing. The Albatros oat variety used in this study is a spring variety, however, very high yield values were obtained when it was sowed in winter with HA and Zn applications. Humic acid and Zn application in regions with mild winter conditions enable to get higher yields using the spring oat varieties. This study should be carried out using different oat varieties in different locations for the identification of elite varieties.

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Authors' contributions

IK carried out the field studies, performed the statistical analysis and drafted the manuscript. NG conceived the study and took part in its design and coordination. Both authors read and approved the final manuscript.

Conflict of interests

Authors have no competing interests to declare.

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