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EFFECT OF GLYCINE BETAINE, CHITOSAN AND SALICYLIC ACID ON PEPPER PLANTS UNDER SALT WATER IRRIGATION CONDITIONS

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Two field experiments were conducted at Wadi El-Natrun, Beheira governorate, Egypt in 2020 and 2021 summer seasons on sweet pepper plants hyprid 1515. The study aims to investigate the effect of glycine betaine, chitosan and salicylic acid as foliar spray at concentrations of 2, 3 and 1 g/l, respectively to ameliorate the adverse effects of salt stress. The results demonstrated that all applied treatments greatly improved all growth parameters, including plant height, leaf area, fresh and dry weight, and mineral composition (nitrogen, phosphorus and potassium) and significantly reduced the impacts of salinity stress. Additionally, foliar spraying with glycine betaine, chitosan, and salicylic acid in this study increased total yield and average fruit weight compared to the control. Glycine betaine therapy outperformed all other treatments in terms of its favourable impact on plant growth, mineral content, average fruit weight, and overall yield. Chitosan treatment came in second. The salicylic acid therapy was shown to have the least beneficial effect. Contrarily, the control group of sweet pepper plants, which received no treatment, had the lowest values of the previous features.

Keywords: sweet pepper, foliar spraying, salinity stress, production, Egypt

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) is known as a favorite and widespread vegetable crop over the world, its fruit is rich in antioxidants, vitamins and minerals for human diet and health (Mateos et al., 2003). Sweet pepper is one of the most significant vegetable crops, belongs to the Solanaceae family, and has a high importance in the exporting market. About 14% of sweet pepper fruit loss is attributed to increased salt levels (Yilmaz et al., 2004). Salt in soil or water is one of the most significant abiotic stresses; as a result, it is estimated that more than 6% of the world's land area is under salinity stress. The main contributors to the salinity issue in agricultural areas include improper soil and water management, poor rainfall, and high evaporation (Arzani, 2008). It is well known that pepper plants are classified sensitive to moderately sensitive to salt stress (Lee, 2006), witch severely inhibits plant growth and yield (Paridam and Das, 2005 and Abdelaal et al., 2020) due to both a salt-specific or ion excess effect of NaCl as well as an osmotic or water-deficit effect of salinity. As a result of oxidative damage to lipids, proteins, and nucleic acids, plants in salinity stress circumstances release cytotoxic activated oxygen that can adversely impair normal metabolism (Abbaspour, 2012). Plants use salicylic acid as a signaling or messenger molecule to help them tolerate a variety of biotic and abiotic stresses (Horvath et al., 2007). Glycine betaine is frequently accumulated during salt stress and is crucial for plants to reset their osmotic balance (Szabados and Savouré, 2010).

A polysaccharide known as 2-amino-2-deoxybeta-D-glucosasmine makes up chitosan (Peniston and Johnson, 1980). A plant's defensive mechanism is sparked by the chitosan molecule, creating chemical and physical barriers to keep invaders out. A naturally occurring polymer created when chitin is deacetylated is chitosan. Chitin is easily obtained from shellfish waste generated during food processing. Chitosan has been used in agriculture to coat seeds, leaves, fruits, and vegetables (Devlieghere et al., 2004), as a fertilizer and in controlled agrochemical release (Sukwattanasinitt et al., 2001), to boost plant product (Wanichpongpan et al., 2001) and to stimulate plant immunity (Hadwiger et al., 2002), to safeguard plants from pathogens (Struszczyk and Pospieszny, 1997), and to promote plant development. In the most recent investigations, chitosan was found to have an advantageous impact on the development of roots, shoots, and leaves in a variety of plants, including gerbera (Wanichpongpan et al., 2001). In tomato studies, the addition of chitosan led to yield gains of about 20% (Walker et al., 2004).

From all the above, it becomes clear to us the role of chitosan in increasing vegetative growth and yield, and therefore, plants treated with chitosan are stronger than others untreated, and therefore they can withstand various stresses, especially salinity.

This work is aiming to study the foliar spray of glycine betaine, chitosan and salicylic acid to ameliorate the stressful effects of salinity stress on sweet pepper plants in sandy soil under drip irrigation system, under the conditions of Wadi El-Natrun, Beheira governorate, Egypt in 2020 and 2021 seasons.

