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**ORIGINAL RESEARCH PAPER**

# Different methods of bacterial inoculation on the yield of chamomile blossoms and essential oil

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**ABSTRACT**

Chamomile is one of the most wide spread medicinal plant cultivated in Egypt. This work aimed at enhancement of blossoms and oil production of chamomile plants via biofertilization with PGPRs under organic farming system. In this study, 6 bacterial strains were applied using two different inoculation techniques. The first application method was throughout soaking the roots of seedlings in the bacterial suspension before transplanting. The second technique was by adding the bacterial inocula to soil 2 weeks after transplantation. The results showed that root dipping method displayed high impact on the yield of chamomile blossoms and essential oil percentage. Furthermore, the soil application of the bacterial inocula didn't show any significant impact in this respect. Where *Paenibacillus polymyxa, Bacillus subtilis*, *Serratia plymuthica* and *Streptomyces subrutilus* increased the dry weight of chamomile blossoms compared to the control, essential oil content increased significantly in case of *Serratia plymuthica, Stenotrophomonas rhizophyla* and *Bacillus subtilis*. The current results also indicated that bacterial strains produced the highest indole-3-acetic acid and gibberellic acid resulted in the highest yield of both flowers and essential oil.

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## INTRODUCTION

Chamomile is one of the most commonly used medicinal plant all over the world, where the main countries producing chamomile are Hungary, Russia, Argentina, Germany, Slovakia, Finland, Egypt and India (Mohammad, 2011). In 2012, the chamomile production in Egypt was 10 thousand tons and the production of chamomile was concentrated in Nubaria, BaniSwaif, Asiout, and Fayoum Governorates (Amal, 2015). Chamomile is used to treat inflammations in the throat and in the stomach if it used as infusion, while externally it can be used for treatment of skin problems (Gosztola, 2012). In addition, it is used to treat insomnia, neuralgia, back pain, rheumatism, flatulence, indigestion, headaches, and gout (Srivastava *et al.*, 2010). Organic farming system is an appreciative technology for safe agroproducts and environment preservation. Application of organic fertilizers under organic farming system is an approach to produce high quality and safe products from medicinal plants. Organic fertilizers

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provide plants with sustainable supply of nutrients. It improves the physiochemical properties of the soil including water holding capacity, texture, structure, and porosity of soil. Organic fertilizers enrich the soil with beneficial microorganisms to maintain the stability between nutrients through the bioprocesses such as mineralization and immobilization (Alvarenga, 2004 and Mapeli *et al.*, 2005). In organic farming system, plant growth promoting rhizobacteria (PGPR) are widely used to replace the chemical fertilizers and pesticides, but their numbers in organic fertilizer or the soil is not enough. Thus, the addition of targeted microorganism could enhance the plant growth and improve the physicochemical properties of the soil (Rodriguez and Fraga, 1999). Chamomile's essential oil, which comprises 0.5% to 1.5% of the flower head, has one hundred twenty chemical constituents including terpenoids (α-bisabolol, α-bisabolol oxide A and B, chamazulene, sesquiterpenes), flavonoids (apigenin, luteolin, quercetin), coumarins (umbelliferone), spiroethers (en-yndicycloether), and other constituents like anthemic acid, choline, tannin and polysaccharides (Newall *et al.*, 1996). The essential oil of both German and Roman chamomile is a light blue color due to the terpenoid chamazulene. The plant growth and essential oil content are highly affected by plant microbiome. Inoculating mycorrhizal fungi and phosphate solubilizing bacteria (*Bacillus coagolance*) on chamomile revealed a significant enhancement in yield quality compared to its quantity (Farkoosh *et al.*, 2011). The overall aim of this study was to increase the quantity as well as the quality of chamomile yield using plant growth promoting rhizobacteria in organic farms using ecofriendly microorganisms avoiding the hazards of chemical fertilizers. Evaluation of microbial inoculation methods was aimed as well. This study was carried out in the organic farms in Sekem, Egypt in 2016.

## MATERIALS AND METHODS

*Microorganisms used*

Six different bacterial strains were tested in this study. Three Gram-positive strains were isolated from the rhizosphere of some medicinal plants in Egypt having promising antagonistic effect on different plant pathogens. Another three Gram-negative European strains known for their efficiency to colonize the rhizosphere/endosphere and considered as biocontrol agents were utilized. All strains were obtained from the Institute of Environmental Biotechnology, Graz University, Austria.

