Plant Archives Vol. 20 No. 1, 2020 pp. 633-638 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF BACTERIAL INOCULUM, SPRAYING WITH CALCIUM NITRATE AND SALICYLIC ACID IN VEGETATIVE AND FLOWERY GROWTH TRAITS OF *GERBERA JAMESONII*

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Abstract

This study was conducted in lath house of the Agricultural researches station (B)/ College of Agricultural Engineering Sciences, University of Baghdad/ Al-Jadriya for 2018 - 2019 year, to study the Effect of Bacterial inoculums, spraying with Calcium nitrate and Salicylic acid in Vegetative and flowery growth traits of *Gerbera jamesonii* cv Bighorn by injected 20 ml plant^{-1} bacteria *Bacillus subtilus* in Rhizospher area and foliar spraying of Calcium nitrate (Ca) was applied in two levels (500 and 1000 mg L^{-1}) while salicylic acid (SA) with 75 and 150 mg L^{-1} levels at three times as foliar spraying during the vegetative period as well as control treatment. A field experiment was carried out according to Randomized Completely Block Design (RCBD). Results indicated that the combination (T_{15}:BCa_1SA_1) distinguished in the most growth characteristics including number leaves (9.750 leaf plant^{-1}), leaf area (680.2 cm^2), total Chlorophyll (151.2), wet and dry weight of leaf (3.124, 0.489 gm), number of days required for the emergence of the flowery bud (113.3 Days), inflorescences number (4.083 flower plant^{-1}), peduncle diameter (7.494 mm), vase life (11.08 Days), and bending Angle of peduncle (2.080º).

Key words: Bacterial inoculum, Calcium nitrate, Salicylic acid, *Gerbera jamesonii*

Introduction

Gerbera jamesonii is a perennial herbaceous plant that belongs to the Compositae family, the original native was Africa and Asia (Zheng *et al.*, 2016). This genus has approximately 50 species. The flower very suitable for cutting, the gerbera flowers vase life can last for 1-4 weeks, Blossoms in the spring and autumn (Acharyya *et al.*, 2012 and Al-Khayat. 2018).

Several experiments and researches have been done to explain the importance of biofertilization (Palagani and Singh (2017 and Osman (2013) on the gerbera plant, which has improved the growth, either directly by assisting in acquisition N, P and essential minerals or modulating plant hormone levels, or indirectly by reducing the inhibitory effects of different pathogens on plant growth (Kandil *et al.*, 2004, Mahdi *et al.*, 2010, and Ahemad and Kibret 2014), Sakr (2017) noted that the addition of *Bacillus megaterium* improved growth of the *Calendula officinalis* as plant height, number of branches, leaves, leaf area, wet and dry weight of the leaves, the stem and the root. Osman (2013) reached to increasing in plant height, leaves number, number of days from planting to first flower bud formation, peduncle height in floresence diameter, vase life when Used *Rhizobium* sp, *Bacillus megaterium* and *Bacillus circulans* on *Gerbera jamesonii* Bolus. Nutrition is an important factor in plant growth and development where nitrogen is a necessary component of plant life, which is absorbed by the plant in form NO_3^- often or NH_4^+ sometimes and enters in the composition of amino acids, proteins, nucleic acids, nucleotides, considered one of the most important components of energy compounds ATP, NADH, NADPH and chlorophyll pigments (Bidwell 1979; Abu Dahi and Al-Yunis 1988; Taiz and Zeiger 2006). Calcium is a major nutrient and important in resisting disease because it is involved in cell walls protection and has an important biochemical function, supports many metabolic processes and activates many enzymatic systems (El-Habbasha and Ibrahim, 2015), It also increases the absorption of ammonium, potassium and phosphorus and stimulates photosynthesis (Feagley and Fenn, 1998), this was what many researchers founded when adding calcium nitrate, Milioni *et al.*, (2019) reported that *Aechmea fasciata* was sprayed with concentrations (0.25, 2.5, 5, 7.7, 10, 12.5 mmol plant^{-1}), the highest concentration led to