MATERIALS AND METHODS

Two successive experiments were conducted at Wadi El-Natrun, Beheira governorate, Egypt in 2020, 2021 seasons. The objective of this work is using glycine betaine, chitosan and salicylic acid to ameliorate the adverse effects of salinity stress on sweet pepper plants during the early summer season. The soil and water irrigation analysis are shown in Tables (1 and 2).

Table (1). Soil chemical analysis of the experimental farm.

Soil depth (cm)	EC (dS/m)	pH	Soluble anions (ppm)			Soluble cations (ppm)			
			CO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
0-30	4.77	7.7	55.85	31.2	10.5	24.00	11	10.52	2.180
30-60	4.16	7.4	51.21	22.5	16.1	16.83	6	17.80	0.097

Table (2). Chemical analysis of irrigation water (underground well) of the experimental farm.

Water sample	EC (dS/m)	pH	Soluble anions (ppm)			Soluble cations (ppm)			
			HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
Average	5.47	7.8	2.50	81.08	16.24	25.29	19.43	54.83	0.45

A number of 1515 hybrid seeds from Holland were sown in foam trays filled with peat and vermiculite (1:1), enriched with different nutrients. After 45 days (beginning of February in the two seasons), sweet pepper transplants were transplanted into one side ridges 0.7 m width and 0.4 m apart, with planting density about 10000 plant/feddan. During the growing season all cultural practices were performed as recommended.

1. Experimental Treatments

Treatments were as follows:

- Salicylic acid (1 g/l)
- Chitosan (3 g/l)
- Glycine betaine (2 g/l)
- Control (spray with tap water)

The plants were applied (foliar spray) with treatments four times, the first application was carried out after 30 days after transplanting with 10 days intervals.

2. Experimental Parameters

The following measurements and determinations of plant growth characteristics were recorded in both seasons.

2.1. Physical characteristics of plant

Ninety days after transplanting, five plants of each plot were randomly taken out with their roots for measuring the following vegetative growth traits:

Plant height

It was measured in cm, starting from the ground level to the tip of the longest branch.

Fresh weight (g/plant)

Total fresh weight of each plant includes tip parts and root was measured.

Dry weight (g/plant)

Oven dried plants were weighted.

Leaf area (m^2/plant)

Fresh leaf samples were collected from each treatment and by a known diameter crock borer twenty disks were made, then oven dried at 70°C till reaching constant weight.

$$\text{Leaf area} = \frac{dry\ weight\ of\ leaves}{dry\ weight\ of\ disks} \times no.of\ disks\ \times \text{disk area}$$

2.2. Chemical characteristics of plant

Macro elements

Sample of fresh leaves (the 6th leaf from the plant top) was taken after 90 days from transplanting, and considered the most representative ones for plant analysis, according to Ward (1963). The leaves were oven dried at 70ºC till a constant weight. The dry matter was finely ground and wet digested with H_2O_2 and concentrated H_2SO_4 for the determination of nitrogen, phosphorus, potassium, calcium and silicon according to the following methods:

Nitrogen was determined in the digestion product, using the Micro-Kjeldahl method (Piper, 1947). Phosphorus was determined colorimetrically in the above-mentioned digestion product, and spectrophotometrically according to the method described by King (1951). Potassium was determined in the above-mentioned digestion product, using the Flame photometer according to the method described by Jackson (1967).

2.3. Fruit Yield and its Components

- Average fruit weight (g).
- Total fruit yield per feddan

3. Experimental Design and Statistical Analysis

All obtained data were statistically analyzed using complete randomized block design with three replicates, main of different treatment were compared by using the least significant difference test (L.S.D.) at 5% level of probability (Snedecor and Cochran (1991).

RESULTS

Two successive experiments were conducted to ameliorate the adverse effects of salinity stress on sweet pepper plants during the early summer season of 2020 and 2021, at Wadi El-Natrun, Beheira governorate. The plant characteristics were recorded after glycine betaine, chitosan and salicylic acid applications.

1. Physical Characteristics of Plant

Data provided in Table (3) demonstrate that under salt stress circumstances, plant height, leaf area per plant, fresh and dry weight per plant, and dry weight per plant, all responded positively and significantly to all administered treatments. However, compared to all other treatments, the treatment with glycine betaine had the most favourable impact on plant height, leaf area per plant, and fresh and dry weight per plant, followed by the treatment with chitosan. The use of salicylic acid gave the least results. On the contrary, that the untreated pepper plants (control) gained the lowest values of the previous characters. These findings were true during both experimental seasons.

