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APPLICATION OF PLANT BIOSTIMULANTS IN TANK MIXTURE WITH IMAZAMOX-CONTAINING HERBICIDAL PRODUCTS AT IGROWTH SORGHUM (*SORGHUM BICOLOR* L.)

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Abstract

Sorghum (*Sorghum bicolor* L.) is the world's fifth-most important grain crop after rice, wheat, maize, and barley. The main limiting factor for the sorghum's growth and development are the uncontrolled weeds. In nowadays farming systems the most distributed method for weed control is the application of herbicides. For decreasing the financial costs, the herbicides may be applied together with plant biostimulants in tank mixture. For evaluation of the combined application of imazamox-containing herbicidal products and plant biostimulants, a field trial was conducted. The experiment was situated at the field of the Agricultural University of Plovdiv, Bulgaria. The variants of the trial were: 1. Untreated control; 2. Twice-hoed control; 3. Pulsar 40 (1.20 l ha^{-1}); 4. Pulsar 40 (1.20 l ha^{-1}) + Amino Expert Balans (1.00 l ha^{-1}); 5. Pulsar 40 (1.20 l ha^{-1}) + Amino Expert Impuls (2.50 l ha^{-1}); 6. Pulsar Plus (2.00 l ha^{-1}); 7. Pulsar Plus (2.00 l ha^{-1}) + Amino Expert Balans (1.00 l ha^{-1}); 8. Pulsar Plus (2.00 l ha^{-1}) + Amino Expert Impuls (2.50 l ha^{-1}). The sorghum hybrid grown in 2023 and in 2024 was “Sentinel” IG (imazamox-tolerant). Several parameters as chlorophyll content index (CCI), plant height, number of leaves, and panicle length at the end of the vegetation, grain yield, 1000 grain weight, and hectoliter seed mass were studied. The combined application of both biostimulant products (Amino Expert Balans and Amino Expert Impuls) together with Pulsar 40 or Pulsar Plus led to increase of all studied parameters.

Keywords: combined application, herbicides, plant biostimulants, growth, yield

INTRODUCTION

Sorghum (S. bicolor L.), is the fifth most important cereal crop in the world (Ottman and Olsen, 2009). Globally, it is considered an important dietary staple for million people in a great number of countries (Charyulu et al., 2024).

Previous studies have indicated that despite its high agronomic potential and nutritional/forage value, the total area with sorghum has declined due to limited weed management options worldwide (Kumar et al., 2012). Climatic variability, particularly fluctuating precipitation and rising temperatures, poses a significant threat to crop productivity and stability. Forage sorghum hybrids are a promising alternative for fodder and bioenergy due to their high level of drought tolerance (Rakić et al., 2025).

Weeds are reported to be a major obstacle to increasing the yield and quality of grain sorghum (Geier et al., 2009). Weeds compete with the crop, harbor pests and compete with sorghum for nutrients, water and light, thereby reducing grain yield and quality and cause problems at harvest. Sorghum is a poor competitor against weeds due to its slow growth and poor early vigor, although it eventually produces a dense crop (Ottman and Olsen, 2009).

The weed competition reduces the yields of sorghum in the USA by millions of dollars per year, and weed control costs about US $ 53 million per year in Australian sorghum fields (Bridges, 1992).

Combined use of pesticides reduces the number of agricultural machinery entries into the cultivated areas, fuel consumption, water use for spraying and working hours (Gazziero, 2015). According to Arrué et al. (2012), treatment with agrochemicals for pest control has a high value. For this reason, farmers prefer to apply pesticides in tank mixtures. Combined pesticide tertiaries can be advantageous, but farmers require information on which products can be mixed and what the consequences would be (Marta Martins Gandini et al., 2020).

Data related to the compatibility of foliar fertilizers, growth regulators, and biostimulants in sorghum are lacking.

Studies have been conducted with different crops, which show positive results after the combined application of different herbicides with different biostimulants.

