Export of N, P and K in barley subjected to fertilizer doses formulated with and without humic substance at sowing

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ABSTRACT. The aim of this study was to evaluate the effect of nitrogen fertilization with and without humic acid in barley cultivars on the export of nitrogen, phosphorus and potassium grains. The experiment was performed in the city of Guarapuava, PR, during the 2015 and 2016 growing seasons. The experimental design was in RCB in factorial 5 x 2, with the following sowing fertilization (kg ha⁻¹): 0, 100 NPK, 100 NPK+HS, 250 NPK, 250 NPK+HS and two cultivars, BRS Brau and BRS Elis. The dose of 250 kg NPK with humic substance increased the nitrogen export to barley grains, with the highest levels occurring in the BRS Brau cultivar under favorable climatic conditions. Under favorable climatic conditions, the export of phosphorus to barley grains in the agricultural crop was not influenced by the humic substance, with the highest levels occurring in the BRS Brau cultivar. The dose of 100 kg NPK with humic substance increased the export of potassium to barley grains, with the highest levels occurring in the BRS Brau cultivar regardless of climatic condition. There were correlations between nitrogen and phosphorus levels in grains and between phosphorus content and grain yield.

Keywords: Hordeum vulgare; NPK fertilizer; nitrogen; phosphorus; potassium.

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Introduction

Barley is the fourth most-produced cereal worldwide behind wheat, rice and maize. Its primary use is to serve as raw material for malt and beer, being the grain type essential for such products (Wei, Dai, Wu, & Zhang, 2009). However, little attention has been focused on the effects of long-term chemical fertilization and the relation between grain yield and nutrient concentration in the grain (Hejcman, Bercová, & Kunzová, 2013).

Plants, mainly winter cereals, have an immediate requirement for nutrients during the early stages of establishment and growth. The primary means to meet this need is mineral fertilization (Mehraban, 2014; Shah, Shah, Mohammad, Shafi, & Nawaz, 2009). However, the use of HS or organic compounds as mineral fertilizers positively affects root growth, nutrient absorption, chlorophyll synthesis, soil CEC, seed germination and microbial activity. This approach also helps reduce the salinity effect (Anwar et al., 2016; Mahmoud & Ibrahim, 2012; Ouni, Ghnaya, Montemurro, Abdelly, & Lakhdar, 2014).

In connection with barley's importance and its several uses, there are many factors that affect its production. Among the most aggravating are the following: low pH, low soil fertility, hydrical stress, poor drainage of soil and precarious agronomic management (Agegnehu, Nelson, & Bird, 2016). Unstable production can compromise the quality of seeds produced by the crop and frequently results in seeds not reaching the quality required for malt production, leading to a need to import raw materials (Grzybowski, Ohlson, Silava, & Panobianco, 2012).

Regarding the nutritional quality of grains, there is controversy over whether to produce organically or conventionally. This controversy arises from different management practices and climatic conditions (Farahani, Chaichi, Mazaheri, Afshari, & Savaghebi, 2011). Wilczewski, Szczepanek, Knapowski, and Rosa (2014) state that the use of humus for seed treatment on barley grown in fertile, mineral-rich soils can improve the content of the macronutrients in the grain. Still, Aşik and Katkat (2013) verified that applying different doses of humic acids through the solid route increased plant dry matter and the N, P and K contents in the plant.

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Based on the foregoing, this research has as its objective to assess the effect of fertilization doses at sowing, with and without the use of humic substance, in barley cultivars on the characteristics of nitrogen, phosphorus and potassium export to the grain in the municipality of Guarapuava, Paraná State, during the 2015 and 2016 growing seasons.

Material and methods

The experiment was conducted in a no-tillage system in the municipality of Guarapuava, Paraná State, Brazil, during the 2015 and 2016 growing seasons. In the 2015 crop, sowing occurred on July 1, and the experiment was conducted in the experimental field of the State University of Central West - UNICENTRO (altitude 1,028 m, latitude 25° 23 '04.83'' S and longitude 51° 29' 44.32 '' W). For the 2016 crop, sowing occurred on June 26, and the experiment was conducted in the experimental field of the Agrarian Foundation of Agricultural Research - FAPA (altitude 1,109 m, latitude 25° 32 '43.52" S and longitude 51° 29' 40.22" W). The soils of both experimental fields are classified as Oxisol (Embrapa, 2013).