increasing plant height, stem diameter, wet and dry weight of leaves and leaves content of calcium, whereas leaves content of phosphorus and nitrogen decreased with increased Calcium nitrate concentration, and the leaves content of potassium was not affected significantly, Saikia *et al.*, 2018 indicated that spraying of *Rhynchostylis retusa* L. with Calcium nitrate $Ca(NO_3)_2$. $4H_2O$ (500 mg L^{-1} concentration) in combination with NPK (19 : 19 : 19) 2 g L^{-1} showed significant superiority in plant height, number of leaves and leaf area. Reddy and Sarka (2016) pointed out that the spraying of *Gladiolus grandiflorus* with 300 mg calcium nitrate increased characters like vase life, fresh weight and diameter of spike, delay the senescence, corm diameter, corm thickness and cormel weight per plot. Salicylic acid is an organic acid that was built from the bio-amino acid Phenylalanine and acts as a natural growth regulator in the plant has several functions and effects in growth and development, Reverse the process of stimulating ABA in closing stomata and leaves fall and inhibiting the construction and formation of ethylene, It has a role in control and defense against diseases and physiological modification of some plants. Kazemi (2012) and Al- Khafaji (2014), This plant hormone has the ability to accelerate the process of chlorophyll and carotene formation and increases the activity of some enzymes (Khan *et al.*, 2003), Thus, some researchers resorted to foliar spray on plants or add to the soil for improve plant growth. Babarabie *et al.*, (2018) have sprayed tuberose (*Polianthus tuberosa* L.) with salicylic acid (50, 100, 150, 200 mg L^{-1}), the concentration 100 mg L^{-1} gave the highest stem diameter and leaf area while the concentration 200 and 50 were gave increasing significant in the highest diameter and height spike as well as 50 mg L^{-1} concentration recorded early flowering, Al-Maathedi *et al.*, (2018) explained that spraying salicylic acid at a concentration 90 mg L^{-1} increased the flower yield in *Dianthus caryophyllus* L. plant, while Sewedan *et al.*, (2018) found that spraying of the *Gladiolus grandiflorus* with concentrations 0, 50, 100, 150 mg L^{-1} resulted in significant differences in vegetative and floral characteristics such as plant height, leaf area, dry weight, flowering time, flower diameter, Spike length, number of florets per spike, corn diameter and total chlorophyll at the higher concentration. Thus, the study aimed to improve the vegetative and flowery characteristics of the gerbira plant and reduce the use of chemical fertilizers.

Materials and methods

This study carried out in lath house of the Agricultural researches station (B)/College of Agricultural Engineering Sciences, University of Baghdad/Al-Jadriya site for 2018-2019 year, was planted plants Gerbera *cv.* Bighorn (six months old) at 15/9/2018 that produced by the company Green Life for Textile Agriculture Ltd./Baghdad, Potted in plastic bags contained (Soil sand + organic fertilizer Fertak waste in the rate 2% of the soil weight). The soil was well washed to reduce the salinity of the organic manure before planting. Table 1 presents the physical and chemical characteristics of the farming medium.

