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TANK MIXTURE OF PLANT PROTECTION PRODUCTS WITH BIOSTIMULANT IN WINTER RYE (*Secale cereale* L.)

Mariyan YANEV[1], Nesho NESHEV[1], Anyo MITKOV[1], Todor MANILOV[1], Maya DIMITROVA[1], Cveta MOSKOVA[2]

[1]Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria
[2]University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria

Corresponding author email: mar1anski@abv.bg

Abstract

The application of various chemical products for plant protection and plant biostimulans increases financial costs, which is a prerequisite for their combined application in tank mixtures. During 2021 and 2022 on the experimental field of the Agricultural University of Plovdiv, Bulgaria, a field experiment with the rye variety "Milenium" was performed. The aim of the trial was to study the application of plant protection products in tank mixture with biostimluant and its influence to the rye grain yield and quality. The trial included the following treatments: 1. Untreated control; 2. Granstar 75 DF - 15 g ha^{-1} (herbicide); 3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} (fungicide); 4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1} (biostimulant); 5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}. The highest rye grain yield, absolute and hectoliter seed mass, grain wet gluten and crude protein for treatment 5 were recorded.

Key words*: rye, yield, quality, pesticides, tank mixture.*

INTRODUCTION

The progressive growth of the population worldwide necessitates an increase in the food production. This necessitates the use of new technologies that minimize problems caused by pests, nutrient deficiencies, competition between plants, water shortages, etc. (Grzebisz, et al., 2022; Georgiev et al., 2019; Shopova & Cholakov, 2015; Shopova and Cholakov, 2014; Bernstein et al., 2011; Calkins and Swanson, 1995). The use of different chemicals to control pests in cultivated areas has high operating costs and therefore their combined application is often applied by farmers (Arru et al., 2012). Approximately 97% of farmers mix 6 or more products which are simultaneously applied (Gazziero, 2015). Surfactants are typical of these mixtures and these products can induce synergistic effects in pest control (Li et al., 2019).

Winter cereal crops occupy a major share of cultivated areas not only in our country, but also abroad.

Mixing pesticides in a tank mix results in lower production costs. The combined application reduces the number of machine entries into the cultivated land, fuel consumption, water use for solution preparation and hours spent, resulting in less compaction of the soil (Gazziero, 2015). However, tank mixtures of pesticides pose environmental challenges as they can cause production loss as well as environmental risks such as cross-source pollution (Vale et al., 2019) and neurotoxic effects on living organisms (Wang et al., 2015). They can undergo three types of interactions (Ikeda, 2013): 1. Enhancing - when the efficacy of the product mix is similar to the efficacy of each product individually; 2. Synergistic - when the tank mixture of certain products gives better results than the application of each separately; 3. Antagonistic - when the result of the products applied in a tank mixture is worse than each individually.

Herbicidal products are widely used in agriculture. Most herbicide mixtures used to control certain weeds, such as those that are tolerant or even resistant to a particular product, have an additive effect. Mixtures of different types of agrochemicals can have antagonistic effects because they include more than one category of substances used to control pests. There is evidence that the use of insecticides or fungicides together with herbicides in tank mixtures can reduce the selectivity of the preparations to crop replantation (Gassen, 2002). Mixtures of different groups of pesticides can also affect plant metabolism (Hartzler et al., 2000). Mixtures of insecticides and herbicides can be toxic to natural bioagents, as for example an increase in *T. podisi* intoxication has been reported following the combined application of the insecticides cypermethrin, thiamethoxam and bifenthrin together with the herbicides cyhalofop-butyl, imazethapyr, imazapic and penoxulam (Pazini et al., 2017).

In recent years, the question of the effect of mixing plant protection products and biostimulants and their use in a tank mixture has been increasingly raised. There is very little scientific research on this issue, relying mainly on data from the companies that offer them. However, these are studies done abroad, with different combinations of plant protection products, depending on the problems posed by practice in the respective countries. It is extremely important to shed light on these questions regarding which component of the mixture has a negative effect on plants, which requires monitoring physical indicators when mixing different plant protection products and foliar fertilizers, biological and physiological indicators that take into account their effect mainly in terms of the cultivated plant. This also defines the aim of the present study.

MATERIALS AND METHODS

The research was conducted in 2020-2022 at the experimental field of the Department of Agriculture and Herbology at the Agricultural University - Plovdiv, Bulgaria with rye, variety "Millennium". The study was based on the method of long plots on an area of 100 m^2.