To confirm this hypothesis, a three-year study was conducted with winter wheat in Karaj, Iran. The treatments included a combination of herbicides at nine levels and urea application by two methods: foliar application and drench application. Mixing urea with the herbicide did not affect the efficacy of the herbicides. Among the combinations of herbicides and urea, Urea + Tribenuron-methyl + Clodinafop-propargil was the best combination for weed control and increased grain yield (Moeini et al., 2006).

In 2007-2010, a study was conducted in Poznan, Poland on the efficacy of mixtures of herbicides and fertilizers applied to maize in the 4-6 leaf phenophase. The results reported selectivity of the mixtures to the studied maize varieties and showed high efficacy of 92-99% against the dominant weed species Chenopodium album L., Solanum nigrum L. and Echinochloa crus-galli L. In addition, the tested mixture contributed to an increase in grain yield (by 34-36%) and hectoliter weight (by 5-8%) (Pietryga and Drzewiecki, 2011).

The aim of the present paper is to study certain possibilities for mixing and combined application of herbicides with biostimulants in igrowth® sorghum.

MATERIALS AND METHODS

The experiment was conducted during the sorghum vegetation periods in 2023 and 2024 at the experimental field of the Department of Agriculture and Herbology, Agricultural University – Plovdiv. The study was carried out using the long-plot method, with a plot size of 60 m^2. The tested sorghum hybrid was "Sentinel" IG.

Characteristics of hybrid "Sentinel" IG: A medium-late sorghum hybrid with high yield potential. It was developed for cultivation under Advanta Seed's igrowth™ technology. The hybrid was bred in Australia using elite grain sorghum genetics from Pacific Seeds®. It can be cultivated under a wide range of conditions, as the igrowth™ technology allows the application of registered herbicides from the imidazolinone group (www.pacificseeds.com).

Herbicides were applied at the 3^{rd}–5^{th} leaf stage using a SOLO manual sprayer. The spray volume was 210 L ha^{-1}.

Variants of the experiment:

1. Untreated control;
2. Twice-hoed control;
3. Pulsar 40 (1.20 l ha^{-1});
4. Pulsar 40 (1.20 l ha^{-1}) + Amino Expert Balans (1.00 l ha^{-1});
5. Pulsar 40 (1.20 l ha^{-1}) + Amino Expert Impuls (2.50 l ha^{-1});
6. Pulsar Plus (2.00 l ha^{-1});
7. Pulsar Plus (2.00 l ha^{-1}) + Amino Expert Balans (1.00 l ha^{-1});
8. Pulsar Plus (2.00 l ha^{-1}) + Amino Expert Impuls (2.50 l ha^{-1}).

Used herbicides were:

1. Pulsar 40 (40 g/L imazamox) – Controls grass and broadleaf weeds. Registered for use in Clearfield sunflower and rice hybrids, and alfalfa (https://preparati.info).
2. Pulsar Plus (25 g/L imazamox) – Controls grass and broadleaf weeds. Registered for use in Clearfield and Clearfield Plus sunflower hybrids (https://preparati.info).

Used Biostimulants:

1. Amino Expert Balans – Nitrogen, amino acids, organic carbon, sulfur trioxide, peptides. (https://ecofol.com).
2. Amino Expert Impuls – Amino acids, phytohormones, nitrogen, magnesium, sulfur, boron, copper, iron, manganese, molybdenum, zinc. (https://ecofol.com)

The sorghum was grown after winter wheat under irrigated conditions. The entire experimental area was fertilized with 250 kg ha^{-1} N:P:K (15:15:15), followed by deep plowing. Before sowing, discing to 15 cm and two cultivations to 8 cm were performed. Spring fertilization with 250 kg ha^{-1} NH_4NO_3 was also done. Sowing took place in May 2023 and May 2024.

Measured indicators:

- Selectivity of treatments – assessed by the 9-score EWRS scale at 7, 14, and 28 days after application.

