

## Enhancing yield and storability of snap bean by bio-fertilizer as a Solutions for climate change-affected sustainable agriculture

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### ABSTRACT

Climate change adaptation and mitigation strategies heavily rely on biotechnology. Thus, the development and application of bio fertilizers containing beneficial bacteria and algae are a potential technique to improve horticulture crops. To evaluate the effects of bio-fertilizer such as seaweed extract, *Azospirillum brasilense* and *Rhizobium phaseolus vulgaris* on promoting the productivity, quality, and storability of two cultivars (Tema and Joty) of snap bean. The experiment was carried out during two summer seasons of 2020 and 2021 at Agricultural Station of Cairo University. There were four treatments in the study: foliar applications of liquid seaweed extract (2 ml/l), soil applications of *Azospirillum baselines* and *Rhizobium phaseolus vulgaris* (300 g/acre per microorganism), and a control treatment (water spraying). Pods after harvest from each treatment were stored in polyethylene packages for 21 days at 5 C. The results revealed that foliar application of seaweed achieved vegetative growth (plant height, Number of leave/plant, chlorophyll content, and leaf area) of both cultivars, yield, and pod quality (pod firmness, diameter, and weight) of Joty while, the highest total soluble sugar was recorded for the Tema cultivar in all the tested treatments compared to control treatment. The highest ascorbic content, TSS, general appearance and antioxidant activity were recorded in a foliar application of seaweed extract after 21 day of storage at 5 C. We can summarized that foliar application of seaweed at 2% can be used as a bio stimulant for improving vegetative growth, yield, and storability of snap beans.

**KEYWORDS:** Biofertilizer, Climate change, Productivity, Storability, Snap bean

### 1. INTRODUCTION

The increase in surface temperature is projected to reach 3.2 °C by the end of the century. Food production systems are impacted by global warming, which can harm all living forms by causing hunger, malnutrition, and increased poverty (Kumar, P. 2018).

One of the most susceptible industries is agriculture since it is intrinsically subject to climate change. Efficiency of agricultural production is directly impacted by climate change. Agriculture is impacted by climate change in terms of production, farming methods, environmental effects, rural space, and adaptation. Mitigation of climate change and adaptation to the changing climate are crucial for reducing the above-mentioned severe effects of climate change (Bakshi 2003; Stringer et al. 2009). Reforestation, a decrease in green house gasws (GHG) emissions, and a decrease in the usage of fossil fuels can all help to mitigate climate change.

The common bean, an extraordinary pulse crop, is the main source of nutritional protein for millions of people around the world. It is grown on more than 35 million ha of land annually (Mulas et al., 2011; FAOSTAT, 2019; Broughton et al., 2003). Legumes are thought to be helpful for reducing climate change because they have a symbiotic relationship with N-fixing bacteria (Herridge et al., 2008), which provides a considerable amount of nitrogen that the crop needs (Jensen et al., 2012).

Due to the scarcity of chemical fertilizers, biofertilizers are viewed as being more environmentally friendly, providing a cheap and sustainable alternative to synthetic fertilizers, enhancing agricultural production, and lowering environmental pollution (Sattar et al., 2018; Mounir et al., 2020). Through a variety of methods, biotechnology approaches play a significant part in climate change adaptation and mitigation. Thus, a possible strategy to

enhance horticulture crops is the creation and use of biofertilizers that include these useful bacteria.

*Azospirillum brasilense*, an alphaproteobacterium that lives in the rhizosphere and promotes plant growth, is one of species that is utilized globally as a commercial bio-fertilizer. This specie has a reputation for promiscuously colonising the roots of numerous different plants. Additionally, *Azospirillum* can promote root growth and root hair formation, increases the amount of soil that the root system explores which aids plants in surviving environmental challenges (Bulegon et al. 2017 and Chibeba et al. 2015).

By encouraging root growth, boosting nutrient absorption from the soil, and reducing nitrogen loss, *Azospirillum brasilense* inoculation of crops can significantly enhance crop yield (Raffi, M.M et al., 2020; Nio Paul, M.B. et al., 2012). *Azospirillum* are plant growth promoting bacteria (PGPB) used as a seed inoculant in grasses, and they offer benefits beyond nitrogen fixing. As a result, they also boost the level of phenols and flavonoids in the fruits (Khan et al. 2017). Also, *Azospirillum* produces auxins, gibberellin, ethylene, siderophores, HCN, and anti-microbial, as well as helping to solubilize the phosphorus in the soil (Suhameena et al., 2020).

Rhizobium is a top generator of IAA and siderophores, which boosts agricultural output and yield, making it a type of rhizobacteria that promotes plant growth (Datta et al., 2015). A biological nitrogen-fixing bacteria called rhizobium collaborates with the roots of legume plants to transform atmospheric nitrogen into ammonia.

In *Phaseolus vulgaris*, Rhizobium encourages plant growth by increasing plant height, fresh and dry weight, and flower bud production. Also, at harvest, the grain yield and the number of pods/plant (Memenza et al., 2016)). Biological nitrogen fixing is the major mechanism by which rhizobia enhance plant growth (BNF). The plant and rhizobia working together can provide the crop's entire nitrogen requirement and guarantee high harvests (Hungria et al. 2017). The nitrogenase BNF enzyme, which is found inside root nodules, is responsible for that; it converts atmospheric nitrogen into ammonia, which the plant subsequently absorbs (Bruijn 2015). Rhizobia inoculation significant increased shoot biomass and root biomass the number of root, yield components, number of pods per plant, number of grain per pod and yield per hactre of the climbing bean (Mmbaga et al., 2015).

Seaweed extracts (SWE) are produced as a source of biofertilizer because of their high nutritional contents, which have an effect on a number of

physiological processes such seed germination, vegetative growth, and productivity. Moreover, it makes plants more disease-resistant (Sathya et al., 2010). Seaweed extracts have a positive effect on plants in a number of ways, including the stimulation of phytohormone synthesis, the uptake and transfer of nutrients, and soil conditioning (achieved through improving water-air conditions and the activity of beneficial soil bacteria) (Van Oosten, M.J. et al., 2017). Seaweed contains large amounts of organic matter, microelements, vitamins, amino acids, auxins, cytokinins, and gibberellins (Khan et al., 2009). Furthermore, using seaweed extracts helps plants tolerate less-than-ideal growing conditions, such as salinity, drought, or severe temperatures (Battacharyya, D. et al., 2015; Sosnowski, J. et al., 2016; Drobek, M. et al., 2019).

Recent studies have tended to using modern and environmentally friendly technologies in fertilizer. The goal of the current study was to determine how bio-fertilizers such seaweed extract, *Azospirillum brasilense*, and *Rhizobium phaseolus vulgaris* affected several physiological and biochemical parameters, as well as the quality and storability of two cultivars of snap beans (Tema and Joty).

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

This study was carried out at the experimental station at Faculty of Agriculture, Cairo University, Giza, during the two consecutive summer seasons 2020 and 2021. The seeds of two cultivars (Tema and Joty) were purchased from Agricultural Research center and Semens Company. The seeds sowing on 9<sup>th</sup> march of both season on 5 cm between plants and 60 cm between ridges. Dripirrigation was used. The plot, which had two ridges that were each 5 m long and 0.85 m wide, measured 8.5 m<sup>2</sup>. Five centimetres apart, seeds were planted in hills on the two sides of the ridge. A hill received three to five seeds. Plants were divided onto two hills ten days after seeding. The number of plot area was 24 (2 Cultivar \* 4 Treatment \* 3 Replication). The experiment was designed in RCBD with two factor as split plot. The cultivars arranged in main plot and biofertilizer treatments in sub blot.

Phosphorus fertilizers were added during soil tillage at rates of 360 kg ha<sup>-1</sup> and 120 kg ha<sup>-1</sup>, respectively. The phosphorus fertilizer was added as calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>) and the potassium fertilizer as potassium sulphate (48% K<sub>2</sub>O). Additionally, several agricultural production techniques, like as irrigation and pest control, were

used in accordance with the Egyptian Ministry of Agriculture's recommendations

### 2.2. Seaweed extract

Seaweed extract (Oligo-X as commercial name) was obtained from Union for Agriculture Development (UAD) Company, Cairo, Egypt; it contains organic matter (6% total amino acid, 35% carbohydrate, 10% alginic acid, 4% mannitol, 0.04% betaines); growth regulators (0.03% IAA, 0.02% cytokinins (adenine)) and some macro and microelements (3.12 % N, 2.61 % P<sub>2</sub>O<sub>5</sub>, 4.71 % K<sub>2</sub>O, 0.25 % Ca, 3.56 % S and 0.58% Mg).

