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THE INFLUENCE OF SELECTED *TRICHODERMA* SPP. STRAINS ON THE SOWING VALUE AND VIGOUR OF WINTER WHEAT SEEDS

Summary

Experiments were conducted at the Seed Laboratory, Department of Agronomy, Poznań University of Life Sciences, Poland. Seeds colonised by Fusarium ssp. fungi to different extent and selected Trichoderma ssp. strains (T1 - T. atroviride, T2 - T. atroviride, T3 - T. harzianum, T4 - T. harzianum, T5 - T. harzianum) were investigated. There were three independent series of bivariate analyses with 4 replicates. The results showed that the infestation with fungi of the Fusarium ssp. genus significantly affected the quality of seeds. The lowest sowing value parameters were observed in the seeds which were most colonised by the fungi (38%). The Trichoderma ssp. strains significantly modified the value and vigour of winter wheat seeds. When strain T2 - T. atroviride was applied, the quality of winter wheat seeds was higher. They were characterised by higher germination energy and capacity, greater vigour (expressed by means of the seedling growth test and the seedling growth rate test), greater fresh weight and longer radicles.
***Keywords**: Trichoderma* ssp., *sowing value, vigour, winter wheat*

WPŁYW WYBRANYCH SZCZEPÓW *TRICHODERMA* SPP. NA WARTOŚĆ SIEWNĄ I WIGOR ZIARNA PSZENICY OZIMEJ

Streszczenie

Doświadczenia laboratoryjne wykonano w Laboratorium Nasiennym Katedry Agronomii Uniwersytetu Przyrodniczego w Poznaniu. Badania uwzględniały ziarno o zróżnicowanym stopniu zasiedlenia przez grzyby z rodzaju Fusarium ssp. oraz wybrane szczepy Trichoderma ssp. (T1 - T. atroviride,T2 - T. atroviride, T3 - T. harzianum, T4 - T. harzianum, T5 - T. harzianum). Badania wykonano w 3 niezależnych seriach jako dwuczynnikowe, w 4 powtórzeniach. Uzyskane wyniki wskazują że zasiedlenie przez grzyby z rodzaju Fusarium ssp. istotnie wpływało na jakość materiału siewnego, najniższe parametry wartości siewnej odnotowano w przypadku ziarna o największym zasiedleniu tym grzybem (38%). Badane szczepy Trichoderma ssp. istotnie modyfikowały wartość siewną i wigor ziarna pszenicy ozimej. Wyższą jakością materiału siewnego wyrażoną energią kiełkowania i zdolnością kiełkowania oraz wigorem ziarna wyrażonym za pomocą testu wzrostu siewki, testu szybkości wzrostu siewki, a także większą świeżą masą i długością korzonka zarodkowego charakteryzowało się ziarno pszenicy ozimej po zastosowaniu szczepu T2 - T. atroviride.
***Słowa kluczowe**: Trichoderma* ssp., *wartość siewna, wigor, pszenica ozima*

1. Introduction

The high share of cereals in the crop structure, simplified sowing and adverse weather conditions favour the development of fungal diseases. Economic loss is caused especially by the higher occurrence and intensity of fungi of the *Fusarium* spp. genus. According to Walkowiak and Krzyśko-Łupicka [13], these pathogens reduce the yield of grains and deteriorate their quality, especially the germination capacity and baking parameters. Therefore, scientists search for new biological methods of pest control due to limitations concerning the use of crop protection products, greater environmental care and greater concern about crops [2, 5].

Many studies discuss the possibilities of using natural compounds and antagonistic microorganisms as an alternative to chemical products. *Trichoderma* spp. fungi are commonly known to aid the growth and development of plants [3, 9]. The studies conducted by Doni et al. [1] on rice, by Okoth et al. [10] on maize and beans, and by Mastouri et al. [8] on tomatoes proved that selected strains of *Trichoderma* spp. fungi improved the sowing value and vigour of seeds.

The aim of the study was to determine the influence of selected *Trichoderma* spp. strains on the sowing value and vigour of winter wheat seeds characterised by diversified degree of colonisation by *Fusarium* spp.

