Influence of Crop Growth Enhancer Bacteria on Yield and Essential Oil Content of Safflower
(*Carthamus tinctorius* L)

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**ABSTRACT:** To examine the effect of crop growth enhancer bacteria on quantitative and qualitative yield of safflower, an experiment in randomized complete block design was conducted in eight levels with four replications. Bacterial factor consisted of control (absence of bacteria), *Azotobacter Chroococcum*, *Azospirillum Brazilians*, *Pseudomonas Fluorescens*, *Azotobacter Chroococcum* + *Azospirillum Brazilians*, *Azotobacter Chroococcum* + *Pseudomonas Fluorescens*, *Azospirillum Brazilians* + *Pseudomonas Fluorescens*, *Azotobacter Chroococcum* + *Azospirillum Brazilians* + *Pseudomonas Fluorescens* respectively. The results showed that the effect of bacteria inoculation on the grain yield was significant at 1% probability level. The highest grain yield with an average of 2284.3 kg ha\(^{-1}\) belonged to the treatment in which the seeds were inoculated with all three crop growth enhancer bacteria at planting time. In contrast, the lowest grain yield with an average of 1643.4 kg ha\(^{-1}\) was related to the control treatment (non-inoculation). Moreover, the percentage and yield of protein and oil were also affected by the application of bio-fertilizers, so that the highest percentage of protein and oil, related to inoculation with *Azotobacter* + *Azospirillum* + *Pseudomonas*, were 17.83% and 29.36% respectively and the lowest of this amounts such as grain yield were related to the control treatment with an average of 15.20% and 25.82% respectively. Based on the obtained results, Safflower seed inoculation with crop growth enhancer bacteria, especially with a combination of bacteria, improved crop’s yield and characteristics.

**Keywords:** Safflower, Crop Growth Enhancer Bacteria, Qualitative Characteristics

**INTRODUCTION**

Safflower cultivation has a long history in many parts of the world. Iran is one of the richest areas of the world in terms of Safflower genetic resources. The main purpose of planting Safflower is to produce oil. Its oil production varies based on the kind of Safflower and the oil quality determines kind of its consumption (Zeinali, 1999).

In many agricultural ecosystems including Safflower, due to the removal of nutrients from the soil through harvest, leaching, or evaporation, constantly high levels of fertilizers should be added to the soil. Since the price of fertilizer as an input in always increasing and since fertilizer leaching contaminates surface and underground water, better cognition of optimum cycles in agricultural ecosystems is essential to achieve long-term stability. Since crops receive nutrients from the soil, providing the soil nutrients is considered as an indicator of fertility of agricultural ecosystems. When the levels of the soil nutrient are below the desirable range, it is said that the soil has faced lack of nutrients and the nutrients must be added to the soil. Farming soil loses considerable level of nitrogen through leaching each year which leads to serious decrease of total nitrogen which is available for crop growth. Under such circumstances, the operation of fixed atmospheric nitrogen in symbiosis with legume plants and biological nitrogen fixation in non-legume plants would be a good alternative for providing the nitrogen input which is required by soil and for replacing soil nitrogen reserves. Various estimates have been given of the contribution of biological nitrogen fixation in providing soil nitrogen as 44-200 kg ha\(^{-1}\) per year and in average 140 kg N ha\(^{-1}\) per year. Nitrogen inputs from biological nitrogen fixation in agricultural systems are the result of symbiotic relationship between legume species and *Rhizobium* bacteria and also non-symbiotic cooperation between molecular free-living, nitrogen-fixing micro-organisms and plants roots. Although there is native population of these bacteria in the soil, they might not be efficient enough in terms of nitrogen fixation. As a result, effective strains of such bacteria are commonly used as bio-fertilizers.
The increase of grain yield of some crops has been reported due to the use of Phosphate solubilizing microorganisms and nitrogen-fixing bacteria (De freitas, 2000, Ferrettin et al., 2004). Results of the experiment conducted by Mirzakhani et al., (2009) showed that the highest grain yield of Safflower was related to the treatment in which the seeds were inoculated with Azotobacter and Mycorrhiza fungus and 100 kg nitrogen was used at the planting time. Moreover, some changes were reported in morphological characteristics of crops such as a change in the crop height due to the use of Azospirillum and Azotobacter (Zahiret al., 2000).

