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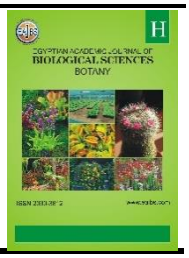

Isozyme Analysis of Jew's Mallow and Garden Rocket Treated with AM Fungi, *Bacillus Megaterium* var. *Phosphaticum* Bacteria and Phosphorous Fertilizer under Sandy Soil Conditions

Mona M. Yousry[1] and Karam A. Elzopy[2]

1-Department of Plant Production - Vegetable, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt.

2-Department of Soil and Agricultural Chemistry, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt.

*****E-mail:karam2016@alexu.edu.eg

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ABSTRACT

Two pot experiments were conducted in the plant house at the faculty of Agriculture (Saba-Basha), Alexandria University, Egypt, during the autumn season of 2017/2018 to estimate the response of Jew`s mallow (*Corchorus olitorius L.*) cv. Balady and garden rocket (*Eruca sativa Mill)* cv. Balady to arbuscular mycorrhizal (AM) fungi inoculation and *Bacillus megaterium* var. *phosphaticum* (BMP) in combination with phosphorous rates on the vegetative growth characters. A completely randomized block design with four replicates was used. The experiments included twelve treatments combination for each of them. The combined effect of AM fungi, BMP bacteria and phosphorus fertilizer rates on plant height, shoot fresh and dry weight, root fresh and dry weight, and N, P, K, Zn, Mn and Fe content in Jew's mallow and garden rocket was significantly superior over the solo effect of each of them and control. Application of 50% of phosphorus in combinations with AM and BMP to Jew's mallow and garden rocket registered the highest growth characters and nutrient content of leaves. The banding pattern activity of the treatment AM+ BMP+ 50% P presented a unique marker band at seven loci Px.C1, Px.C2, Px.C3 and Px.C4 locus indicating that Px.A1, Px.A2 and Px.A3 loci are polymorphic specifically to the Garden rocket. Similarly, the peroxidase patterns in the Jew's mallow leaves showed in total of three loci ($Px.A_2$, $Px.C_1$ and $Px.A_1$) under AM+ BMP+ 50% P treatment. Biofertilizers such as AM fungi and BMP bacteria can reduce the inorganic phosphorous fertilizer requirements of Jew's mallow and garden rocket up to 50% under the conditions of the present study. Moreover, the peroxidase isoenzyme activity could be a reliable indicator for testing the genetic variability of biofertilizers and phosphorous fertilizers in Jew's mallow and garden rocket (Balady *sp.*) germplasm.

INTRODUCTION

Jew's Mallow (*Corchorus olitorius* L), family *Tiliaceae*, is extremely consumed as a health vegetable, as it contains abundant β- carotene and other carotenoids, vitamins (B_1, B_2, C and E) and minerals (Haridy *et al*., 2019). Jew's Mallow also has a varying proportion of dietary fiber and protein required for health. It is cultivated for the stem bark which is used in fiber production (Jute) and for its leaves which are also used as food vegetables (Neveen *et al.,* 2017). The composition of Jew's Mallow leaves per 100 g fresh edible portion

is water 80.4 g (74.2– 91.1), energy 243 kJ (58 kcal), protein 4.5 g, fat 0.3 g, carbohydrate 12.4 g, fiber 2.0 g, Ca 360 mg, P 122 mg, Fe 7.2 mg, β-carotene 6410 µg, thiamin 0.15 mg, riboflavin 0.53 mg, niacin 1.2 mg, ascorbic acid 80 mg (Asmaa *et al*., 2014).Also, polysaccharides are extracted from Jew's Mallow leaves (Mohammed, 2018).

Garden rocket(*Eruca sativa* Mill),family *Brassicaceae,* is probably consumed due to the spicy taste of its leaves which are used as a garnish to flavour salads, snacks and a large variety of meals. Garden rocket contains high ascorbic acid, fibres, polyphenols, carotenoids and glucosinolates (Bell and Wagstaff, 2014).The economic potential of garden rocket is steadily increasing thanks which called 4th Generation of vegetables, i.e. these leafy vegetables are marketed after cleaning, leaf-cutting and packaging which contribute to a longer shelf life (Natasha *et al*., 2016).Oil from *Eruca* plants is commonly used in India; plant parts are used in the traditional pharmacopoeia for various purposes (Awatif *et al*., 2018) depurative, diuretic, emollient, tonic, stimulant, laxative, anti-inflammatory.

The application of phosphorus fertilizers is essential for crop growth and adequate food and fiber production. Soils in arid and semi-arid zones are often low in the available phosphorus and therefore require different inputs of phosphorus (P) for optimum plant growth; especially for the rapid growth of crops such as leafy vegetables (Van Kauwenbergh, 2002). Arbuscular mycorrhizal (AM) fungi can improve the capacity of the root system in absorbing and translocating phosphorus (P) and microelements via an extensive net of mycelium. AM fungi are associated with most plants and can improve nutrient uptake (Requena *et al*., 2001 and Liu *et al*., 2021).

