ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Agricultura 10(1) 2011, 79-86*

EFFECT OF HUMIC SUBSTANCES ON GERMINATION OF WHEAT AND BARLEY UNDER LABORATORY CONDITIONS

Małgorzata Szczepanek, Edward Wilczewski
University of Technology and Life Sciences in Bydgoszcz

Abstract. The effect of grain dressing with humic substances contained in the preparation Humistar (Humifirst) on the germination energy and capacity, as well the dynamics of the initial growth and development of wheat and barley were assessed in laboratory study. It was showed that the application of the preparation did not affect the proportion of kernals germinating normally in the laboratory assessment of germination energy and capacity of the tested cereal species. Dressing barley grain with Humistar stimulated elongation and growth in the germ mass as early as in the first week of germination, and of the root mass in the second week. A positive effect of the preparation on the growth of wheat seedling fresh mass was observed after 14 and 21 days of development.

Keywords: coleoptile, germ, germination capacity, root, seedling

INTRODUCTION

Humic substances exert an influence on the physical, chemical and biological properties of soil, and consequently, they indirectly affect the growth and development of plants. The research of the application of these substances to soil or on leaves indicated that they have also a direct effect, positive or limiting. The plant response depends on the source of humus and the chemical composition related to it [Muscolo et al. 2007], as well as molecular mass [Marino et al. 2008]. Fractions with a small mass easily get to the plasmalemma of higher plant cells, whereas heavier fractions can cooperate only with the cell wall [Nardi et al. 2002]. The dose is also of some importance. It was indicated that humic substances applied in small amounts had a positive effect on growth in barley shoots and roots, but their too high concentration limited growth [Ayuso et al. 1996].

The effect on metabolism is not well known and it may refer to both respiration and photosynthesis [Nardi et al. 2002]. Humic substances have also an effect similar to that of hormones, whereas it is not clear whether it depends on their chemical structure or

Corresponding author – Adres do korespondencji: dr inż. Małgorzata Szczepanek, Department of Plant Cultivation of University of Technology and Life Sciences in Bydgoszcz,
Ks. A. Kordeckiego 20, 85-225 Bydgoszcz, e-mail: szczepan@utp.edu.pl

the molecular mass [Nardi et al. 2002]. Using them at various developmental stages stimulates rooting [Cooper et al. 1998], as well as the growth of aboveground parts [Ayuso et al. 1996]. They can also have a positive effect on nutrient uptake [Cooper et al. 1998, Asik et al. 2009] and yielding of field crops, e.g. potatoes or grasses [Verlinden et al. 2009, 2010].

Humic preparations of various origin are applied in vegetable and agricultural crops [Ayuso et al. 1996, Cooper et al. 1998], in the solid form, e.g. built in the granules of mineral fertilizers, or in the liquid form [Verlinden et al. 2010]. They can be applied into soil or on leaves [Fernandez-Escobar et al. 1996, Cooper et al. 1998]. Dressing seed material with them can be one of easy and cheap ways of their introduction into soil.

The aim of this study was to assess the effect of humic substances on germination energy and capacity and the dynamics of the initial growth and development of spring barley and spring wheat under laboratory conditions.

The working hypothesis assumed that the application of humic substances directly on wheat and barley grains can have a significant effect on germination process, because of their large concentration in the outer part of the grain,

MATERIAL AND METHODS

A laboratory study carried out in the Department of Plant Cultivation, the Faculty of Agriculture and Biotechnology UTP in Bydgoszcz, in 2006-2008, aimed at assessing the effect of dressing grain of the spring wheat 'Monsun' and spring barley 'Mauritia' with humic substances contained in the liquid form of the preparation on germination energy and capacity and the dynamics of plant initial growth and development. The carrier of humic substances was Humistar (= Humifirst). The preparation in the liquid form was used, with black color and acid reaction, containing 12% of humic acids and 3% fulvic acids. It is an extract of leonardite – a highly oxidized form of brown coal from Canada [Verlinden et al. 2010]. Seed material of cereals dressed with a fungicide, intended for field experiments being the continuation of the research subject realized, was thoroughly covered with Humistar in a seed dresser for 5 minutes. A dose of 1 dm^3 of the preparation was applied per 50 kg of grain. Samples of the grain were placed in plastic cuvettes lined with two layers of medium filter paper, saturated with distilled water. In each cuvette, 100 grains dressed with the humic extract or undressed (the control) were placed, in four replications, separately for wheat and barley. The cuvettes were placed in a room with a temperature of 20+/–1°C. Based on daily control, the constant moisture of paper was maintained by adding distilled water.

