



## Peroxidase Isozyme Activity in Parsley and Celery Using Efficient Microbes, Kitchen Wastes and Other Combinations of Bio and Organic Fertilizers

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### ABSTRACT

Pot experiments were carried out in the greenhouse at the faculty of Agriculture (Saba Basha), Alexandria University, Egypt, in 2017/2018 season to estimate the response of parsley (*Petroselinum crispum* Mill) and celery [*Apium graveolens* L.) Balady *sp.*] to different combinations of bio and organic fertilizers i.e. [1-Chicken manure, 2- (Kitchen wastes + LAB (*lactobacillus spp.*), 3- (Peat moss+ Yeast + Molasses), 4-Seaweed algae, 5- plant compost, and 6- Control NPK ] on seed germination period, percentage and vegetative growth characters. A completely randomized block design with four replicates for each of them were carried out. According to the results, vegetative growth parameters, shoot length (cm), shoot and root fresh and dry weight, and concentrations of N, P, K, Zn, Mn and Fe in parsley and celery leaves significantly increased by the application of Chicken manure, (Kitchen wastes + LAB), (peat moss +Yeast+ Molasses), Seaweed algae and plant compost treatments, gave the highest mean values of the given characters, meanwhile the control treatment gave the lowest mean values. Also, these combinations of bio and organic fertilizers reduced the long time of seed germination period and increased seed germination percentages of parsley and celery. Isozyme analysis discussed the performance of peroxidase activities on two types of leafy vegetables mentioned above. The results showed that the banding pattern activity of the treatment (A-1, 2 and 3), displayed a unique marker bands at four loci (Px.C<sub>1</sub>and Px.C<sub>2</sub>) and (Px.A<sub>1</sub>and Px.A<sub>2</sub> loci) are polymorphic specifically to parsley plant. In the same trend the treatment (B-1, 2 and 3) can be conducted that the peroxidase patterns in celery plant leaves showed in total of four loci (Px.C<sub>1</sub>, Px. C<sub>2</sub>, Px. A<sub>1</sub> and Px.A<sub>2</sub>).Therefore, the best treatments were detected in parsley and celery when treated with Chicken manure, (Kitchen wastes + LAB), (Peat moss+ Yeast + Molasses) and seaweed algae to obtain healthy and safety food.

**Keywords:** Peroxidase isozyme analysis, Chicken manure, (Kitchen wastes + Lactic acid bacteria), (Peat moss +Yeast + Molasses), Seaweed algae, Plant compost, Parsley and Celery.

### Introduction

Celery (*Apium graveolens* L) is a plant from the *Apiaceae* family, and is one of the annual or perennial plants that grow throughout Europe and the tropical and subtropical regions of Africa and Asia (Gauri *et al.*, 2015). Celery is cultivated as a leafy vegetable crop or medicinal herb crop (Kolarovic *et al.*, 2009). Seeds are important in human nutrition as they are rich in fatty acids, proteins, phytochemicals and micronutrients (Sarah and Diana 2021). Active compounds such as alkaloids, coumarins, flavonoids, glycosides, limonene, myrcene, phenols, phthalides, and steroids present in celery seeds are responsible for different medicinal properties (Danijela *et al.*, 2021). Medicinal properties of celery seeds include anticancer, antidiabetic, anti-hyperlipidemic, antioxidant, antimicrobial and anti-inflammatory activity (Sarah and Diana 2021). The parts are used in this plant include seeds, leaves, and essential oils (Bhattacharjee 2004). Celery can prevent cardiovascular diseases, (Sowbhagya *et al.*, 2010) jaundice, liver and lien diseases, (Nadkarni 2010; Grzanna *et al.*, 2005; Zare *et al.*, 2016). Celery reduces glucose, blood lipids and blood pressure, which can strengthen the heart (Kooti *et al.*, 2014).

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Parsley (*Petroselinum crispum* Mill.) is one of the most important vegetable crop belongs to the family *Apiaceae*. It imparts a wonderful savory taste and herbaceous flavor. Parsley is the most valuable for the nutritional benefit, mineral matter, ascorbic acid and other biologically active compounds, which supply human food allowances with the stuff of natural origin (Orav *et al.*, 2003; Vokk *et al.*, 2011; Danijela *et al.*, 2021). The plants are cultivated extensively for the purpose of sending their fresh leaves to markets. It is also available in dried and powdered form to be used as a culinary flavouring, especially in the winter months. Chopped parsley fresh leaves are used in soups, stuffings, minces, rissoles and also as a garnish over vegetables and salads. Furthermore, its leaves are used widely in folk herbal medicine, pharmaceutical industries and cosmetics (Khan and Abourashed, 2011; Peter, 2012; Aburto-Gill, 2014).

The use of effective microorganism as (*Lactobacillus spp.*) has become an important part of nature farming. Because most of the microorganisms in EM cultures are heterotrophic (CAI *et al.*, 2012), i.e., they require organic sources of carbon and nitrogen. Efficient microbes (EM) has been most effective when applied in combination with organic amendments to provide carbon, nitrogen and energy (Kengo and Xu 2001). The genus (*Lactobacillus spp.*), belongs to the phylum Firmicutes, family *Lactobacillaceae* (Felis and Delagio, 2007; Herbel *et al.*, 2013). This group of microorganisms are found in different environments, such as natural niches like plants, soil, and vegetables. It can also be isolated from beverages and foods such as wine, milk, kefir, meat, fruit, vegetables, cereals and dairy products, mainly in yogurts and cheeses (Pál *et al.*, 2012). Soil is one of the key elements for supporting life on Earth. It delivers multiple ecosystem services, which are provided by soil processes and functions performed by soil biodiversity. In particular, soil efficient microbes are one of the fundamental components in the sustainment of plant biomass production and plant health (Marta *et al.*, 2021).

Peat moss as growth substrate, is an important component in the growth of plant seedlings (Schmilewski, 2008). The peat moss can become a major carbon emitter (Gaudig, 2008). Peat moss is noted for its qualities to retain moisture, good structure, and sterility (Schmilewski, 2008), but it is a non-renewable and very expensive substrate. Good germination rates for peat moss can be attributed among other factors, to high biological stability and low salt levels (Zapata *et al.*, 2005). The advantages from using peat moss are as follow: (1) making soilless systems more inclusive of sustainable and eco-friendly growing substrates, possibly available at a local level, (2) replacing chemicals with more sustainable products (e.g., organic active compounds) which is possible for plant nutrition and protection (Giulia *et al.*, 2021).