Bacillus megaterium var. *phosphaticum* belongs to plant growth-promoting rhizobacteria (PGPR) and is known for its ability to solubilize rock phosphate material (Amin *et al*., 2017 and Anna *et al.,* 2021). PGPR are root colonizing bacteria with beneficial effects, which include plant growth promotion, biological disease control and induced systemic resistance (Zehnder *et al*., 2001; Viruel *et al*., 2014; Mona and Abou El-Goud, 2020). This group of bacteria includes Phosphate-Solubilizing Bacteria that can convert insoluble phosphates into soluble forms through acidification, chelation, exchange reactions and production of organic acids (Akgül and Mirik 2008). They are found in soil but usually, they are not enough in population, therefore inoculation of plants by a target microorganism at a higher concentration than normally found in soil is necessary (Vessey, 2003).

Peroxidases (E.C. 1.11.1.7) are commonly found in plants, micro-organisms and animals, where they catalyze the reduction of hydrogen peroxide (H_2O_2) to water, rendering it harmless (Hameed Akbar *et al*., 2018). Thus, peroxidases help to alleviate plant cells of extra H_2O_2 under standard and stress conditions (Hameed Akbar *et al*., 2018). Peroxidases are found largely as haemoproteins and utilize hydrogen peroxide as the oxidizing substrate, although further different peroxidases have been exhibited lately to include either metal ions or a flavin group. Varieties of electron donors have been used, including ascorbic acid, phenols and aromatic amines to investigate peroxidase activity in the plant extract.

The current study aims to inspect the impact of AM Fungi and *Bacillus megaterium* var. *phosphaticum* Bacteria with two rates of phosphorus fertilizer (50% and 100% of recommended dose) under sandy soil conditions on vegetative growth of Jew's mallow and Garden rocket. Also, evaluating the enzymatic reaction and peroxidase enzyme activity of Jew's mallow and garden rocket treated by biofertilizers with two phosphorous rates.

MATERIALS AND METHODS

Two pot experiments were carried out in the greenhouse at the Faculty of Agriculture, Saba Basha, Alexandria University, during the autumn season of 2017/ 2018 to estimate the response of Jew's mallow (*Corchorus olitorius* L.) cv. Balady and garden rocket

(*Eruca sativa* Mill) cv. Balady to inoculation of arbuscular mycorrhizal (AM) fungi and *Bacillus megaterium* var. *phosphaticum* (BMP) Bacteria with two different rates of phosphorous. The experiment's soil was collected from Borg El-Arab City, Alexandria, Egypt. Surface soil samples were combined from the surface layer to plough depth. The soil was air-dried and sieved through a 2 mm sieve. The physical and chemical properties of the selected soil according to the methods outlined in Page *et al.* (1982) are shown in Table (1).

Table 1: Physical and chemical properties of the experimental soil.

Sand (%)	96.5
Silt (%)	1.7
Clay (%)	1.8
Soil texture	Sand
pH	8.1
Ec (dSm^{-1})	1.84
O.M (%)	0.08
Calcium carbonate (%)	0.26
Available N (ppm)	6.9
Available P (ppm)	6.2
Available K (ppm)	64

Inoculum Preparation of AM Fungi:

AM Fungi (*Glomus intraradices*) was obtained from Hanover University, Germany and activated in the Soil Microbiology Lab., Soil and Agriculture Chemistry Dep., Faculty of Agriculture, Saba Basha, Alexandria University, Egypt. Mycorrhiza was mixed with seeds two hours before planting.

Inoculum Preparation of BMP Bacteria:

The commercial product of BMP bacteria (*Bacillus megaterium* var. *phosphaticum*) was obtained from Hanover University, Germany. The obtained bacteria were mixed with seeds two hours before planting.

AM Fungi and BMP Bacteriawere applied as biofertilizersin combination with two different rates of phosphorous (50% and 100% of P) to Jew's mallow and garden rocket cultivars (cv. Balady). Both crops were planted on the 10th of September, during the autumn season of 2017/2018 in the greenhouse at the Faculty of Agriculture (Saba-Basha), Alexandria University. Phosphorous was applied as calcium super phosphate (15.5%).Twelve treatments for each crop were carried out in this investigation as follow:

Treatment	Jew's mallow	Garden rocket
50% P (calcium super phosphate 50%)	A1	B1
100% P (calcium super phosphate 100%)	A2	B2
AM	A3	B3
AM+ 50% P	A4	B4
AM+ 100% P	A5	B5
BMP	A6	B6
BMP+ 50% P	A7	B7
BMP+ 100% P	A8	B8
AM + BMP	A9	B9
AM+ BMP+ 50% P	A10	B10
AM+ BMP+ 100% P	A11	B11
Control (recommended doses of chemical fertilizer N, P and K).	A12	B12

Pot Experiments:

Plastic pots with a diameter of 15 cm and deep 12 cm, were uniformly filled with 1000 g of the prepared soil and compacted to a bulk density of about 1.37 $g.cm^{-3}$ and placed in a greenhouse. A small amount of seeds of Jew's mallow (*Corchorus olitorius* L.*)* and garden rocket (*Eruca sativa* Mill) were planted in the pots directly. Thinning was done at 7 days after sowing by retaining ten healthy seedlings per pot. The recommended amount of chemical N. P. K fertilizers was added only in control pots as follow: For Jew's mallow**,** 100 kg/fed of ammonium sulphate (20%), 50 kg/ fed of super calcium phosphate (15.5%) and 25 kg/ fed of potassium sulphate (48%). For garden rocket,50 kg/fed of ammonium sulphate (20%), 50 kg/ fed of super calcium phosphate (15.5%). All plants were harvested after45days from planting.

