Efficacy and phytotoxicity of various thiabendazole and tebuconazole ratios in a fungicidal mordant

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Abstract. When developing combined preparations, a possible phytotoxic effect should be considered, depending on the dose of the mordant. *Fusarium oxysporum* and *Pyrenophora graminea* strains were cultured in a liquid enrichment culture with the addition of fungicides at a concentration of 1.5; 2.5; 3.3 *ppm*. Wheat grains of the Avesta variety were treated with thiabendazole and tebuconazole in the following doses: 20 g a.d./ t, 30 g a.d./t, 60 g a.d./t, 80 g a.d./t, 100 g a.d./t of grain. The effectiveness of tebuconazole and thiabendazole on mycelium growth slightly depended on their concentration. A.d. mutually reinforced each other. The synergism of the action of two a.d. is revealed. The analysis of variance showed that the effect of tebuconazole was greater than the thiabendazole effect. For both strains, the optimal ratios of tebuconazole and thiabendazole are 1.5:2.5 *ppm*. The addition of salicylic acid is effective at ratios of 2.5:0 and 2.5:3.3. The mixture of thiabendazole and tebuconazole reduced the germination energy of the grains mainly due to the appearance of abnormal seedlings. A composition of active substances for seed etching is proposed, including the following ratios of components, pts. wt.: tebuconazole 20-100, thiabendazole 20-150.

1 Introduction

The use of chemical fungicides in agriculture provides sufficiently reliable protection of agricultural plants from fungal infections that reduce their yield [22]. Fungicides have become the main means of chemical plant protection due to low cost, manufacturability and efficiency [24].

The disadvantage of fungicides is that they can be toxic not only to phytopathogenic fungi, but also to protected plants, as well as to humans and animals [20].

Numerous studies have shown that the use of fungicides can disrupt photosynthesis, the synthesis of sterols, gibberellins, transpiration, reduce the assimilation of CO_2 and biomass, affect the total content of pigments [18], inhibit the growth of seedlings, especially abnormal

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ones [6]. Fungal metabolites can lead to the accumulation of malondialdehyde and enhanced synthesis of antioxidants in plants [7]. Some metabolites exhibit such pronounced phytotoxic properties that they can be used as bioherbicides [5].

There are five main chemical classes of fungicides [18]. The largest group of them are triazoles. Fungicides of this class are widely used against pathogens. Nevertheless, the systematic use of triazoles leads to the appearance of resistant strains of fungi. Triazoles can damage the photosynthetic apparatus [15; 20], cause a retarding effect (disrupt the synthesis of gibberellins, contribute to an increase in the content of ABA) [21]; disrupt the synthesis of sterols, reduce plant transpiration, inhibit mitochondrial respiration [1]. Tebuconazole has a pronounced suppressive effect on germination and shoot growth [9].

Benzimidazole preparations were among the first systemic fungicides, nevertheless, their use contributes to the reduction of pigments in leaves and, as a consequence, to a decrease in plant biomass [17; 16].

Fungicides have a slight species-specificity of phytotoxic action. When choosing a mordant and developing combined preparations, a possible phytotoxic effect should be considered, depending on the dose of the mordant [2].

Fungicides, having a direct toxic effect on plants, enhance the production of toxins by fungi, which causes similar toxic effects. In this case, we should not talk about toxicity, but about the toxigenicity of the fungicide [14].

Indeed, phytopathogenic fungi are capable of producing substances toxic to plants (phytotoxins), animals, and microorganisms. Phytotoxins include representatives of various classes of organic compounds. These are mainly low-molecular-weight secondary metabolites that cause damage and death of plant cells. Symptoms are usually expressed in wilting, inhibition of growth, as well as in chlorosis, necrosis, and leaf spotting. Biotests are used to detect phytotoxins and assess their toxicity; fungi are cultivated on liquid or, less often, dense nutrient media [12, 3].

Tebuconazole (Tebuconazole) [(RS)-1p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazole-1-yl-methyl) pentane-3-il] is an effective systemic fungicide for the treatment of grain seeds in the fight against phytopathogens transmitted with seeds. It belongs to the third generation triazoles. A wide range of systemic action puts the preparation on one of the first places in the assortment of protectants [4].

