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Original article

Effect of Storm Fertilizer on Germination and Seedling Vigor in Rice (*Oryza sativa* L.)

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Abstract

The effect of Storm (fertilizer with 3% growth regulators from *Sargassum* and *A. nodosum* algae and 6% organic N) on germination parameters and seedling vigor in rice was examined in seed from San Andrea cultivar produced in 2017 and 2018, treated with three concentrations: 0.1%, 0.5% and 2.5% against a control (no fertilizer added). Germination energy, total germination, germination speed, seedling (root, shoot and total) length and dry weight and vigor indexes (I and II) were examined. The results were statistically evaluated by two-way ANOVA and LSD test. The total germination (94.38% to 96.75%) was not affected by the treatments, while the germination energy and the germination speed were significantly negatively affected by the 2.5% treatment-83.00% and 21.53 accordingly against 90.63% and 23.94 in the control). All Storm treatments significantly increased the seedling length. With the increase in concentration, the root length decreased while the shoot length increased. Even thought all of the Storm treatments significantly increased the Vigor index I (1587.44 to 1644.75) and Vigor index II (35265.38 to 37753.75) compared to the control (1362.07 and 29984.50 respectively), they affected the seedling development differently. The 2.5% treatment negatively affected the development of the seedling, as promoted increased development of the seedling shoot, but inhibited the development of the root. Best results were obtained with 0.1% concentration, as it significantly promoted both the root and shoot development and resulted in significantly higher index I, index II, shoot, root and total seedling length compared to the control. With this treatment were obtained the longest root (7.98 cm), the highest root dry weight (3.89 mg) and the highest root volume (50 μL) in the trial.

Keywords: Germination, Vigor indexes, Seedling length, Seedling dry weight, Fertilizer, Growth regulators, Rice.

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INTRODUCTION

In rice production good germination and seedling emergence in field conditions are necessary for obtaining healthy and uniform crop stand. In order to obtain good germination and growth of young seedlings, the seeding material can be treated with materials that affect germination, such as nutrients and growth regulators. In the past years, studies on different agricultural plant species have shown the beneficial effect of algae extracts on germination, young seedling growth, as well as plant yield and quality. Extracts from *Sargassum* algae have shown a positive effect on seedling growth of crops such as tomato *Lycopersicon esculentum* (Kumari, Kaur & Bhatnagar, 2011), black lentil *Vigna mungo* (Kalaivanan & Venkatesalu, 2012), green gram *Vigna radiata* (Kumar, Vanlalzarzova, Sridhar & Baluswami, 2012), corn *Zea mays* (Sutharsan, Nishanthi & Srikrishna, 2017) and wheat *Triticum aestivum* (Kumar & Sahoo, 2011). *Ascophytum nodosum* extracts positively affected seed priming and germination in pepper *Capsicum annuum* (Sivritepe & Sivritepe, 2008), the vigor and emergence of dry bean seeds *Phaseolus vulgaris* (Carvalho, Castro, Novembre & Chamma, 2013.). In watermelon *Citrullus lantus* L., extracts from *A. nodossum* positively affected the plant growth, yield and quality (Abdel-Mawgoud, Tantaway, Hafez & Habib, 2010) as well as the growth and production of seedlings (Júnior et al., 2019).

In rice, solid fertilizer of macroalgae *Sargassum crassifolium* and *Sargassum aquifolium* induced significantly plant height and number of tillers, whereas liquid macroalgae fertilizers proved more affective in promoting yield content in rice plants. *Sargassum crassifolium* showed promising effects in promoting yield content in rice plants (Sunarpi et al., 2019).

In this study was examined the effect of Storm fertilizer on germination and young seedling growth in rice. The fertilizer contains growth promoting regulators from *Sargassum* and *A. nodosum* algae.

Materials and Methods

Materials

Storm is a commercially available fertilizer (Agros, 2018). It is a brown liquid with a pH of 5 to 6.5 and contains 6% organic Nitrogen and 3% plant growth regulators from *Sargassum* and *A. nodosum* algae (according to the fertilizer product label). The effect of the fertilizer was tested in three concentrations (0.1%, 0.5% and 2.5%), compared to a control treatment with no fertilizer added. Instead of fertilizer solution, distilled water was used in the control (Table 1).