1. *Streptomyces subrutilus* 1Wb2n-11 isolated from desert soil from Sinai Peninsula. Strain 1 (Köberl *et al.*, 2011).
2. *Bacillus subtilis* subsp. *subtilis* Co1-6 isolated from the rhizosphere of *Calendula officinalis.* Strain 2 (Köberl *et al.*, 2011).
3. *Paenibacillus polymyxa* Mc5Re-14 isolated from the endorhiza of *Matricaria recutita*. Strain 3 (Köberl *et al.*, 2011).
4. *Pseudomonas fluorescens* L13-6-12 isolated from the endorhiza of *Solanum tuberosum*. Strain 4 (Lottmann and Berg, 2001)
5. *Stenotrophomonas rhizophila* P69 isolated from the rhizosphere of *Brassica napu.* Strain 5 (Wolf *et al.*, 2002)
6. *Serratia plymuthica* 3Re4-18 isolated from the endorhiza of *Solanum tuberosum*. Strain 6 ( Grosch *et al.*, 2005)

*Preparation of bacterial inocula*

According to Mehnaz *et al.* (2010), the inoculum was prepared by inoculation of the sterilized LB medium with tested organism and incubated for 48 hours at 30 °C. Cells were harvested by centrifugation at 12000 rpm for 20 min. Pellets were resuspended in 2 ml sucrose solution (1%) as cryoprotectant agent, and then were frozen to -70°C for 5 h. Tubes containing the frozen bacterial suspension were put into ampules and connected to a freeze-dryer (Labconco Free Zone 4.5 Liter Benchtop, USA) for 12 h under vacuum at <0.1 Pa.

*Inoculation*

Two inoculation techniques were followed in this regard. In the first experiment, dipping of chamomile's seedlings root in the bacterial suspension for 30 minutes was applied. On the other hand, the injection method was carried out by adding 50 ml of the bacterial suspension using sterile syringe in the zone around the root system of the plants which were previously transplanted two weeks before the treatment. The bacterial count was not less than $10^6$ CFU/mL in both techniques.

*Experimental design*

Each inoculation technique was carried out in separate field experiment using Randomized

Complete Block Design. In both experiments, six treatments plus a control were running in 5 replicates as shown in Fig. 1.

*Soil and compost*

The application of plant growth promoting inocula were applied to guarantee the nutritional needs of both microorganisms and the plant to make healthy and beneficial plant-microbe interaction. Physicochemical properties of soil and compost are shown in Table 1. The compost was applied at 10 tons/ feddan (4200 $m^2$).

*Chamomile growth parameters*

The blossoms of the chamomile plant were harvested after 3 months from transplanting. The harvest was carried out every 2 weeks and 4 harvests were collected during the season. The fresh and dry weights of blossoms as well as essential oil percentage were evaluated in all the treatments and the control.

*Fresh and dry weight estimation*

The fresh weight of blossoms was estimated for every 2 square meters which contain 14 plants in 2 rows. The collected blossoms were dried in a hot air oven at 40 °C then weighted to represent the dry weight.

*Oil content*

The oil content was estimated by steam distillation using Clevenger's apparatus on dried weight basis according to Eur. pharmacopeia 8/2014 test 2.8.12.

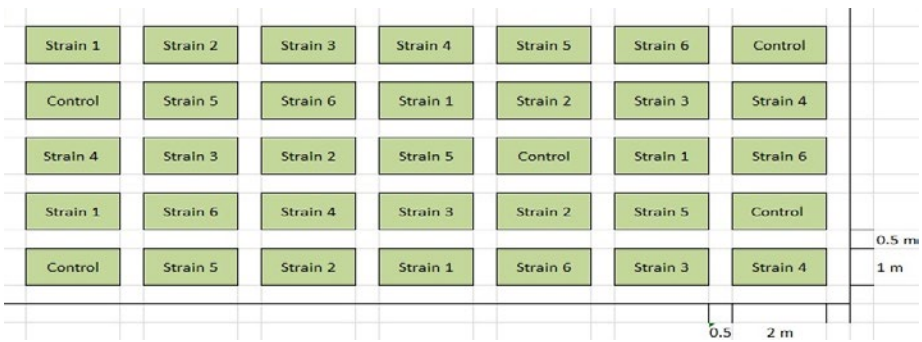


Fig. 1. The design of chamomile open field experiments

Table 1. Physicochemical properties of soil and compost used in the study (performed in the department of soil analysis, Heliopolis University, Cairo, Egypt)