Plant height was measured at the end of the growing season from the ground level to the tip of the tallest leaf using a meter ruler (Fageria *et al.*, 2006). The fresh and the dry weight of shoot and root ($g.plant^{-1}$) were recorded for harvested plants. In order to determine the mineral contents of each of Jew's mallow and garden rocket leaves, dry samples of leaves were finely ground for chemical analysis. The oven-dried plant material samples were wet digested by using a concentrate of H_2SO4/H_2O_2 (Lowther, 1980). Mineral N, P and K and micronutrients (Fe, Mn and Zn) were determined in leaves using the method described by A.O.A.C. (1990), Chapman and Pratt (1978). The Fe, Mn and Zn concentration solutions were measured by Atomic Absorption Spectrophotometer (Perkin Elmer-3300) according to Chapman and Pratt (1978).

Biochemical Genetic Analysis (isozymes analysis):

This study was investigated in the Genetic laboratory at the faculty of Agriculture Saba- Basha to study the profile of proxidase isozymes expressed in leaves of Jew's mallow (*Corchorus olitorius* L.) and Garden rocket (*Eruca sativa* Mill) cv. Balady were used in the present study as gene markers for studying the genetic polymorphism. 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 twenty different treatments 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 performed according to Jonathan and Norman (1989).

Samples from 12 explants for each of them were separately ground using cooled mortar with a 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-polyvinyl-pyrrolidone gel and Tris-borate or Tris-acetate separation buffers (Sabrah, 1980). Electrophoresis was conducted at 270v, 4O^{o}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_2O_2) were layered over the gel immediately before staining.

Statistical Methods:

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).

RESULTS AND DISCUSSION

Vegetative Growth of Jew's Mallow:

The vegetative attributes of Jew's mallow were improved significantly by AM fungi, BMP bacteria and phosphorous application rates as compared to control (Table. 2). Inoculation of AM fungi and BMP Bacteria as biofertilizers along with different rates of phosphorous (50% and 100% P) registered the highest plant height (17.53 and 18.00 cm), fresh weight of shoot (4.45 and 4.68 g plant^{-1}), dry weight of shoot (0.89 and 0.94 g plant^{-1}), fresh weight of root (3.44 and 3.27 g plant^{-1}) and dry weight of root (0.89 and 0.77 g) in A10, A11 treatments, respectively, with no critical difference among them. While, the solo effect of AM fungi, BMP bacteria or phosphorous fertilizer rates on the vegetative attributes of Jew's mallow was significantly less as presented by A1, A2, A3 and A6 in Table (2).

Table 2: Effect of AM Fungi, *Bacillus megaterium* var. *phosphaticum* and phosphorus rates on vegetative growth characters of Jew's mallow (cv. Balady).

Treatments	Plant height (cm)	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)
A1	12.38cd	1.20 efg	0.24 efg	2.04 cd	0.39 de
A2	13.93bc	1.80 e	0.36 e	2.53bc	0.49cde
A3	12.73cd	1.23 efg	0.25 efg	1.77 d	0.30 e
A4	16.53 a	3.58 c	0.72 c	3.19 a	0.59bcd
A5	16.80 a	3.90 bc	0.78 bc	2.93ab	0.69abc
A6	11.68 d	1.13 fg	0.23 fg	2.08 cd	0.38 de
A7	13.15 bcd	1.70 ef	0.34 ef	2.35bcd	0.71ab
A8	14.58 b	2.68 d	0.54 d	2.90ab	0.68abc
A9	13.95 bc	2.65 d	0.53 d	1.98 cd	0.45 de
A10	18.00 a	4.68 a	0.94 a	3.27 a	0.77ab
A11	17.53 a	4.45 ab	0.89 ab	3.44 a	0.89 a
A12	8.00 e	0.68 g	0.14 g	1.02 e	0.29 e
L.S.D 0.05	1.654	0.585	0.117	0.65	0.21
Significant	***	***	***	***	***

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

Chemical Composition of Jew's Mallow:

The individual and the combined effect of biofertilizers and phosphorus were significant on the chemical composition of Jew's mallow leaves (Table 3). The highest N (2.91 %), P (0.33%), K (2.07 %), Zn (47.40 mg kg^{-1}) and Mn (25.21 mg kg^{-1}) were obtained by A10. While the maximum Fe content in Jew's mallow leaves (55.04 and 64.68 mg kg^{-1}) was recorded by A3 and A6, respectively. All the treated fertilizers showed statistically higher N, P, K, Zn and Mn as compared to control. While leaves content of Fe in control was significantly on par with A10 and A11.

Table 3: Effect of AM Fungi, *Bacillus megaterium* var. *Phosphaticum*and Phosphorus rates on chemical composition in leaves of Jew's mallow (cv. Balady).