The results of soil chemical analysis at a depth of 0-20 cm for the 2015 crop were as follows: pH (CaCl₂): 4.5, MO: 30.8 g dm⁻³, P: 4.62 mg dm⁻³, K: 0.3 cmol_c dm⁻³, Ca: 3.0 cmol_c dm⁻³, Mg: 1.2 cmol_c dm⁻³, Al: 0.27 cmol_c dm⁻³, H+Al: 8.36 cmol_c dm⁻³, CEC: 12.86, and V(%): 34.99. For the 2016 crop, the results of soil chemical analysis were as follows: pH (CaCl₂): 5.1, MO: 44.5 g dm⁻³, P: 2.7 mg dm⁻³, K: 0.48 cmol_c dm⁻³, Ca: 3.0 cmol_c dm⁻³, Mg: 2.0 cmol_c dm⁻³, Al: 0.0 cmol_c dm⁻³, H+Al: 4.59 cmol_c dm⁻³, CEC: 10.05, and V(%): 54.3.

The experiment was performed in randomized blocks in a factorial scheme (5 fertilizations x 2 cultivars) with four repetitions, for a total of 40 plots. The plots consisted of nine lines of plants (5.0 m length x 0.2 m interline), with a total area of 9 m² and the useful area constituted by the three central lines. The following fertilizations at sowing were assessed: fertilization 1: (control) 0.0 kg ha⁻¹; fertilization 2: 100 kg ha⁻¹ NPK; fertilization 3: 100 kg ha⁻¹ NPK with 5% HS (NPK+HS); fertilization 4: 250 kg ha⁻¹ NPK; fertilization 5: 250 kg ha⁻¹ NPK+HS, for BRS Elis and BRS Brau barley cultivars.

The fertilizer used was NPK 10-20-12 formulated with and without HS, which includes 0.5% HS in the formulation and whose source leonardite had 85% HS content. For cover fertilization, 60 kg N ha⁻¹ was applied in the form of urea when the plots were in the tilling period. For sowing, a Semina $^{\circ}$ seed drill was used, with a final population goal of 320 plants m⁻² for BRS Brau and 280 plants m⁻² for BRS Elis.

The cultivars used were of small size, had high productive potential (reaching up to 6,000 kg ha⁻¹) and had early maturity (approximately 88 days until earing and 130 to 132 days until maturation). The low height provides a good level of resistance to lodging, and both cultivars meet the main specifications of the brewing industry, whose preferred cultivation regions are Rio Grande do Sul (RS), Santa Catarina (SC), and Parana (PR) States, Brazil (Lunardi, 2009; 2010).

The following characteristics of the barley grain were assessed: export of macronutrients in g kg⁻¹ of nitrogen (N), phosphorus (P), and potassium (K), with N determined by indophenol blue spectrophotometry, P by vanadate-yellow spectrophotometry and K by flame photometry, according to the methods described by Silva (2009). The outcomes were subsequently extrapolated to export kg ha⁻¹ in agreement with grain yield per hectare, as described by Ferrari Neto, Crusciol, Soratto, and Costa (2011).

The assessed characteristics data were submitted to the homogeneity test of variances using the Harley test. Later, the averages were subjected to analysis of individual and joint variance for the different growing seasons. The averages were compared by the Tukey test at the 5% probability level. Subsequently, nine non-orthogonal contrasts were performed (0 vs. 100 NPK, 0 vs. 100 NPK+HS, 0 vs. 250 NPK, 0 vs. 250 NPK+HS, 100 NPK vs. 100 NPK+HS, 250 NPK vs. 250 NPK+HS, NPK vs. NPK+HS, C1 vs. C2 and SF1 vs. SF2) using SISVAR® statistical software (Ferreira, 2014).

To determine the degree of association between the assessed characters, for each pair of characteristics, the Pearson coefficient correlation was estimated at the 5% significance level by the t test, with the assistance of GENES® statistical software (Cruz, 2013).

Results and discussion

The climatic conditions, including rainfall and average temperature, from the seeding of the two crops to harvest (i.e., June to November) were obtained from the meteorological station of the Agronomic Institute of Parana, located at Unicentro, and from the Fapa climatic station. The values are provided in Figure 1.