The bacterial vaccine of *Bacillus subtilus* was prepared in the Department of Agricultural Research / Ministry of Science and Technology, was injected 20 ml plantÉ¹ in the Rhizosphere area for the determent treatments after 18 days of cultivation. The bacterial vaccine was repeated after 1 month and the bacterial density was 2.4×10^{11} and 4×10^{11} For the first and second time respectively. The foliar spraying with the calcium nitrate solution (Ca) was on11/10/2018 while the salicylic acid (SA) was sprayed on 18/10/2018 and repeated three times for calcium nitrate and salicylic acid alternately, (NPK) Sprayed foliar as recommendation for the Company on 22/10/2018 and repeated three times every 30 days. The treatments contained seventeen combinations of fertilizers (T_0 Control: no addition, T_1: NPK, T_2: *Bacillus subtilus* (B), T_3: Ca_1 500 mg L^{-1}, T_4: Ca_2 1000 mg L^{-1}, T_5:SA_1 75 mg L^{-1}, T_6:SA_2 150 mg L^{-1}, T_7:B+Ca_1, T_8:B+Ca_2, T_9:B+SA_1, T_{10}:B+SA_2, T_{11}: Ca_1+SA_1, T_{12}: Ca_1+SA_2, T_{13}:Ca_2+SA_1, T_{14}:Ca_2+SA_2, T_{15}:B+Ca_1+SA_1, T_{16}:B+Ca_2+SA_2). Add to the combinations (T_2-T_{16}) half of the fertilizer recommendation (NPK). The studied traits were total number of leaves (leaf $plant^{-1}$), leaf area (cm^2), total chlorophyll ratio (mg 100g wet $weight^{-1}$), wet and dry leaf weight (g), number of days for emergence flower bud (Day), inflorescences number (flower $plant^{-1}$), peduncle diameter(mm), vase life (Day) and Determination of peduncle bending angle. The experiment was designed as Randomized Completely Bloke Design (Elsahookie and Wuhaib, 1990). The results were statistically analyzed using the GenStat program with 17 combinations.

Results and discussion

Number of leaves(leaf $plant^{-1}$)

As shown in Table 2 T_{15} is characterized by an

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

Soil texture	Clay	Silt	Sand	O.M	Ca Ready	K Ready	P Ready	N Ready	EC	pH	Soil contents
Sandy	14	4	82	0.15	53.10	123.01	3.85	14.70	3.20	7.31	Concentration
loam	%	%	%	%	mMl kg $soil^{-1}$	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	dS m^{-1}	----	measuring unit

increase the number of leaves to 9.750 leaf plant^{-1}, by increasing 46.24% over control treatment, that not different significantly from the T_6 treatment, while T_{16} treatment gave the lowest number of leaves (6.000 leaf plant^{-1}) and not differ significantly from the treatment T_{14}.

Leaf area (cm^2)

The leaf area of the plant is one of the most important criteria for the nutritional response of the plant, which increase the efficiency of photosynthesis and the production of carbon compounds (Taiz and Zeiger, 2006). Table 2 showed that the treatment T_{15}, appeared significant superiority over the rest treatments in giving the largest leaf area 680.2 cm^2 and an increase of 189.57% over the control treatment. There was no significant difference between the T_0 and T_1 treatments in recorded the lowest leaf area 234.9 cm^2 and 254.9 cm^2 respectively.

Leaf content of total chlorophyll (mg 100 g wet weight^{-1})

Although the difference between T_1 and T_{15} was not significant (Table 2), it was superior significantly on other treatments in the chlorophyll content, T_1 (158.5 mg 100g wet weightÉ¹). The treatment T_{16} gave the lowest chlorophyll in the leaf (33.2 mg 100 g wet weight^{-1}) compared to the rest treatments.

Wet weight of leaf(g)

The results in Table 2 showed response T_2 to addition biofertilization that was not differ significantly from T_{15} and T_6 treatments However, it was significantly increased the wet weight of the leaves to 3.618g compared to the rest treatments, control treatment T_0, gave a lower wet weight (1.934 g).

Dry weight of leaf (g)

Results of Table 2 indicated that the T_{15} treatment was distinguished in giving maximum dry weight (0.489g) and an increase of 64.65% than the control treatment that was not different significantly from the T_{13} treatment (0.453g), The treatments of T_0 and T_1 were not significantly different in giving them a minimum dry weight 0.297 and 0.323g respectively.

Number of days for emergence flowery bud (day)

The results of statistical analysis in Table 2 showed that T_{15} was recorded the lowest number of days required for the emergence of flowery bud (113.3 days) which significantly higher than the rest of the treatments, comparison with control treatment T_0 and a decrease of 43.69 %, but not differ significantly from the treatment T_{13} and T_9. The treatment T_1 gave more number of days was 166.2 days, which not significantly different from control treatment T_0 (162.8 days).