| Item | Unit | Soil | Compost |
|---|---|---|---|
| Bulk density | $Kg/m^3$ | 1120.3 | 845.6 |
| pH | - | 7.5 | 7.5 |
| Soil texture | Sand% | 42 | - |
| | Clay% | 5 | - |
| | Silt% | 53 | - |
| EC | dS/m | 1.1 | 2.9 |
| Organic matter | % | 0.6 | 19 |
| Organic carbon | % | 0.34 | 11.02 |
| C/N ratio | % | 2.4: 1 | 19.7: 1 |
| Ash | % | 99.4 | 81 |
| Total nitrogen | % | 0.14 | 0.8 |
| Total phosphorus | % | 0.21 | 0.41 |
| Total potassium | % | 0.10 | 0.32 |
| Nitrate nitrogen ($NO_3^-$) | % | 0.0033 | 0.025 |
| Ammonical nitrogen ($NH_4^+$) | % | Nil | 0.046 |
| Available phosphorus | % | 0.011 | 0.041 |
| Available potassium | % | 0.0085 | 0.031 |
| $Na^+$ | Meq/L | 5.64 | 5.64 |
| $Mg^{++}$ | Meq/L | 3.8 | 3.8 |
| $Ca^{++}$ | Meq/L | 2.3 | 2.3 |
| $SO_4^{--}$ | Meq/L | 1.84 | 1.84 |
| $Cl^-$ | Meq/L | 10.4 | 10.4 |
| $HCO_3^-$ | Meq/L | 1.34 | 1.34 |
| $CO_3^{--}$ | Meq/L | Not detected | Not detected |

*Phytohormones estimation using GC-MS*

Phytohormones analysis was carried out using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000) and equipped with a polar Agilent HP-5ms (5%-phenyl methyl poly siloxane) capillary column (30 m × 0.25 mm i.d. and 0.25 µm film thickness). The carrier gas was helium with the linear velocity of 1 mL/min. The injector and detector temperatures were 200ºC and 250ºC, respectively. Volume injected 1µl of the sample. The MS operating parameters were as follows: ionization potential 70 eV, interface temperature 250ºC, and acquisition mass range 50–800. The identification of components was based on a comparison of their mass spectra and retention time with those of the authentic compounds and by computer matching with NIST and WILEY library, as well as by comparison of the fragmentation pattern of the mass spectral data with those reported by Muller *et al.,* (2002).

*Statistical analysis*

Statistical analysis of the data was treated by one way analysis of variance (ANOVA) as described by Snedecor and Cochran, (1969), the mean values were compared by LSD at 5% using the computer program SPSS, ver. 12.

## RESULTS AND DISCUSSION

During this research two microbial inoculation techniques, root dip and soil injection, were applied to enhance the production of chamomile blossoms as well as the essential oil percentage as affected by bacterial inoculation. The observations and results indicated a general improvement in both vegetative and blossom yield of chamomile regarding root dipping inoculation method over soil injection method (Fig. 2).

*Dipping seedlings in the bacterial inoculum*

Table 2 shows the results of chamomile blossoms fresh weight, dry weight and oil content percentage affected by microbial inoculation through dipping the seedlings in the bacterial suspension.

*Fresh weight*

Results in Fig. 3 showed the fresh weight of chamomile blossoms influenced by applying bacterial inocula in organic farming system. *Paenibacillus polymexa*, *Bacillus subtilis*, and *Serratia plymuthica* showed significant increase in fresh weight of blossoms when compared to *Stenotrophomonas rhizophyla, Pseudomonas fluorescence* and the control treatment.


Fig. 2. (a) Differences in vegetative growth of chamomile plants inoculated by dipping method; (b) Injection method

Table 2. The fresh weigh, dry weight and oil content of the treatments and the control of seedling dip method.

| Treatments | Fresh weight (g) | Dry weight (g) | Oil content (%) |
|---|---|---|---|
| Control | 365.80 | 78.33 | 0.8 |
| *S. subrutilus* | 648.98 | 132.89 | 0.90 |
| *B. subtilis* | 699.08 | 145.68 | 1.00 |
| *P. polymyxa* | 733.30 | 149.67 | 0.70 |
| *Ps.fluorescence* | 306.74 | 65.40 | 0.75 |
| *S. rhizophyla* | 427.20 | 86.44 | 1.03 |
| *S. plymuthica* | 682.23 | 138.06 | 1.20 |
| LSD (P ≤ 0.05) | 234.65 | 47.93 | 0.238 |

On the other hand, no significant differences were found between *Paenibacillus polymyxa, Bacillus subtilis, Serratia plymuthica* and *Streptomyces subrutilus*. Similarily, results indicated that no significant differences ($P \leq 0.05$) were found in fresh weights of blossoms due to the inoculation with *Streptomyces subrutilus* and *Stenotrophomonas rhizophyla*.