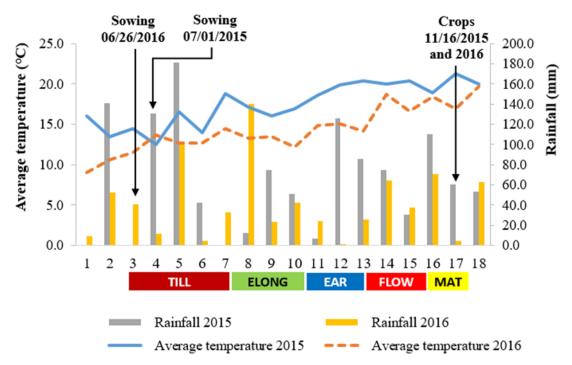


Figure 1. Average temperature data (°C) and rainfall (mm) per 10-day period starting at the 1° ten-day period of June (1) until the last 10-day period of November (18) during the 2015 and 2016 growing seasons in the municipality of Guarapuava, Paraná State. TILL: tilling, ELONG: elongation, EAR: earing, FLOW: flowering, MAT: maturation. **Source:** UNICENTRO and FAPA meteorological stations.

The accumulated rainfall index for the 2015 crop during its cycle was 962 mm. However, this volume was low during the stage that included tilling, and in the final stage of development, this volume was high, reaching up to 120 mm per 10-day period, negatively affecting grain yield. Regarding temperature, out-of-season frosts occurred during the 75th and 76th days after sowing (i.e., the final stages of elongation and the start of earing), with temperatures of 0.8 and 3.8°C, respectively. At the end of the crop cycle, temperatures were higher than the optimal for barley development.

Regarding the 2016 crop, the accumulated rainfall index throughout the barley development was 581 mm, with well-distributed rains, which favored crop development. Similarly, temperatures during the crop cycle were optimal (Figure 1).

The climatic conditions of the two crops were contrasting. The 2015 crop suffered unfavorable conditions, including frosts at the end of elongation and the start of the earing period (75th and 76th day after sowing) and high temperatures and rainfall volume during the grain filling and maturation stages. The 2016 crop enjoyed optimal conditions for cultivation. This difference between the growing seasons was evident for most assessed characteristics. This effect can be explained by the instability of barley production. As stated by Gezahegn and Kefale (2016), even with the use of the same species, management and cultivation sites, there might be differences between crops due to climatic variations. These circumstances emphasize the importance of medium to long-term studies.

According to the results of joint variance analysis, for the characteristics of grain quality used in the experiment, significant effects were observed 1) for the double interactions cultivar x fertilization, cultivar x crop and fertilization x crop for the export of nitrogen (N), 2) for the export of phosphorus (P) in the interaction cultivar x crop, and 3) in the cultivar x fertilization and cultivar x crop interactions for potassium export (K).

In the export data for N, P, and K in g kg⁻¹, only N responded to sowing fertilization, whereby the association with HS had a positive effect on the nutrient levels. When these data were related to the data for grain yield, the export in kg ha⁻¹ was obtained. The best results were achieved with a dose of 250 kg ha⁻¹ associated with HS for N, and for P and K, the association of HS was independent of the fertilization dose at sowing. In this sense, we can infer that for the assessed characteristics of macronutrient export in the grain, there was a difference between the treatments with the sowing formulations and the agricultural growing season.

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Regarding nitrogen export (N), there was a significant difference between the crops and treatments. For the BRS Brau cultivar, the 2015 crop was superior to the 2016 crop. For the treatments during the 2015 growing season, the use of HS resulted in an increase in the extraction of N. For the 2016 crop, only the 250 kg ha⁻¹ dose with HS differed from the treatment without fertilization (Table 1).

Table 1. Average values for nitrogen export (N) for different cultivars of barley subjected to different doses of fertilizer application at sowing, with NPK formulated with and without humic substance during the 2015 and 2016 growing seasons in the municipality of Guarapuava, Paraná State.

