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The Effect of Application Type and Composition of Growth Stimulating Bacteria on Quantitative and Qualitative Characteristics of Medicinal Plant Calendula (*Calendula officinallis* L.)

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Abstract

Stimulating Bacteria use to enhance the growth and development of plants. This study focuses on "The Effect of Application Type and Composition of Growth Stimulating Bacteria on Quantitative and Qualitative Characteristics of Medicinal Plant Calendula". An experimental test was conducted as a factorial randomized complete block design with three replications at the Agricultural Research Institute of Zabol University. The first factor is types of growth stimulating bacteria in octahedral: control (no inoculation), *Azotobacter chroococcum*, *Azosperilium brasilense*, *Pesudomonans putida*, *Azotobacter chroococcum* plus *Azosperilium brasilense*, *Pesudomonans putida* plus *Azotobacter chroococcum*, *Pesudomonans putida* plus *Azosperilium brasilense*, *Pesudomonans putida* plus *Azotobacter chroococcum* plus *Azosperilium brasilense* and the second factor is the type of consumption including two levels (Seed and Foliar application). The results showed that growth stimulating bacteria and application type had a significant effect on all analyzed traits. Foliar traits on the flower yield, the flower number per square meter, ratios of total biomass to flowers, total biomass to grain yield, flower harvest index, flower extract yield and Seed application affected on grain yield, grain harvest index, oil percentage, and oil yield had the greatest impact. The foliar application treatment is better to increase the performance of quantitative characteristics and seed treatments be used to enhance the quality performance. Azospirillum + Pseudomonas treatment brought great impact on the qualitative and quantitative characteristics of Calendula.

Keywords: Extract Yield (EY), Flower Yield (FY), Oil Content (OC)

1. Introduction

In recent years, due to the growing trend of diseases and shortage of raw material for production of synthetic drugs medicinal plants have been taken humans' attentions[1]. In plant classification, Calendula (*Calendula officinalis* L.) belongs to family *Asteraceae*[2]. Calendula is annual with stems consisting of many branches that are covered with soft lint. Its height reaches 45 to 75 cm. The leaves are simple, oval, and located on and behind the fluff. Its capitols are large with a diameter of 3 to 5 cm. the flowers located in the middle of the capitol are tubular with rows of tab- shaped yellow to orange flowers[3]. Calendula has long been cultivated as an ornamental plant until its medicinal properties became known and it has been used as a medicinal plant[2]. Its active ingredient, which most notably include water-soluble flavonoids (0.04 to 0.1) and oil (0.02 to 0.1) is built and is stored in its flowers[4]. Bio-fertilizers not only refer to organic materials derived from animal fertilizers, plant residues, green manure etc., but they also

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include bacterial and fungal micro-organisms, especially micro bacteria plant growth (Plant Growth Promoting Rizobacteria) or so-called PGPR and materials derived their activities among which bio-fertilizers are the most important[5]. They use one or more particular systems to enhance the growth and development of plants[6]. Studies have shown that some growth-stimulating bacteria secret phosphates and organic to increase absorbable phosphorus through dissolution of the inorganic compounds of phosphate and turning insoluble forms of phosphorus into forms soluble for plants. The other positive effect of growth-stimulating bacteria is production of plant growth regulators such as auxin, cytokinin and gibberellins[7,8].

The results of a study by Koocheki et al.[9] suggest that the use of bio-fertilizers containing microorganisms, bacteria or fungus (either alone or in combination) has positive effect on improving growth characteristics and yield of the herb, hyssop (*Hyssopus officinalis*). By far, analysis of the effects of different plants inoculated with growth stimulating bacteria has been considered important, whereas the foliar effect of these bacteria on crop growth and yield has been ignored. In fact, due to foliar of these bacteria on the aerial organs, the direct effect of the plant on plant growth in comparison with seeding method appears darker. On the other hand, it seems that combining a variety of growth stimulating bacteria can provide a synergistic and resonating relationship resulting in increased effects of type of bacteria and its application type on plant growth and its qualitative and quantitative characteristics in Calendula.

