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The Effect of Funaben T Seed Dressing on the Occurrence of Bacteria in Soil under Peas

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Abstract

The aim of this study was to investigate the effect of the fungicide Funaben T (s.a. thiuram - 45%, carbendazym - 20%) on the occurrence of various ecotrophic groups of bacteria in soil under pea cultivation. In the examined developmental stages of the pea, in the rhizospheric and non-rhizospheric soil, the counts of oligotrophic, copiotrophic, methyltrophic, amylolytic, pectinolytic, *Pseudomonas*, phosphate solubilizing and Azotobacter bacteria were determined using culture methods. The obtained results showed that the applied fungicide, Funaben T, did not have any major effect on the growth of most investigated groups of bacteria in the non-rhizospheric zone. However, in the rhizosphere (in the majority of cases) it stimulated the growth of the investigated groups of bacteria except for the periods of budding and flowering. In those stages the fungicide inhibited the growth of bacteria.

Keywords: fungicide seed dressing, Funaben T, pea, rhizosphere, bacteria.

Introduction

Fungicides have not only a direct effect on soil microorganisms, but also an affect through plants, disturbing the processes of phosphorylation, photosynthesis or plant respiration [2,3]. Fungicidal preparations - through changes in plant metabolism - modify root secretion and indirectly contribute to quantitative and qualitative changes in the composition of soil microorganisms. These changes may manifest themselves especially in the rhizospheric zone of plants as seeds are dressed with these chemical compounds. Fungicides are nowadays commonly used to control fungi parasitizing on crop plants, including legumes. A lot of information is found in literature on the effect of fungicidal preparations on the activity of atmospheric nitrogen fixation both by free-living bacteria and those fixing N_2 in the symbiosis with legumes [13], while reports concerning their effect on soil microorganisms connected directly with the rhizosphere of legumes [19,20] are scarce.

This study presents investigations aimed at the comparison of the effect of Funaben T on the occurrence of microorganisms belonging to different ecotrophic groups of bacteria in the rhizospheric and non-rhizospheric zones of pea plants. The investigations concerned both oligotrophic and copiotrophic bacteria. Within the group of copiotrophic microorganisms, special attention was paid to methyltrophic, amylolytic and pectinolytic bacteria, as well as those solubilizing phosphates belonging to the *Pseudomonas* and *Azotobacter* genera. Observations were conducted in a one-year field experiment in 2000.

Material and Methods

The experimental site was a field under pea cultivation belonging to the Złotniki Experimental Didactic Station, part of the August Cieszkowski Agricultural University of Poznań.

Table 1. Characterization of some physico-chemical properties.

Soil level (cm)	pH_{KCl}	C g·kg^{-1}	N g·kg^{-1}	C:N	Percentage proportion of fraction with diameter in mm				Texture group*
0 - 30	5.1 -6.2	5.93	0.611	9.70	1- 0.1	0.1- 0.02	< 0.02	(< 0.02)	ps (sand)
					75 - 80	18 - 24	7	(2)	

*According to BN-78/91 180-11

The experiment was conducted (in the randomized block design in four replications) on soil belonging to group of grey-brown podzolic soils with grain composition of the layer of humus sand (ps), some physico-chemical characteristics of which are presented in Table 1.

The cultivated crop was a pea cultivar AGRA, sown in the amount of 300 kg·ha^{-1} on plots of 48 m^2.

The fungicide applied in the investigations as seed dressing was Funaben T (s.a. thiuram - 45%, carbendazym - 20%) in the amount of 400 g·100 kg^{-1} seeds and nitrogen fertilization in the amount of 30 kg N·ha^{-1}. Samples for analysis were collected at different developmental stages of pea plants: late emergence, shooting tendrils, budding, flowering and pods (green maturity and full maturity).

Microbiological analyses were conducted on the soil samples of the rhizospheric and non-rhizospheric zones of pea plants, the seeds of which either had previously been dressed with Funaben T or had not been dressed (control plots).