Many attempts were made to prepare a bio-fertilizer using effective microorganisms including bacteria and yeast. Recently, yeast became a positive alternative to chemical fertilizers safely used for humans, animals, plants and environment (Omran, 2000). Many studies indicated that plant root growth may be directly enhanced by yeast in the rhizosphere (Nassar *et al.*, 2005; Cloete *et al.*, 2009). A wide diversity of yeast has been researched for its potential as a bio-fertilizer (Gomaa and Mohamed, 2007; Eman *et al.*, 2008). Several yeast strains with different metabolic activities were investigated for their ability to reduce the sugar content in red beet juice, which resulted in a decrease in the extract level corresponding to sugar content for diabetes. This strategy was found to have the additional advantage of increasing the mineral and microbial stability of the red beet juice (Dawid *et al.*, 2021).

Molasses is a primary by-product in the fermentation industry and can be used in the food industry such as sugar and yeast production (Liang *et al.*, 2008; Babu *et al.*, 2012). Molasses is characterized by a dark brown color and exhibit a high content of substances that are found in nature, such as melanoidins, and its pigment is difficult to be degraded by microorganisms (Satyawali and Balakrishnan 2008; Shan *et al.*, 2016). Moreover, Molasses has a high concentration of organic materials and soluble, as well as high biological oxygen demand (BOD) and chemical oxygen demand (COD) [Robles-Gonzalez *et al.*, 2012]. Due to the presence of poor bio-degradable compounds, such as melanoidins. The treatment of molasses has become the best. Moreover, molasses is rich in mineral elements including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), micronutrients and organic matter (Anish *et al.*, 2019 ; Srivastava *et al.*, 2012).

Seaweed extracts have been marketed as organic fertilizers and have beneficial results (Khan *et al.*, 2009). They observed that the value of seaweeds as fertilizers were not only due to nitrogen, phosphorus and potash content, but also because of the presence of trace elements. These extracts enhance seed germination, growth, yield and uptake of nutrients by the plants. Thus, these extracts when applied to seeds or when added to the soil, stimulate growth of the plants (Taha *et al.*, 2011; Mona and

Abou El-Goud 2021). The extract also contained hormones like (IAA and IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, Ni), vitamins and amino acids (AbouEl-Yazied *et al.*, 2012).

Successful organic production depend on strong and healthy seedlings. Accordingly, usages of organic fertilizers like kitchen wastes, compost tea, vermicompst etc. (Abou El-Goud *et al.*, 2021; Mona and Abou El-Goud 2020; Mohammad *et al.*, 2015), could possibly be one of the effective techniques for raising seedlings in organic production system. Organic fertilizers can be an effective alternative to chemical fertilizers as they contain high levels of nutrients and organic matter (Shabani *et al.*, 2011). This technique has the potential to use in adverse condition as the pots can be easily moved in safe places and ultimately facilitates the production of seedlings in due time and enhance seed germination and percentage.

Peroxidase isozyme analysis is useful in expanding knowledge and application of crop genetics with regard to linkage relationships and the distribution of variation (Lauri and Joseph, 1990). The term isozyme was first introduced by Market and Moller, (1959) which refers to multiple molecular forms of an enzyme with similar or identical substrate specificity in the same organism. Later stated that isozyme is conceived as an operational term, (Duchovskiene and Siksnianiene 2001) had defined isozymes as multiple forms of enzymes in the same organism and having similar or identical catalytic activities. It is important to notice that peroxidase enzyme analysis by electrophoresis offers very well defined and effective tools for detection of genetic differences among individuals (Francine *et al.*, 2021).

The aims of this study are to focus on the Peroxidase isoenzyme printing reaction of peroxidase enzyme activity in parsley and celery which activated by treated with the combinations of bio and organic fertilizers by using isozyme analysis which used in the present study -as gene markers- for studying the variation between individual treatments. In addition to study the influence of these bio and organic fertilizers to reduce the long time of seed germination period and increased germination percentages of parsley and celery. Also, the use of bio and organic combinations which is an environment-friendly, economical and ergonomically sound practice which has already been established recently (Van der Vossen, 2005).

## 2. Materials and Methods

### 2.1. Experimental location and arrangement

Pot experiments were carried out in the greenhouse at the Faculty of Agriculture (Saba Basha) Alexandria University, during the growing season of 2017/ 2018. The sandy soil used in this study was collected from Burg El-Arab City, Alexandria, Egypt from the surface layer to plough depth (down to 20 cm). The soil was air-dried, sieved through a 2 mm sieve to homogenize. The experimental soil was sampled at the beginning of the growing season and analyzed for some physical and chemical properties according to the methods described by Page *et al.* (1982) are presented in Table (1), the Chemical analysis of plant compost illustrated in Table 2.

**Table 1:** Some physical and chemical properties of the experimental soil at the growing season of 2017/2018.

Physical and chemical properties	
Sand (%)	96.5
Silt (%)	1.7
Clay (%)	1.8
O.M (%)	0.08
Soil texture	Sand
Available N (ppm)	6.9
Available P (ppm)	6.2
Available K (ppm)	64
Calcium carbonate (%)	0.26
pH	8.1

**Table 2:** Chemical analysis of plant compost used during season, 2017 / 2018.

Parameters	Sample	Unit
PH (1:10 Water extract)	9.5	
EC (1:10 Water extract)	1.6	ds/m
O.M	15.40	%
O.C	8.93	%
C/N	34.3:1	
<b>Total nutrients</b>		
Nitrogen (N)	0.26	%
Phosphorus (P)	0.58	%
Potassium (K)	0.40	%

**Table 3:** Chemical analysis of chicken manure used during season 2017/2018.

Chicken manure properties			
pH (1:10)	7.2	KCl -extractable (N)	4.7%
EC (dS m <sup>-1</sup> ) (1:10)	6.2	NH <sub>4</sub> -Ac- extractable (k)	1.0%
O.M	16%	NaHCO <sub>3</sub> - extractable (P)	1.64%
O.C	9.28%	Soluble cations (meq / L)	
C/N	1.97	Ca <sup>2+</sup>	3.4
Moisture	41.0%	Mg <sup>2+</sup>	6.8
		Na <sup>+</sup>	8.15
		K <sup>+</sup>	17.95
		Soluble anions (meq / L)	
		HCO <sub>3</sub> <sup>-</sup>	40.0
		SO <sub>4</sub> <sup>2-</sup>	22.93

## 2.2. Experimental materials

Program of organic fertilizers in parsley and celery (cultivar: Balady *sp.*) were applied during fall season 2017/ 2018 in the greenhouse pot experiments at the Faculty of Agriculture (Saba-Basha), Alexandria University. Seeds were planted on 5<sup>th</sup> of September for each of them. Six treatments were carried out in this investigation for parsley plant i.e.