2. Materials and Methods

This study was designed and conducted to investigate the effect of application type and composition of growth-stimulating bacteria on quantitative and qualitative characteristics of the medicinal plant Calendula (*Calendula officinallis* L). The first factor is types of growth stimulating bacteria in octahedral: A_1: control (no inoculation), A_2: *Azotobacter chroococcum*, A_3: *Azosperilium brasilense*, A_4: *Pesudomonans putida*, A_5: *Azotobacter chroococcum* plus *Azosperilium brasilense*, A_6: *Pesudomonans putida* plus *Azotobacter chroococcum*, A_7: *Pesudomonans putida* plus *Azosperilium brasilense*, A_8: *Pesudomonans putida* plus *Azotobacter chroococcum* plus *Azosperilium brasilense* and the second factor is the type of consumption including two levels (B1: Seed application B2: Foliar application). The seeds were first disinfected with 10% white-x for 15 minutes, and washed three times with sterile distilled water. They were implanted in a depth of 1 cm with the seminal fluid provided by the Soil and Water Research Institute of Biology of the country. After seedling establishment, thinning takes place. Foliar application occurs when the plant has 4 leaves. Harvest is carried out after flowering once a week at the determined area. To calculate the feature of number of flowers per square meter, withdrawal was done once a month in each of the units for four plants depends on the flowering duration, once a week for 3 weeks. Next, their average was considered as a representative for the number of flowers in each test unit. Harvested petals of each unit were weighed with a digital weighing scale and flower field was calculated based on their average. The biomass yield was also obtained from the weight of the harvested plants for each plot. The flower harvest invest was later determined by considering the ratio of economic yield (dry weight of flowers or flower field) and the biologic field. For extraction and measurement of total flavonoids, flowers were taken from plants and were dried at room temperature and in the shade. 0.5 g petal was soaked with %80 ethanol at a ratio of 10:1 and then, it was placed for 48 hours in a dark area at room temperature. It was filtered with a filter paper (Whitman 42) and was placed in a rotary device to remove the ethanol and dry it. This study was conducted as a factorial experiment in a completely random block design with three replications, data analysis was performed with SAS software version 9.1, and the averages were compared at 5% level by Duncan's multiple range test.

3. Results and Discussion

3.1 Flower Yield

Flower field was significantly affected by growth-stimulating bacteria, type of application and their interaction (Table 1). Comparison of the average data showed that foliar treatment (3097.27 kg.ha) was better than seeding treatment (1842.31 kg. ha) and in comparison with seeding treatment, the foliar treatment increased %68.1 (Table 2). Moreover, the comparison of these treatments showed that there is a significant difference in levels of growth-stimulating bacteria, so that flower field in treatment of Azospirillum plus Pseudomonas (4013.06 kg. ha), was about 25% more than Pseudomonas plus Azotobacter plus Azospirillum treatment (3205.10 kg.ha), about 36.5% more than the Azotobacter treatment (2937.69 kg.ha),

Table 1. Analysis of variance of quantitative and qualitative characteristics of Calendula plants affected by Growth promoting *Rhizobacteria* and Type of Application

S.O.V	df	Flower yield	Grain yield	Number of flowers	Total Biomass yield with flowers	Total biomass yield with grain	HI flower	HI Grain	Oil content	Oil yield	Extracts yield
		MS									
Replication	2	694880.32**	312190.59**	195690.27**	17187919ns	2078305 ns	13.705ns	2.512**	61.01**	4578.29ns	17.105**
Type of Application (A)	1	18899208.17**	16798363.7**	4020802.08**	557289947**	180819446**	73.038**	731.64**	1165.25**	2751247.050**	92.963**
Growth promoting rhizobacteria (B)	7	4141536.09**	1483205.49**	922207.35**	234962080**	232080189**	60.253**	20.05**	52.79**	153927.78**	63.567**
A*B	7	2788697.41**	874809.77**	650879.92**	205451711**	201613152**	34.273**	15.67**	15.13ns	77416.99**	35.220**
Error	30	10604.45	14886.71	3454.98	8324030	1985142	9.74648	0.30501	7.404823	3909.322	3.15738
C.V.	-	4.16	6.93	4.91	14.20	7.12	24.74	5.92	10.71	12.87	18.17

ns: non-signification, **: P<0.01

Table 2. Means comparisons of quantitative and qualitative characteristics of Calendula plants affected by Growth promoting *Rhizobacteria* and Type of Application