- Chlorophyll content index (CCI) – measured in 2024 with a CCM-200 Plus chlorophyll meter, 7,

14, and 21 days after treatment (average of 5 plants per variant).

- Plant height (cm);
- Panicle length (cm);
- Number of leaves;

The biometric parameters were performed on thirty plants total per treatment.

- Grain yield (t ha^{-1});
- 1000 grain weight (g) – measured in three replications;
- Hectoliter weight (kg) – measured in three replications.

Statistical processing was done using Duncan's test in SPSS 19. Differences were considered significant at $p<0.05$.

Climatic Characteristics.

Figures 1 and 2 present rainfall and temperature data during sorghum vegetation. Both experimental years showed critical dry periods, leading to reduced grain yields.

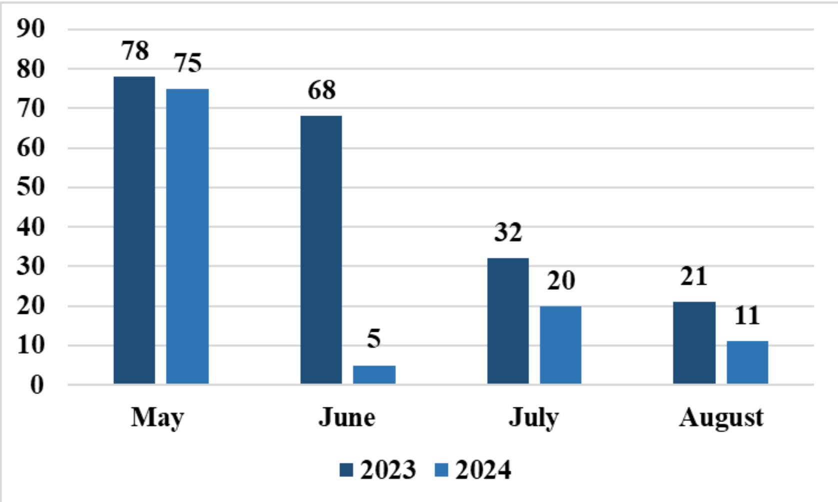

Figure 1. Amount of precipitation during the sorghum vegetation, mm

Rainfall was more favorable in 2023 (the first experimental year) (Figure 1). July and August were critical with 32 and 21 mm rainfall, respectively, resulting in yield reduction.

In 2024, drought conditions were even worse, starting in June with only 5 mm of rainfall, followed by insufficient precipitation in July (20 mm) and August (11 mm).

Temperatures (Figure 2) were higher in 2024 than in 2023, making it a more unfavorable agro-meteorological year for sorghum growth.

Based on meteorological data analysis, both experimental years were unfavorable for growth, development, and realization of sorghum productive potential, with the second year being less favorable.

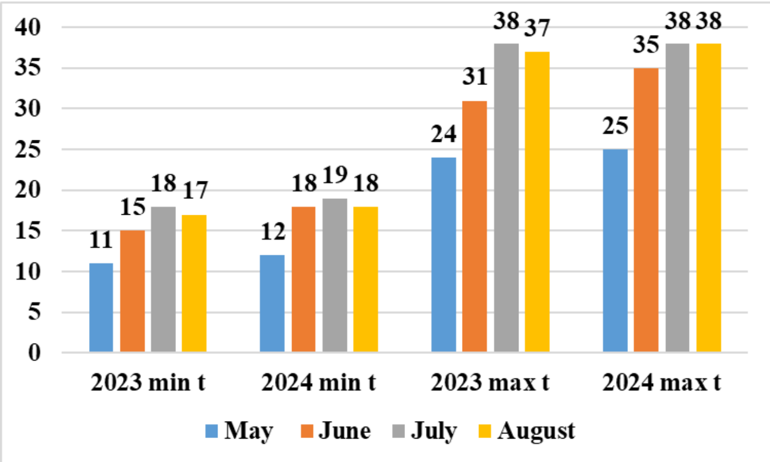

Figure 2. The lowest and highest temperatures during the sorghum vegetation, °C

RESULTS AND DISCUSSION

During the reporting (7, 14 and 21 days after application of the products alone or in a tank mix) to assess the selectivity of the treatments, no phytotoxic symptoms were observed in any of the trial variants. For this reason, the values in Table 1 are only ones, meaning that the plants were healthy. The results are identical in both experimental years.

