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The effect of the Fitostemin-WP® biostimulator on yield and quality characteristics of rice (*Oryza sativa* L.)

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Abstract

In order to avoid yield and quality reduction of rice, it is essential to provide nutrition rice plants require. Therefore, a field experiment was conducted at the research area of the Institute of Agriculture - Skopje, Department of Field Crops, Ss. Cyril and Methodius University in Skopje, Republic of Macedonia, in Kochani, a rice production region. The experiment was set up to determine the effects of the Fitostemin-WP® biostimulator on some yield and quality properties of an Italian rice variety Opale during the vegetative seasons in 2020 and 2021. Standard agrotechnical methods for crop production were applied. Fitostemin-WP® is an herbal product in the form of powder and it consists of herbal extracts and minerals. It is used for increasing crop yield. The field experiment was arranged in a Zade method with a Fitostemin-WP® treatment and a control one in 3 replications. Only basic fertilizers (350kg/ha NPK and 200kg/ha Urea (46% N)) were applied on the control plot, while the treatment plot, besides basic fertilization, was also treated with Fitostemin-WP® with 100 g/ha dosage once during the vegetation period (tillering stage). The following parameters were examined in this study, namely the biological, grain and straw yield, the number of productive tillers/m2, panicle weight, and milling fractions. Results were analyzed with the ANOVA and LSD test. According to the results from this study, statistically significant differences were not found in these two years of field experiments for the examined parameters. However, the values for the brokens have shown some significant differences between the years, but not between the treatment and the control. This can result from the environmental impact during the vegetative period. More frequent usage of

Fitostemin-WP® during the vegetation period of the rice plant may show more significant results.

Key words: herbal fertilizer, biological yield, productive tillers, milling fractions

Introduction

Rice (Oryza sativa L.) is one of the major staple foods for global consumption. In the Republic of Macedonia, a centuries old tradition of rice cultivation persists. The first written sources date back to the middle of the XV century (Atanasova, 2015). Rice holds significant strategic importance as a cereal crop in the Republic of Macedonia, which has a cultivation tradition spanning centuries. The primary area for rice production is concentrated in the eastern region of the country, specifically the Kochani valley along the Bregalnica river, covering an average cultivation area of 4,926 hectares. Historical data from 1939 to 2014 indicate an average yield of 4,692 kg per hectare, resulting in an annual paddy production averaging 23,145 t per year (Andreevska and Andov, 2015). Yield is a complex trait, which is influenced by several quantitative traits and is governed by polygenes. Tillering is an important agronomic trait for rice population quality and grain production (Counce and Wells, 1990). The number of tillers has been reported to have a positive association with plant biomass and economic yields in rice (Deng et al. 2015). Rice yield is closely related to the number and proportional area of rice panicles. The panicle number is directly related to rice yield, so panicle detection and counting has always been one of the most important scientific research topics (Wang et al., 2022; Zhou et al., 2022). Rice grain quality is the primary determinant of market acceptability. Since whole grain rice is mostly consumed as a staple meal, rice grain quality is quite more complicated than other cereals (Samantara et al., 2022). In recent years, there has been growing interest in foliar application of fertilizers, driven not only by scientific inquiry but also by practical considerations in crop production. Some researchers suggest that foliar nutrition is gaining importance due to its ability to quickly deliver minerals for immediate impact on cellular turnover. Additionally, foliar fertilization complements conventional soil-based fertilization, thereby integrating into comprehensive plant nutrition strategies for crop production (Vukadinović and Lončarić, 2011). These fertilizers are typically rich in nutrients, encompassing major elements (such as N, P₂O₅, K₂O, MgO), trace elements (including Fe, Mn, Cu, Zn, B, Mo, Co), and occasionally growth promoters like indole acetic acid and vitamins. Megeed et al. (2022) have reported that the usage of stimulating substances is one of the effective means of improving rice grain quality and enhancing the nutritional value of milled rice. Pan et al. (2013) indicated that foliar application with plant growth regulators enhanced yield and grain quality characteristics. Standard rice production practices in Macedonia involve initial fertilization with mineral NPK