Thiabendazol (Thiabendazole) [2-(4-thiazolyl)-1H-benzimidazole] is an active ingredient of pesticides, a highly effective fungicide of the benzimidazole class, a systemic mordant. It is used for aerosol treatment against pathogens on fruit, vegetable, and grain crops when stored [4].

The combination of two active substances related to azoles is effective, but causes relatively rapid adaptation of pathogens [13].

Due to the inherent resistance to chemical protection agents inherent in pathogenic fungi that infect agricultural crops, there is a need to update the compositions of pre-sowing preparations [1].

The constant use of pesticides for plant protection reduces the stability of agrocenoses due to the toxicity of components for soil microbiota and insect pollinators. Accumulation and migration of toxic components of pesticides leads to an increase in the environmental burden on surface reservoirs and aquatic organisms [19]. The impossibility of abandoning the use of fungicidal drugs and the need to develop ways to reduce the environmental and toxic load determine the tasks of the work. The development of new products is aimed at preparing a composition of several substances to achieve the effect of mutual enhancement of the action and reducing the dose of individual active substances [23].

High phytotoxicity of tebuconazole and thiabendazole has been established. The necessity of using growth-stimulating substances in the composition of a mordant containing these active substances of fungicides is substantiated [10].

The purpose of the study was to identify the minimum doses of tebuconazole and thiabendazole in a complex preparation that effectively inhibit the growth of phytopathogenic fungi and avoid the phytotoxic effect of a mordant based on them.

2 Materials and Methods of research

The strains MFG58518 *Fusarium oxysporum* and b/n *Pyrenophora graminea* were used provided by the Laboratory of Mycology and Phytopathology of the FSBSI All-Russian Institute of Plant Protection. The strains provided were cultured in Petri dishes on a dense nutrient medium (Chapek medium, V4 medium) used for growing and storing strains. The dishes were illuminated by an erythema lamp with a power of 30 watts, which is a low-pressure fluorescent mercury lamp with radiation in the region of 350-370 nm for the sporulation induction.

To obtain a static culture of phytopathogenic fungi, 100 ml of a suspension of fungus spores containing 10^3 CFU were added to sterile flasks with 50 ml of sterile liquid nutrient medium. Solutions of fungicides were introduced into the same flasks so that their concentration in the medium was 1.5; 2.5; 3.3 ppm. The following a.d. ratios were used (Table 1).

Table 1. Concentrations of tebuconazole and thiabendazole in enrichment cultures of *F. oxysporum* and *P. graminea*, ppm.

Tebuconazole	Thiabendazole			
	0.0	1.5	2.5	3.3
0.0	0:0 (control)	0:1.5	0:2.5	0:3.3
1.5	1.5:0	1.5:1.5	1.5:2.5	1.5:3.3
2.5	2.5:0	2.5:1.5	2.5:2.5	2.5:3.3
3.3	3.3:0	3.3:1.5	3.3:2.5	3.3:3.3

Deliberately low concentrations of a.d. were used to obtain fungus growth even at the most effective ratios of a.d.

Since tebuconazole and thiabendazole contribute to the growth inhibition [10], the phytohormone salicylic acid was added to the fungicide mixture in separate experiments at a dose of 20 g a.d./t of grain.

Mycelium was grown for 10-14 days. Then the culture liquid and mycelium were filtered through pre-dried at a temperature of 55 °C and weighted filters (white or red tape), the resulting filters with mycelium were dried at a temperature of 55 °C to a constant mass, re-weighed and the mass of the mycelium was calculated. The data obtained are shown in the diagrams.

The contamination of seeds was determined according to GOST 12044-93 Seeds of agricultural crops. Methods for determining infection with diseases. In sterile Petri dishes with a diameter of 9.5-10 cm, 10 cm^3 of sterilized agarized Chapek medium was poured. The thickness of the medium layer in the Petri dish should be 3-4 mm. The filling of nutrient media into cups and the planting of seeds was carried out in a sterile box. The seeds were laid out on a frozen nutrient medium with tweezers, periodically sterilizing it by firing on an alcohol lamp. The seeds were washed with a jet of water under a tap for 1-2 hours and disinfected with 96% alcohol for 1-2 minutes. Then the seeds were washed in sterile water and dried between sheets of sterile filter paper. Seeds were placed in Petri dishes of 10-15 pcs and they were put for germination in a thermostat at a temperature of 22-25°C. Seed germination was carried out during the period specified for determining the germination of seeds.