According to the assumptions concerning investigation of germination energy and capacity comprised in the ISTA method [Anonim 2008], the first counting of germinated grains (germination energy) was carried out after 4 days from sowing. The proportion of normally germinating grains, forming germs with a length of at least 2 mm, was determined.

Germination capacity (final counting) in the case of barley was determined after 7 days, and of wheat after 8 days from the beginning of the test. The number and proportion of normally and abnormally germinating, fresh (swelling but not forming a root or germ) and dead (decaying and mildewing) grains was determined.

The dynamics of the initial growth and development of cereals was assessed after 7, 14 and 21 days from sowing. Each time 20 normal seedlings from each replication

were collected randomly. After cutting off grains, the length of each germ (coleoptile or the first leaf) was measured and the germs and roots of the whole sample collected were weighed separately. To determine the absolutely dry mass of roots and germs, the plant material was dried at 50°C and after cooling the samples in an exsiccator it was weighed on a laboratory balance enabling making the reading to an accuracy of 0.001g.

The results obtained were normalized and subject to the analysis of variance using the computer software AWAR, worked out by IUNG in Puławy, for the completely randomized design. The significance of differences was determined with Tukey's confidence half-interval at the significance level P = 0.05.

RESULTS AND DISCUSSION

Spring wheat germination energy observed in the study was high, and that of spring barley was very high (Fig. 1). This was not depended on dressing grain with Humistar. A significant effect of this preparation on the proportion of normally germinating grains in the assessment of germination capacity was not observed either (Fig. 2). The proportion of spring wheat grains germinating abnormally was significantly lower, and that of fresh grains – higher in samples dressed with the humic extract, as compared with the control. A significant effect of Humistar on the number of dead grains of spring wheat was not found. In the case of spring barley, only a significantly higher number of dead grains in samples dressed with Humistar was observed as compared with the samples without the preparation.

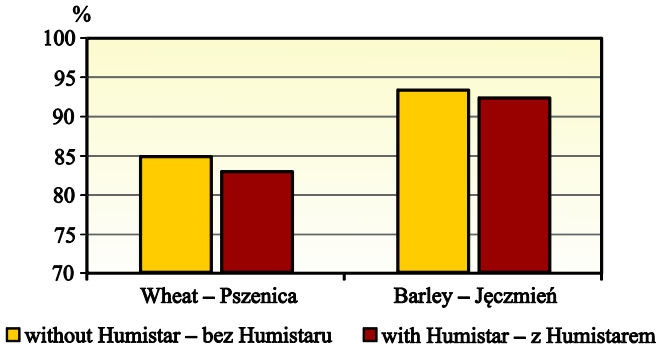
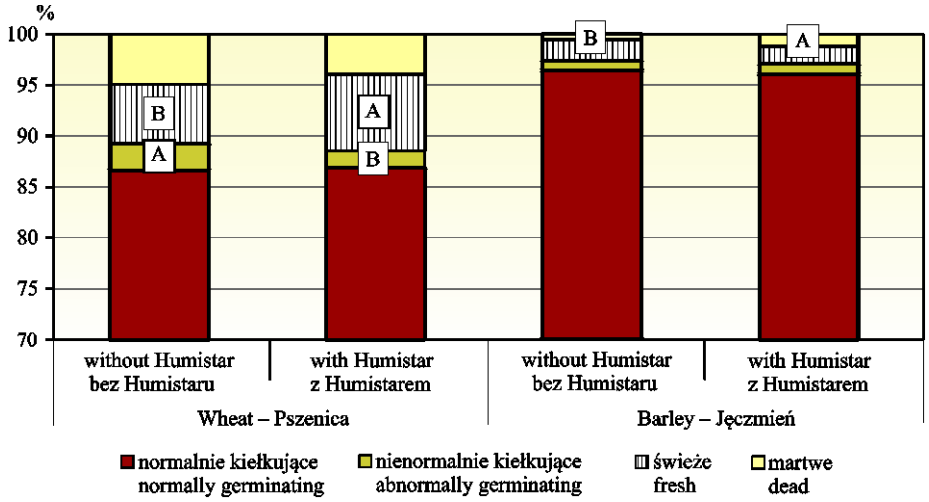


