Application of agglomerative granulation of plant seeds Part 1: Survival of the fungus *Trichoderma viride* spores

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Introduction

Pelleted vegetable seeds are known and commonly used, especially on commodity and seed plantations. Large mass volume of the pellet allows for adding natural substances to seeds which favourably influence the germination and growth of seedlings [4]. Pellets include mixtures of mineral or organic materials which facilitate germination, plant protection agents or smoothing materials which constitute a mechanical protective layer for seeds. The pellet enables also the introduction of microorganisms which positively influence the condition of plants together with additives which allow for their survivability [5]. Germination of pelleted seeds depends on the water saturation level of the surface for germination [1].

The tight layer applied to seeds protects them mechanically during the first days after their sowing. For the purpose of preparing seeds for sowing on an organic plantation, seed inoculation with *Trichoderma viride* fungus spores was applied [3], followed by a pelleted pressure-free agglomeration method on a granulation disc.

The amount of the preparation per pelleted seeds was calculated from the balance (Domoradzki 2012)

$$M \cdot N = \Omega \cdot m \cdot n$$

hence $$M = \frac{\Omega \cdot m \cdot n}{N} \qquad (1)$$

where:

M – preparation mass

N – number of spores in 1 g of preparation

Ω – number of spores per 1 seed

m – seed mass

n – number of seeds in 1 g, quantity

The survivability factor of spores was calculated based on the dependence:

$$\eta = \frac{\Omega_{determined}}{\Omega_{calculated}}$$

It was assumed that a certain surplus of spores should be applied per each seed because of their non-survivability, e.g. approx. 10 spores, which, with the amount of spores in 1 g of preparation equal to $N = 1 \cdot 10^5$, gives different amounts of preparation added to seeds with their differing content in 1g of seeds. This dependence was presented in Figure 1.

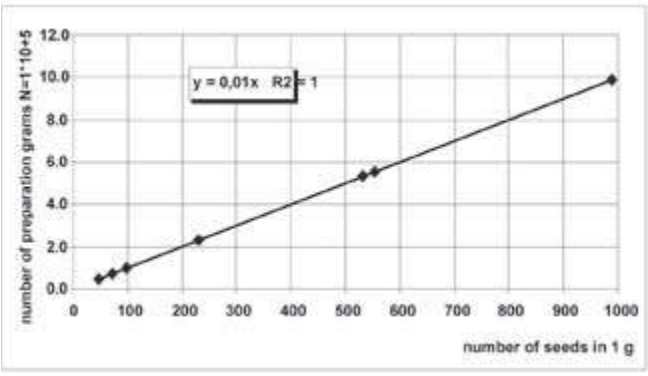

Fig. 1. Amount of preparation added to seeds in 1 g/100 g of seeds depending on the quantity

Aim of the work

The aim of the work was to test the survivability degree of *Trichoderma viride* fungus spores during storage, the thermal resistance of the preparation and simultaneous inoculation, pelleting and drying of organic seeds, as well as the impact of inoculation on the germination capability.

Materials and methods

Storing the preparation with *Trichoderma viride* fungus spores

Before testing, a determination had to be made as to the amount of units creating the colony in the used preparation and as to the impact of the lapse of time on the decay of survivability of *Trichoderma viride* fungi. Changes were presented in Figure 2.

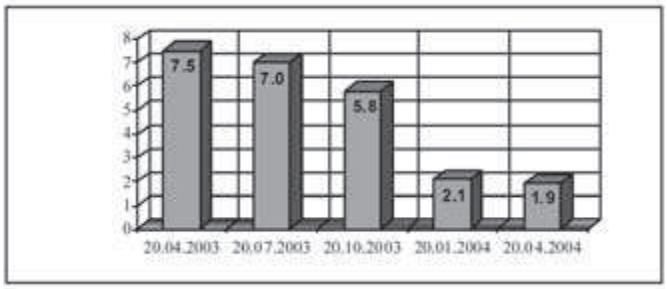

Fig. 2. Change of the number of *Trichoderma viride* units in 1 g of preparation during long storage

An analogous test was performed using fungi spores applied onto seeds during storage. For this purpose, seeds were inoculated using an aqueous suspension of spores and dried at 30°C. Results were presented in Figure 3. The maximum storage time of inoculated seeds should not exceed 7 months.