Treatments	N %	P %	K %	Zn mg kg^{-1}	Mn mg kg^{-1}	Fe mg kg^{-1}
A1	1.24 ef	0.16 fg	0.94 h	27.12 f	12.54 d	35.90 bcd
A2	1.20 f	0.18 e	1.26 e	31.68 e	14.39 d	26.40 cd
A3	1.16 f	0.17 efg	1.08 g	27.48 f	14.12 cd	55.04 ab
A4	1.68 c	0.23 c	1.74 c	42.00 b	17.16 bcd	24.42 cd
A5	1.76 c	0.25 b	1.86 b	43.20 b	20.06 abc	26.53 cd
A6	0.93 g	0.16 g	0.79 i	25.44 f	11.09 de	64.68 a
A7	1.17 f	0.18 ef	1.17 f	30.96 e	14.39 cd	40.00 bc
A8	1.49 d	0.22 cd	1.44 d	38.40 c	16.10 cd	28.51 cd
A9	1.36 df	0.21 d	1.42 d	34.80 d	14.65 cd	29.04 cd
A10	2.91 a	0.33 a	2.07 a	47.40 a	25.21 a	17.03 de
A11	2.04 b	0.26 b	1.91 b	44.52 b	23.76 ab	17.71 de
A12	0.36 h	0.03 h	0.43 j	16.67 g	7.22 e	15.84 e
L.S.D 0.05	0.145	0.016	0.07	2.68	6.57	18.63
Significant	***	***	***	***	***	***

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

Biochemical Genetic Analysis of Jew's Mallow:

The zymogram and photograph showing mobility pattern of peroxidase isozymes are illustrated in Figures (1and 2 dendrogram), cleared that the peroxidase patterns in the Jew's mallow plants leaves showed in a total of three loci. First, it was clear that all plants presented the Px.A2 and Px.C1 (fast and slow), these two loci indicated that these common loci were consistently monomorphic expressed. Second, some plants under different fertilization rates displayed extra one common locus (Px.A1) for the following A-2, A-3, A-6, A-8 and A-10 locus indicating that (Px.A1) locus is polymorphic specifically to these treatments.

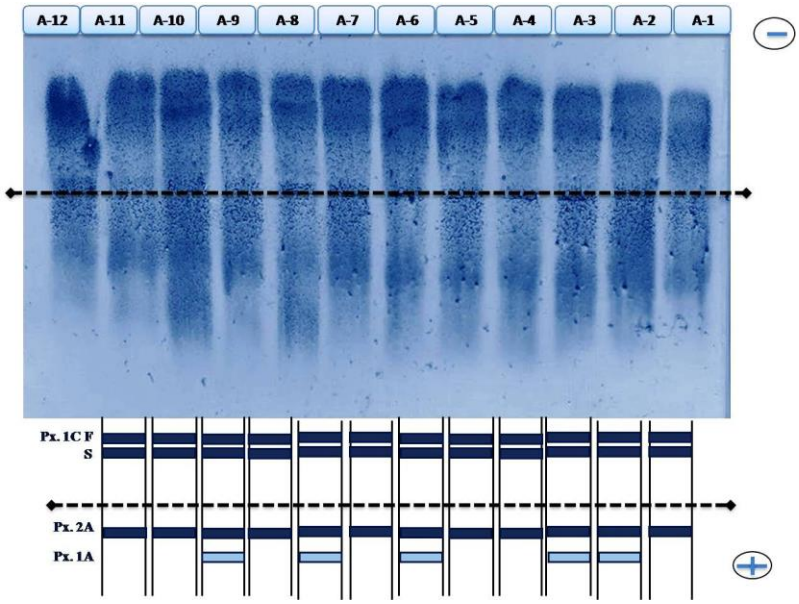

Fig.1: Zymograms showing electrophoretic profiles of Peroxidase isozyme in Jew's mallow as follow from right to left i.e. (A-1) 50%P, (A-2) 100%P, (A-3) AM, (A-4) AM+50%P, (A-5) AM+100%P, (A-6) BMP, (A-7) BMP+50%P, (A-8) BMP+100%P, (A-9) AM+BMP, (A-10) AM+BMP+50%P, (A-11) AM+BMP+100%P and (A-12) Control.

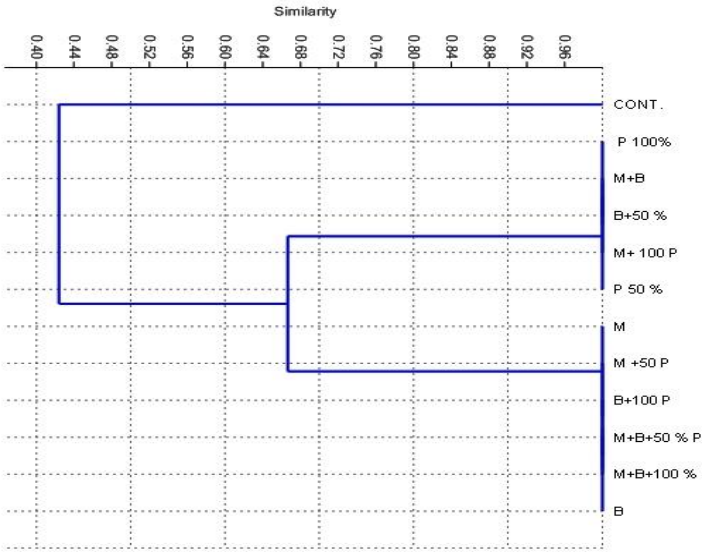

Fig.2: Dendrogram of different treatments of Jew's mallow based on peroxidase enzymes (isozyme).