Increase of dry weight of maize plant due to inoculation of seeds with Azotobacterchroococcum bacteria (Tilak et al., 1982), increase of dry and wet weight of leaves and the height of maize plant due to inoculation of seeds with Azospirillum Baselines bacteria (Kapulink et al., 1982) and increase of wet weight, number of leaves and the height of maize plant due to inoculation of seeds with Pseudomonas Fluorescens bacteria have been reported.

Zahir et al., (1998) reported 19.8% increase of maize grain yield due to inoculation of seeds with both Azotobacter and Pseudomonas bacteria, Tilak et al., (1982) reported that the increase of maize grain yield due to inoculation of seeds with both Azotobacterchroococcum and Azospirillum Brazilians bacteria. Zahiret al., (2000) observed the increase of dry weight of maize plant due to PGRP.

This research aims to study the effect of bacterial growth enhancers like Azotobacter, Azospirillum, and Pseudomonas on grain yield and qualitative traits of Safflower and also to determine the best bacterial combination for improving the yield so that some steps could be taken to achieve the goals of sustainable agriculture with regard to the lack of adverse environmental impacts of their consumption.

**MATERIALS AND METHODS**

To examine the effect of crop growth enhancer bacteria on quantitative and qualitative yield of safflower, an experiment in randomized complete block design was conducted with four replications. In this experiment 8 different bacterial combinations and one safflower variety was implemented with four replications. Therefore, there were 32 experimental units or plots with regard to the number of treatments and replications. Bacterial factor consisted of control (absence of bacteria), Azotobacterchroococcum, Azospirillum Brazilians, Pseudomonas Fluorescens, AzotobacterChroococcum+ Azospirillum Brazilians, AzotobacterChroococcum+ Pseudomonas Fluorescens, Azospirillum Brazilians+ Pseudomonas Fluorescens, AzotobacterChroococcum+ Azospirillum Brazilians+ Pseudomonas Fluorescens respectively. After preparing and providing the seed bed, the seeds of each treatment was planted. The distance between planting lines was 60 cm, the distance between two seeds in every row was 5 cm, the distance between two neighbor plots was 50 cm and in order to avoid the mixture of irrigation water between two replications, two streams were prepared. Before planting, the seeds were impregnated with bacteria by means of adhesive materials.

The final harvest was reaped after the removal of two lateral lines of each plot and one meter of the top and bottom of each line and then the samples were separated to test the seed quality and the grain yield per unit. Data was analyzed by means of SAS statistical software.

**RESULTS AND DISCUSSIONS**

**Grain yield**

The effect of inoculated seeds with bacteria on the grain yield was significant at 1% probability level, the highest grain yield was related to the treatment in which the seeds of Safflower were inoculated with Azotobacter, Azospirillum, and Pseudomonas at the planting time (Tables 1 and 2). Nitrogen fixation increase in this treatment led to increase of leaf area and more assimilate production and finally increase of dry matter production and grain yield in the crop.

Beech and Norman (2002) suggested the treatment of the consumption of 80 kgNha⁻¹ with as much grain yield as 2150 kg as the most economical treatment in relation to lack of nitrogen consumption with as much grain yield as 1410 kg.

Engel and Bergman (1997) reported that consumption of 0-150 kgNha⁻¹ increased Safflower grain yield.

The results of the research by Mirzakhani et al., (2002) on the effects of dual inoculation of Azotobacter and Mycorrhiza with nitrogen and phosphorus levels on yield and yield components of spring Safflower displayed that the highest grain yield was related to the treatment in which Safflower seeds were inoculated with Azotobacter and Mycorrhiza and 100 kg nitrogen and 50 kg phosphorus were used at the planting time.

**Biological yield**

Variance analysis results indicated that the effect of inoculation of seeds with bacteria on biological yield was significant at 1% probability level; the highest biological yield belonged to the treatment in which the seeds of Safflower were inoculated with all three bacterial growth enhancers i.e. Azotobacter, Azospirillum, and Pseudomonas at the planting time (Tables 1 and 2). Nitrogen fixation increase in this treatment led to increase
of leaf area and more assimilate production and finally increase of dry matter production in the crop. Through the production of various amino acids and hormones and the increase of absorption and solubility of nutrients such as nitrogen, phosphorus, iron and zinc, the bacteria enhanced the growth and resistance of the plant which led to increase of plant biomass (AsadiRahmani and Falah, 2001).