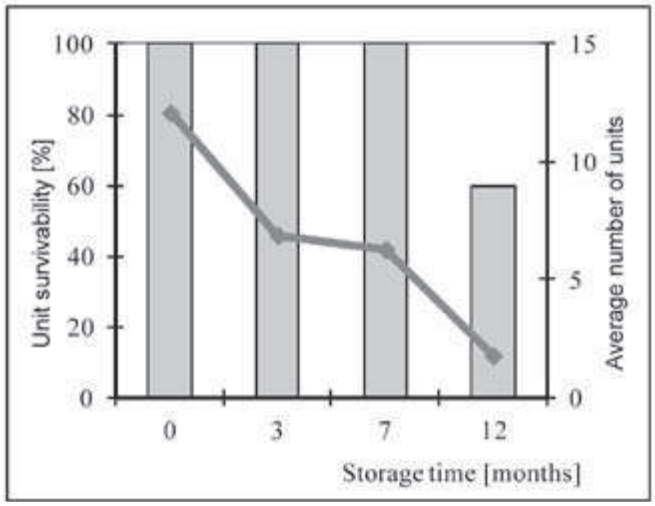

Fig. 3. Decay of shelf life of the preparation with *Trichoderma viride* fungus spores applied onto seeds

Thermal resistance of the preparation with Trichoderma viride fungus spores

In order to determine the resistance of the biopreparation to temperature and heating time, the preparation was applied (onto sterilized seeds) by immersing for 5 minutes in a 5% aqueous suspension of *Trichoderma viride*. Such prepared seeds were placed in incubators at temperatures of 22°C, 30°C and 35°C and were stored from 0.5 to 5 hours; every hour a sample was collected for determining the unit amount.

Table 1

Content of seeds grown with *Trichoderma viride*

Time [hours]	Content of covered seeds [%]		
	22°C	30°C	35°C
1.0	100.0	100.0	33.3
2.0	100.0	100.0	23.3
4.0	100.0	100.0	16.7
6.0	100.0	100.0	10.3
8.0	100.0	100.0	7.3

Based on test results presented in Table 1, the limiting temperature of processing seeds with *Trichoderma viride* spores was adopted at t = 30°C, while the maximum wet processing time – at 6÷8 hours.

Table 2

Unit number of *Trichoderma viride* in pellets for N=1·10^{+5}

Dose of biopreparation with *T. viride* calculated acc. to the number of seeds added to the peat				
Seed species	Dose g/100 g of seeds	% of pellets from units with ***T. viride***	Average number of units in pellet	Range of units in pellets
Parsley	5.52	100	8.6	1-6
Dill	5.32	88	3.9	1-5
Carrot	9.90	100	4.7	2-8

The dose of the biopreparation should be specified based on the seed sample quantity assuming min. 10 pcs of spores per one seed in pellet.

Technique for pelleting seeds with *Trichoderma viride* fungus spores

Dusts. For the purpose of seed pelleting, peat and dust with the following composition were used: kaolin 45%, dolomite 35%, wood dust 20%. The seeds were first pelleted with peat and then with an organic mixture with wood dust.

Spores. For inoculation, a preparation was used with fungus spores, containing 1·10^5 of spores in one gram. The calculated portions of the preparation were mixed with peat used for pelleting seeds during the second stage.

In order to determine the minimum dose of the preparation, tests were performed with seeds onto which different preparation doses with *Trichoderma viride* were applied; next, seeds were pelleted in a disc granulator (using the method described below). Based on the determined quantity of seeds, a dose of the preparation calculated from formula (1) was added, assuming 10 spores per seed. The biopreparation was added to the peat and seeds were pelleted using this mixture. 10 samples were made in each series. Results were presented in Table 2.

Methods

During seed pelleting with spores, the time of the biopreparation's contact with water was shortened to a minimum in order to prevent germination of the spore form of the fungus.

In addition, the drying temperature of pelleted seeds was lowered to 25°C in order to avoid damaging the spore form. The process of pelleting with peat is the slowest at the initial stage and it lasts approx. 6 hours, while the spores germinate within approx. 6÷8 hours; therefore, the granulation process was divided into two stages.

First stage. 70 g of sterile peat was added per 100 g of seeds. Seeds were pelleted with peat for 4 hours and then dried at 25°C, this way obtaining coats for applying spores.