2. Material and methods

Experiments were conducted at the Seed Laboratory, Department of Agronomy, Poznań University of Life Sciences, Poland. Winter wheat grains of the Tonacja cultivar were harvested in 2013 and used as the research material. The caryopses varied in the degree of colonisation by fungi of the *Fusarium* spp. genus. The winter wheat grains were harvested at four different locations: A - Gałowo, West-Pomeranian Voivodeship, brown earth, soil valuation class IVa; B - Hulcze, Lublin Voivodeship, chernozem, soil valuation class Ia; C - Jarząbkowice, Lower Silesian Voivodeship, brown earth, soil valuation class IIIb; D - Karolew, Greater Poland Voivodeship, brown earth, soil valuation class IVa. Winter rape was the forecrop in the first two locations, sugar beets – in the third location, and maize – in the last one. Wheat was grown according to applicable agrotechnical rules.

The following factors were analysed in the research: seed colonisation by fungi of the *Fusarium* spp. genus and inoculation with selected *Trichoderma* spp. strains: T1 – *T. atro-*

viride (isolated from soil - field Skierniewice), T2 - *T. atroviride* (isolated from soil – field Radzanów), T3 – *T. harzianum* (isolated from soil- field Balcerów), T4 – *T. harzianum* (isolated from the substrate under champignons), T5 – *T. harzianum* (isolated from soil under organic plantations). Caryopses soaked in deionised water were used as the control variant. There were three independent series of univariate analyses with 4 replicates. The *Trichoderma* spp. isolates came from the collection of strains of the Department of Microbiology, Institute of Horticulture in Skierniewice, Poland. The strains were proliferated in a potato dextrose agar -PDA, (Sigma) on 90 mm Petri dishes at the Department of Environmental Microbiology, Poznań University of Life Sciences, Poland. The strains were incubated for 7 days at a temperature of 25°C. Initially they were stored in darkness and then they were exposed to visible light to increase the fungal sporulation efficiency. After incubation the dishes were flooded with 10 ml of sterile 0.85% NaCl solution. Mycelia were removed from the surface of the substrate with a sterile scalpel and transferred to sterile flasks. A hand-held blender (Braun) was used to separate conidial spores from mycelial hyphae. The spore density was measured with a haemocytometer. The final spore density of the isolates in a 100 ml NaCl solution amounted to 107 ml^{-1}. Strain isolates were used as spore suspensions.

After harvesting winter wheat grains were stored under controlled conditions, i.e. at 4°C, without access to light. After 4-month storage respective to the combination clean caryopses were soaked for 10 minutes and placed on filter paper of medium filtration rate in order to assess the sowing quality. The sowing value was expressed by means of the first count (germination energy) and the final count (germination capacity). The seed vigour was measured [4] by conducting vigour tests, i.e. the seedling growth test and the seedling growth rate test. Additionally, the seed vigour index was calculated by multiplying the mean length of the sprout (cm) and the mean germination capacity (%).

The results were analysed statistically. The least significant difference was estimated by means of Student's t-test, where the significance limit was $\alpha = 0.05$.

3. Results and discussion

The Table 1 shows the occurrence and percentage colonisation of winter wheat seeds by fungi of the *Fusarium* spp. genus. The following strains of *Fusarium* spp. fungi were found in the winter wheat seeds: *F. avenaceum* (Corda ex FR.) Sacc., *F. graminearum* Schwabe, *F. poae* (Peck) Wollenweber, *F. culmorum* (W.G.Sm.) Sacc., *F. equiseti* (Corda) Sacc. The highest percentage of fungal colonisation was found in the seeds from Lublin Voivodeship (38%), followed by the seeds from Lower Silesian Voivodeship (30%), Greater Poland Voivodeship (24%) and West-Pomeranian Voivodeship (11%). The most seeds from Lublin Voivodeship and Greater Poland Voivodeship were colonised by *F. graminearum* Schwabe. The most seeds from Lower Silesian Voivodeship were colonised by *F. avenaceum* (Corda ex FR.) Sacc., whereas those from Greater Poland Voivodeship were mostly colonised by *F. poae* (Peck) Wollenweber.