Table 1. Content of nitrogen and growth regulators in the fertilizer and in the prepared solutions (0.1%, 0.5% and 2.5%).

Fertilizer concentration	**Undiluted**	**0.1%**	**0.5%**	**2.5%**	**Control**
N content	6%	0.006%	0.030%	0.150%	0%
Growth regulators content	3%	0.003%	0.015%	0.075%	0%
Volume of solution used per replication of 100 seeds	/	15 ml	15 ml	15 ml	15 ml dH_2O

Content of N and growth regulators is based on product label information.

Seed material from the cultivar San Andrea produced in two latest production years (2017 and 2018) was used.

Methods

The study was carried out during 2019 in the Rice research station of the Institute of Agriculture Skopje, located in the Kochani rice production region. The following parameters were evaluated: germination energy (GE), total germination (TG), germination speed (GS), root length (RL), shoot length (SL) and total seedling length – root + shoot (TSL), dry root weight (DRW), dry shoot weight (DSW) and the total dry seedling weight – root + shoot (TSW), seedling vigor index I (VI–I) and vigor index II (VI–II).

For each treatment (0.1%, 0.5%, 2.5% and control) and seed production year (2017 and 2018) combination (8 variants in total) four replications of 100 seeds were made. The seeds in the control were presoaked in distilled water for 24 hours before testing, while the seeds in the treatments with Storm were treated with 0.1%, 0.5% and 2.5% solution from the fertilizer respectively. Each replication was germinated in Petri dish between filter paper wetted with distilled water. The Petri dishes with seed samples were incubated in a germination cabinet at 25 ^oC. The germinated seeds for each replication were daily calculated after incubation until the end of the trial (day 14). A seed was considered germinated when both the coleoptile and the radicle were emerged, and radicle was minimum 2 mm long. The GE was determined on the fifth day, while the TG on the fourteenth day after incubation. These parameters were calculated as % from the total number of inoculated seeds in the Petri dish (Official gazette of the Republic of Macedonia No 61/2007). The GS was calculated according to Maguire (1962) and AOSA (1983), by calculating the germinated seeds every day from the first day to the last day (day 14) of trial:

GS = Sum (Ni seeds/ Ni days), where i = 1 to 14; Ni seeds = number of seeds germinated on day Ni; Ni days = number of days (1 to 14).

In order to calculate the VI–I and VI–II, seeds from each treatment were germinated in open Petri dish in the germination cabinet along with the replications. The RL and SL of 40 random seedlings in each of the variants developed in dark conditions were measured at the end of the trial (day 14). After the RL and SL were measured, the roots and shoots of the measured plants were separated and grouped by treatments. The root volume of the group of 40 roots in each variant was determined by the actual volume displacement method, which measures the volume of water that is displaced when plant tissue is submerged in a vessel of water (Harrington, Mexal & Fisher, 1994; Novoselov 1960). The average seedling root volume in each variant was determined by the formula:

Root volume = Volume of group of 40 roots (ml)/ 40

The 40 roots and shoots in each variant (treatment-year combination) were dried in air-oven at 100 °C to constant weight. The dry root, shoot and the total seedling dry weight were measured for each variant.

Dry root weight = m_{roots} in each variant/40

Dry shoot weight = m_{shoots} in each variant/40

Dry seedling weight = dry root + dry shoot weight

The seedling vigor was determined on the basis of the vigor indexes (Abdul-Baki & Anderson 1973):

Vigor index I: VI–I = Seedling length (cm) × Germination (%)

Vigor index II: VI–II = Seedling dry weight (mg) × Germination (%)

The results were statistically analyzed by two-way ANOVA with replication (Table 2) and LSD test at 0.05 and 0.01 significance level. Correlation analysis was determined by calculating the Pearson's correlation coefficient. The two way ANOVA was calculated based on the statistical methods for agricultural research provided by Hadživuković (1973).

