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Research Article

Roselle Responsiveness to Application of Certain Bio and Mineral Fertilizers in Relation to Plant Parasitic Nematodes

[1]Mohamed Mohamed Moussa and [2]Mohamed Said Abo-Korah

[1]Department of Horticulture, Menoufia University, Shebin El-Kom, Egypt
[2]Department of Economic Entomology and Agricultural, Menoufia University, Shebin El-Kom, Egypt

Abstract

Background and Objective: Roselle, *Hibiscus sabdariffa* L. var. *sabdariffa* is considered one of the most important medical plants all over the world. The application of different strains of bio fertilizers can help to control parasitic nematodes and to reduce the use of chemical nitrogen fertilizers and enhance the plant growth to decrease the production cost and environmental risk. The objective of this study was to maximize bio fertilizer and urea for controlling plant-parasitic nematode infected rosella in relation to improve growth and active constituents. **Methodology:** Field experiments were carried out to explore the role of three bio fertilizers: *Azotobacter chroococcum*; *Bacillus polymyxa* and *Pseudomonas fluorescens* singly and in combination with different doses of urea (0, 30, 60, 90 and 120 kg/feddan) in the control programs of plant parasitic nematodes infected roselle plants (*Hibiscus sabdariffa* L.) in relation to its growth characters under field conditions during two successive seasons. The experiment included 20 treatments, singly and combined between five levels of urea and different strains of bio fertilizers. The obtained data were subjected to analysis of variance (ANOVA) one way direction with LSD 5% using CoStat Software, Version 6.4 (2008). **Results:** All tested treatments significantly at 5% reduced soil and root population of the plant parasitic nematodes and increased the growth, yield and chemical constituents of roselle plants compared with untreated control. The highest reduction percentages in parasitic nematode population (84.3 and 85.9%) in the two successive seasons, respectively, occurred at the application of; *A. chroococcum*+60 kg urea/feddan. In addition, effectiveness of this combined gave a better enhancement in plant height (218.80 and 231.44 cm), fresh weight of whole plant (1227.22 and 1264.18 g) and seed yield (676.89 and 661.06 kg/feddan), in the two successive seasons, respectively, compared with untreated control. **Conclusion:** Finally, it could be concluded that, the best conformity of urea plus bio-fertilizers are *A. chroococcum*+60 kg urea/feddan. Which descendingly effective on parasitic nematodes reduction and roselle plant growth characters.

Key words: Parasitic nematodes, *Azotobacter chroococcum*, *Bacillus polymyxa*, *Pseudomonas fluorescens*, *Hibiscus sabdariffa*

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Corresponding Author: Mohamed Said Abo-Korah, Department of Economic Entomology and Agricultural, Menoufia University, Shebin El-Kom, Egypt
Tel: 00201006601496

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Roselle, *Hibiscus sabdariffa* L. var. *sabdariffa* is considered as one of the most important medical plants of the family Malvaceae[1]. In addition, its drink has a laxative effect and the calyx extraction is a great therapeutic action for curing heart and nerve diseases, high blood pressure and calcified arteries[2]. It has been mentioned that it is beneficial for cathartic, cancer, protective, restorative, sexual stimulator, appetizer, refrigerant and anti-cough[3].

The annual global loss in agriculture due to damage by plant-parasitic nematodes has been estimated as US $100 billion worldwide. Most of plant parasitic nematodes attack the roots or other parts in the soil such as bulbs and tubers and interrupt the uptake of water and nutrients by plants[4]. The dominant nematode genera attacking Roselle plants are *Meloidogyne*, *Pratylenchus* and *Xiphinema* which occurred in different numbers. One or more *Meloidogyne* species have been reported as pathogens of roselle. Roselle is generally susceptible to *M. arenaria* and *M. incognita* infection[5,6].

Many popular fertilizers incorporate nitrogen in their composition or made specifically as a nitrogen fertilizer is necessary and critical for plant growth. The application of ammonia (NH_3) has been shown to control parasitic nematodes, with exposure of J_2 to ammonia at 9.3 mg plant^{-1} for 40 h causing 95% mortality[7]. Plants require nitrogen as it is a major component of chlorophyll, which is essential for the process of photosynthesis[8].

It is also a major component of amino acid, which required in making proteins and that essential for the structural elements in the plant. Some soil amendments (e.g., with low C/N ratios of organic matter, in addition to NH_4OH or urea) have been proposed for controlling parasitic nematodes[9].

The application of different strains of bio fertilizers considered an important factors that can help to reduce the use of chemical nitrogen fertilizers and enhance the plant growth to decrease the production cost and environmental risk[10,11].

The *A. chroococcum*; *B. polymyxa* and *P. fluorescens* strains are implicated in the control of plant parasitic nematodes, such products may be toxic to nematodes directly or it may be indirectly suppress nematode population by modifying the rhizosphere environment[12,13].

Azotobacter chroococcum has been reported to inhibit hatching of juveniles of *Meloidogyne incognita* and its penetration in roots[14]. *Bacillus polymyxa* was previously found to have the ability, not only inhibit parasitic nematode growth but also facilitate colonization of the root and induce host resistance[15]. *Pseudomonas fluorescens* was found to exert toxic effects towards eggs and juveniles of *M. javanica in vitro* and contributes in reducing nematode densities and consequently root-knot disease in tomato[16].

Inoculating seeds with bio fertilizers secrete some organic acid which can convert important elements from unvalued to available form through biological process[17]. One of the most troublesome limiting factors in increasing the cultivation lands for roselle plants in Menoufia Governorate are nematodes. Therefore, objective of this field study was reduction of plant parasitic nematodes infected Roselle plants and improvement in its growth characters.

MATERIALS AND METHODS

Field experiments were carried out as Randomized Complete Block Design (RCBD); 20 treatments with three replicates for each during two successive seasons 2013 and 2014 at the experimental farm of the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt, to study the effect of bio and chemical fertilizers singly and in combination on plant parasitic nematodes infected roselle plants and measures growth, yield and chemical composition of *Hibiscus sabdariffa* L. plants.