A-1 Chicken manure

A-2 Kitchen wastes + LAB [Lactic acid bacteria (*Lactobacillus spp.*)]

A-3 Peat moss + Yeast + Molasses

A-4 Seaweed algae

A-5 plant compost

A-C Control treatment (recommended doses of chemical fertilizer N, P and K).

And six treatments were carried out in this investigation for celery plant i.e.:

B-1 Chicken manure

B-2 Kitchen wastes + LAB [Lactic acid bacteria (*Lactobacillus spp.*)]

B-3 Peat moss+ Yeast + Molasses

B-4 Seaweed algae

B-5 plant compost

B-C Control treatment (recommended doses of chemical fertilizer N, P and K).

## 2.3. The composition and amount of each material.

Each plastic pot (15cm in diameter and 12cm deep) was washed, labeled, and a filter paper placed on the bottom of each pot to prevent soil leakage. The soil was mixed with the organic and bio materials of each pot on a weight per weight (w/w). After homogenization, each sample was transferred into pots in 4 replicates for each application and for each cultivar at the greenhouse pot experiments. The soil was mixed with the combinations of bio and organic fertilizers four days before planting. The pots were seeded with small amount of seeds of parsley (*Petroselinum crispum* Mill) and other pots with celery (*Apium graveolens* L) Balady *sp.*, on the 5<sup>th</sup> of September. The pots were watered to 80% field capacity until the end of vegetative growth period. Thinning was done at 10 days after sowing for organic treatments meanwhile after 20 days for control treatment by retain ten healthy seedlings per pot.

1- Chicken manure: was added at 4 ton/fed.

2- Kitchen wastes +LAB: It was added at 6 ton/fed.

## 3.3. Preparation of Lactic acid bacteria (*Lactobacillus spp.*).

### I. Then preparation of Kitchen wastes + LAB (Lactic acid bacteria) treatment.

Lactic acid bacteria was prepared by adding rice (1 kg), in water (1000 ml) and then filtered from rice and took rice water washing and put it in a big jar then covered with a piece of cloth and then put it in a worm place for 7 days for carry out the fermentation process. After that it was added about 5 liters of milk to the fermented rice water washing to investigate another fermentation process, then covered and put this jar in a worm place for another 10 days, then took the precipitate that contained billions of (*lactobacillus spp.*) bacteria (Kanbe, 1990; Koto, 1992; Uchimura and Okada, 1992). At planting date the seeds were mixed with the precipitate of (*lactobacillus spp.*) bacteria two hours before

planting. Kitchen wastes material were collected from houses, then air dried and grinded into small pieces (Alok 2010). These grinded wastes material were mixed with LAB prepared above and were subjected aerobic composting to initiate microbial activity. Moisture content of the mixture was maintained to 60% to 70% and this mixture was then kept in plastic containers covered with paper having holes to facilitate aeration in order to get final composted material. This mixture was hand manipulated at regular time intervals for sufficient microbial activity. Kitchen wastes +LAB were mixed with the sand soil (1:1) four days before planting.

## II. Preparation of (Peat moss +Yeast + Molasses).

Firstly, 250 g of bread yeast (*Saccharomyces cerevisiae*) were dissolved into 400 ml of molasses with good flipping and put them in a warm place two hours before using it. The seeds of parsley and celery were soaked in the mixture of yeast and molasses two hours before planting. Secondly, Peat moss + yeast+ molasses were prepared by mixed the peat moss with the mixture of yeast and molasses mentioned above and mixed with the sand soil (1:1) four days before planting. After planting in a growth chamber for 45 days plant height, shoot and root fresh and dry weight and plants isozyme activity were measured and recorded.

**Seaweed algae:** was added at 4kg/fed. It was mixed with the soil four days before planting.

**Plant compost:** was added at 6 ton/fed. It was mixed with the soil four days before planting.

**Control** (recommended doses of chemical fertilizer N, P and K for celery consist of Ammonium sulphate 33.5% = 100 kg/fed. + Super calcium phosphate 15.5%=300 kg/fed. + Potassium sulphate 48% = 50 kg/fed).And for parsley consist of Ammonium sulphate 33.5% = 100 kg/fed. + Super calcium phosphate 15.5%=50 kg/fed. + Potassium sulphate 48% = 25 kg/fed).

### 3.4. Vegetative growth characters:

Six plants were randomly taken from each experimental unit for determination of growth characteristics. Shoot length (cm), shoot and root fresh and dry weight. After 45 days from planting, Shoot length was measured from the ground level to the tip of the tallest leaf (Fageria *et al.*, 2006) using a meter ruler, as well as fresh and dry weights (g/plant) were recorded After 45 days from planting for shoots and roots as a whole plant.

### 3.5. Plant chemical analysis

#### I. N, P, K and (Zn, Mn and Fe) contents in leaves

In order to determine the mineral contents for parsley and celery leaves, dry samples of leaves were finely ground for chemical analysis. The oven dried plant material samples were wet digested by using concentrate of H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O<sub>2</sub> (Lowther, 1980). Mineral elements N, P and K and micronutrients (Zn, Mn and Fe) were determined in leaves using the method described by A.O.A.C. (1990), Chapman and Pratt (1978). The Zn, Mn and Fe concentration the solutions were measured by Atomic Absorption Spectrophotometer (Perkin Elmer-3300) according to Chapman and Pratt (1978).

### 3.6. Seed germination of parsley and celery

Seed germination period (days) and seed germination percentage (%) were investigated in this study in order to determine the effect of bio and organic fertilizers on enhancing and improving of seed germination period and percentage of parsley and celery.