Table 2. Two-way ANOVA with replication (analysis of variance).

Source	**df**	**SS**	**MS**	**F exp.**	**F crit. ($\alpha_{0.05}$; $\alpha_{0.01}$)**
Replication	dfr	SSR	MSR= SSR/ dfr		
Storm treatment	dft	SST	MST = SST /dft	MST/ MSE	r_1 = dft; r_2 = dfe
Production year	dfy	SSY	MSY = SSY/ dfy	MSY/ MSE	r_1 = dfy; r_2 = dfe
Interaction	dfi	SSI	MSI= SSI/ dfi	MSI/ MSE	r_1 = dfi; r_2 = dfe
Error	dfe	SSE	MSE= SSE/ dfe		
Total	dft	SS_{Total}			

df- degrees of freedom; SS – sum of squares; MS – mean of squares; dfr = n_r – 1; dft = n_t – 1; dfy= n_y – 1; dfi = dft × dfy; dfe = dft − (dfr + dft + dfy + dfi); dft= $n_r \times n_t \times n_y - 1$, where n_r, n_t and n_y are the number of replications, treatments with Storm fertilizer and seed production years respectively.

SSR = Σ (Sum in each replication)²/ ($n_t \times n_y$) – C; SST = Σ (Sum in each Storm treatment)²/ ($n_r \times n_y$) – C;

SSY = Σ (Sum in each year)²/ ($n_r \times n_t$) – C; SSI = SSV – SST – SSY; SSE = SS_{Total} – SSR – SSV;

SS_{Total} = Σ (individual result)² – C; SSV = Σ (Sum in each variant)²/ n_r − C; C = (Σ individual result)²/ ($n_r \times n_t \times n_y$).

Results and Discussion

The two-way ANOVA showed significant effect of the Storm treatment and the production year on most of the examined properties (Table 3). The total germination was not affected by the examined factors, and the interaction was not significant. The germination energy was significantly affected only by the Storm treatment, while the rest of the examined properties were significantly affected by both factors and there was also significant treatment × year interaction.

Table 3. Mean squares of the examined properties.

Source	Replications	Treatments	Year	Interaction	Error
Df	3	3	1	3	21
GE	19.08	99.42*	60.50	5.08	14.58
TG	1.50	10.25	2.00	0.25	5.81
GS	0.55	9.20**	13.46**	3.01*	0.84
RSL	0.17	6.33**	10.89**	3.52**	0.25
SSL	1.44	22.02**	1.45**	1.52**	0.17
SL	2.49	14.25**	4.40*	2.68*	0.62
SVI–I	351.65	128846.68**	30613.28**	23574.58**	1574.72
SVI–II	184755.42	85836383.58**	61966278.13**	22733445.54**	756415.82

*significant at 0.05 level of probability, ** significant effect at 0.1 level of probability

The Storm treatments had no significant effect on the standard germination test (the total germination). The TG ranged from 94.38% in the 0.1% and 2.5% treatments to 96.75 in the 0.5% treatment. The germination energy was affected only by the 2.5% treatment which significantly decreased the GE (83.00%) compared to the control (90.63%).

Table 4. Germination energy and total germination.

Germination energy (%)					
	0.1%	**0.5%**	**2.5%**	**Control**	**AVG**
2017	90.50 ± 3.70	90.50 ± 3.11	85.00 ± 2.58	92.00 ± 4.08	89.50
2018	86.75 ± 7.41	90.00 ± 1.83	81.00 ± 3.74	89.25 ± 1.50	86.75
AVG	**88.63b**	**90.25b**	**83.00 a**	**90.63b**	
LSD	5.62 ($\alpha_{0.05}$); 7.64 ($\alpha_{0.01}$)				
Total germination (%)					
	0.1%	**0.5%**	**2.5%**	**Control**	**AVG**
2017	94.75 ± 2.06	96.75 ± 1.71	94.75 ± 2.22	95.75 ± 1.89	95.50
2018	94.00 ± 4.24	96.75 ± 2.06	94.00 ± 1.41	95.25 ± 1.50	95.00
AVG	**94.38 a**	**96.75 a**	**94.38 a**	**95.50 a**	
LSD	3.55 ($\alpha_{0.05}$); 4.82($\alpha_{0.01}$)				