Table 1. Selectivity of the treatments (average for the period), scores by EWRS

Variants of trial/days	7 days	14 days	21 days
1. Untreated control	-	-	-
2. Twice-hoed control	-	-	-
3. Pulsar 40 (1.20 l ha^{-1})	1	1	1
4. Pulsar 40 (1.20 l ha^{-1}) + AEB (1.00 l ha^{-1})	1	1	1
5. Pulsar 40 (1.20 l ha^{-1}) + AEI (2.50 l ha^{-1})	1	1	1
6. Pulsar Plus (2.00 l ha^{-1})	1	1	1
7. Pulsar Plus (2.00 l ha^{-1}) + AEB (1.00 l ha^{-1})	1	1	1
8. Pulsar Plus (2.00 l ha^{-1}) + AEI (2.50 l ha^{-1})	1	1	1

Quantification of plant leaf pigments using non-invasive optical methods is fast and easy and provides reliable data on relative chlorophyll content compared to traditional and chemical methods (Richardson et al., 2002). Miri (2009) reported that the chlorophyll content index (CCI) is directly and positively correlated with grain yield. Leaf chlorophyll content is used to measure leaf nitrogen content and serves as a significant indicator of nitrogen deficiency in plants (Cerovic et al., 2012). Furthermore, the chlorophyll content index (CCI) can be used as a tool to support decision-making on nitrogen fertilization of crops and can be used to improve the assessment of crop yield and biomass. The index varies widely among crops, particularly in the sorghum of the present experiment. The aim of monitoring the index over time is to identify any potential disturbances in plants resulting from treatment with Pulsar 40 and Pulsar Plus together with the various biostimulants in a tank mixture. The results of the experiment are presented in Table 2. The data obtained from the dynamic measurements showed that the CCI index increased over time. During all measurements, the plants with the lowest values for the studied index were the those of the untreated control.

On the 7th day after treatment, the CCI index varied from 25.2 in the untreated control to 33.9 CCI in variant 4 (Pulsar 40 – 1.20 l ha^{-1} + AEB – 1.00 l ha^{-1}). In variants 3 and 6, in which the plants were treated only with the herbicide, the chlorophyll index had lower values – 31.8 and 30.8 for variant 3 (Pulsar 40 – 1.20 l ha^{-1}) and variant 6 (Pulsar Plus – 2.00 l ha^{-1}) respectively.

Table 2. Chlorophyll Index, CCI

Variants of trial/days	**7 days**	**14 days**	**21 days**
1. Untreated control	25.2 c	29.9 c	30.1
2. Twice-hoed control	30.7 b	34.2 b	38.0
3. Pulsar 40 (1.20 l ha^{-1})	31.8 b	33.0 b	38.7
4. Pulsar 40 (1.20 l ha^{-1}) + AEB (1.00 l ha^{-1})	33.9 a	36.3 a	40.3
5. Pulsar 40 (1.20 l ha^{-1}) + AEI (2.50 l ha^{-1})	33.7 a	36.4 a	40.9
6. Pulsar Plus (2.00 l ha^{-1})	30.8 b	33.7 b	39.6
7. Pulsar Plus (2.00 l ha^{-1}) + AEB (1.00 l ha^{-1})	33.2 a	36.5 a	40.0
8. Pulsar Plus (2.00 l ha^{-1}) + AEI (2.50 l ha^{-1})	32.9 a	36.8 a	40.7

Figures with different letters are significantly different according to Duncan's test ($p < 0.05$).