*Dry weight*

Regarding the effect of dipping seedlings treatment with different bacterial inocula on blossoms dry weight, data in Fig. 4 demonstrated similar trends to that of blossoms fresh weight. The highest dry weights of chamomile blossoms (149.67 g, 145.68 g and 138.06 g) were obtained when dipped in *P. polymyxa, B. subtilis* and *S. plymuthica*, respectively. On the other hand, when the chamomile seedling were dipped in *Ps. fluorescence*, *S. rhizophyla* and *S. subrutilus*, the plants exhibited the lowest blossoms dry weights (65.4 g, 86.44 g and 132.89 g, respectively). Biofertilizers were reported to decrease the hazards effects of chemical fertilizers to get the same yield. In this respect, Khalil and Agah, (2017) indicated that applying *Trichoderma* and *Bacillus* as biofertilizing agents for strawberry led to decrease 50% of the recommended dose of chemical fertilizers. They found that treatment of strawberry plants with 50% of the mineral fertilizers together with biofertilizers caused significant increase in the fruit yield units indicating the role of biofertilizers to compensate plants nutritional requirements even more efficiently than 100% mineral fertilizers. The utilized microorganisms (as biofertilizers) could provide plant with not only their nutritional requirements but also with some growth promoters *e.g.,* indole acetic

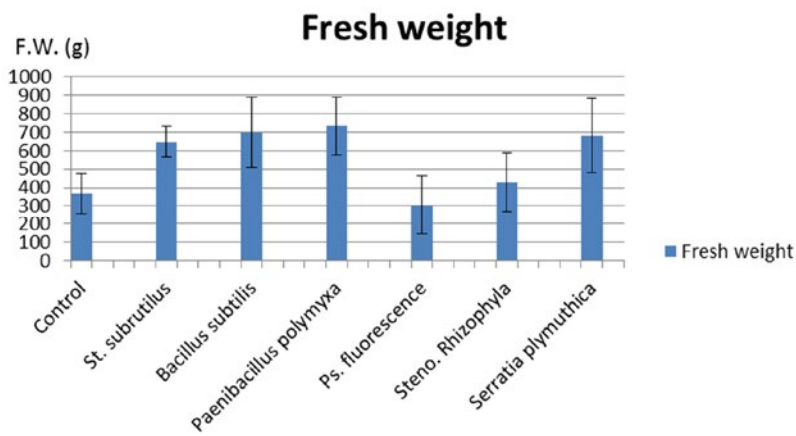


Fig. 3. The fresh weight of chamomile blossoms as affected by bacterial inoculants using dip method

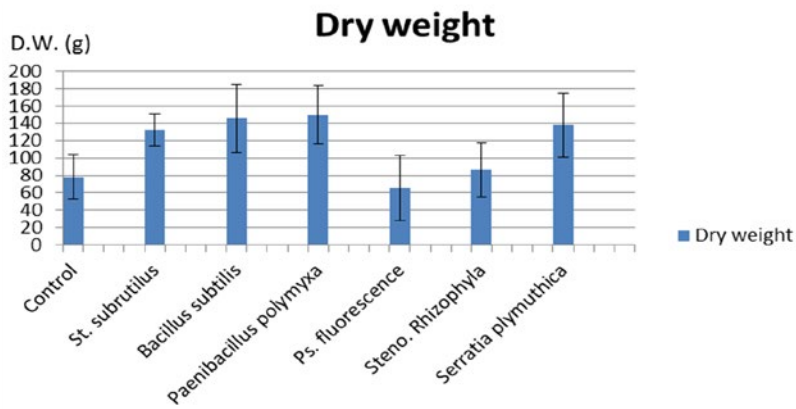


Fig. 4. The dry weight of chamomile blossoms as affected by bacterial inoculants using dip method

