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ORIGINAL ARTICLE

The influence of *Hermetia illucens* L. frass on the health, stress, and development of barley

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Abstract

Barley cultivation faces challenges from changing climate conditions, including the increasing threat of drought. This study explored the potential of a fertilizer derived from *Hermetia illucens* L. frass to enhance the development and health of spring barley under optimal and drought conditions. The experiment, conducted in a controlled greenhouse environment, employed various fertilization treatments, including cattle manure and two doses of *H. illucens* L. frass-based fertilizer. Comprehensive assessments were made through visual observations and physiological measurements, including chlorophyll fluorescence, leaf gas exchange, and CO_2 exchange between the soil and the atmosphere. The results demonstrated that the application of *H. illucens* L. frass-based fertilizer significantly improved barley vigor and health compared to the control and cattle manure treatments, especially under drought stress. Physiological measurements revealed positive effects on chlorophyll fluorescence parameters, indicating enhanced photosynthetic efficiency. Leaf gas exchange parameters also reflected improved photosynthetic activity, with the *H. illucens* L. frass-treated plants outperforming others. This study provides valuable insights into the potential of insect-derived fertilizers, particularly *H. illucens* L. frass, as a sustainable and effective way to enhance crop resilience to drought. As climate change continues to pose challenges to agriculture, incorporating such novel fertilizers may offer a promising avenue for sustainable crop production.

Keywords: drought, fertilizer, insect frass, seedling blight

Introduction

Barley (*Hordeum vulgare* L.) is an important crop species (Langridge 2018). Both spring and winter forms can be found (von Zitzewitz *et al.* 2005). It is used in the production of groats, animal feed as well as in the brewing industry (Lukinac and Jukić 2022). The yield of cultivated plants depends on many factors, such as, climatic and soil conditions as well as the occurrence of diseases, pests, and weeds (Ngoune and Charles 2020; Galon *et al.* 2022). For proper development barley has specific water and fertilization requirements (Cossani *et al.* 2012). Droughts are becoming one of the biggest problems in many parts of the world (Balti *et al.* 2020). Water deficiency adversely affects the course of photosynthesis and limits the uptake and transport of nutrients (Rouphael *et al.* 2012). One way to prevent the effects of water shortage is balanced fertilization of crops (Nawaz *et al.* 2020). It is also important in the context of influencing plant disease resistance (Huber *et al.* 2012). Currently, more and more attention is being paid to reducing the use of mineral fertilizers (Montanarella and Panagos 2021). However, in many countries there is a decrease in the number of livestock (Popescu *et al.* 2022).

The rearing of insects is gaining in importance on a global scale (van Huis 2020). Among the most commonly used are those belonging to the species *Hermetia illucens* L. They process organic waste into nutrients that can be used in animal nutrition (Siddiqui *et al.* 2022). They are also used in the chemical industry (De Smet *et al.* 2018). The fat fraction obtained from these insects can be used in the production of biofuel (Franco *et al.* 2021). Various species of insects can also be used in human nutrition (Hawkey *et al.* 2021). The European Union has recently introduced additional regulations enabling the use of insects in human nutrition (Commission Implementing Regulation 2023). Insect frass, on the other hand, can become a valuable fertilizer that can be used in agriculture. Attention is drawn here to their valuable properties, which include the content of nutrients easily absorbed by plants, the impact on reducing plant stress and the content of microorganisms and biomolecules that stimulate plant growth (Poveda *et al.* 2019). Chitin contained in such fertilizers may have a beneficial effect on plant health (Chavez and Uchanski 2021).

The aim of this research was to evaluate the effect of a fertilizer based on *H. illucens* L. frass on the development and health of spring barley under optimal and drought conditions

Materials and Methods

The experiment was carried out in a greenhouse belonging to the Department of Agronomy of the Poznań University of Life Sciences. Spring barley (variety Penguin) was sown in 1-liter pots, which were filled with a soil BIOBIZZ LIGHT-MIX (Biobizz Worldwide SL, Bizkaia Basque Country, Spain). Soil was determined using the ThetaProbe Soil Moisture Sensor (ThetaProbe, Eijkelkamp, The Netherlands). The photoperiod was kept at the level of 16 h day: 8 h night. Natural sunlight was supplemented with sodium lamps (HPS) with a capacity of 400 W (Elektro-Valo Oy. Netafim. Avi: 13473, Uusikaupunki, Finland). Air humidity in the greenhouse was maintained at 60–80%. The air temperature was 25 ± 2°C during the day and 20 ± 2°C during the night.

