Evaluation of germination capacity and selected biometric parameters (length and dry weight of roots and coleoptile) of sunflower seeds (*Helianthus annuus*) after application of preparations containing effective microorganisms (EM)

Ocena zdolności kiełkowania nasion oraz wybranych parametrów biometrycznych (długość oraz sucha masa korzeni i koleoptyla) słonecznika zwyczajnego (*Helianthus annuus*) po zastosowaniu preparatów zawierających efektywne mikroorganizmy (EM)

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Abstract

Seed germination and early growth microbiotest Phytotoxkit™ was used in the experiment, which consisted of 3 independent laboratory experimental series with one month intervals each and 3 replications. The aim of this study was to evaluate germination dynamics and capacity as well as selected biometric parameters after seed treatment with two preparations containing effective microorganisms: EM Farma (EMF) and EM Farma Plus (EMFP). Sunflower seeds *(H. annuus)* were chosen as the experimental material. Seeds soaked in distilled water were control objects (K) in these experiment. Apart from control (K), reference material was prepared in the form of two biostimulants: Kelpak SL (KSL) and gibberellic acid (GA_3). The effect of the two biopreparations EM Farma (EMF) and EM Farma Plus (EMFP) was beneficial related to germination capacity and biometric parameters of sunflower (*H. annuus*).

Key words: biometric parameters, effective microorganisms (EM), germination dynamics and capacity, microbiotest Phytotoxkit™, sunflower (*H. annuus*)

Streszczenie

Doświadczenie przeprowadzono z użyciem mikrobiotestu kiełkowania i wczesnego wzrostu roślin – Phytotoxkit™. Testy obejmowały 3 niezależne serie doświadczeń laboratoryjnych w odstępach miesięcznych po 3 powtórzenia. Celem doświadczenia była ocena dynamiki i zdolności kiełkowania oraz wybranych parametrów biometrycznych nasion po zastosowaniu dwóch preparatów zawierających efektywne mikroorganizmy: EM Farma (EMF) oraz EM Farma Plus (EMFP). Materiałem do badań były nasiona słonecznika zwyczajnego (*H. annuus*). Obiekt kontrolny (K) stanowiły nasiona, moczone jedynie w wodzie destylowanej. W doświadczeniu oprócz kontroli absolutnej (K), zastosowano również jako obiekty porównawcze dwa biostymulatory: Kelpak SL (KSL) oraz kw. giberelinowy (GA_3). W przeprowadzonym doświadczeniu stwierdzono korzystny wpływ zastosowanych biopreparatów EM Farma (EMF) i EM Farma Plus (EMFP), związany z polepszeniem dynamiki kiełkowania oraz wybranych parametrów biometrycznych słonecznika zwyczajnego (*H. annuus*).

Słowa kluczowe: efektywne mikroorganizmy (EM Farma i EM Farma Plus), mikrobiotest Phytotoxkit™, parametry biometryczne, słonecznik zwyczajny (*H. annuus*), zdolność i dynamika kiełkowania

Streszczenie szczegółowe

Badania obejmowały 3 serie doświadczeń laboratoryjnych, które przeprowadzono przy użyciu zmodyfikowanego mikrobiotestu Phytotoxkit™. Materiał do badań stanowiły nasiona słonecznika zwyczajnego (*H. annuus*), które moczono przez okres 6h w następujących roztworach preparatów: EM Farma (EMF), EM Farma Plus (EMFP), Kelpak SL (KSL) oraz w kwasie giberelinowym (GA_3). Obiekt kontrolny (K), stanowiły nasiona słonecznika zwyczajnego (*H. annuus*), które moczono jedynie w wodzie destylowanej, w takim samym czasie jak w roztworach preparatów. Eksperyment miał na celu zbadanie wpływu biopreparatów zawierających efektywne mikroorganizmy (EMF i EMFP) na dynamikę kiełkowania oraz wybrane parametry biometryczne (długość oraz suchą masę korzeni i koleoptyla) słonecznika zwyczajnego (*H. annuus*). Pierwszej oceny zdolności kiełkowania nasion słonecznika zwyczajnego (*H. annuus*) dokonano po 24 h od momentu założenia biotestu, a kolejne odczyty wykonywano po 48, 72 i 96 h, rejestrując obraz pojedynczej płytki testowej, przy pomocy aparatu cyfrowego. Natomiast do pomiarów długości korzeni i koleoptyla użyto programu analizy obrazu „Image Tools”. Po 120 h od chwili założenia biotestu, określono suchą masę korzeni oraz koleoptyla słonecznika zwyczajnego (*H. annuus*). Uzyskane wyniki badań posłużyły do sporządzenia wykresów, na których przedstawiono efekt zastosowania biopreparatów EM Farma (EMF) i EM Farma Plus (EMFP) w odniesieniu do dynamiki kiełkowania, długości oraz suchej masy korzeni i koleoptyla słonecznika zwyczajnego (*H. annuus*).

