



The Effect of Biofertilization and the Addition of Canola Seed Residues on the Growth of Pomegranate Transplants

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ABSTRACT

The investigation was carried out in pots at the greenhouse of the Horticulture Department at the Desert Research Center (DRC) on Wonderful pomegranate (*Punica granatum* L.) transplants during 2019 and 2020 seasons. This study contained three doses of canola seeds residues (CSR) 5, 10 and 15g/transplants) each with or without one of two kind of bacteria *Pseudomonas aerogenosa* (B1) and *Pseudomonas genoculate* (B2) beside control treatment in twelve treatments. Data were recorded on seedling growth parameter, leaf mineral content, organic carbon (OC) and organic matter (OM). Also targeted was to measure nitrogenase activity, CO₂ evolution, and indole acetic acid (IAA) production in rhizosphere under pomegranate transplants. Also anticipated was to assess the content of N, P, and K in the shoots and roots of those Seedlings. The current study revealed that the greater the applied amount of canola seed residues caused, the higher the soil N, P, K, OC and OM, and in addition, the higher the shoot and root contents of N, P, and K. This is attributed to higher nitrogenase activity, CO₂ evolution, and IAA production in rhizosphere below the pomegranate seedlings.

Keywords: canola seed residues, bio fertilizer, pomegranate transplants, vegetative growth, leaf mineral content, nitrogenase activity, CO₂ evolution and IAA production.

1. Introduction

The pomegranate is a one of main crops for newly reclamation area in Egypt. Pomegranate (*Punica granatum* L.) is a fruit shrub well adapted to arid and semi-arid zones, where the winter is cold and the summer is long, hot and dry. This plant well succeeds as far as the 35 the degree latitude north but during extreme cold periods, the plant are sometimes injured by cold. The trees with stand a temperature of 10 F to 15 F, a rather large amount of summer heat is required to ripen the fruits the Standard and Cochran (1970). In Egypt, pomegranate cultivated areas reached about 35983.9 Hectares (85676 feddans) with fruit production of 381426 metric tons, according to Ministry of Agriculture and Land Reclamation (2016).

Gajender *et al.* (2008), revealed that, a significant improvement in the plant height, plant canopy, pruned material and fruit yield was evident in 5-year-old pomegranate plants in field conditions. The use of biofertilizer technology may be adopted for the establishment and development of other horticultural plant species in arid regions. Kohler *et al.* (2007) recorded that biofertilizers improving the microbiological activity in the rhizosphere. Though there are many reports on the effect of different biofertilizers on various fruit plants, no information is available on usefulness of these biofertilizers with respect to pomegranate. Greeshma *et al.* (2017) working on the application of organics (neem + pongamia cake in 1:1) to supplement 50 per cent N & P₂O₅ along with bio-inoculants (*Trichoderma harzianum* and *Pseudomonas fluorescens*). They recorded that, Pomegranate growth parameters did not record significant variation, however, the plant height and secondary branches were relatively more in bio-inoculants and organics supplemented treatments. Also, Bacterial biofertilizers, its associations with plants and transformations of nutrients in soil, would be the using for farmers to be very beneficial in the plant health improvement and increase productivity, it play a key role in productivity and sustainability of soil and also in protecting the environment as eco-friendly and cost-effective inputs for the farmers (Prabakaran *et al.* 2020).

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2. Materials and Methods

This experiment was carried out during two successive seasons (2019 & 2020) respect., Wonderful pomegranate seedlings (*Punica granatum* L.) under green house of pomology unit at Plant Production Department, Desert Research Center, Cairo, Egypt., uniformity selected as possible in growth. Its grown in black plastic bags 5 L in the size, filled with washed sand. Experiment began at first week of marsh of each season and the following twelve treatments were done as follow:

1. Control.
2. Bacteria 1 (B1).
3. Bacteria 2 (B2).
- 4 Canola seed residues (CSR) at 5g/ plant
5. Canola seed residues (CSR) at 10g/ plant.
6. Canola seed residues (CSR) at 15g/ plant 6. Bacteria 2 (B2).
7. Canola seed residues at 5g/ plant + Bacteria 1.
8. Canola seed residues at 10g/ plant + Bacteria 1.
9. Canola seed residues at 15g/ plant + Bacteria 1.
10. Canola seed residues at 5g/ plant + Bacteria 2.
11. Canola seed residues at 10g/ plant + Bacteria 2.
12. Canola seed residues at 15g/ plant + Bacteria 2.

