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Original Article

Unconventional Bio-fungicides for the Seed Treatment Used in the Control of Main Wheat Diseases

PUIA Carmen, Loredana SUCIU, Raluca MICLEA*

University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture, 3-5 Manastur St. 400372, Cluj-Napoca, Romania

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Abstract

The objective of the present paper was the study of the unconventional treatment behaviour and the influence of these treatments on the wheat diseases. In this research there has been tested the efficacy of different seed treatments before sowing and during the vegetation. Before sowing the products were tested in the laboratory - the spring wheat kernels of Pădureni variety being treated and analysed in whet chamber. The pathogens observed on the wheat kernels were from the genus *Penicillium*, *Fusarium*, *Alternaria* and also a bacterial strain, in different percents. In the experiments 19 variants of biological products were applied on the wheat kernels, by immersion for 3-5 minutes, followed by the powdering with zeolitic dust. At these variants is also added an untreated variant (no 20) – the control. The biological products are constituted of various combinations of plant extracts, essential oils, antagonistic and symbiotic microorganisms, commercial products and other substances. During the vegetation period, observations were made on the emergence of the plants, the incidence of foliar and ear diseases. The testing of the efficacy of the BIONEC products under real field conditions was carried out in the experimental field of ADRS Turda, where an experience was established in which the products were tested.

Keywords: wheat, bio-products, plant extracts, kernel, pathogens, unconventional.

1. Introduction

In agriculture, currently it is pursuit the growth of the quality by increasing biological protection in order to eliminate toxic and polluting chemical pesticides. In an attempt to reduce the use of chemicals to control main plant pathogens had been developed safer alternative that does not have negative consequences on the environment, humans and on animals and that is part of the sustainable agriculture concept, this involving the rational and integrated control of plant diseases.

In an attempt to reduce the use of chemicals to control plant pathogens have been developed safer alternatives that do not have negative consequences on the environment, human and animal, and that are part of the concept of sustainable agriculture [1].

Romania has favourable conditions for the promotion of organic farming because has a favourable agro-ecological potential being able to convert at least 15% of the agricultural area of the country for this type of agriculture. The future of agriculture is mainly focused on achieving healthy products, maintaining soil fertility, optimizing agricultural production and the environment without neglecting and food security issues [3].

In cereal crops, to increase productivity it is necessary to use a healthy biological material with

* Corresponding author.
Tel: +40-264-596384
Fax: ++40-264-593792
e-mail: miclearalucavasilica@yahoo.com

high cultural value and with a high degree of biological purity.The research conducted is part of a research project „Biofungicide and unconventional treatment methods for cereal seed used to control the main toxigenic fungi with impact on the quality in sustainable agriculture context BIONEC". The research aims to obtain an unconventional bio fungicides for the treatment of cereals kernels used in the control of main toxigenic fungi, a protocol to achieve unconventional bio fungicides cereals seed treatment used to combat the major toxigenic fungi, an appropriate method of application of this type of treatment, with impact on quality and to study the behaviour and influence on wheat diseases.

2. Material and Method

The biological material used in his experiments was a zeolite material treated with plant extracts and also essential oils.

The preparation of the plant extracts was made from dry vegetable material (dry herb or dry rhizome) or, in some cases, fresh material was used. Essential oils have been purchased already-made.

The hydroalcoholic extracts were made according to the methodology of Pârvu [1] by macerating the whole plant or certain parts of the plant in alcohol of various concentrations for 12 days.In order to observe the in vivo efficacy of the bio products on wheat seeds, 19 treatment combinations (Table 1) were designed with whom series of experiments were made.

The treatment variants were carried out by immersing the seeds in the biocontrol solution for 3-5 minutes followed by powdering with zeolite dust (volcanic tuff) - which also serves as soil improver for agricultural soils with reduced ion exchange capacity and also is a source of potassium for plant culture.

