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THE EFFECT OF SELECTED BIOSTIMULANTS ON SEED GERMINATION OF FOUR PLANT SPECIES

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Abstract. A laboratory experiment was conducted in 2015 to evaluate the germination energy and capacity of seeds of four ornamental plant species, treated with various biologically active substances. Three replications of the experiment were performed in three independent series. The experimental materials comprised seeds of four ornamental plant species: China aster (*Callistephus chinensis* (L.) Nees), scarlet sage (*Salvia splendens* Sellow ex Roemer & J.A. Schultes), common zinnia (*Zinnia elegans* Jacq.) and French marigold (*Tagetes patula* L.), which constituted the first experimental factor. The second experimental factor were biostimulants used for seed pre-conditioning: Effective Microorganisms, *Trichoderma* spp. and Goëmar Goteo. Seeds soaked in distilled water were the control. French marigold seeds were characterised by the highest average germination energy and capacity in three experimental series (mean values) and in each of the series. Scarlet sage seeds had the lowest germination energy, and common zinnia seeds had the lowest germination capacity in experimental series 1 and 3 and in three series (mean values). The biostimulants used for pre-conditioning exerted varied effects on the germination energy and capacity of seeds of the analysed ornamental plant species. The plant species compared in the study responded differently to the tested biostimulants. The germination energy and capacity of seeds of *Callistephus chinensis* (L.) Ness and *Tagates patula* L. decreased in response to the applied biostimulants.

Keywords: germination, bioconditioning, Effective Microorganisms, *Trichoderma* spp., Goëmar Goteo

INTRODUCTION

The ultimate goal of growing seed-bearing plants is to achieve high sowing value. Delayed and uneven emergence poses a serious problem in the production of horticultural crops, particularly during drought and under adverse weather conditions (Grzesik *et al.* 2012). Effective methods of improving seed quality have been continuously searched for in order to increase germination capacity (Borowski and Michałek 2006, Dziąg and Szczech 2011, Małuszyńska *et al*. 2012). Due to the presence of numerous soil-borne pathogens, pre-sowing conditioning of seeds is becoming a necessity. Undressed seeds are characterised by poor germination and infected seedlings die, which reduces the number of plants per stand, crop yield and quality. Seed priming is the most common and cheapest treatment applied in agricultural and horticultural practice (Juszczak *et al.* 2001). The use of synthetic substances is not allowed in organic farming, which is why alternative seed pre-conditioning agents, including biostimulants, have been gaining popularity. Such products can be applied to soil or in the form of spray, seed dressing or a seed conditioner. Pre-conditioning involves increasing seed moisture content to approximately 40% by soaking in water or solutions of biological compounds, followed by incubation. Such treatment accelerates seedling emergence, improves germination uniformity and seedling health under different weather conditions (Grzesik *et al.* 2013). Various seed priming methods have been extensively researched and discussed in the literature, but there is insufficient information on the parameters of the seed priming process in different plant species (Grzesik and Janas 2013). According to Janas (2009), biological agents can be successfully used in the production of ornamental plants. The most widely used are Effective Microorganisms (EM), Goëmar Goteo and *Trichoderma* spp.

Fungi of the genus *Trichoderma* spp. have disinfecting properties and produce compounds that accelerate seed germination, such as gibberellins and zeatins (Małuszyńska *et al.* 2012). Those microorganisms are potent antagonists of parasitic soil-inhabiting fungi. Effective Microorganisms (EM), a combination of approximately 80 species of anaerobic and aerobic microorganisms, improve seed germination. Goëmar Goteo is intended for use as an aqueous solution. The product contains mainly phosphorus and potassium. These elements are important during the rooting process. Organic substances support cell division.

The objective of this study was to evaluate, in laboratory tests, the germination energy and capacity of seeds of four ornamental plant species, treated with the following biologically active substances: EM, *Trichoderma* spp. and Goëmar Goteo.

MATERIALS AND METHODS

A two-factorial experiment was conducted in November and December of 2015 in the laboratory of the Department of Horticulture at the University of Warmia and Mazury in Olsztyn. Three replications of the experiment were performed in three independent series to determine the germination energy (GE) and germination capacity (GC) of seeds.