The grain of winter soft wheat *Triticum aestivum* L. of Avesta variety was used. A variety of *lutescens*. The bush is intermediate. The plant is short - of medium length. Mid-early. The growing season is 226-278 days. Susceptible to hard smut, brown rust, septoria. In the field, powdery mildew was affected on average.

After surface sterilization, Avesta wheat grains were treated with thiabendazole and tebuconazole in the following doses: 20 g a.d./t, 30 g a.d./t, 60 g a.d./t, 80 g a.d./t, 100 g a.d./t of grain. The control was left without treatment with fungicides. 20 variants of fungicide ratios were used according to Table 2.

Table 2. Ratio of active substances, 1 series, g a.d./t of grain.

Thiabendazole	Tebuconazole			
	0	30	60	100
0	0:0 control	0:30	0:60	0:100
20	20:0	20:30	20:60	20:100
30	30:0	30:30	30:60	30:100
40	40:0	40:30	40:60	40:100
80	80:0	80:30	80:60	80:100

Laboratory germination of seeds was determined according to GOST 12038-84. – 01.07.86. Seeds of agricultural crops. Methods for determining germination ability.

When considering the energy of viability and germination, normally sprouted; swollen, hard, which made up ungrown seeds and abnormally sprouted – non-germinating seeds were counted separately. The arithmetic mean of the results of determining the germination of all analyzed samples was taken as the result of the analysis.

The obtained results were subjected to statistical processing. The arithmetic mean (M), the mean square deviation (δ), the error of representativeness of the arithmetic mean (m_m), the Student's criterion (t) were calculated. The reliability of the difference was assessed by comparing the obtained value with the standard t_{st}.

The results of the study are shown in tables and figures.

3 Results and Discussion

As can be seen from Fig. 1, in most cases, an increase in the dose of a.d. led to a paradoxical accumulation of biomass. An increase in the mass of filtrate in a culture with high concentrations of a.d. could be associated with a protective accumulation of mucus. In this case, there is a possibility of the formation of mycotoxins, as there is a change in synthetic processes.

The effectiveness of tebuconazole and thiabendazole slightly depended on their concentration. A.d. mutually enhanced each other, except for those variants in which the concentration of tebuconazole was 3.3 ppm. The synergism of the action of two a.d. is revealed. The analysis of variance showed that the effect of tebuconazole was greater than the thiabendazole effect. Optimal ratios should be considered 1,5:1,5; 1,5:2,5; 2,5:2,5 ppm. When using a given ratio of 2.5:3.3, the accumulation of fungus biomass paradoxically increases.

As can be seen from Fig. 2, salicylic acid as the only additive slightly suppresses the growth of mycelium *F. oxysporum*. The addition of salicylic acid is effective at ratios of 2.5:0 and 2.5:3.3.

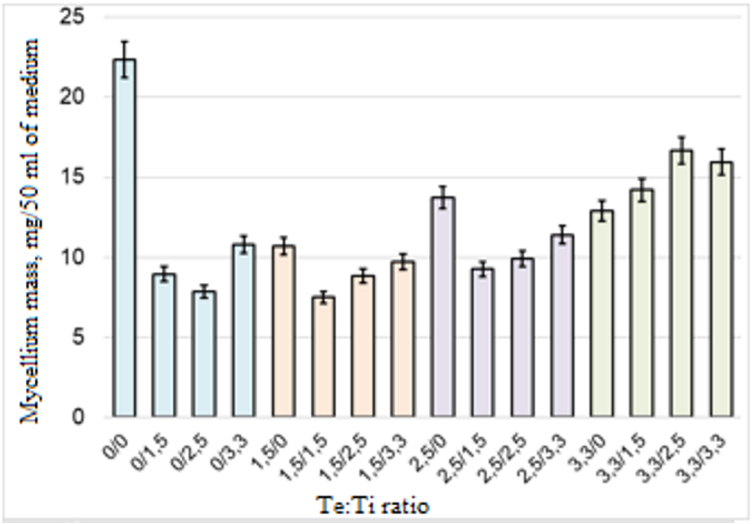

Fig. 1. The effect of tebuconazole and thiabendazole on the growth of *F. oxysporum* in the enrichment culture.