The chemical and physical properties of the experimental soil were determined according to the method of Cottenie *et al.*[18] as follows: pH 7.9, EC 1.73 dS m^{-1}; 2.80% organic matter, 44.24% silt, 3.84% coarse sand, 27.40% fine sand, 23.20% clay, the texture grade was a clay loam soil. The available macronutrients were 2.30% $CaCO_3$, 0.12% N and 0.25% P_2O_5.

Preparation strains of bio fertilizers: Efficient local strains of *A. chroococcum*, *B. polymyxa* and *P. fluorescens* were obtained kindly from Pharmaceutical Department National Research Center, Giza, Egypt. The aforementioned bacterial strains were prepared for application according to the method described by Mahfouz[19]. The prepared culture from each bacterial strain contained 10^7cell mL^{-1}.

Experimental preparation and design: Seeds of Roselle, *Hibiscus sabdariffa* L. were soaked in different bacterial strains solely for 12 h, in addition, the control soaked in distilled water. Seeds were sown on 14th of April in the first and second seasons. The soil was prepared and divided into plots of 2×1.8 m with three rows at 60 cm apart and 40 cm between hills. Beside inoculation the seeds, *A. chroococcum*; *B. polymyxa* and *P. fluorescens* were applied (10^6 CFU mL^{-1}) at a rate of 10 mL per plant after 15 days form planting[20].

The experiment included 20 treatments, singly and combined between five levels of urea (46% N) and different strains of bio fertilizers (*A. chroococcum*; *B. polymyxa*; *P. fluorescens* plus un-inoculated seeds). Roselle plants were harvested at the first of October during maturity stage through two successive seasons (2013 and 2014).

Obtained data showed that all measurements of roselle such as: plant height (cm); number of main branches; fresh weight of whole plant (g per plant); number and fresh weight of fruits (g per plant); fresh weight of sepals (g per plant); dry weight of sepals (g per plant and kg per feddan); seed yield (g per plant and kg per feddan); fixed oil% and fixed oil yield (L. feddan).

Nematode extraction and enumeration: Each composite soil sample was carefully mixed and an aliquot of 100 cm^3 was processed for nematode extraction according to methods described by Southey[21], each treatment was replicated three times. An aliquant of 1 mL each of nematode suspensions were pipetted off, placed in a Hawksley counting slide and examined by using a stereomicroscope.

Nematode counts were carried out before treatment and after 1-6 months of application and the identification to generic level were based on morphology of the adult and larval forms, according to the description of Mai and Lyon[22]. Roots were carefully washed and the nematode galls were counted and rated as mentioned in Table 1 as well as one gram per root was stained by acid fuchsin lactophenol to counted root knot nematode stages inside the roots with the aid of a dissecting microscope.

Egg masses were assessed by staining the roots with Phloxin-B solution (0.15 g L^{-1} tap water) for 20 min according to Daykin and Hussey[23].

Chemical analysis: Total anthocyanin content in the sepals was determined by using Du and Francis[24] method. Total carbohydrate percentages in the dried leaves were determined by using the colorimetric method of DuBois *et al.*[25]. Nitrogen, phosphorus and potassium percentages were determined as follows: in the dried leaves by Kjeldahl methods, using Spectrophotometrically (Benchtop CS-800 series) and by Flame photometer (model 360, 420 series), respectively[18]. The pH values of sepals were determined according to Diab[26]. Fixed oil percentages were determined according to the AOAC [27].

Statistical analysis: The obtained data were subjected to analysis of variance (ANOVA) one way direction with LSD 5% using CoStat Software, Version 6.4 (2008).

Reduction percentages were counted according to the formula of Henderson and Tilton[28] and Fleming and Retnakaran[29]:

$$\text{Reduction (\%)} = \left[1 - \left(\frac{\text{Treatment after}}{\text{Treatment before}} \times \frac{\text{Control before}}{\text{Control after}}\right)\right] \times 100 \quad (1)$$

$$\text{Increase or decrease (\%)} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Effect of bio and mineral fertilizers on plant parasitic nematodes infected roselle plants

In the first season (2013): The obtained results presented in Table 2 show the effect of bio and mineral fertilizers on the average numbers of plant parasitic nematodes infected roselle plants in the first season (2013) before treatment and after 1-6 months of application under field conditions.

Statistical analysis of data in Table 2 indicated that there were significant differences among treatments at 5%. Nematode population was suppressed in the treated soils after 1-6 months in comparison with control treatment. The combined treatment of (*A. chroococcum*+60 kg urea/feddan) recorded the least number of parasitic nematode (375.0 individual's/100 g) in the 6th month (October, 2013),

Azotobacter spp. is aggressive colonizers of the rhizosphere of various crop plants and has broad spectrum antagonistic activity against plant pathogens[30].

The highest reduction percentages of the nematode in the soil were recorded in the combined treatments of (*A. chroococcum*+60 kg urea/feddan) followed by (*B. polymyxa*+90 kg urea/feddan) and (*P. fluorescens*+30 kg urea/feddan) with (84.3, 73.0 and 59.1%), respectively, descendingly as shown in Table 3.

Some species of *Azotobacter* and *Bacillus* are reported to induce systemic resistance in plants against invading pathogens and antagonists to root-knot nematodes[31].

Such findings could lead us to make some restrictions in choosing the suitable fertilizers level to, fit with our requirements. The main tool in this respect must be the behavior of soil fertilizer under different environmental conditions.