	Flower yield (Kg/ha)	Grain yield (Kg/ha)	Number of flowers (m²)	Total Biomass yield with flowers (Kg/ha)	Total biomass yield with grain (Kg/ha)	HI flower (%)	HI Grain (%)	Oil content (%)	Oil yield (Kg/ha)	Extracts yield (%)
Type of Application										
Spraying	3097.27 a	1168.07 b	1485.53 a	23712.4 a	21713.4 a	13.8517 a	5.4196 b	20.4729 b	246.17 b	11.1708 a
Seed inoculation	1842.31 b	2351.23 a	906.68 b	16897.7 b	17831.6 b	11.3846 b	13.2279 a	30.3271 a	724.99 a	8.3875 b
Growth promoting rhizobacteria										
No Inoculation	2038.72 d	1871.25 c	1003.91 d	18352 bc	18054.1 c	10.46 cd	10.46 bc	20.183 d	392.17 de	7.433 c
Azotobacter(Azoto)	2937.69 c	1754.94 c	1433.12 c	18025 bc	16842.6 cd	15.44 ab	11.33 a	26.525 bc	454.62 cd	8.733 c
Azospirillum(Azosp)	1879.62 e	1191.82 e	909.88 e	13704 d	13016.4 e	13.64 bc	8.85 d	23.358 cd	316.00 e	7.833 c
Psedomunas(Psedo)	2100.28 d	2283.29 b	1020.80 d	28798 a	28882.2 a	8.00 b	10.28 bc	24.392 bc	595.47 b	6.650 c
Azoto+Azosp	2016.47 d	1572.50d	975.23 de	15825 cd	15380.6 d	12.70 bc	9.83 c	26.933 ab	436.99 d	7.167 c
Azoto+Psedo	1567.39 f	1256.64 e	774.01 f	16267 cd	15906.7 b	9.90 cd	7.23 e	24.667 bc	357.89 e	11.733 b
Azosp+Psedo	4013.06 a	2637.48 a	1928.10 a	30934 a	29558.8 a	12.89 bc	10.61 b	30.142 a	816.54 a	15.267 a
Azoto+Azosp+Pseudo	3205.10 b	1509.30 d	1523.79 b	20534 b	20538.4 b	17.9 a	6.04 f	27.000 ab	514.96 c	13.417 ab

Similar letters at each column explain non significance based on Duncan examination.

about 95.5% more than the Pseudomonas treatment (2100.28 kg.ha), control (2038.72 kg.ha) and Azotobacter plus Azospirillum treatment (2016.47 kg per ha) which are in a similar statistical level and about 113.5% more than Azospirillum treatment (1879.62 kg.ha) and about 156% more Pseudomonas plus Azotobacter treatment (1567.39 kg.ha) (Table 2).

The results indicated that the use of these fertilizers, which leads to an increase of flower yield and improves its medicinal qualities, apparently helps growth-stimulating bacteria produce ACC Di-amines enzyme. 1-Cycle propane Amino-1-Carboxylic Acid (ACC) Di-amines enzyme degrades ACC into ammonia and catalyzes acid Alfa Butyric acid to[10]. Since ACC is the precursor of ethylene production in higher plants, removing this provision reduces the amount of ethylene in plants and increases root growth, nutrient absorption, and flower yield. Interaction of application type and growth- stimulating bacteria has a significant meaning flower yield. Comparison of treatment means showed that the highest yield (5538 kg.ha) was achieved by Azospirillum pluse Pseudomonas treatments and foliar, which compared to control treatment (3178 kg.ha) had an increase of 74.2% (Table 3).