Table 1. The treatment variant used in the experiments

Variant code	Materials used
V1	e.o. *Malaleuca alternifolia* + *Trichoderma* sp.+humic acids
V2	e.o. *Origanum vulgare*
V3	e.o. *Satureja hortensis*
V4	h.e. *Allium sativum* + e.o. *Cinamomum zeylanicum*+ h.e. *Satureja hortensis*+Glomax
V5	h.e. *propolis + e.h. Capsicum annuum*+ mycorhiza dust
V6	*Trichoderma spp.strains*
V7	h.e. *Picea* + *Abies* + h.e. *Allium sativum*+ Radiforce
V8	h.e. *Aloe marlothi*+ h.e. *Allium sativum*+ humus
V9	h.e. *Aloe paradisiacum* + e.o. *Satureja hortensis +Trichoderma sp.*
V10	h.e. *Satureja hortensis + Trichoderma spp.*
V11	h.e. *Tagetes* sp + h.e. *Allium sativum*+ mycorhiza dust
V12	h.e. *Armoracia rusticana* + *Trichoderma* spp.
V13	h.e. *Arnica montana* + h.e. propolis+ humic acids
V14	h.e. *Achillea millefolium*+ e.o. *Satureja hortensis*
V15	h.e. *Rubus idaeus* + e.o. *Cinnamomum zeylanicum*
V16	e.o. *Origanum vulgare* e.o. *Cinnamomum zeylanicum*+ e.o. *Satureja hortensis*
V17	h.e. *Allium sativum* + h.e. *propolis + Trichoderma sp.*
V18	h.e. *Capsicum annuum*+ *Trichoderma spp.*
V19	h.e. *Chelidonium majus*+ e.h. *Allium cepa*+ e.o. *Malaleuca alternifolia*
V20	Untreated variant

*Legend: e.o. – essential oil; h.e. – hydroalcoholic extract

Biological products (named BIONEC products) are various combinations of plant extracts, essential oils, microorganisms, commercial products and other substances such as humic acids, humus, mycorrhizal dust (from Mykosoil) and two commercial products Glomax and Radiforce produced by Agrifutur (Table 1).

Field testing was carried out in spring using PADURENI spring wheat variety as a biological material in the experimental field of ADRS Turda

Before sowing in the field, the products were tested in the laboratory.

To determine the effectiveness of the products tested against the caryopses pathogens the wet chamber method was used. After 48h the kernels were subjected to the macroscopic and microscopic examination.

The characteristics of the Pădureni wheat variety are as follows: extensive variety with medium production potential; plant height 105-120 cm; red ear with arista, 9-11 cm; small oval, red with MMB 29-38 g; sowing density of 400-550 germinal grains/ m^2; average twinning ability of 1.5-2 brothers/ plant; good resistance to spice grains; vegetation period 113-130 days; medium resistant to powdery mildew, yellow rust, septoriosis and sensitive to brown rust and fusariosis; good quality of bakery and milling; protein content 14.2%, dry gluten 9.1% and wet gluten 29.1%; production capacity of 3.910 - 5.630 kg/ ha [4]. Under favourable conditions, the production of this variety may exceed 6500 kg/ ha. At ADRS Turda, in the recent years, for Padureni spring wheat variety, were obtained comparable yields to those obtained from autumn wheat [4].

Prior to sowing, a milling work was done to level the ground and destroy the weeds that emerged in the spring.

Sowing was done on 27 March on an argiloiluvium chernozem soil and the pre-plant was pea. The experimental variants were sown in open gutters - each variant being sowed in three replicates. The sowing density was 550-640 grains / m^2 depending on the germination capacity determined in the laboratory at each variant, so that the number of germinal grains/m^2 would be according to the variety parameters.

After sowing a fertilization with nitrogen in the form of 200 kg of ammonium nitrate a 61 herbicides SDMA 0,5 l/ha and Sekator Progres 0,15 l/ha were applied. For pest management was made a treatment with Fastac 0.1 l/ ha.

To determine the degree of attack of phytopathogens was calculated the frequency and the severity of the attack.During the vegetation eriod observations were made regarding the occurrence and incidence of foliar diseases. The data from the observations were interpreted using the POLIFACT program.

3. Results and Discussions

The results obtained after the observations made in the laboratory are presented in Table 2. Pathogens traced during laboratory observation of the wheat kernels were: *Fusarium* spp., *Alternaria* spp., *Helmintosporium* spp., *Epiccocum* spp., *Cladosporium* spp., *Xanthomonas* spp. The others species of fungi identified on the spring wheat kernels were *Penicillium* sp., *Rhizopus* sp., *Gonatobotrys* sp., *Mycotypha* sp. and peritecia of *Erysiphe* sp.

From all the 20 variants seeded only 6 variants have emerged:

- V1 – e.o. *Malaleuca alternifolia* + *Trichoderma* sp. + humic acids,
- V3 - e.o. *Satureja hortensis*,
- V4 - h.e. *Allium sativum* + e.o. *Cinamomum zeylanicum* + h.e. *Satureja hortensis* +Glomax,
- V6 - tulpini de *Trichoderma spp.*,
- V10 – h.e. *Satureja hortensis* + *Trichoderma spp.* and
- V20 – untreated control, these variants being the same that germinated in the laboratory conditions on wet chamber.

