

**Effects of a Bio-Stimulant and Salinity Stress on Growth
and Quality of Ryegrass (*Lolium preenne* L.), an Urban Desert
Landscape and Forage Crop, for Sustainable Agriculture in Arid Regions**

Mohammad Pessarakli

School of Plant Sciences, Forbes Bldg., Room 303, College of Agriculture
and Life Sciences, The University of Arizona, Tucson, AZ 85721, USA

Abstract: Perennial ryegrass (*Lolium perenne* L.) cultivar Covet was studied hydroponically in a greenhouse to evaluate its growth responses in terms of shoot and root lengths, shoot fresh and dry weights and grass quality under EC = 10 and 15 dSm⁻¹ sodium chloride (NaCl) stress at various Bio-stimulant application rates. Results showed shoot lengths and weights of ryegrasses were stimulated under all levels of Bio-stimulant applications at the lower (10 dSm⁻¹) salinity level. However, at the higher (15 dSm⁻¹) salinity level, the beneficial effect of the Bio-stimulant was inconsistent. There was no difference on the effect of the compound between ½ the recommended rate and the recommended rate on the shoot lengths and weights of ryegrass. Doubling recommended rate of the product resulted in insignificant increase in shoot length compared to the untreated plants. Bio-stimulant had a significant effect on enhancing root lengths and weights at the lower level of salinity, but did not have any beneficial effect on the root length of the grasses under the higher salinity level. Grass quality followed essentially the same pattern as the shoot dry weights, it was more beneficially affected under ½ the recommended rate or the recommended rate of the Bio-stimulant with no difference under these two application rates. These application rates of the product significantly improved the grass quality. Doubling the recommended rate of the product did not have any significant beneficial effect on grass quality, especially at the higher salinity level. Based on the results of this study, the Bio-stimulant generally exhibited a significant beneficial effect on ryegrass growth under salinity stress. Therefore, application of the Bio-stimulant that resulted in increasing survival and salt tolerance of this grass species will make the grass more suitable as a sustainable cover grass for desert regions with high soil salinity and saline water resources.

Key words: Ryegrass • Salinity stress • Bio-stimulant • Shoot and root growth • Grass quality

INTRODUCTION

Proper types and adequate rates of chemicals and fertilizer applications are critical issues in agricultural practices, particularly considering the general public's growing concern on the interactive effects of any chemicals and the environment in the USA and other countries. There are numerous new chemicals, fertilizers and growth stimulants continuously manufactured and used in agriculture with very limited or no tests done prior to their release for broad applications. Proper scientific tests on these products before their release for general use results in at least two major advantages as follows:

- Better results on plant growth by using proper type and optimum quantity of the products (lower quantities will not result in optimum growth and higher quantities will be harmful to the plants and waste of the chemicals/fertilizers and unnecessary economic loss).
- Prevent any environmental issues and problems related to the chemicals and fertilizers use (i.e., ground water contamination with nitrate due to over application of nitrogenous substances).

With the new policies and regulations regarding the chemicals and fertilizers use in agriculture, the researchers and the scientists in agriculture are constantly looking into strategies that lead to

optimum or minimum chemicals and fertilizers use by plants, yet no adverse effects on the plant growth and development.

Abiotic environmental (i.e., salinity, drought and heat) stresses are also major concerns in agriculture and any cultural practices. Among the environmental stresses, salinity stress is one of the major issues in agriculture and crop production and almost nowhere the plants/grasses are immune to the adverse effects of salinity. Therefore, to find the optimum application rate of any new product, the product must be tested on plants under various salinity stress levels. To find the optimum application rate of the products on the most tolerant plant species/cultivars to salinity stress and their uses under such conditions would probably be one of the most logical and effective solutions of the salinity stress problems. Researchers are continuously working on finding the most tolerant plants/crops [1-5] and turfgrasses species/cultivars and other crops [6-28] to environment (i.e., salinity and drought) stresses. Using proper types of plant species/cultivars and proper rate of the products for cultural practices under such conditions will prevent unexpected surprises and unwanted results. This is a critical issue in agricultural practices, particularly considering the agricultural practitioners growing concerns on the adverse effects of any stress on plants/grasses growth and development as well as the concerns of the general public in the improper use of the chemicals (i.e., Bio-stimulants) on the environment.

