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The Impact of Sowing Time and Biostimulant Application on Seed Production in Italian Ryegrass

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ABSTRACT

This research was performed to determine the effects of different sowing times (ST1, ST2, and ST3) and biostimulant (Pi-NFS) doses (0, 100, 250, 500 ml da-1) on seed yield components of Italian ryegrass ryegrass (*Lolium multiflorum* L.) in the continental condition of Eskişehir, Turkey. The results indicated that the main factors significantly affected the seed yield components except for the effect of sowing time on plant height, however, the interaction effect of these factors was significant only on the number of the reproductive tillers and seed yield. There was no significant difference between the ST1 and ST2 in terms of the number of the reproductive tillers, thousand seed weight, and seed yield. Likewise, the values of these components did not differ significantly in the doses of 250 and 500 ml da-1. The highest seed yield was derived from the dose of 500 ml da-1 at ST2 and the values obtained from the doses of 250 and 500 ml da-1 at the ST1 were in the high seed yield group. It was concluded that early sowing time and the doses of 250 to 500 ml da-1 biostimulant application resulted in high seed yield in Italian grass. On the other hand, the dose of 500 ml was more effective in case of delayed sowing time like ST2.

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Introduction

Italian ryegrass (*Lolium multiflorum* Lam.) has attracted in recent years due to its high yield and nutritive value. It does not become coarse quickly and preserves its form, which livestock will like to consume, during the vegetation period (Darvishi, 2009). Its cultivation areas have increased 8 times in five years and reached 374,000 da in Turkey (TUIK, 2021) and this has increased the demand for its seeds. It is grown for hay production or grazing as a winter crop and for hay or to produce seeds as the main summer crop in the coastal and central regions of Turkey, respectively (Kuşvuran and Tansı, 2005).

Seed production and quality of Italian ryegrass are affected by various factors such as sowing time, variety, fertilization, harvesting, and especially climatic and soil conditions (Rolston et al. 2018). Botha et al. (2015) determined that sowing time was very important for high production in Italian ryegrass in the spring and early summer and it should be sowed in May or June in the Western Cape province of South Africa.

Biostimulants; which may include organic or inorganic compounds, positively affect plant growth, plant nutrition, quality and yield in order to increase the resistance to plant stress. They are applied to plants from leaves, soil or seeds, and some of them also have soil structure regulating effects (EBIC, 2013). The positive effects of the applications of different biostimulants on Italian ryegrass (Khaleda et al., 2017; Ciepiela and Godlewska, 2019; Godlewska and Ciepiela, 2020) and perennial grass (*Lolium perenne*) cultivation (Luca et al. 2020; Qiu et al. 2020; Talar-Krasa et al. 2021) and lawn (Radkowski et al. 2020; Sever Mutlu et al., 2019) have been reported. The use of biostimulants in *Lolium* species and grasses has recently been started and precise statistical information on the subject is not available.

In this study, the effect of Pİ-NFS brand biostimulant on seed yield of Italian ryegrass for the first time at different sowing times.

Material and Methods

The study was performed in Eskişehir, Tukey, at 390 45' 23' N, 300 28' 40'' E, between March and July 2021. The *L. italicum* cv. İlkadım, which is currently the most common seed production in Eskişehir conditions, as the seed material and Pi-NFS brand (Registration number: 13687) biostimulant as foliar liquid fertilizer were used.

The physical and chemical properties of soil samples in the experimental area from 0-30 and 30-60 cm depths are given in Table 1. According to this table, the amount of organic matter is very low with 0.93% at 0-30 cm and low with 1.39% at 30-60 cm (Dewi and Arbawa, 2019). Also, the soil shows a moderately calcareous or calcareous and moderately alkaline reaction, and has sufficient available P2O5 and high K2O (Aydeniz, 1985).

While the total precipitation of the research area is 441 mm in 2021, the long-term average of this period is 365 mm (Table 2). Especially between January and May, higher precipitation was observed compared to the long-term average. On the other hand, there was no difference in monthly temperature values and relative humidity was low compared to the long term between March to July 2021.

The experiment was designed in split plots in a randomized block with four replications. The main plots were three sowing times (ST1: 06.03.2021, ST2: 21.03.2021, and ST3: 28.03.2021) and the sub-plots were four biostimulant doses [(control, 100, 250 (recommended dose), and 500 ml da^{-1})].

The seeding rate was 2 kg da^{-1} and row spacing was arranged to 30 cm. Diammonium phosphate (20 kg da^{-1}) was applied with sowing and 6-8 hours of sprinkler irrigation was performed for the emergence. Herbicide (Corvette, Registrated number: 7371) application was conducted on 09.05.2021 for weed control. Urea as a top-dressing (26 kg da^{-1}) was applied to crops at beginning of tillering and a week after top-dressing, liquid fertilizer (Pi-NFS) was sprayed on the foliage of crops at 3 different doses.