### 2.3. Inocula preparation

In the broth culture, each bacterium was grown and maintained to a density of at least 10<sup>8</sup> cells ml<sup>-1</sup> on its own medium (tables 1 and 2). Each bacterial liquid culture was mixed separately with sterilising fine

peatmoss that had been previously neutralised with 5% CaCO<sub>3</sub> (2:1 w/v), with the final product's moisture content adjusted to around 50%. Arabic gum was used as an adhesive material to coat bean seeds with peat-based inocula of the bacteria. Inoculation was done on the same day as sowing and allowed to dry in the shade before planting. Following planting, the inoculated plots were given a boost inoculum of liquid bacterial culture. Seeds were planted in hills 5 cm apart, either inoculated or uninoculated. Each hill received three seeds. The plants were thinned to two plants per hill ten days after sowing. Rhizobia and *Azospirillum* were used as inoculants at a rate of 300 g/acre per microorganism (50% when seeds were inoculated and 50% after 15 days). Plants were sprayed with seaweed liquid extract (SLE) three times; the first time was 25 days after sowing and was repeated every 10 days.

**Table 1. The composition (g l<sup>-1</sup>) of yeast extract mannitol (YEM) medium for growing and maintaining Rhizobia.**

Composition	Weight (g)
Yeast Extract	1.0
Mannitol	10.0
NaCl	0.1
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2
K <sub>2</sub> HPO <sub>4</sub>	0.5
Agar	15 – 20
Distilled water added up to	1.0 Liter
pH	7.0

**Table 2. The composition (g l<sup>-1</sup>) of Dobereiner medium for growing and maintaining Azospirillum.**

Composition	Weight (g)
Malic acid	5.0
Mn SO <sub>4</sub>	10.0
NaCl	0.1
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.2
K <sub>2</sub> HPO <sub>4</sub>	0.5
FeSO <sub>4</sub>	0.5
CaCl <sub>2</sub>	0.02
KOH	4.0
BTB	0.002
Na <sub>2</sub> MoO <sub>4</sub>	0.002
Agar	1.75
Distilled water added up to	1.0 Liter
pH	7.0

**The ability of bacteria to produce some plant growth regulators in a qualitatively in the laboratory**

**1. Indole acetic acid estimation**

Use of amended Luria- Bertani agar medium (LB) containing 5 mM L-tryptophan (Bric et al., 1991)

**2. Siderophore production assay**

Siderophore production by using chrome azural S (CAS) assay according to Schwyn and Neilands (1987).

**3. Phosphate solubilization production assay**

Using tricalcium phosphate in Pikovskaya medium as described by Jasim *et al.* (2013). The endophytes were spot inoculated in the center of the Pikovaskay's agar medium amended with bromophenyl blue (2.4 mg.ml<sup>-1</sup>).

Bacteria	IAA concentration (µg/mL)	Siderophores	Solubilizing phosphorus
<i>Rhizobium leguminosarum</i>	+	+	+
<i>bv phaseoli</i>	+	+	+
<i>Azospirillum brasilense</i>	+	+	+

**2.4. Treatments**

The four combination treatments were as follows:

1. Plants received the recommended NPK levels without biofertilizer considered as controls.
2. Plants inoculated with *Rhizobium leguminosarum biovar phaseoli* (Rh) + 50% (NPK).
3. Plants inoculated with *Azospirillum brasilense* (Ab) + 50% (NPK).
4. Seaweed liquid extracts (SLE) 2ml/l + 50% (NPK).

**2.5. Data recorded:**

**2.5.1. Vegetative growth:**

Ten randomly chosen plants from each plot were chosen after 60 days of planting to measure plant height, number of leaves per plant, leaf area per plant, and total greenish chlorophyll (SPAD reading).

**2.5.2. Pod quality and yield components:**

During the harvesting time, green pods were harvested at the appropriate maturation stage (12-14 cm in length) in order to estimate the yield characteristics of pod firmness, pod diameter, average pod weight, number of pods per plant, and total yield (g)/plant and m<sup>2</sup>.

**2.5.3. Pod chemical composition:**

Chemical properties of pods i.e., total soluble solid (TSS) was determined by digital refractometer ((model PR101, Co. Ltd., Atago, Tokyo, Japan), Ascorbic acid content was determined as described by (A.O.A.C. 2000), According to Dubois et al., total carbohydrates were calculated (1956). Total chlorophyll was extracted by dimethyl formamide and measured by spectrophotometer.

**2.5.4. Storage experiments**

Effect of some biofertilizer on quality attributes of snap beans during storage and shelf life conditions. Snap bean pods free from disease, uniform in length, straight and dark green color from each plot were selected to determine. The effect of biofertilizer of storability of snap bean. Pods were packed in perforated polyethylene pags (length 22 cm, width 16.5 cm, and thickness 1 mm). The samples were weighted (200 g) and stored at 5 C and 95% R.H for three weeks. Each treatment was performed three times, and the entire experiment was repeated. For each treatment, the samples were divided into two groups. One group was used to assess weight loss and general appearance during the storage period, while Another was used to determine the total soluble solids %, pod firmness, gas changes, ascorbic acid and total chlorophyll contents, total phenolic compounds, and antioxidant activity after 7, 14, and 21 days (shelf life) of storage, while the other was used to determine the pod quality parameters.

**2.6. STATISTICAL ANALYSIS**

Using the computer programme "MSTATC," all the collected data from six replicates over the two consecutive growing seasons was analysed using a randomised complete block design with two factors. Gomez, A.A. and K.N. (1984). At a 5% probability level, the LSD test was employed to evaluate variations between treatment methods. G.W. Snedecor and W.G. Cochran (1980).

### 3. RESULTS AND DISCUSSION

#### 3.1. Vegetative growth parameters

Plant height, no.of leaves/plot, leaf area and chlorophyll content (SPAD reading) were influenced by soil or foliar application of bio-fertilizer in both

cultivars. The information in table (3) demonstrated that, in both seasons, the highest plant heights (56.33 and 54.4 cm) were obtained when seaweed extract was combined with the Joty cultivar. By applying foliar seaweed extract in both seasons, the Joty and Tema cultivars' respective numbers of leaves reached their

**Table 3. Effects of biofertilizer applications on snap bean cultivars' vegetative growth 60 days after sowing during the 2020–2021 seasons.**

Treatment	Plant height (cm)		No. of leaves/plant		leaves area (cm <sup>2</sup> )		Total chlorophyll (SPAD)		
	2020	2021	2020	2021	2020	2021	2020	2021	
Joty cv.	<i>Azospirillum brasilense</i>	52.00	49.69	17.28	16.52	37.05	35.40	38.97	37.25
	<b>Rhizobium</b>	45.33	43.53	16.08	15.45	31.52	30.29	37.30	35.84
	<b>Seaweed</b>	56.33	54.46	18.65	18.02	32.50	31.41	39.82	38.49
	<b>control</b>	42.33	43.23	12.51	12.81	21.77	22.28	36.43	37.27
Tema cv.	<i>Azospirillum brasilense</i>	46.67	47.73	15.94	16.31	33.82	34.51	37.82	38.65
	<b>Rhizobium</b>	42.67	43.27	14.14	14.33	36.81	37.43	36.77	37.27
	<b>Seaweed</b>	50.67	51.77	17.66	18.04	35.25	36.02	39.21	40.07
	<b>control</b>	40.67	41.94	12.10	12.49	21.51	22.17	34.65	35.75
	<b>LSD 0.05</b>	2.712	3.888	0.9891	1.613	3.36	4.383	0.6895	2.902

greatest values (18.65, 18) and (17.66, 18.04), respectively. All bio-fertilizer treatments achieved maximum leaf area compared to control. Soil application of *Azospirillum brasilense* with both cultivars recorded the highest values of leaf area (37.5, 35.40 cm<sup>2</sup>) in both seasons respectively, with no significant difference with the foliar application of *Azospirillum brasilense* or seaweed in Tema cultivar in both seasons. Total chlorophyll (SPAD) maximum content was observed with both cultivar with seaweed foliar spray (39.82, 39.21), respectively in first season. As of the second season, *Azospirillum brasilense* had the lowest values of total chlorophyll (SPAD), but there were otherwise no significant differences between any of the treatments.