The germination speed was highest in the control (23.94, Table 5). With the increase of Storm concentration the GS decreased and was significantly affected by the highest concentration (2.5%), which decreased the GS down to 21.53. This results are in accordance with reports from other researchers which showed that lower concentrations of the *Sargassum* and *Ascophylum nodusum* extracts are more efficient in stimulating germination, while higher concentration had inhibitory effect (Hidangmayum & Sharma, 2017; Kalaivanan & Venkatesalu, 2012; Kumar et al., 2012).

Table 5. Germination speed.

	0.1%	**0.5%**	**2.5%**	**Control**	**AVG**
2017	24.46 ± 1.15	22.69 ± 1.19	22.30 ± 0.13	25.15 ± 1.22	**23.65**
2018	22.78 ± 1.34	23.14 ± 0.43	20.75 ± 0.18	22.73 ± 0.40	**22.35**
AVG	**23.62b**	**22.91b**	**21.53a**	**23.94b**	
LSD	1.35 ($\alpha_{0.05}$); 1.83($\alpha_{0.01}$)				

All Storm treatments resulted in significantly higher vigour index I and vigour index II compared to the control, where the lowest VI–I (1362.07) and VI–II (749.61) were determined. The highest VI–I (1669.44) and VI–II (943.84) were determined in the 2.5% treatment (Table 6).

Table 6. Seedling vigor index I and seedling vigor index II.

Seedling VI–I					
	0.1%	**0.5%**	**2.5%**	**Control**	**AVG**
2017	1606.01 ± 34.94	1657.33 ± 29.26	1533.06 ± 35.88	1275.39 ± 25.21	1517.95
2018	1568.86 ± 70.81	1632.17 ± 34.78	1669.44 ± 25.12	1448.75 ± 22.82	1579.81
AVG	**1587.44b**	**1644.75b**	**1601.25b**	**1362.07a**	
LSD	58.36 ($\alpha_{0.05}$); 79.44 ($\alpha_{0.01}$)				
Seedling VI–II					
	0.1%	**0.5%**	**2.5%**	**Control**	**AVG**
2017	950.34 ± 20.68	948.15 ± 16.74	971.19 ± 22.73	727.70 ± 14.39	899.35
2018	815.92 ± 36.83	815.60 ± 17.38	916.50 ± 13.79	771.53 ± 12.15	829.89
AVG	**883.13b**	**881.88b**	**943.84c**	**749.61a**	
LSD	31.99 ($\alpha_{0.05}$); 43.54 ($\alpha_{0.01}$)				

The total seedling length (root + shoot) of 14 days old seedlings was significantly affected (increased) by the Storm treatments compared to the control (14.26 mm, Table 7). The highest average was determined in the 0.5% treatment (17.00 mm). The differences in seedling length among the Storm concentrations were not significant, yet the different concentrations differently affected the root and the shoot development. The control had the shortest stem (7.25 mm) and a root length of 7.01 mm. The