The first experimental factor were four ornamental plant species: China aster (*Callistephus chinensis* (L.) Nees), scarlet sage (*Salvia splendens* Sellow ex Roemer & J.A. Schultes), common zinnia (*Zinnia elegans Jacq*.) and French marigold (*Tagetes patula* L.). The second experimental factor were biostimulants used for seed pre-conditioning: Effective Microorganisms (EM) at a concentration of 20%, *Trichoderma* – 2.4 g dm^{-3}, and Goëmar Goteo – 1%. Seeds soaked in distilled water were the control.

Seeds were conditioned in flasks; 150 seeds of each plant species were soaked in solutions of biological compounds for 30 minutes. Seeds with visible mechanical damage were discarded. The seeds were dried on sterile filter paper, under relatively aseptic conditions, and they were placed in PS 100 mm Petri dishes (50 seeds per dish) lined with two layers of filter paper soaked with conditioning substances. Wet seeds were incubated at a temperature of 24/20^{o}C (day/night) with the methodology developed by the Main Inspectorate of Plant Health and Seed Inspection. Seeds that produced a sprout with a minimum length of 1 mm were classified as germinated (ISTA 2003). Germination energy and germination capacity were evaluated after 3 days and 8 days, respectively.

The results of the study were analysed statistically by analysis of variance (ANOVA). The significance of differences was determined by creating confidence intervals in Tukey's test at a significance level of $\alpha = 0.05$. All calculations were performed in the program STATISTICA 12.

RESULTS AND DISCUSSION

Seed germination is defined as the ability to produce a seedling. The rate of germination is determined by temperature, relative humidity, moisture content, exposure to light, concentrations of O_2 and CO_2 in air, and the substrate used (ISTA 2003). A dormant seed is one that is unable to germinate under normally optimal conditions. Seed dormancy and germination are also regulated by endogenous factors, primarily growth regulators, hormones and the allelopathic effects of microorganisms. Under adverse environmental conditions, the efficiency of crop production is determined by the sowing value of seeds. The laboratory procedures used for evaluating the germination capacity of seeds are limited to counting the number of seeds that germinated within a specified period of time. Researchers emphasize the importance of germination energy and germination capacity as a combination of indices that characterize a seed at the moment of sowing. According to Baranowski (2008), a decrease in germination capacity is preceded by a decrease in seed vigour. A key factor in assessing seed quality is viability, i.e. the period of time a seed remains capable of germination after dormancy (Borowski and Michałek 2006, Grzesik and Janas 2013a, b). In the present study, French marigold (*Tagetes patula* L.) seeds were characterised by the highest average germination energy and germination capacity in three experimental series (mean values) and in each of the series. Scarlet sage (*Salvia splendens* Sellow ex Roemer & J.A. Schultes) seeds had the lowest germination energy, and common zinnia (*Zinnia elegans Jacq*.) seeds had the lowest germination capacity in experimental series 1 and 3 and in the three series (mean values) (Table 1).

Attempts have also been made to develop effective methods for accelerating seed germination and improving emergence uniformity, such as hydropriming/hydroconditioning (Lewak and Kopcewicz 2009, Grzesik *et al.* 2013b). According to Grzesik *et al.* (2012), one of the problems encountered in organic crop production is a shortage of registered protection agents that could be used to protect seeds and seed plantations, and to prevent and control the contamination by microflora. To maximise its effectiveness, seed priming needs to be tailored to each plant species or even variety. The seed priming process has been well documented in the literature, but detailed data on the effects of seed pre-conditioning with biologically active substance are scarce (Borowski and Michałek 2006, Dziąg and Szczech 2011). Previous research has shown that the high vigour of seeds subjected to bio-priming, manifested in the ability to produce healthy seedlings, is probably associated with the disinfecting properties of the tested agents. Similar conclusions were formulated by Nowakowska (2005) who analysed the antagonistic activity of solutions containing fungal strains against the causative agents of damping off of sugar beet seedlings. Grzesik *et al.* (2012), and Janas and Grzesik (2006b) also demonstrated that biologically active substances increased seed vigour and reduced the number of mouldy seeds.