The foliar application of seaweed (SWE), increased No. of leaves /plan, leaf area and chlorophyll reading (SPAD). Abbas (2013), Abo-Seder et al (2016), Abou El-Yazied et al (2012), Boghdady (2016), and Zewail almost all had values that were identical (2014). The ability of SWE to stimulate growth may be due to the presence of macro components and growth regulators. Because they have been found to contain growth-regulating compounds including Ioxin (IAA and IBA), gibberelin, cytokines, betenes, and macronutrients,

fruits, vegetables, and other crops have been known to develop more quickly when subjected to SWE. Endogenous hormones that promote cell division and cell elongation, including IAA, GA3, and active cytokinins, are responsible for improving the vegetative growth of plants treated with seaweed (Awad et al., 2006). Also, because it encourages the uptake of nutrients by plants, it lessens the growth-inhibiting effects of Na toxicity and promotes growth (Nelson and Van-Staden, 1984). According to Sridhar and Regasany (2010), the promotion of vegetative growth by seaweed is caused by the activation of nitrate reductase, a crucial enzyme in nitrogen metabolism.

#### 3.2. Pod quality parameters

The information in table (4) Foliar application with seaweed extract and *Azospirillum* soil application gave the highest average pod weight in both seasons (9.42 , 8.99) and (9.10 , 8.59), respectively with joty cultivar, additionally *Azospirillum* and seaweed had the highest values of average pod weight with tema cultivar in the second season (8.46, 8.81).

The most effective treatment for increasing pod firmness (28.52, 29.14) and pod diameter (7.89, 8.07) in both seasons was foliar spraying with seaweed and

**Table 4. Effects of biofertilizer applications on snap bean cultivars' pod quality 60 days after sowing during the 2020–2021 seasons.**

Treatment	Average Pod weight (g)		Pod diameter (mm)		Pod firmness (gm/cm <sup>2</sup> )		
	2020	2021	2020	2021	2020	2021	
Joty cv.	<i>Azospirillum brasilense</i>	8.99	8.59	7.08	6.77	26.83	25.65
	Rhizobium	7.71	7.40	6.70	6.43	24.34	23.36
	Seaweed	9.42	9.10	7.15	6.91	27.95	27.02
	control	5.79	5.94	5.45	5.58	22.60	23.12
Tema cv.	<i>Azospirillum brasilense</i>	8.28	8.46	7.27	7.43	27.40	27.99
	Rhizobium	7.16	7.24	6.21	6.30	25.26	25.60
	Seaweed	8.61	8.81	7.89	8.07	28.52	29.14
	control	5.33	5.51	5.35	5.51	23.12	23.85
<b>LSD 0.05</b>		0.6338	0.9719	0.3323	0.6338	1.072	1.941

Tema cultivar. According to Abdulraheem (2009), using seaweed increased pod diameter and stiffness. These results were acquired over the course of two seasons and concurred with those on snap bean obtained by Abou El-Yazied et al. (2012) for Seaweed extract.

### 3.3. Yield component

Table (5) results illustrate how applying bio-fertilizers to snap bean plants affected their overall yield and its component yields. The results show that plants treated with foliar or soil applications had significantly more pods, total yield per plant, and total

yield per square meter of soil than untreated plants. The number of pods/plant (39.64, 38.32), total yield/plant (373.2, 360.8 g), and total yield/m<sup>2</sup> (2.43, 2.35 kg/m<sup>2</sup>) were all improved by the foliar spray of seaweed with the joty cultivar.

The harvestable bean (*Phaseolus vulgaris* L.) yield was reported to be improved by foliar seaweed extract treatment in this regard (Temple and Bomke, 1989) In terms of the quantity of pods per plant, seed yield (Kg/Fed), and seed yield per plant (g), Abou El-Yazied et al. (2012), Zodape et al. (2010), Ramya et al. (2010), and Boghdady almost all had comparable findings (2016). Several studies have shown that topically

**Table 5. Effects of biofertilizer applications on snap bean cultivars' yield component after 60 days of sowing date during 2020-2021 seasons.**

Treatment	Total yield (kg)/m <sup>2</sup>		Total yield (g)/plant		No. of pods/plant		
	2020	2021	2020	2021	2020	2021	
Joty cv.	<i>Azospirillum brasilense</i>	2.22	2.12	340.90	325.90	37.91	36.23
	Rhizobium	1.73	1.66	265.90	255.50	34.49	33.15
	Seaweed	2.43	2.35	373.20	360.80	39.64	38.32
	control	1.07	1.10	165.10	169.50	28.51	29.19
Tema cv.	<i>Azospirillum brasilense</i>	1.87	1.91	287.30	293.70	34.68	35.44
	Rhizobium	1.49	1.51	228.60	231.60	31.95	32.41
	Seaweed	2.04	2.09	313.80	321.10	36.44	37.25
	control	0.96	0.99	147.70	152.80	27.66	28.57
<b>LSD 0.05</b>		0.179	0.247	27.5	37.93	2.467	3.923

applying seaweed extract enhanced bean output by 24%. (Nelson and van Staden, 1984; Wajahatullah Khan et al., 2009).

The higher yield in plants treated with seaweed is thought to be caused by the hormonal components in the extracts, especially the cytokinins (Featonby-Smith

and van Staden 1983, 1984). While cytokinins are linked to nutrient partitioning in vegetative plant organs, high levels of cytokinins in reproductive organs may be linked to nutrient mobilisation. As a result of its beneficial components, including as growth stimulators and nutrients, plants treated with SEW produce higher biomass (Mansori et al. 2015).

### 3.4. pod chemical composition

As shown in table (6) there were non-significant effect between treatments on the total chlorophyll in the first season. The largest value of total chlorophyll (42.42) was produced by the Rhizobium and tema cultivar interaction in the first season. However, the second season's total chlorophyll (43.12) was significantly raised by the interaction between *Azospirillum brasilin* and the Tema cultivar.

The interaction between Rhizobium and Tema cultivar increased total carbohydrates content (28.56, 29.19), respectively in both seasons compared to control.

Foliar spray with seaweed extract were significantly increased protein with Joty cultivar (19.73, 19.08) , respectively in both seasons, in addition

foliar spray with seaweed with tema cultivar in the second season significantly increased protein (19.08).

The important role that seaweed played in the production of chlorophyll molecules had an impact on the amount of total carbohydrates because it increased the transfer of photosynthates from source to sink and other growth factors, may explain the reported increases in crude protein and carbohydrate in broad bean (*Vicia faba* L.) (Thomas et al, 2009). Seaweed Increase the manufacture of the carotenoids, ascorbic acid, and to copherols that protect PSII's photosynthetic machinery (Zhang and Schmidt 2000)

SWE may have a beneficial effect on raising the amount of chlorophyll in pods since it contains magnesium, which is a key component in the production of chlorophyll (Ramya et al., 2010). Moreover, Richardson et al. (2004) found that SWE contains significant amounts of cytokinins, auxin, and betaines, which boost plant resistance, delay leaf senescence, and control cell division as well as enhance the amount of chlorophyll in the leaves (Schwab and Raab, 2004). Seaweed can enhance the accumulation of total protein, total chlorophyll, and total carbs in snap bean pods (Hamed, 2012).