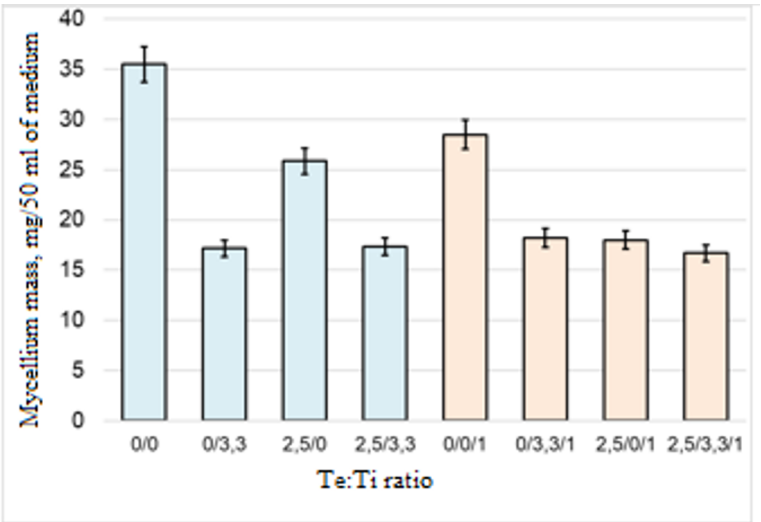

Fig. 2. The effect of tebuconazole, thiabendazole, and salicylic acid, 20 g a.d./t of grain, on the growth of *F. oxysporum* in the enrichment culture.

As can be seen from Fig. 3, the effectiveness of tebuconazole slightly depended on its concentration, the greatest inhibition of mycelium growth was noted at a concentration of 2.5:0 ppm. The effectiveness of thiabendazole also depended on its concentration, the most effective concentration was 3.3 ppm. A.d. mutually reinforced each other. Optimal ratios should be considered 1,5:2,5; 1,5:3,3; 2,5:3,3; 3,3:3,3.

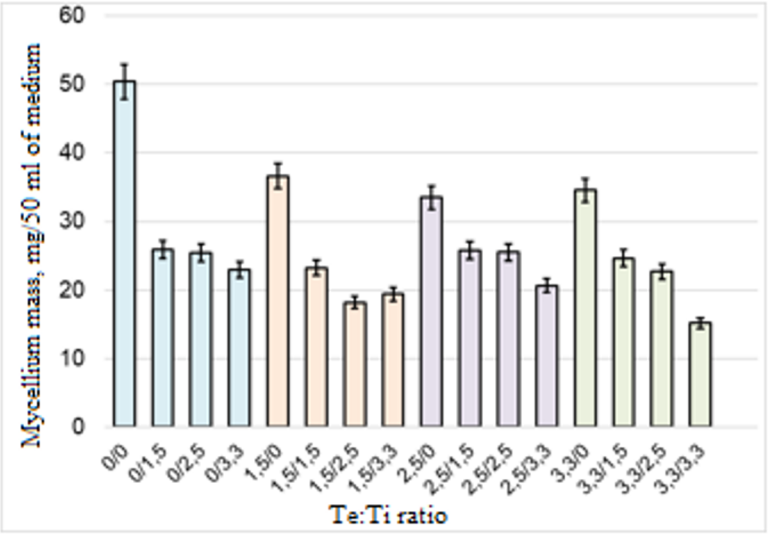

Fig. 3. The effect of tebuconazole and thiabendazole on the growth of *P. graminea* in the enrichment culture.