The agricultural investigators, particularly those involved in plant nutrition and fertilizer use efficiency of plants and plant/grass stress tolerances usually consider the above issues in the initiation and conducting of their research work.

Ryegrass is a major turf and landscaping species used in cool climates and as an over seeding turfgrass in warm climates as well as a major pasture and rangeland plant species for animal feed in both climates. There is a wide range of salt tolerance among various cultivars of ryegrass. My extensive salinity stress tolerance research on various cultivars of ryegrass at the University of Arizona found the salt tolerance of this species ranging between 6 to 20 dSm⁻¹.

The objectives of this study were to compare growth responses in terms of shoot and root lengths and weights as well as general quality of ryegrass, a common turfgrass/landscaping and rangeland/pasture grass species grown under different salinity stress levels and at various application rates of Encapsalt Bio-stimulant and recommend the most beneficial application rate of the Bio-stimulant for cultural practices of this grass and the similar grass species.

MATERIALS AND METHODS

Perennial ryegrass (*Lolium perenne* L.), variety Covet was studied hydroponically in a greenhouse to evaluate its growth responses in terms of shoot and root lengths, shoot fresh and dry matter (DM) weights and grass general quality under two levels of sodium chloride (NaCl) salinity stress (10 and 15 dSm⁻¹) at various (0, 32, 64, 128 ml/100m²) Encapsalt Bio-stimulant application rates.

The grasses were grown from seeds in cups, 9 cm diameter and 7 cm height. Silica sand was used as the plant anchor medium. Each cup was fitted into one of the 9 cm diameter holes cut in a rectangular plywood sheet 46 cm (length) X 37 cm (width) X 2 cm (thickness). The plywood sheets served as lids for the hydroponics tubs and supported the cups above the solution to allow for root growth. The lids were placed on 42 cm (length) X 34 cm (width) X 12 cm (depth) Carb-X polyethylene tubs, containing half strength Hoagland solution No.1 [29].

Prior to the salinity treatments and the Bio-stimulant applications, the grasses were grown in this nutrient solution for 60 days. During this period, the grass shoots were harvested weekly in order to allow the grass to reach full maturity and develop uniform and equal size plants. The harvested plant materials (clippings) were discarded. The culture solutions were changed bi-weekly to ensure adequate amount of plant essential nutrient elements for normal growth and development. After 60 days growing in this nutrient solution, the salinity treatments were started by adding NaCl to the culture solution equivalent to EC of 2 and 3 dSm⁻¹ per day to reach the desired salinity levels of EC 10 and 15 dSm⁻¹, respectively. Treatments included control (no Bio-stimulant) and NaCl at 2 salinity levels (EC = 10 and 15 dSm⁻¹). Four Bio-stimulant levels (0, 32, 64 and 128 ml/100 m⁻²) were used at each salinity level. A randomized complete block design (RCBD) with 4 replications was used in this study. The culture solution levels in the tubs were marked at the 10 liter volume level and maintained at this level by adding distilled water and adjusted the salinity levels of the culture solutions as needed. During this period, also, the culture solutions were changed bi-weekly to maintain the desired plant nutrient levels and the respected salinity levels as well as the Bio-stimulant application rates were used.

The grass shoots were harvested weekly for the evaluation of the dry matter (DM) production. At each weekly harvest, shoot and root lengths were measured and recorded. The grass canopy general quality was also evaluated every other day or weekly at each salinity stress level and at each Bio-stimulant application rate. The harvested plant materials were

weighed (fresh weight), then oven-dried at 65°C and DM weights measured and recorded. The recorded data were considered the weekly plant DM production. At the termination of the experiment, the last harvest, plant roots were also harvested, fresh weights recorded and then oven dried at 65°C and DM weights determined and recorded.

The data were subjected to Analysis of Variance (ANOVA), using SAS statistical package [30]. The means were separated, using Duncan Multiple Range test.