Table 1: Rating scale levels of resistance or susceptible by gall numbers

No. of galls	Galling index	Resistance rating
0	0	Immune
1-2	1	Highly resistant
3-10	2	Resistant
11-30	3	Moderately resistant
31-70	4	Moderately susceptible
71-100	5	Susceptible
<100	6	Highly susceptible

Source: Southey[21]

Table 2: Effect of mineral urea levels and bio-fertilizers on the population density of plant parasitic nematodes infected roselle plants under field conditions (2013)

Months	Urea levels (kg/feddan)	Average no. of parasitic nematodes/100 g soil				
		Control	*A. chroococcum*	*B. polymyxa*	*P. fluorescens*	Mean
Pre-treatment						
	0	1687.8	1653.4	1589.0	1612.9	-
	30	1320.0	1592.0	1490.0	1703.9	-
	60	1805.2	1689.8	1638.0	1700.7	-
	90	1425.5	1463.2	1388.3	1409.6	-
	120	1369.0	1411.2	1397.3	1500.0	-
May						
	0	1695.7	1601.0	1522.0	1572.0	1597.68
	30	1331.0	1422.0	1388.5	1670.0	1452.88
	60	1817.0	1402.0	1508.0	1663.0	1597.50
	90	1448.5	1380.0	1163.3	1348.3	1335.03
	120	1392.0	1388.0	1348.3	1473.4	1400.43
	Mean	1536.84	1438.60	1386.02	1545.34	-
LSD at 0.05		Urea = 18.12, Biofertilizer = 14.34, Urea×Biofertilizer = 32.12				
June						
	0	1750.0	1501.0	1443.3	1503.0	1549.33
	30	1415.5	1212.5	1297.5	1523.2	1362.18
	60	1893.0	1165.0	1367.0	1601.0	1506.50
	90	1512.0	1210.0	1079.6	1281.0	1270.65
	120	1446.2	1300.0	1323.0	1461.0	1382.55
	Mean	1603.34	1277.70	1302.08	1473.84	-
LSD at 0.05		Urea = 24.15, Biofertilizer = 20.11, Urea×Biofertilizer = 45.05				
July						
	0	1898.0	1298.5	1262.0	1423.0	1470.38
	30	1601.2	1050.0	1147.3	1402.0	1300.13
	60	1978.0	992.0	1119.0	1532.3	1405.33
	90	1680.6	1015.0	1002.0	1210.3	1226.98
	120	1603.3	1102.0	1297.9	1428.0	1357.80
	Mean	1752.22	1091.50	1165.64	1399.12	-
LSD at 0.05		Urea = 61.16, Biofertilizer = 52.53, Urea×Biofertilizer = 117.66				
August						
	0	2098.6	1023.0	1137.0	1340.1	1399.68
	30	1750.5	980.0	1019.0	1316.0	1266.38
	60	2100.0	638.0	1026.0	1417.0	1295.25
	90	1806.0	972.5	830.9	1128.5	1184.48
	120	1958.0	1022.0	1207.0	1357.0	1386.00
	Mean	1942.62	927.10	1043.98	1311.72	-
LSD at 0.05		Urea = 73.25, Biofertilizer = 65.35, Urea×Biofertilizer = 146.38				
September						
	0	2211.0	909.5	1023.0	1301.9	1361.35
	30	1980.0	732.0	975.0	1240.4	1231.85
	60	2310.5	501.0	866.3	1347.0	1256.20
	90	1998.0	789.5	693.0	1003.0	1120.88
	120	2201.0	1097.0	1159.0	1314.0	1442.75
	Mean	2140.10	805.80	943.26	1241.26	-
LSD at 0.05		Urea = 95.13, Biofertilizer = 82.97, Urea×Biofertilizer = 185.85				
October						
	0	2450.0	897.0	839.0	1210.0	1349.00
	30	2203.0	702.0	840.0	1162.0	1226.75
	60	2545.0	375.0	791.0	1170.0	1220.25
	90	2290.0	700.0	602.0	970.0	1140.50
	120	2479.0	1115.0	1117.3	1287.5	1499.70
	Mean	2393.40	757.80	837.86	1159.90	-
LSD at 0.05		Urea = 99.73, Biofertilizer = 86.26, Urea×Biofertilizer = 193.22				

Second season (2014): Data presented in Table 4 showed that the side effect of applied treatments on the population density of plant parasitic nematodes infected Roselle plants under field conditions.

Table 3: Reduction percentages of plant parasitic nematodes infected roselle plants as affected by bio and mineral levels fertilizers under field conditions (2013)

		Reduction (%)		
Months	Urea levels (g/feddan)	*A. chroococcum*	*B. polymyxa*	*P. fluorescens*
May				
	0	3.6	4.7	3.0
	30	11.4	7.6	2.8
	60	17.6	8.5	2.9
	90	7.2	17.5	5.9
	120	3.3	5.1	3.4
June				
	0	12.4	12.4	10.1
	30	29.0	18.8	16.6
	60	34.3	20.4	10.2
	90	22.0	26.7	14.3
	120	12.8	10.4	7.8
July				
	0	30.2	29.4	21.5
	30	45.6	36.5	32.2
	60	46.4	37.7	17.8
	90	41.2	38.8	27.2
	120	33.3	20.7	18.7
August				
	0	50.2	42.5	33.2
	30	53.6	48.4	41.8
	60	67.5	46.2	28.4
	90	47.5	52.8	36.8
	120	49.4	39.6	36.7
September				
	0	58.0	50.9	38.4
	30	69.3	56.4	51.5
	60	76.8	58.7	38.1
	90	61.5	64.4	49.2
	120	51.6	48.4	45.5
October				
	0	62.6	63.6	48.3
	30	27.3	66.2	59.1
	60	84.3	65.7	51.2
	90	70.2	73.0	57.2
	120	56.4	55.8	52.6

Statistical analysis indicated that all treatments significantly at 5% suppressed nematode population in the soil treated after 1-6 months in comparison with control treatment. The combined treatment of (*A. chroococcum*+60 kg urea/feddan) recorded the least population density of parasitic nematode (239.5 ind's /100 g) in the 6th month (October, 2014).

Followed by that found in combined treatments of (*B. polymyxa* and *P. fluorescens*+90 kg urea/feddan) for each, resulted nematode population density of (597.0 and 829.0 ind's/100 g), respectively.

The application of ammonia (NH_3) has been shown to control nematodes, with exposure of J2 to ammonia at 9.3 mg per l for 40 h causing 95% mortality[7]. Some soil amendments (e.g., with low C/N ratios of organic matter, in addition to NH_4OH or urea) have been proposed for controlling nematodes[9].

Results in Table 5 showed that, the highest reduction percentages of the nematode in the soil, occurred in the combined treatment of *A. chroococcum*+60 kg urea/feddan followed by *B. polymyxa*+90 kg urea/feddan and *P. fluorescens*+30 kg urea/feddan with 85.9, 67.8 and 58.8% respectively, descendingly.

