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A PRELIMINARY EXAMINATION OF TIMOREX GOLD 24 EC AS A NATURAL SEED DRESSING FOR WINTER WHEAT

Summary

Timorex 24EC is a commercial, natural fungicide containing 23.8% of oil extracted from tea tree [Melaleuca alternifolia (Maiden & Betche) Cheel], registered in more than 30 countries around the world, including Poland, to control some fungal diseases of vegetables grown in conventional and organic systems. The aim of this preliminary work was to establish concentrations of this product applied as seed dressing, at which it would be effective in reducing fungal contaminations of winter wheat seeds. Results of this study indicate that 1:10 water dilution of Timorex Gold 24 EC can be recommended as a seed dressing at the rates of 1-2 ml/100g of seed as at this rate it effectively inhibited development of fungi contaminating winter wheat seeds and did not show any adverse effects on seed germination.
***Key words**: natural fungicide, seed dressing, winter wheat, germination, fungi, inhibition*

WSTĘPNE BADANIA NAD WYKORZYSTANIEM TIMOREXU GOLD 24 EC JAKO NATURALNEJ ZAPRAWY NASIENNEJ DLA PSZENICY OZIMEJ

Streszczenie

Timorex 24 EC jest handlowym preparatem zawierającym 23,8% olejku z krzewu herbacianego [Melaleuca alternifolia (Maiden & Betche) Cheel], który zarejestrowany jest w ponad 30 krajach na świecie, także w Polsce, jako naturalny fungicyd do zwalczania niektórych chorób grzybowych w konwencjonalnych i ekologicznych uprawach warzyw. Wyniki uzyskane w tych badaniach wskazują, że Timorex Gold 24 EC rozcieńczony wodą w stosunku 1:10 może być rekomendowany do przedsiewnego zaprawiania nasion pszenicy ozimej w ilości 1-2 ml/100 g nasion. W wymienionej koncentracji fungicyd ten skutecznie ograniczał rozwój grzybów zanieczyszczających ziarno pszenicy i nie wpływał ujemnie na kiełkowanie nasion.
***Słowa kluczowe**: naturalny fungicyd, zaprawa nasienna, pszenica ozima, kiełkowanie, grzyby, hamowanie*

1. Introduction

Pre-planting treatment of seeds with preparations containing fungicides or insecticides, or both, is one of the most important practices widely used in modern agriculture. These pesticides applied as seed dressings protect germinating seeds and young seedlings of agricultural crops against numerous fungal pathogens and pests [1, 4, 5, 6]. A choice of synthetic seed protectants for commercially grown crops is abundant [9], but for the organic crop production system these products are not allowed [1]. For this reason various microorganisms or substances of a natural origin have been tested as seed dressings for their effectiveness in the protection of germinating seeds against fungal pathogens. For example, Panasewicz et al. [7] reported that two fungal species belonging to the genus *Trichoderma*: *T. artroviride* and *T. harzianum* applied as seed dressings improved sowing value of blue lupine seeds and that *T. artroviride* was more effective in this respect than *T. harzianum*. Wielgusz et al [8] have shown that selected formulations of hemp essential oil applied as to seeds effectively protected flax plants against *Fusarium oxysporum* f. sp. *lini* and significantly increased seed yields of this crop. Wiewióra [9] tested various methods of controlling fungal pathogens occurring on grain of organically grown cereals and found that seeds treated with hot water (50^{o} C, 30 min.) or soaked for 24 hours in 10% solution of sodium bicarbonate were significantly less contaminated with fungi, particularly those belonging to the genus *Fusarium*, than untreated seeds.

Timorex Gold 24 EC is a natural fungicide containing oil extracted from tea tree, registered in more than 30 countries around the world, including Poland [10], to control some fungal diseases of vegetables grown in conventional and organic systems. Effects of this product on germination and fungal contaminants occurring on winter wheat grain have not been studied so far. The aim of this preliminary work was to establish concentrations of Timorex Gold 24 EC at which it is effective in reducing fungal contaminations of winter wheat seeds, when applied as seed dressing.

2. Materials and methods

Timorex Gold 24 EC, a commercial product, according to the producer it contains 23.8% water solution of natural oil extracted from tea tree [*Melaleuca alternifolia* (Maiden & Betche) Cheel], as an active substance. To test its usefulness as a seed dressing two laboratory experiments with winter wheat grain (cultivar Tonacja) were conducted under laboratory conditions.