RESULTS AND DISCUSSION

Shoot and Root Lengths: The shoot growth (length) of ryegrass was stimulated under any level of the Bio-stimulant application rate at the lower (10 dSm⁻¹) level of salinity. However, at the higher level of salinity (15 dSm⁻¹), the beneficial effects of the Bio-stimulant was inconsistent. Pessaraki and Dehghani Bidgoli [17] found similar results on the effects of this Bio-stimulant on another salt tolerance cultivar (Galileo) of ryegrass under lower levels of salinity stress (8 and 12 dSm⁻¹). The effect of the Bio-stimulant on shoot length was shown from the first harvest at all application rates, including the lowest (½ the recommended rate of the Bio-stimulant, 32 ml Encapsalt/100 m² that is equal to 1 oz Encapsalt/1000 ft²). There was no difference on the effect of the compound between ½ the recommended rate and the recommended rate (64 ml Encapsalt/100 m² that is equal to 2 oz/1000 ft²) treatments on the shoot growth of ryegrass (Table 1). Doubling the recommended rate (128 ml Encapsalt/100 m² that is equal to 4 oz/1000 ft²) of the product also resulted in some increase in shoot growth compared to the untreated control plants. However, the effect of doubling the product rate was less than the ½ or the recommended application rates of the Bio-stimulant. Therefore, under high saline (soil or water) conditions, the lower or the recommended application rates of the product are recommended. Under such conditions, although the higher application rates of the product stimulates the plant growth, but economically may not be beneficial, therefore not recommended.

The Bio-stimulant had a significant effect on enhancing root length at the lower level of salinity (10 dSm⁻¹), but did not have any beneficial effect on the root length of the grasses under the higher level (15 dSm⁻¹) of salinity stress (Table 1). In fact, at the high (15 dSm⁻¹) of salinity stress, the root length decreased at the beginning of the stress period under the Bio-stimulant application at all application rates. As the stress period

progressed at this level of salinity (15 dSm⁻¹), doubling the recommended rate of the product (128 ml Encapsalt/100 m² that is equal to 4 oz/1000 ft²) either reduced the root length or did not have any effect on it compared to the untreated control plants. However, the effect of the product on the root length of ½ the recommended rate (32 ml Encapsalt/100 m² that is equal to 1 oz/1000 ft²) and the recommended rate (64 ml Encapsalt/100 m² that is equal to 2 oz/1000 ft²) treated plants were significantly higher than that of the untreated control plants (Table 1).

Shoot Fresh and Dry Weights: The shoot fresh and dry weights of the plants were better indicators in showing the beneficial effects of the Bio-stimulant. For both fresh and dry weights of the shoots, ryegrass was more beneficially affected under the ½ the recommended rate (32 ml Encapsalt/100 m² that is equal to 1 oz/1000 ft²) and the recommended rate (64 ml Encapsalt/100 m² that is equal to 2 oz/1000 ft²) of the product with no difference under these two application rates (Table 2). These Bio-stimulant application rates increased shoot fresh and dry weights more at the lower (10 dSm⁻¹) level of salinity stress. However, at the higher (15 dSm⁻¹) level of salinity, doubling the product rate had less effect in enhancing shoot growth in terms of fresh and dry weights compared to the other application rates of the product. In some cases, at this higher level of salinity (15 dSm⁻¹), there was no significant difference between this application rate (doubling the product, 128 ml Encapsalt/100 m² that is equal to 4 oz/1000 ft²) of the Bio-stimulant and the untreated control plants (Table 2).

There was a linear reduction found in shoot fresh and dry weights as exposure time to salinity and Bio-stimulant progressed (Table 2). The degree of reduction in the plants fresh weights was more than that of the dry weights. Also, this reduction was more pronounced under the EC 15 compared to that of EC 10 dSm⁻¹ (Table 2).