It is very interesting to know that, volatile compounds, fatty acids, hydrogen sulfide, enzymes, hormones, alcohol and phenolic compounds are among the bacterial products implicated in the control of plant parasitic nematodes[13].

Such products may be toxic to nematodes directly or it may be indirectly suppress nematode population by modifying the rhizosphere environment [12].

This study results are in agreements with those obtained by Wen *et al.*[32], who found that urea+bacteria to be effective against RKN and ammonia may act by disrupting cell membranes.

Table 4: Effect of mineral urea levels and bio-fertilizers on the population density of plant parasitic nematodes infected roselle plants under field conditions (2014)

		Average no. of parasitic nematodes/100 g soil				
Months	Urea levels (g/feddan)	Control	*A. chroococcum*	*B. polymyxa*	*P. fluorescens*	Mean
Pre-treatment						
	0	1572.0	1512.3	1490.0	1576.6	-
	30	1496.0	1426.0	1412.0	1496.2	-
	60	1437.5	1398.5	1479.0	1465.3	-
	90	1380.4	1329.0	1399.0	1458.2	-
	120	1479.0	1496.0	1412.9	1397.0	-
May						
	0	1597.0	1320.0	1413.0	1511.3	1460.33
	30	1520.3	1341.1	1368.2	1449.0	1419.65
	60	1469.0	1310.0	1412.0	1413.2	1401.05
	90	1398.0	1249.0	1314.0	1319.0	1320.00
	120	1514.0	1465.9	1379.2	1382.0	1435.28
	Mean	1499.66	1337.20	1377.28	1414.90	
LSD at 0.05		Urea = 23.12, Biofertilizer = 18.87, Urea×Biofertilizer = 42.27				
June						
	0	1670.0	1249.0	1354.0	1475.0	1437.00
	30	1560.5	1223.3	1319.2	1379.0	1370.50
	60	1512.0	1114.5	1345.0	1306.0	1319.38
	90	1413.5	1205.0	1289.0	1267.0	1293.63
	120	1535.0	1414.0	1341.0	1312.0	1400.50
	Mean	1538.20	1241.16	1329.64	1347.80	
LSD at 0.05		Urea = 23.27, Biofertilizer = 19.36, Urea×Biofertilizer = 43.76				
July						
	0	1803.0	1153.6	1290.0	1417.2	1415.95
	30	1589.0	1098.0	1236.0	1297.2	1305.05
	60	1564.0	945.0	1212.3	1196.0	1229.33
	90	1548.5	1104.3	1103.0	1141.2	1224.25
	120	1589.0	1379.0	1319.6	1298.2	1396.45
	Mean	1618.70	1135.98	1232.18	1269.96	
LSD at 0.05		Urea = 51.34, Biofertilizer = 45.54, Urea×Biofertilizer = 102.01				
August						
	0	1970.4	1037.0	1231.1	1315.0	1388.38
	30	1614.0	1001.5	1173.7	1223.0	1253.05
	60	1597.0	712.0	1103.7	1055.0	1116.93
	90	1632.3	989.9	1042.1	1071.3	1183.90
	120	1610.0	1358.0	1276.4	1231.3	1368.93
	Mean	1684.74	1019.68	1165.40	1179.12	
LSD at 0.05		Urea = 53.60, Biofertilizer = 48.67, Urea×Biofertilizer = 109.02				
September						
	0	2036.0	987.5	1097.0	1206.0	1331.63
	30	1689.0	869.4	1012.0	1099.3	1167.43
	60	1690.0	416.0	997.4	1003.0	1026.60
	90	1674.2	802.0	836.0	984.2	1074.10
	120	1815.0	1268.9	1147.2	1194.2	1356.33
	Mean	1780.84	868.76	1017.92	1097.34	
LSD at 0.05		Urea = 67.27, Biofertilizer = 53.37, Urea×Biofertilizer = 119.54				
October						
	0	2230.0	905.0	902.4	1111.0	1287.10
	30	1860.0	712.0	873.2	989.3	1108.63
	60	1812.0	239.5	801.2	903.0	938.93
	90	1903.0	709.2	597.0	829.0	1009.55
	120	2041.6	1218.0	1029.3	1001.0	1322.48
	Mean	1969.32	756.74	840.62	966.66	
LSD at 0.05		Urea = 120.11, Biofertilizer = 107.43, Urea×Biofertilizer = 240.64				

All plant treatments decreased the gall index and egg-masses production (Table 6). The combined treatment of *A. chroococcum*+60 kg urea/feddan recorded the highest decrease percentage in root gall index (-80 and -80%) and egg

Table 5: Reduction percentages of plant parasitic nematodes infected roselle plants as affected by bio and mineral levels fertilizers under field conditions (2014)

		Reduction (%)		
Months	Urea levels (kg/feddan)	*A. chroococcum*	*B. polymyxa*	*P. fluorescens*
May				
	0	14.1	6.7	5.6
	30	7.5	4.7	4.7
	60	8.3	6.6	5.6
	90	7.2	7.3	10.7
	120	4.3	4.6	3.4
June				
	0	23.7	13.9	7.6
	30	35.0	25.1	31.5
	60	21.3	2.0	8.3
	90	16.9	6.4	9.4
	120	10.6	14.4	22.0
July				
	0	34.7	24.0	17.7
	30	42.7	31.1	36.8
	60	35.5	14.6	18.8
	90	30.5	26.9	25.5
	120	15.8	18.6	25.4
August				
	0	46.3	33.6	30.2
	30	48.6	35.6	41.3
	60	52.4	23.8	29.9
	90	40.9	34.4	33.6
	120	18.2	22.3	30.2
September				
	0	50.5	42.8	38.0
	30	57.3	46.9	49.6
	60	73.7	35.0	37.0
	90	53.3	48.7	40.6
	120	32.2	38.1	40.0
October				
	0	58.6	57.0	47.9
	30	68.3	58.4	58.8
	60	85.9	51.3	47.1
	90	63.7	67.8	55.9
	120	42.1	50.6	55.3

masses production (-86.0 and -88.8%) in the two seasons, respectively. Our results are in agreements with those obtained by Li *et al.*[33] and Anwar-ul-Haq *et al.*[34].