On the 14th day after the treatment, the results were identical to those reported on the 7th day. On the 14th day, the CCI index varied from 29.9 in the untreated control to 36.8 CCI in variant 8 (Pulsar Plus – 2.00 l ha^{-1} + AEI – 2.50 l ha^{-1}). In variants 3 and 6, in which the plants were treated only with the herbicides, the chlorophyll index had lower values – 33.0 and 33.7 for variant 3 (Pulsar 40 – 1.20 l ha^{-1}) and variant 6 (Pulsar Plus – 2.00 l ha^{-1}), respectively.

More pronounced differences in the CCI index values were found 21 days after treatment. The highest index values were measured in the variants that had a biostimulant in the reservoir mixture. Again, lower values for the index were measured in the variants where the plants were treated only with Pulsar 40 or Pulsar Plus, with the results being followed by the hoed control, which had a CCI of 38.0. On the 21st day after treatment, the untreated control again had the lowest index for chlorophyll content in the leaves – 30.1 CCI.

Regarding the data on the studied biometric indicators, it should be noted that in the second experimental year the results were lower, which is due to the worse weather conditions in 2024. Table 3 presents the results for the height of the plants at the end of the sorghum vegetation period. The lowest plants were measured in the untreated control - 81.36 cm on average.

The height of the plants in the control is significantly different compared to the Duncan test with all other variants in the individual years.

The highest plants were measured in treatment 7 (Pulsar Plus – 2.00 l ha^{-1} + AEB – 1.00 l ha^{-1}) – 108.16 cm on average for the two years of the experiment. The plants to which only herbicides without a biostimulant were applied in a tank mixture, as well as the twice - hoed control, had lower values for the plant height.

Table 3. Plant height, cm

Variants of trial/days	2023	2024	Average
1. Untreated control	85.47 d	77.25 c	81.36
2. Twice-hoed control	102.56 c	95.41 b	98.99
3. Pulsar 40 (1.20 l ha^{-1})	109.63 b	97.27 b	103.45
4. Pulsar 40 (1.20 l ha^{-1}) + AEB (1.00 l ha^{-1})	111.28 a	99.49 a	105.39
5. Pulsar 40 (1.20 l ha^{-1}) + AEI (2.50 l ha^{-1})	112.69 a	101.28 a	106.99
6. Pulsar Plus (2.00 l ha^{-1})	103.39 c	94.75 b	99.07
7. Pulsar Plus (2.00 l ha^{-1}) + AEB (1.00 l ha^{-1})	113.44 a	102.88 a	108.16
8. Pulsar Plus (2.00 l ha^{-1}) + AEI (2.50 l ha^{-1})	114.17 a	101.54 a	107.86

Figures with different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 4 presents the results for the panicle length of sorghum from the experiment. The plants with the shortest measured panicles are those from the untreated control – 10.89 cm on average for the two experimental years. Identical to the height of the plants, the length of the panicles in the control is significantly different compared to the Duncan test with all other variants in the individual years.

The longest panicles were measured in the plants of variant 8 (Pulsar Plus – 2.00 l ha^{-1} + AEI – 2.50 l ha^{-1}) – 16.06 cm on average for the two years of the study. The plants to which only herbicides without a biostimulant were applied in a tank mixture, as well as the twice - hoed control, were with shorter panicles.

Table 4. Panicle length, cm

Variants of trial/days	2023	2024	Average
1. Untreated control	12.42 c	9.35 c	10.89
2. Twice-hoed control	16.27 b	11.30 b	13.79
3. Pulsar 40 (1.20 l ha^{-1})	16.33 b	11.98 b	14.16
4. Pulsar 40 (1.20 l ha^{-1}) + AEB (1.00 l ha^{-1})	17.87 a	12.34 a	15.11
5. Pulsar 40 (1.20 l ha^{-1}) + AEI (2.50 l ha^{-1})	18.36 a	12.57 a	15.47
6. Pulsar Plus (2.00 l ha^{-1})	16.59 b	10.69 b	13.64
7. Pulsar Plus (2.00 l ha^{-1}) + AEB (1.00 l ha^{-1})	17.69 a	12.57 a	15.13
8. Pulsar Plus (2.00 l ha^{-1}) + AEI (2.50 l ha^{-1})	18.94 a	13.18 a	16.06