acid and antimicrobial compounds. Additionally, *Bacillus* and *Paenibacillus* sp. have a great ability to colonize the rhizosphere of plants due to several physiological properties including its multilayered cell wall, formation of stress resistant endospore and antibiotic secretion provide these species with high advantage to survive for long periods of time under various environmental conditions. Besides, *Bacillus* and *Paenibacillus* sp. were reported to have mechanisms to promote plant growth by phytohormones production, mineralization and mobilization of phosphorus, siderophore production and nitrogen fixation in case of *Paenibacillus polymyxa* (Richardson *et al.,* 2009; Idris *et al.,* 2007; Gutierrez-Manero *et al.,* 2001; Whipps 2001). This could explain the positive impact of *Bacillus subtilus* and *Paenibacillus polymyxa* on the fresh and dry blossom yield of chamomile. *Serratia plymuthica* is known as biocontrol agent against *Botrytis cinerea* and *Sclerotinia sclerotiorum* (Kamensky *et al.,* 2003). Furthermore, Wei *et al.,* (1991) reported that *Serratia plymuthica* activates Induced Systemic Resistance (ISR) in cucumber which decrease the symptoms caused by *Colletotrichum orbiculare.* In addition, *Serratia plymuthica* produces phytohormones, modulate phytohormonal balance and solubilize phosphate. 'Additive hypothesis' postulate that these coordinated mechanisms may explain the growth promotion caused by the bacteria towards the plant (Bashan and Holguin, 1997).

*Oil content*

Obvious significant differences among treatments in oil content were shown in Fig. 5. It was observed that both *Bacillus subtilis* and *Serratia plymuthica* were the common players giving the highest yield in case of fresh and dry weight of blossoms and the oil content ensuring their beneficial impact on chamomile plants rhizosphere. Hamed *et al.,* (2017) used *Azotobacter chroococcum, Bacillus megaterium* and *Saccharomyces cerevisiae* as biofertiliers to enhance the yield of lemon grass and its essential oil content. These results were in agreement with the current study where the biofertilization increased the essential oil content per feddan and per plant significantly compared to the non-inoculated plants.

The previous results indicated that the most efficient bacterial inocula used in our study were *B. subtilis* and *S. plymuthica* where the biomass of blossoms and oil content were the highest. On the other hand, treating plants with *P. polymyxa* gave the

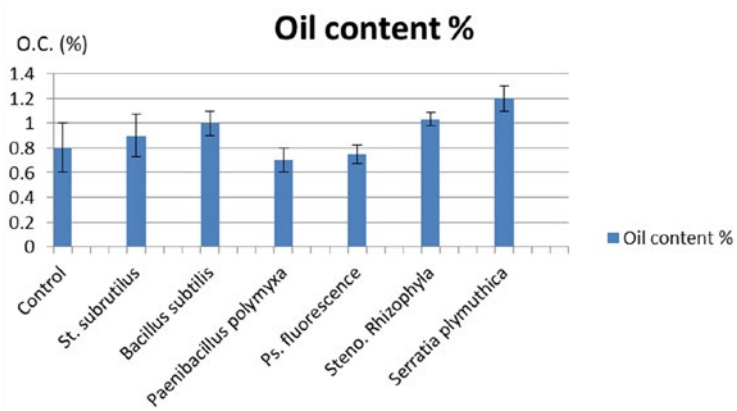


Fig. 5. Essential oil content of chamomile blossoms as affected by tested bacterial strains using dip method

Table 3. the production of gibberellic acid (GA3), abscisic acid (ABA) and indole acetic acid (IAA) by *B. subtilis*, *S. plymuthica* and *P. polymyxa* in nutrient broth

| Treatments | GA3 (PPM) | ABA (PPM) | IAA (PPM) | Dry weight | Oil % |
|---|---|---|---|---|---|
| Control | 11.8 | 0.12 | 0.77 | 78.33 | 0.8 |
| *B. subtilis* | 409.89 | 1.83 | 3.056 | 145.68 | 1 |
| *P. polymyxa* | 26.12 | 1.95 | 3.7 | 149.67 | 0.7 |
| *S. plymuthica* | 238.77 | 2.88 | 5.35 | 138.06 | 1.2 |
| LSD (P ≤ 0.05) | 13.68 | 0.2 | 0. 32 | 47.93 | 0.238 |

highest blossoms biomass with poor oil content. To explain these results, the capability utilized microbes to produce phytohormones was tested. These results were shown in Table 3. In this regard, both *B. subtilis* and *S.plymuthica* produced gibberellic acid (GA3) much higher than that of *P. polymyxa.* In the case of indole acetic acid (IAA), *S. plymuthica* produced the highest concentration, then *P. polymyxa* and *B. subtilis*. On the other hand, Abscesic acid (ABA) production was nearly equal in the case of *B. subtilis* and *P. polymyxa*, while *S. plymuthica* showed the highest result.