Statistical analysis showed that, there are significant differences at 5% among all of experimental factors between each of months; bio and mineral fertilizers; population density of plant parasitic nematodes and the interactions within the experimental factors.

Vegetative growth characteristics

Effect of nitrogen application: The obtained data in Table 7 indicated that, the application of urea levels had a significant at 5% effected on vegetative growth characters i.e., plant height, number of main branches as well as fresh weight of whole plant which reached to optimum values as a results of using 90 kg/feddan during the two seasons (2013 and 2014).

These results could be explained by using nitrogen in building protein molecules and enhancing cell division and cell elongation which was reflected in promoting the growth of lateral buds and producing more branches, fresh weight and increasing the length of the plant and was similar to those obtained by Sakr *et al.*[35] on rosella plants.

Effect of bio-fertilizers application: The three bio-fertilizers gave significantly at 5% increased in growth characters of roselle plants (Table 7). The *A. chroococcum* produced the best results followed by *B. polymyxa* and *P. fluorescens*, during the two successive seasons, respectively. This effectiveness may be due to producing adequate amounts of IAA, gibberellins, cytokinins and vitamins which improve roots capacity, length, hair branching and mineral up take from the soil[36]. This study results stated the same treat with those obtained by Aly *et al.*[37] on dill plants.

Effect of interaction: Interaction occurred within most of combined treatments caused significant differences at 5%

Table 6: Decrease of gall index and egg-masses production on roselle as influenced by treatments application in the two seasons (2013 and 2014)

		1st season				Decrease (%)		
Root gall and egg-masses	Urea levels	Control	*Azoto.*	*Baci.*	*Pseud.*	*Azoto.*	*Baci.*	*Pseud.*
Root gall index								
	0	6.0	3.0	3.0	4.0	-50.0	-50.0	-33.3
	30	6.0	2.0	3.0	4.0	-66.6	-50.0	-33.3
	60	5.0	1.0	2.5	4.0	-80.0	-50.0	-20.0
	90	6.0	2.5	2.0	3.0	-58.3	-66.6	-50.0
	120	6.0	4.0	4.0	5.0	-33.3	-33.3	-16.6
Egg-masses production per root								
	0	58.0	24.0	26.0	34.0	-58.6	-55.1	-41.3
	30	54.0	15.0	25.0	32.0	-72.2	-53.7	-40.7
	60	43.0	6.0	17.0	33.0	-86.0	-60.4	-23.2
	90	51.0	19.0	11.0	19.0	-62.7	-78.4	-62.7
	120	54.0	29.0	31.0	40.0	-46.2	-42.5	-25.9
		2nd season				Decrease (%)		
Root gall and egg-masses	Urea levels	Control	*Azoto.*	*Baci.*	*Pseud.*	*Azoto.*	*Baci.*	*Pseud.*
Root gall index								
	0	6.0	3.0	3.5	4.0	-50.0	-41.6	-33.3
	30	6.0	2.5	2.5	3.5	-58.3	-58.3	-41.6
	60	5.0	1.0	2.5	3.5	-80.0	-50.0	-30.0
	90	6.0	2.0	2.0	3.0	-66.6	-66.6	-50.0
	120	6.0	4.0	3.5	4.0	-33.3	-41.6	-33.3
Egg-masses production per root								
	0	57.0	22.0	25.0	28.0	-61.4	-56.1	-50.8
	30	58.0	16.0	18.0	26.0	-72.4	-68.9	-55.1
	60	45.0	5.0	17.0	25.0	-88.8	-62.2	-44.4
	90	53.0	13.0	11.0	19.0	-75.4	-79.2	-64.1
	120	55.0	26.0	25.0	28.0	-52.7	-54.5	-49.0

level in all vegetative characters during both seasons under study, reached the maxima with application of the combined treatment of *A. chroococcum*+60 kg urea/feddan. Its values were varied within both seasons, with higher levels in 2014 than that found in 2013 seasons. Such findings may be affected by different environmental factors [38]. Results obtained are in harmony with that found by Mohamed *et al.*[39] on sweet basil plants.

Yield parameters and oil production

Effect of nitrogen application: Data recorded in Table 8 and 9 showed that, all urea levels significantly at 5% increased number of fruits, fresh weight of fruits (g per plant), fresh weight of sepals (g per plant), dry weights of sepals (g per plant and kg per feddan), seed yield (g per plant and kg per feddan), fixed oil% and fixed oil yield (L per feddan). Level of 90 kg urea per feddan was the best treatment and this may be due to the direct effect of fertilization on increasing the photosynthetic activity and different metabolic processes which encourage the yield of plants. These results are in agreements with those obtained by Khalil and Yousef[40] on roselle plants.

Effect of bio-fertilizers application: Bacterial strain of *A. chroococcum* was the best treatment which significantly at 5% increased both total yield parameters and oil production, while *P. fluorescens* caused the higher value of fixed oil% in both seasons.

This may be due to that bio-fertilizers improve physical, chemical, microbiological characteristics of the soil and increase its hold capacity which led to increase the yield[41]. These results are in harmony of that found by Patel *et al.*[42] on *Plumbago zeylanica*.

Effect of interaction: All combined treatments caused significantly at 5% increased of total yield parameters and oil production, reached the maximum by application *A. chroococcum*+60 kg urea/feddan in both seasons, followed by the application of 90 kg urea/feddan plus of each *A. chroococcum* and *P. fluorescens* which resulted the highest fixed oil yield and fixed oil percentage in both seasons, respectively. Similar results were found by Mohamed *et al.*[38] on dill plants.