**Table 6. Effects of biofertilizer applications on snap bean cultivars' pod chemical composition after 60 days of sowing date during 2020-2021.**

Treatment	Total chlorophyll		Total carbohydrates (%)		Protein (%)		
	2020	2021	2020	2021	2020	2021	
Joty cv.	<i>Azospirillum brasilense</i>	40.21	42.07	26.57	25.40	18.44	17.62
	Rhizobium	40.79	39.92	27.87	28.49	17.36	16.68
	Seaweed	38.91	40.50	24.48	23.53	19.73	19.08
	control	39.50	38.97	26.72	27.09	15.99	16.36
Tema cv.	<i>Azospirillum brasilense</i>	41.68	43.12	27.51	26.59	17.32	17.70
	Rhizobium	42.42	41.51	28.56	29.19	16.45	16.66
	Seaweed	40.75	39.83	23.34	23.86	18.90	19.31
	control	38.22	37.05	23.33	24.06	15.92	16.43
	LSD 0.05	3.1	0.6895	0.91	2.251	0.3229	1.237

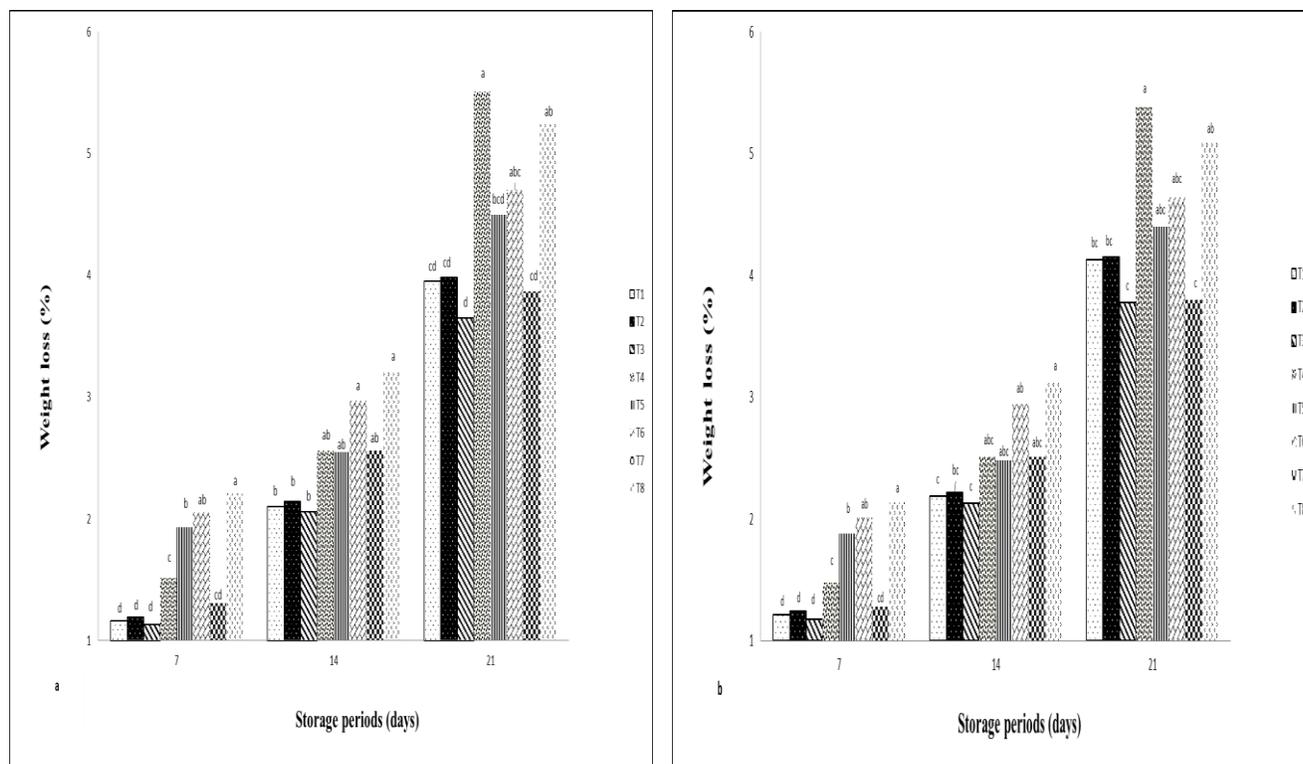
### 3.5. Storage experiments

#### 3.5.1. Weight loss

Weight loss % increased gradually of both cultivars with increasing prolongation of storage period (Fig. 1). All tested bio-fertilizer treatments reduced the weight loss % of both cultivars after 7, 14 and 21 days of storage period compared to untreated treatment. Soil application of *Azospirillum*, Rhizobium and seaweed foliar spray recorded the lowest weight loss % in Joty cultivar after 7 days of storage period in both seasons with no significant difference with seaweed treatment

in Tema cultivar in both seasons. Following 14 days of storage, there were no significant differences in the percentage of weight loss between any of the biofertilizer treatments and the control treatments in the same cultivar. The control treatment using the Joty cultivar had the highest value of weight loss% in both seasons after 21 days of storage.

Respiration, transpiration, and other senescence-related metabolic activities may be the cause of the increasing weight loss percentage during storage (Wills, 1998SWE material is abundant in both organic



**Fig 1. Effects of biofertilizer applications on snap bean cultivars' weight loss % during storage seasons 2020-2021.**

T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + Rhizobium), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + Rhizobium), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).

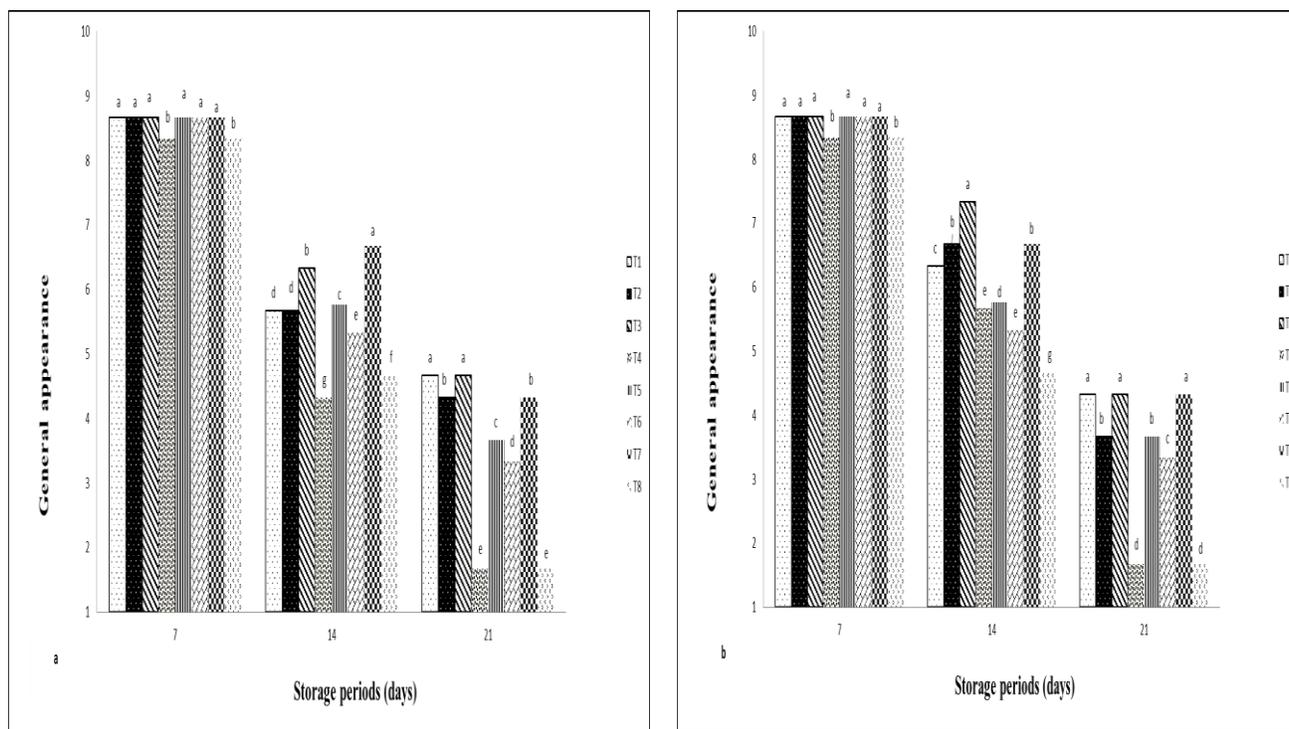
and mineral compounds, and these minerals prevent weight loss and retain green colour during storage, which may be the reason for the enhanced effect in both seasons, according to Khan et al. (2009) and Gad El-Hak et al. (2012). (Shehata et al., 2015). To maintain metabolic homeostasis after harvest and reduce pod dehydration, it may be necessary to supplement seaweed extract with other nutrients (Abou El-Yazied, 2012).