W przeprowadzonych badaniach, stwierdzono wyraźny wpływ zastosowanych biopreparatów EM Farma (EMF) i EM Farma Plus (EMFP), na dynamikę kiełkowania nasion słonecznika zwyczajnego (*H. annuus*) w porównaniu do obiektu kontrolnego (K) oraz do jednego ze środków referencyjnych, którym był kwas giberelinowy (GA_3). Zastosowanie biopreparatu EM Farma Plus (EMFP) wpłynęło istotnie jedynie na zwiększenie średniej długości oraz suchej masy korzeni słonecznika zwyczajnego (*H. annuus*). Natomiast zastosowanie preparatu EM Farma (EMF) spowodowało istotne, stymulujące działanie ale tylko w odniesieniu do średniej długości oraz suchej masy koleoptyla słonecznika zwyczajnego (*H. annuus*) w porównaniu do kontroli (K) i środków referencyjnych (KSL i GA_3)

Introduction

The amount and quality of the gained yield in the form of seeds depend on many factors i.e. genotype, environmental conditions, seed storage, natural or unnatural selection as well as conscious seeds ennoblement (Ertekin, 2011; Florez, et al., 2012; Grzesik, et al., 2012; Grzesiuk and Kulka, 1981; Seliga and Żurawicz, 2011; Szajsner and Drozd, 2007). Moreover, for at least two decades biopreparations which stimulate mainly plant and seed metabolic processes have been successfully used, resulting not only in yield increase but also in higher quality and plant health. Among the most important biopreparations containing algae Bio-Algeen 90 and Kelpak SL should be mentioned, based on humic acids - Humiplant and Rosahumus and on effective microorganisms (EM) i.e. EM-1, EM-4 (Augustynowicz, et al., 2008; Boligłowa and Gleń, 2008; Ertekin, 2011; Grzesik, et al., 2012; Javaid and Bajwa, 2011; Klama, et al., 2010; Kocira, et al., 2013; Matysiak, et al., 2012; Naqvi, et al., 2000).

EM preparations are biological mixtures containing naturally-occurring, beneficial microorganisms, that may belong to a few distinctive groups i.e. lactic acid bacteria, photosynthetic bacteria, mould fungi, yeast or ray-fungi (Higa, 1991; Higa and Wididana, 1991). As a result of the use of these preparations, an improvement of the biological activity of the soil may be noticed as well as faster seed germination (e.g. of field crop). Nowadays biopreparations are applied mainly in the organic and integrated farming, not only to improve the physico-chemical soil properties but also to stimulate seed dressing (Faltyn and Miszkieło, 2008; Iwaishi, 2003; Janas, 2009; Kucharski and Jastrzębska, 2005).

The aim of the conducted research was the evaluation of the influence of the biopreparations containing effective microorganisms (EM) on the dynamics and germinating capacity as well as selected biometric parameters of sunflower (*H. annuus*).

Material and methods

In the experiment that was carried out in the laboratory conditions, the influence of biopreparations (EMF and EMFP) containing effective microorganisms on the dynamics and germination capacity and selected biometric parameters (length and dry weight of roots and coleoptile) of sunflower (*H. annuus*) was analysed.