Fig. 1. Proportion of normally germinating kernels of wheat and barley after 4 days of test (germination energy) depending on application of Humistar

Rys. 1. Udział ziaren normalnie kiełkujących pszenicy i jęczmienia po 4 dniach testu (energia kiełkowania) w zależności od zastosowania Humistaru



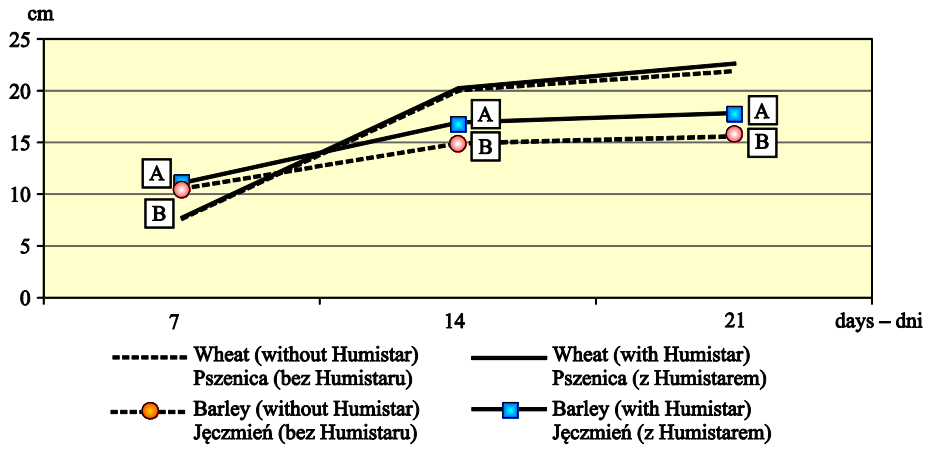
A, B – means followed different letters differ significantly – średnie oznaczone różnymi literami różnią się istotnie

Fig. 2. Proportion of normally and abnormally germinating, fresh and dead kernels of wheat and barley, after 7 and 8 days of test, respectively (germination capacity) depending on application of Humistar

Rys. 2. Udział ziaren normalnie i nienormalnie kiełkujących, świeżych i martwych pszenicy i jęczmienia, odpowiednio po 7 i 8 dniach testu (zdolność kiełkowania) w zależności od zastosowania Humistaru

A differentiation of the growth rate of the germ (coleoptile) or the first leaf was observed in the tested species. In barley, as early as after 7 days the germ obtained 67% of the length obtained during three weeks of development, whereas in wheat it was only 35%. Wheat germs grew more intensively in the second week. During the last seven days the growth of both species was small and accounted for 5-10% of the length obtained during three weeks of germination.

Grain dressing with the humic preparation stimulated the germ growth only in the case of quickly developing barley seedlings (Fig. 3). The difference was significant after 7, 14 and 21 days of the test. However, a significant effect of Humistar on the length of wheat germs was not found. It was indicated that spring barley grain dressed with Humistar formed significantly heavier germs than not dressed grain (Table 1). A positive effect of this preparation on the fresh and dry mass of germs was observed at all the measurement dates. A quicker growth of the above-ground part of barley after the application of humic substances derived from leonardite was already shown earlier in hydroponic cultivation [Ayuso et al. 1996].



A, B – for explanations, see Fig. 2 – objaśnienia pod rys. 2

Fig. 3. Germ length of wheat and barley after 7, 14 and 21 days of test
Rys. 3. Długość kiełka pszenicy i jęczmienia po 7, 14 i 21 dniach testu

Table 1. Germ and root mass of 20 barley seedlings after 7, 14 and 21 days of the test (mean of 2006-2008), g
Tabela 1. Masa kiełków i korzonków 20 siewek jęczmienia po 7, 14 i 21 dniach testu (średnia z lat 2006-2008), g