Fertilization ¹	Dose (kg ha ⁻¹)	Nitrogen				
		BRS Brau		BRS Elis		
		2015	2016	2015	2016	
Control	0	52.83 cA	42.95 bA	31.20 cB	49.70 abA	
NPK	100	65.54 bcA	49.53 abB	35.33 bcB	50.05 abA	
NPK+HS	100	87.32 aA	48.67 abB	46.51 abA	39.50 bA	
NPK	250	86.26 aA	40.81 bB	46.24 abcA	49.33 abA	
NPK+HS	250	77.50 abA	61.89 aB	57.89 aA	60.15 aA	
CV%			12	.04		

Averages followed by the same lowercase letter in the column and uppercase in the line do not differ according to the average comparison Tukey test (p < 0.05). 'NPK: Fertilization at sowing with formulated NPK; NPK+HS: Fertilization at sowing with formulated NPK+HS.

Similar results were found for wheat by Verma, Shivay, Kumar and Ghasal (2016), who used half of the recommended fertilization dose associated with organic compounds, which increased the N content in the grain.

For the BRS Elis cultivar, during the 2015 growing season, the 250 kg ha⁻¹ dose of the formulated NPK and the association with HS regardless of the dose increased the N export to the grain, highlighting the benefits of HS use when growing conditions are unfavorable. During the 2016 growing season, the formulated NPK at a dose of 250 kg ha⁻¹ associated with HS increased the export of N. However, this difference was only found for the 100 kg ha⁻¹ dose of the formulated NPK associated with HS.

In Table 1, one can observe cultivar responses according to whether the growing season was favorable or unfavorable. When comparing both cultivars, we find that the BRS Brau cultivar was more efficient with respect to N export during the unfavorable growing season, which reduced the grain yield. Similarly, Wilczewski, Szczepanek, Piotrowska-Dlugosz, and Wenda-Piesik (2013) verified high levels of N in wheat grains with low grain yield under conditions of good fertilizer supplementation and temperatures that were unfavorable for crop development.

Regarding the average values of phosphorus (P) and potassium (K) export, there were significant differences between cultivars and growing seasons. For P and K, during the unfavorable growing season, the BRS Brau cultivar was more efficient in the export of these nutrients. Different results were found by Verma et al. (2016), who obtained P and K increments in grain by using half of the recommended fertilization associated with wheat organic compounds. When the growing seasons were compared, the BRS Elis cultivar exhibited better performance for the export of P during the favorable growing season. For K, BRS Brau was better during the unfavorable growing season, while the BRS Elis cultivar performed better under favorable conditions (Table 2).

Table 2. Average values for the export of phosphorus (P) and potassium (K) for different barley cultivars subjected to different doses of fertilization at sowing, with NPK formulated with and without humic substance during the 2015 and 2016 growing seasons in the municipality of Guarapuava, Paraná State.

Cultivar	Phosp	ohorus	Potas	ssium
Cuitivai	2015	2016	2015	2016
BRS Brau	10.08 aA	10.97 aA	7.55 aA	5.54 aB
BRS Elis	5.51 bB	10.65 aA	4.28 bB	5.77 aA
CV%	13	.85	18	.80

Averages followed by the same lowercase letter in the column and uppercase in the line do not differ according to the average comparison Tukey test (p < 0.05).

For the data analysis, the use of contrasts was a simple way to obtain results for main and comparison effects between the assessed treatment groups (Nogueira, 2004).

Table 3 presents the non-orthogonal contrasts (0 vs. 100 NPK, 0 vs. 100 NPK+HS, 0 vs. 250 NPK, 0 vs. 250 NPK+HS, 100 NPK vs. 100 NPK+HS, 250 NPK vs. 250 NPK+HS, NPK vs. NPK+HS, C1 vs. C2, and SF1 vs. SF2).

The table compares the different levels of fertilization at sowing, with the NPK with and without HS, with both growing seasons being assessed for the characteristics of nitrogen (N), phosphorus (P) and potassium (K) ha⁻¹ export in the municipality of Guarapuava, Paraná State, Brazil.

Table 3. Estimation and probability of contrast significance for grain yield (PROD), classification of grain class 1 (1C), class 2 (2C), class 3 (3C), grain protein content (PROT) and export of nitrogen (N), phosphorus (P) and potassium (K) ha⁻¹ for different barley cultivars subjected to different doses of fertilization at sowing with NPK formulated with and without humic substance (HS) during the 2015 and 2016 growing seasons in the municipality of Guarapuava, Paraná State (UNICENTRO – 2017).