Table (3). Effect of glycine betaine, chitosan and salicylic acid on plant height, leaf area/plant and fresh and dry weight/plant of sweet pepper in the two seasons of 2020 and 2021.

Treatments	Plant height (cm)		Leaf area/plant (m^2)		Fresh weight/plant (g)		Dry weight/plant (g)	
	2020	2021	2020	2021	2020	2021	2020	2021
Salicylic acid (1 g/l)	67.13	59.55	2.258	2.246	627.33	481.43	120.22	115.05
Chitosan (3 g/l)	69.12	61.11	2.483	2.407	691.82	537.14	127.85	125.27
Glycine betaine (2 g/l)	70.00	61.88	2.512	2.806	734.27	579.76	132.79	129.78
Control	62.11	57.00	1.991	1.943	550.24	397.86	97.15	93.60
L.S.D. 5%	1.12	1.01	0.01	0.02	15.12	17.32	3.45	4.12

2. Chemical Characteristics of Plant

Concerning the effect of glycine betaine, chitosan and salicylic acid on N, P and K in sweet pepper plants, it is clear from data in Table (4) that a considerable rise in N, P and K percentages was seen after treatments were applied, if compared with the control. Moreover, the maximum N, P and K percentages were recorded via using glycine betaine, followed in decreasing order by those plants supplied with chitosan. But the lowest positive effect of N, P and K percentages was recorded by salicylic acid. On the contrary, untreated plants (control) gained the lowest N, P and K percentages. These findings were true during both experimental seasons.

3. Yield and its Components

As for the effect of glycine betaine, chitosan and salicylic acid on average fruit weight and total yield of sweet pepper plants, it is clear from data in Table (5) that spraying glycine betaine, chitosan and salicylic acid caused a significant effect on average fruit weight and total yield of sweet pepper. In contrast, as compared to the control treatment, all the treatments utilized in this study raised the average fruit weight and total yield values. Moreover, the application of glycine betaine resulted the highest values of average fruit weight and total yield, followed in descending order by that plants which was sprayed with chitosan. The lowest positive effect was recorded by salicylic acid. On the contrary, that untreated plants (control) gained the lowest sweet pepper average fruit weight and total yield. The above-mentioned results are similar in both seasons of 2020 and 2021.

Table (4). Effect of glycine betaine, chitosan and salicylic acid on nitrogen, phosphorus and potassium percentages of pepper plants in the two seasons of 2020 and 2021.

Treatments	N %		P %		K %	
	2020	2021	2020	2021	2020	2021
Salicylic acid (1 g/l)	2.40	2.42	0.11	0.12	0.25	0.27
Chitosan (3 g/l)	2.73	2.75	0.13	0.14	0.30	0.32
Glycine betaine (2 g/l)	3.12	3.15	0.17	0.18	0.37	0.39
Control	2.15	2.17	0.10	0.12	0.19	0.20
L.S.D. 5%	0.11	0.12	0.001	0.002	0.002	0.003

Table (5). Effect of glycine betaine, chitosan and salicylic acid on average fruit weight and total yield of pepper plants in the two seasons of 2020 and 2021.

Treatments	Average fruit weight (g)		Total yield (ton/feddan)	
	2020	2021	2020	2021
Salicylic acid (1 g/l)	62.47	58.53	16.53	17.41
Chitosan (3 g/l)	63.59	59.71	17.44	18.53
Glycine betaine (2 g/l)	64.87	61.43	18.31	19.63
Control	55.11	51.34	14.31	15.32
L.S.D. 5%	0.48	0.37	0.55	0.62

DISCUSSION

This study was carried out to investigate some possible alternatives that may alleviate the negative effect of salinity on sweet pepper. The findings demonstrated that salinity has widespread adverse impacts on plant growth metrics like plant height and leaf area as well as fresh and dry weights of the plant. Salinity is well known to negatively affect plant water status (Livett,

1980) and the latter is reflecting on little elongation of plant cells and hence shorter plants and smaller leaf areas.

Smaller canopy (plant height and leaf area) means smaller light interception hence, lower photo assimilate production and ending with lower fruit yield which has been recorded in this study. Not only water status, salinity also negatively affects nutrients uptake (Grattana and Grieve, 1999), which has been observed in this study as well. This also contributes to the negative effect of salinity on overall plant performance and production. On the other hand, all trails that have been carried out earlier (Haghighi et al., 2012; Tantawy et al., 2014 and 2015) as well as this study are attempting to improve these situations of plant water and nutritional status.