### 3.5.2. General appearance

Results show that snap bean pods' overall look drastically declined as storage time was extended. (Fig. 2). After 7 days of storage with both cultivars, there were no discernible variations between any of the studied treatments in regard to their general appearance. After 14 and 21 days of storage in both seasons, all treatments preserved the overall appearance

compared to the control of both cultivars. After 14 days from storage the interaction between foliar spray with seaweed in Tema cultivar had the highest score of general appearance in the first season, as well as the interaction between seaweed with Joty cultivar had the highest score of general appearance in the second season. After 21 days of storage Joty cultivar and foliar seaweed spray was significantly maintained general appearance in both seasons. In addition, the interaction between foliar spray with seaweed and Tema had significantly effect on general appearance in the second season.

Shehata et al. (2015) showed similar outcomes for snap bean pods. Snap bean pods' general appearance may suffer during storage due to shrivelling, withering, colour changes, and deterioration (El-Mogy, 2001).



**Fig 2. Effects of biofertilizer applications on snap bean cultivars' General appearance during storage seasons 2020-2021.**

T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + Rhizobium), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + Rhizobium), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).

### 3.5.3. Pod firmness

Figure (3) illustrates the modifications in snap bean pod firmness after bio-fertilizer treatment. At longer storage times, snap bean pod firmness significantly decreased across all studied treatments. Rhizobium treatment kept the pod firmness in both seasons in both cultivars' pods. The lowest pod firmness values, on the other hand, were discovered in both seaweed cultivars treated and untreated throughout both seasons' worth of storage. Ubhi et al. (2014) on snap beans achieved comparable results. According to a direct correlation between the rate of fruit softening and the reduction in firmness, proto-pectin may gradually break down into lower molecular fractions that are more soluble in water (Wills et al., 1998).

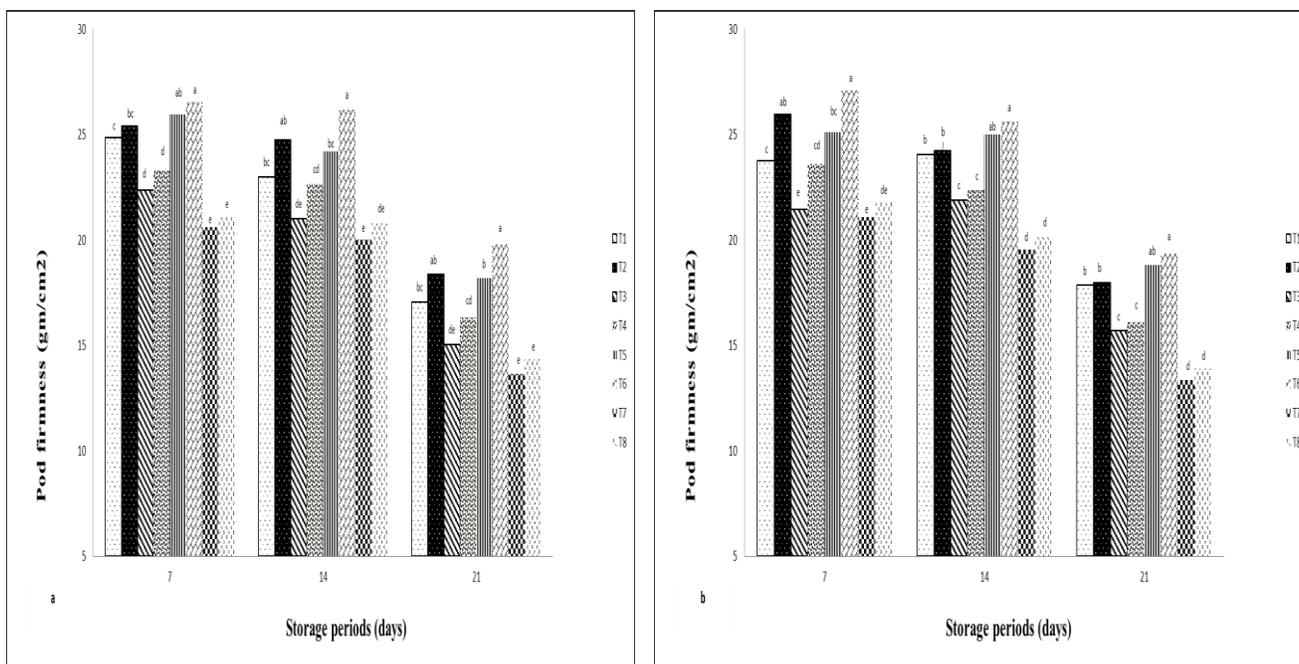
### 3.5.4. Total chlorophyll

As shown in fig (4), the total chlorophyll was decreased with increasing the storage period in both cultivate in the both seasons. The only treatment that showed a significant difference in the first season, after

7 days of storage, was foliar spraying with seaweed extract and the Tema cultivar, which had the greatest value of total chlorophyll content. The *Azospirillum* treatment with both cultivars after 14 days of storage significantly affected the amount of total chlorophyll. The greatest values of total chlorophyll content were found in *Azospirillum* with both cultivars. The highest overall chlorophyll concentration was found in Tema cultivar with *Azospirillum* at the conclusion of storage.

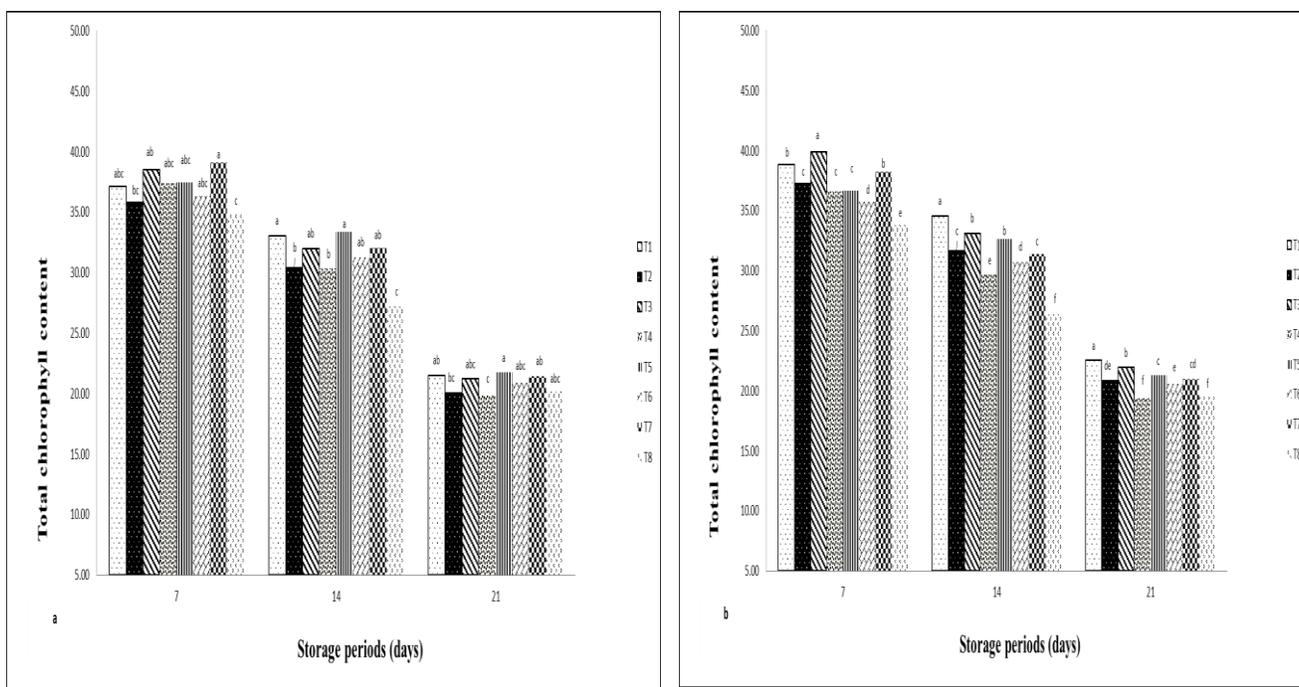
While, in the second season the interaction with foliar pray extract with Joty cultivar had positive effect on total chlorophyll content, while, after (14, 21 days) of storage the highest values of total chlorophyll content were recorded with *azospirillum* with Joty cultivar

High levels of glycinebetaine are present in seaweed extracts. By inhibiting the breakdown of chlorophyll in isolated chloroplasts under storage conditions, this chemical slows the loss of photosynthetic activity (Genard and al., 1991).