In our study, the biostimulants used for pre-conditioning exerted varied effects on the germination energy and germination capacity of the analysed seeds (Table 1). The plant species compared in the study responded differently to the tested biostimulants. The germination capacity of seeds was significantly reduced by Effective Microorganisms (EM) in experimental series 2, and by Goëmar Goteo in series 3. Our results contradict the findings of Janas and Grzesik (2005), Janas and Grzesik (2006a), and Małuszyńska *et al.* (2012), who reported a significant improvement in the vigour and health of seeds treated with EM. In a study by Janas (2013), EM and Goëmar Goteo had a beneficial influence on dill seeds. According to Faltyn and Miszkiełο (2008), germination capacity is affected primarily by the dose of the seed priming agent. When applied at the concentrations recommended by the manufacturer, such agents increase the germination energy and germination capacity of seeds, whereas the same agents used at a double dose decrease the values of those indices. However, Okorski and Majchrzak (2008) did not observe any influence of EM solutions on the germination capacity of pea seeds. Szydłowska and Małuszyńska (2011) demonstrated that EM did not significantly improve seed germination. Varied effects of biostimulants on the germination energy and germination capacity of seeds of selected ornamental plant species were also noted in our experiment. The efficacy of biostimulants varied across the analysed species. An analysis of the responses of seeds of the four examined species to the tested biostimulants (interactions) revealed that French marigold seeds were characterised by the highest germination energy and germination capacity (74.6 and 96.6%, respectively), scarlet sage seeds from the control treatment had the lowest germination energy (2.6%), and China aster seeds pre-conditioned with EM had the lowest germination capacity (40.0%).

An analysis of mean values in the three experimental series indicated that the germination energy of China aster seeds was significantly reduced by the applied biostimulants, in comparison with the control treatment with distilled water. The germination capacity of China aster seeds decreased in response to EM, and it was the highest when seeds were pre-conditioned with *Trichoderma* spp., but at the same level of significance as the control seeds. Dziąg and Szczech (2011) also demonstrated that fungi of the genus *Trichoderma* spp. produce compounds that accelerate seed germination, such as gibberellins and zeatins. The effectiveness of *Trichoderma* spp. was also confirmed by Pięta *et al.* (2002) who analysed soybean seeds, and by Sadowski *et al.* (2006) who investigated the effect of the above fungi on the vigour and health of radish seeds. However, in a study by Domaradzki *et al.* (2014) the germination capacity of vegetable seeds was not significantly altered by fungi of the genus *Trichoderma* spp., relative to the control treatment.

The analysed biostimulants had a positive effect on the germination energy of scarlet sage seeds. The most beneficial influence was exerted by *Trichoderma* spp. The biostimulants reduced seed germination capacity, in comparison with the control treatment.

Common zinnia seeds treated with Goëmar Goteo were characterised by the highest germination energy (27.3%). The germination capacity of common zinnia seeds was the highest (69.3%) when they were pre-conditioned with EM. *Trichoderma* spp. reduced both the germination capacity and germination energy of common zinnia seeds.

The germination energy and germination capacity of French marigold decreased in response to treatment with the analysed biostimulants, and the highest decrease was noted for EM.

Bioconditioning of seeds aimed at improving their sowing value, accelerating germination and seedling growth is recommended in all crop production systems, but further research is needed to select the most appropriate priming agents, determine their doses, treatment time, and effects on seeds of different plant species (Borowski and Michałek 2006, Dziąg and Szczech 2011, Małuszyńska *et al.* 2012).

Table 1. The effect of biologically active substances used for pre-conditioning on the germination energy (GE) and germination capacity (GC) of seeds of China aster (*Callistephus chinensis* (L.) Nees), scarlet sage (*Salvia splendens* Sellow ex Roemer & J.A. Schultes), common zinnia (*Zinnia elegans Jacq.*) and French marigold (*Tagetes patula* L.)