During the second stage 30g of peat mixed with fungus spores was applied onto seeds with a peat coating; next, a 200 g dust mixture for pelleting was applied onto the peat coating.

Pelleting was conducted using 5% of dextrin solution for approx. 1÷4 hours. The surface layer of pellets was created from a mixture of 20 g of talc and dye (to differentiate between coats) within approx. 30 min. After granulation, pellets were quickly dried using large air stream intensity with a temperature of 30°C for approx. 3÷4 hours. The total time of granulation and drying (wet operations) with spores did not exceed 6 hours.

The mass of seeds after granulation increased 4 times, i.e. the mass of the dried portion of pelleted seeds equalled approx. 400 g.

Results

After pelleting and drying seeds, an inspection was made of the number of units creating a colony on pelleted seeds, the survivability factor of spores was specified and a sample was collected for determining the energy and germination capability. Germination results were gathered in Tables 3÷6, while attempts of germinating pelleted seeds were conducted at different degrees of saturating the germination coating with water.

Table 3

Number of Trichoderma viride spores on seeds prepared for sowing

Item no.	Species, Variety	Quantity [pcs/g]	Amount of preparation with *Trichoderma viride* [g/100 g of seeds]	Number of *Trichoderma viride* spores on a seed		Survivability factor η [%]
				calcu-lated	deter-mined	
1	Perfekcja carrot	990	9.90	10.0	6.0	60
2	Ołomuniec parsley	552	5.52	10.0	8.7	87
3	Szmaragd dill	532	5.32	10.0	3.2	32

Table 3 presents a comparison of the number of *Trichoderma viride* spores introduced onto seeds and the number of spores after pelleting and drying. The determined number of spores is an average

value from the number of colonies germinated from the elution of 10 seeds. An unevenness of covering seeds with spores for carrot was observed – from 5 to 8.

The number of *Trichoderma viride* spores in the process of wet pelleting undergoes reduction as a result of the germination of fungus spores and their damaging during further drying.

Table 4

Germination capability of seeds inoculated with *Trichoderma viride*

Item no.	Species, variety	Amount of preparation with *Trichoderma viride* [g/100 g of seeds]	Germination capability in %	
			of controlled seeds	of seeds with *Trichoderma viride*
1	Szmaragd dill	5.32	91	95
2	Perfekcja carrot	9.90	82	85
3	Ołomuniec parsley	5.52	80	83

For the process of pelleting seeds with *Trichoderma viride* spores according to the presented pelleting technology, the survivability factor of spores is high and equals from 87% for parsley pellets to 32% for dill pellets.

Table 5

Germination capability of pelleted seeds with fungus spores (0.7 water saturation degree of surface for germination)

Item no.	Species, variety	Germination capability of seeds [%]	
		controlled	with Trichoderma.
1	Szmaragd dill	74	88
2	Perfekcja carrot	67	71
3	Ołomuniec parsley	80	83

Table 6

Germination capability of pelleted seeds with fungus spores (1.0 water saturation degree of surface for germination)

Item no.	Species, variety	Germination capability of seeds [%]	
		controlled	with Trichoderma.
1	Szmaragd dill	74	77
2	Perfekcja carrot	67	72
3	Ołomuniec parsley	80	79

Pelleted seeds with *Trichoderma viride* spores germinate better than controlled seeds with a 0.7 water saturation of the surface for germination and not worse than controlled seeds with a 1.0 saturation of the surface for germination.

Summary

In order to introduce *Trichoderma viride* fungus spores onto pellets, it is necessary to determine the survivability of biopreparations directly before their use and, based on this, the dose of the preparation must be determined taking into account the quantity of seeds.

In order to obtain a desired degree of covering all seeds with fungus spores it is recommended to use at least a tenfold surplus of the biopreparation with regard to the calculated amount. This surplus should be even higher if the seeds are subjected to processing under conditions which are unfavourable for spores.

The maximum wet processing and drying time of inoculated seeds at 30°C cannot exceed 6÷8 hours because pelleting and drying of inoculated seeds leads to the decrease of survivability of *Trichoderma viride* fungus spores on seeds.

Germination tests show an improvement of the germination capability of inoculated seeds.

An inhomogeneity was observed of covering seeds with *Trichoderma viride* spores as well as a different survivability factor of the inoculated fungus' spores.

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