fertilizers, typically applied at rates between 400 to 600 kg/ha. During the vegetative growth stage, additional nitrogen is often applied as a supplementary fertilization (second split application) ranging from 150 to 250 kg/ha, using fertilizers such as urea, ammonium nitrate, or calcium ammonium nitrate (CAN) (Andov and Andreevska, 2010). Spaseva et al. (2021) found that Orgalife, an organic foliar fertilizer, had a beneficial impact on seed germination and seedling growth. Also, Mitrushev et al. (2022) noticed in their research a significant bio stimulant impact of Orgalife on some yield and quality properties of rice such as the biological, grain and straw yield, harvest index, the number of productive tillers per meter squared, and 1000 grain mass of white rice. Various other foliar fertilizers have been studied in rice production research in the Republic of Macedonia. Research outcomes have indicated that foliar applications of water soluble NPK fertilizers containing chelated micronutrients, organic acids, and amino acids such as KristalonTM Special (Andreevska et al., 2006) and Lactofol O (Andreevska et al., 2012), have also demonstrated positive effects on rice yields. However, calcium-based foliar fertilizers like Megagreen and Herbagreen generally did not show significant effects on rice production (Dimitrovski et al., 2017, 2016). Since fertilization plays a crucial role in rice production, various types of fertilizers and techniques have been studied over time. Therefore, the aim of this study was to assess the effect of the Fitostemin-WP® biostimulator on some yield and quality rice properties. Notably, Fitostemin-WP® has not been previously utilized for fertilizing rice in the Republic of Macedonia.

Material and Methods

Experimental design

In order to examine the effect of the ecological Fitostemin-WP® biostimulator on the yield properties of rice, a field experiment was set up and arranged in design according to the Zade method (trial with plot arrangement in long stripes developed by Adolf Zade), with a Fitostemin-WP® treatment and a control one in 3 replications. The experimental plot size was 1000 m² (500 m² for the control and 500 m² for the treatment with Fitostemin-WP®).

The experimental site

The experiment was conducted during two vegetative crop seasons in 2020 and 2021, at the Department of Field Ccrops, Institute of Agriculture, Ss. Cyril and Methodius University in Skopje, the Republic of Macedonia, in the rice production region of Kochani at 350 m above the sea level. The soil at the experimental site was alluvial type, with a pH value of 5.65.

Plant material

For the purpose of this field experiment Opale, an Italian rice variety introduced in the Republic of Macedonia in 2010 and widely used in the country's rice production, was studied.

Agronomic operations

In the early spring season of both years the first plowing operation and preparation of the experimental plot was done. Early spring plowing of the rice fields is a common practice in this region in order to expose the soil to air and to facilitate unfavourable conditions for weed seed, s such as freezing or drying (Melikhov and Popov, 2017). The following operations included bordering the rice field edges, laser land leveling, and disking the ground. Prior to sowing, 350 kg/ha NPK fertilizer (16:16:16) as a basic fertilizer and 200 kg/ha Urea 46% N were applied to the whole experimental field. Afterwards, the Oxadiazon herbicide (1.5 L/ha) against weeds such as *Heteranthera* sp. and other broadleaf weeds was applied pre-sowing on dry surface. Subsequently, the experimental plot was filled with water and direct manual sowing was performed in the middle of April. The rice was sown at a seed rate of 200-250 kg/ha. The distance between the experimental blocks was 70 cm. In order to control pests and diseases at different growth stages, agronomical management was performed. At the end of vegetation when the grains totally ripened, three sheaves of rice plants were harvested according to the Quadrat method (1 x 1 m of rice area) in 3 replications of the treatment and the control. The grain moisture average for the 2020 Fitostemin-WP® treatment was 12.27% and it was 11.73% for the control, while 2021 averages were 12.33% and 12.03%, respectively.

Experimental Treatments

Fitostemin-WP® is a plant vaccine, i.e., a plant-based preparation in the form of powder and it consists of herbal extracts and minerals. Suggested usage is 100g of the agent which is dissolved in 3-5 liters of water and then mixed with about 300 liters of water, sufficient for 1 ha. It can be mixed with all pesticides and it is used for foliar application. Two different experimental variants included a foliar application of the Fitostemin-WP® fertilizer (once during the vegetation in the tillering phase) and a control treatment (only basic fertilizers applied).

Evaluation of Agronomic Traits

The following rice plant parameters were measured and analyzed in this study, namely the biological yield (paddy + straw) (kg/ha), grain yield (kg/ha) and straw yield (kg/ha), the number of productive tillers per meter squared, panicle weight (kg/ha), and milling fractions (white rice yield, brokens, hull and bran) (%). All of the examined parameters were determined under the laboratory conditions. Milling fractions (white rice yield, brokens, total milled rice, rice bran, and hull) were assessed by milling average sample of 100 g paddy rice of each repetition with a laboratory milling machine during 1.40 min.