When crops reached maturity with a drying rate of 35-40%, important seed yield components for ryegrass such as plant height (cm), spike length (cm), spikelet number per spike, reproductive tiller (number), thousand seed weight (g), and seed yield (kg da^{-1}) were observed.

The data were analyzed with the JMP version 14 program for variance analysis and those with significant differences between the means were compared with the LSD test.

Results and Discussion

In this study, the effect of three sowing times and four doses of liquid foliar fertilizer application on the seed yield of Italian ryegrass was investigated. All seed yield components were significantly affected by main factors except for plant height at sowing time (Table 3). However, the interaction of those factors made a significant difference only reproductive tiller and seed yield.

Although plant height was not affected by sowing time, it increased with the high doses of biostimulant (Table 3). Contrary to these findings, Botha et al. (2015) stated that sowing time affected the production potential of Italian grass. On the other hand, Khaleda et al. (2017) found that biostimulants like humic and synthesized humic mimic acid had a positive effect on the plant growth of Italian grass.

Table 1. Physical and chemical properties of the soil in the experimental area.

Years	Depth (cm)	pH	Lime (%)	Salt (%)	Organic matter (%)	The amount of nutrients available to the plant (kgda^{-1})		Soil structure
						Phosphorus	K_2O	
2020	0-30	7.90	6.14	0.02	0.93	6.52	152	Loam
2021	30-60	7.94	4.76	0.03	1.39	8.14	191	Loam

Table 2. Climatic data of 2021 year and long-term at the experimental area.

	Rainfall (mm)		Temperature (°C)		Relative humidity (%)	
	Long-term	2021	Long-term	2021	Long-term	2021
January	44.63	48	-0.1	-0.1	84	77
February	29.49	47	1.6	1.6	79	72
March	30.46	54	5.3	5.2	73	66
April	32.43	53	9.9	9.7	70	62
May	39.66	49	14.9	14.6	70	58
June	63.11	34	18.9	18.5	67	56
July	15.53	12	21.9	21.7	62	51
August	16.36	14	21.9	21.7	64	51
September	14.01	16	17.5	17.6	68	54
October	25.14	36	12.1	12.2	77	62
November	24.09	35	5.8	6.4	80	68
December	30.06	54	1.7	1.6	85	76
Total	365.0	441	-	-	-	-
Mean	30.42	36.75	11.0	10.9	73.2	62.8

Table 3. The analysis of variance and differences between mean values of the effect of sowing time and biostimulant doses on seed yield and yield components in Italian grass.

Source	Plant height (cm)	Spike length (cm)	Number of spikelet per spike	Number of reproductive tiller	Thousand- seeds weight (gr)	Seed yield (kg da^{-1})
Sowing Times (ST)						
ST1	102.4±0.7	30.1±0.3^{c}	20.6±0.4^{b}	1126.8±56.8^{a}	2.62±0.03^{a}	334.3±6.7^{a}
ST2	103.0±0.5	31.9±0.3^{a}	21.5±0.2^{a}	1120.3±35.7^{a}	2.63±0.05^{a}	324.5±8.7^{a}
ST3	101.1±0.5	30.9±0.4^{b}	20.9±0.3^{b}	700.3±17.0^{b}	2.44±0.04^{b}	146.9±6.5^{b}
Biostimulant Doses (BD) (ml da^{-1})						
0	99.4±0.2^{d}	29.5±0.2^{c}	19.6±0.2^{c}	835.2±47.3^{b}	2.40±0.03^{c}	236.8±23.8^{c}
100	101.7±0.4^{c}	30.8±0.2^{b}	20.7±0.2^{b}	866.5±48.9^{b}	2.53±0.04^{b}	253.6±25.0^{b}
250	103.0±0.5^{b}	31.6±0.4^{ab}	21.4±0.3^{b}	1100.3±72.7^{a}	2.64±0.04^{ab}	293.2±22.4^{a}
500	104.4±0.6^{a}	31.9±0.4^{a}	22.3±0.2^{a}	1127.8±76.7^{a}	2.69±0.06^{a}	290.7±29.7^{a}
Analysis of variance						
ST	ns	*	*	*	*	*
BD (ml/da)	*	*	**	*	**	*
ST×BD	ns	ns	ns	*	ns	*
CV (%)	1.42	3.01	3.51	6.37	5.47	4.18

*, **: significant level of 5% and 1%, respectively, ns: non-significant.

