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MICROBIOLOGICAL DRESSING OF SPRING BARLEY SEEDS AS A METHOD OF IMPROVEMENT IN PLANT DEVELOPMENT

Summary

Seeds treatments before sowing (dressing) with e.g. the microorganisms can stimulate their germination, the health of the seedlings and to improve the development of young plants. The search for alternative means and methods to seeds dressing was the purpose of the research. A seed dressing in dry and wet way was evaluated for finding the most effective. The experiments were conducted in greenhouse and field conditions with barley (Hordeum vulgare L.) seeds. Commercial products based on Pythium oligandrum, Trichoderma asperellum and Saccharomyces cerevisiae in doses of 10 g/1 kg of seeds were used. Also the product containing a complex of microorganisms (EM Farm) in dose 1 ml/100 ml of water was included. The number of seedlings, health and development of plants according to microbial dressing were evaluated. In glasshouses conditions non positive effect on number of emergence was noted in EM combination It was found that especially S. cerevisiae the most improved development of seedlings in the pots with the soil. Effect of seeds dressing was the most visible in the tests with the soil compared to the tests with perlit. In field conditions we observed a positive effect on the parameters of the development of young plants after the application S. cerevisiae and T. asperelleum comparing to untreated plants and P. oligandrum.
***Key words**: Pythium oligandrum, Saccharomyces cerevisiae, Trichoderma asperellum, seed dressing*

MIKROBIOLOGICZNE ZAPRAWIANIE JAKO METODA POLEPSZENIA ROZWOJU MŁODYCH SIEWEK JĘCZMIENIA JAREGO

Streszczenie

Zabiegi przedsiewne (np zaprawianie) z mikroorganizmami mogą stymulować kiełkowania nasion, polepszać zdrowotność sadzonek i ich rozwój. Poszukiwanie alternatywnych środków i metod do zaprawiania nasion było celem badań. Stosowano zaprawianie nasion na sucho i na mokro. Badania przeprowadzono w warunkach polowych i szklarniowych stosując jęczmień (Hordeum vulgare L.). Wykorzystano produkty handlowe oparte na Pythium oligandrum, Trichoderma asperellum i Saccharomyces cerevisiae w dawce 10 g / 1 kg nasion. Stosowano także produkt zawierający kompleks mikroorganizmów (EM Farm) w dawce 1 ml / 100 ml wody. Oceniana była liczba wschodów oraz rozwój sadzonek. W szklarni nie stwierdzono pozytywnego wpływu na liczbę wschodów stwierdzono zastosowaniu EM. Ponadto, stwierdzono, że S. cerevisiae wpłynął na polepszenie parametrów rozwoju sadzonek w glebie. Wpływ zaprawiania nasion był najbardziej widoczny w testach z gleba w porównaniu do badań z perlitem. W warunkach polowych obserwowano pozytywny wpływ na parametry rozwoju młodych roślin zabiegi z S. cerevisiae i T. asperellum porównując z wyniki z kombinacji, gdzie stosowano P. oligandrum i brak zaprawiania.
***Słowa kluczowe**: Pythium oligandrum, Saccharomyces cerevisiae, Trichoderma asperellum, zaprawianie nasion*

1. Introduction

The studies were conducted in the years 2014–2015 in the glasshouse and at the Field Experimental Station of IPP-NRI. Commercial products based on *Pythium oligandrum* (Polyversum), *Trichoderma asperellum* (Trifender), *Saccharomyces cerevisiae* (yeast, bakery product) in doses of 10 g/1 kg of seeds were used. Each experimental combination included 4 repetitions (big plots). One gram of Polyversum is registered in Poland as biological plant product and it consists of $1x10^6$ of zoospores *P. oligandrum*. Trifender WP (product of Biovéd, Hungary) is used in Poland as microbial plant growth promoter. One gram of the product contained $5x10^8$ of conidium isolate T1. As source of *S. cerevisiae* was used trade bakery product with lyophilized fungus in concentrate $11x10^6$ of *S. cerevisae*in in one gram. In glasshouse conditions combinations with each microorganism were made with 100 seeds (5 seeds in 20 pots), which were sown in the sterile perlit or soil. Pots sowed seeds were kept in a greenhouse at 20 ° C giving optimal growth of seedlings. For dry dressing seeds were placed in a Petri dish and mixed with microbial product in accurate dose in relation to weight of seeds. Petri dishes were shaken for 10 s and after next 5 minutes seeds were sown. In way of wet dressing the fixed dose of Polyversum was mixed with warm water (100 ml). Seeds were put into solution for 0.5 h. EM Farm in dose 1 ml/100 ml of water was used, also. Dressed seeds were out and slightly drained on paper towel for 24 hrs. In glasshouse experiments the number of seedlings was evaluated during 2 weeks after sowing. After 4 weeks, the parameters of development of the seedlings were measured (fresh weight of the total, weight of the roots). In field conditions the experiment was established using the random block design on plots of 400 m^2 area where barley cv. Eunova was sown. The field experiments were performed in 2015. Date of sowing was 10th of April, number of plant emergence on 1 m in four locations in each combination was evaluated on 16th of April. Seeds were treated with powder of microbial product, only EM was used as water treatment. The same doses as in glasshouse were used.