Vegetative Growth of Garden Rocket:

The growth performance of garden rocket was statistically superior under AM fungi, BMP Bacteria and/or phosphorus application treatments as compared to control (Table 4). The highest plant height (18.00 cm), shoot fresh weight (4.05 g plant^{-1}), shoot dry weight (0.81 g plant^{-1}) were recorded by B10. The highest root fresh weight (4.25 g plant^{-1}) was recorded by B11 which was statistically on par with B8 and B10. While, under B7, B8, B10 and B11 treatments, root dry weight was significantly higher as compared to the rest treatments.

Table 4: Effect of AM Fungi, *Bacillus megaterium* var. *phosphaticum* and Phosphorus rates on vegetative growth characters of garden rocket (cv. Balady).

Treatments	Plant height (cm)	Shoot fresh weight (g/plant)	Shoot dry weight(g/plant)	Root fresh weight (g/plant)	Root dry weight (g/plant)
B1	10.30 g	1.00 efg	0.20 efg	1.94 e	0.56 de
B2	12.40 def	1.30 ef	0.26 ef	2.71 d	0.72 cd
B3	11.00 fg	1.18 ef	0.24 ef	1.87 e	0.42ef
B4	13.70 cd	1.98 d	0.40 d	3.03 cd	0.80bc
B5	14.45 c	2.78 c	0.56 c	3.49bc	0.87abc
B6	10.30 g	0.98 fg	0.20 fg	2.96 cd	0.58 d
B7	12.15 ef	1.25 ef	0.25 ef	3.64 b	0.91ab
B8	13.43 cde	1.85 d	0.37 d	3.75ab	0.97 a
B9	13.05 cde	1.35 e	0.27 e	2.80 d	0.60 d
B10	18.00 a	4.05 a	0.81 a	3.94ab	0.94ab
B11	16.05 b	3.35 b	0.67 b	4.25 a	0.97 a
B12	9.70 g	0.78 g	0.16 g	1.07 f	0.30 f
L.S.D 0.05	1.386	0.327	0.065	0.54	0.16
Significant	***	***	***	***	***

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

Chemical Composition of Garden Rocket:

As presented in table (5) the chemical composition of garden rocket leaves showed statistically lower values in N, P, K, Zn, Mn and Fe content under control treatment as compared to other treatments. The treatment B10 produced maximum N (1.41 %), P (0.71

%), K (1.89 %), Zn (36.27 mg kg^{-1}) and Mn (17.45 mg kg^{-1}). While the highest Fe content was registered by B6.

Table 5: Effect of AM Fungi, *Bacillus megaterium* var. *phosphaticum* and Phosphorus rates on chemical composition in leaves of garden rocket (cv. Balady).

Treatments	N %	P %	K %	Zn mg kg^{-1}	Mn mg kg^{-1}	Fe mg kg^{-1}
B1	1.16 g	0.58 ef	1.44 d	20.79 g	15.99 c	56.50 ab
B2	1.23 ef	0.59 e	1.59 c	29.52 d	16.54 bc	53.32 cde
B3	1.14 g	0.59 e	1.57 c	22.68 f	16.32 bc	55.06 bc
B4	1.32 c	0.65 c	1.76 b	30.87 c	16.91 ab	51.10 def
B5	1.34 bc	0.67 bc	1.87 a	30.87 c	17.02 ab	50.68 ef
B6	1.09 h	0.56 f	1.29 e	16.47 h	15.85 c	58.27 a
B7	1.20 f	0.59 e	1.58 c	24.57 e	16.43 bc	53.68 cd
B8	1.29 cd	0.64 cd	1.61 c	30.51 cd	16.63 bc	52.87 cde
B9	1.24 e	0.62 d	1.60 c	30.33 cd	16.62 bc	53.17 cde
B10	1.41 a	0.71 a	1.89 a	36.27 a	17.45 a	46.30 g
B11	1.37 b	0.68 b	1.88 a	33.39 b	17.44 a	49.33 f
B12	1.04 i	0.49 f	0.55 f	13.95 i	15.06 d	40.12 h
L.S.D 0.05	0.03	0.03	0.08	1.21	0.79	2.95
Significant	***	***	***	***	***	***

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

Biochemical Genetic Analysis of Garden Rocket:

The result of peroxidase isozyme activity cleared that garden rocket showed the highest specific activity of peroxidase isozyme locus. The zymogram and photograph showing the mobility pattern of peroxidase isozymes are illustrated in Figures (3 and 4dendrogram). From this data, it can be conducted that the peroxidase patterns in the garden rocket plants leave shown in a total of seven loci. First, it was clear that all plants presented the Px.A3 and Px.C2, these two loci indicated that these common loci were consistently monomorphic expressed.Second, all treatments displayed extra five common loci (Px.C1, Px.C3, Px.C4, Px.A1and Px.A1). The banding pattern activity of (B-10), displayed a unique marker band at Px.C3 locus indicating that (Px.C1, Px.C4, Px.A1 and Px.A2) loci are polymorphic specifically to the garden rocket plant.