### 3.7. Isozymes analysis

This study was investigated in the Genetic laboratory at the faculty of Agriculture Saba- Basha, to study the profile of peroxidase isozyme expressed in leaves of Celery (*Apium graveolens var. dulce*) and Parsley (*Petroselinum crispum* Mill) Balady *sp.*, after 45 days from planting was used in the present study -as gene markers- for studying the genetic polymorphism between bio-organic fertilizers and inorganic fertilizers (control) of parsley and celery plants. As conventional symbols in electrophoretic analysis, a pattern was first described in terms of Anodal (A) and Cathodal (C) zones according to their direction of mobility in the electrophoretic field. Each zone is assigned for a locus coding for an Peroxidase isozyme. Twenty four plants for each cultivar were examined individually for their isozyme patterns. A combination of agar-starch gel electrophoresis and enzyme activity attaining was used to

screen for polymorphisms of peroxidase. The laboratory methods were performing according to Jonathan and Norman (1989). Samples from 10 seedlings were separately ground using cooled mortar with pestle by addition of 0.23 M Tris-acetate, pH 5.0. Homogenate was extracted by the solution containing Tris (27.7 g) and citric acid (11.0 g) in 1L volume adjusted with distilled water. Electrophoresis was carried out by the prescriptions recommending 1% agar-starch-polyvinylpyrrolidone gel and Tris-borate or Tris-acetate separation buffers (Sabrah, 1980). Electrophoresis was conducted at 270 v, 40°C for 100 min. 100 ml of 0.01 M acetate buffer, pH 5.0, containing 0.1% benzidine and 0.5% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were layered over the gel before staining.

### 3.8. Statistical Analysis

The experiments were structured following a randomized complete block design (RCBD) with four replications. Data were statistically analyzed using Costat Software (Steel and Torrie, 1980), and treatments means were compared using Duncan's Multiple Range test at 5% level of probability. Also, SAS Institute Inc 2013. SAS/ACCESS® 9.4. were used for analysis of seed germination period (days) and percentage (%).

## 3. Results

The results of the soil analysis before cultivation (Table 1) showed that the soil used in the experiment was sand texture. The amounts of available macro and micro-elements were in the range of low availability to plants as compared to standard values (Soltanpour, 1985). The amount of available phosphorus of the soil was below the range of high availability according to Landon (1991). This finding further signifies that the soil requires external application of nutrients for high growth and yield of the crop.

### 3.1. Vegetative Growth of (Parsley and celery)

Data in Table (4 and 5) showed that all vegetative growth characters of parsley and celery (*Balady sp.*) were significantly affected by applying different bio and organic fertilizers compared with the control. The results showed that all treatments of bio and organic combinations significantly increased vegetative growth including shoot length, fresh and dry weight for shoots and roots. Among the treatments data in Table (4 and 5) showed that, application of chicken manure and (Kitchen wastes + LAB) recorded the highest mean values for shoot length without significant difference as (14.05 and 12.85 cm for parsley) and as (14.25 and 14.20 cm for celery) respectively. Also highest mean values for fresh weight of shoots and roots (0.725 for A<sub>1</sub> and A<sub>2</sub> then 0.525 for A<sub>3</sub> g plant<sup>-1</sup> for parsley) and (B<sub>1</sub> as 2.68, then followed by B<sub>2</sub> and B<sub>3</sub> as 2.18, 2.10 g plant<sup>-1</sup> for celery) regularly, these data were produced from the treatments of chicken manure, (Kitchen wastes + LAB) and (Peat moss+ Yeast + Molasses). In the same trend the highest mean values of shoot and root dry weight recorded when applied with chicken manure, (Kitchen wastes + LAB) and (Peat moss+ Yeast + Molasses) as (145 for both treatments and 105 g plant<sup>-1</sup> for parsley) and (0.54 then 0.44 and 0.42 g plant<sup>-1</sup> for celery).

**Table 4:** Effect of different combinations of bio and organic fertilizers on vegetative growth characters of parsley (*Balady sp.*) for season 2017/2018.

Treatments	Shoot length (cm)	Shoot and root fresh weight (g/plants)	Shoot and root dry weight (g/plants)
A-1 Chicken manure	14.05 a	0.725 a	0.145 a
A-2 Kitchen wastes + LAB	12.85 ab	0.725 a	0.145 a
A-3 Peat moss+ Yeast + Molasses	11.35 bc	0.525 ab	0.105 ab
A-4 Seaweed algae	11.35 bc	0.475 b	0.095 b
A-5 plant compost	10.98 c	0.450 b	0.090 b
A-C Control (N,P,K)	7.75 d	0.30 b	0.060 b
L.S.D 0.05	1.580	0.226	0.045

Values with the same alphabetical letters, within a comparable group of means, don't significant differ, using L.S.D test at 0.05 level.

**Table 5:** Effect of different combinations of bio and organic fertilizers on vegetative growth characters of celery (*Balady sp.*) for season 2017/2018.

Treatments	Shoot length (cm)	Shoot and root fresh weight(g/plants)	Shoot and root dry weight (g/plants)
B-1 Chicken manure	14.25 a	2.68 a	0.54 a
B-2 Kitchen wastes + LAB	14.20 a	2.18 b	0.44 b
B-3 Peat moss + Yeast + Molasses	12.50 b	2.10 b	0.42 b
B-4 Seaweed algae	12.50 b	1.63 c	0.33 c
B-5 plant compost	12.40 b	1.45 c	0.29 c
B-C Control (N,P,K)	7.25 c	0.88 d	0.18 d
L.S.D 0.05	1.097	0.426	0.085

Values with the same alphabetical letter (s), within a comparable group of means, don't significant differ, using L.S.D test at 0.05 level.

### 3.2 Chemical Analysis of parsley and celery

As shown in Table (6 and 7), the macro elements (N, P and K) and micronutrients (Zn, Mn and Fe) contents in leaves of parsley and celery plants under investigation were indicated as follow: treatment of chicken manure significantly, produced higher values of nitrogen content in leaves of parsley and celery as (2.01 and 2.11 g/kg) respectively. On the same trend, the highest phosphorus in leaves significantly obtained from (A-1, A-2 and A-3 for parsley) and (B-1, B-2 and B-3 for celery), compared with the control which gave the lowest values.

**Table 6:** Effect of different combinations of bio and organic fertilizers on chemical constitutes in leaves of parsley (*Balady sp.*) for season 2017/2018.