2. Statistical methods

Differences in observation between treated and untreated were determined using Tukey's multiple range test at $P \leq 0.05$ for selected combination. The data are presented as untransformed means.

3. Results and discussion

Effective microorganisms (EM) consist of about 70 species of microorganisms belonging to five groups, namely lactic acid bacteria, photosynthetic bacteria, actinomycetes, yeast fungi and filamentous fungi [23]. The application of EM has a beneficial effect on soil texture and quality [7]. In Poland the biopreparation EM is registered as a soil enhancer recommended for use in organic farming [13].

As described by Hu and Qi [6], the inoculant called EM was first described in non-refereed presentations in the early 1900s. The contents of EM were subsequently summarized [6]. Field experiments with cotton showed that EM increased the efficiency of mineral and organic fertilizers, and the combination of EM with organic matter increased yields by 23 % compared to treatment with organic matter alone [7]. In contrast, it was found that EM did not increase yield or soil quality in trials conducted over 4 years in Central Europe [12].

Table 1. Barley seeds wet dressing with EM – impact on plants emergence, cv. Eunova in glasshouses conditions

Sowing into pots with soil	Barley Seed treated	Barley Untreated seeds
4th day after sowing	11	21
5th day after	21	36
6th day after	31	64
7th day after	67	91
8th day after	75	90
9th day after	79	**94**

Source: own work

On the basis of data included in table 1 it can be stated that that non positive effect on number of emergence was noted in EM combination, however the use of EM should cause increased resistance of plants to conditions environment. EM is not an optimal seed treatment against common bunt, since other treatments like acetic acid or milk powder give the same or better disease control with less harm to germination vigour.

If however EM is used as a seed treatment in Kyusei Nature Farming for other reasons, i.e. to increase nutrient availability in the soil, the EM can in combination with other measures contribute to the regulation of common bunt [3].

After wet seed treatment with EM a weaker emergence is clearly seen, compared to not treated seeds. This phenomenon is difficult to explain and confirms the supposition that the use of EM does not always give results. The positive effects were obtained for pea seeds, which are provided in another paper [9].

The effect of dressing is much more visible in experiments using soil as compared to the perlite. In tests with the soil yeast and *P. oligandrum* stated to improve almost all evaluated parameters (tab. 2).

Poor effect of barley seed treatment and its effect on emergence was confirmed under field conditions using all microorganisms. Only in the case of EM there were a higher numbers of emergences compared to the control (tab. 3).

In other tests symptoms of downy mildew (*Peronospora viciae*) on pea leaf, Ascochyta pod spot and Fusarium root significantly was affected by dressing of seeds with EM in 0.2% concentration of solution. [13]. EM reduced the incidence of pea diseases. In tests of Solarska [2014] winter wheat seeds were dressed with wet Em Farma formulation in an amount of 1.5 liters / 100 kg of grain. In addition, the day before sowing of winter wheat soil was sprayed using 20 liters of Em Farma in 300-400 liters of water per 1 ha. It was concluded that seed treatment using microbiological preparations has no effect on infestation of cereals by Fusarium blight and contamination of winter wheat grains by fungi of the genera *Penicillium* and *Aspergillus.* Małuszyńska et al. [2012] evaluated germination capacity after seed treatment with formulations containing effective microorganisms: EM-Farming, Ema Plus. The experimental material among others consisted of the seeds of spring wheat, barley and oats from organic farming. Seed treatment with effective microorganisms had no effect on germination capacity of wheat and barley. Only for one cultivar oat, (Polar) germination capacity was improved after seed treatment, but the difference was not statistically significant.