Quality of sepals and chemical composition

Effect of nitrogen application: The obtained results show that, all urea levels increased the quality of sepals including anthocyanin content and pH values which arrived to the greatest values by added urea at 60 and 90 kg/feddan in

Table 7: Effect of mineral urea, bio-fertilizers and their interaction on vegetative characters of roselle plants during the seasons (2013 and 2014)

	Urea levels (kg/feddan) First season (2013)					
Bacterial strains	0	30	60	90	120	Mean
Plant height (cm)						
Control	152.05	156.33	161.66	169.93	171.72	162.33
A. chroococcum	204.22	212.44	218.80	215.50	209.50	212.09
B. polymyxa	195.30	199.33	203.26	208.72	202.04	201.73
P. fluorescens	172.39	179.67	179.85	185.14	181.50	179.71
Mean	180.99	186.94	190.89	194.82	191.19	-
LSD at 5%	U = 1.96, B = 1.81, UB = 4.05					
No. of main branches per plant						
Control	6.67	7.05	7.55	8.92	9.78	7.99
A. chroococcum	11.36	12.11	13.33	12.72	12.04	12.31
B. polymyxa	10.14	11.14	11.50	12.50	12.00	11.46
P. fluorescens	8.86	9.56	9.98	11.78	10.11	10.06
Mean	9.25	9.96	10.59	11.48	10.98	-
LSD at 5%	U = 0.28, B = 0.20, UB = 0.45					
Fresh weight/whole plant (g)						
Control	532.65	570.41	614.32	643.22	686.16	609.35
A. chroococcum	1065.79	1114.14	1227.22	1173.36	1131.52	1142.40
B. polymyxa	942.73	986.45	1071.44	1148.98	1053.18	1040.55
P. fluorescens	882.41	920.70	965.81	1026.93	1001.11	959.39
Mean	855.90	897.93	969.70	998.12	967.99	-
LSD at 5%	U = 26.37, B = 21.3, UB = 47.70					
	Urea levels (kg/feddan) 2nd season (2013)					
Bacterial strains	0	30	60	90	120	Mean
Plant height (cm)						
Control	159.22	163.69	163.95	175.67	182.13	168.93
A. chroococcum	215.85	222.83	231.44	220.50	213.41	220.81
B. polymyxa	193.37	203.83	203.67	213.82	207.14	204.37
P. fluorescens	177.50	185.67	185.20	193.40	189.62	186.28
Mean	186.49	194.01	196.07	200.85	198.08	-
LSD at 5%	U = 2.05, B = 1.94, UB = 4.34					
No. of main branches per plant						
Control	7.66	7.67	8.05	9.36	9.92	8.53
A. chroococcum	12.11	12.47	14.50	12.47	11.86	12.68
B. polymyxa	10.17	10.58	11.86	12.66	11.33	11.32
P. fluorescens	9.47	9.86	10.56	11.98	11.11	10.59
Mean	9.85	10.14	11.24	11.61	11.05	-
LSD at 5%	U = 0.23, B = 0.18, UB = 0.40					
Fresh weight/whole plant (g)						
Control	565.15	612.71	648.72	713.63	736.50	655.34
A. chroococcum	1132.41	1187.80	1264.18	1240.73	1195.69	1204.16
B. polymyxa	1020.39	1065.57	1114.24	1175.17	1096.20	1094.31
P. fluorescens	916.29	942.95	981.15	1012.33	787.62	928.06
Mean	908.56	952.25	1002.07	1035.46	954.00	-
LSD at 5%	U = 23.34, B = 20.37, UB = 45.63					

U: Urea, B: Biofertilizers, UB: Interaction

both seasons, respectively (Table 10). Total carbohydrate N and K (%) increased by using different levels of urea except phosphors percentage which increased at 30 kg urea/feddan and then decreased gradually by increasing urea level up to 120 kg/feddan. These results are in accordance with those found by Abo-Baker and Mostafa[43] on roselle plants.

Effect of bio-fertilizers application: The highest mean value in this trail were obtained by using *B. polymyxa* in the two seasons except anthocyanin content which reached its maxima by using *P. fluorescens.* Similar results were obtained by Sakr *et al.*[35] on roselle plants.

Effect of interaction: High increments in total anthocyanin content, pH values in roselle leaves by the application of *P. fluorescens*+90 kg urea/feddan and *B. polymyxa*+60 kg urea/feddan in both seasons, respectively (Table 10). While the combined treatments

Table 8: Effect of mineral, bio-fertilizers and their interactions on roselle fruits during two seasons (2013 and 2014)