3.2 Number of Flowers per Square Meter

Number of flowers per square meter significantly influenced growth- stimulating bacteria, application type, and their interaction (Table 1). Comparison of average data showed that foliar treatment (1485.53) was better than seed treatment (906.68) foliar treatment had an increase of 63.8% (Table 2). Also, comparing treatment means showed that there is a significant difference between the levels of growth- stimulating bacteria, so that the quantity of flowers per square. In Azospirillum plus Pseudomonas treatment (1928.10) was about 26.5% more than Pseudomonas plus Azotobacter plus Azospirillum treatment (1523.79), about 34.5% more than Azotobacter treatment (1433.12), about 90.4% more than Pseudomonas treatment (1020.80). Control treatment (1003.91), which is statistically identical was about 94.7% more than Azotobacter plus Azospirillum treatment (975.23), about 111.9% more than Azospirillum treatment (909.88) and about 149.1% more than Pseudomonas plus Azotobacter treatment (774.01) (Table 2).

It seems that through the production of substances such as in dole acetic acid, growth- stimulating bacteria enhance plant growth and the early stages of plant growth resulting in a greater volume of soil occupied by the roots and absorption increase[11], and may lead to an increased number of flowers. Interaction of application type and growth-stimulating bacteria on flower quantity per square. Comparison of treatment means showed that the maximum amount of flowers in each square. (2628) was achieved from Azospirillum plus Pseudomonas treatments and foliar which increased by 69.6 % in comparison with control treatment (1549) (Table 3).

3.3 Grain Yield

Grain yield was significantly affected by growth-stimulating bacteria, type of application and their interaction (Table 1). Comparison of data means showed grain yield in seeding treatment (2351.23 kg.ha) better than foliar treatment (1167.07 kg.ha) and seeding treatment increased by 101.2% compared with foliar treatment (Table 2). The results of Akbari et al.[12] showed that in sunflower, seeds inoculated with growth-stimulating bacteria had a 9% seed yield increase rather than seeds without inoculation. Moreover, the comparison of the treatment averages showed that there is a significant difference between different layers of growth-simulating bacteria, so that grain yield in Azospirillum plus Pseudomonas treatment (2637.48 kg.ha) was about 15.5% more than Pseudomonas treatment (2283.29 kg.ha), about 45.4% more than the control treatment (1871.25 kg.ha). Azotobacter treatment (1754.94 kg.ha), which is in the same statistical level, is about 71.1% more than Azotobacter plus Azospirillum treatment (1572.50 kg.ha) and Pseudomonas plus Azotobacter plus Azospirillum treatment (1509.30 kg.ha) is in a statistical level and is about 115.4% of Azotobacter plus Pseudomonas (1256.64 kg.ha) and Azospirillum treatment (1191.82 kg.ha), which are in a similar statistical level (Table 2).

Roesti et al.[13] stated that the probable reason of increased yield in pretreatment of seed with bacteria is related to increased absorption of available nutrients, increased root health during the growing season to compete with root pathogens. Interaction of application type and growth stimulating bacteria had a significant effect on grain yield. Comparison of treatment means showed that the highest grain yield (3272 kg.ha) was obtained from Azospirillum + Pseudomonas treatments and seeding which were located in a similar statistical group with pseudomonas and seeding treatments, and in comparison

Table 3. Comparison of mean quantitative and qualitative characteristics of evergreen influenced by type of application× Growth promoting *Rhizobacteria*