The predominant auxin in plants is IAA which is considered as the plant growth hormone. IAA has an important role in the cell elongation. GA3 is responsible for stem elongation and flowering process. ABA has an important role in the adaptation of plants under stress conditions such as salinity, drought and low temperature (Bano *et al.,* 2016). In this regard, Singh *et al.,* (2015) stated that treating chamomile plants with 100 ppm GA3 by foliar spraying gave the highest essential oil content (0.26%) compared to the untreated plants (0.12%), while treating with 100 ppm IAA increased oil content by 0.2 %. Moreover, treatment of chamomile plants with 100 ppm GA3 also gave the highest fresh and dry weight of blossoms (35.56 g and 9.96 g, respectively). Similarly, treating plants with 100 ppm IAA increased the flower fresh and dry weight of chamomile plants (31.66 g and 6.38 g, respectively). Reda *et al.,* (2010) demonstrated that applying IAA on chamomile plants has no influence on the dry weight of chamomile flowers, where applying GA3 increased the dry weight of flowers significantly compared to the untreated ones. With respect of the oil percentage, results in this study showed that GA3

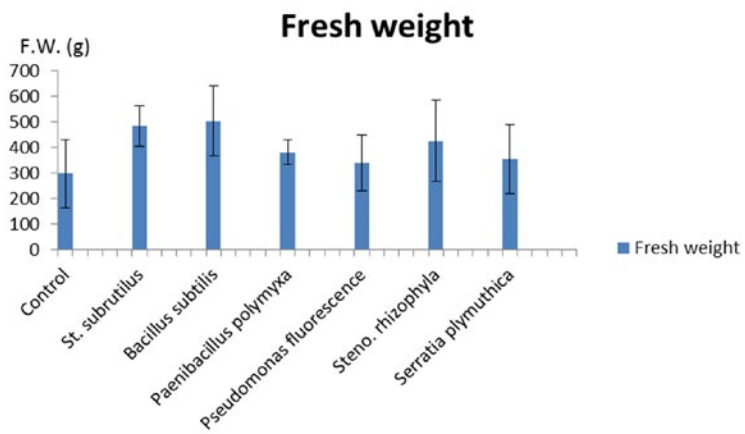


Fig. 6. The fresh weight of chamomile blossoms as affected by injection of bacterial inocula

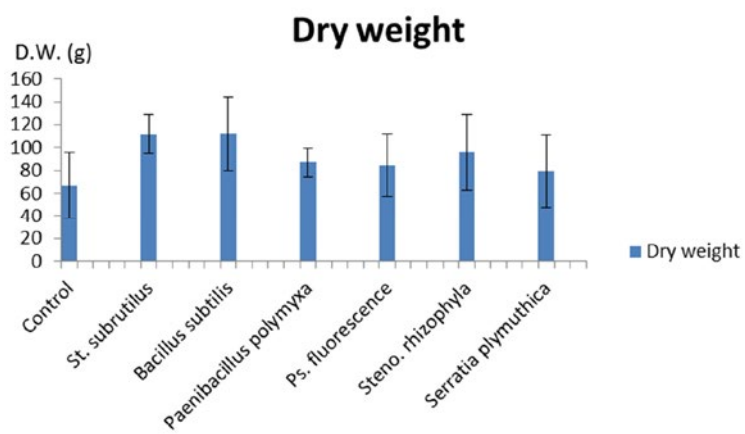


Fig. 7. The dry weight of chamomile blossoms as affected by injection of bacterial inocula

has higher influence on the oil content compared to IAA. Amiri *et al.,* (2014) recorded that 50 ppm of GA3 increased fresh and dry weight of chamomile flowers as well as the percentage of essential oil. In case of palmarosa plant, Khan *et al.,* (2015) stated that the influence of GA3 on the oil content and the herbage yield was higher than that of IAA. On the other hand, GA3 in basil plants caused mild decrease in the content of the essential oil, while IAA increased the oil percentage in the herb (Hazzoumi *et al.,* 2014). In a study on carnation plant, 4 levels of each of GA3 and IAA were used to investigate their effect on the fresh weight of flowers/ plant. Results showed that the highest yield was obtained by applying 150 ppm of GA3 together with 300 ppm of IAA (Kumar *et al.,* 2012). This explains the influence of the microbial inocula producing both GA3 and IAA in sufficient quantities in increasing the fresh and dry weight of blossoms as well as oil percentage of chamomile plants.