In Table 3 it can be observed the behaviour of the treated variants that have risen compared with the untreated control.

The analysis of the results presented in Table 3 shows that from the point of view of emergence, variants V1, V3 and V4 were below the potential of the Pădureni variety, which requires a minimum of 400 germinal kernels (germ)/m^2.

Compared to the control variant in which 438.67 plants emerged, V6 and V10 treatment variants recorded values close to the maximum potential of the Pădureni variety. This result reveals the antagonistic effect on soil pathogens of *Trichoderma* species.The twinning degree of all variants falls within the upper limit of the parameters characteristics of the variety; reaching values ranging from 1.75 to 2.01.Regarding the number of ears at harvest/ m^2 is observed the correlation of these values with the level of emergence. The highest number of ears was recorded at V6 and V10 treatment variants and the smallest number of spices was observed in variant V4. In this variant, also was observed the lowest number of emerged plants.

The MMB (mass of one thousand grains) values were over 30 grams for all the variants, the V1 variant recording the highest value of 38.30 g, slightly above the maximum potential of the variety.

We note that the V10 variant which although had the highest density at seeding, at harvest had an MMB level of 34.8 g.

Table 2. Treatment of seed by immersion - Padureni variety

Var.	Germination		Pathogenic agents %						
	G.F. %	A.G. %	*Fusarium* spp.	*Alternaria* spp.	*Helminth.* sp.	*Epicoccum* sp.	*Cladosporium sp.*	*Xanthomonas* spp.	Other species
V1	13.3	-	-	-	-	-	-	-	13.3
V2	-	-	-	-	-	-	-	66.7	-
V3	60.0	6.7	46.7	53.3	-	8.3	-	16.7	-
V4	13.3	6.7	6.7	13.3	13.3	-	-	-	13.3
V5	-	-	26.7	-	-	-	-	26.7	20.0
V6	100	-	-	6.7	-	6.7	-	-	-
V7	-	-	46.7	26.7	6.7	6.7	6.7	33.3	26.7
V8	-	-	66.7	6.7	-	33.3	13.3	6.7	93.3
V9	-	-	13.3	-	-	-	-	86.7	-
V10	93.3	-	-	-	-	13.3	-	-	13.3
V11	8.3	-	46.7	6.7	-	13.3	13.3	66.7	40.0
V12	-	-	20.0	-	-	-	-	20.0	-
V13	-	-	73.3	-	-	-	-	33.3	20.0
V14	-	-	-	13.3	-	-	-	46.7	6.7
V15	-	-	20.0	6.7				60.0	20.0
V16	-	-	6.7					80,0	13.3
V17	-	-	60.0	13.3	-	-	-	66.7	20.0
V18	-	-	13.3	-	-	-	-	-	-
V19	-	-	60.0	13,3	-	-	-	46.7	73.3
V20	93.3	20.0	66.7	60.0	-	-	20.0	13.3	-

*Legend: G.F. - Germinative faculty; A.G. - Abnormal germs; *Helminth*. - *Helminthosporium*

Table 3. Initial observations made in the field

Variant	No. of rise plants	Twinning No of twins/plant	Ear no at harvest/ m^2	Grain mass at harvest (g/m^2)	MMB (g)	Yield (Kg/ha)
V1	382.67	2.00	768.00	435.33	38.30	4353.33
V3	381.33	1.99	760.00	461.87	32.70	4618.67
V4	308.00	2.00	616.00	344.67	32.00	3446.67
V6	526.00	1.75	920.00	527.87	32.10	5278.67
V10	550.67	2.01	1104.00	638.93	34.80	6389.33
Mt	438.67	2.01	880.00	467.87	32.90	4678.67

In terms of production, the variants V6 and V10 recorded the highest values compared to the untreated control and V10 exceeded the maximum potential of the variety. At the opposite pole, the productions of variants V1 and V4 were well below the untreated control level. Pathogens traced during vegetation were *Puccinia graminis* – black rust, *Puccinia recondita* – brown rust, *Zymoseptoria tritici* c.f. *Septoria tritici* and *Phaeosphaeria nodorum* f.c. *S. nodorum* – septoriosis and *Blumeria* (*Erysiphe*) *graminis* c.f *Oidium monilioides* –powdery mildew.