The first experiment was designated to study effects of two concentrations of Timorex Gold 24 EC on germination and inhibition of fungal contaminants occurring on winter wheat kernels. To obtain these concentrations Timorex Gold 24 EC was 5-times and 10-times diluted with distilled water by mixing 1 volume of the bio-fungicide and 4 volumes of water (1:5) and 1 volume of the bio-fungicide and 9 volumes of water (1:10). Portions of winter wheat seeds (100 g) were weighed into glass beakers and thoroughly mixed with 5 ml of Timorex Gold 24 EC 1:5 or Timorex Gold 24 EC 1:10 solutions. Control seeds were treated with

the same volume of distilled water. Seeds were also treated with Vitavax 200 FS (carboxin + thiuram) – a common commercial preparation registered mainly to control fungal contaminants occurring on cereal grain. Vitavax 200 FS was mixed with water (1:1 v/v) and applied to winter wheat seeds at the rate of 0.6 ml/100 g seed. Germination of the treated seeds was tested in glass Petri plates lined with water moistened filter paper. There were 3 replicated plates per each treatment and each plate contained 50 seeds. The plates were placed in an incubator adjusted to 20 ± 2ºC. After 4 days and 8 days numbers of germinating seed (with coleoptiles and roots) were counted. Timorex-treated seeds and control seeds were also placed on the surface of PDA medium in Petri plates (5 plates, 15 seeds per plate) to determine inhibitory effects of the treatments on fungi contaminating winter wheat grains. After 7 days of incubation at 22ºC seeds with fungi out-growing from them were counted.

In the second experiment Timorex Gold 24 EC at 1:10 dilution was farther tested with respect to its effects on germination and fungal contaminants of winter wheat seeds. In this experiment 100 g portions of seeds were treated with the following volumes of Timorex Gold 24 EC (1:10) - 1 ml, 2 ml and 4 ml. Seed treatments with water and Vitavax 200 FS at the rate of 0.3 ml/100 g seeds were also included. Further experimental procedures concerning germination and testing fungal contaminants on PDA were the same as in the first experiment described above.

In the second experiments perlite was also used as a substrate for germination of winter wheat seeds. The seeds treated as described above were placed on the bottom of Petri plates and covered with 1.5 cm layer of perlite moistened with water. After 4 days of germination at 20 ± 2º C visible coleoptiles were counted and the plates were left open for further 4 days. After 8 days germinating seedlings were counted and the coleoptiles were cut at the seed to determine their fresh weight in each plate.

The data were subject to the analysis of variance (ANOVA) with significance of differences assessed at p <0.05. Data expressed in percentages were log transformed before statistical analyses.

3. Results and discussion

In testing sowing quality of cereal seeds their germination ability is usually assessed by counting numbers of germinating seeds after 4 days (germination energy) and after 8 days of incubation (germination capacity) at 20 ± 2ºC [2, 3, 6]. Results of the first experiment shown in Table 1 indicate that Timorex Gold 24 EC applied at higher concentration of (1:5) significantly reduced germination of winter wheat kernels, both after 4 and 8 days of incubation, as compared to control seeds. Germination of seeds treated with 1:10 dilution of Timorex Gold 24 EC in water was reduced only after 4 days of incubation. Vitavax 200 FS also significantly decreased germination energy (after 4 days) of winter wheat seeds treated with this fungicide. In this experiment we treated seeds with larger volume of Vitavax 200 FS (0.6ml instead of 0.3 ml/100 g of seeds, as advised by the producer) and this was probably the main reason of the inhibitory effect of this fungicide on seed germination shown in Table 1.

Figure 1 shows that more than 50% of control seeds were contaminated with different fungi as indicated by fungal mycelium out-growing from these seeds on PDA medium, while only on 2% or 7% of seeds treated with Timorex Gold 24 EC 1:5 or Timorex Gold 24 EC 1:10, respectively, fungal growth could be detected on PDA medium.