Species	Biostimulant	Series 1		Series 2		Series 3		Mean of three series	
		GE	GC	GE	GC	GE	GC	GE	GC
		%							
Callistephus chinensis (L.) Ness	Control	68.0	84.0	46.0	82.0	58.0	90.0	57.3	85.3
	EM	0.0	34.0	40.0	0.0	18.0	86.0	19.3	40.0
	Trichoderma spp.	36.0	82.0	40.0	86.0	72.0	90.0	49.3	86.0
	Goëmar Goteo	42.0	92.0	48.0	90.0	30.0	64.0	40.0	82.0
	Mean	36.5	73.0	43.5	64.5	44.5	82.5	41.6	73.3
Salvia splendens Sellow ex Roemer & J.A. Schultes	Control	6.0	76.0	2.0	80.0	0.0	88.0	2.6	81.3
	EM	10.0	94.0	14.0	58.0	14.0	80.0	12.6	77.3
	Trichoderma spp.	16.0	76.0	16.0	58.0	20.0	66.0	17.3	66.6
	Goëmar Goteo	0.0	84.0	10.0	68.0	6.0	72.0	5.3	74.6
	Mean	8.0	82.5	10.5	66.0	10.0	76.5	9.8	74.9
Zinnia elegans Jacq.	Control	22.0	62.0	10.0	66.0	36.0	72.0	22.6	66.6
	EM	14.0	56.0	6.0	66.0	48.0	86.0	22.6	69.3
	Trichoderma spp.	14.0	66.0	6.0	56.0	30.0	70.0	16.6	64.0
	Goëmar Goteo	30.0	70.0	26.0	76.0	26.0	54.0	27.3	66.6
	Mean	20.0	63.5	12.0	66.0	34.8	70.5	22.3	66.6
Tagetes patula L.	Control	88.0	98.0	66.0	94.0	70.0	98.0	74.6	96.6
	EM	58.0	94.0	32.0	74.0	64.0	82.0	51.3	83.3
	Trichoderma spp.	72.0	88.0	64.0	92.0	72.0	92.0	69.3	90.6
	Goëmar Goteo	66.0	90.0	70.0	94.0	48.0	90.0	61.3	91.3
	Mean	71.0	92.5	58.0	88.5	63.5	90.5	64.1	90.4
Mean	Control	46.0	80.0	31.0	80.5	.40.8	87.5	39.3	82.4
	EM	20.5	69.5	23.0	49.5	36.0	83.5	26.5	67.5
	Trichoderma spp.	34.5	78.0	31.5	73.0	48.5	79.5	38.1	76.8
	Goëmar Goteo	34.5	84.0	38.5	82.0	27.5	70.0	33.5	78.6
LSD 0.05 for:									
Species (a)		12.02	10.67	7.91	17.16	11.18	8.00	9.70	9.54
Biostimulant (b)		n.s.	n.s.	n.s.	15.59	n.s.	8.64	n.s.	n.s
Interaction (a×b)		1.48	0.83	0.83	18.80	0.80	8.30	0.83	0.70

Matysiak *et al.* (2010) demonstrated that marine algae extracts exerted a positive influence on crop and ornamental plants. According to Reitz and Trumble (1996), the biostimulating effects of marine algae extracts, including *Ascophyllum nodosum* (L.), result from high concentrations of macronutrients, micronutrients and plant hormones, mostly cytokinins which are responsible for breaking dormancy (Czerpak and Piotrowska 2003).

In the present study, the analysed ornamental plant species responded differently to the tested pre-conditioning agents. In all experimental series, seeds began to germinate on the third day, but germination rates varied across the species (Figs 1a, b, c, d.). French marigold was characterised by the highest number of germinated seeds and the most uniform germination. Pre-conditioning with EM delayed germination and reduced germination capacity, to the highest extent in China aster and French marigold.