Table 4. The phytosanitary status in the field

Diseases	Observation	V1	V3	V4	V6	V10	Control
Black rust	F%	50.95	53.13	45.41	44.52	33.96	68.28
Brown rust	F%	23.74	64.44	48.96	55.68	35.83	64.97
	GA%	0.20	1.22	0.58	0.73	0.18	1.51
Septoriosis	F%	9.07	12.63	15.50	9.86	10.53	15.58
	GA%	0.03	0.08	0.08	0.04	0.05	0.14
Powdery mildew	F%	32.64	37.02	30.13	37.45	36.99	35.69
	GA%	2.38	2.82	2.17	3.19	3.82	4.75

*Legend: F%- frequency of the attack; GA % - Degree of attack %

The black and brown rust had manifested at a high frequency (over 20%) in all tested variants but with values below the untreated control. The lowest frequency and degree of rust attack was observed in variants in V10 and V1. Regarding the *Septoria* spp. attack it was observed that the lowest frequencies were recorded in the variants V1, V6 and V10, compared to the untreated control, and in all the experimental variants the attack rate has lower values below 0.15%.From all the foliar diseases encountered on vegetation, the highest degree of attack was recorded in the case of powdery mildew that had the value between 2% and 5%.

The frequency of attack on this disease was between 30% and 37%.As can be seen in Table 5, powdery mildew attack (*Blumeria graminis* f.c. *Oidium monilioides*) was reported in all the studied variants. The lowest values of the frequency of attack were recorded in variants V1 and V4, V4 being the single variant with a statistically ensured difference.

The frequency of the mildew attack was higher at variants 3, 6 and 10 but in insignificant limits compared to the untreated control.

Figure 2 shows the intensity and the degree of attack of the powdery mildew, on vegetation in the treated wheat variants of Pădureni. We can observe the low intensity of attack, below 15% in all variants, confirming the characteristic of the variety – medium resistant variety.

Table 5. Influence of test variants on the frequency of powdery mildew

Var. code	Variant	F %	% to control	Difference to control	Significance of the difference
20	Untreated control	35.70	100.0	0.00	Control
1	e.o. *Malaleuca alternifolia* +*Trichoderma* sp.+humic acids	32.64	91.4	-3.05	-
3	e.o. *Satureja hortensis*	37.02	103.7	1.33	-
4	h.e. *Allium sativum*+ e.o. *Cinamomum zeylanicum*+ h.e. *Satureja hortensis*+Glomax	30.13	84.4	-5.57	0
6	*Trichoderma strains*	37.45	104.9	1.76	-
10	h.e. *Satureja hortensis*+*Trichoderma spp.*	37.00	103.6	1.30	-
DL (p 5%)				5.49	
DL (p 1%)				7.80	
DL (p 0.1%)				11.29	