In these types of the experiments most researchers compare their results only to control (Klama, et al., 2010; Kleiber, et al., 2013; Szydłowska and Małuszyńska,

2010). However, it seems more reasonable to compare the results not only to control but also to other reference preparations. Therefore, in this experiment apart from control (K), additional agents were used i.e. Kelpak SL (KSL) and gibberellic acid (GA_3), in the doses recommended by the producer. The research consisted of 3 experimental series, 10 replicates each, with the use of Phytotoxkit^{TM} microbiotest. Sunflower (*H. annuus*) seeds gained from one batch were soaked in the following preparations for 6 h: EM Farma (EMF), EM Farma Plus (EMFP), Kelpak SL (KSL) and gibberellic acid (GA_3). Control (K) were sunflower (*H. annuus*) seeds soaked in distilled water also for 6 h. In Table 1 an experimental scheme can be seen.

Table 1. The biopreparations used in the experiment to prepare sunflower (*H. annuus*) seed dressing
Tabela 1. Biopreparaty zastosowane w doświadczeniu do zaprawiania nasion słonecznika zwyczajnego (*H. annuus*)

	Treatments Obiekty	Solution concentration Stężenie roztworu	Dressing time Czas zaprawiania (h)
K	Check Kontrola absolutna	–	6
EMF	EM Farma	10.0 (%)	
EMFP	EM Farma Plus	10.0 (%)	
KSL	Kelpak SL	1.0 (%)	
GA_3	Gibberellic acid Kwas giberelinowy	20 (ppm)	

Seeds were planted in plates containing washed sand (particle size diameter 0.6 – 0.8 mm), moistened with distilled water. Microbiotest plates were covered with paper filter on which treated seeds of sunflower *(H. annuus)* were sown, 5 grains per plate. Thereafter, microbiotest was incubated in vertical position, in 25°C, no light, during 120 h. Additional information regarding the Phytotoxkit^{TM} microbiotest implementation can be found in the standard procedure (Phytotoxkit, 2004).

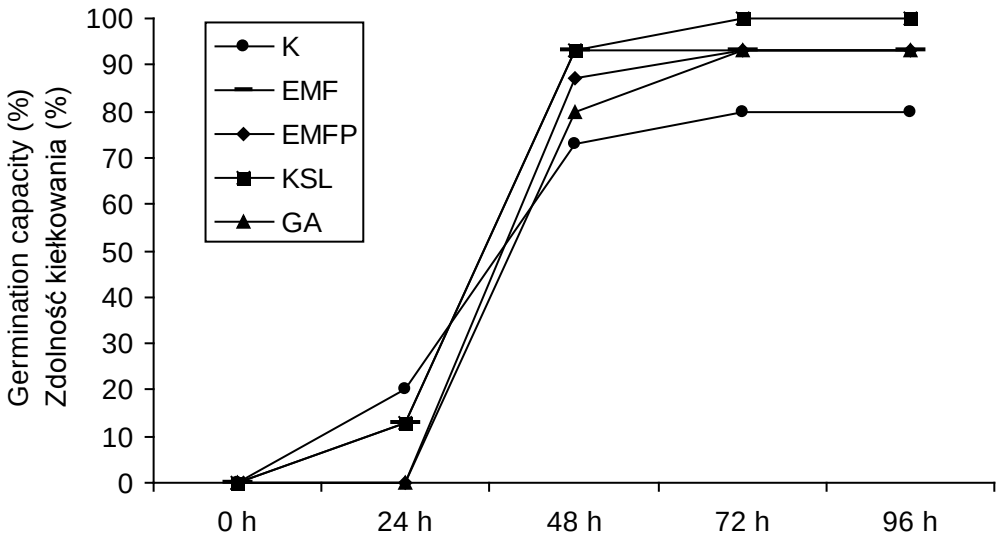
The first evaluation of germination capacity of sunflower (*H. annuus*) was done after 24 h after setting up the test and the next readouts were done after 48, 72 and 96 h, recording the individual images using camera. Moreover, the „Image Tools" programme, analysing image, was used to measure roots and coleoptile length, provided by MicroBioTest. After 120 h of setting up the test, the dry weight of roots and coleoptile of sunflower (*H. annuus*) was determined after using seed drier at 105°C. Gained results were analysed statistically, using ARM8 programme, comparing differences between the means for each variant for the level of significance P≤0.05.

Results

Germination dynamics of the sunflower *(H. annuus)* seeds according to the biopreparations used

In the experiment a significant influence has been noticed resulting from the use of preparation containing effective microorganisms influence on the dynamics and germination capacity of the sunflower (*H. annuus*) seeds.