0.1% treatment positively affected both the root and shoot development, resulting in a significantly longer root and shoot compared to the control. This treatment had to longest root (7.98 mm). The 0.5% treatment significantly increased the shoot length but did not affected the root length. The 2.5% treatment significantly increased the shoot length and significantly decreased the root length, resulting in the longest shoot (11.16 mm) and the shortest root (5.82 mm). Similarly, when treated with *Sargassum* extract, as concentration increased, a decline in root length and number of lateral roots among other parameters in wheat *Triticum aestivum* (Kumar & Sahoo, 2011) and decline in rooting of *Vigna mundo* seedlings (Kumari et al. 2011) were observed. According to Anisimov, Chaikina, Klykov & Rasskazov (2013), who studied the effect of different algal extracts including *Sargassum* sp., all algal extracts in concentration higher than 10^{-3} gSW mL^{-1} inhibited the development of buckwheat *Fagopyrum esculentum* seedling roots. Similarly, aqueous extracts of most algae had an inhibitory effect on the growth of seedling roots of soybean in concentrations 10^{-2} gSW mL^{-1} (Anisimov & Chaikina, 2014). In a study conducted by Hidangmayum & Sharma (2017), the 5500 ppm concentration of *Ascophylum nodusum* extract resulted in longer roots and shoots of onion (*Allium cepa*) seedlings, as compared to higher concentrations of 6500 ppm and 7500 ppm. Similarly, in a study evaluating the effect of *A. nodosum* extract on the growth of watermelon plants (Júnior et al., 2019), the 3 and 4 mL/L doses were more efficient in the watermelon seedlings production compared the highest dose of 5 mL/L.

Table 7. Root. shoot and total seedling length.

Root seedling length (cm)					
	0.1%	**0.5%**	**2.5%**	**Control**	**AVG**
2017	7.39 ± 0.36	7.32 ± 0.50	5.19 ± 0.23	5.64 ± 0.46	6.39
2018	8.58 ± 0.88	6.81 ± 0.27	6.44 ± 0.41	8.38 ± 0.52	7.55
AVG	**7.98c**	**7.06b**	**5.82a**	**7.01b**	
LSD	0.73 ($\alpha_{0.05}$); 1.00($\alpha_{0.01}$)				
Shoot seedling length (cm)					
	0.1%	**0.5%**	**2.5%**	**Control**	**AVG**
2017	9.56 ± 0.90	9.81 ± 0.41	10.99 ± 0.20	7.68 ± 0.41	9.51
2018	8.11 ± 1.05	10.06 ± 0.36	11.33 ± 0.43	6.83 ± 0.25	9.08
AVG	**8.83b**	**9.94c**	**11.16d**	**7.25a**	
LSD	0.61 ($\alpha_{0.05}$); 0.83($\alpha_{0.01}$)				
Total seedling length (cm)					
	0.1%	**0.5%**	**2.5%**	**Control**	**AVG**
2017	16.95 ± 1.11	17.13 ± 0.70	16.18 ± 0.36	13.32 ± 0.69	15.89
2018	16.69 ± 1.76	16.87 ± 0.61	17.76 ± 0.76	15.21 ± 0.68	16.63
AVG	**16.82b**	**17.00b**	**16.97b**	**14.26a**	
LSD	1.16 ($\alpha_{0.05}$); 1.58($\alpha_{0.01}$)				

Similar effect of Storm fertilizer concentrations was observed regarding the seedling dry weight (Figure 1) and the root volume (Figure 2). The 0.1% treatment had the highest root dry weight (3.89 mg) and the highest root volume (50 μL). The 2.5% treatment had the highest shoot dry weight (7.16 mg) and total seedling weight (10.00 mg), the lowest root dry weight (2.84 mg) and the lowest root volume (31.25 μL).