Root Fresh and Dry Weights: Bio-stimulant at any application rate did not show any significant effect on root growth in terms of root fresh and dry weights at the high (15 dSm⁻¹) level of salt stress (Table 3). However, at the lower (10 dSm⁻¹) level of salinity stress, there were some significant enhancement effects of the product on the root fresh weight at both ½ the recommended rate (32 ml Bio-stimulant/100 m² that is equal to 1 oz/1000 ft²) and the recommended rate (64 ml Bio-stimulant/100 m² that is equal to 2 oz/1000 ft²) of the product (Table 3). This enhancement on the root dry weight was not

Table 1: Ryegrass shoot and root lengths influenced by Bio-stimulant under salinity stress conditions

Salinity (dSm ⁻¹)	Bio-stimulant* (ml/100 m ²)	Treatments				Harvest			
		1		2		3		4	
		Shoot**	Root**	Shoot**	Root**	Shoot**	Root**	Shoot**	Root**
------(cm)***-----									
10	0	2.86b	1.90d	1.72ab	1.92d	3.85c	5.22e	3.32a	5.86d
10	32	2.84b	4.64a	1.95a	4.70a	4.52b	12.78a	3.64a	14.26a
10	64	3.48a	4.24a	1.98a	4.60a	5.65a	13.42a	3.78a	15.00a
10	128	3.46a	2.70c	1.77a	3.06b	4.10bc	10.90b	2.88b	13.80a
15	0	2.70bc	3.54b	1.32b	3.55b	3.67cd	9.38c	2.50b	10.18b
15	32	2.46c	1.80d	1.38b	2.50c	3.82c	10.33b	2.40b	13.08a
15	64	2.98b	3.10c	1.48b	3.30b	4.21bc	8.46d	3.30a	10.08b
15	128	2.44c	2.14d	1.30b	2.66c	3.28d	7.89d	2.48b	8.12c

*Bio-stimulant application rates (0, no Bio-stimulant; 32, ½ the recommended rate; 64, recommended rate; 128, double the recommended rate).

**The values are the averages of 4 replications of each treatment.

***The values followed by the same letters in each column are not statistically different at the 0.05 probability level.

Table 1(Cont.): Ryegrass shoot and root lengths influenced by Bio-stimulant under salinity stress conditions

Salinity (dSm ⁻¹)	Bio-stimulant* (ml/100 m ²)	Treatments				Harvest			
		5		6		7		8	
		Shoot**	Root**	Shoot**	Root**	Shoot**	Root**	Shoot**	Root**
------(cm)***-----									
10	0	2.80a	10.56d	2.48ab	12.50d	1.98b	16.16c	1.58a	16.90d
10	32	2.64a	16.20b	2.76a	20.24b	2.18b	21.00b	1.70a	22.56b
10	64	3.08a	21.50a	2.90a	25.40a	2.66a	25.80a	1.94a	26.16a
10	128	2.68a	16.54b	2.42ab	17.30c	2.13b	17.58c	1.76a	18.56c
15	0	2.14b	13.10c	1.86c	13.54d	1.56c	13.66d	1.18b	14.22e
15	32	2.00b	15.30b	2.26b	20.30b	2.20b	20.40b	1.44ab	20.60b
15	64	2.18b	13.10c	2.24b	13.40d	2.14b	14.22d	1.78a	14.30e
15	128	2.52ab	11.80d	2.30b	13.00d	1.94b	13.66d	1.66a	13.80e

*Bio-stimulant application rates (0, no Bio-stimulant; 32, ½ the recommended rate; 64, recommended rate; 128, double the recommended rate).

**The values are the averages of 4 replications of each treatment.

***The values followed by the same letters in each column are not statistically different at the 0.05 probability level.

Table 2: Ryegrass shoots fresh and dry weights influenced by Bio-stimulant under salinity stress conditions

Salinity (dSm ⁻¹)	Bio-stimulant* (ml/100 m ²)	Treatments				Harvest			
		1		2		3		4	
		Fresh**	Dry**	Fresh**	Dry**	Fresh**	Dry**	Fresh**	Dry**
------(g)***-----									
10	0	0.372c	0.156b	0.402b	0.157ab	0.423b	0.233b	0.452c	0.181b
10	32	0.494b	0.181a	0.400b	0.155ab	0.625a	0.298a	0.684b	0.243a
10	64	0.624a	0.184a	0.438a	0.179a	0.656a	0.288a	0.824a	0.260a
10	128	0.506b	0.142b	0.326c	0.137b	0.386c	0.178c	0.342d	0.087d
15	0	0.352c	0.112c	0.300c	0.107c	0.266d	0.173c	0.298d	0.134c
15	32	0.500b	0.147b	0.255d	0.095c	0.499b	0.247b	0.412c	0.198b
15	64	0.472b	0.133b	0.303c	0.109c	0.496b	0.221b	0.504c	0.183b
15	128	0.328c	0.100c	0.262d	0.102c	0.268d	0.141c	0.414c	0.149c

*Bio-stimulant application rates (0, no Bio-stimulant; 32, ½ the recommended rate; 64, recommended rate; 128, double the recommended rate).