Transplants received the same all nursery practices managements for about 8 months for each season. The analyses of canola seed residues shown in table A.

The complete completely randomized design with four replicates for each treatment every replicate was represented as a plant.

Table A: The analyses of canola seed residues.

Nutrient	Canola meal
Crude protein	36.31
Crude fat	1.61
Crude fiber	12.80
Amino acid	
Lysine	2.01
Methionine	0.78
Threonine	1.57
Tryptiphan	0.49
Arginine	2.42
Leucine	2.65
Histidine	1.01
Elements	
N%	1.32 mg/l
P%	70.07 mg/l
K%	42.0 mg/l
Ca%	152.8 mg/l
Mg%	46.39 mg/l
Zn ppm	1.14 mg/l
Fe ppm	45.04 mg/l
Mn ppm	5.21 mg/l

At last week of August of each season the following data were recorded:

1. Stem height increment percentage:

Plant height in the end of August - initial plant height on the first of April/ initial plant height on the first of April X 100.

- Average number of lateral shoots /seedling at end of August.

- Stem thickness increment percentage: at 10 cm above soil surface zone = stem thickness in the end of August - initial stem thickness on the first of April/ initial stem thickness on the first of April X 100.

2. Root length was recorded as cm. at the end of each season.

- Stem and leaves dry matter percentage was calculated at the end of each season.

- Root dry matter percentage was calculated at the end of each season.

- Leaf mineral content was determined as follow: leaves of each seedling as a replicate at the end of each season were collected from each. The leaf samples were washed several times with tap water then rinsed with distilled water, dried at 70°C in an electric oven till a constant weight, grounded in electric mill and digested according to Chapman and Prat (1961). Nitrogen was determined by MicroKjeldahl method. While, phosphorus was determined colorimetrically by spectrophotometer (Lambda 1A, Perkin Elmer, Inc., MA, USA), using the ascorbic acid method. Potassium was determined by the method of the flame photometer (JENWAY, PFP-7, ELE Instrument Co. Ltd., UK). All elements were determined according to Page *et al.* (1982).

Data obtained throughout this study were statistically analyzed using the analysis of variance method as reported by Snedecor and Cochran (1980) and the differences between means were differentiated by using Tukey's multiple range test.

6- Microbiological data: the enzyme activity for nitrogenase and cellulose in soil sample at the end of each season were.

3. Results and Discussion

Data presented in Table (1) showed that the stem height increment percentage significantly affected by treatments, CSR at 15g/plant alone and at all doses with B2 gave higher values than all other treatments in first season. Meanwhile, in the second season CSR at 10 g/plant alone and CSR at 15g/plant with B2 gave higher values than the control and all other treatments.

Average number of lateral shoots/plant showed that, CSR at 5g/plant had lowest values in both seasons, but inconstant trend could be noticed among treatments.

Stem thickness increment percentage cleared that, CSR at 15g/plant in first season and at 10g/plant in second season recorded highest values but without any significance with most of other treatments.

Root length values, treatments of CRS at 15g/plant alone or with B2 in first season and at 15g/plant plus B2 in second season had higher significant vales than most of other treatments.

Dry matter percentage of leaves and stem, insignificant differences among treatments could be noticed among treatments in both seasons.

Dry matter percentage of roots, CSR plus B2 showed higher significant value than control and all CRS alone in first season. But in the second season differences among treatments lack significance.

Total leaf chlorophyll content, control treatment recorded lowest significant values in both seasons. The highest values noticed by treatments of CSR at 5g/plant plus B2 in first season and at 10g/plant plus B1 in second season.

Nitrogen, phosphorus and potassium percentage in stem and root: all treatments of CSR with or without Bacteria showed higher percentages of N, P and K in stem and root than control. The eighth treatment (Canola 5 g. X Bacteria 2) had highest significant values of N, P and K.

Acar *et al.* (2022) found two bacterial strains such as *Pseudomonas* sp. HV 5 and *Micrococcus luteus* GC- subgroup B MFDV3 on pomegranate, that stimulate different growth to be used in organic production in pomegranate plants should be tried. Also, use of organic and bio-fertilization sources an increase the activity of micro flora, improves the soil structure a, soil organic matter, water holding capacity, soil humus content and the availability of most nutrients and use to organic farming products El-Salhy *et al.* (2022).