Figures with different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 5 presents the results for the number of leaves that developed in plants from the different variants. The plants that developed the lowest number of leaves were in the untreated control – 5.75 leaves on average. Identical to the height of the plants and the length of the panicles, the results for the number of leaves in the control are significantly different from all other variants in the individual years according to the Duncan’s test.

The highest number of leaves was counted in plants from variant 7 (Pulsar Plus – 2.00 l ha^{-1} + AEB – 1.00 l ha^{-1}) – 11.57 cm on average for the two years of the study. The plants to which only herbicides were applied and twice-hoed plants were with lower number of leaves.

Table 5. Leaf number

Variants of trial/days	2023	2024	Average
1. Untreated control	6.24 c	5.25 c	5.75
2. Twice-hoed control	10.39 b	8.67 b	9.53
3. Pulsar 40 (1.20 l ha^{-1})	10.98 b	8.57 b	9.78
4. Pulsar 40 (1.20 l ha^{-1}) + AEB (1.00 l ha^{-1})	12.25 a	10.38 a	11.32
5. Pulsar 40 (1.20 l ha^{-1}) + AEI (2.50 l ha^{-1})	12.58 a	10.24 a	11.41
6. Pulsar Plus (2.00 l ha^{-1})	10.37 b	9.21 b	9.79
7. Pulsar Plus (2.00 l ha^{-1}) + AEB (1.00 l ha^{-1})	12.53 a	10.60 a	11.57
8. Pulsar Plus (2.00 l ha^{-1}) + AEI (2.50 l ha^{-1})	12.84 a	10.23 a	11.54

Figures with different letters are significantly different according to Duncan's test ($p < 0.05$).

It should be noted that as the biometric indicators, the productive parameters in the second experimental year were lower, which is again due to the worse weather conditions in 2024. Table 6 presents the results for sorghum grain yields in t ha^{-1}. The lowest yields were recorded for plants from the untreated control – 1.92 t ha^{-1} on average for the two experimental years.

In a trial with winter rye Manilov et al., (2024) found that the yield was positively influenced by the combined application of herbicide and biostimulant. In the present trial, the highest yields of 3.46 t ha^{-1} for variant 8 (Pulsar Plus – 2.00 l ha^{-1} + AEI – 2.50 l ha^{-1}) were recorded.

The twice - hoed control had higher yields (3.25 t ha^{-1}) of grain on average compared to the variants treated only with the herbicides Pulsar 40 (3.21 t ha^{-1}) and Pulsar Plus (3.10 t ha^{-1}).

Table 6. Sorghum grain yield, t ha^{-1}

Variants of trial/days	2023	2024	Average
1. Untreated control	2.38 c	1.45 c	1.92
2. Twice-hoed control	3.74 b	2.75 b	3.25
3. Pulsar 40 (1.20 l ha^{-1})	3.70 b	2.71 b	3.21
4. Pulsar 40 (1.20 l ha^{-1}) + AEB (1.00 l ha^{-1})	3.89 a	2.95 a	3.42
5. Pulsar 40 (1.20 l ha^{-1}) + AEI (2.50 l ha^{-1})	3.95 a	2.93 a	3.44
6. Pulsar Plus (2.00 l ha^{-1})	3.64 b	2.56 b	3.10
7. Pulsar Plus (2.00 l ha^{-1}) + AEB (1.00 l ha^{-1})	3.99 a	2.88 a	3.43
8. Pulsar Plus (2.00 l ha^{-1}) + AEI (2.50 l ha^{-1})	4.02 a	2.90 a	3.46

Figures with different letters are significantly different according to Duncan's test ($p < 0.05$).