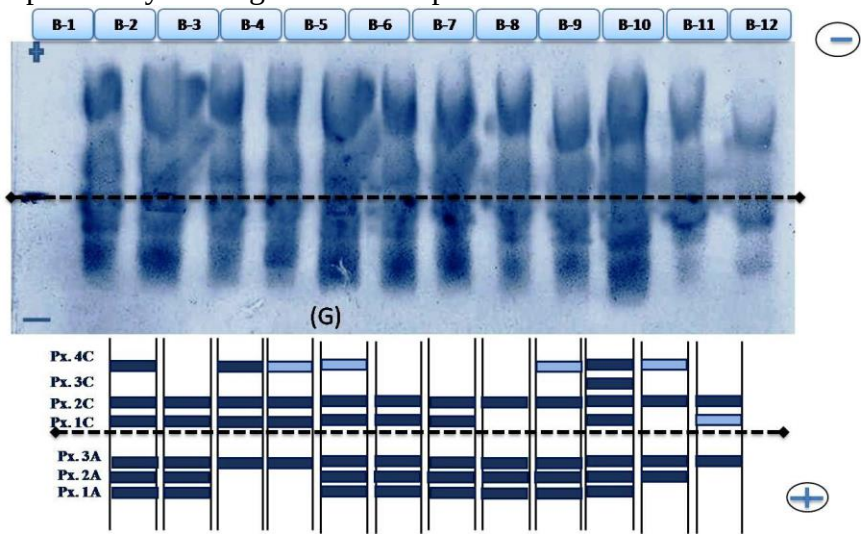

Fig.3: Zymograms showing electrophoretic profiles of Peroxidase isozyme in garden rocket as follow from left to right i.e. (B-1) 50%P, (B-2) 100%P, (B-3) AM, (B-4) AM+50%P, (B-5) AM+100%P, (B-6) BMP, (B-7) BMP+50%P, (B-8) BMP+100%P, (B-9) AM+BMP, (B-10) AM+BMP+50%P, (B-11) AM+BMP+100%P and (B-12) control.

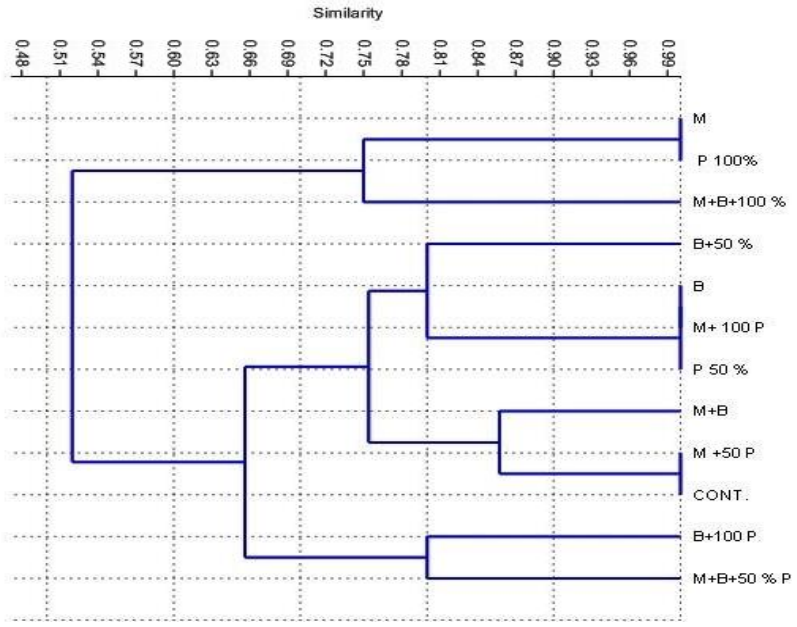

Fig.4: Dendrogram of different treatments of garden rocket based on peroxidase enzymes (isozyme).

DISCUSSION

The aforesaid results revealed significant increases in growth attributes of Jew's mallow and garden rocket with AM fungi and BMP Bacteria inoculation under the different rates of P fertilizers. BMP bacteria are known for their ability to solubilize rock phosphate material (Anna *et al.,* 2021). Application of phosphate solubilizing bacteria to soil or seed helps to enhance solubilization of unavailable soil phosphorus and to induce plant growth (Anna *et al.,* 2021). These are in accordance with Abd-El-Monaim *et al*. (2012), who said that BMP bacteria increased plant growth, quantitative and qualitative parameters of tomato fruits growing under field conditions and increased fresh and dry weight of survival tomato plants growing in pots compared with control. Plant height, seed weight per plant and 1000-seed weight increased in sesame plants as a result of the use *Bacillus megaterium* (Mahrous *et al.,* 2015). Also, BMP bacteria showed the potential to repress a range of diseases on all of the plant parts (Jock *et al*., 2002, Jung and Kim, 2005). AM fungi symbiotic relationships retain many advantages to the plants. These advantages include promoting plant growth and enhancing plant tolerance to various diseases. Inoculation of *Glomus spp*. significantly reduced nematode population, a number of root-knot index besides increasing the growth, plant biomass, p uptake and yield (Shreenivasa *et al.,* 2007 and Liu *et al*., 2021).