It is clear from table (2) that application at Bacteria 2 plus 5g Canola /pot gave the highest significant of nitrogenase activity, CO₂-evolution, and IAA production than the control Treatments. Same table (2) depict the follow up of nitrogenase enzyme activity, the CO₂-evolution, and indolacetic acid (IAA)

Table 1: Adding of canola seed residues and bio Fertilization and its effect on some vegetative parameters and leaf chlorophyll of Pomegranate Seedlings in seasons of 2018 and 2019.

Treatments	Plant height increment %	No. branches /seedling	Branch ht. increment %	Root length	% Dry matter of leaves and stem	% Dry matter of roots	Total Chlorophyll
1st season							
Control	11.79 D	31.10 A	13.97 D	8.70 E	58.27 A	68.33 BC	46.60 D
Canola 5 g.	16.94 CD	23.65 AB	29.99 B	23.86 CD	61.40 A	69.10 BC	59.23 BC
Canola 10 g.	22.21 BCD	25.00 AB	29.01 AB	25.79 CD	65.63 A	70.97 BC	62.83 AB
Canola 15 g.	40.90 A	20.00 AB	47.59 A	21.02 DE	60.77 A	62.27 C	62.53 AB
Bacteria 1	16.87 CD	24.52 AB	28.63 BCD	35.38 BC	66.90 A	74.07 ABC	60.47 BC
Bacteria 2	20.62 BCD	20.56 AB	24.39 BCD	20.75 DE	57.80 A	71.77 ABC	55.73 C
Canola 5 g. X Bacteria 1	17.95 BCD	21.67 AB	23.90 CD	25.85 CD	59.67 A	73.00 ABC	64.13 AB
Canola 5 g. X Bacteria 2	23.94 BCD	12.23 B	26.13 BCD	29.09 BCD	66.63 A	83.70 A	67.80 A
Canola 10 g. X Bacteria 1	20.71 BCD	31.11 A	39.71 AB	54.57 A	60.00 A	76.20 AB	62.00 B
Canola 10 g. X Bacteria 2	32.20 ABC	24.44 AB	30.30 BC	39.07 B	58.17 A	70.80 BC	58.63 BC
Canola 15 g. X Bacteria 1	34.59 AB	23.33 AB	45.46 A	55.54 A	60.47 A	73.90 ABC	55.97 C
Canola 15 g. X Bacteria 2	41.09 A	24.44 AB	45.49 A	41.08 B	61.63 A	77.90 AB	55.20 C
2nd season							
Control	15.70 D	12.23 B	16.28 C	12.17 D	60.87 A	70.60 A	46.20 D
Canola 5 g.	23.29 abc	19.25 B	47.23 A	19.04 CD	66.00 A	73.60 A	58.53 ABC
Canola 10 g.	28.44 A	37.22 AB	23.13 BC	38.02 AB	66.40 A	69.53 A	64.37 AB
Canola 15 g.	23.64 ABC	17.78 B	34.95 ABC	27.43 BCD	66.67 A	75.13 A	61.40 ABC
Bacteria 1	22.11 BC	26.11 AB	29.39 ABC	19.70 CD	61.83 A	76.37 A	62.47 ABC
Bacteria 2	26.26 AB	23.33 AB	21.92 BC	24.47 BCD	57.87 A	74.57 A	60.27 ABC
Canola 5 g. X Bacteria 1	20.03 CD	36.11 AB	17.81 C	18.03 CD	69.47 A	75.10 A	61.30 ABC
Canola 5 g. X Bacteria 2	22.60 ABC	14.49 B	33.65 ABC	31.00 ABC	65.37 A	84.03 A	62.97 ABC
Canola 10 g. X Bacteria 1	26.24 AB	30.56 AB	34.71 ABC	29.45 BC	62.83 A	75.33 A	65.43 A
Canola 10 g. X Bacteria 2	24.74 ABC	46.22 A	30.93 ABC	31.66 ABC	59.10 A	77.13 A	56.80 BC
Canola 15 g. X Bacteria 1	25.53 ABC	19.45 B	42.73 AB	29.77 ABC	58.53 A	72.53 A	55.60 C
Canola 15 g. X Bacteria 2	28.62 A	23.33 AB	29.49 ABC	45.64 A	66.63 A	81.60 A	56.27 BC