The following variants were under evaluation:

1. Untreated control
2. Granstar 75 DF - 15 g ha^{-1};
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1};
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1};
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}.

Studied plant protection products and product with biostimulant mode of action:

Herbicide: Granstar 75 DF (750 g/kg tribenuron-methyl);

Fungicide: Zantara 216 EC (50 g/l *bixafen* + 166 g/l tebuconazole);

Plant biostimulant: Amino Expert Impuls (Amino acids, phytohormones, nitrogen, magnesium, sulfur, boron, copper, iron, manganese, molybdenum, zinc);

The application of plant protection products with the biostimulant is in a tank mixture with a volume of the working solution of 210 l ha^{-1}. The treatment was carried out at the end of March, in the phenophase of the end of tillering - the beginning of spindleing of the crop (BBCH 29-31).

The following indicators were studied:

- Absolute seed mass (g), (Dimitrova et al., 2006);
- Hectoliter seed mas (kg) (Dimitrova et al., 2006);
- Seed crude protein content (%) (By Kjeldahl's method, Tomov et al., 2009);
- Seed wet gluten conent (%) (ISO 21415-2:2015).
- Rye grain yields (t ha^{-1}). The harvest was carried out with a Wintersteiger® field trial harvester.

Levels of phytotoxicity for the crop by the 9-score scale of EWRS (European Weed Research Society) were reported (at score 1 - there is no visible damages to the crop, and at score 9 - the crop plants are completely destroyed). The evaluations were done four times - on the 7^{th}, 14^{th}, 28^{th}, and 56^{th} day after treatments.

In both experimental years the winter rye was grown as a monoculture.

The tillage carried out before rye's sowing was deep plowing, followed by harrowing. Before sowing, fertilization with 250 kg ha^{-1} with NPK 15:15:15 and spring dressing with 250 kg ha^{-1} NH_4NO_3 was accomplished.

Duncan's method with the SPSS 19 program (Duncan, 1955) was used for the statistical processing of the obtained data. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Table 1 shows the amounts of precipitation during the rye's growing seasons (2020/2021 and 2021/2022). The precipitations measured are a prerequisite for relatively good moisture storage and normal vegetation during both experimental years. Rye was sown in October, with germination and emergence taking place at high soil moisture. During the vegetation periods, no water deficit is observed.

Table 1. Average monthly precipitation (mm) and average monthly minimum and maximum temperatures during the vegetation seasons of rye (°C)

Months	2020/2021			2021/2022		
	Precipitation, mm	Average monthly temperature, min. t°	Average monthly temperature, max. t°	Precipitation, mm	Average monthly temperature, min. t°	Average monthly temperature, max. t°
October	62.3	9.2	22.3	180.0	9.0	12.6
November	50.7	1.6	13.3	26.5	6.0	11.4
December	51.8	2.4	9.0	124	0.4	8.1
January	29.3	-1.4	10.1	48.8	0.8	5.8
February	32.8	-0.7	18.4	58.8	3.5	5.6
March	43.3	0.8	13.9	76.0	1.0	13.5
April	67.4	4.8	18.8	52.0	10.7	16.9
May	58.1	11.4	26.5	35.5	14.1	24.2
June	51.7	15.3	30.9	106.8	20.8	26.1

Temperatures (min. and max.) were favourable for plant development as well. Despite the high winter temperatures, no negative influence of the warm winter months on the growth and development of the crop plants was found. The analysis of meteorological data shows that the experimental years are favourable for the growth, development and realization of the productive possibilities of rye. During the two experimental years, the selectivity of the applied products to the crop was also studied. Under the conditions of the experiment and during the four reporting dates of the two years, no visible symptoms of phytotoxicity were found after all treatments - score 1 by the EWRS scale.

Table 2 presents the results of the rye grain yield, the absolute and hectoliter seed mass.