Number of inflorescences (flower plant^{-1})

The plant's response to treatments primarily affects in improvement the vegetative growth, which results amelioration the flowery growth traits. As Table 2 noted that the T_{15} treatment is characterized in increasing the number of flowers to 4.083 flower plant^{-1}, This was not differ significantly from the T_5 treatment where it was superior to the other treatments the T_{16} treatment gave the minimum number of flowers (2.083 flower plantÉ¹) and was not differ significantly from T_4 treatment (2,167 flower plant^{-1}).

Peduncle diameter(mm)

Increasing the Peduncle diameter is caused by responding to nutrient factors and increasing cell division and elongation activity. The results in Table 2 indicated that the treatment T_{15} was significantly different from the rest treatments, in giving the maximum Peduncle diameter (7.494 mm), while T_{16} recorded the minimum Peduncle diameter, it was 5.910 mm.

Vase life (day)

From Table 2 it was clear the significant difference between treatments. If the T_8 treatment exceeds the vase life to 11.33 days, It was not differ significantly from the T_{15} and T_9 treatments but surpassed the rest treatments, the T_0 control treatment was not significantly different from the T6 in giving the lowest vase life that reached to 8,000 days for both treatments.

Peduncle bending angle (degree)

The treatment T_{16} exceeded in determination the bending angle (Table 2) where the bending value was 0.000º followed treatment T_{14}, T_{15} 1.670º and 2.080º, This was superior to the rest treatments, control treatment T_0 which gave the maximum peduncle bending angle (14.89º).

The results of the statistical analysis showed significant differences between the combinations in vegetative, flowery and post-harvest growth traits (Table 2). The T_{15} (BCa_1SA_1) treatment was characterized by an increasing in the number of leaves, leaf area, total chlorophyll, wet and dry weight of leaf, early flowering, number inflorescences, Peduncle diameter, vase life and reducing Peduncle bending angle, This might be due to addition of biofertilizer which improved, both physical and chemical soil properties, it has directly affected by increased growth and of root hairs number, then improved the absorption of nutrients, like nitrogen, phosphorus, and potassium, It's secretions of organic acids like formic,

acetic, propionic, lactic, glycolic, fumaric, succinic, vitamins and growth regulators such as Gibberellin and IAA have played an important role in improving vegetative and flowrey traits of the plants (Gharib *et al.,* 2008; Abdulhameed *et al.,* 2012; Osman , 2013; Pansuriya *et al.,* 2018 and Dhakar *et al.,* 2019). This was in line with the findings Krushnaiah *et al.,* (2018) on the Italian asters *Aster amellus* and Pratap (2018) on the Ester plant *Callistephus chinensis*.

Improving most growth traits when sprayed with Calcium nitrate could be attributed to the nitrogen that involved in the construction of auxins , thereby increasing activity of cellular division and elongation, as well as entrance to the bio-construction of chlorophyll as an input to the building of porphyrins, enzymes, then affect in photosynthesis, the formation of Energy compounds, proteins, nucleic acids and co-enzymes (Al-Doghachi *et al.,* 2011, Altaee, 2013, Kumari *et al.,* 2014; Mazed *et al.,* 2015; Hassanain *et al.,* 2018), generaly this was reflected positively in improvement of the characteristics of vegetative and flowery growth, The role of Calcium promotes photosynthesis, cells division and elongation, its effect in cell membrane, the plasma membrane, absorption of other elements such as nitrogen, potassium, zinc, boron and magnesium, and its effects on the process of metabolism it also shares with nitrogen in strengthening and flexibility of cell walls, High calcium concentrations reduce the building of carbohydrates because of its oxalate activation, It also competes on the absorption sites in the root between ammonia ions and calcium, Calcium is also a messenger for the response of the plant to hormonal and environmental signals (Sharma *et al.,* 2013; Reddy and Sarka 2016; Azeez *et al.,* 2017; Saikia *et al.,* 2018; Milioni *et al.,* 2019).