The highest yields, absolute and hectoliter seed mass, were reported for the treatment with herbicide and plant biostimulant in tank mixture (Yanev et al., 2023). These findings are in confirmation with our results discussed below.

Table 7 shows the results for the 1000 grain weight of the sorghum seeds. The lowest 1000 grain weight was recorded in the untreated control – 26.94 g on average for the two years of the trial. The variants with a biostimulant in the tank mixture were with a higher 1000 grain weight of ranging from 31.95 to 32.54 g. The 1000 grain weight at the plants treated only with the herbicide products, as well as in those from the hoed control, showed lower results.

Table 7. 1000 grain weight, g

Variants of trial/days	2023	2024	Average
1. Untreated control	28.19 c	25.68 d	26.94
2. Twice-hoed control	32.23 b	30.58 c	31.41
3. Pulsar 40 (1.20 l ha^{-1})	32.12 b	30.54 c	31.33
4. Pulsar 40 (1.20 l ha^{-1}) + AEB (1.00 l ha^{-1})	33.65 a	31.20 a	32.43
5. Pulsar 40 (1.20 l ha^{-1}) + AEI (2.50 l ha^{-1})	33.74 a	31.33 a	32.54
6. Pulsar Plus (2.00 l ha^{-1})	31.87 b	29.87 c	30.87
7. Pulsar Plus (2.00 l ha^{-1}) + AEB (1.00 l ha^{-1})	33.68 a	30.22 b	31.95
8. Pulsar Plus (2.00 l ha^{-1}) + AEI (2.50 l ha^{-1})	33.89 a	30.52 b	32.21

Figures with different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 8. Hectoliter seed mass, kg

Variants of trial/days	2023	2024	Average
1. Untreated control	60.20 c	55.50 d	57.85
2. Twice-hoed control	64.50 b	59.50 c	62.00
3. Pulsar 40 (1.20 l ha^{-1})	64.20 b	60.20 b	62.20
4. Pulsar 40 (1.20 l ha^{-1}) + AEB (1.00 l ha^{-1})	65.70 a	60.30 b	63.00
5. Pulsar 40 (1.20 l ha^{-1}) + AEI (2.50 l ha^{-1})	65.90 a	61.20 a	63.55
6. Pulsar Plus (2.00 l ha^{-1})	64.10 b	59.20 c	61.65
7. Pulsar Plus (2.00 l ha^{-1}) + AEB (1.00 l ha^{-1})	65.10 a	61.30 a	63.20
8. Pulsar Plus (2.00 l ha^{-1}) + AEI (2.50 l ha^{-1})	65.30 a	61.00 a	63.15

Figures with different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 8 shows the results for the hectoliter seed mass of sorghum from the present experiment.

Both for this productive indicator, as well as for the 1000 grain weight, the lowest results were found for the untreated control – 57.85 kg on average for the two years of the study.

The variants with a biostimulant in the tank mixture were with higher hectoliter seed mass, which varies from 63.00 to 63.55 kg.

As for the mass of 1000 seeds, the hectoliter seed mass for the variants treated only with herbicides, as well as for those from the twice-hoed control, was with lower values.

CONCLUSIONS

No visual phytotoxicity symptoms were observed in plants treated with Pulsar 40 and Pulsar Plus, either alone or in combination with Amino Expert Balance and Amino Expert Impulse. Combined application of Amino Expert Balance or Amino Expert Impulse with Pulsar 40 and Pulsar Plus in tank mixtures increased the chlorophyll content index in leaves. The combined use of Amino Expert Balance and Amino Expert Impulse with Pulsar 40 and Pulsar Plus enhanced plant height, panicle length, leaf number, grain yield, thousand kernel weight, and hectoliter weight. Independent application of Pulsar 40 or Pulsar Plus, as well as double hoeing, resulted in lower values for all measured parameters. The lowest results for all studied indicators were recorded in the untreated control.

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