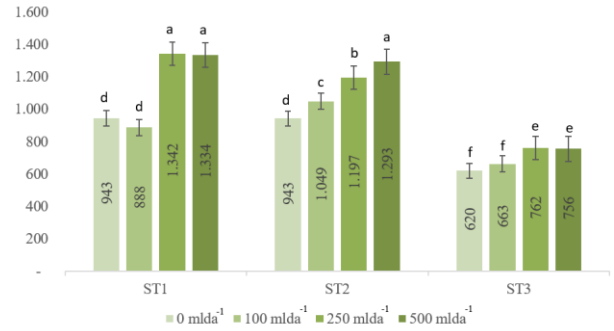

Figure 1. The effect of different sowing times and biostimulant applications on the number of reproductive tiller in Italian ryegrass

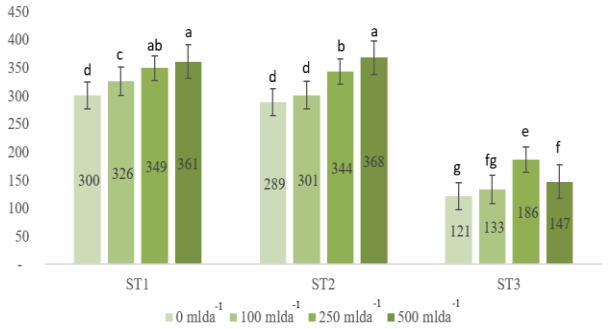

Figure 2. The effect of different sowing times and biostimulant applications on seed yield in Italian ryegrass (kg da-1).

Spike length was found to be statistically significant in biostimulant dose and sowing time as indicated in Table 3. In early and late sowings, spike length had lower values, but the increased biostimulant doses affected it positively. The highest values were recorded in ST2 and 500 ml da^{-1} dose. It has been confirmed in many studies that early sowing (Fayed et al., 2015; Farooq et al., 2016; Madhu et al., 2018) and the use of biostimulants (Sarmin 2014; Kumbhar et al. 2017) increased the length of the spike. Contrary to these findings, biostimulant had no significant effect on ear length of maize (Ferreira et al. 2018).

The number of spikelets per spike differed by sowing time and biostimulant dose (Table 3). The highest values were obtained from ST2 and 500 ml da^{-1}. In accordance with these findings, Arduini et al. (2009) reported that there was a decrease in the number of spikelets in late sowing time. The results confirmed by Kandil et al. (2016) that the foliar spraying with mixture of humic and amino acids increased the number of spikelets of wheat cultivars. Also, Hristov and Kolev (2019) indicated that mineral and organic bio stimulators have a positive effect on the spikelet number of wheat compared to control application.

Both main factors and their interaction had a statistically significant effect on the number of reproductive tiller (Table 3). In general, late sowing decreased in reproductive tiller number, while high biostimulant doses increased them. The higher values was obtained at 250 and 500 ml doses at the ST1 (Figure 1). Similar to these findings, late sowing negatively affected the number of spike in wheat (Hossain et al. 2021) and barley (Razzaque and Rafiquzzaman, 2006). In addition, the positive effects of biostimulant applications on the number of fertile tillers have been reported in Marundu grass (Oliveira et al., 2019) and wheat (Al Majathoub, 2004; Szczepanek and Grzybowski, 2016).

Different sowing times and biostimulant doses had significant effect on thousand-seeds weight, however the interaction effect of these factors occurred insignificant (Table 3). The higher values were derived in early sowing times like ST1 and ST2 than late sowing. The increasing biostimulant doses resulted in positive effect on thousand-seeds weight and the high seed weights was measured in 250 and 500 ml da^{-1} doses. Similar to these findings, early sowing (Fayed et al., 2015; Farooq et al., 2016) and biostimulant application (Al Majathoub, 2004) increased 1000-seed weight in wheat.

Seed yield, which is the main subject of the research, was significant affected by sowing time, and biostimulant doses and their interaction (Table 3). Early sowing and high doses of biostimulant increased the seed yield. The seed yield increased at the ST2 with the highest application dose, and there was no significant difference between this value and the seed yields obtained from 250 and 500 ml da^{-1} doses at the ST1 (Figure 2). In perennial ryegrass,

Chynoweth and Moot (2013) found that the seed yield in perennial grass was affected by the sowing date, and PGR application, but their interaction was insignificant. Although there is not much data on the use of biostimulant in seed production of Italian ryegrass, it has been determined that the use of plant growth regulators increases seed yield in some previous studies (Rijckaert, 2007; Rolston et al., 2012; Sveˇcnjak et al., 2022).

In conclusion, early sowing times (ST1 and ST2) and the use of the biostimulant named Pi-NFS had a positive effect on the seed yield of Italian grass in continental climates. The higher seed yields were produced at ST1 and ST2 with recommended or high dose of biostimulant (250 and 500 ml da^{-1}). Delaying of germination and emergence due to low temperature during the first sowing time (ST1) could not show the expected effect in terms of seed yield components that directly affect seed production and seed yield. As a result, it should be sowed at the earliest possible time considering low temperatures and 250-500 ml da doses of biostimulant should be applied for high seed yield in Italian ryegrass.

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