**The values are the averages of 4 replications of each treatment.

***The values followed by the same letters in each column are not statistically different at the 0.05 probability level.

Table 2 (Cont.): Ryegrass shoot fresh and dry weights influenced by Bio-stimulant under salinity stress conditions

Salinity (dSm ⁻¹)	Bio-stimulant* (ml/100 m ²)	Treatments				Harvest			
		5		6		7		8.	
		Fresh**	Dry**	Fresh**	Dry**	Fresh**	Dry**	Fresh**	Dry**
		(g)***							
10	0	0.588c	0.142b	0.532c	0.166a	0.340d	0.122bc	0.254bc	0.102a
10	32	0.702b	0.174a	0.660b	0.178a	0.404c	0.133b	0.356a	0.120a
10	64	0.806a	0.182a	0.682b	0.178a	0.566a	0.171a	0.360a	0.114a
10	128	0.522c	0.121bc	0.734a	0.190a	0.476b	0.142b	0.378a	0.113a
15	0	0.244f	0.058d	0.310de	0.092b	0.112f	0.039d	0.110d	0.031d
15	32	0.424d	0.108c	0.336d	0.114b	0.294de	0.134b	0.256bc	0.071b
15	64	0.438d	0.109c	0.340d	0.100b	0.280de	0.121bc	0.280b	0.081b
15	128	0.356e	0.082cd	0.288e	0.088b	0.248e	0.103c	0.232c	0.051c

*Bio-stimulant application rates (0, no Bio-stimulant; 32, ½ the recommended rate; 64, recommended rate; 128, double the recommended rate).

**The values are the averages of 4 replications of each treatment.

***The values followed by the same letters in each column are not statistically different at the 0.05 probability level.

Table 3: Ryegrass root fresh and dry weights influenced by Bio-stimulant under salinity stress conditions

Salinity (dSm ⁻¹)	Bio-stimulant* (ml/100 m ²)	Harvest 8 (last harvest), Root Weight**	
		Fresh	Dry
		(g)***	
10	0	0.406b	0.041a
10	32	0.498a	0.049a
10	64	0.462a	0.047a
10	128	0.408b	0.041a
15	0	0.201c	0.031c
15	32	0.184c	0.033c
15	64	0.190c	0.032c
15	128	0.188c	0.030c

*Bio-stimulant application rates (0, no Bio-stimulant; 32, ½ the recommended rate; 64, recommended rate; 128, double the recommended rate).

**The values are the averages of 4 replications of each treatment.

***The values followed by the same letters in each column are not statistically different at the 0.05 probability level.

Table 4: Ryegrass general quality scores affected by different salinity stress levels and various Bio-stimulant application rates

Salinity (dSm ⁻¹)	Bio-stimulant* (ml/100 m ²)	Observation Dates**								
		11/01	11/04	11/08	11/12	11/16	11/20	11/24	11/28	12/02
10	0	10	9	8.5	8	7.5	7	6.5	6	6
10	32	10	10	9	8.5	8	8	7.5	7	7
10	64	10	10	9	8.5	8	8	7.5	7	7
10	128	10	10	8.5	8	7.5	7	6.5	6	6
15	0	10	9	8	7	6.5	6	6	6	5.5
15	32	10	10	8.5	8	7.5	7	7	6.5	6
15	64	10	10	8.5	8	7.5	7	7	6.5	6
15	128	10	9	7.5	7	6.5	6	6	5.5	5.5

*Bio-stimulant application rates (0, no Bio-stimulant; 32, ½ the recommended rate; 64, recommended rate; 128, double the recommended rate).