Two doses of fertilizer containing insect frass (HI frass) and one reference fertilizer were used in the experiment. Cattle manure was dosed in accordance with the manufacturer's (GRUPA INCO S.A., Warsaw Poland) recommendations, at the dose 10 g · l^{-1}. The composition of cattle manure was: organic N – (%) of dry matter (d.m.) – 2.8; P(P_2O_5) – (%) d.m. – 2.8; K (K_2O) – (%) d.m. – 2; Mg (MgO) – (%) d.m. – 0.8; organic substances – (%) d.m. – 60. HI frass was applied at two doses: 10 and 12.5 g · l^{-1}. Analysis of the composition of the HiProMinie fertilizer indicated a high content of sulfur, potassium and iron (Table 1).

Table 1. Composition of fertilizer based on HI frass

Component	Unit average content	Unit average content
Zinc	mg kg d.m.$^{-1}$	2.1
Phosphorus	% d.m.	146
Magnesium	% d.m.	1.88
Fat	%	0.79
Copper	mg kg d.m.$^{-1}$	15.8
Potassium	% d.m.	2.62
Sulfur	mg kg d.m.$^{-1}$	7672
Calcium	% d.m.	0.65
Iron	mg kg d.m.$^{-1}$	389
Nitrogen	% d.m.	4.24
Organic substances	% d.m.	79.3
Dry mass	%	69.9
Ash in analytical state	%	12.1

Barley seeds were sown at the rate of 10 seeds · pot^{-1}. Five days after the emergence of the plants, their infection with seedling blight was assessed (0% – no plants with symptoms of the disease; 100% – complete death of the seedlings). During plant growth, the soil was watered every 48 h with 100 ml · pot^{-1}. Two parallel cultures were performed, one under the optimal constant irrigation system ('Control') and the other under drought stress ('Drought'). In order to induce drought stress, watering was discontinued at 25 days after sowing the seeds. All combinations were tested in four replicates.

After 7 days of such imposed drought, during which the soil volume moisture content was monitored daily using a probe (ThetaProbe, Eijkelkamp, Netherlands), the soil moisture level of 6 to 8 volume % was achieved. The control plants were provided with the optimum soil moisture for all analyzed variants at the level of 20 to 22 vol. %. At that time, a visual assessment of the effect of fertilizers on the development of barley under drought conditions was made. The control was treated as 100% (results are given in %). Using the Fluorometer OS5p OPTISCIENCES.INC., Hudson, USA, the following parameters were measured after dark adaptation: F_0 – minimum fluorescence of dark-adapted state, Fm – maximum fluorescence of dark-adapted state, Fv/Fm – maximum quantum yield of PSII photochemistry. In light: yield – quantum yield of photosynthetic energy, and ETR – electron transport rate were measured. The leaf gas exchange of plants was assessed using the LCpro-SD apparatus, ADC BioScientific Ltd., UK, based on the following parameters: A – CO_2 assimilation level, E – transpiration, Gs – stomatal conductance, Ci – intercellular CO_2 concentration. In addition, this apparatus is equipped with special cylinders for measuring soil gas exchange based on changes in CO_2 and H_2O concentrations per unit of time. A cylinder with a soil respiration measurement chamber installed is used to enclose a volume of air to measure the gas exchange between the soil and the atmosphere due to biomass activity. The construction of the chamber consists of an acrylic dome with a built-in fan for mixing air and a relief valve to prevent an excessive pressure gradient inside the chamber. The parameter measured and calculated by the apparatus is NCER- netto CO_2 exchange rate. It was assessed after harvesting the barley, 10 days after drought stress was initiated.

Results were analyzed with Statistica 13 software (StatSoft Ltd., Kraków, Poland). Analysis of variance (two-way ANOVA) to determine significant differences between treatments was used. Means were separated by protected Tukey`s HSD test at $p = 0.05$.

Results

Table 2. Visual assessment of plant infection with seedling blight

Fertilizer	Dose [g · l⁻¹]	Visual assessment of plant infection [%]
Control without fertilization	–	8.8 a
Cattle manure	10.0	0.0 b
HI frass	10.0	0.0 b
HI frass	12.5	0.0 b
Tukey's HSD *p* = 0.05		1.54
SD		1.02
CV		69.99

Different letters a–b indicate statistically different mean values (α = 0.05)

The visual assessment of plant infection indicated that the plants were infected with seedling blight only in control combinations (Table 2). Application of all fertilization variants had a positive effect on the development of barley, both under optimal water conditions and during drought stress (Fig. 1). The most beneficial effect on vigor was found with a higher dose of fertilizer based on HI frass, which was confirmed statistically.