Table 2. Impact of dry dressing with different microorganisms and substratum, in glasshouses conditions

		perlit			**soil**		
Microbial agent/dose		number of plants emergence (pcs)	mean mass of whole plant (g)	mean mass of roots (g)	number of plants emergence (pcs	mean mass of whole plant (g)	mean mass of roots (g)
S. cerevisae	10 g	94	**0,7**	**0,3**	**97**	**1,6**	**0,5**
untreated	100		0,5	0,2	90	1,1	0,2
P.oligandrum	10 g	**100**	0,6	0,3	**93**	**1,3**	0,2
untreated	93		1,2	0,3	91	1,2	0,3

Source: own work

Tab. 3. The number of plants emergence after seed barley dressing in the field conditions

Mean number /1 m	Microbial agent	Location 1	Location 2	Location 3	Location 4
49 pcs	EM	52	46	46	51
40 pcs	untreated	42	50	39	31
30 pcs	*P. oligandrum*	29	33	29	31
31 pcs	*T. asperellum*	31	29	34	30
33 pcs	*S. cerevisiae*	44	33	28	27

Source: own work

Table 4. Mean values of development parameters of plants collected from different combinations in the field conditions

Microbial dressing	weight of whole plant [g]	weight of roots [g]	number of leaves [pcs]
Trichoderma asperellum	**1.61 a**	**0.08 a**	**6.3 a**
Pythium oligandrum	0.79 c	0.06 b	5.0 b
Effective microorganisms (EM)	**0.93 b**	0.04 c	5.15 b
S. cerevisae	**1.45 a**	**0.10 a**	5.35 b
untreated	0.8 c	0.05 b	5.2 b

Values with letters are statistically different within column

Source: own work

In studies of Zbroszczyk and Kordas [2012] there was the assessment of a potential effect of tillage systems and EM treatments (seed dressing and soil application) on reduction of plant diseases infection. The three-year studies did not show any explicit positive effects of EM on health status of spring wheat grown in a short-term monoculture.

In field conditions it was observed a positive effect on the parameters of the development of young plants after the application of yeast and *T. asperelleum* comparing to untreated plants (tab. 4). The positive effect of the yeast impact observed in the greenhouse conditions was also confirmed in the field. *Trichoderma* isolates are known for their ability to control plant pathogens and have been used in different fields of production and protection in agriculture [5, 8]. *Trichoderma* is able not only to produce toxic compounds with a direct antimicrobial activity against pathogens, but also generates fungal substances which are able to stimulate plant to produce its own defense metabolites.

When used seeds barley and various microbiological agents, it can be concluded that only *T. asperellum* and *S. cerevisiae* confirmed positive impact on young seedlings in the field condtitions (tabl. 4). The influence of the various microorganisms on the development of young plants of various species will depend on the crop species and the microorganism, probably.

Fungi of the genus Trichoderma are often used as seed treatments influencing favorably the characteristics of seedlings [11, 18, 19]. It may be assumed that such treatments are favourable only for a short time and few further reports of Trichoderma impact on plants during their development are available. Table 2 clearly points out that effects of seed treatments are more visible in the experiments with soil than in the perlit. The best effect for development of young plants in the soil was observed for *S. cerevisae.*.

In the field conditions seed treatments with *T. asperellum* had the most positive effect on development of seedlings and young plants in all evaluated parameters. Two microbial treatments were influencing on increase of leaves number (tab. 4). These impacts were statistically confirmed.

No effect of phytotoxicity of *Trichoderma* spp. isolates was observed. [20].

In experiments Rochalska et al. [2010] showed that natural substances based on powdered species provided positive effect on germination and morphological development. The various species and even cereal varieties react differently on dressing natural plant substances. Kind of substances to dressing has impact on the weight of thousand grains of tested cereals. In the case of oats and wheat the best effects were obtained after treatments with chamomile. However, in the case of barley both cv. Antek and Nadek it was garlic.The aim of work conducted by Horoszkiewicz and Jajor [2007] was to determine establishment of pathogenic and saprotrophic fungi in barley seeds dressed with bioproducts of different composition and mechanism of action. The following products were used for seed's dressing: Biochikol 020 PC, Biosept 33 SL, Bioczos BR, Cedomon EO, Polyversum, Biolux - New and Cropaid. All products applied effectively controlled an occurrence of pathogenic fungi of *Fusarium* genus in barley seeds.

Antagonistic fungi such as *Trichoderma harzianum*, *T.* v*iride* and *T. hamatum, Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* were tested in dose $2x10^4$cfu/mL by El-Mougy et al. [2012]. All used seed dressing treatments reduced significantly root rot incidence at both pre-, and post-emergence growth stages of cucumber, cantaloupe, tomato and pepper plants comparing with untreated check control.

4. Conclusions

1. In glasshouses conditions non positive effect on number of emergence was noted in EM combination It was found that especially *S. cerevisiae* the most improved development of seedlings in the pots with the soil. Effect of seeds dressing was the most visible in the tests with the soil compared to the tests with perlit.
2. In field conditions we observed a positive effect on the parameters of the development of young plants after the application *S. cerevisiae* and *T. asperelleum* comparing to untreated plants and *P. oligandrum.*

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