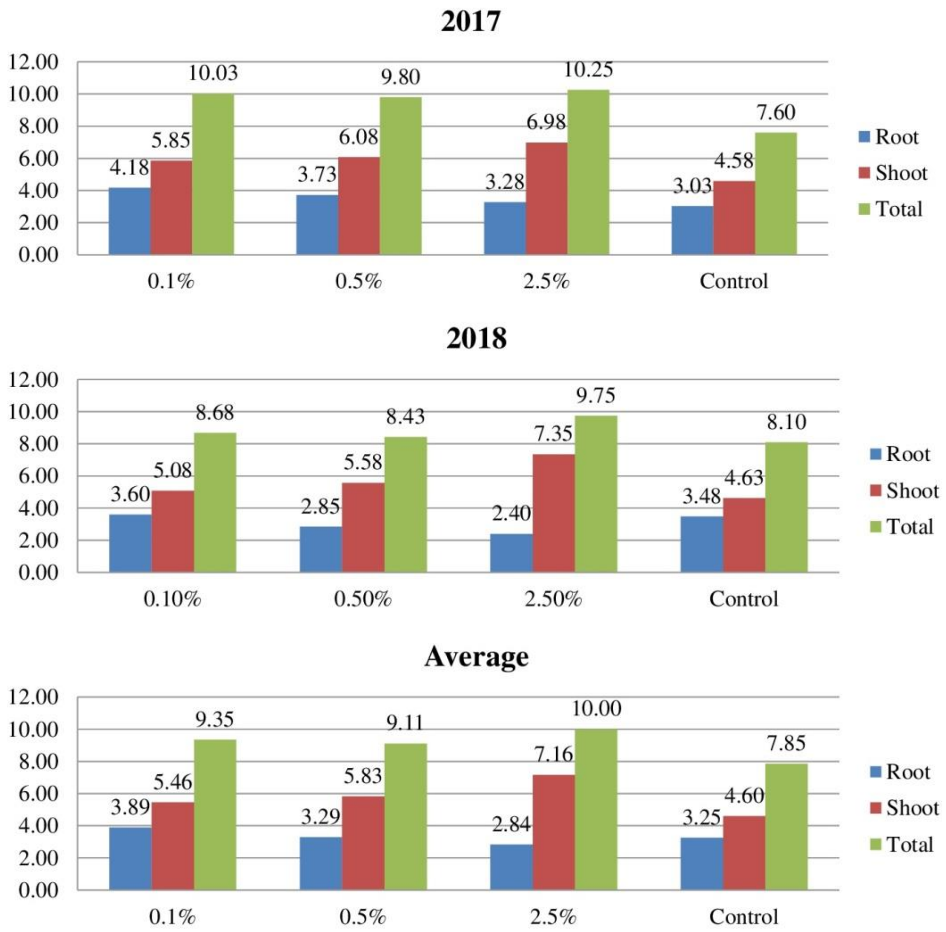

Figure 1. Root, shoot and total seedling dry weight (mg).

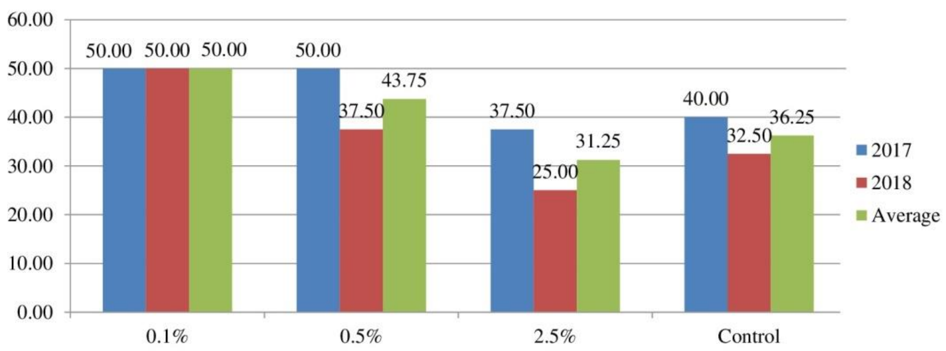

Figure 2. Root volume (μL).

Conclusion

The Storm treatments in this study had no significant effect on the standard (total) germination test, but they did affect the other germination parameters – the germination energy, germination speed, as well as the early rice seedling development (14 days old seedlings) in controlled laboratory conditions. Based on the obtained results, the recommended concentration is 0.1%, as this treatment significantly positively stimulated the seedling development, including both root and shoot. Although the highest concentration (2.5%) resulted in the highest seedling vigor indexes, it is not recommended as it disbalanced the seedling growth by inhibiting the root and over stimulating the shoot development. Additionally, this concentration significantly decreased the germination energy and the germination speed.

In the recommended dosage the Storm fertilizer could be used for optimization of the development of rice seedlings, at least in controlled conditions, such as seedlings production.

Additional Declaration

Research and publication ethics principles were comply with in this study. Authors contributed equally to the study.

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