An analysis of the results of the plant chlorophyll fluorescence showed that there was a statistically significant effect of both doses of fertilizer based on HI frass on the parameters measured after plant adaptation to dark (Table 3). The decrease in the value of the minimum fluorescence (F_0), the increase in the value of the maximum fluorescence (Fm) as well as the maximum quantum yield of PSII photochemistry (Fv/Fm) indicated that the test plants were in better condition. Parameters measured in the light (Yield and ETR) (Table 4) under optimal conditions and with drought stress also showed a beneficial effect of the applied fertilizers on the condition of spring barley. The analysis of most of the studied fluorescence parameters showed no significant differences between the two doses of the fertilizer based on HI frass. However, significantly

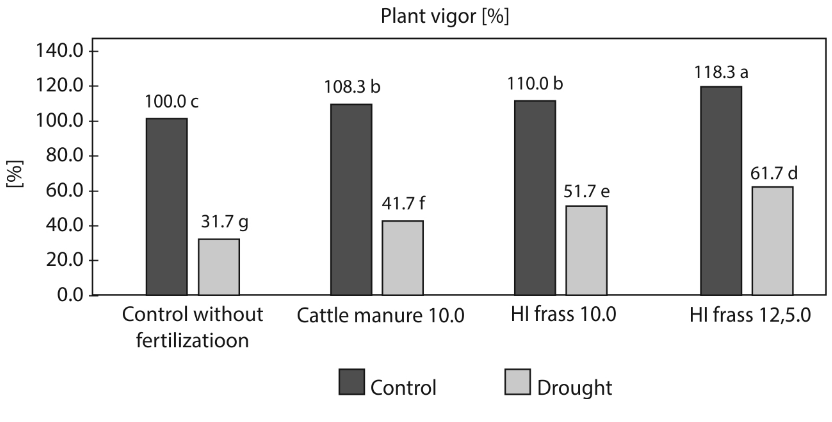

Fig. 1. Visual assessment of plant vigor in selected fertilization variants. The values given next to the fertilizer names are doses (g · l⁻¹). Different letters a-g indicate statistically different mean values (α = 0.05). Tukey's HSD(0.05) = 7.37

Table 3. Parameters of plant chlorophyll fluorescence measured after dark adaptation under optimal conditions and drought stress depending on the fertilizer used

Fertilizer	Dose [g · l^{-1}]	F_0 – minimum fluorescence	
		control	drought
Control without fertilization	–	316.0 ± 6.9 a	320.5 ± 6.4 a
Cattle manure	10.0	268.0 ± 7.0 bcd	278.5 ± 6.5 b
HI frass	10.0	261.0 ± 7.4 cd	268.3 ± 6.1 bc
HI frass	12.5	255.2 ± 3.8 d	260.0 ± 7.3 cd
Tukey's HSD *p* = 0.05		13.08	
SD		4.54	
CV		1.63	

Fertilizer	Dose [g · l^{-1}]	Fm – maximum fluorescence	
		control	drought
Control without fertilization	–	1214.3 ± 11.6 e	1170.7 ± 5.5 f
Cattle manure	10.0	1286.2 ± 5.7 c	1201.8 ± 5.8 e
HI frass	10.0	1394.5 ± 1.6 a	1291.2 ± 8.3 c
HI frass	12.5	1313.5 ± 3.4 b	1251.8 ± 10.1 d
Tukey's HSD *p* = 0.05		16.91	
SD		5.87	
CV		0.46	

Fertilizer	Dose [g · l^{-1}]	Fv/Fm – maximum quantum yield of PSII photochemistry	
		control	drought
Control without fertilization	–	0.729 ± 0.006 d	0.7312 ± 0.004 d
Cattle manure	10.0	0.778 ± 0.002 c	0.777 ± 0.004 c
HI frass	10.0	0.799 ± 0.004 a	0.788 ± 0.004 b
HI frass	12.5	0.800 ± 0.007 a	0.791 ± 0.003 b
Tukey's HSD *p* = 0.05		0.0074	
SD		0.0026	
CV		0.33	

Different letters a-f indicate statistically different mean values (α = 0.05)

Table 4. Plant chlorophyll fluorescence parameters measured in light under optimal conditions and drought stress depending on the fertilizer used (unnominated units)

Fertilizer	Dose [g · l^{-1}]	Yield – quantum yield of photosynthetic energy	
		control	drought
Control without fertilization	–	0.302 ± 0.004 c	0.174 ± 0.004 f
Cattle manure	10.0	0.312 ± 0.004 b	0.172 ± 0.004 f
HI frass	10.0	0.314 ± 0.004 b	0.181 ± 0.003 e
HI frass	12.5	0.336 ± 0.006 a	0.193 ± 0.006 d
Tukey's HSD *p* = 0.05		0.0073	
SD		0.0025	
CV		1.02	