**The values are the averages of 4 replications of each treatment.

***The values followed by the same letters in each column are not statistically different at the 0.05 probability level.

Table 4 (Cont.): Ryegrass general quality scores affected by different salinity stress levels and various Bio-stimulant application rates

Salinity (dSm ⁻¹)	Bio-stimulant* (ml/100 m ²)	Observation Dates**						
		12/05	12/08	12/12	12/16	12/20	12/24	12/28
10	0	6	5.5	5.5	5.5	5	5	4.5
10	32	7	6.5	6.5	6.5	6	6	5.5
10	64	7	6.5	6.5	6.5	6	6	5.5
10	128	6	5.5	5.5	5.5	5	5	4.5
15	0	5	5	5	5	4.5	4.5	4
15	32	6	5.5	5.5	5.5	5	5	5
15	64	6	5.5	5.5	5.5	5	5	5
15	128	5.5	5	5	5	4.5	4.5	4

*Bio-stimulant application rates (0, no Bio-stimulant; 32, ½ the recommended rate; 64, recommended rate; 128, double the recommended rate).

**The values are the averages of 4 replications of each treatment.

***The values followed by the same letters in each column are not statistically different at the 0.05 probability level.

statistically significant. At this level of salinity (10 dS m⁻¹), there was no difference in root fresh and dry weights at the highest application rate (doubling the product, 128 ml Bio-stimulant/100 m² that is equal to 4 oz/1000 ft²) of Bio-stimulant compared with the untreated control plants (Table 3).

Grass General Quality: The grass general quality followed essentially the same pattern as the shoot dry weights. Ryegrass general quality was more beneficially affected under the ½ the recommended rate (32 ml Bio-stimulant/100 m² that is equal to 1 oz/1000 ft²) or the recommended rate (64 ml Bio-stimulant/100 m² that is equal to 2 oz/1000 ft²) of the product with no difference under these two application rates (Table 4). These application rates of the product improved the quality scores of the grasses with score of 1 (scores of 4.5 and 4 to scores of 5.5 and 5, respectively, Table 4). Doubling the recommended application rate of the product (128 ml Bio-stimulant/100 m² that is equal to 4 oz/1000 ft²) did not have any significant beneficial effect on grass quality, especially at the higher (15 dSm⁻¹) level of salinity stress (Table 4).

Overall, since all the studied parameters showed a positive growth response under salt stress by this grass due to the Bio-stimulant application, it can be concluded that this compound is beneficial to this grass and enhances its growth and development and improves the grass quality under salt stress conditions.

CONCLUSIONS

Shoot lengths and weights of ryegrass were stimulated under any level of the Bio-stimulant application rate at the lower (10 dSm⁻¹) level of salinity. However, at the higher level of salinity (15 dSm⁻¹), the beneficial effects of the Bio-stimulant was inconsistent. There was

no difference on the effect of the compound between the ½ the recommended rate and the recommended rate treatments on the shoot lengths and weights of ryegrass. Doubling the recommended rate of the product resulted in insignificant increase in shoot length compared to the untreated plants. The Bio-stimulant had a significant effect on enhancing root lengths and weights at the lower (10 dSm⁻¹) level of salinity, but did not have any beneficial effect on the root length of the grasses under the high (15 dSm⁻¹) level of salinity stress. The grass general quality followed essentially the same pattern as the shoot dry weights. Ryegrass general quality was more beneficially affected under the ½ the recommended rate and the recommended rate of the Encapsalt with no difference under these two application rates. These application rates of the product changed the unacceptable quality scores of 5 and 4.5 to acceptable scores of 6.5 and 5.5, respectively. Doubling the recommended application rate of the product did not have any significant beneficial effect on grass quality, especially at the higher level of salinity. Therefore, under high saline (soil or water) conditions, the lower or the recommended application rates of the product are recommended. Under such conditions, although the higher application rates of the product stimulates the plant growth, but economically may not be beneficial, therefore not recommended. Based on the results of this study, Encapsalt bio-stimulant generally exhibited a significant beneficial effect on ryegrass growth under salinity stress condition.

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