Treatments	N (g/kg)	P (g/kg)	K (g/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
A-1 Chicken manure	2.01 a	0.28 a	10.62 a	2.85 a	3.01 a	3.82 a
A-2 Kitchen wastes + LAB	1.91 b	0.27 b	9.85 b	2.72 ab	2.80 a	2.47 b
A-3 Peat moss+ Yeast + Molasses	1.72 c	0.26 b	9.60 bc	2.34 bc	2.55 a	2.46 b
A-4 Seaweed algae	1.55 d	0.20 c	9.22 c	2.27 c	1.68 b	2.01 bc
A-5 plant compost	1.32 e	0.17 d	8.59 d	2.24 c	1.44 b	1.93 bc
A-C Control (N,P,K)	0.46 f	0.06 e	5.33 e	1.34 d	1.39 b	1.39 c
L.S.D 0.05	0.007	0.004	0.390	0.390	0.758	0.722

Values with the same alphabetical letters, within a comparable group of means, don't significant differ, using L.S.D test at 0.05 level.

**Table 7:** Effect of different combinations of bio and organic fertilizers on chemical constitutes in leaves of celery (*Balady sp.*) for season 2017/2018.

Treatments	N (g/kg)	P (g/kg)	K (g/kg)	Zn (mg/kg)	Mn (mg/kg)	Fe (mg/kg)
B-1 Chicken manure	2.11 a	0.27 a	16.03 a	3.62 a	3.96 a	3.89 a
B-2 Kitchen wastes + LAB	1.98 b	0.27 a	13.29 b	3.17 b	3.90 a	3.72 b
B-3 Peat moss+ Yeast + Molasses	1.89 c	0.26 b	12.00 c	3.06 b	3.75 a	2.22 b
B-4 Seaweed algae	1.88 d	0.25 c	10.39 d	2.63 c	3.51 a	2.13 c
B-5 plant compost	1.55 e	0.21 d	9.23 e	2.58 c	2.75 b	1.63 cd
B-C Control (N,P,K)	1.31 f	0.16 e	8.68 f	1.37 d	1.33 c	1.42 d
L.S.D 0.05	0.009	0.003	0.345	0.261	0.530	0.508

Values with the same alphabetical letter (s), within a comparable group of means, don't significant differ, using L.S.D test at 0.05 level.

For potassium content in parsley and celery leaves, the highest values obtained from using treatments (A-1 as (10.62),A-2 as (9.85),A-3 as (9.60) and A-4 as (9.22) g/kg for parsley) and (B-1 as (16.03), B-2 as (13.29),B-3 as (12.00) and B-4 as (10.39) g/kg for celery), meanwhile the lowest values obtained from the control treatments. Also, the same trend were found in the content of leaves of Zn, Mn and Fe.

As for zinc content in parsley and celery leaves, the highest values obtained from using treatments (A-1 as (2.85) and A-2 as (2.72) mg/kg for parsley) and (B-1 as (3.62), B-2 as (3.17) and B-3 as (3.06) mg/kg for celery). At the same trend for manganese content in parsley and celery leaves, the highest values obtained from using treatments (A-1 as (3.01), A-2 as (2.80), A-3 as (2.55) and A-4 as (1.68) mg/kg for parsley) and (B-1 as (3.96), B-2 as (3.90), B-3 as (3.75) and B-4 as (3.51) mg/kg for celery). Meanwhile for iron content in parsley and celery leaves, the best values obtained from using treatments (A-1 as (3.82), A-2 as (2.47) and A-3 as (2.46) mg/kg for parsley) and (B-1 as (3.89), B-2 as (3.72) and B-3 as (3.22) mg/kg for celery) compared with control treatments which gave the lowest values.

### 3.3. Effect of bio and organic fertilizer treatments on seed germination period and percentages of Parsley and celery

As shown in table (8) there are some problems in seed germination of parsley and celery especially celery that behave in taking a long time in seed germination period for about 15 to 18 days or more but when used some of these combinations investigated in this study, happened as follow:

**Table 8:** Effect of different combinations of bio and organic fertilizers on seed germination period (days) and seed germination percentage (%) of parsley and celery (*Balady sp.*) for season 2017/2018.

Treatments	Parsley		Celery	
	S.G. (days)	S.G. (%)	S.G. (days)	S.G. (%)
Chicken manure	7.00 bc	73.50 b	9.00 c	66.50 b
Kitchen wastes + LAB	6.00 c	76.50 a	8.50 c	72.25 a
Peat moss+ Yeast + Molasses	6.25 c	77.50 a	9.25 c	64.50 bc
Seaweed algae	6.75 bc	69.50 c	9.75 bc	62.25 cd
Plant compost	7.75 b	64.75 d	11.50 b	60.25 d
Control (N,P,K)	12.50 a	59.75 e	14.50 a	55.75 e
L.S.D 0.05	1.392	2.224	2.022	2.308

Values with the same alphabetical letter (s), within a comparable group of means, don't significant differ, using L.S.D test at 0.05 level.

#### I. Reducing time of germination period and increased seed germination percentage of parsley plant)

Plants which treated with bio and organic fertilizers germinated at 6 to 8 days and gave the highest mean value of germination percentage obtained from treatments: (chicken manure, Kitchen wastes + LAB, Peat moss + yeast + molasses and seaweed algae). Meanwhile parsley plant that treated with chemical fertilizers (recommended doses of chemical fertilizer N, P and K) germinated at 10 to 12 days and gave the lowest mean value of germination percentage as shown in fig (1 and 2).

#### II. Reducing time of germination period and increased seed germination percentage of celery plant

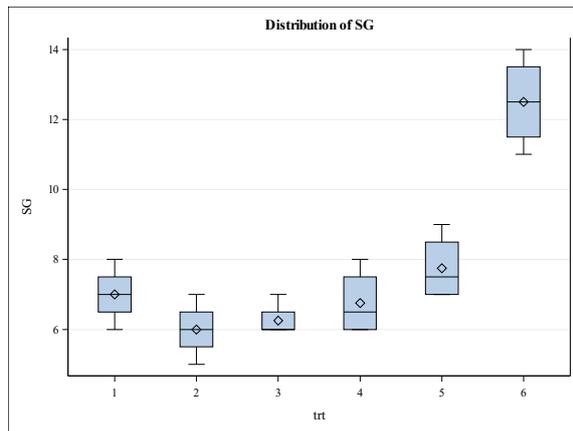
Plants which treated with bio and organic fertilizers germinated at 8 to 10 days and gave the highest mean values of germination percentage produced from treatments: (chicken manure, Kitchen wastes + LAB, Peat moss + yeast + molasses and seaweed algae). Meanwhile celery plant that treated with chemical fertilizers (recommended doses of chemical fertilizer N, P and K), germinated at 15 to 18 days and gave the lowest mean value of germination percentage as shown in fig (3 and 4).