During the first readout that was done after 24 h after the test start, the most germinated seeds were seen on the control (K) – 20%, then on the EM Farma (EMF) and Kelpak SL (KSL), both 13%, while on the objects with EM Farma Plus (EMFP) and with gibberellic acid (GA_3), no germinated seeds were found. After the next readout (48 h), a significant increase in the number of germinated seeds has been noticed independent on the object tested. However, the biggest change has been noticed for the seeds soaked in EMF and KSL – 93%, then EMFP – 87% and GA_3 - 80% and the least on the control – 73%. After 72 h after the test start only seeds soaked in KSL gained 100% of the germination capacity, whereas for the rest of the preparations i.e. EMF, EMFP and GA_3, the germination capacity was also high and amounted 93%. Only the seeds from the control object gained lower results i.e. only 80%. During the last readout (96 h) no differences were noticed between percentage of germinated seeds in comparison to the previous readout .The final readout after 96 h showed that in comparison to control, seed inoculation with EMF and EMFP preparation, increased the germination capacity of 16%. However, in relation to the reference preparation KSL, lower germination capacity was noticed amounting 7%, and with reference to GA_3 no differences were reported (Figure1).



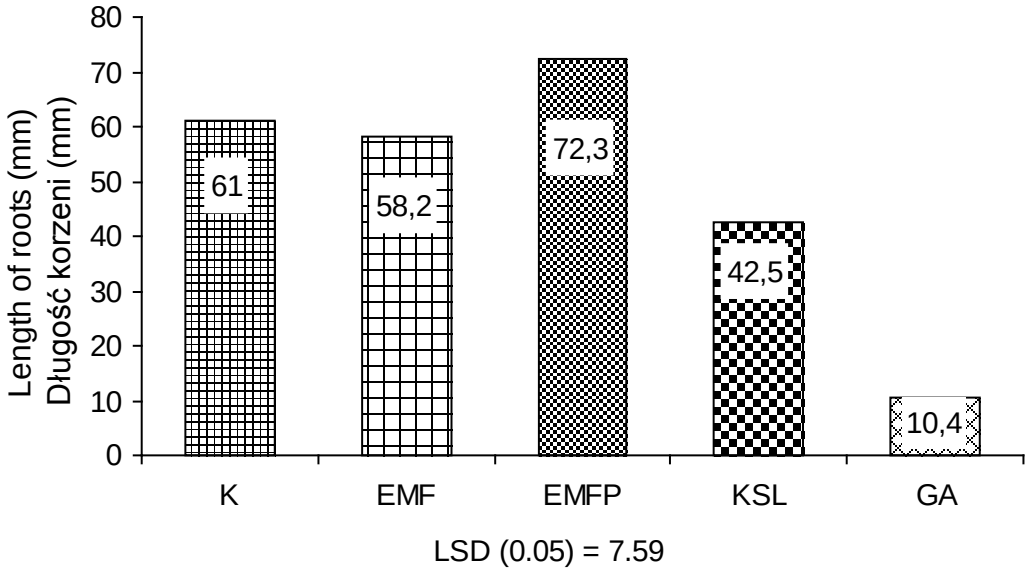
K – Check, EMF – biopreparation EM Farma, EMFP – biopreparation EM Farma Plus, KSL – biopreparation Kelpak SL, GA_3 - gibberellic acid
K – kontrola absolutna, EMF – biopreparat EM Farma, EMFP – biopreparat EM Farma Plus, KSL – biopreparat Kelpak SL, GA_3 – kwas giberelinowy

Figure 1. Sunflower (*H. annuus*) seed germination dynamics depending on the applied biopreparations
Rysunek 1. Dynamika kiełkowania nasion słonecznika zwyczajnego (*H. annuus*) w zależności od zastosowanych biopreparatów

Influence of biopreparations on the chosen biometric parameters of sunflower (*H. annuus*)