Type of Application	Growth promoting rhizobacteria	Flower yield (kg/ha)	Grain yield (kg/ha)	The flowers (m^2)	Total Biomass yield with flowers (kg/ha)	Total biomass yield with grain (kg/ha)	HI flower (%)	HI Grain (%)	Oil content (%)	Oil yield (kg/ha)	Extracts yield (%)
	No Inoculation	3178 c	1582e	1549 cd	20660 bcd	18800 de	15.36 ab	8.39 f	15.08 h	238.6 f	5.56 h
	Azotobacter	4412 b	1963 cd	2147 b	25130 b	22680 c	17.53 a	8.64 f	22.05 def	433.3 e	11.96 cde
	Azospirillum	1586 gh	578.2 fg	764.7 hi	13120 fg	12110 hi	12.09 abc	4.76 g	16.86 gh	98.19 g	8.76 efgh
	Psedomunas	2515 d	1397 e	1210 e	40460 a	39140 a	6.21 d	3.56 h	20.01 efg	280.0 f	6.90 fgh
Spraying	**Azotobacter + Azospirillum**	2535 d	777.4 f	1209 e	15870 defg	14110 gh	15.92 a	5.46 g	24.76 cde	190.2 fg	7.10 fgh
	Azotobacter + Psedomunas	1849 f	480.3 g	911.5 g	14530 efg	13060 ghi	12.68 abc	3.63 h	18.16 fgh	84.23 g	15.63 b
	Azospirillum + Psedomunas	5538 a	2003 cd	2628 a	42300 a	38760 a	13.07 ab	5.16 g	26.48 bcd	529.5 de	20.40 a
	Azotobacter + Azospirillum + Psedomunas	3165 c	564.3 fg	1466 d	17640 def	15040 fg	17.93 a	3.74 h	20.35 efg	115.4 g	13.03 bcd
	No Inoculation	899.8 j	2161 c	458.9 k	16050 defg	17310 ef	5.56 d	12.41 d	25.28 cd	545.8 de	9.30 efg
	Azotobacter	1446 h	1547 e	719.5 ij	10920 g	11000 i	13.35 ab	14.02 c	31.00 ab	475.9 e	5.50 h
	Azospirillum	2173 e	1805 d	1055 f	14290 efg	13920 gh	15.19 ab	12.94 d	29.85 abc	533.8 de	6.90 fgh
Seed inoculation	**Psedomunas**	1685 fg	3170 a	831.9 gh	17140 def	18620 de	9.79 bcd	17.00 a	28.76 abc	910.9 b	6.40 gh
	Azotobacter + Azospirillum	1498 h	2368 b	741.5 hi	15780 defg	16650 ef	9.47 bcd	14.20 c	29.10 abc	683.8 c	7.23 fgh
	Azotobacter + Psedomunas	1286 i	2033 c	636.5 j	18010 def	18760 de	7 .1 2 cd	10.83 e	31.16 ab	631.5 cd	7.83 fgh
	Azospirillum + Psedomunas	2488 d	3272 a	1228 e	19570 cde	20360 cd	12.70 abc	16.06 b	33.80 a	1104 a	10.13 def
	Azotobacter + Azospirillum + Psedomunas	3245 c	2454 b	1582 c	23430 bc	26040 b	17.86 a	8.34 f	33.65 a	914.5 b	13.80 bc

Similar letters at each column explain non significance based on Duncan examination with control treatment (2161 kg.ha), it had about 10.5% increase (Table 2).

3.4 Oil Content

Oil content was significantly affected by growth stimulating bacteria and type of application and their interaction had no significant effect on oil content (Table 1). Comparison of data means showed that the percentage of oil in the seeding treatment (30.3271%) was better than foliar treatment (20.4729%) and seeding treatment had an increase of 48.1 % rather than foliar solution (Table 2). Akbari et al.[12] reported that seeds inoculated with growth stimulating bacteria had an increased oil percentage than control group. Shehata and El-Khawas[14] reported a significant increase in percentage of sunflower oil in case of using bio-fertilizers. Likewise, comparison of treatment means showed that there is significant difference among various levels of growth simulating bacteria so that the percentage of oil in the treatment of Pseudomonas plus Azospirillum (30.142%) is about 11.7% more than the treatment of Pseudomonas plus Azotobacter plus Azospirillum (27.000%), and Azotobacter plus Azospirillum treatment (26.933) which is statistically at the same level is about 19.6% more than Azotobacter treatment (26.525%), and Pseudomonas plus Azotobacter treatment (24.667%) and Pseudomonas treatment (24.392%) which are statistically at the same level are about 29.04% more than Azospirillum treatment (23.358%) and about 49.3% more than the control treatment (20.183%) (Table 2).