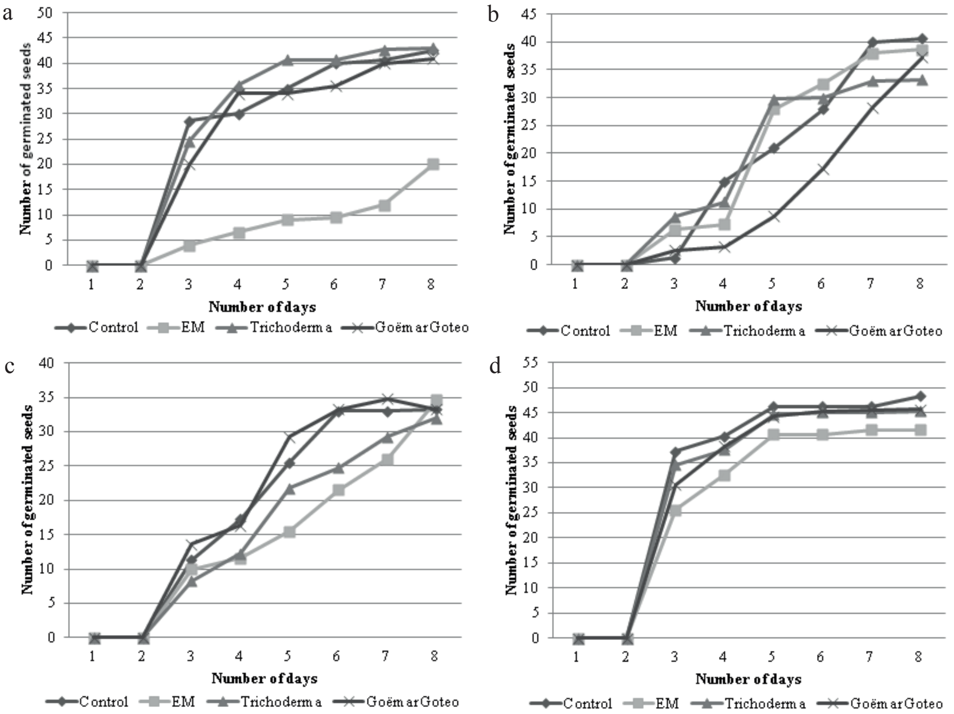

Fig. 1. The effect of biologically active substances used for pre-conditioning on seed germination rate: a – China aster (*Callistephus chinensis* (L.) Nees), c – scarlet sage (*Salvia splendens* Sellow ex Roemer & J.A. Schultes), c – common zinnia (*Zinnia elegans Jacq*.), d – French marigold (*Tagetes patula* L.); means of three experimental series

CONCLUSIONS

1. The germination energy and germination capacity of seeds of *Callistephus chinensis* (L.) Ness and *Tagates patula* L. decreased in response to the applied biostimulants.

2. Analysis of the mean of three series of experiments showed that biostimulants reduced energy (GE) and germination capacity (GC).

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WPŁYW WYBRANYCH BIOSTYMULATORÓW NA KIEŁKOWANIE NASION CZTERECH GATUNKÓW ROŚLIN

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Streszczenie. W 2015 r. przeprowadzono eksperyment laboratoryjny w celu oceny energii kiełkowania i zdolności kiełkowania nasion czterech gatunków roślin ozdobnych, w zależności od zastosowanej zaprawy biologicznej. Trzy powtórzenia eksperymentu przeprowadzono w trzech niezależnych seriach. Materiał badawczy stanowiły nasiona czterech gatunków roślin: aster chiński pomponowy (*Callistephus chinensis* (L.) Nees), szałwia błyszcząca (*Salvia splendens* Sellow ex Roemer & J.A. Schultes), cynia wytworna (*Zinnia elegans* Jacq.) i aksamitka rozpierzchła (*Tagetes patula* L.), które stanowiły pierwszy czynnik doświadczenia. Drugim czynnikiem eksperymentu były biostymulatory stosowane do prekondycjonowania nasion: Efektywne Mikroorganizmy, *Trichoderma* spp. i Goëmar Goteo. Kontrolę stanowiły nasiona moczone w wodzie destylowanej. Materiał siewny aksamitki rozpierzchłej charakteryzowały się najwyższą średnią energią kiełkowania i zdolnością kiełkowania w każdej serii doświadczenia oraz średnio z trzech serii. Nasiona szałwii szkarłatnej miały najniższą energię kiełkowania, a nasiona cynii wytwornej najniższą zdolność kiełkowania w 1 i 3 serii eksperymentu oraz średnio z trzech serii. Biostymulatory stosowane do kondycjonowania wywierały różny wpływ na energię kiełkowania i zdolność kiełkowania nasion analizowanych gatunków roślin ozdobnych. Porównane w badaniu gatunki roślin reagowały odmiennie na badane biostymulatory. Zastosowanie biostymulatorów zmniejszało energię kiełkowania i zdolność kiełkowania nasion *Callistephus chinensis* (L.) Nees i *Tagetes patula* L.

Słowa kluczowe: kiełkowanie, biokondycjonowanie, Efektywne Mikroorganizmy, *Trichoderma* spp., Goëmar Goteo