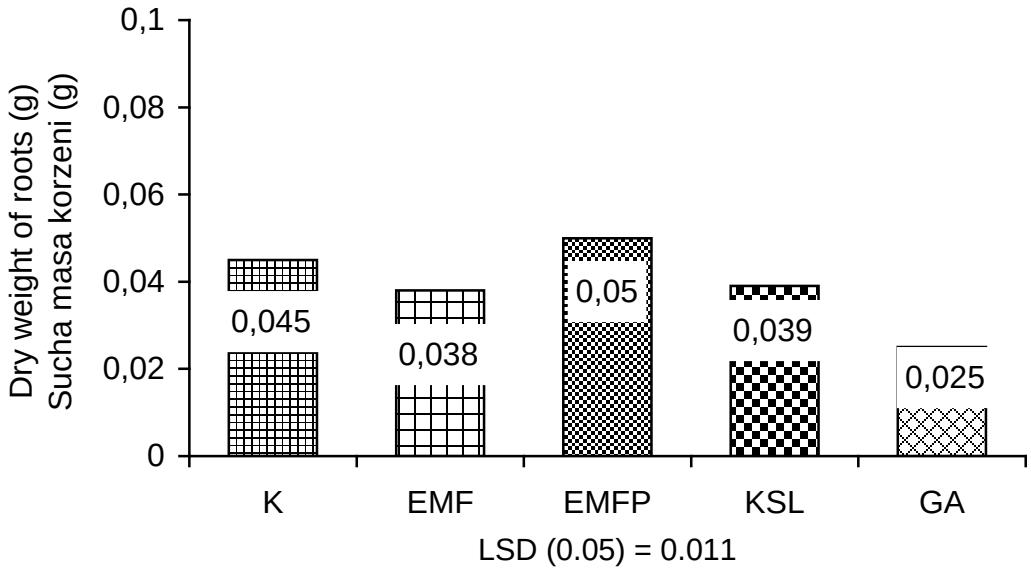
As a result of the experiment, significant influence of the biopreparations EMF on the coleoptile mean length and dry weight and EMFP on the mean length and dry weight of sunflower (*H. annuus*) root, comparing to the control as well as to reference preparation i.e. KSL and GA_3 was observed.

Gained results clearly indicate that EMFP has a favourable effect on the mean length and root dry weight of sunflower *(H. annuus)* in comparison to reference preparations (Figure 2-3).



K – Check, EMF – biopreparation EM Farma, EMFP – biopreparation EM Farma Plus, KSL – biopreparation Kelpak SL, GA_3 - gibberellic acid
K – kontrola absolutna, EMF – biopreparat EM Farma, EMFP – biopreparat EM Farma Plus, KSL – biopreparat Kelpak SL, GA_3 – kwas giberelinowy

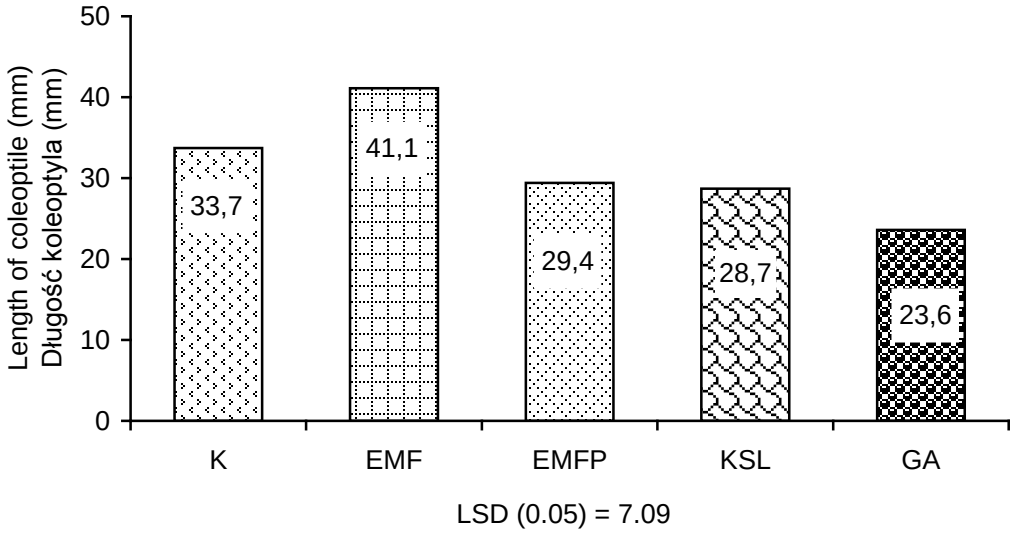
Figure 2. Influence of the selected biopreparations on the sunflower (*H. annuus*) roots length
Rysunek 2. Wpływ badanych biopreparatów na długość korzeni słonecznika zwyczajnego (*H. annuus*)