production in the rhizosphere under Pomegranate seedlings. Nitrogenase enzyme activity points to how much the atmospheric N₂ fixation is in the rhizosphere under the Pomegranate seedlings. CO₂-evolution refers to how active the cellulose decomposition is. While, indolacetic acid production refers to the extent to which the rhizobacteria could invade and colonize the roots and other plant parts. These observations go along with those by Spaepen *et al.* (2007) and Shraddha *et al.* (2019) They stated that roots produce various auxins such as Indole-3-acetic acid (IAA). IAA is a product of L-tryptophan which signals the bacteria to interact with plant roots and to colonize the plant parts.

Also, strains of Rhizobium, Azorhizobium is a stem nodule-forming symbiotic bacteria that form stem nodules and fixes nitrogen there (Gourion *et al.*, 2015), they also produce large amount of indole acetic acid (IAA) that promote plant growth. Bradyrhizobium is an efficient nitrogen fixer and enhances total organic carbon, N₂, phosphorus, and potassium contents in the soil efficiently. Thus, it significantly increases plant growth, soil microbial population

Table 2: Adding canola seed residues and bio Fertilization and its effect on Nitrogenase activity, CO₂ evolved and IAA producing of Pomegranate Seedlings in seasons of 2020.

Treatments	Nitrogenase activity in Rhizosphere	CO ₂ evolved	IAA producing by bacteria
Control	59.03 J	20.92 L	13.33 J
Bacteria 1	139.72 I	25.88 K	40.00 I
Bacteria 2	182.43 E	32.73 F	49.67 E
Canola 5 g.	187.54 D	34.75 E	61.67 D
Canola 10 g.	158.60 J	29.53 J	47.33 F
Canola 15 g.	150.93 H	30.90 H	42.67 H
PS.1+ K1	169.53 F	31.92 G	48.33 F
PS.2+ K1	233.48 A	40.68 A	58.33 A
PS.1+ K2	161.98 G	29.93 I	44.67 G
PS.2+ K2	192.70 C	28.12 D	52.67 CD
PS.1+ K3	193.90 C	39.60 C	53.67 C
PS.2+ K3	213.60 B	40.33 B	55.33 B

In response to the significant advantages of activities in the rhizosphere under pomegranate seedlings data in table (3) expose the N, P, and K uptake in the shoots and retained in the roots of pomegranate seedlings. Again, the rate of kanola application 5g/pot with Bacteria 2 have taken up significantly higher N, P, and K in the shoots and roots in pomegranate transplants (Table 3). Marathe *et al.* (2012) found that soil organic matter plays a vital role in improving the physicochemical attributes of fruits, it has been shown to affect nutrient fluxes in sweet orange and increase microbial biomass in pomegranate. Soil microbes are capable of converting insoluble soil phosphorus into plant available form(s) through various mechanisms of solubilization and mineralization (Alori *et al.*, 2017). Also, the increase in microbial may be attributed to readily available micronutrients and organic manures as sources of organic carbon for microbial population buildup. It may also be attributed to favourable excretions by root exudates like carbohydrates, amino acids, organic acids (Farooq *et al.*, 2021).

Table 3: Adding of canola seed residues and bio Fertilization and its effect on N, P, and K in the shoots and roots of pomegranate seedlings.

Treatments	Percentage of some macro elements in shoot			Percentage of some macro elements in root		
	Nitrogen	phosphorus	potassium	Nitrogen	phosphorus	potassium
Control	0.91 K	0.235 J	0.433 I	0.883 I	0.172 E	0.210 H
Bacteria 1	1.52 J	0.281 I	0.567 H	0.967 H	0.192 D	0.297 G
Bacteria 2	1.74 F	0.333 EF	0.727 E	1.117 DE	0.207 A-D	0.377 DE
Canola 5 g.	1.81 E	0.338 DF	0.760 D	1.57 CD	0.211 A-D	0.387 D
Canola 10 g.	1.67 H	0.318 FG	0.680 F	1.013 GH	0.200 BCD	0.340 F
Canola 15 g.	1.59 I	0.292 HI	0.643 G	0.997 GH	0.194 CD	0.307 G
PS.1+ K1	1.71 G	0.325 EFG	0.707 E	1.080 EF	0.202 BCD	0.363 E
PS.2+ K1	2.19 A	0.392 A	0.880 A	1.273 A	0.221 A	0.490 A
PS.1+ K2	1.67 H	0.308 GH	0.657 G	1.033 FG	0.195 CD	0.327 F
PS.2+ K2	1.87 D	0.352 CD	0.773 D	1.190 BC	0.213 ABC	0.410 C
PS.1+ K3	1.97 C	0.365 BC	0.793 C	1.200 BC	0.214 AB	0.420 C
PS.2+ K3	2.10 B	0.371 B	0.820 B	1.217 B	0.217 AB	0.440 B