CONTRAST ¹	N	P	K
0 vs. 100 NPK	(-) 0.03	(-) 0.44	(+) 0.66
0 vs. 100 NPK+HS	(-) 0.01	(-) 0.08	(-) 0.08
0 vs. 250 NPK	(-) 0.01	(-) 0.01	(-) 0.01
0 vs. 250 NPK+HS	(-) 0.01	(-) 0.01	(-) 0.01
100 NPK vs. 100 NPK+HS	(-) 0.05	(-) 0.30	(-) 0.03
250 NPK vs. 250 NPK+HS	(-) 0.01	(-) 0.37	(+) 0.29
NPK vs. NPK+HS	(-) 0.01	(-) 0.18	(-) 0.41
C1 vs. C2	(+) 0.01	(+) 0.01	(+) 0.01
SF1 vs. SF2	(+) 0.01	(-) 0.01	(+) 0.36
CV (%)	11.72	14.46	18.77

¹0 (Control); 100 NPK (100 kg ha⁻¹ formulated NPK), 100 NPK+HS (100 kg ha⁻¹ formulated NPK+HS), 250 NPK (250 kg ha⁻¹ formulated NPK), 250 NPK+HS (250 kg ha⁻¹ formulated NPK+HS), NPK (fertilization with formulated NPK), NPK+HS (fertilization with formulated NPK + HS), C1 (cultivar BRS Brau), C2 (cultivar BRS Elis), SF1 (2015 growing season) and SF2 (2016 growing season).

For the contrasts that involve the characteristic N ha⁻¹ export (Table 3), the following contrasts with more than 95% probability were significant: 0 vs. 100 NPK, 0 vs. 100 NPK+HS, 0 vs. 250 NPK, 0 vs. 250 NPK+HS, 100 NPK vs. 100 NPK+HS, 250 NPK vs. 250 NPK+HS, NPK vs. NPK+HS, C1 vs. C2 and SF1 vs. SF2. The contrasts estimative for the N export involving the treatments 100 NPK, 100 NPK+HS, 250 NPK, 250 NPK+HS and NPK+HS were negative, indicating the numerical superiority of the same. That is, fertilization increased the N ha⁻¹ export, particularly fertilization with NPK formulated in association with HS, which presents a positive effect for this nutrient in barley. Regarding C1 and SF1, the estimates were positive, indicating their superiority. Studying the use of HS in wheat, Yassen, Hellal, and Abo-Basha (2011) also reported a positive effect for N when using foliar application with a 2% rate of HS associated with 0.5% zinc (Zn).

For N, a significant difference between growing seasons was verified. The 2015 season with climatic conditions unfavorable for development appeared superior to the 2016 season. In addition, BRS Brau presented a better response for the export of this nutrient than the BRS Elis cultivar, indicating that different cultivars present distinct responses with respect to N export by the grain.

Researching barley, Hejcman et al. (2013) discovered that the application of fertilization at sowing with N, P, and K associated with organic fertilizers in the same area for 9 years increased N, P and K levels in grain and consequently increased the export of these substances in relation to a control treatment. However, these levels did not differ from those achieved with organic fertilizer treatments, thus agreeing with this research, in which fertilization obtained a positive export response, particularly for N.

Regarding the contrasts that involved the characteristic P export, the following outcomes with more than 95% probability were significant: 0 vs. 250 NPK, 0 vs. 250 NPK+HS, C1 vs. C2 and SF1 vs. SF2. The contrasts estimative for the export of P that involved 250 NPK, 250 NPK+HS and SF2 were negative and for C1 positive, indicating the numerical superiority of the same. That is, regarding P, fertilization with NPK formulated with HS only has an effect when higher doses are used under favorable climatic conditions. This outcome agrees with Yassen, Khaled, and Zaghloul (2010), who observed that manure application incremented the level of P in grain.

This effect was observed in other crops, for example, by Osman, El-Masry, and Khatab (2013), whose results agree with those presented here. Those authors found that two different forms of N supplementation in rice associated with the use of HS and fulvic substance (FS) increased P levels. They state that HS increases the permeability of cell membranes, enabling faster passage of nutrients and thus an increased increment of this and other macronutrients. However, in research on carrots, Kaseker, Bastos, Consalter, and Mógor (2014) tested organo-mineral doses applied via the foliar method and did not observe an effect of fertilizer doses on the contrasts compared to a control treatment without the application and between doses for P and K export.