Number of days Liczba dni	Seedling element – Element siewki					
	germ – kiełek		root – korzonek		total – razem	
	control kontrola	Humistar	control kontrola	Humistar	control kontrola	Humistar
Fresh mass – Świeża masa						
7	B 1.66	A 1.85	A 1.45	B 1.27	3.11	3.12
14	B 2.20	A 2.62	B 1.96	A 2.13	B 4.16	A 4.75
21	B 2.25	A 2.82	B 1.96	A 2.10	B 4.21	A 4.93
Dry mass – Sucha masa						
7	B 0.137	A 0.158	0.141	0.126	0.277	0.284
14	B 0.142	A 0.173	B 0.156	A 0.161	B 0.298	A 0.334
21	B 0.127	A 0.163	0.152	0.156	B 0.281	A 0.318

A, B – for explanations, see Fig. 2 – objaśnienia pod rys. 2

Fresh mass of barley roots which grew from grains treated with the humic preparation was after 7 days from sowing significantly lower and after 14 and 21 days significantly higher than that in the control (Table 1). The effect of Humistar on dry mass of roots was less distinct, as under the influence of this preparation it was significantly higher only at the second measurement date. A stimulating effect of the extract of leonardite on the root mass was also observed in marsh bent grass (*Agrostis stolonifera*) [Cooper et al. 1998]. After the introduction of humic substances into soil the root mass in the layer 0-10 cm increased by 45%, and the maximal root length by 15%, as compared with the control. Canellas et al. [2002], in turn, obtained a higher number of lateral roots and the increase in total radicular length in seedlings of maize treated with humic acids, which had a direct influence on an increase in the total root

area. Additionally, the study showed an increase in the activity of H^+-ATP-ase and a fast growth of mitosis areas at the zone of elongation and differentiation of maize roots. There were no differences until 3 day, whereas from 4 day there were 7-12 times more of mitosis areas (hyperinduction) in the variant with humic acids, as compared with the control.

The effect of Humistar on the fresh mass of wheat germs after 7 days of germination was not observed (Table 2). After 14 and 21 days germs grown from dressed grains were by 4.9% and 13.7% heavier, respectively, than in the control. A significant, positive effect of Humistar on the fresh mass of the whole wheat seedlings was also found at these dates. A positive effect of the humic preparation on the fresh mass of spring wheat roots was statistically proved at the second measurement date.

Table 2. Germ and root mass of 20 wheat seedlings after 7, 14 and 21 days of the test (mean of 2006-2008), g
Tabela 2. Masa kiełków i korzonków 20 siewek pszenicy po 7, 14 i 21 dniach testu (średnia z lat 2006-2008), g

Number of days Liczba dni	Seedling element – Element siewki					
	germ – kiełek		root – korzonek		total – razem	
	control kontrola	Humistar	control kontrola	Humistar	control kontrola	Humistar
Fresh mass – Świeża masa						
7	1.27	1.24	0.94	0.97	2.20	2.21
14	B 2.23	A 2.34	B 2.10	A 2.34	B 4.32	A 4.68
21	B 2.41	A 2.74	2.37	2.57	B 4.77	A 5.31
Dry mass – Sucha masa						
7	A 0.126	B 0.122	A 0.100	B 0.098	A 0.225	B 0.220
14	0.242	0.228	A 0.200	B 0.195	A 0.442	B 0.423
21	B 0.226	A 0.238	B 0.189	A 0.205	B 0.415	A 0.443

A, B – for explanations, see Fig. 2 – objaśnienia pod rys. 2

The effect of Humistar on the dry mass of wheat seedlings was varied in time. At the first measurement date (after 7 days) it was significantly negative both for the mass of germs and roots. Similarly, after 14 days of development, the dry mass of roots and whole seedlings which grew from grains treated with Humistar was significantly smaller than that in the control. At the last measurement date a statistically significant, positive effect was observed of this preparation on the mass of dried germs and roots of spring wheat. The inhibition of the dry mass growth of seedling elements in the first and second week of germination may indicate too large absorption of substances contained in Humistar by naked grains of wheat. These compounds may have limited the action of gibberellins and decomposition of starch into small-particle substances transported to new cells. The Receptor of gibberellins responsible for the synthesis of α-amylase (the enzyme hydrolyzing starch in the endosperm of cereal grains) is the aleurone layer, occurring directly under the fruit-seed coat of the grain [Lewak 2005].