*Injection method*

No significant differences were shown between any of the treatments and the control in dry or fresh weight or oil content respecting to delivering microbial inocula through injection method. Figs. 6 to 8 show the fresh weight, dry weight and oil content of chamomile plant using the injection method of inoculation percentage, respectively.

*Fresh weight*

Injecting the seedlings rhizosphere with the bacterial inocula had no significant effect on the flower fresh weight. However, *B. subtilis* showed the highest fresh of the chamomile blossoms. Additionally, the non-inoculated plants showed the least fresh weight (Fig. 6).

*Dry weight*

With the same trend, blossoms dry weight was the highest in the case of applying *B. subtilis* as biofertilizer, while the control showed the least dry weight (Fig. 7).

*Oil content*

Fig. 8 shows that there were no significant differences between the treatments regarding the oil content percentage. Generally, *Serratia plymuthica* showed the highest oil content and *P. polymyxa*

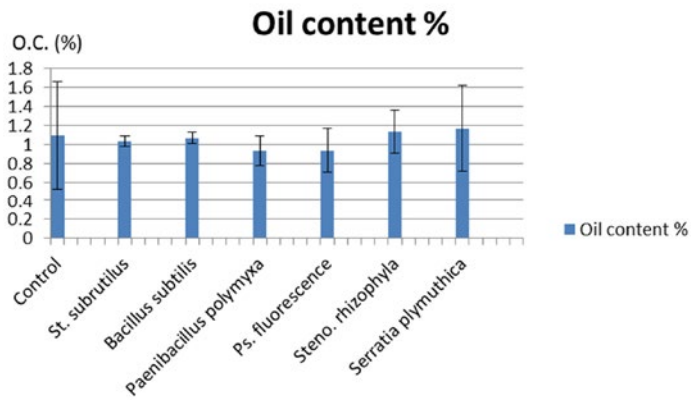


Fig. 8. Essential oil content of chamomile blossoms as affected by injection of tested bacterial strains.

Table 4. Fresh weight, dry weight, and oil content of the treatments and the control of the injection method of inoculation

| Treatments | Fresh weight (g) | Dry weight (g) | Oil content (%) |
|---|---|---|---|
| Control | 297.02 | 66.83 | 1.03 |
| *S. subrutilus* | 484.27 | 111.77 | 1.03 |
| *B. subtilis* | 503.74 | 112.10 | 1.07 |
| *P. polymyxa* | 381.76 | 87.01 | 0.93 |
| *Ps.fluorescence* | 359.91 | 84.63 | 0 93 |
| *S. rhizophyla* | 426.29 | 96.01 | 1.13 |
| *S. plymuthica* | 355.10 | 79.06 | 1.17 |
| LSD (P ≤ 0.05) | 212.64 | 51.29 | 0.588 |

Table 5. Comparison between the results of recent study and results of previous ones

| Bioferterlization | Dry weight (g)/2 $m^2$ | Oil content (%)/2 $m^2$ | Reference |
|---|---|---|---|
| No-inoculation | 328.4 | 0.90 | Dastborhan *et al.,* (2012) |
| *Azotobacter chroococcum* | 340.20 | 0.85 | |
| *Azospirillum lipoferum* | 358.00 | 0.91 | |
| Mixture of two bacteria | 355.88 | 0.87 | |
| Non-inoculated | 65.24 | 0.60 | Salehi *et al.,* (2018) |
| Inoculated (A. chroococcum, A. lipoferum and Ps. fluoresence) | 74.9 | 0.68 | |
| Control | 78.33 | 0.80 | Present study |
| *S. subrutilus* | 132.89 | 0.90 | |
| *B. subtilis* | 145.68 | 1.00 | |
| *P. polymyxa* | 149.67 | 0.70 | |
| *Ps.fluorescence* | 65.40 | 0.75 | |
| *S. rhizophyla* | 86.44 | 1.03 | |
| *S. plymuthica* | 138.06 | 1.20 | |

displayed the lowest oil content as observed in the dipping method as illustrated in Table 4.