In the obtained suspensions of rhizospheric and non-rhizospheric soils - after a series of 10-times dilutions - counts of the following groups of bacteria were determined using the plate method:

- oligotrophic bacteria - on the DNB broth medium (diluted 100 times) according to Ohta and Hattory [16], after 21 days of plate incubation.
- copiotrophic bacteria - on the NB broth medium (undiluted) according to Ohta and Hattory [16], after 7 days of plate incubation.
- methyltrophic bacteria - on the medium prepared according to Urakami and Kamagata [22] with the addition of methanol in the amount of 10ml l^{-1} of medium, after 21 days of plate incubation.
- amylolytic bacteria - on the medium with starch according to Rodina [18], on the basis of the staining reaction with Lugol solution, after 3 and 5 days of plate incubation.
- pectinolytic bacteria - on the medium prepared according to Kreisel and Schraner [11], on the basis of the reaction with 1% hexadecyltrimethylammonia solution, after 5 days of plate incubation.
- bacteria of the genus Pseudomonas on the medium promoting the growth of these bacteria according to Rodina [18], after 36 hours of plate incubation.
- bacteria solubilizing phosphates on the medium prepared according to Rodina [18], after 5 days of plate incubation.
- bacteria from the genus *Azotobacter* on Jensen's medium modified by Fenglerowa [6], after 2 days of plate incubation.

All the investigated groups of bacteria were incubated at 28°C. Inoculations were performed in five replications. Bacterial counts were given in colony forming units per 1 gram of dried soil (cfu·g^{-1}).

Results concerning the bacterial counts were verified using the analysis of variance for factorial experiments in the independent design. The least significant differences were calculated using Tukey's t-test at the level of significance α=0.05.

Results and Discussion

Oligotrophic Bacteria

In the soil coming from the zone outside of rhizosphere no significant changes were observed in the counts of this group of bacteria in the period of pea vegetation and the applied fungicide did not affect their numbers. However, the number of oligotrophic bacteria in the rhizosphere of the control soil and that with applied fungicide was many times higher (up to 64 times) than outside the rhizosphere (Table 2). An advantageous effect was found of both the plant and the applied fungicide on the growth and development of this group of bacteria - characterized by relatively small nutrient requirements. During similar investigations [9] on the effect of Oxafun T on oligotrophic bacteria in the soil under barley cultivation such high numbers of these bacteria as obtained in this study were not observed. These differences were a result of other conditions than those existing in the root zone of barley and pea. The effect of the fungicide depended on the developmental stage of pea plants. Funaben T stimulated the growth of oligotrophs in rhizosphere during late emergence and maturing of pea plants. The rhizosphere effect in the presence of the fungicide increased more than two times during these stages of plant growth. On the other hand, in the period of budding and flowering of the pea plants, the number of oligotrophic bacteria (in comparison to the control) was reduced by the fungicide.

Copiotrophic Bacteria

Copiotrophic bacteria reacted to the presence of the fungicide similarly to oligotrophic bacteria (Table 3). No effect of the fungicide was observed on the development of

Table 2. Effect of Funaben T seed dressing on the number of oligotrophic bacteria in the rhizosphere and non-rizosphere soil under pea crop.

Pea growth-stage (C1-C6)	Bacteria number [cfu·g^{-1} DM of soil]·10^6				R:S relation	
	Control (A1)		Funaben T (A2)		Control	Funaben T
	S (B1)	R (B2)	S (B1)	R (B2)		
Late emergence	12.9	119.6	12.0	254.8	9.2	21.3
Shooting tendrils	17.0	675.0	11.8	647.0	39.7	53.9
Budding	12.0	266.9	20.9	70.9	22.3	3.4
Full flowering	6.6	137.0	9.0	60.8	22.8	6.8
Pod-green maturity	12.8	396.0	19.7	780.2	30.1	39.0
Pod-full maturity	35.0	1124.9	25.6	1684.7	32.1	64.8
LSD for A/BC comparison = 48.0 ·10^6 LSD for B/AC comparison = 48.0 ·10^6 LSD for C/AB comparison = 71.7 ·10^6					not analysed	

Explanations: S - non-rhizospheric soil, R - rhizospheric soil

Table 3. Effect of Funaben T seed dressing on the number of copiotrophic bacteria in the rhizosphere and non-rizosphere soil under pea crop.