	First season (2013)						Second season (2014)					
	0	30	60	90	120	Mean	0	30	60	90	120	
Bacterial strains	kg/feddan urea						kg/feddan urea					Mean
No. of fruits per plant												
Control	38.20	41.33	43.14	46.28	47.67	43.32	42.27	42.77	45.19	48.64	52.52	46.27
A. chroococcum	81.80	84.91	89.18	84.77	80.64	84.26	79.50	80.60	85.93	85.21	78.82	82.01
B. polymyxa	73.15	73.11	75.23	78.37	74.32	74.83	69.18	71.22	71.60	74.62	70.19	71.36
P. fluorescens	65.80	68.66	70.54	70.15	65.29	68.08	61.54	61.67	62.20	65.29	65.80	63.30
Mean	64.73	67.00	69.52	69.89	66.98	-	63.12	64.06	66.23	68.44	66.83	-
LSD at 5%	U = 1.01, B = 0.85, UB = 1.90						U = 1.22, B = 1.00, UB = 2.24					
Fresh weight of fruits (g plant^{-1})												
Control	208.62	219.23	227.40	229.64	236.55	224.28	210.17	225.69	231.82	240.74	245.53	230.79
A. chroococcum	401.35	409.18	434.07	418.17	405.46	413.64	405.61	414.66	447.37	420.29	410.80	419.74
B. polymyxa	355.47	365.18	374.66	395.86	371.40	372.51	364.66	372.51	385.34	400.48	387.11	382.02
P. fluorescens	315.20	334.54	348.72	366.49	357.50	344.49	318.27	339.66	351.54	372.20	360.18	348.37
Mean	320.16	332.03	346.21	352.54	342.72	-	324.67	338.13	354.01	358.42	350.90	-
LSD at 5%	U = 8.27, B = 6.58, UB = 14.73						U = 11.80, B = 10.05, UB =22.51					
Fresh weight of sepals (g plant^{-1})												
Control	103.48	108.41	111.82	112.80	115.68	110.43	107.14	112.50	114.92	119.40	120.09	114.81
A. chroococcum	195.60	198.54	208.65	200.18	197.56	200.10	199.50	202.94	216.99	201.92	201.19	204.50
B. polymyxa	174.87	178.04	181.64	192.68	183.66	182.17	181.09	184.15	189.65	196.07	189.70	188.13
P. fluorescens	152.17	162.13	169.75	178.53	175.86	167.68	155.12	166.17	172.90	182.35	178.21	170.95
Mean	156.53	161.78	167.96	171.04	168.19	-	160.71	166.44	173.61	174.93	172.29	-
LSD at 5%	U = 3.11, B = 2.08, UB = 4.66						U = 5.54, B = 3.97, UB = 8.89					
Dry weight of sepals (g plant^{-1})												
Control	15.28	16.02	16.84	17.13	17.67	16.58	15.80	16.32	17.41	17.90	18.01	17.08
A. chroococcum	25.05	25.89	27.54	26.28	25.79	26.11	25.89	26.64	28.30	27.52	26.96	27.06
B. polymyxa	21.45	22.13	22.86	24.23	23.16	22.76	22.17	22.66	23.40	24.91	24.15	23.45
P. fluorescens	18.22	19.45	20.62	21.79	21.15	20.24	18.45	18.92	20.88	21.95	21.40	20.32
Mean	20.00	20.87	21.96	22.35	21.94	-	20.57	21.13	22.49	23.07	22.63	-
LSD at 5%	U = 0.53, B = 0.41, UB = 0.92						U = 0.75, B = 0.63, UB = 1.41					
Dry weight of sepals (kg/feddan)												
Control	254.65	266.98	280.55	285.38	294.27	276.36	263.22	271.89	290.05	298.21	300.04	284.68
A. chroococcum	417.33	431.32	458.81	437.82	429.66	434.98	431.32	443.82	471.47	458.48	449.15	450.84
B. polymyxa	357.35	368.68	380.68	403.67	385.84	379.24	369.35	377.51	389.84	415.00	402.33	390.80
P. fluorescens	303.54	324.03	343.52	363.02	352.35	337.29	307.37	315.20	347.86	365.68	356.52	338.52
Mean	333.21	347.75	365.89	372.47	365.53	-	342.81	352.10	374.80	384.34	377.01	-
LSD at 5%	U = 10.12, B = 8.07, UB = 18.08						U = 11.21, B = 9.35, UB = 20.94					

U: Urea, B: Biofertilizers, UB: Interaction

Table 9: Effect of mineral urea, bio-fertilizers and their interactions on seed yield, fixed oil and fixed oil yield of roselle plants during the two seasons (2013 and 2014)

	First season (2013)						Second season (2014)					
	0	30	60	90	120		0	30	60	90	120	
Bacterial strains	kg/feddan urea					Mean	kg/feddan urea					Mean
Seed yield (g plant^{-1})												
Control	21.82	22.60	23.40	24.10	24.51	23.28	21.15	21.22	22.36	23.14	25.25	22.62
A. chroococcum	38.40	38.62	40.63	39.06	38.79	39.10	37.20	38.14	39.68	39.73	37.62	38.47
B. polymyxa	34.73	34.92	35.12	36.79	34.90	35.29	33.20	33.28	34.18	34.69	32.20	33.51
P. fluorescens	29.35	29.39	31.40	31.72	28.92	30.15	28.11	29.23	31.17	32.80	29.81	30.22
Mean	31.07	31.38	32.63	32.91	31.78	-	29.91	30.46	31.84	32.59	31.22	-
LSD at 5%	U = 0.51, B = 0.32, UB = 0.72						U = 0.55, B = 0.40, UB = 0.89					
Seed yield (kg/feddan)												
Control	363.52	376.51	389.84	401.50	408.33	387.94	352.35	353.52	372.51	385.51	420.66	376.91
A. chroococcum	639.74	643.40	676.89	650.73	646.24	651.40	619.75	635.41	661.06	661.90	626.74	640.97
B. polymyxa	578.60	581.76	585.09	612.92	581.43	587.96	553.11	554.44	569.43	577.93	536.45	558.27

Table 9: Continue

	First season (2013)						Second season (2014)					
	0	30	60	90	120		0	30	60	90	120	
Bacterial strains	kg/feddan urea					Mean	kg/feddan urea					Mean
P. fluorescens	488.97	489.63	523.12	528.45	481.80	502.39	468.31	468.97	519.29	546.44	496.63	503.52
Mean	517.70	522.82	543.73	548.40	529.45	-	498.38	507.58	530.57	542.94	520.12	-
LSD at 5%	U = 10.11, B = 7.34, UB = 16.44						U = 11.64, B = 9.47, UB = 21.12					
Fixed oil (%)												
Control	14.20	14.35	14.95	15.00	15.00	14.70	13.10	13.25	14.20	15.15	14.85	14.11
A. chroococcum	15.35	16.25	16.80	17.00	16.20	16.32	14.60	15.35	15.80	16.25	16.00	15.60
B. polymyxa	16.40	17.25	18.90	18.30	17.95	17.76	15.65	16.20	17.75	17.45	16.60	16.73
P. fluorescens	17.25	18.80	19.95	20.00	19.35	19.07	16.85	17.30	18.10	18.95	17.80	17.80
Mean	15.80	16.66	17.65	17.57	17.12	-	15.05	15.52	16.46	16.95	16.31	-
LSD at 5%	U = 0.63, B = 0.46, UB = 1.03						U = 0.58, B = 0.41, UB = 0.92					
Fixed oil yield (L/feddan)												
Control	51.61	54.02	58.28	60.22	61.24	57.07	46.15	46.84	52.89	58.40	62.46	53.34
A. chroococcum	98.20	104.55	113.71	110.62	104.69	106.35	90.48	97.53	104.44	107.55	100.27	100.05
B. polymyxa	94.89	100.35	110.58	112.16	104.36	104.46	86.56	89.81	101.07	100.84	89.05	93.46
P. fluorescens	84.34	92.05	104.36	105.69	93.22	95.93	78.91	84.24	93.99	103.55	88.40	89.81
Mean	82.26	87.74	96.73	97.17	90.87	-	75.52	79.60	88.09	92.58	85.04	-
LSD at 5%	U = 3.88, B = 2.47, UB = 5.53						U = 4.62, B = 3.37, UB = 7.55					