Shaukat et al.[15] showed while inoculating growth stimulating bacteria with sunflower seed concluded that in most used bacteria strains, oil percentage increased. Kandeel et al.[16] also mentioned that the highest percentage of volatile oils in fennel plant results from inoculation of these plants with Azospirillum and Azotobacter combinations and the total intake of recommended amounts of nitrogen, phosphorus and potassium. Akbari et al.[12] investigated the effects of biological fertilizers on sunflower and observed a significant increase of oil after using bio fertilizers. It appears that growth stimulating bacteria have increased nutrient uptake sufficient for plant and consequently, have made better filling of grain and have increased oil percentage. Interaction of application type and growth stimulating bacteria on oil percentage was not significant, but comparison of treatment means showed that the highest oil percent (33.65%) is obtained from treatment of Azotobacter plus Azospirillum plus Pseudomonas and which were in the same statistical level with Azospirillum plus Pseudomonas and seeding treatment treatments which in comparison with the control group (25.28%) increased by about 33.4% (Table 3).

3.5 Oil Yield

Oil yield was significantly affected by growth stimulating bacteria, type of application type and their interaction (Table 1). Comparison of data means showed that oil yield in seed treatment (724.99 kg.ha) was better than foliar treatment (246.17 kg.ha) and seeding treatment had a 194.5% increase more than foliar treatment (Table 2). Seed inoculation with growth stimulating bacteria has positive and significant impact on increase of sunflower oil[17].

Moreover, comparison of treatment means showed that there is a meaningful difference between various levels of growth stimulating bacteria, so that oil yield in the treatment of Pseudomonas plus Azospirillum (816.54 kg.ha),was about 37.1% more than the treatment of Pseudomonas (595.47 kg.ha). It was about 58.7% more than the treatment of Pseudomonas plus Azotobacter plus Azospirillum (514.51 kg.ha), and about 79.6% more than Azotobacter treatment (454.62 kg.ha). It was also, about 86.8% more than Azotobacter plus Azospirillum treatment (436.99 kg.ha), about 108.2% more than the control treatment (392.17 kg.ha), about 142.3% more than the treatment of Pseudomonas plus Azotobacter (357.89 kg.ha) and Azospirillum treatment (316.00 kg.ha) that are in the same statistical level (Table 2).

In their studies on inoculating effect of sunflower seeds with Azotobacter at different levels of nitrogen, Soleimanzadeh et al.[18] showed that due to inoculating effect of Azotobacter, oil yield increased significantly so that oil yield in inoculated seeds with Azotobacter was 7% more than un-inoculated seeds. Badran and Safwat[19] and El-Ghadban et al.[20] found that because of using bio fertilizers, plant growth, and fennel oil yield increased and its chemical composition changed.

The higher the yield and oil percentage, the higher oil yields per ha. Shehata and El-Khawas[14] observed a significant increase in sunflower oil yield with application of bio fertilizers through increased oil percentage and grain yield. Interaction of application type and growth stimulating bacteria on oil yield was significant. Comparison of the treatment means showed that the maximum oil yield (1104 kg.ha) was obtained from Azospirillum + Pseudomonas and seeding treatments which compared to the control treatment (545.8 kg.ha), approximately increased by 102.2% (Table 3).

3.6 Total Biomass Yield with Flowers

Total biomass yield with flowers significantly affected growth stimulating bacteria, type of application type and their interaction (Table 1). Comparison of data means showed that foliar treatment (2371.4 kg.ha) was better than seeding treatment (16897.7 kg.ha) and compared with seeding treatment, foliar treatment had an increase of 40.3% (Table 2). Likewise, comparison of treatment means showed there is a significant difference between various levels of growth promoting bacteria. Therefore, the total biomass yield with flowers in the treatment of Azospirillum plus Pseudomonas (30934.30 kg.ha) and the treatment of Pseudomonas (28798.28 kg. ha), both at the same statistical level, were about 45.44% more than the treatment of Pseudomonas plus Azotobacter plus Azospirillum (20534.20 kg. ha), and about 64.2% more than the control treatment (18352.18 kg.ha). Azotobacter treatment (18025 kg per ha) which are in the same statistical level, are about 86.12% more than the treatment of Pseudomonas plus Azotobacter (16267 kg.ha). Azotobacter plus Azospirillum treatment (15825 kg.ha), which are located at the same statistical level, are around 117.9% more than Azospirillum treatment (13704 kg.ha) (Table 2).