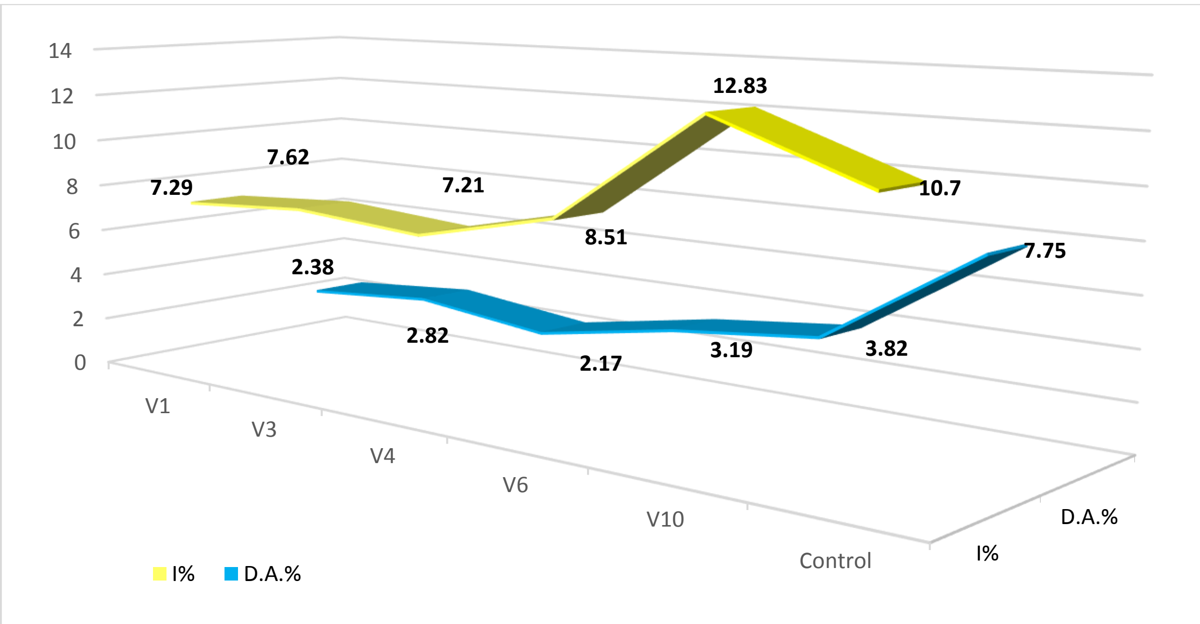

Figure 2. Powdery mildew – Intensity and Degree of attack

The degree of attack reached low values (below 4%) for all treatment variants, and a higher value of 7.75% for the untreated control.In the climatic conditions of the experimental year, *Septoria* spp. attack was manifested at a low frequency, below 16% in all the variants (Table 6). The lowest frequency values were recorded in variants V1 and V6, the difference from the untreated control of variant V1 being statistically assured.The highest efficacy on septoriosis was observed in the variant treated with *Trichoderma* strains, the frequency being the lowest compared to the untreated control. Concerning the attack of brown rust (*Puccinia recondita*) it can be seen in Table 7 that the lowest values of the attack frequency has been recorded in the V1 and V10 variants, the differences being significant and distinct significant negative compared to the control (Table 7). On the opposite side, variant 3 is noticeable with the approximate the same frequency as the untreated control.

In the case of *Puccinia graminis* producing black rust attack on wheat, in all the treatment variants the observed frequency of the attack was lower than that of the untreated control - Table 8.

There were also noticed frequency differences, statistically assured in the case of variants V4, V6 and V10.

Table 6. Influence of test variants on the frequency of *Septoria* spp.

Var. code	Variant	F %	% to control	Difference to control	Significance of the difference
20	Untreated control	15.58	100.0	0.00	Control
1	e.o. *Malaleuca alternifolia* +*Trichoderma* sp.+humic acids	9.07	58.2	-6.51	0
3	e.o. *Satureja hortensis*	12.64	81.1	-2.94	-
4	h.e. *Allium sativum*+ e.o. *Cinamomum zeylanicum*+ h.e. *Satureja hortensis*+Glomax	15.50	99.5	-0.08	-
6	*Trichoderma strains*	9.86	63.3	-5.72	-
10	h.e. *Satureja hortensis*+*Trichoderma spp.*	10.54	67.6	-5.04	-
DL (p 5%)				6.15	
DL (p 1%)				8.74	
DL (p 0.1%)				12.66	

Table 7. Influence of test variants on the frequency of brown rust

Var. code	Variant	F %	% to control	Difference to control	Significance of the difference
20	Untreated control	64.97	100.0	0.00	Control
1	e.o. *Malaleuca alternifolia* +*Trichoderma* sp.+humic acids	23.74	36.5	-41.23	00
3	e.o. *Satureja hortensis*	64.44	99.2	-0.53	-
4	h.e. *Allium sativum*+ e.o. *Cinamomum zeylanicum*+ h.e. *Satureja hortensis*+Glomax	48.96	75.4	-16.01	-
6	*Trichoderma strains*	55.68	85.7	-9.29	-
10	h.e. *Satureja hortensis*+*Trichoderma spp.*	35.83	55.1	-29.14	0
DL (p 5%)				21.20	
DL (p 1%)				30.14	
DL (p 0.1%)				43.64	

Table 8. Influence of test variants on the frequency of black rust

Var. code	Variant	F %	% to control	Difference to control	Sgnificance of the difference
20	Untreated control	68.28	100.0	0.00	Control
1	e.o. *Malaleuca alternifolia* +*Trichoderma* sp.+humic acids	50.95	74.6	-17.33	-
3	e.o. *Satureja hortensis*	53.13	77.8	-15.15	-
4	h.e. *Allium sativum*+ e.o. *Cinamomum zeylanicum*+ h.e. *Satureja hortensis*+Glomax	45.41	66.5	-22.87	0
6	*Trichoderma strains*	44.52	65.2	-23.75	0
10	h.e. *Satureja hortensis*+*Trichoderma spp.*	33.96	49.7	-34.32	00
DL (p 5%)				19.83	
DL (p 1%)				28.19	
DL (p 0.1%)				40.82	

4. Conclusions

In conclusion the seed treatments had an impact on the seed transmitted pathogens – it had reduced the attack of *Septoria* spp., *Fusarium* spp. and it had a fungistatic effect against *Helminthosporium* genus which has not appeared in the field (observed on the kernel before sowing).

On the foliar diseases the effect of the treatments was indirect, the humic acids and the mycorrhizal fungi from the composition of the treatment had an fertilizing effect, increasing the vigour of the plants and gave them a bigger resistance to the foliar infections during vegetation (powdery mildew, rust).

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