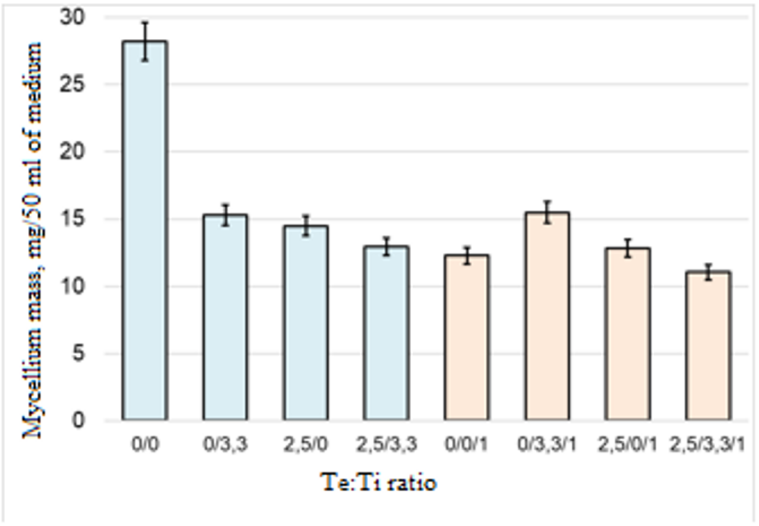

Fig. 4. The effect of tebuconazole, thiabendazole, and salicylic acid, 20 g a.d./t of grain, on the growth of *P. graminea* in the enrichment culture, 10 days.

For both strains, the optimal ratios of tebuconazole and thiabendazole are 1.5:2.5.

As can be seen from Fig. 4, salicylic acid as the only additive suppressed the growth of mycelium *P. graminea*. The addition of salicylic acid is effective at ratios of 2.5:0 and 2.5:3.3. The same tendency is characteristic of *F. oxysporum*.

As can be seen from Fig. 5, on day 4, the number of normally sprouted grains in the control was 80%, while 20% of the grains were infected with phytopathogenic fungi – mycelium growth and inhibition of the seedling were detected. Tiabendazole without the addition of tebuconazole at a dose of 20 g/t had no effect on phytopathogenic fungi, and at a dose of 30 g/t did not completely suppress the growth of fungi; 5% of the grains were infected. In other variants, mycelium growth was not detected.

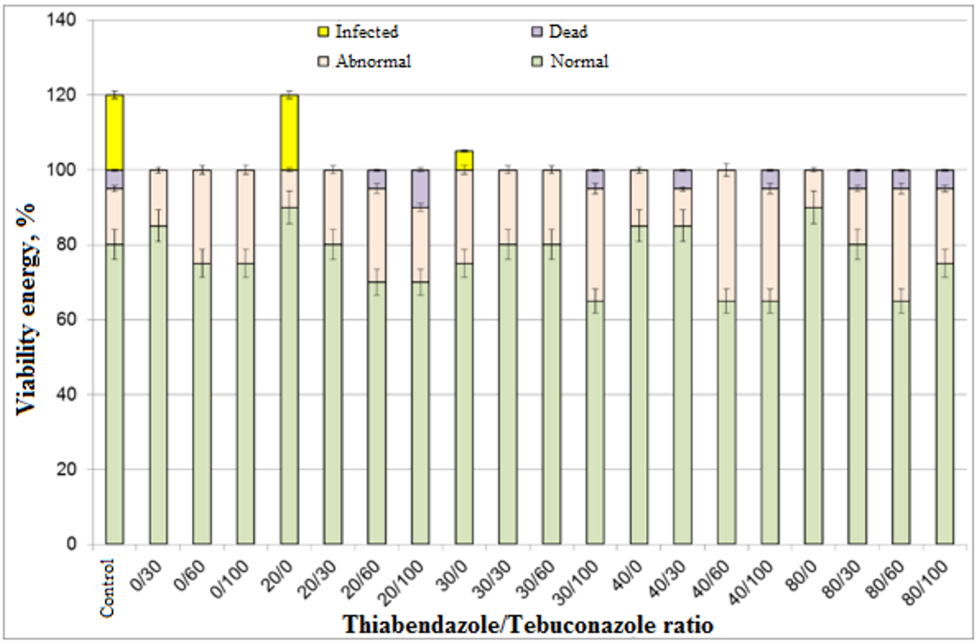

Fig. 5. The effect of thiabendazole and tebuconazole on the germination energy of Avesta wheat grains, 4 days.