Fertilizer	Dose [g · l^{-1}]	ETR – electron transport rate	
		control	drought
Control without fertilization	–	44.217 ± 0.313 c	23.967 ± 1.189 f
Cattle manure	10.0	44.800 ± 0.219 c	25.253 ± 0.709 ef
HI frass	10.0	47.250 ± 0.327 b	26.072 ± 0.407 e
HI frass	12.5	48.683 ± 0.671 a	28.985 ± 1.102 d
Tukey's HSD *p* = 0.05		1.40	
SD		0.49	
CV		1.35	

Different letters a-f indicate statistically different mean values (α = 0.05) more favorable values of the tested parameters were found, showing a higher efficiency of photosystem II under drought conditions in plants treated with HiProMine fertilizer, than in plants not fertilized and fertilized with cattle manure. These differences were less visible in plants kept under appropriate humidity conditions.

The assessment of the leaf gas exchange was based on CO_2 assimilation (A), H_2O transpiration (E), stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) (Table 5). The highest level of the value of the first of the mentioned parameters, and thus the highest efficiency of photosynthesis, both under optimal conditions and drought, was recorded for plants to which fertilizer based on HI frass was applied. The application of 10 g · l^{-1} of the mentioned fertilizer turned out to be the most beneficial. For the level of transpiration, stomatal conductance and intercellular CO_2 concentration, no statistically significant differences were found for plants growing under conditions of optimal hydration. For plants exposed to drought, the highest level of transpiration was found for those fertilized with HI frass. The use of all types of fertilizers had a positive effect on stomatal conductance under drought stress conditions, but did not have a statistically significant effect on intercellular CO_2 concentration.

The analysis of measurements of CO_2 emissions, indicating the formation of the root mass, showed significant differences under the conditions of optimal hydration (Fig. 2). The highest level of soil respiration was found for a dose of 12.5 g · l^{-1} of fertilizer based on HI frass. With drought stress, no statistically significant differences were found for this parameter, however, an upward trend was observed for the fertilized objects.

Table 5. Parameters of barley leaf gas exchange under optimal conditions and drought stress depending on the fertilizer used

Fertilizer	Dose [g · l^{-1}]	A – CO_2 assimilation level [µmol $m^{-2} \cdot s^{-1}$]	
		control	drought
Control without fertilization	–	13.367 ± 1.038 bc	0.713 ± 0.070 e
Cattle manure	10.0	13.857 ± 1.080 bc	3.137 ± 0.313 e
HI frass	10.0	16.993 ± 1.686 a	12.427 ± 1.245 cd
HI frass	12.5	15.667 ± 1.213 ab	9.310 ± 0.640 d
Tukey's HSD $p = 0.05$		3.1205	
SD		1.0830	
CV		10.14	

Fertilizer	Dose [g · l^{-1}]	E – transpiration [mmol $m^{-2} \cdot s^{-1}$]	
		control	drought
Control without fertilization	–	3.177 ± 0.431 a	0.230 ± 0.026 c
Cattle manure	10.0	3.313 ± 0.495 a	0.553 ± 0.029 c
HI frass	10.0	3.680 ± 0.171 a	2.047 ± 0.145 b
HI frass	12.5	3.530 ± 0.488 a	1.543 ± 0.025 b
Tukey's HSD $p = 0.05$		0.780	
SD		0.271	
CV		11.99	

Fertilizer	Dose [g · l^{-1}]	Gs – stomatal conductance [mol $m^{-2} \cdot s^{-1}$]	
		control	drought
Control without fertilization	–	0.213 ± 0.025 a	0.003 ± 0.006 c
Cattle manure	10.0	0.220 ± 0.060 a	0.020 ± 0.000 bc
HI frass	10.0	0.280 ± 0.030 a	0.100 ± 0.010 b
HI frass	12.5	0.243 ± 0.061 a	0.070 ± 0.010 bc
Tukey's HSD $p = 0.05$		0.0823	
SD		0.0286	
CV		19.87	

Different letters a–e indicate statistically different mean values (α = 0.05)