Table 2. Rye grain yield (t ha^{-1}), absolute seed mass (g) and hectoliter seed mass (kg)

Treatments	Grain yields (t ha^{-1})		
	2021	2022	Average
1. Untreated control	2.36 c	2.24 d	2.30
2. Granstar 75 DF - 15 g ha^{-1}	3.21 b	3.06 c	3.14
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	3.25 b	3.12 c	3.19
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	3.29 ab	3.32 b	3.31
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	3.38 a	3.42 a	3.29
Treatments	Absolute seed mass (g)		
	2021	2022	Average
1. Untreated control	26.07 e	25.26 c	25.67
2. Granstar 75 DF - 15 g ha^{-1}	29.23 b	26.37 b	27.80
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	27.47 c	26.20 b	26.84
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	29.27 b	26.34 b	27.81
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	30.67 a	27.40 a	29.04
Treatments	Hectoliter seed mass (kg)		
	2021	2022	Average
1. Untreated control	65.67 d	64.33 c	65.00
2. Granstar 75 DF - 15 g ha^{-1}	66.67 de	65.67 b	66.17
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	67.33 cd	66.00 b	66.67
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	68.67 b	67.16 ab	67.92
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	70.00 a	67.83 a	68.92

Figures with different letters are with a proven difference by Duncan's multiple range test ($p < 0.05$).

After the application of herbicides and biostimulants, Matysiak et al. (2018) found an increase in wheat grain yield. The highest grain yields in the current trial in option 5 were recorded (after the combined applicacion in a tank mixture of Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 3.29 t ha^{-1}, both in the two experimental years and on average over the study period. These data were statistically proven. The lowest yields were obtained in the untreated control.

The absolute seed mass is of great importance for yields (Georgiev et al., 2014).

The absolute seed mass differed among the treatments. In the first experimental year, in relation to this indicator, there were proven differences between the treated variants, as well as with the untreated control, while in the second year the absolute seed mass of treatments 2, 3 and 4 showed close values that were not statistically significant.

The highest absolute seed mass for variant 5 was obtained (Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 29.04 g on average for the period, and the lowest - in the untrained control was recorded - 25.67 g.

As both yields and absolute seed mass, the hectoliter mass was affected by the treatments as well.

The highest hectoliter seed mass for the rye seeds of variant 5 was measured (Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 68.92 kg , and this indicator was the lowest for the control - 65.00 kg.

Crude protein content varies depending on the variety and is affected by nitrogen fertilization (Bártová et al., 2013). The highest content of this indicator was reported in the seeds at treatment 5 (Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 13.99 %, average for the experimental conditions, while in the control the lowest crude protein content was 10.97% recorded. In treatments 2 and 3 values were approximately similar in the different years and were not statistically proven.

Xhaferaj et al. (2023) examined samples of 32 rye varieties, and the results showed that the gluten content of rye flour varied from 3.0 to 7.8 g/100 g. The data from the present experiment showed that this indicator of the rye, variety “Millennium”, varies from 5.07 to 7.78%. The lowest results were found for the untreated control and were reliable compared to the treated variants (2, 3, 4 and 5). The highest results for this indicator were obtained for treatment 5 (Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}) - 7.78%.

Table 3. Rye grain crude protein (%) and wet gluten (%)

Treatments	Crude protein (%)		
	2021	2022	Average
1. Untreated control	11.15 c	10.79 c	10.97
2. Granstar 75 DF - 15 g ha^{-1}	13.47 b	12.53 b	13.00
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	13.24 b	12.76 b	13.00
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	14.09 ab	13.40 a	13.75
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	14.28 a	13.69 a	13.99
Treatments	Wet gluten (%)		
	2021	2022	Average
1. Untreated control	5.60 c	4.54 c	5.07
2. Granstar 75 DF - 15 g ha^{-1}	7.50 a	7.83 a	7.67
3. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1}	7.10 ab	6.26 b	6.68
4. Granstar 75 DF - 15 g ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	6.90 b	7.37 a	7.14
5. Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}	7.74 a	7.81 a	7.78

Figures with different letters are with a proven difference by Duncan's multiple range test ($p < 0.05$).

CONCLUSIONS

After the treatment of the rye with the herbicide Granstar 75 DF - 15 g ha^{-1} alone, or in combination with the fungicide Zantara 216 EC - 1.25 l ha^{-1} or with the amino stimulant Amino Expert Impuls - 3.00 l ha^{-1}, no visible symptoms of phytotoxicity were detected on the crop. The highest yields, absolute and hectoliter mass of the seeds, crude protein and gluten content were reported for variant 5 (tank mixture of Granstar 75 DF - 15 g ha^{-1} + Zantara 216 EC - 1.25 l ha^{-1} + Amino Expert Impuls - 3.00 l ha^{-1}), which are statistically proven, compared to the untreated control.

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