Availability of water and nutrients leads to optimal plant growth. The most basic requirement to produce a high yield is to produce more dry matter per unit area. The results of this experiment showed that growth-stimulating bacteria increased the number of lateral branches and flowers per square. It also increased dry plant weight and in return, increased total biomass yield with flowers. Interaction of application type and growth stimulating bacteria was significant on total biomass yield with flowers. Comparison of treatment means showed that the highest total biomass yield (42300 kg.ha) is obtained from Azospirillum plus Pseudomonas treatments and foliar which are in the same statistical level with treatment of Pseudomonas and foliar. In comparison with the control (20660 kg.ha), they had about 104.7% increase (Table 3).

3.7 Flower HI

Flower HI was significantly affected by growth stimulating bacteria, type of application type and their interaction (Table 1). comparison of data means showed that foliar treatment (13.8517%) was better than seeding treatment (11.3846%) and foliar treatment increased 21.6% more than seeding treatment (Table 2.) in addition, comparison of the treatment means showed that there is a significant difference between various levels of growth stimulating bacteria, so that flower HI in Azospirillum plus Azotobacter plus Pseudomonas (17.90%) was about 16% more than Azotobacter treatment (15.44%), about 36.95% more than Azospirillum treatment (13.64%). The treatment of Azospirillum plus Pseudomonas (12.89%) and Azotobacter plus Azospirillum treatment (12.70%) which are at the same statistical level are approximately 75.8% more than the control treatment (46.10%) and Pseudomonas plus Azotobacter treatment (9.90%) which are statistically at the same level is about 123% more than Pseudomonas treatment (8%) (Table 2).

Harvest index expresses the distribution ratio of photosynthesis matters between flower yield and its biological function. Since the growth stimulating bacteria, particularly the composition of Azospirillum and Pseudomonas, enhance performance in comparison with the control, it is expected to increase flower HI. Interaction of application type and growth stimulating bacteria was significant for flower harvest index. Comparison of treatment means showed that the highest harvest index (17.93%) was obtained in the treatment of Azotobacter plus Azospirillum plus Pseudomonas and foliar that were at the same statistical level with Azotobacter plus Azospirillum plus Pseudomonas, Azotobacter and foliar treatments and Azotobacter plus Azospirillum and foliar solution all at the same statistical level which compared with control group (15.36%) had about 16.70% increase (Table 3).

3.8 Total Biomass Yield with Grain

Total biomass yield with grain was significantly affected by the growth stimulating bacteria, type of application and their interaction (Table 1). The data means showed that foliar treatment (21713.4 kg.ha) was better than seeding treatment (17931.7 kg.ha) and foliar treatment had an increase of 21.7% more than seeding treatment (Table 2). Moreover, comparison of treatment means showed that there is a significant difference between various levels of growth stimulating bacteria, so that the total biomass of seed in treatments with Pseudomonas plus Azospirillum (29558.8 kg.ha) and treatment of Pseudomonas (28882.2 kg.ha), which are in the same statistical level, are about 42.2% more than the treatment of Pseudomonas plus Azotobacter plus Azospirillum (20538.4 kg.ha), about 61.8% more than the control treatment (18054.1 kg.ha), about 73.4 more than Azotobacter treatment (16842.6 kg.ha), about 86.7% more than the treatment of Pseudomonas plus Azotobacter (15906.7 kg.ha) and treated with Azotobacter plus Azospirillum (15380.6 kg.ha) are in the same statistical level and are about 124.4% more than Azospirillum treatment (13016.4 kg.ha) (Table 2).