So, Chicken manure, Kitchen wastes + LAB, Peat moss + yeast + molasses and seaweed algae treatments led to reduce the long time of germination period and enhanced seed germination percentage of parsley (76.50 and 77.50 %) and celery (72.25 %) after planting.

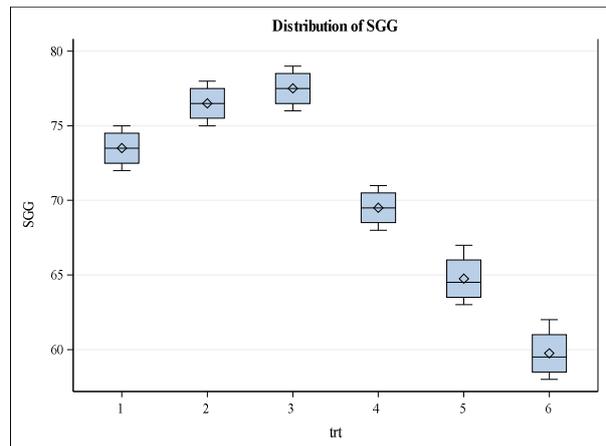
### 3.7. Isozymes analysis for Parsley and Celery

The zymogram and cluster showing mobility pattern of peroxidase isozymes are illustrated in Figure (5 and 6). From this data it can be concluded that the peroxidase patterns in the parsley and celery plants leaves showed in total of four loci. First, it was evident that all plants expressed the Px.A<sub>2</sub>,

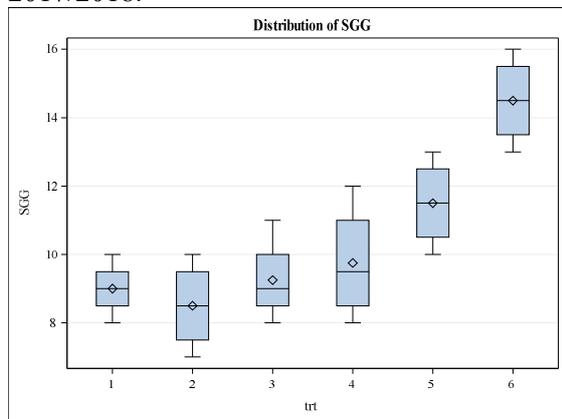
this locus indicated that, these common loci were consistently monomorphic expressed. Second, some treatment under different organic and bio fertilization displayed extra two common loci for treatment B-1 (Px.c<sub>1</sub>) for celery plant and specific unique locus for the treatment B-2 (Px.C<sub>2</sub>) locus polymorphic specifically to this treatment. While three specific unique locus for the treatment B-C, B-5 and B-4 (Px.A<sub>1</sub>). That is mean B-1 and B-3 activated two loci (PX.C<sub>1</sub>, PZ.A<sub>2</sub>) meanwhile, B-2 activated three loci as (PXC<sub>2</sub>, PXC<sub>1</sub> and PXA<sub>2</sub>) and the treatment of B-4, B-5 and B-C activated three loci as (PXC<sub>1</sub>, PXA<sub>2</sub> and PXA<sub>1</sub>). The specific unique locus were detected also in the plants of parsley except the control treatment such as the treatment of A-1,A-2,A-3,A-4 and A-5 activated three loci as (PXC<sub>2</sub>,PXA<sub>2</sub> and PXA<sub>1</sub>) meanwhile the control activated two loci only as (PX.C<sub>1</sub> and PX.A<sub>2</sub>).



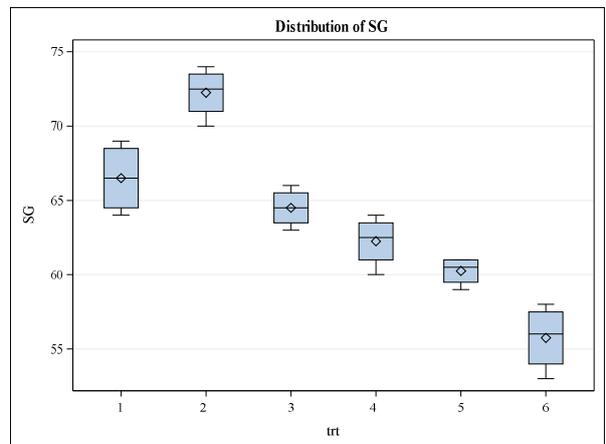
**Fig. 1:** Diagram of seed germination period (days) of parsley (*Balady sp.*) for season 2017/2018.



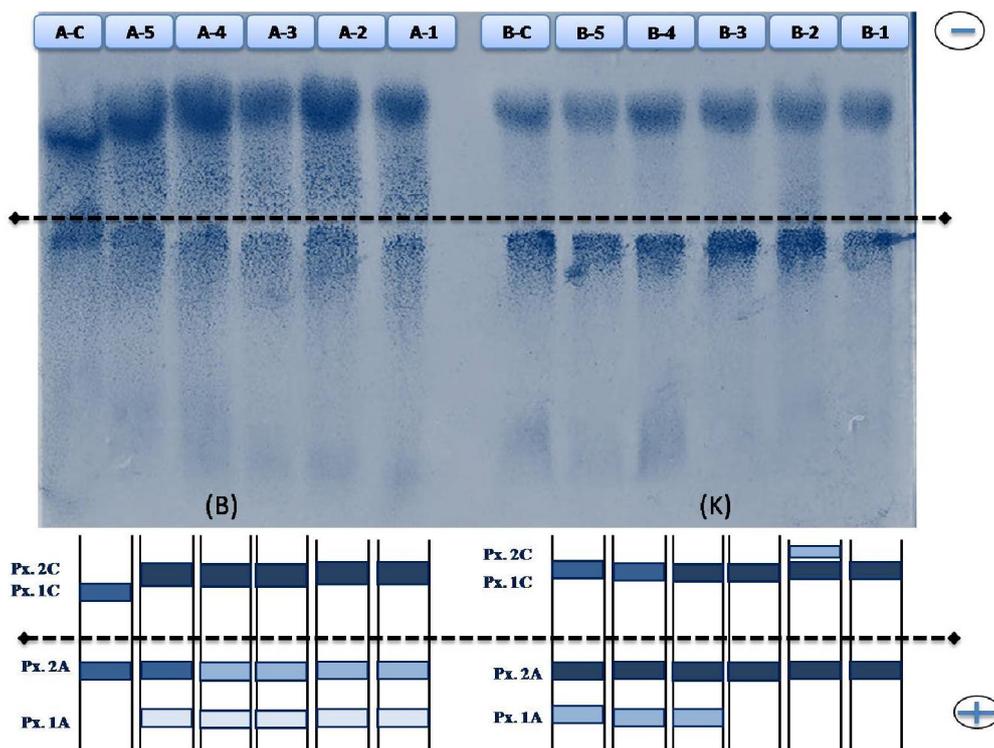
**Fig. 2:** Diagram of seed germination percentage (%) of parsley (*Balady sp.*) for season 2017/2018.