Statistical Analysis

A statistical analysis was performed by using XLSTAT. Results were analyzed by ANOVA and mean comparison of traits was performed by least significant difference (LSD) tests, at the p<0.05 and p<0.01 probability level to interpret the differences among the treatments.

Results and Discussion

Biological yield, paddy rice yield, and straw yield (kg/ha)

The appropriate combination of organic and inorganic nutrient sources was found to enhance their efficiency and ultimately increase yield attributes of rice (Akter et al. 2022). The results for biological yield, paddy rice yield, and straw yield are shown in Table 1. Thus, they have shown that the biological yield determines 8.1% higher average values for the Fitostemin-WP® treatment for both years compared with the control averages. Paddy rice yield for the control in 2020 was 4% higher compared with the Fitostemin-WP® treatment.

Opale	Fitostemin- WP® treatment	Control	Average	LSD _{0.05}	LSD _{0.01}
Biological yield					
2020	16666.67	14383.33	15525a	4377.30	6626.21
2021	21916.67	21000	21458.33ª		
Mean	19291.67ª	17691.67ª			
CV (%)	0.19	0.26			
Paddy rice yield					
2020	9000	10066.67	9533.33ª	1280.24	1820.94
2021	10683.33	10416.67	10550 ^a		
Mean	9841.67ª	10241.67ª			
CV (%)	0.12	0.02			
Straw yield					
2020	7666.67	4300	5983.33 ^a	3880.57	5519.66
2021	11233.33	10583.33	10908.33ª		
Mean	9450 ^a	7441.67 ^a			
CV (%)	0.27	0.60			

Tab. 1 Biological yield, paddy rice yield, and straw yield (kg/ha)

Note: ^{abc} differences between values in a given row are statistically significant if the labels do not contain the same letter

The critical nutrients for the rice crop production are N, P, K, S, and Zn (Asten et al. 2004; Buri et al. 2000; Quijano-Guerta et al. 2002; Shah et al. 2008; Kumar and Yadav 2005). Many studies in the environmental conditions of the Republic of Macedonia have shown that basic rice fertilization before sowing with nitrogen or NPK fertilizers is necessary for obtaining good yield results (Gjorgijev and Andreevska, 1990; Andreevska, 2000). Andreevska et al. (2012) reported that the application of NPK foliar fertilizers and some trace elements a showed positive effect on rice yield.

The number of productive tillers per m² and panicle weight

The quantity and quality of tillers determine the number and structure of panicles, which determine the rice yield (Zhou et al., 2022). The mean value differences for the number of productive tillers/m2 between the Fitostemin-WP® treatment and the control were not statistically significant for both years. The average value for the Fitostemin-WP® treatment was 561 tillers/m² and it was 541.83 tillers/m² for the control. The same situation was for the panicle weight parameter: 1065.83 kg/ha for the Fitostemin-WP® treatment average and 1068.33 kg/ha for the control average (Tab. 2). Dimitrovski et al. (2017) also found that the ecological fertilizers used in their experiment did not show statistically significant differences between the treatments and the control in regards with the number of productive and non-productive tillers.

Opale	Fitostemin- WP® treatment	Control	Average	LSD _{0.05}	LSD _{0.01}
Number of productive tillers/m ²					
2020	505.67	497.33	501.50 ^b	90.84	129.20
2021	617.33	586.33	601.83 ^a		
Mean	561.50 ^a	541.83 ^a			
CV (%)	0.16	0.13			
Panicle weight					
2020	1008.33	1055	1031.67 ^a	84.80	120.62
2021	1123.33	1081.67	1102.50 ^a		
Mean	1065.83 ^a	1068.33 ^a			
CV (%)	0.08	0.02			

Tab. 2 The number of productive tillers per m² and panicle weight (kg/ha)

Note: ^{abc} differences between values in a given row are statistically significant if the labels do not contain the same letter

Milling fractions

Rice milling is a crucial step to determine the final rice quality in the production process. Rice grain quality is a key factor that determines its economic value in the international market as well as consumer approval. The results from Table 3 about the milling fractions parameters did not show a significant difference between the average values of the treatment and control for the whole grains for both years (69.55-70.50%). These findings are in correlation with the results of Dimitrovski et al. (2017) about the non-significant effect of the ecological foliar fertilizer on the white rice yield.

Brokens had statistically significant differences between the years, while between the treatment and the control there were no significant differences at all. The percentage of brokens after the Fitostemin-WP® treatment (2.97%) in 2021 was higher compared with the control (2.13%), while in 2020 the fertilizer achieved a

lower value (0.80%) than the control (1.03%). This implies that rice breakage is related to milling conditions, particularly the prevailing relative humidity, the temperature, and the extent of milling (Kashi, 1969).