**Fig 3. Effects of biofertilizer applications on snap bean cultivars' Pod firmness during storage seasons 2020-2021.**

T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + Rhizobium), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + Rhizobium), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).



**Fig 4. Effects of biofertilizer applications on snap bean cultivars' Total chlorophyll during storage seasons 2020-2021.**

T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + Rhizobium), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + Rhizobium), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).

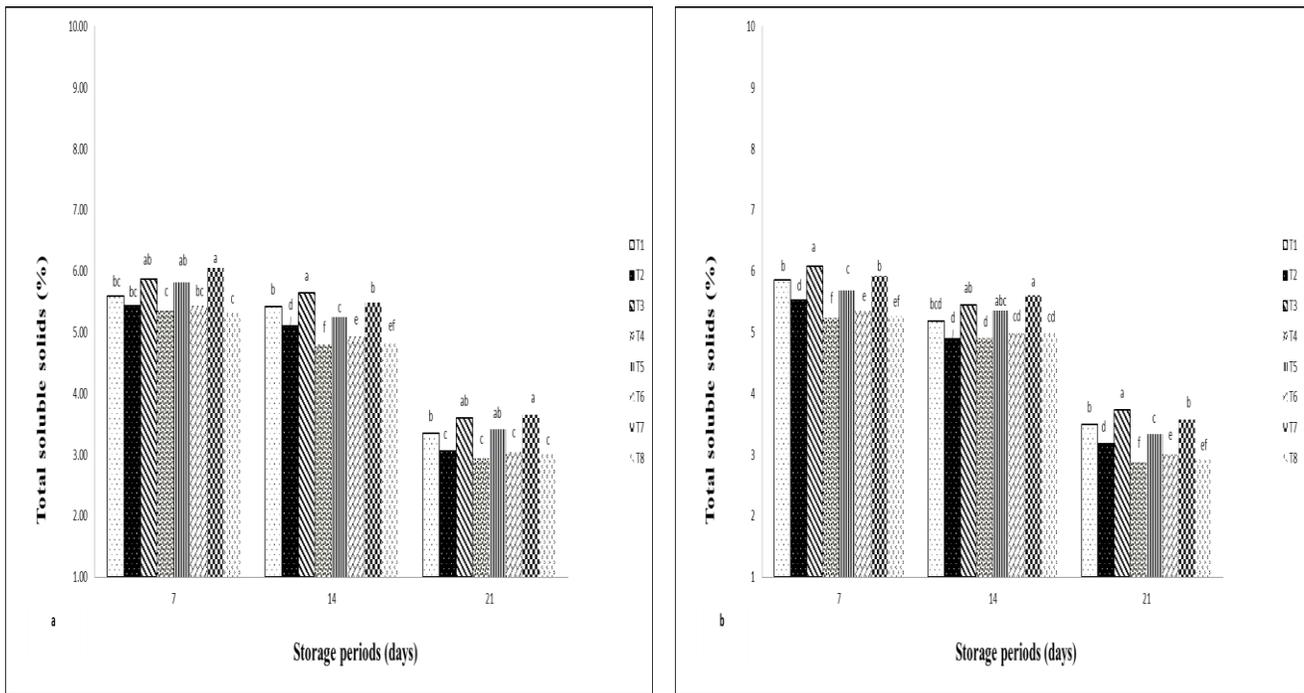
**3.5.5. Total soluble solid (TSS)**

The changes in total soluble solid (TSS) of snap bean pods during storage is shown in fig. (5). TSS content decreased with increasing the storage period time.

The interaction between foliar spray with seaweed extract and Tema cultivar had the highest value of TSS (6.05) after 7 days of the interaction between seaweed extract and Joty cultivar had a positive effect on total soluble solid (5.65) after 14 days of storage. At the end of storage the interaction between seaweed extract and Tema cultivar had the highest value of total soluble solid (3.65) in the first season but

in the second season after (7, 21 days) the interaction between foliar spray with seaweed and Joty cultivar had the highest values of TSS (6.089, 3.73), respectively. Meanwhile after 14 days of storage the interaction between seaweed extract and Tema cultivar had the highest value of total soluble solid (5.45).

Total soluble solids are reduced due to the loss of pectin's and carbohydrates, the partial hydrolysis of proteins, and the breakdown of glycosides into smaller units during respiration (Ball.1997). TSS decreased in this parameter as expected because organic acids and sugars are the two main substrates for respiration Gerasopoulos and Drogoudi, (2005).



**Fig 5. Effects of biofertilizer applications on snap bean cultivars' Total soluble solid (TSS) during storage seasons 2020-2021.**

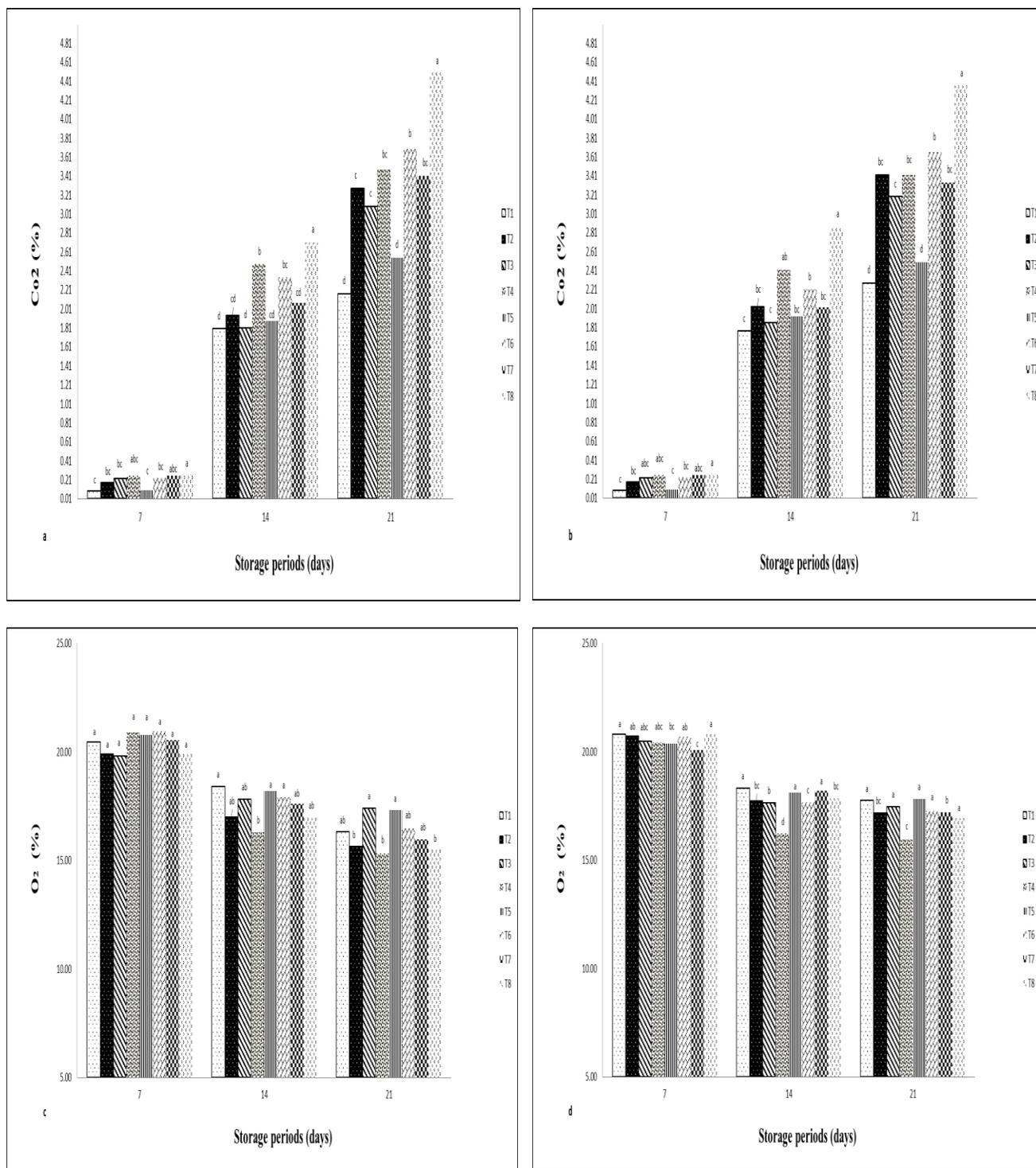
T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + Rhizobium), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + Rhizobium), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).