AM fungi can increase P uptake in plants was documented in many studies (Goussous and Mohammad 2009 and Bouwmeester *et al*., 2007). P and N uptake were higher in mycorrhizal treated plants than in control treatment. Plant P is the main controlling factor in the plant-fungal relationship, which plays a significant role in increasing the total uptake of nutrients which leads to the increase in growth and yield (Gosling *et al*., 2006 and Thomsen *et al*., 2021). AM inoculation stimulated the plant growth and it was attributed to enhance photosynthesis which is associated with increased P uptake in leaves, stems (Al-Karaki and Al-Momany 1997). These are in accordance with Abou El-Goud and Yousry(2019), who stated that AM fungi increased vegetative growth characters in summer squash and enhance the uptake of phosphorous by the plants and improve soil fertility. Some researchers have indicated that AM fungi inoculation tends to decrease pH in the rhizosphere, and leads to producing more carbon dioxide (Goussous and Mohammad 2009;

Liu *et al*., 2021). AM fungi have been considered to be a significant mechanism for increasing soil carbon and conserving soil organic carbon from decay by aggregation (Cheng *et al*., 2012). Many reports have shown that AM fungi are capable to avoid soil erosion by increasing the stability of soil aggregates through the combined action of extra-radical hyphae and their exudates.

Generally, in the current study, the application of AM fungi and BMP Bacteria in combinations with two phosphorus rates (50 and 100 %) increased the chemical composition of Jew's mallow and garden rocket. Bio-stimulants work by increasing the uptake of minerals by plants, including N, P, K, Zn. Mn and Fe, and improve the efficiency of nutrient use (Calvo *et al.*, 2014 and Parađiković *et al.*, 2013). Kocira *et al.* (2013) demonstrated that biofertilizers' application with mineral phosphorus fertilizer improved soil properties, which is associated with an increase in nutrient availability for plants. This, in turn, was manifested in their concentration and accumulation in crops (Vessey 2003). Additionally, Adesemoye *et al*. (2009), Sharma *et al.* (2011), and Kumar *et al*. (2014) claimed that the application of biological products in cereal cultivation reduced chemical fertilizer rates. According to Emilsson *et al.* (2007), Wu *et al.* 2008 and Kumar *et al*. (2012), the application of BMP bacteria contributes to lower losses of nutrients in the soil environment and guarantees a steadfast supply of nutrients for plants throughout the growing season, which ensures a high accumulation of the nutrients. These findings were confirmed in the present study as the highest nutrient concentration were recorded in Jew's mallow and garden rocket leaves treated with BMP bacteria and AM fungi under different rates of P fertilizers. Also, Glomalin is a fungal component, insoluble and hydrophobic proteinaceous substance, which has been reported to improve soil stability by avoiding disaggregation by water, so AM could be used as a promoter of soil in agro-ecosystems (Liu *et al*., 2012; Bedini *et al*., 2009 and Liu *et al*., 2021). Soil types, such as clay loam and sandy soils facilitate the buildup of *Glomus spp.* population, this is an influential factor in the introduction and reproduction of AM spores (Al-Momany 1989).

Inoculation of BMP bacteria increases P solubility and availability for roots (Han and Lee, 2006). Growth enhancement by *Bacillus spp.* mayb*e* associated with its ability to produce hormones, especially indole acetic acid (IAA)(Sheng and Huang 2001). Additionally, phosphate availability in soil is essential for N uptake and assimilation in the plant (Kim *et al*., 2003; Al-Taweil *et al*., 2009). Finally, increasing soil organic nitrogen and other essential nutrients in the soil-plant system declines the requirement for fertilizers and improves the release of soil nutrients (Baset *et al*., 2010).

Three peroxidase isozyme loci were activated in tissue extracts from Jew's mallow leaves. This diversity of isozymes could provide plants with flexibility in dealing with common environmental threats such as herbivory, fungal and bacterial pathogen infection, mechanical stress, damage and air pollutants (Hameed Akbar *et al*., 2018). In addition, the complex phenolic chemistry (Ragab *et al.*, 2010), may require specific peroxidase isozymes for synthesis or activation (Calder´on *et al*., 1990). These results came in alignment with Yunshu *et al.* (2019) and Ila and Mahanta (2012), who found that peroxidase was seen as useful in developing protein markers for the identification of inbred lines, for the electropherograms of the cathodal and anodal isozymes of leaf peroxidases.

Peroxidase shows a potential to use for biotechnological application with the advance in the purification method of plant peroxidases (Hameed Akbar *et al*., 2018).The results of peroxidase isozyme activity of garden rocket showed the highest specific activity of the peroxidase isozyme at seven loci, These are in accordance with Duchovskiene and Siksnianiene (2001), Eunyoung and Kim(2013) and Hee *et al.*(2019), who studied the dynamics of peroxidase isozymes composition in various organs of cabbage (*Brassica oleracea* var. *capitata*) cv. early golden acre, kohlrabi (*Brassica oleracea* var. *gongylodes*)