Dipping seedlings and soil application techniques were studied on tomato plant using *Pseudomonas fluorescens* by Eltayeb, (2017). His results indicated that root dipping in the bacterial suspension has much more influence on the fresh and dry weight of shoot which increased 124% and 31.32%, respectively compared to the control. He stated that soil application increased the fresh and dry weight of shoot only 56.59% and 6.29%, respectively over the control. Accordingly, in agreement of our results, Eltayeb, (2017) found dipping method was more efficient in inoculating plants with biofertilizers than soil application technique. This results may owe to increasing root-associated microorganisms (in case of dipping method) resulted in reducing its competition with the inhabitant microbial communities. The low efficacy of microbial inoculation via injection method could refer to the competition between the added inocula and the native soil microbiome which affect adversely its role in growth promoting and biofertilization. In this regard, Thomas and Sekhar, (2016) stated that soil inoculation disturb the balance in inherent microbial community in soil which make fought back to restore its population. The used inoculum couldn't survive for more than a week when added to the soil rhizosphere, while it survived for 28 days in sterilized soil. These findings clarify the lower efficacy of soil applied microorganisms compared to rood dipping method. Few previous studies were carried out to evaluate the effect of biofertilizers on chamomile plants. Table 5 summarizes the comparison between the current study results and the findings of earlier researches. Table 5 illustrated changes in dry weight of blossoms and its oil content as a result of several biofertilizing agnates compared to control. It was observed that our biofertilizing agents greatly affected the dry yield compared to the influence of the treatment applied by Salehi *et al.,* (2018). In spite of using 3 different genera as biofertilizing agents (*Azotobacter chroococcum*, *Azospirillum lipoferum* and *Pseudomonas fluoresence*), our findings indicated using single inoculation (one microbe) was much easier and less expensive and more effective. In addition, applying *B. subtilis, P. polymyxa* and *S. plymuthica* separately showed much higher influence in the chamomile productivity. Although the results of Dastborhan *et al.,* (2012) indicated no significant effect on neither dry yield nor oil content of chamomile, our inocula significantly improved the parameters under evaluation.

## CONCLUSION

It could be concluded that microbial inoculation method has a great influence on yield of chamomile plant for both the blossoms and the essential oil. Soaking the seedling's roots in the bacterial suspension increase the load of the targeted organism resulting in high advantage to the microorganism to compete, survive and promote the plant growth. On the other hand, adding the microorganism in the soil decrease its ability to compete with other native microbial flora in the rhizosphere of the plant, decreasing the total impact of the biofertilization on the yield of chamomile. Likewise, using *Serratia plymuthica* and *Bacillus subtilis* as biofertilizers

following the root dip method in chamomile plant showed superior significant influence in both yields of blossoms and the oil content percentage. Dipping inoculation method could be recommended for improving chamomile production via biofertilization under organic farming system.

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## CONFLICT OF INTEREST

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

## ABBREVIATIONS

| | |
|---|---|
| *ABA* | Abscisic acid |
| *$Ca^{++}$* | Calcium ion |
| *CFU* | Colony forming unit |
| *$Cl^{-}$* | Chloride ion |
| *C/N* | Carbon/nitrogen ratio |
| *$CO3^{--}$* | Carbonate ion |
| *D.W.* | Dry weight |
| *dS/m* | Decisiemens per meter |
| *EC* | Electric conductivity |
| *eV* | Energy value |
| *Fig.* | Figure |
| *F.W.* | Fresh weight |
| *GA3* | Gibberellic acid |
| *GC-MS* | Gas chromatography- mass spectroscopy |
| *G* | Gram |
| *H* | Hour |
| *$HCO_3^{-}$* | Bicarbonate ion |
| *IAA* | Indole acetic acid |
| *i.d* | Inner diameter |
| *ISR* | Induced systemic resistance |
| *$Kg/m^3$* | Kilogram per cubic meter |
| *LB* | Luria broth |
| *LSD* | Least significant difference |
| *Meq/L* | Milliequivalent per liter |
| *$Mg^{++}$* | Magnesium ion |
| *M* | Meter |
| *Min* | Minuets |
| *mL* | Milliliter |
| *$Na^{+}$* | Sodium ion |
| *$NH_4^{-}$* | Ammonium ion |
| *$NO_3^{-}$* | Nitrate ion |
| *NPK* | Nitrogen, phosphorus, potassium fertilization |
| *O.C.* | Oil content |
| *Pa* | Pascal |
| *PGPR* | plant growth promoting rhizobacteria |
| *pH* | Potential of hydrogen (measure of acidity or alkalinity of a substance) |
| *Ppm* | Part per million |
| *PSB* | Phosphate solubilizing bacteria |
| *RDF* | Recommended dose of fertilizer |
| *Rpm* | Round per minute |
| *$SO_4^{--}$* | Sulfate ion |
| *SPSS* | Statistical package for the social sciences |
| *ver.* | Version |
| *%* | Percent |
| *°C* | Degree Celsius |
| *ml* | Microliter |
| *µm* | Micrometer |

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