U: Urea, B: Biofertilizers, UB: Interaction

Table 10: Effect of mineral urea, bio-fertilizers and their interactions on chemical composition of roselle plants during the two seasons (2013 and 2014)

	First season (2013)						Second season (2014)					
	0	30	60	90	120		0	30	60	90	120	
Bacterial strains	kg/feddan urea					Mean	kg/feddan urea					Mean
Nitrogen (%)												
Control	0.88	1.25	1.33	1.47	1.40	1.26	0.90	1.06	1.24	1.56	1.49	1.25
A. chroococcum	1.80	1.89	1.92	2.11	2.03	1.95	1.65	1.59	1.82	1.94	1.96	1.79
B. polymyxa	1.93	2.11	2.17	2.34	2.29	2.16	2.05	2.17	2.31	2.35	2.40	2.25
P. fluorescens	1.85	1.90	2.00	2.00	1.93	1.93	1.71	1.82	2.05	1.91	1.86	1.87
Mean	1.61	1.78	1.85	1.98	1.91	-	1.57	1.66	1.85	1.94	1.92	-
Phosphors (%)												
Control	0.37	0.41	0.41	0.44	0.44	0.41	0.35	0.40	0.43	0.41	0.38	0.39
A. chroococcum	0.48	0.46	0.44	0.43	0.40	0.44	0.50	0.52	0.47	0.43	0.44	0.47
B. polymyxa	0.54	0.64	0.57	0.55	0.49	0.55	0.60	0.60	0.51	0.47	0.48	0.53
P. fluorescens	0.49	0.42	0.42	0.39	0.38	0.42	0.52	0.54	0.55	0.47	0.40	0.49
Mean	0.47	0.48	0.46	0.45	0.42	-	0.49	0.51	0.49	0.44	0.42	-
Potassium (%)												
Control	1.75	1.77	1.82	1.95	2.04	1.86	1.62	1.59	1.74	1.83	1.95	1.74
A. chroococcum	2.10	2.16	2.70	2.46	2.36	2.35	2.20	2.31	2.69	2.78	2.70	2.53
B. polymyxa	2.25	2.44	2.96	2.60	2.56	2.56	2.55	2.80	2.91	3.02	3.00	2.85
P. fluorescens	2.12	2.23	2.61	2.38	2.31	2.33	2.20	2.41	2.52	2.61	2.48	2.44
Mean	2.05	2.15	2.52	2.34	2.31	-	2.14	2.27	2.46	2.56	2.53	-
Total carbohydrate (%)												
Control	12.11	14.25	15.37	14.90	14.07	14.14	11.34	12.37	13.61	13.20	12.80	12.66
A. chroococcum	15.20	16.70	16.40	15.89	15.35	15.90	14.50	15.70	15.32	14.90	14.73	15.03
B. polymyxa	16.00	16.44	18.12	17.64	17.15	17.07	15.80	16.11	17.40	17.10	16.80	16.64
P. fluorescens	14.90	16.50	15.92	15.88	15.11	15.66	15.10	15.35	15.00	14.68	14.48	14.92
Mean	14.55	15.97	16.45	16.07	15.42	-	14.18	14.88	15.33	14.97	14.70	-
pH value												
Control	2.82	2.82	2.86	2.90	2.87	2.85	2.80	2.84	2.87	2.89	2.89	2.85
A. chroococcum	2.90	2.99	3.01	2.94	2.92	2.95	2.92	2.95	3.05	3.05	2.90	2.97
B. polymyxa	3.01	3.11	3.16	3.10	3.04	3.08	2.99	3.05	3.14	3.12	3.07	3.07
P. fluorescens	2.92	2.95	3.05	3.01	2.89	2.96	3.00	3.05	3.07	2.95	2.90	2.99
Mean	2.91	2.96	3.02	2.98	2.93	-	2.92	2.97	3.03	3.00	2.94	-
LSD at 5%	U = 0.011, B = 0.009, UB = 0.020						U = 0.013, B = 0.011, UB = 0.024					

Table 10: Continue

Bacterial strains	First season (2013) 0	30	60	90	120	Mean	Second season (2014) 0	30	60	90	120	Mean
	kg/feddan urea						kg/feddan urea					
Total anthocyanin content (mg g⁻¹)												
Control	6.27	6.40	6.81	7.09	6.90	6.69	6.05	6.15	6.45	6.83	7.72	6.64
A. chroococcum	7.15	7.26	7.30	7.51	7.39	7.32	6.95	7.11	7.41	7.47	7.05	7.19
B. polymyxa	7.55	7.72	7.90	7.74	7.45	7.67	7.22	7.40	7.71	7.53	7.15	7.40
P. fluorescens	7.70	8.15	8.40	8.91	8.27	8.28	8.09	8.40	8.65	9.11	9.00	8.65
Mean	7.16	7.38	7.60	7.81	7.50	-	7.07	7.26	7.55	7.73	7.73	-

U: Urea, B: Biofertilizers, UB: Interaction

of *B. polymyxa*+each of 30, 60, 90 and 120 kg urea/feddan produced the highest value of P (%), total carbohydrate, K (%) and maximum N (%), respectively, in the two successive seasons. These results conformity with those obtained by Abo-Baker and Mostafa[43] on roselle plants.

CONCLUSION

Finally, it could be concluded that the best and optimum combined treatments among bio-fertilizers and urea levels occurred by application (*A. chroococcum*+60 kg urea/feddan). This application gave the highest plant parasitic nematodes reduction percentages and consequently flourished all vegetative growth characteristics; yield parameters and chemical composition of roselle plants through both seasons (2013 and 2014).

SIGNIFICANCE STATEMENTS

This study discovers that the use of *Azotobacter chroococcum* as bio-fertilizer combined with urea in roselle plantation gave adequate control of plant parasitic nematodes as well as improved growth characters.

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