Pea growth-stage (C1-C6)	Bacteria number [cfu·g^{-1} DM of soil]·10^6				R:S relation	
	Control (A1)		Funaben T (A2)		Control	Funaben T
	S (B1)	R (B2)	S (B1)	R (B2)		
Late emergence	8.2	37.9	3.5	99.8	4.6	28.8
Shooting tendrils	11.9	294.6	21.4	382.2	24.8	17.8
Budding	15.0	328.2	14.6	122.4	21.9	8.4
Full flowering	16.2	133.3	19.6	108.7	8.2	5.5
Pod-green maturity	15.3	157.7	11.9	608.4	10.3	51.1
Pod-full maturity	20.0	520.0	18.4	644.6	26.0	35.1
LSD for A/BC comparison = 11.5 ·10^6 LSD for B/AC comparison = 11.5 ·10^6 LSD for C/AB comparison = 17.1 ·10^6					not analysed	

Explanations as in Table 2.

copiotrophs in the zone outside of rhizosphere of pea plants. The dynamics of changes in the counts of copiotrophs in rhizosphere during the vegetation of pea was similar to that observed in case of oligotrophs, along with the effect of the fungicide on the bacterial counts. The rhizosphere effect under the influence of the applied fungicide increased several times during germination and maturing. A relatively large increase in the number of copiotrophs at the stage of full maturity of pea plants may be linked with an increased supply of nutrients reaching them from decaying root tissues.

Methylotrophic Bacteria

Literature data indicate a wide variety of methyltrophic bacteria in the rhizosphere of plants (at limited supply of oxygen) [17, 8, 4], as well as their significant contribution to the fixation of N_2 [1, 5]. In the zone outside the rhizosphere the biggest numbers of methyltrophs were found at the beginning of the vegetative period of pea plants, while at the later stages of plant development the numbers of bacteria from this group were lower (Table 4). In the soil treated with the fungicide (outside rhizosphere), initially the number of bacteria decreased, whereas subsequently it grew at the stage of pea maturity.

An advantageous effect of the crop plant on the count of methyltrophic bacteria was observed in the rhizosphere soil, especially in the period of shooting tendrils. The dressing of seeds with fungicide increased the size of the methylotrophs population in this zone in the period preceding budding and during maturing, whereas it reduced their number during budding and flowering, as it did also in the case of oligotrophs and copiotrophs. The obtained

Table 4. Effect of Funaben T seed dressing on the number of methylotrophic bacteria in the rhizosphere and non-rhizosphere soil under pea crop.

Pea growth-stage (C1-C6)	Bacteria number [cfu·g^{-1} DM of soil]·10^6				R:S relation	
	Control (A1)		Funaben T (A2)		Control	Funaben T
	S (B1)	R (B2)	S (B1)	R (B2)		
Late emergence	28.3	27.5	11.4	67.8	1.0	5.9
Shooting tendrils	4.4	255.7	2.4	279.1	57.9	117.3
Budding	1.1	37.8	2.9	21.8	35.3	7.7
Full flowering	2.2	54.4	2.9	13.9	25.2	4.9
Pod-green maturity	5.4	91.1	66.3	85.7	16.7	1.3
Pod-full maturity	14.3	106.3	15.5	119.8	7.5	7.7
LSD for A/BC comparison = 13.5·10^6 LSD for B/AC comparison = 13.5·10^6 LSD for C/AB comparison = 20.2·10^6					not analysed	

Explanations as in Table 2.

Table 5. Effect of Funaben T seed dressing on the number of amylolytic bacteria in the rhizosphere and non-rhizosphere soil under pea crop.

Pea growth-stage (C1-C6)	Bacteria number [cfu·g^{-1} DM of soil]·10^6				R:S relation	
	Control (A1)		Funaben T (A2)		Control	Funaben T
	S (B1)	R (B2)	S (B1)	R (B2)		
Late emergence	7.8	85.2	6.4	129.8	10.9	20.1
Shooting tendrils	4.3	56.9	4.2	57.9	13.3	13.9
Budding	8.7	398.2	4.9	172.3	45.8	35.0
Full flowering	6.1	234.1	4.6	103.5	38.5	22.5
Pod-green maturity	4.1	267.8	3.1	489.1	64.9	159.8
Pod-full maturity	11.6	635.0	13.6	844.5	54.9	62.3
LSD for A/BC comparison = 23.4·10^6 LSD for B/AC comparison = 23.4·10^6 LSD for C/AB comparison = 35.0·10^6					not analysed	

Explanations as in Table 2.

results were different from those obtained by Kaszubiak and Durska [9], who did not observe a reduction in the oligotrophs, copiotrophs and methyltrophs population under the influence of Oxafun T in the rhizosphere of barley at the stage of flowering. Considering that fact, it should be assumed that the plant species had an effect on the development of the investigated groups of bacteria in that zone.