Salicylic acid also played a role in regulating physiological, nutritional and hormonal processes, as well as its effect on photosynthesis, increased cell division, absorption of nutrients and maintenance of cellular membranes as wll as increased nitrate reductase, which increased representation of nitrates and content total nitrogen and thus improves vegetative and flowery traits (Horvth *et al.,* 2002; Sibgha *et al.,* 2009; Al-Rubaee *et al.,* 2012; Hamood and Majeed, 2017 and Basit *et al.,* 2018).

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Table 2: Effect of Bacterial inoculum , spraying with Calcium nitrate and Salicylic acid in vegetative, flowery growth traits of *Gerbera jamesonii* Bighorn.

Post harvest parameters		Flowery parameters			Vegetative parameters					Treatment
Peduncle bending angle (degree)	Vase life (Days)	Peduncle diameter (mm)	Number of inflores-cences (flower plant^{-1})	Number of days for emergence flowery bud (day)	Dry weight of leaf (g)	Wet weight of leaf (g)	Leaf content of total chlorophyll (mg 100 g wet weight^{-1})	Leaf area (cm^2)	Number of leaves (leaf plant^{-1})	
14.89	8.000	6.397	2.833	162.8	0.297	1.934	89.3	234.9	6.667	$T_{0\ control}$
5.700	9.333	6.363	2.833	166.2	0.323	2.542	158.5	254.9	7.000	$T_{1\ NPK}$
4.790	9.583	6.628	2.583	125.0	0.398	3.618	124.9	381.2	7.000	$T_{2\ B}$
4.240	9.875	6.733	2.917	129.0	0.399	2.578	104.1	437.8	8.333	$T_{3\ Ca1}$
7.780	8.667	5.950	2.167	153.8	0.388	2.597	135.5	351.0	6.567	$T_{4\ Ca2}$
3.650	9.444	6.483	4.083	135.1	0.427	2.739	111.2	492.0	8.667	$T_{5\ SA1}$
4.170	8.000	6.440	3.000	141.0	0.430	2.888	71.9	489.6	9.333	$T_{6\ SA2}$
3.610	8.833	6.828	3.500	129.9	0.393	2.584	94.9	424.6	7.833	$T_{7\ B\ Ca1}$
7.080	11.33	6.415	3.167	140.3	0.355	2.265	85.8	377.9	8.667	$T_{8\ B\ Ca2}$
4.420	10.83	6.631	3.250	116.8	0.403	2.629	86.5	491.2	8.667	$T_{9\ B\ SA1}$
6.020	9.708	6.483	3.500	127.2	0.403	2.786	106.3	444.5	8.333	$T_{10\ B\ SA2}$
4.440	10.08	6.798	3.583	135.3	0.403	2.505	121.8	425.9	8.417	$T_{11\ Ca1\ SA1}$
6.140	8.583	6.746	3.167	159.0	0.370	2.283	138.7	371.7	8.917	$T_{12\ Ca1\ SA2}$
3.540	9.542	6.709	3.000	114.0	0.453	2.571	105.0	379.4	7.500	$T_{13\ Ca2\ SA1}$
1.670	9.985	6.403	3.417	126.1	0.387	2.367	120.4	354.3	6.500	$T_{14\ Ca2\ SA2}$
2.080	11.08	7.494	4.083	113.3	0.489	3.124	151.2	680.2	9.750	$T_{15\ B\ Ca1\ SA1}$
0.000	8.083	5.910	2.083	131.5	0.423	2.324	33.2	330.5	6.000	$T_{16\ B\ Ca2\ SA2}$
2.185	0.667	1.185	0.347	7.569	0.042	0.755	16.93	24.53	0.562	LSD

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