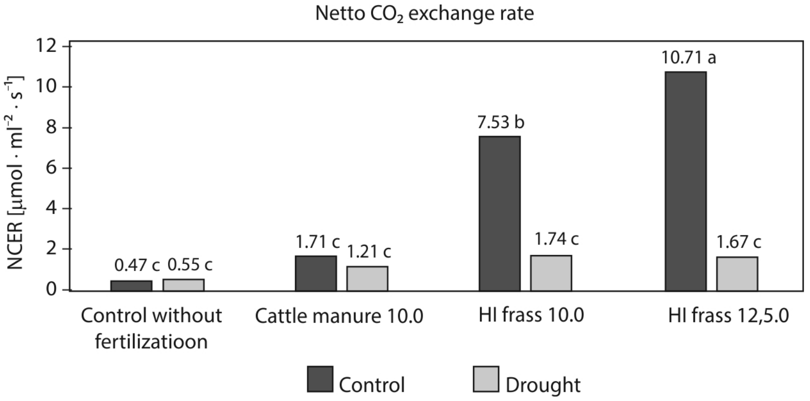

Fig. 2. CO_2 exchange between the soil and the atmosphere under optimal conditions and drought stress depending on the fertilizer used. The values given next to the fertilizer names are doses (g · l^{-1}). Different letters a-c indicate statistically different mean values (α = 0.05). Tukey's HSD(0.05) = 2.480

Discussion

Drought may contribute to a significant decrease in the yield of spring barley (Trnka *et al.* 2007). One of the factors that make it possible to reduce the negative impact of meteorological conditions is a balanced level of fertilization (Sanina 2020). In studies by Radzikowska *et al.* (2023) and Poveda *et al.* (2019) the use of a fertilizer based on insect frass had a positive effect on drought tolerance of plants. In the described experiment, the application of HI frass influenced the reduction of plant stress caused by drought to a greater extent than cattle manure. In the experiment, the use of HI frass contributed to an increase in plant health. Chitin contained in insect frass may have a beneficial effect on plant disease resistance (Barragán-Fonseca *et al.* 2022).

Iron in plants is involved in many key processes, such as photosynthesis and respiration, by participating in the transport of electrons (Rout and Sahoo 2015). Potassium is an element involved in the water management of plants (Hasanuzzaman *et al.* 2018). It increases tolerance to water stress (Cai *et al.* 2019). The deficiency of this nutrient is one of the greatest challenges for agriculture worldwide (Al Azzawi *et al.* 2021). Sulfur, and other elements, in plants is a component of proteins and amino acids (Čolović *et al.* 2018). Sulfur compounds are involved in photosynthesis and respiration (Nakai and Maruyama-Nakashita 2020). This macro-element affects the uptake and use of nitrogen (Capaldi *et al.* 2015). The high content of these elements in the test fertilizer could have a positive effect on the development of the tested plants. As reflected in their development these ingredients perform many important functions in plants which were assessed visually and by physiological measurements. In the described experiment, the test fertilizer supported the development of spring barley to a greater extent than cattle manure. In research conducted by Borkent and Hodge (2021), it was found that a fertilizer based on *H. illucens* L. frass has a positive effect on dry matter, the development of roots and aboveground shoots of herbs and vegetables.

In the present research, the use of a fertilizer based on HI frass had a positive effect on the parameters of plant chlorophyll fluorescence. This applied to both optimal conditions and drought stress. The more favorable results of plant chlorophyll fluorescence achieved in the described experiment for the fertilized combinations than the control testify to the plants being in better condition than those that grew in soil without fertilizers. The research of other scientists also indicate that appropriate fertilization gave beneficial plant chlorophyll fluorescence results (Sturzeanu *et al.* 2017; Veres *et al.* 2019).

The use of a fertilizer based on HI frass had a positive impact on most leaf gas exchange parameters. This proves the better condition of plants and higher efficiency of photosynthesis (Xiaoli and Weikai 2011; Haworth *et al.* 2018). Other scientists also found a beneficial effect of balanced fertilization on such parameters (Ullah *et al.* 2020). The application of fertilizers, especially the higher dose of HI frass, had a positive effect on soil respiration and CO_2 exchange between the soil and the atmosphere. This indicates a high level of development of the root system of the test plants, as the level of soil respiration is related to the root biomass (Lee 2018). Deficiency of certain nutrients can significantly affect the development of underground parts of plants (Forde and Lorenzo 2001). In this experiment, this effect was leveled after the application of the test fertilizer.

Conclusions

Research on new sources of fertilizers is an important part of agricultural science. The results of the present research indicated that the fertilizer based on *H. illucens* L. frass had a positive effect on the development and health of spring barley under optimal conditions and under drought stress. This was confirmed both by visual assessment of plant vigor and by measurements of plant chlorophyll fluorescence, leaf gas exchange and soil respiration. This gives hope for the possibility of using waste from rearing insects in agricultural production, protecting plants against drought stress in the context of current climate change.

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