Consequently, total milled rice had statistically significant differences between the years; in 2020 the mean value was lower (70.19%) than in 2021 (73.33%). Overall, this situation may be a result of the climatic conditions during the vegetative seasons. Considering the hull and bran fractions, the Fitostemin-WP® treatment did not show any statistically significant differences between the years.

Opale	Fitostemin- WP® treatment	Control	Average	LSD _{0.05}	LSD _{0.01}
Whole grains					
2020	68.67	69.87	69.27ª	1.17	1.67
2021	70.43	71.13	70.78 ^a		
Mean	69.55 ^a	70.50 ^a			
Brokens					
2020	0.80	1.03	0.92 ^b	1.27	1.81
2021	2.97	2.13	2.55ª		
Mean	1.89 ^a	1.58 ^a			
Total milled rice					
2020	69.47	70.90	70.19 ^b	1.17	1.67
2021	73.40	73.26	73.33ª		
Mean	71.44 ^a	72.08 ^a			
Hull					
2020	19.80	15.33	17.57 ^a	3.99	5.67
2021	17.57	17.83	17.70 ^a		
Mean	18.68 ^a	16.58 ^a			
Bran					
2020	8.93	12.27	10.60 ^a	4.57	6.51
2021	8.63	7.67	8.15 ^a		
Mean	8.78 ^a	9.97ª			

Tab. 3 Shade of milling fractions (%)

Note: ^{abc} differences between values in a given row are statistically significant if the labels do not contain the same letter

Conclusion

This study was conducted to evaluate the effects of the Fitostemin-WP® biostimulator on some yield and quality properties of the Opale rice variety. During the highest nutrient demand and consumption of the rice plant, fertilizer nutrients should be easily available in order to avoid yield reduction. High-yield rice varieties require more nutrients compared with the traditional ones. Therefore, in this study the statistical analysis has clarified that the Fitostemin-WP® treatment did not show

a significant impact on the yield and quality characteristics of rice, such as the biological yield (paddy + straw), paddy rice yield and straw yield, the number of productive tillers per meter squared, panicle weight, and white rice yield. The statistical analysis has shown some significant differences between the year only for the brokens, but not between the treatment and the control. Thus, the reason for this situation may result from the influence of many environmental factors (temperature amplitudes, relative humidity, rainfalls, etc.) during the vegetative period of rice production that determine the rice grain quality. More field experiments should be performed with more frequent use of Fitostemin-WP®, e.g. 2-3 times during the rice vegetation period.

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Утицај биостимулатора Фитостемин-WP® на карактеристике приноса и квалитета пиринча (*Oryza sativa* L .)

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Сажетак

У граду Кочани, Источне Македоније, спроведен је теренски експеримент да би се утврдили ефекти биостимулатора Фитостемин-WP® на неке особине приноса и квалитета једне сорте пиринча - Opale, током 2020-2021. У пољском огледу изведеном методом Цаде, проучавана су два третмана: контролна и варијанта 1. Као основно ђубриво примењено је 350 kg/ha NPK (16:16:16) и 200 kg/ha урее (46% N) у фаза формирања бочне стабљике, на целој огледној површини пре сетве. Контролни третман има само основна ђубрива, док је варијанта 1 третирана и са Фитостемин-WP® у дози од 100 g/ha, једнократно током вегетационог периода (фаза формирања бочне стабљике). Параметри који су испитивани у овој студији су: биолошки, принос зрна и сламе, број продуктивних метлица/m², маса метлице и фракције љуштење пиринча. Резултати су анализирани са ANOVA и LSD тестом. Резултати о просецима биолошког приноса варирају од 19261,67 kg/ha за варијанту 1 до 17691,67 kg/ha за контролу. По броју продуктивних метлица/m² просеци за варијанту 1 (561) били су изједначени са контролом (541), као и маса метлице (варијанта 1 - 1065,83 kg/ha и контрола - 1068,33 kg/ha). Фитостемин-WP® је показао приближно исте просечне вредности о укупним фракцијама љуштење пиринча испитиваних у обе године, без обзира на третмане. Према резултатима ове истраживачке студије, у ове две године теренског експеримента нису утврђене статистички значајне разлике за испитиване параметре. Неопходно је наставити теренски експеримент са чешћом употребом Фитостемин-WP® (2 или 3 пута током вегетационог периода производње пиринча).

Кључне ријечи: биљна вакцина, биолошки принос, продуктивне метлице, фракције за љуштење.

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