Data in table (4) showed that the three applied rates of Canola 5 g/pot + Bacteria 2 significantly accumulated higher N, P, K, OC and OM than the control treatment in pomegranate transplants. It could be said that the greater the rate of kanola application, the greater the N, P, K, OC and OM accumulation in the rhizosphere under pomegranate transplants. The results of Raynaud and Nunan (2014) found that Bacteria in the soil depends upon the physical and chemical properties of the soil, organic matter, and phosphorus contents, as well as cultural activities. However, nutrient fixation and plant growth enhancement by bacteria are key components for achieving sustainable agriculture goals in the future. In addition, Ramesh *et al.* (2022) who recorded that use of organic sources has been more helpful in improving the nutritional status and microbial population of the soil, which aids in the growth, yield, and quality of pomegranate.

Microbes also facilitate various nutrient cycles in the ecosystem. Microorganisms play a central role in the natural N, P and K cycles. The use of N₂-fixers, phosphate and potassium solubilizers contribute in enhancing uptake of plant nutrients (Afifi *et al.*, 2014). Beneficial microorganisms are a tool that enhances plant growth and nutrient uptake. Chamila (2018) Application of phosphorus mobilizing microorganisms to soils can therefore be a promising approach for improving phosphorus fertilization efficiency in agriculture. Mycorrhizal fungi use as phosphorus mobilizing biofertilizers and they form a bridge between the roots and the soil, gathering nutrients including phosphorus from the soil and giving them to the roots.

Potassium is another vital nutrient and considered as a key parameter of soil fertility and plant growth. Also, Some rhizobacteria are able to solubilize insoluble potassium bearing phyllosilicates. Some *Bacillus* spp. and *Aspergillus niger* are used as Potassium solubilizing biofertilizers.

Table 4: Adding of canola seed residues and bio Fertilization and its effect on N, P, K, organic carbon (OC) and organic matter (OM) in the rhizosphere of pomegranate seedlings.

Treatments	Nitrogen in Soil(ppm)	Phosphors in Soil (ppm)	Potassium in Soil (ppm)	Organic carbon	Organic matter
Control	38.03 L	4.11 L	85.20 J	0.127 K	0.267 I
Bacteria 1	83.20 K	13.35 K	142.21 I	0.340 J	1.050 H
Bacteria 2	715.67 F	16.92 F	371.2 E	0.500 F	0.603 E
Canola 5 g.	720.97 E	17.58 E	361.0 E	0.580 E	1.963 D
Canola 10 g.	256.9 H	15.97 I	209.9 G	0.417 H	1.280 FG
Canola 15 g.	88.00 J	15.48 J	173.0 H	0.393 I	1.183 GH
PS.1+ K1	648.8 G	16.42 G	259.5 F	0.460 G	1.477 EF
PS.2+ K1	1298.7 A	30.35 A	923.6 A	2.007 A	3.830 A
PS.1+ K2	98.55 I	16.10 H	198.8 G	0.417 H	1.250 GH
PS.2+ K2	807.4 D	17.88 D	428.5 D	0.780 D	2.137 D
PS.1+ K3	1162.2 C	19.03 C	642.6 C	0.950 C	2.503 C
PS.2+ K3	128.1 B	19.57 B	720.3 B	1.170 B	2.717 B

4. Summary and Conclusion

Finally, it could be summarized that all treatments of canola seed residues addition as fertilizer improved the vegetative growth and stem, the height the shoot and root contents of N, P, and K. Also, the higher nitrogenase activity, CO₂ evolution, and IAA production in the rhizosphere below the pomegranate seedlings.

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