The quality of seeds largely depended on variable weather conditions in the research years [6, 12]. According to Lenc et al. [7], the standard protection of wheat from diseases does not always limit the colonisation of seeds by fungi of the *Fusarium* spp. genus. Crops are usually infected by the pathogen at the phase of florescence. In consequence, the yield may be reduced and its quality may be worse.

Table 1. The colonisation of winter wheat seeds by fungi of the *Fusarium* spp. genus (%) according to the locations
Tab. 1. Zasiedlenie ziarna pszenicy ozimej przez grzyby z rodzaju Fusarium spp. (w %) według lokalizacji

Colonisation	Location			
	A	B	C	D
F. avenaceum (Corda ex FR.) Sacc.	2	5	12	6
F. graminearum Schwabe	1	26	6	13
F. poae (Peck) Wollenweber	4	1	2	5
F. culmorum (W.G.Sm.) Sacc.	3	5	3	-
F. equiseti (Corda) Sacc.	1	1	7	-
Others	7	4	4	1
Total - *Fusarium* spp.	11	38	30	24

Source: own work / Źródło: opracowanie własne

The infestation by fungi of the *Fusarium* spp. genus had diversified influence on the seed sowing value (Table 2). The lowest germination energy (86%) and the highest percentage of rotting grains (6%) was noted in the combination which was the most colonised by fungi of the *Fusarium* spp. genus (combination B). The highest germination energy was noted in the seeds from combinations A and D (93%). The share of abnormally germinating seeds and rotten seeds did not differ significantly. The percentage of these seeds was similar.

Table 2. Seeds sowing value of winter wheat depending on infested grain by *Fusarium* spp. and strain of *Trichoderma* ssp.
Tab. 2. Wartość siewna ziarna pszenicy ozimej w zależności od porażenia przez Fusarium spp. i szczepu Trichoderma ssp.

Factor	Level	Energy capacity [%]	Share of abnormally germinating kernels [%]	Share of rotting kernels [%]	Share of healthy and not germinated kernels[%]
Infested grain *Fusarium* spp.	*A*	93	4	0	1
	B	86	4	6	2
	C	91	4	1	1
	D	93	3	1	1
$LSD_{0.05}$		1.9	*n.s.	1.1	n.s.
Strain of *Trichoderma* ssp.	control object	89	5	3	1
	T1	89	4	3	1
	T2	94	3	1	2
	T3	91	4	2	1
	T4	91	3	2	1
	T5	91	3	2	1
$LSD_{0.05}$		2.4	1.6	n.s.	1.2

* n.s. – not significant differences

Source: own work / Źródło: opracowanie własne

The inoculation of seeds with fungi of the *Fusarium* spp. genus significantly modified the sowing value of winter wheat seeds. In comparison with the uninoculated combination, the germination energy improved significantly (by 94%) in the combinations treated with strain T2. Panasiewicz et al. [11] observed a similar increase in the germination energy of narrow-leafed lupine when the T. *atroviride* strain was applied.

Our study revealed that the share of abnormally germinating seeds improved in all the combinations inoculated with *Trichoderma* spp., but there were no significant differences between the control variant and strains T1 and T3 or strains T2, T4 and T5. The *Trichoderma* spp. strains did not significantly modify the share of rotting seeds or healthy non-germinating seeds.

Our study showed that despite infestation with fungi of the *Fusarium* spp. genus winter wheat seeds exhibited germination capacity and met the requirements of qualified material, i.e. at least 85% (Table 3). The colonisation of seeds by fungi of the *Fusarium* spp. genus significantly influenced the germination capacity. Similarly to the germination energy, the highest germination capacity was observed in the combinations which were infested by the pathogen to a lesser extent (A and D). The lowest germination capacity was observed in the seeds where the percentage of infestation was the highest (B).