**3.5.6. Gas composition**

As shown in fig. (6) after 7 days from storage there were no significant differences between all biofertilizer treatments on CO<sub>2</sub> composition with both cultivar control treatment with Tema cultivar had the highest values of CO<sub>2</sub> in both seasons. After 14 days of storage the interaction between Azoprillium, seaweed with Joty cultivar had the lowest value of CO<sub>2</sub> in both seasons. At the end of storage the interaction between Joty and Tema cultivars with Azosprillium treatment

had the lowest values of CO<sub>2</sub> composition in both seasons.

In the first season, after 7 days of storage there were no significant differences between all treatments on O<sub>2</sub> campsites. After 14 days the highest value of O<sub>2</sub> composition were recorded with the interaction between azosprillium and Joty cultivar, the interaction between azosprillium and rhizobium with Tema cultivar, and there were no significant differences between all other treatments.



**Fig 6. Effects of biofertilizer applications on snap bean cultivars' Gas composition during storage seasons 2020-2021.**

T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + *Rhizobium*), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + *Rhizobium*), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).

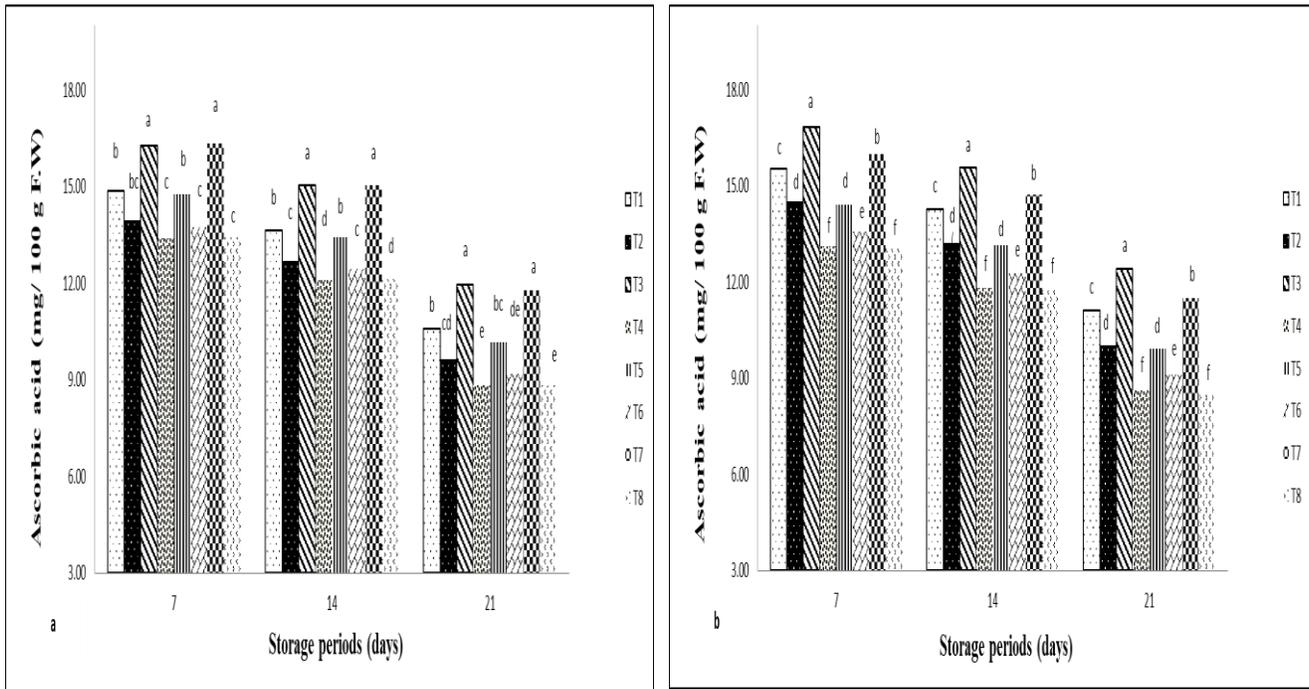
After 14 days of storage, the highest values of O<sub>2</sub> composition was observed with Azospirillum with both cultivar while, the foliar spray with seaweed with Joty cultivar had positive effective on O<sub>2</sub> composition. At the end of storage the foliar application by seaweed with Joty cultivar had the lowest values of O<sub>2</sub> composition followed by Rhizobium with Joty cultivar and foliar spray with seaweed and Tema cultivar

**3.5.7. Ascorbic acid**

As shown in fig. (7), the total ascorbic acid of all snap bean pods significantly decreased with the

prolongation of storage period. The foliar spray with seaweed extract with both cultivar significantly increased ascorbic acid in first season after (7, 14, 21days) from storage, while in the second season the interaction between foliar spray with seaweed and Joty cultivar increased the ascorbic acid during all storage period (7, 14, 21 days).

The decrease in ascorbic acid level over the period of storage may have been caused in large part by the fact that sugar respiration losses were greater than water losses during storage (Wills et al., 1998).



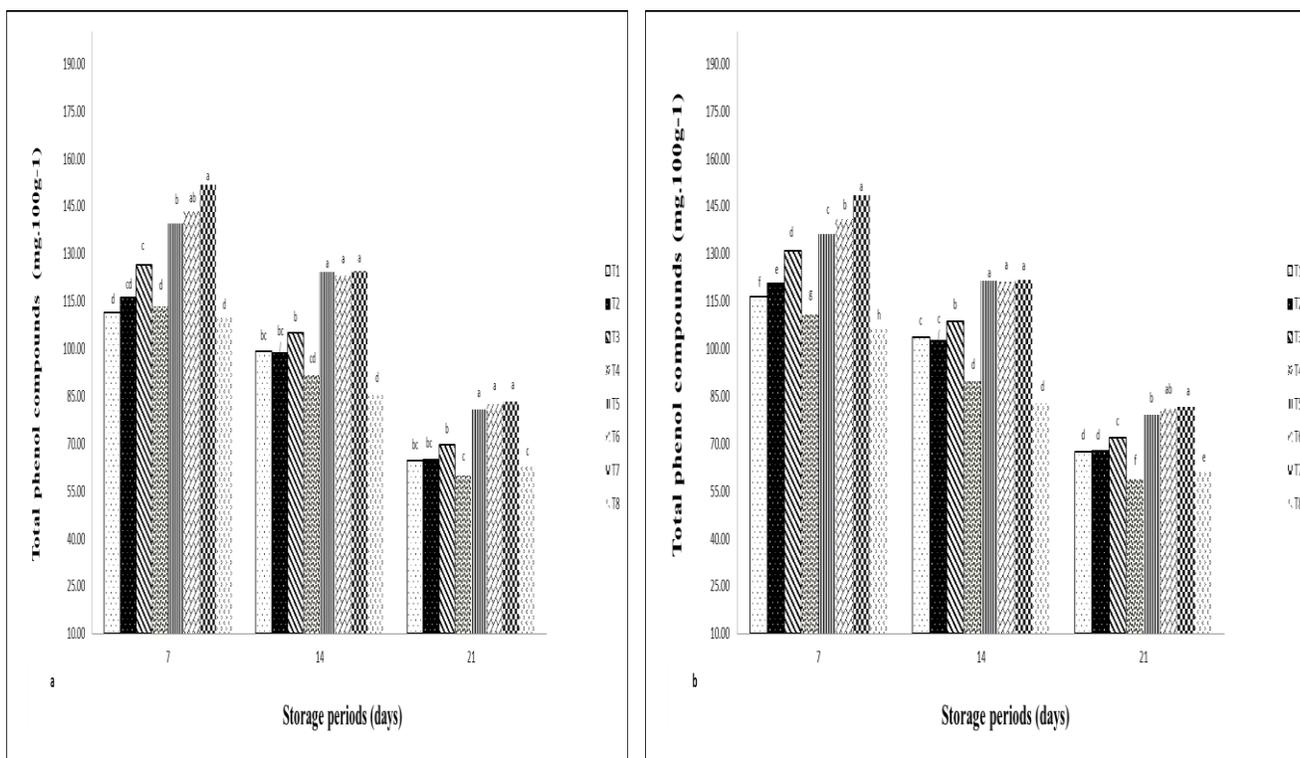
**Fig 7. Effects of biofertilizer applications on snap bean cultivars' Ascorbic acid during storage seasons 2020-2021.**

T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + Rhizobium), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + Rhizobium), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).