cv. white vienna and red beet (*Beta vulgaris* var. *rubra*) cv. kamuoliai during ontogenesis and radish. They found that the largest number of isoforms was detected from the juvenile stage until the end of evocation in cabbage (5 isoforms), at the end of evocation and during flower initiation in kohlrabi (7 isoforms), from the beginning of flowering inducing until flower initiation in red beet (13 isoforms). Activation of seven loci of peroxidase isozyme activity from garden rocket germplasm due to the application of PGPR such as BMP bacteria + AM fungi had a stimulation effect on the population of rhizosphere microorganisms and increased their numbers significantly (Mahrous *et al.,* 2015; Mona and Abou El-Goud. 2020). The effect of PGPR as bio-agents may be due to attacking and binding the pathogenic organisms by sugar linkage and beginning to secrete extracellular protease and lipase (Zaghloul *et al.,* 2007) and produce antibiotics products of secondary metabolites such as phenazine-1-carboxilic acid (PCA), 2, 4-Pyrrolnitrin and Oomycin, which lead to activate seven loci at peroxidase isozyme activity in garden rocket germplasm (Ehteshamul-Haque and Ghaffar, 1993 and Knudsen *et al*., 1995).

CONCLUSION

The obtained results concluded that the combined effect of BMP bacteria and AM fungiin combinations with two rates of phosphorus (50% and 100%) especially 50% was significantly superior over the solo effect of each and control, with respect to plant height, shoot fresh and dry weight, root fresh and dry weight, and N, P, K, Zn, Mn and Fecontent in Jew's mallow and garden rocket leaves. Moreover, the isozyme studies could be a reliable marker for testing the genetic variability of biofertilizers and phosphorous rates treated plants and for evaluating the activation of peroxidaseisozyme activity in the tissues of the Jew'smallow and Garden rocket germplasm.

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ARABIC SUMMARY

أختبار الأيزوزيم للملوخية والجرجير المعاملة بفطر الميكوريزا والبكتريا المحررة للفسفور مع جرعات الفسفور المعدنى تحت ظروف الأراضى الر ملية

منى يسري[1] ، كرم الزوبي[2]

[1]قسم الإنتاج النباتي (خضر) – كلية الزراعة – سابا باشا – جامعة الإسكندرية

[2]قسم الأراضي والكيمياء الزراعية – كلية الزراعة – سابا باشا – جامعة الإسكندرية

تم إجراء تجربتين في الأصص فى صوبة كلية الزراعة ساباباشا للموسم الخريفى2017- 2018 لتقييم استجابة الملوخية والجرجير(الصنف البلدى) تجاه التلقيح بفطر الميكوريزا arbuscular mycorrhizal fungi (AM) والبكتيريا المحررة للفسفور *Bacillus megaterium* var. *phosphaticum* (BMP) مع معدلات مختلفة من السماد الفوسفوري (50٪ و100٪ من الجرعة الموصى بها) وتأثيره على النمو الخضرى. تم استخدام تصميم القطاعات العشوائية الكاملة بإستخدام أربعمكررات، تم تنفيذ عدد 12 معاملة من التوليفات المختلفة. البكتيريا المحررة للفسفور يمكنها إذابة فوسفات الصخور و الميكوريزا تساعد الجذور فى إمتصاص الفسفور بالاضافة إلى أن نشاط إنزيم البيروكسيديز فى أوراق النباتات يعتبر مؤشرا للكشف عن الأختلاف أو أوجه الشبه بين النباتات المعاملة بالأسمدة الحيوية والفسفور المعدنى والتوليفات بينهم. كان التأثير المشترك لفطر AM، بكتريا BMP ومعدلات سماد الفوسفور المعدنى متفوقًا بشكل ملحوظ في صفات طول النبات، ووزن الساق الطازج والجاف، ووزن الجذر الطازج والجاف، ومحتوى أوراق الملوخية والجرجير من العناصر N وP وK وZn وMn وFeعلى التأثير الفردي لكل منهم ومعاملة المقارنة. وجد أن إضافة 50٪ من الجرعة الموصى بها من الفسفور مع AM وBMP للملوخية والجرجير سجل أعلى معدل في صفات النمو المدروسة ومحتوى العناصرفى الأوراق. بالنسبة لتأثيرنشاط المعاملة (الميكوريزا + البكتريا + 50% من جرعة الفسفور الموصى بها) قدمت علامة فريدة من نوعها لسبع مواقع تنشيطية لإنزيم البيروكسيديز على وجه الخصوص فى أوراق نبات الجرجير, و بصورة مماثلة أظهرت المعاملة نشاط إنزيم البيروكسيديز فى أوراق نبات الملوخية فى ثلاث مواقع تنشيطية لإنزيم البيروكسيديز . لذا يمكن للأسمدة الحيوية مثل فطر الميكوريزا AM وبكتيريا BMP أن تساعد في خفض متطلبات الأسمدة الفوسفورية غير العضوية للملوخية والجرجير بنسبة تصل إلى 50٪ في ظل ظروف الدراسة الحالية. علاوة على ذلك ، يمكن أن يكون نشاط إنزيم البيروكسيديز مؤشرًا موثوقًا به لاختبار التباين للنشاط الإنزيمى بواسطة إختبار الأيزوزيم للأسمدة الحيوية والأسمدة الفوسفورية في الأصول الوراثية (الأصناف البلدية) للملوخية والجرجير.