The inoculation with *Trichoderma* spp. improved the germination capacity in all the combinations under analysis. The highest germination capacity was noted in combination T2 - T. *atroviride*. In comparison with the uninoculated variant the germination capacity increased by 4 pp. The germination capacity increased by 3 pp in combinations T4 and T5 and by 2 pp in combinations T1 and T3.

In almost all the combinations infected with *Fusarium* spp. the inoculation of caryopses with *Trichoderma* fungi increased the germination capacity. The only exception was combination T3, which was characterised by the lowest colonisation of seeds by fungi of the *Fusarium* spp. genus. Among the seeds colonised by *Fusarium* spp. the greatest improvement in germination capacity was noted in the seeds which were the most infested by the pathogen (B). In comparison with the control variant the greatest increase was noted in strain T4 (8 pp). Okoth et al. [10] conducted a study on maize and observed that the inoculation of seeds with *Trichoderma* fungi improved the germination capacity. However, it did not have significant influence on the germination of beans. Mukhtar et al. [9] noted that the germination capacity of soy seeds improved when they were inoculated with *T. harzianum* and *T. hamatum* strains.

The vigour of winter wheat seeds was assessed according to the degree of colonisation by fungi of the *Fusarium* spp. genus. The analysis showed that this factor significantly influenced the values of the seedling growth test, the seedling growth rate test, the vigour index, the fresh weight of seedlings and the length of radicles. The analysis of the seedling growth test showed that the longest seedlings were found in combinations C and B, which were the most infested with *Fusarium* spp. The shortest seedlings were found in combination D, where the percentage of infestation with *Fusarium* spp. amounted to 24%. In comparison with the control variant, the seed inoculation with *Trichoderma* fungi caused a significant increase in this parameter in each combination. The greatest increase was noted in the combination inoculated with strain T2 - T. *atroviride*. However, this combination did not differ significantly from combination T3 - T. *harzianum* (Table 4). When the seeds colonised by *Fusarium* spp. were inoculated with *Trichoderma* fungi, the length of seedlings increased in nearly all combinations, except C and D, which had been inoculated with strain T5 - T. *harzianum*. As far as the seeds which were the least colonised by *Fusarium* spp. (A) are concerned, the most effective increase in the length of seedlings was observed in those inoculated with *Trichoderma* spp. strains T4 and T2, i.e. 14.7% and 13,1%, respectively. As far as the seeds which were the most colonised by *Fusarium* spp. (B) are concerned, strains T3 and T2 caused the greatest increase in the length of seedlings, i.e. 29.4% and 27.4%, respectively. 30% of the seeds in combination C were colonised by *Fusarium* spp. – the length of seedlings increased most after treatment with strains T2 and T3, i.e. by 17.2% and 16.9%, respectively. 24% of the seeds in combination D were colonised – the length of seedlings increased most after treatment with strains T2 and T1, i.e. by 18.4% and 13.7%, respectively.

The analysis of the dry weight of seedlings (the seedling growth rate test) showed the greatest weight in combination B (colonisation by *Fusarium* spp. – 38%) (Table 5). The smallest dry weight was observed in combination D (colonisation by *Fusarium* spp. – 24%). As far as the vigour index is concerned, the greatest value was noted in combination C (colonisation by *Fusarium* spp. – 30%), i.e. 648. The smallest fresh weight and the shortest radicle were noted in combination D (colonisation by *Fusarium* spp. – 24%). The assessment of the dry weight of winter wheat seedlings (the seedling growth rate test), the vigour index, the fresh weight of seedlings and the radicle length according to the *Trichoderma* spp. strain showed that the highest values of these parameters were noted after applying strain T2 – T. *atroviride*.

Doni et al. [1] compared seven *Trichoderma* spp. strains and identified seedlings characterised by the greatest weight, length and vigour index when *Trichoderma* spp. strain SL2 was applied. According to Zheng and Shetty [14], the seed vigour improved after inoculation with *Trichoderma* ssp. because phenolic compounds were induced during germination.