CONCLUSIONS

1. Dressing of seed material of wheat and barley with Humistar did not have an effect on the proportion of grains germinating normally in the laboratory assessment of germination energy and capacity.
2. The application of humic substances contained in Humistar on grain caused the elongation and mass growth of barley germs as early as in the first 7 days of germination, as well as of root mass in the second week of the seedling development. A positive effect of the preparation on the growth of fresh mass of wheat roots and germs was shown after 14 and 21 days of development.
3. A favorable effect of dressing wheat and barley grain with Humistar on the development of seedlings mainly justifies the need for further research into plant response under field conditions. This will enable the full assessment of usefulness of this treatment for agricultural practice.

REFERENCES

Anonim, 2008. International Rules for Seed Testing. International Seed Testing Association (ISTA), Polska Wersja Wydania, IHAR Radzików.

Asik B., Turan M., Celik H., Katkat A., 2009. Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihli) under conditions of salinity. Asian J. Crop Sci. 1(2), 87-95

Ayuso M., Hernandez T., Garcia C., Pascual J., 1996. Stimulation of barley growth and nutrient absorption by humic substances originating from various organic materials. Biores. Technol. 57, 251-257.

Canellas L., Olivares F., Okorokova-Facanha A., Facanha A., 2002. Humic acids isolated from earthworm compost enhance root elongation, lateral root emergence, and plasma membrane H+-ATPase activity in maize roots. Plant Physiol. 130, 1951-1957.

Cooper R., Chunhua L., Fisher D., 1998. Influence of humic substances on rooting and nutrient content of creeping bentgrass. Crop Sci. 38, 1639-1644.

Fernandez-Escobar R., Benlloch M., Barranco D., Duenas A., Guterrez Ganan J., 1996. Response of olive trees to foliar application of humic substances extracted from leonardite. Sci. Hortic. 66, 191-200.

Lewak S., 2005. Kiełkowanie nasion [Seed germination]. [In:] Fizjologia roślin, J. Kopcewicz, S. Lewak (eds), PWN Warszawa, 485-497 [in Polish].

Marino G., Francioso O., Carletti P., Nardi S., Gessa C., 2008. Mineral content and root respiration of in vitro grown kiwifruit plantlets treated with two humic fractions. J. Plant Nutr. 31, 1074-1090.

Muscolo A., Sidari M., Attina E., Francioso O., Tugnoli V., Nardi S., 2007. Biological activity of humic substances is related to their chemical structure. Soil Sci. Soc. Am. J. 71(1), 75-85.

Nardi S., Pizzeghello D., Muscolo A., Vianello A., 2002. Physiological effects of humic substances on higher plants. Soil Biol. Biochem. 34, 1527-1536.

Verlinden G., Coussens T., Vliegher A., Baert G., Haesaert G., 2010. Effect of humic substances on nutrient uptake by herbage on production and nutritive value of herbage from sown grass pastures. Grass Forage Sci. 65, 133-144.

Verlinden G., Pycke B., Mertens J., Debersaques F., Verheyen K., Baert G., Bries J., Haesaert G., 2009. Application of humic substances results in consistent increases in crop field and nutrient uptake. J. Plant Nutr. 32(9), 1407-1426.

WPŁYW SUBSTANCJI HUMUSOWYCH NA KIEŁKOWANIE PSZENICY I JĘCZMIENIA W WARUNKACH LABORATORYJNYCH

Streszczenie. W badaniach laboratoryjnych oceniano wpływ zaprawienia ziarna substancjami humusowymi zawartymi w preparacie Humistar (Humifirst) na energię i zdolność kiełkowania, a także dynamikę początkowego wzrostu oraz rozwoju pszenicy i jęczmienia. Laboratoryjna ocena energii i zdolności kiełkowania badanych gatunków zbóż wykazała, że zastosowanie preparatu nie wpływało na udział ziaren kiełkujących normalnie. Zaprawianie ziarna jęczmienia Humistarem stymulowało wydłużanie i przyrost masy kiełków już w pierwszym tygodniu kiełkowania, natomiast masy korzeni w drugim. Pozytywny wpływ preparatu na przyrost świeżej masy siewek pszenicy wykazano po 14 i 21 dniach rozwoju.

Słowa kluczowe: kiełek, koleoptyl, korzeń, siewka, zdolność kiełkowania

Accepted for print – Zaakceptowano do druku: 25.01.2011