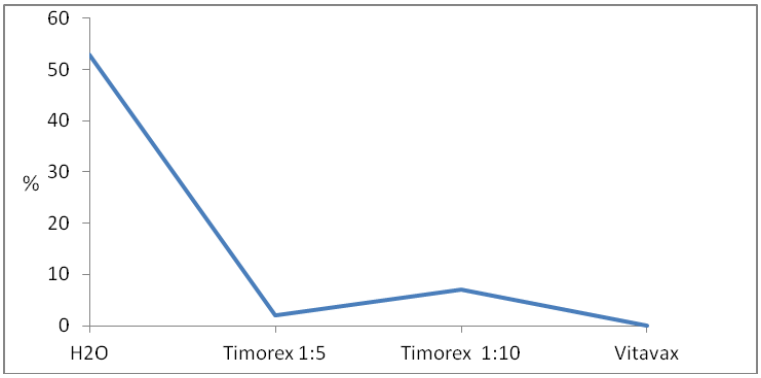
Table 1. Influence of different concentrations of Timorex Gold 24 EC on germination of winter wheat seeds on filter paper and on PDA medium

Tab. 1. Wpływ różnych koncentracji preparatu Timorex Gold 24 EC na kiełkowanie nasion pszenicy ozimej na bibule filtracyjnej i na pożywce PDA

Treatments	Germination on filter paper (%)		Germination on PDA medium (%)	
	After 4 days	After 8 days	After 4 days	After 8 days
Control (H_2O)	86 a*	92 a	94 a	95 a
Timorex Gold 24 EC (1:5)	42 c	67 b	95 a	95 a
Timorex Gold 24 EC (1:10)	73 b	89 a	94 a	94 a
Control (Vitavax 200 FS)	73 b	89 a	93 a	95 a

*Numbers in columns with the same letter are not significantly different at p = 0.05

Source: own work / Źródło: opracowanie własne



Source: own work / Źródło: opracowanie własne

Fig. 1. Percentages of winter wheat seeds with fungi growing on PDA after seed treatment with 1:5 or 1:10 dilutions of Timorex Gold 24 EC as compared to control seeds treated with water or Vitavax 200 FS

Rys. 1. Procentowy udział nasion z grzybami na pożywce PDA po zaprawieniu ziarna preparatem Timorex Gold 24 EC rozcieńczonym 1:5 lub 1:10 w porównaniu do nasion kontrolnych(H_2O) i zaprawionych zaprawą Vitavax 200 FS

Vitavax 200 FS at the rate applied completely inhibited the growth of fungi on seeds treated with this fungicide (Fig. 1). It is interesting to note that when the treated seeds were placed on PDA medium to monitor growth of fungi contaminating winter wheat seeds no negative effects of the tested fungicides on germination could be detected (Table 1). These results indicate that the kind of medium used for assessing seed germination is important. For this reason in the second experiment perlite was also included as the medium for seed germination.

Based on the results of the first experiment for further experimentation 1:10 water dilution of Timorex Gold 24 EC was chosen as at this concentration it markedly inhibited development of fungi contaminating wheat kernels and had no significant effect on germination capacity of the tested seeds (Fig. 1, Tab. 1). Slight reduction in germination energy (4 days germination) found in this experiment for winter wheat seeds treated with Timorex Gold 24 EC diluted 10-times might result from too large volume (5 ml/100 g seed) of this solution used. Therefore, in the second experiment seeds were treated with different volumes of Timorex Gold 24 EC 1:10 – 1, 2 and 4 ml/100 g of seeds. For comparison, a portion of winter wheat seeds was also treated with Vitavax 200 FS at the rate of 0.3 ml/100 g of seeds. As mentioned above, in this experiment effects of the fungicides on germination was tested both on filter paper and in perlite (Table 2). As it is shown in this table, irrespective of the medium used (filter paper or perlite) germination capacity after 8 days was statistically similar in all the treatments and ranged from 92% to 97% in the case of Timorex Gold 24 EC treated seeds. In the case of control seeds germination capacity was 97% and only for Vitavax 200 FS treated seeds it amounted to 100%. Also fresh weight of coleoptiles grown from untreated seeds and seeds dressed with Timorex Gold 24 EC and Vitavax 200 FS did not differ significantly (Table 2).

Timorex Gold 24 EC 1:10 applied at the rate of 4 ml significantly reduced germination energy (after 4 days) both on filter paper and in perlite, and this result indicates that Timorex Gold 24 EC at this rate should not be recommended for practical use. Slight reduction of germination energy could also be seen in the case of seeds treated with 2 ml of Timorex Gold 24 EC, but only when seeds were germinating on filter paper (Table 2).