Amylolytic and Pectinolytic Bacteria

The fungicide was also investigated in terms of its effect on microorganisms utilizing organic carbon from starch and pectins.

The number of amylolytic bacteria (Table 5) showed considerable stability in the zone outside of rhizosphere, irrespective of fungicide application. However, in the rhizosphere the fungicide stimulated the size of the population of these bacteria at the stages of late emergence and maturity, while it reduced the number of bacteria in the period of budding and flowering of pea plants.

No significant effect of the fungicide on the number of pectinolytic group of bacteria was observed in the zone outside the rhizosphere (Table 6).These bacteria in the rhizosphere under the influence of the applied fungicide reached at the stage of shooting tendrils the highest of the observed values, indicating that their growth was stimulated by the fungicide (at that stage of plant development). This group of bacteria was characterized by the biggest effect of plant presence during the whole period of pea vegetation, reaching the value of the rhizosphere effect amounting to 250.

Table 6. Effect of Funaben T seed dressing on the number of pectinolytic bacteria in the rhizosphere and non-rhizosphere soil under pea crop.

Pea growth-stage (C1-C6)	Bacteria number [cfu·g^{-1} DM of soil]·10^5				R:S relation	
	Control (A1)		Funaben T (A2)		Control	Funaben T
	S (B1)	R (B2)	S (B1)	R (B2)		
Late emergence	10.1	192.1	8.3	204.8	19.1	24.6
Shooting tendrils	7.1	770.4	4.1	1020.0	107.9	250.0
Budding	5.9	646.8	4.3	435.1	109.9	101.6
Full flowering	2.7	321.6	1.0	152.5	119.1	147.1
Pod-green maturity	2.6	97.2	2.6	337.9	38.1	132.5
Pod-full maturity	14.4	445.0	21.6	369.6	30.8	17.1
LSD for A/BC comparison = 27.3·10^5 LSD for B/AC comparison = 27.3·10^5 LSD for C/AB comparison = 40.8·10^5					not analysed	

Explanations as in Table 2.

Table 7. Effect of Funaben T seed dressing on the number of Pseudomonas bacteria in the rhizosphere and non-rhizosphere soil under pea crop.

Pea growth-stage (C1-C6)	Bacteria number [cfu·g^{-1} DM of soil]·10^6				R:S relation	
	Control (A1)		Funaben T (A2)		Control	Funaben T
	S (B1)	R (B2)	S (B1)	R (B2)		
Late emergence	3.9	32.1	4.7	46.2	8.3	9.9
Shooting tendrils	2.8	59.6	2.9	76.6	21.6	26.7
Budding	44.9	336.4	10.3	105.1	7.5	10.2
Full flowering	14.4	130.0	29.4	108.7	9.0	3.7
Pod-green maturity	8.2	148.7	5.4	757.9	18.2	139.3
Pod-full maturity	21.4	702.3	11.5	862.6	32.8	74.9
LSD for A/BC comparison = 13.3·10^6 LSD for B/AC comparison = 13.3·10^6 LSD for C/AB comparison = 19.9·10^6					not analysed	

Explanations as in Table 2.

From the ecological point of view, a lack of inhibitory action of the fungicide towards these groups of microorganisms, especially pectinolytic bacteria, may be significant, as these bacteria participate in the colonization of plant roots by rhizobia [7].

Pseudomonas Bacteria

It is common knowledge that bacteria from the genus *Pseudomonas*, metabolizing 90% of nutrients supplied by the roots, are also closely associated with the rhizosphere of plants [23]. Liste [14] and Kundu, and Gaur [12] showed a synergistic interaction between dinitrogen fixing bacteria and *Pseudomonas* that stimulated plant growth. The mechanism of the synergistic co-inoculation effect manifested itself in the increase of root surface area, the stimulation of nitrogenase activity, the uptake of phosphorus and potassium in shoots, and bigger microbial populations in the rhizosphere.