K – Check, EMF – biopreparation EM Farma, EMFP – biopreparation EM Farma Plus, KSL – biopreparation Kelpak SL, GA_3 - gibberellic acid
K – kontrola absolutna, EMF – biopreparat EM Farma, EMFP – biopreparat EM Farma Plus, KSL – biopreparat Kelpak SL, GA_3 – kwas giberelinowy

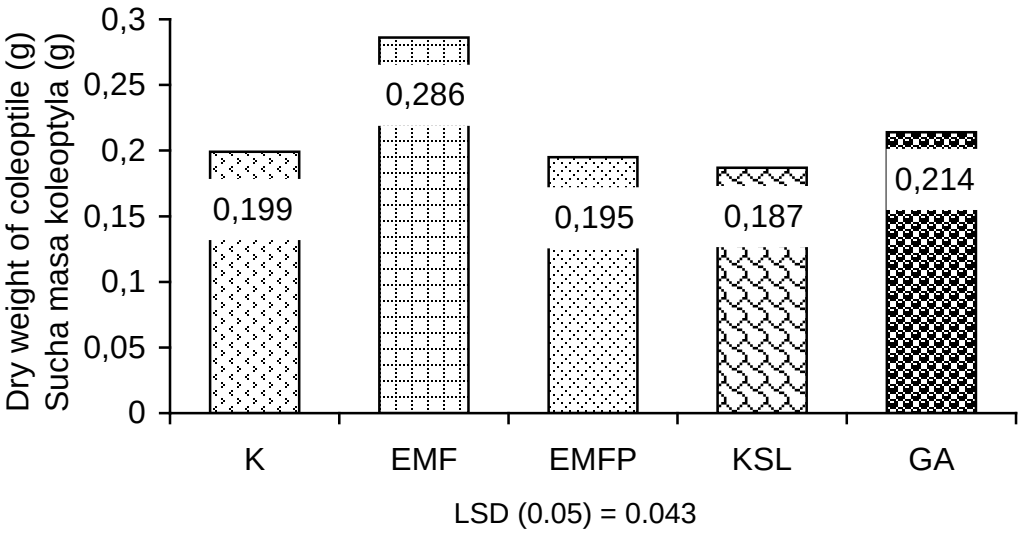
Figure 3. Influence of the selected biopreparations on the sunflower (*H. annuus*) roots dry weight
Rysunek 3. Wpływ badanych biopreparatów na suchą masę korzeni słonecznika zwyczajnego (*H. annuus*)

However, taking other biometric parameters into consideration, i.e. length and dry weight of the coleoptile, decrease has been noticed among these parameters, yet this difference has not been statistically confirmed. Converse effect was noticed for EMF biopreparation, thus after its application significant, stimulating effect was seen with regard to mean length and dry weight of coleoptile of the sunflower *(H. annuus)* in comparison to control and reference preparations (Figure 4-5).



K – Check, EMF – biopreparation EM Farma, EMFP – biopreparation EM Farma Plus, KSL – biopreparation Kelpak SL, GA_3 - gibberellic acid
K – kontrola absolutna, EMF – biopreparat EM Farma, EMFP – biopreparat EM Farma Plus, KSL – biopreparat Kelpak SL, GA_3 – kwas giberelinowy

Figure 4. Influence of the selected biopreparations on the sunflower (*H. annuus*) coleoptile length
Rysunek 4. Wpływ badanych biopreparatów na długość koleoptyla słonecznika zwyczajnego (*H. annuus*)



K – Check, EMF – biopreparation EM Farma, EMFP – biopreparation EM Farma Plus, KSL – biopreparation Kelpak SL, GA_3 - gibberellic acid
K – kontrola absolutna, EMF – biopreparat EM Farma, EMFP – biopreparat EM Farma Plus, KSL – biopreparat Kelpak SL, GA_3 – kwas giberelinowy

Figure 5. Influence of the selected biopreparations on the sunflower (*H. annuus*) coleoptile dry weight
Rysunek 5. Wpływ badanych biopreparatów na suchą masę koleoptyla słonecznika zwyczajnego (*H. annuus*)

Whereas, the mean root length of sunflower (*H. annuus*) after the EMF biopreparation application was comparable to control (K). Only in comparison to the reference preparations, stated difference in root length was statistically confirmed. Also the dry roots weight of the sunflower (*H. annuus*) was lower than on the control and on the reference preparation with KSL but once again these differences were not statistically confirmed. Only taking GA_3 into consideration, difference between the dry root weight was statistically relevant in favour of EMF biopreparation.