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Regarding the contrasts that involve the characteristic K export, the following outcomes with more than 95% probability were significant: 0 vs. 250 NPK, 0 vs. 250 NPK+HS, 100 NPK vs. 100 NPK+HS and C1 vs. C2. The estimative for the K export that involved 250 NPK, 250 NPK+HS and 100 NPK+HS was negative and for C1 positive, indicating the numerical superiority of the same. That is, the NPK formulated with HS influenced the export of K, with the BRS Brau cultivar the most responsive. A similar outcome was observed by Yassen et al. (2011), who found that the application of HS, cattle manure and Zn improved the increment of this nutrient in wheat, and by Osman et al. (2013), who observed the same in rice crops with the association of HS with nitrogen forms applied as coverage.

Studies by Nogalska, Czapla, and Skwierawska (2011) on barley and the mineral fertilizers did not find significant differences for the K export between growing seasons for three consecutive cultivations. Their outcome agrees with this study, where no significant difference between growing seasons was observed.

Because contrasting climatic conditions occurred during the studied growing seasons, it was determined to perform the Pearson correlation analysis separately to avoid masking the obtained results, given that the effect of the growing season was evident for nearly all assessed characteristics.

Table 4 presents the correlation coefficient values between nitrogen (N), phosphorus (P) and potassium (K) ha⁻¹ export and grain yield (YIELD) for the 2015 and 2016 growing seasons.

Table 4. Correlation coefficient between export of nitrogen (N), phosphorus (P) and potassium (K) ha⁻¹ and grain yield (PROD) for the 2015 and 2016 growing seasons in the municipality of Guarapuava, Paraná State (UNICENTRO – 2017).

-		2015			2016	
	P	K	PROD	P	K	PROD
N	0.950*	0.733	0.994**	0.975**	0.420	0.862
P		0.901*	0.962**		0.612	0.949*
K			0.772			0.795

** $p \le 0.01$; * $p \le 0.05$.

For N export during the growing season with unfavorable climatic conditions, significant positive correlations at 95% were found between the export of P and YIELD. That is, there was interaction between these macronutrients such that the larger that the amount of N was, the higher the levels of P and the higher the grain yield. Tigre, Worku, and Haile (2014) state that N and P are nutrients of the utmost importance. Their insufficiency limits crop growth and development, which is reflected in a reduced grain yield. For the P export, a positive correlation at 99% was found between the export of K and YIELD. Thus, the larger that the amount of P was, the higher the content of K and the higher the grain yield. These results agree with the findings of Wilczewski et al. (2014) for barley, who observed a positive correlation of export by the grain between the macronutrients P and K and between P and grain yield. Wilczewski et al. (2013) found a similar correlation between N and P but not between P and YIELD.

For N export during the favorable growing season, a significant positive correlation at 99% was found with the export of P. That is, the larger that the amount of N was, the higher the levels of P. It is known that N and P interact in the plant because of their functions. In this sense, Miller (1974) states that the greater absorption of P in the presence of N is caused by the interaction of N in the processes of absorption and transport of P, as observed in this research.

For the P export, a significant positive correlation at 95% was found with YIELD. Thus, the larger that the amount of P is, the higher the grain yield. This nutrient appears to be closely linked to grain yield, both in favorable or unfavorable growing seasons. Regarding climatic conditions, P was strongly correlated with yield, as reported by Wilczewski et al. (2014).

Barley is subject to climatic instabilities, which act directly on the growth and development of the crop, affecting the yield and quality of the produced grain. According to this research, the responses for macronutrient export and grain yield differ depending on the cultivar and between favorable and unfavorable growing seasons. However, even in distinct growing seasons, the export of N and P nutrients are positively correlated, and the export of P and grain yield are positively correlated, indicating that these parameters are closely linked.

Conclusion

The 250 kg dose the NPK formulated with HS increased nitrogen export in grain. The highest levels were found for BRS Brau cultivar and under favorable climatic conditions.

The barley under favorable climatic conditions and export of phosphorus in grain was not influenced by the HS, with the higher levels found for BRS Brau cultivar.

At 100 kg dose with NPK formulated with HS, the potassium export to grain increased, with highest levels found for BRS Brau cultivar regardless of climatic conditions.

There is correlation between the nitrogen and phosphorus levels in grain and between phosphorus levels and grain yield.

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