A mixture of thiabendazole and tebuconazole had an effect on the germination energy of the grains (4 days, Fig. 5) mainly due to abnormal seedlings. In most variants of treatment with the mordant, there were no dead grains, which indicates the fungicidal effect of the mordant. The germination energy significantly decreased under the action of the mordant with the following doses of a.d.: 20:60 g/t, 20:100 g/t, 30:100 g/t, 40:60 g/t, 40:100 g/t, 80:60 g/t. In these and some other variants, there were dead grains, but their number corresponded to the control (except 20:100 g/t). Thiabendazole at doses of 20:0 g/t, 30:0 g/t, 40:0 g/t, 80:0 g/t did not inhibit germination, but in a mixture with tebuconazole, even small doses of it contributed to a decrease in germination energy. Tebuconazole at doses of 0:60 g/t and 0:100 g/t slightly reduced the germination energy. This pattern also was manifested in mixtures with thiabendazole.

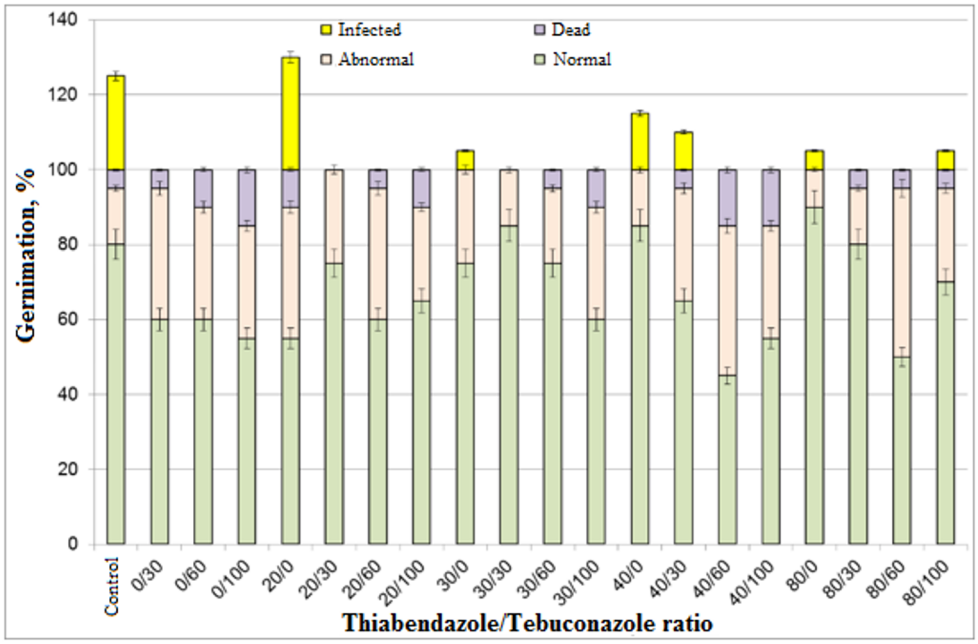

Fig. 6. The effect of thiabendazole and tebuconazole on the germination of wheat grains of the Avesta variety, 8 days.

As can be seen from Fig. 6, on the 8th day of germination, germination in the control was 80%, the number of infected grains increased to 25%. Tiabendazole without the addition of tebuconazole at a dose of 20 g/t had no effect on phytopathogenic fungi, the number of infected grains increased to 30%. At a dose of 30 g/t, it still did not completely suppress the growth of fungi; 5% of the grains were infected. Mycelium growth was detected in other variants: 40:0 g/t, 40:30 g/t, 80:0 g/t, 80:100 g/t. Even high doses of the mordant did not stop the growth of phytopathogenic fungi.

4 Conclusion

Based on the conducted studies, a composition of active substances for seed etching was proposed, including the following ratios of components, pts. wt.: tebuconazole 20-100, thiabendazole 20-150, clothianidine 150-450, sodium p-aminosalicylate 10-40, sodium benzoate 5-25 [8]. The proposed composition of active substances for seed pickling provides an increase in the germination of seed material and a decrease in its infestation, as well as ensures the resistance of seed material to the effects of various pathogens.

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