The number of *Pseudomonas* under pea cultivation (Table 7) was 10 times higher when compared to the data for the soil under barley cultivation [9]. In the zone outside of rhizosphere the population of these bacteria reached the highest values during budding and full maturity. The effect of the fungicide was relatively small and variable, although it reduced the number of bacteria at the stage of budding and maturity of pea plants. In the rhizosphere, the fungicide increased the number of *Pseudomonas* bacteria during most of the vegetation stages of pea, except for the stages of budding and flowering.

Table 8. Effect of Funaben T seed dressing on the number of phosphate solubilizing bacteria in the rhizosphere and non-rhizosphere soil under pea crop.

Pea growth-stage (C1-C6)	Bacteria number [cfu·g^{-1} DM of soil]·10^5				R:S relation	
	Control (A1)		Funaben T (A2)		Control	Funaben T
	S (B1)	R (B2)	S (B1)	R (B2)		
Late emergence	14.8	289.3	21.8	651.9	19.5	29.9
Shooting tendrils	-	-	-	-	-	-
Budding	13.5	312.5	26.4	244.1	23.2	9.2
Full flowering	10.4	96.0	12.0	94.6	9.3	7.9
Pod-green maturity	5.6	130.0	2.9	218.4	23.2	75.6
Pod-full maturity	15.5	63.7	34.2	82.1	4.1	2.4
LSD for A/BC comparison = 20.4·10^5 LSD for B/AC comparison = 20.4·10^5 LSD for C/AB comparison = 28.9·10^5					not analysed	

Explanations as in Table 2.

Table 9. Effect of Funaben T seed dressing on the number of *Azotobacter* bacteria in the rhizosphere and non-rizosphere soil under pea crop.

Pea growth-stage (C1-C6)	Bacteria number [cfu·g^{-1} DM of soil]				R:S relation	
	Control (A1)		Funaben T (A2)		Control	Funaben T
	S (B1)	R (B2)	S (B1)	R (B2)		
Late emergence	18	13	7	2	0.7	0.3
Shooting tendrils	12	14	5	4	1.2	0.9
Budding	8	9	5	9	1.2	1.9
Full flowering	8	16	2	2	1.9	1.5
Pod-green maturity	19	16	6	3	1.0	0.4
Pod-full maturity	20	24	7	7	1.2	1.1
LSD for A/C comparison = 6.0 LSD for B/C comparison = 6.0 LSD for C/A and C/B comparison = 8.9					not analysed	

Explanations as in Table 2.

Phosphate Solubilizing Bacteria

Quite a significant role in the stimulation of the growth and metabolic activity of plants is played by microorganisms in the soil participating in the process of facilitating the assimilation of phosphorus from non-assimilable orthophosphates [10, 24].

The highly quantitative stability of these bacteria was found in the zone outside the rhizosphere, irrespective of the application of fungicide (Table 8).

However, in the rhizosphere the number of phosphate solubilizing bacteria was higher at the beginning of vegetation stages. The fungicide stimulated the development of these bacteria after germination and in the period of maturing, whereas it inhibited it during budding. In the investigations, for reasons outside the control of the author, the stage of shooting tendrils was omitted, which may affect the interpretation of results.

Azotobacter Bacteria

Taking into consideration high significance of dinitrogen fixing bacteria from *Azotobacter* genus, free-living in the rhizosphere of plants, they were also included in our investigations. The found numbers of these bacteria ranged from several to several dozen cells per one gram of dried soil (Table 9). The size of the *Azotobacter* population was similar in both rhizosphere and non-rhizosphere soil, and it showed a decreasing tendency until the stage of budding, whereas a growing trend was observed after

that stage. The fungicide - Funaben T reduced the number of *Azotobacter* cells, irrespective of the soil zone; only during budding no inhibitory effect of the fungicide was observed.

The obtained results confirm the data reported earlier by Niewiadomska [15], who in the presence of Funaben T also found the inhibition of the growth of *Azotobacter*. Similar results were also obtained by Swędrzyńska and Sawicka [21] in their studies on *Azospirillum*.

Conclusions

1. No significant effect of the fungicide was observed on the growth of most investigated groups of ecotrophic bacteria in the zone outside of rhizosphere of pea plants (except for bacteria from *Azotobacter* genus).
2. The addition of the fungicide to the seeds had an inhibiting effect on the growth of the analyzed groups of bacteria during budding and flowering stages in the rhizosphere soil, whereas in most cases it was stimulated at the stages of late emergence, shooting tendrils and maturity.

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