**3.5.8. Total phenol compounds**

Data in fig. (8) Showed that the trend of total phenol content was significantly decreased with increasing storage period. In the first season after 7 days of storage, the highest values of total phenol were recorded with seaweed foliar application and rhizobium with Tema cultivar. After 14 and 21 days all biofertilizer treatment with Tema cultivar had the

highest values of total phenol content. While, in the second season after 7 days the interaction between seaweed extract with Tema cultivar had a significant effect on total phenol content. After 14 days of storage Tema cultivar with all biofertilizer had the highest values of total phenol content. At the end of storage seaweed extract had the highest values of total phenol content.



**Fig 8. Effects of biofertilizer applications on snap bean cultivars' Total phenol compounds during storage seasons 2020-2021.**

T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + Rhizobium), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + Rhizobium), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).

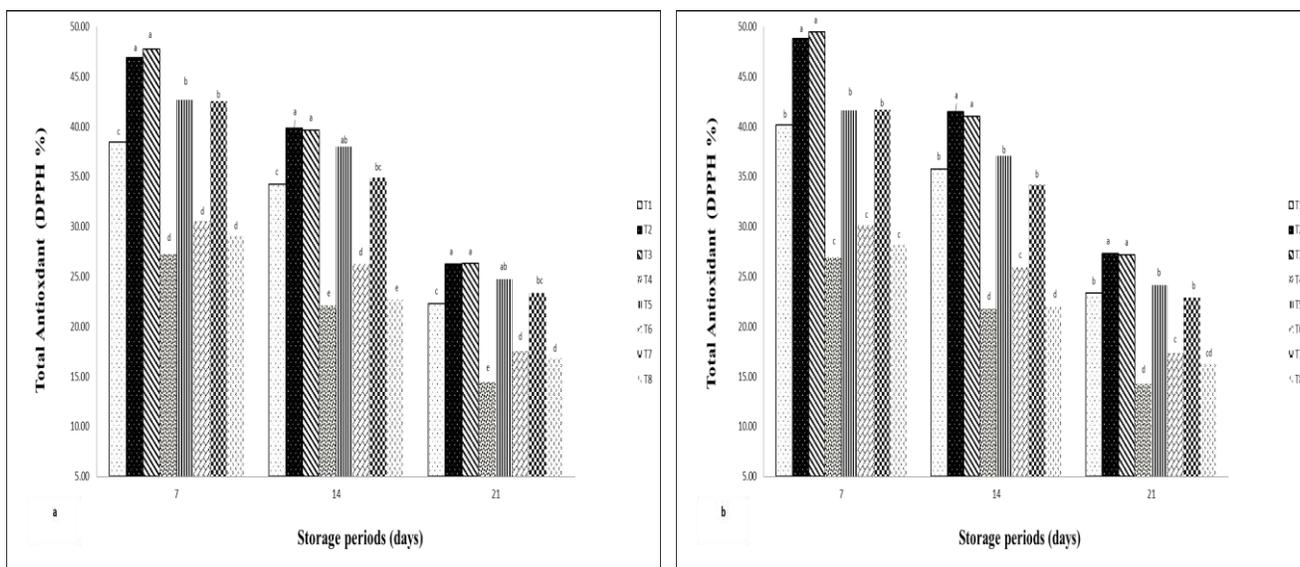
### 3.5.9. G-Total antioxidant

In all of the studied treatments, the total antioxidant content of pods in the treated plants with various bio-fertilizers considerably decreased with longer storage times, as seen in fig. (9). The highest value of total antioxidant content was observed with joty cultivar and seaweed extract after (7days) days in the first seasons. After 14 and 21 days of storage in the both seasons, the highest values of antioxidant capacity % were recorded in rhizobium and seaweed extract of joty cultivar with no significant of azospirillum treatment tema cultivar. According to numerous studies, seaweed extracts increase ascorbate peroxidase activities (Ayad, 1998), demonstrating the powerful antioxidant effects of seaweeds, which have been

associated with bioactive compounds (Meenakshi et al., 2009; O'Sullivan et al., 2011).

### 3. CONCLUSION

The findings of this study offer fresh insight into the usefulness of biofertilizer in reducing the adverse effects of climate change on horticultural crops. The two cultivars of snap beans reacted to each treatment differently. Seaweed foliar spray improves Joty cultivar's vegetative growth (plant height, number of leaves per plant, leaf area per plant, total greenish chlorophyll (SPAD reading), yield, and its components). After 21 days of storage at 5C, the foliar spray of seaweed extract preserved the storageability of snap beans.



**Fig 7. Effects of biofertilizer applications on snap bean cultivars' Total antioxidant during storage seasons 2020-2021.**

T1 (Joty cv. + *Azospirillum brasilin*), T2 (Joty cv. + Rhizobium), T3 (Joty cv. + Seaweed), T4 (Joty cv. + control), T5 (Tema cv. + *Azospirillum brasilin*), T6 (Tema cv. + Rhizobium), T7 (Tema cv. + Seaweed), T8 (Tema cv. + control).

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## الملخص العربي

# تحسين إنتاجية و قدرة الفاصوليا علي التخزين بواسطة الأسمدة الحيوية كحلول للزراعة المستدامة المتأثرة بتغير المناخ

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تعتمد استراتيجيات التأقلم مع تغير المناخ والتخفيف من حدته بشكل كبير على التكنولوجيا الحيوية. وبالتالي ، فإن تطوير واستخدام الأسمدة الحيوية التي تحتوي على البكتيريا والطحالب المفيدة هي تقنية محتملة لتحسين الحاصلات البستانية. لتقييم تأثير الأسمدة الحيوية مثل مستخلص الطحالب البحرية ، *Azospirillum brasilense* و *Rhizobium phaseolus vulgaris* على تعزيز الإنتاجية والجودة وقابلية التخزين لصنفين (تيا وجوتي) من الفاصوليا. أجريت التجربة خلال موسمي صيف ٢٠٢٠ و ٢٠٢١ بمحطة التجارب الزراعية بجامعة القاهرة. تم دراسة أربعة معاملات: رش ورقي لمستخلص الأعشاب البحرية السائلة (٢ مل / لتر) ، معاملة ارضيا لكلا من *Azospirillum* و *Rhizobium phaseolus vulgaris* (٣٠٠ جم / فدان لكل كائن حي دقيق) ، ومعالجة التحكم (رش الماء). تم تخزين القرون بعد الحصاد من كل معاملة في عبوات بولي إيثيلين لمدة ٢١ يوماً عند ٥ درجة مئوية. وأظهرت النتائج أن التطبيق الورقي للأعشاب البحرية حقق أعلى (ارتفاع النبات ، عدد الأوراق / النبات ، محتوى الكلوروفيل ، ومساحة الأوراق) لكلا الصنفين. المحصول وجودة القرون (ثبات القرون ، القطر ، الوزن) للصنف جوتي بينما تم تسجيل أعلى محتوى للمواد الصلبة الذائبة في صنف تيا في جميع المعاملات المختبرة مقارنة بمعاملة الكنترول. تم تسجيل أعلى محتوى من حمض الاسكوريك و المواد الصلبة الذائبة والمظهر الخارجي ونشاط مضاد للأكسدة عند التخزين لمدة ٢١ يوماً علي ٥ درجة مئوية. ويمكن تلخيص ذلك بأن الرش الورقي لمستخلص الأعشاب البحرية ٢% يمكن استخدامه كمحفز لتحسين النمو الخضري والمحصول وقابلية تخزين الفاصوليا

**الكلمات المفتاحية:** التسميد الحيوي، تغيرات المناخ، الإنتاجية، قدرة علي التخزين، الفاصوليا