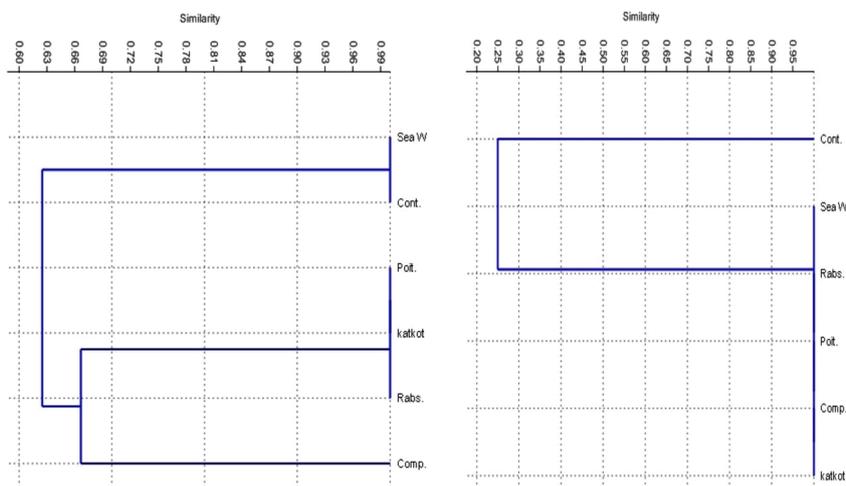
**Fig. 3:** Diagram of seed germination period (days) of celery (*Balady sp.*) for season 2017/2018.



**Fig. 4:** Diagram of seed germination percentage (%) of celery (*Balady sp.*) for season 2017/2018.



**Fig. 5:** Zymograms showing electrophoretic profiles of Peroxidase isozyme in Parsley as follow: from right to left i.e. (A-1) Chicken manure, (A-2) Kitchen wastes + LAB, (A-3), Peat moss+ Yeast + Molasses (A-4) Seaweed algae, (A-5) Plant compost and (A-C) control, and Celery as follow: from right to left i.e. (B-1)Chicken manure, (B-2)Kitchen wastes + LAB,(B-3), Peat moss+Yeast+Molasses (B-4) Seaweed algae, (B-5) Plant compost, (B-C) control .



**Fig. 6:** Dendrogram of different treatments of parsley and celery based on peroxidase enzymes (isozyme).

#### 4. Discussion

The results showed that all growth parameters of parsley (*Petroselinum crispum* Mill) and celery [*Apium graveolens* L], (*Balady sp.*), were significantly enhanced with the application of Chicken manure, (Kitchen wastes + LAB), (Peat moss + Yeast + Molasses), Seaweed algae and plant compost. Increasing in vegetative growth of parsley and celery cultivar (*Balady sp.*) in response to the application of bio and organic fertilizers were reported in previous works (Mahdi *et al.*, 2010). Saber (1994) stated that this increment might be due to the availability of soil microorganisms to convert the unavailable forms of nutrient elements to available forms from bio-fertilizers (Kurtzman and Fell, 2005). Chicken

manure added to the soil increased the vegetative growth characters of parsley and celery plants, due to the beneficial effect of organic matter in improving the nutritional status particularly nitrogen form in the soil, this is in accordance with (Mona and Amal, 2020), they stated that application of (Chicken manure only) recorded the highest average values of vine length character in watermelon. Also, (Amal and Mona 2019) reported that chicken manure application resulted in considerable increase on the productivity of summer squash fruits and the most of vegetative growth parameters including; plant height, number of leaves / plant. Statistical analysis showed that N, P and K concentration due to the application of chicken manure addition were significant. These are in agreement with other studies (Adeniyi and Ojeniyi, 2005; Davis *et al.*, 2006), several investigators reported about the positive effect of applying organic fertilizers on the soil (El Etr *et al.*, 2004). They ascribed to the mineralization of N from chicken manure during its composition and might be the biological fixation of atmospheric N and its reflection on soil fertility. Increasing K content of parsley and celery due to the application of organic fertilizers might be a result of its decomposition and producing organic acids, which increases the nutrients availability in the soil. This is in agreement with those (Ahmed, 2001; Ahmed and Ali, 2005). About high content of total trace elements in chicken manure and its effect on Zn, Mn and Fe uptake by parsley and celery, could be due to that the organic carbon acts as a source of energy for soil microorganism, which upon mineralization releases organic acids that decreased soil pH and improves the availability of micronutrients up take (Bokhtiar and Sakurai, 2005). Parsely and celery considered as medicinal plants and crops that can up- or down-regulate bioactive compounds production. Furthermore, future perspectives should be turned towards the production of genotypes with a lower potential for toxic elements accumulation, therefore, the health benefits will be more prominent (Danijela *et al.*, 2021).

It is evident from the previous research (Alok, 2010) that Kitchen wastes material characterized with high values of pH (9.21), organic carbon (7.25%) and organic matter (12.49%). However, other nutrients such as total nitrogen (0.214%), available phosphorus (0.11%) and exchangeable potassium (0.086%) were found in Kitchen wastes components. The composting activity significantly modified the physical and chemical properties of Kitchen wastes material that can be an important tool for organic farming. The present research suggest that lactic acid fermentation in the treatment of Kitchen wastes +LAB led to the highest values of the all given characters due to its ability to provide the plants with N, P and K, because EM contains 0.1% mineral N, 1% available P and has a C: N ratio of 10: 3 the quality and maturity of EM can be simply estimated by changes in pH and EC (Marta *et al.*, 2021; Kengo and Xu 2001). Therefore, EM that contain *Lactobacillus spp.* can be considered as a “living fertilizer” or “microbial fertilizer, the high available phosphorus content suggests that EM can be a good nutrient source for plants. Consequently, the intermediate substances like lactic acid, amino acid and others increased due to the activities of *Lactobacillus spp.* (CAI *et al.*, 2012). The pH of the soil decreased as the lactic acid concentration increased (Kengo and Xu 2001; Kanbe, 1990; Uchimura and Okada, 1992).