In the first experiment inhibitory effects of the tested fungicidal dressings on germination energy were also more pronounced when seeds were germinating on filter paper than on PDA medium (Table 1). Collectively, the results of both experiments would indicate that in studies on effects of various chemicals on seed germination, particularly on germination energy, substrates such as perlite or sand instead of filter paper should be used, because it seems that such substrates to a greater extent imitate soil conditions.

Similarly to the results of the first experiment, also in the second experiment 1:10 solution of Timorex Gold 24 EC effectively inhibited growth of fungi contaminating winter wheat seed (Fig. 2). While on 60% of control seeds growth of different fungi on PDA medium was detected, in the case of seeds treated with 4 ml of Timorex Gold 24 EC 1:10 or with Vitavax 200 FS no fungal growth was found. Also Timorex Gold 24 EC applied at the rates of 1 ml and 2 ml per 100 g of seeds almost completely eliminated fungal contaminants occurring on winter wheat seeds, as only on 10% or 6% of these seeds, respectively, fungal growth could be detected on PDA medium (Fig. 2).

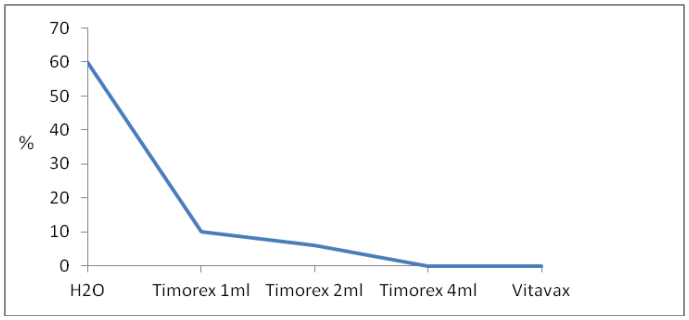
Table 2. Influence of different volumes of Timorex Gold 24 EC 1:10 on germination of winter wheat seeds on filter paper and in perlite

Tab. 2. Wpływ różnych objętości preparatu Timorex Gold 24 EC 1:10 na kiełkowanie nasion pszenicy ozimej na bibule filtracyjnej i w perlicie

Treatments	Germination on filter paper (%)		Germination in perlite (%)		Fresh weight of coleoptiles
	after 4 days	after 8 days	after 4 days	after 8 days	after 8 days
Control (H_2O)	96 a*	97.5 a	95 a	97 a	242 a
Timorex Gold 24 EC 1 ml	94 a	97.5 a	94 a	97 a	248 a
Timorex Gold 24 EC 2 ml	85 b	95 a	90 ab	97 a	247 a
Timorex Gold 24 EC 4 ml	87 b	95 a	87 b	92 a	258 a
Control (Vitavax 200 FS)	87 b	95 a	94 a	100 a	252 a

*Numbers in columns with the same letter are not significantly different at $p = 0.05$.

Source: own work / Źródło: opracowanie własne



Source: own work / Źródło: opracowanie własne

Fig. 2. Percentages of winter wheat seeds with fungi growing on PDA after seed treatment with 1 ml, 2 ml or 4 ml of 1:10 solution of Timorex Gold 24 EC as compared to control seeds treated with water or Vitavax 200 FS

Rys. 2. Procentowy udział nasion ze wzrostem grzybów na pożywce PDA po zaprawieniu ziarna 1 ml, 2 ml lub 4 ml 1:10 roztworem preparatu Timorex Gold 24 EC w porównaniu do nasion kontrolnych (H_2O) i zaprawionych zaprawą Vitavax 200 FS

4. Conclusions

Results of this study have shown that 1:10 water solution of Timorex Gold 24 EC, a commercial natural fungicide, can be recommended as a seed dressing at the rates of 1-2 ml/100g of seed, which correspond to 1-2 L of Timorex Gold 24 EC 1:10 per 100 kg of seeds. At these rates the tested fungicide effectively inhibited development of fungi contaminating winter wheat seeds and did not show any adverse effects on seed germination.

It was also found that in studies on effects of various chemicals on seed germination, particularly on germination energy, substrates such as perlite instead of filter paper should be used, because such substrates to a greater extent mimic soil conditions.

5. References

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