Table 3. Germination capacity depending on infested grain by *Fusarium* spp. and strain of *Trichoderma* ssp. (%)
Tab. 3. Zdolność kiełkowania w zależności od porażenia przez Fusarium spp. i szczepu Trichoderma ssp.

Infested grain *Fusarium* spp.	Strain of *Trichoderma* ssp.						Average
	control object	T1	T2	T3	T4	T5	
A	94	96	97	91	96	97	95
B	84	88	90	91	92	89	89
C	92	94	96	95	95	95	94
D	93	94	97	96	95	96	95
Average	91	93	95	93	94	94	-

LSD for infested grain *Fusarium* spp. – 1.9; LSD for Strain of *Trichoderma* ssp. – 1.5; LSD for infested grain *Fusarium* spp. x strain of *Trichoderma* ssp.– 3.1 *Source: own work / Źródło: opracowanie własne*

Table 4. Seedling growth test depending on infested grain by *Fusarium* spp. and strain of *Trichoderma* ssp. (cm)
Tab. 4. Test wzrostu siewki w zależności od porażenia przez Fusarium spp. i szczepu Trichoderma ssp. (cm)

Infested grain *Fusarium* spp.	Strain of *Trichoderma* ssp.						Average
	control object	T1	T2	T3	T4	T5	
A	6.04	6.63	6.83	6.52	6.93	6.39	6.56
B	5.95	7.31	7.58	7.70	6.82	7.29	7.11
C	6.32	7.05	7.41	7.39	6.86	6.43	7.08
D	5.09	5.79	6.03	5.69	5.70	5.32	5.60
Average	6.10	6.69	6.96	6.82	6.58	6.36	-

LSD for infested grain *Fusarium* spp. – 0.11; LSD for Strain of *Trichoderma* ssp. – 0.16; LSD for infested grain *Fusarium* spp. x strain of *Trichoderma* ssp.– 0.33 *Source: own work / Źródło: opracowanie własne*

Table 5. Seed vigour of winter wheat depending on infested grain by *Fusarium* spp. and strain of *Trichoderma* ssp.
Tab. 5. Wigor ziarna pszenicy ozimej w zależności od porażenia przez Fusarium spp. i szczepu Trichoderma ssp.

Factor	Level	Seedling growth rate test [mg/seedling]	Vigour index	Fresh mass of seedling [mg/seedling]	Length of seedling roots [cm]
Infested grain *Fusarium* spp.	*A*	5.48	611	60.0	13.1
	B	5.66	614	60.0	13.3
	C	5.36	648	57.0	13.4
	D	5.13	520	51.0	12.2
$LSD_{0.05}$		0.13	21.5	1.00	0.28
Strain of *Trichoderma* ssp.	control object	5.43	547	54.0	12.1
	T1	5.28	596	57.0	13.2
	T2	5.54	653	58.0	13.5
	T3	5.49	616	58.0	13.1
	T4	5.38	601	57.0	13.0
	T5	5.33	575	56.0	12.9
$LSD_{0.05}$		*n.s.	22.8	1.00	0.26

* n.s. – not significant differences *Source: own work / Źródło: opracowanie własne*

4. Concluding remarks

The results show that the *Trichoderma* ssp. strains had diversified influence on the basic parameters of the sowing value and vigour of winter wheat seeds, which varied in the degree of colonisation by fungi of the *Fusarium* spp. genus. The colonisation by fungi of the *Fusarium* spp. genus significantly influenced the quality of the sowing material. The lowest sowing value parameters were noted in the seeds which were the most colonised by the fungi (38%). The *Trichoderma* spp. strains significantly modified the sowing value and vigour of winter wheat seeds. When strain T2 – *T. atroviride* was applied, the quality of winter wheat seeds was higher. They were characterised by higher germination energy and capacity, greater vigour (expressed by means of the seedling growth test and the seedling growth rate test), greater fresh weight and longer radicles. Among the seeds with diversified degree of colonisation by *Fusarium* spp., the application of *Trichoderma* ssp. strains caused the greatest improvement in the germination capacity and the seedling growth test in the seeds which were most infested by the pathogen.

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