In agreement with the results, Wali (2010) indicated that yeast has good efficiency on growth characters of wheat plants. The positive effect of yeast is supported by the findings of Mekki and Ahmed (2005), they stated that the increase in vegetative growth because of yeast treatment is mainly attributed to the effect of yeast, which can play a very significant role in making available nutrient elements for plants. In addition, yeast content of macro and micronutrients, growth regulators and vitamins stimulate the plant to build up dry matters (Mirabal *et al.*, 2008; Hesham and Mohamed, 2011).The promoting effect of yeasts could be due to the biologically active substance produced by this treatment such as auxins, gibberellins, cytokinins, amino acids and vitamins (Bahr and Gomaa, 2002). Also, (Mustafa 2014) found that yeasts, especially *Saccharomyces cerevisiae* and other non-saccharomyces yeasts today are increasingly used for the heterologous production of enzymes and pharmaceutical proteins also, *S. cerevisiae*, decreased pH (Sintija *et al.*, 2021).Yeasts have important roles in environmental applications such as bioremediation and removal of heavy metals from wastewaters. Yeasts are also used in agriculture as biocontrol agents. Several chemicals can be produced using yeast as a biocatalyst.

Molasses is rich in mineral elements and is often used primarily as a source of K, due to containing high concentrations. Moreover, it has other significant advantages such as increasing organic matter in the soil and microbial activity associated with nitrification (Wynne and Meyer, 2002). These are in accordance with the results in this study which cleared that treatment of (peat moss + yeast + molasses) gave the best results in vegetative growth characters and chemical composition of N, P, K,

Zn, Mn and Fe. And enhanced seed germination of parsley and celery plants, these are in agreement with (Turner *et al.*, 2002).

The peat moss has an acid pH (Brown *et al.*, 2000) and low nutrient content in comparison with other substrates so it must add to it several combinations of bio and organic fertilizers and mixed with sand soil. Composted organic and bio-fertilizers generally vary greatly in their characteristics, and may be influenced by a diverse number of factors such as geography, management, time of year, and others (Carlile, 2008). Maturity refers to the degree of decomposition of organic substances that will not cause adverse effects on the plants (Bernal *et al.*, 2009). Stability is related to the levels of activity of the microbial biomass ((Giulia *et al.*, 2021; Fuente *et al.*, 2006). According to Prat (1999), peat moss with a high organic content and low biological stability are more exposed to microbial degradation, which may increase the release of CO<sub>2</sub> and affect the absorption of water and nutrients by the roots, these are in accordance with the investigated study of parsley and celery.

Seaweed algae also, have a great role in plant germination and nutrition that contained macro and micronutrients which are very essential for the growth and development of the plant, and enhance seed germination (Attememe, 2009; Ahmed and Shalaby 2012). Seaweed extract treatments increased content of phosphorus which may be due to the high content of phosphorus in seaweed commercial source used in this investigation, these are in agreement with (Mona and Abou El-Goud 2021; Al-shakankery *et al.*, 2014). As well as seaweed algae, which contains some growth regulators, hormones such as auxins, cytokinins and gibberellins and vitamins (Al-shakankery *et al.*, 2014 and Abul Faiz Md.Jamal Uddin *et al.*, 2019).

#### **4.1. Germination of parsley and celery**

The best germination percentages was observed from (Kitchen wastes + LAB) followed by (peat moss + yeast + molasses) and seaweed algae treatments. Seedling emergency was significantly better compared with control treatment that need much more time for about twice the time of what organic fertilizer treatments need. The variation in crop emergence reflects the sensitivity of various plants to various organic matters (Rojas *et al.*, 2007; Webb *et al.*, 2010; Doydora *et al.*, 2011). Thses are in agreements with Eliab (2010), who said that seeds can be germinated in a good way by many different organic additives. Also, these in accordance with (Mohammad *et al.*, 2015), they said that organic fertilizers gave the best performance of tomato seed germination due to the synergistic effect of compost and trichoderma in increasing the root surface area per unit of soil volume, water use efficiency and photosynthetic activity of seedlings in addition to higher nutrient contents in organic fertilizers.

#### **4.2. Isozyme of parsley and celery**

Isozyme technique has been used to score the variability between organic and inorganic (control) treated plants of parsley and celery (cultivar: Balady *sp.*) in this study as agreement with those: (Sahijram *et al.*, 2003; James *et al.*, 2007). The peroxidase isozyme based markers can be used for the identification of cultivars, species and the activity of proteins and enzymatic reactions (Sugandh *et al.*, 2015; Francine *et al.*, 2021). In the present study, isozyme namely peroxidase have been used to test the activity of peroxidase in different loci of parsley and celery pages. The differentiated parsley and celery plants by peroxidase isozyme activity variations detected by polyacrylamide gel electrophoresis when treated with different combinations of bio and organic fertilizers as [Chicken manure, (Kitchen wastes + LAB (*lactobacillus spp.*), (Peat moss+ Yeast + Molasses), Seaweed algae and plant compost], which gave the best results and activated different loci in peroxidase isozyme activity as compared with control treatments. Mendioro *et al.* (2007) studies the isozymes of malate dehydrogenase (MDH), 6-phosphoglucose dehydrogenase (PGI), phosphoglucose isomerase (PGI) and phosphoglucose mutase (PGM) for genome identification of 20 table-type and 6 cooking type of banana (*Musa spp.*).

#### **5. Conclusion**

It can be concluded that the use of bio and organic combinations i.e. chicken manure, Kitchen wastes+ LAB, peat moss + Yeast+ Molasses, seaweed algae and plant compost gave the highest values of most plant germination period and percentage, growth characteristics and nutrient contents of parsley and celery plants compared with the control treatment which gave the lowest values of all tested characters. Improvement in parsley and celery growth were principally due to increase in nutrients availability in soil because of these combinations caused an increase in soil available N, P, K, Zn, Mn

and Fe compared with control treatment. Hence the use of bio and organic fertilizers instead of inorganic fertilizers would be a better and practical approach to sustain soil fertility and productivity. Another performance of using bio and organic fertilizers to improve Balady cultivars of vegetable crops such as parsley and celery. Moreover the isozyme studies could be a reliable marker for testing the genetic variability of organic treated plants and for evaluating the diversity between organic and inorganic plants of the parsley and celery germplasm.

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