Discussion

After the seed germination and early growth test Phytotoxkit™ use, positive effect was stated after the EMF and EMFP biopreparations application on the sunflower *(H. annuus)* seeds germination. Also Kleiber, et al. (2013), showed significant influence of the lettuce *(Lactuca sativa)* inoculation with the EM-A preparation with the simultaneous use of the culture medium II on the seed germination dynamics improvement during the first 5 days of the hydroponic experiment. These researchers also stated that integrated application of culture medium II and EM-A preparation had beneficial effect on lettuce (*L. sativa*) leaves forming.

On the other hand, Faltyn and Miszkieło (2008), stated that in relation to spring wheat, single dose of the EM preparation has advantageous effect, while double dose cause germination suppression. Similar results were reported by Ertekin (2011) with reference to *Koelreuteria paniculata* seeds soaked in EM-1 preparation during 72 h. This author stated that germination capacity of the hard seeds of this tree species was significantly higher in comparison to other biostimulants. Also Sinqueira, et al. (1993) stated significant germination capacity increase of the carrot, tomato, pea, cucumber and beet seeds after effective microorganisms (EM) application.

Quite the contrary results were stated by Małuszyńska, et al. (2012) who did not obtain significant differences in germination capacity of wheat, barley and oats after the EM-Farming and Ema Plus biopreparations application. Also Szydłowska and Małuszyńska (2010) did not confirm significant increase in the germination capacity in oat, blue and yellow lupine, phacelia and serradella seeds after EM-Farming and EM Plus biopreparations application. What's more, these authors observed less abnormal seedlings and less dead seeds of the tested species after these preparations application.

EMFP biopreparation application in the own study clearly indicate that the mean length and dry weight of sunflower (*H. annuus*) roots undergo extension. Moreover, EMF preparation application caused increase of the mean length and dry weight of sunflower *(H. annuus)* coleoptile. Also Muthaura, et al. (2010) observed favourable effect after EM biopreparation application, manifesting in *Amaranthus dubians* fresh as well as dry weight of leaves and roots increase.

Interesting results were also obtained by Mohammed, et al. (2013), who conducted research on the coffee seeds (*Coffea arabica*) germination capacity and selected biometric parameters after different soaking periods and concentration of EM biopreparation. According to these authors, the best results were obtained after soaking coffee seeds *(C. arabica)* in 100% concentrated EM preparation, during 4.5 h.

According to the authors of this work, the advantageous effect, which can be observed after soaking sunflower *(H. annuus)* seeds in biopreparations, is most likely

connected with the stimulative interactions of secondary metabolites from microorganisms contained in these preparations. Similar opinion was put forward by Klama, et al. (2010), who showed that the crucial factor responsible for biostimulative effect could be caused by microorganisms cell secretions, which have beneficial influence for plants and lead to physiological condition improvement.

Whereas, Okorski and Majchrzak (2008) are of a different opinion and did not confirm the protective properties of EM-1 biopreparation to dress the seeds. These authors gained only slight effect on the microflora diversity of pea seed. Also soil applied EM-1 biopreparation did not cause significant differences in the community of soil fungi.

Conclusion

In the experiment carried out with the aid of microbiotest Phytotoxkit^{TM}, relevant influence was noticed after the application of EM Farma (EMF) and EM Farma Plus (EMFP) preparations on the sunflower (*H. annuus*) seeds germination dynamics in comparison to control (K) and one of the reference preparation gibberellic acid (GA_3). Moreover, after EMF and EMFP preparations application significant increase in seed germination capacity was reported only with reference to control.

Moreover, EMFP application resulted in increased mean root length and dry weight of the sunflower (*H. annuus*). Also the application of EMF preparation resulted in significant, stimulating effect with regard to the mean length and dry weight of the sunflower (*H. annuus*) coleoptiles.

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