According to Salisbury and Ross (1992), glycine betaine is a compatible solute in the osmotic adjustment of the cytoplasmic compartments where it may accumulate while ions are sequestered in the vacuole, which is why it has a positive effect on tomato plant growth and productivity when irrigated with saline water. Additionally, glycine betaine was discovered to act as an anti-transpiration agent, protecting protein and membrane functions from stress conditions like drought and salt stressors (Hanson et al., 1995). Additionally, glycine betaine treatment boosted the net photosynthesis of water-stressed tomato plants. This was mostly because the treated plants increased stomatal conductance and decreased photorespiration (Makela et al., 1999). According to Seneoka et al. (1995), leaf tissue of a maize isoline synthesis in glycine betaine maintained higher relative water content and turgor when grown in salt stress than an isoline deficient in glycine betaine synthesis. This finding provided another explanation for how glycine betaine has been reported to directly affect the water content of the plants. Additionally, glycine betaine increased the growth of maize plants under stress conditions like drought and salt stress. These improvements likely came about as a result of glycine betaine application that has a well-known physiological function as a cytoplasmic osmoticum, which increases drought tolerance by allowing plants to maintain photosynthetic activity under osmotic stress conditions, stabilize the enzymes involved in amino acid metabolism, and maintain turgor pressure even at low temperatures (Laurie and Stewart, 1990). Chickpea and maize have both been observed to benefit from salicylic acid (Hussein et al., 2007 and Farjam et al., 2015). Presoaking cucumber seeds in salicylic acid increased growth and reduced the seedlings sensitivity to the damaging effects of salinity (Wahid et al., 2017). Exogenous salicylic acid has been modified to lessen the detrimental impact of water stress (Shao et al., 2018) and salt stress (Jini and Joseph, 2017) on plant performance.

According to a research, chitosan has a positive impact on tomato plant development and productivity when it is irrigated with saline water. This is because chitosan contains amino groups that expand the photosynthetic area of plants and maximize photosynthesis, which boosts plant growth (Sofy et al., 2020). The increase in plant growth following chitosan treatment may be attributable to its effect on boosting mineral uptake and transport, particularly N, P and K.

CONCLUSION

Foliar spraying by glycine betaine, chitosan and salicylic acid on sweet pepper irrigated with saline water improved plant growth and production in this study. Salinity decreased plant growth and yield. Glycine betaine, at a rate of 2 g/l, entered the first rink in reducing the detrimental effects of salinity compared to other treatments, but the application of the prior treatments reduced the adverse effects of salty water irrigation.

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تأثير الجليسين بيتاين والشيتوسان وحمض السيلسيلك على نباتات الفلفل تحت ظروف الري بالماء المالح

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أجريت تجربتان حقليتان بوادي النطرون بمحافظة البحيرة بمصر في موسمي صيف ٢٠٢٠ و٢٠٢١ على نباتات الفلفل الحلو هجين ١٥١٥. تهدف الدراسة إلى معرفة تأثير رش الجليسين بيتاين والشيتوسان وحمض السيلسيلك بتركيز ٢، ٣ و١ جم / لتر، على التوالي وذلك لتخفيف الأثر الضار للري بالماء المالح. أظهرت النتائج أن جميع المعاملات المطبقة حسنت بشكل كبير جميع صفات النمو، بما في ذلك ارتفاع النبات، مساحة الأوراق، الوزن الطازج والجاف، والتركيب المعدني (النيتروجين والفوسفور والبوتاسيوم)، وقللت بشكل كبير من آثار إجهاد الملوحة. بالإضافة إلى ذلك، أظهرت النتائج أن الرش الورقي بالجليسين بيتاين والشيتوسان وحمض السيلسيلك في هذه الدراسة أدى إلى زيادة كل من المحصول الكلي ومتوسط وزن الثمرة مقارنةً بالكنترول، هذا وقد تفوقت المعاملة بالجليسين بيتاين على جميع المعاملات الأخرى من حيث تأثيره الإيجابي على نمو النبات، والمحتوى المعدني، ومتوسط وزن الثمرة، والإنتاج الكلي، بينما جاءت معاملة الشيتوسان في المرتبة الثانية، وجاءت المعاملة بحمض السيلسيلك في المرتبة الثالثة (أقل تأثير مفيد)، بينما أظهرت معاملة الكنترول أدنى القيم للخصائص السابقة من حيث صفات النمو الخضري، المحتوى المعدني وكذلك المحصول.