Rezvani Moghaddam et al.[21] investigated bio fertilizers impression on the savory herb and concluded that bacteria increase photosynthesis and improve plant dry matter and biological performance I in savory herb. Interactions between type of application and growth-promoting bacteria on total biomass grain yield were found meaningful. Comparison of treatment means showed that the highest seed biomass yield (39140 kg/ha) was obtained in the treatment of Pseudomonas and foliar which are in the same statistical level with Azospirillum + Pseudomonas and foliar treatment and compared with the control treatment (18800 kg/ha), had increased by about 108.1% (Table 3).

3.9 Grain HI

Grain harvest index was significantly affected by growth stimulating bacteria, type of application and their interaction (Table 1). Comparison of the data means showed that grain harvest index in seeding treatment (13.2279%) was better than foliar treatment (5.4196%) and compared with seeding treatment, foliar treatment increased by 144.07% (Table 2). Moreover, comparison of treatment means showed that there is a significant difference between various levels of growth stimulating bacteria. Therefore, the grain harvest index was higher in Azotobacter treatment (11.33%) and about 6.7% more than the Azospirillum plus Pseudomonas treatments (10.61%), about 9.2% higher than the control treatment (10.46%) and Pseudomonas treatment (10.28%) which are statistically at a level, approximately 15.2% more than Azotobacter plus Azospirillum treatment (9.83%), about 28.02% more than Azospirillum treatment (8.85%) and about 56.7% more than Azotobacter plus Pseudomonas treatments (7.23%), and approximately 87.5% more than the treatment of Pseudomonas plus Azotobacter plus Azospirillum (6.04%) (Table 2).

With a specific mechanism, bacteria change cleavage patterns of plant photosynthesis matters in favor of the reproductive organs and seeds. Diagnosis requires careful investigation of the action mechanism of bacteria in relation to production of growth regulators and other substances that are somehow involved in plant growth. Mirzai and Maleki et al.[22] that research about the effect of phosphate solubilizing bacteria on wheat (*Triticum aestivum* L.) concluded that these micro-organisms had a significant effect on harvest index. Interaction of application type and growth stimulating bacteria had a significant effect on the grain harvest index. Comparison of treatment means showed that the highest grain harvest index (17.00%) was obtained from the treatment of Pseudomonas and seeding which in comparison to the control (12.41%) increased by 36.9% (Table 3).

3.10 Extract Yield

Extract yield was significantly influenced by growth stimulating bacteria, type of application and their interaction (Table 1). Comparison of data means showed that extract yield of solution sprat treatment (11.1708 mg) was better than seeding treatment (8.3875 mg) and foliar treatment had an increase of 33.18% more than seeding treatment (Table 2). Likewise, comparison of treatment means showed that there is a significant difference between various levels of growth stimulating bacteria. Therefore, extract yield in the treatments of Zvsprlyvm + Pseudomonas (15.267 mg) was approximately 13.78% more than the treatment of Pseudomonas plus Azotobacter plus Azospirillum (13.417 mg) and approximately 30.12% more than Pseudomonas plus Azotobacter treatment (11.733 mg). It was approximately101.85% more than Azotobacter treatment (8.733 mg), Azospirillum treatment (7.833 mg), control treatment (7.433 mg), Azotobacter plus Azospirillum treatment (7.167 mg) and the treatment of Pseudomonas (6.650 mg) which are at the same statistical level (Table 3).

Application of growth stimulating bacteria increased extract yield. This increase was primarily due to production of plant growth regulators by bacteria and its effect on root growth, which in return improved absorption of water and nutrients from soil[23]. Interaction of type of application and growth stimulating bacteria had a significant effect on extract yield. Comparison of treatment means showed that the highest extract yield (20.40mg) was obtained from Azospirillum plus Pseudomonas treatments and foliar treatment, which compared to control (5.56 mg), had increased by approximately 266.9% (Table 3).

4. Conclusion

The foliar application treatment is better to increase the performance of quantitative characteristics and seed treatments be used to enhance the quality performance.

Azospirillum + Pseudomonas treatment brought great impact on the qualitative and quantitative characteristics of Calendula.

5. References

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