**Protein yield and percentage**

The effect of inoculation of seeds with bacteria on protein yield and percentage was significant at 1% probability level, the highest protein yield and percentage belonged to the treatment in which safflower seeds were inoculated with all three growth enhancer bacteria i.e. Azotobacter, Azospirillum, and Pseudomonas at the planting time (Tables 1, 2 and 3).

Nitrogen is the basic part of protein structure. Increase of nitrogen content affects protein synthesis (Rathkeet et al., 2005). Nitrogen content of crop increases by consuming more nitrogen and bio-fertilizers. These results were consistent with the results obtained by other researchers. The results of the research conducted by Kuceyet et al., (1989) and Dubey et al., (1994) indicated that protein content of Canola increased by consuming more nitrogen.

Since protein yield is obtained through multiplying grain yield by protein percentage, the highest protein yield, like grain yield, belongs to this treatment.

**Oil yield and percentage**

The effect of inoculation of seeds with bacteria on oil yield and percentage was significant at 1% level, the highest oil yield and percentage belonged to the treatment in which safflower seeds were inoculated with all three growth enhancer bacteria i.e. Azotobacter, Azospirillum, and Pseudomonas at the planting time (Tables 1, 2 and 3).

Lots of researches indicated that the percentage of grain oil decreases by consuming more nitrogen fertilizers. Nitrogen consumption decreases the percentage of fatty acids through relative increase of amino acids and other components (Marschner, 1995). Since the application of cooperative bacteria Azotobacter and Azospirillum causes gradual production of nitrogen in soil, they could not produce much nitrogen in soil at once and thus could not reduce oil percentage. On the other hand, the increased availability of phosphorus in the treatment of inoculation with all three bacteria increased the oil percentage through increasing synergistic property of Pseudomonas with Azotobacter and Azospirillum.

Rajput and Gautam (1992) reported that the Safflower oil and crop growth increased by consuming nitrogen and the highest percentage of grain oil was obtained by using 80 kg nitrogen. Since oil yield is obtained through multiplying grain yield by oil percentage, the highest oil yield, like grain yield, belongs to this treatment.

**Stearic acid**

With regard to variance analysis (Table 1), the effect of inoculation of seeds with bacteria on Stearic acid was not significant. Also according to the means (Table 3) the lowest rate of Stearic acid was related to the treatment in which Safflower seeds were not inoculated with any crop growth enhancers i.e. Azotobacter, Azospirillum, and Pseudomonas at the planting time.

**The unsaturated fatty acids, Oleic acid and Linoleic acid**

The effect of inoculation of seeds with bacteria on Oleic acid and Linoleic acid was significant at 1% probability level, the highest rate of these two acids belonged to the treatment in which safflower seeds were inoculated with all three growth enhancer bacteria i.e. Azotobacter, Azospirillum, and Pseudomonas at the planting time (Tables 1 and 3).

It seems like any factor which lengthens crop growth period will cause the grains to experience the cool weather of the area during the ripening season and thus the percentage of unsaturated fatty acids will increase. Some researchers such as Samanci and Ozkaynakm (2003) claimed that planting delay would decrease the rate of Stearic and Palmitic acids but it would increase Oleic acid and Linoleic acid. Moreover, Gecgel et al., (2007) stated that different times of planting affect the quality and quantity of safflower oil so that planting delay increases the formation rate of Oleic acid and Linoleic acid while Palmitic acid decreases in the the same period of time. Therefore, the inoculation of seeds with all three bacteria at the planting time will cause nitrogen fixation increase in this treatment and through increasing crop growth time which is coincided with the ripening of the grains in cool weather in the area, it will increase the percentage of unsaturated fatty acids i.e. Oleic acid and Linoleic acid.

According to the obtained results, inoculation of Safflower seeds with growth enhancer bacteria, especially with a combination of them will improve morphological and physiological traits of the crop.
Table 1. Summary of variance analysis results of some measurable traits

<table>
<thead>
<tr>
<th>S.O.V</th>
<th>df</th>
<th>Grain yield</th>
<th>Biological yield</th>
<th>Protein yield</th>
<th>Oil yield</th>
<th>Stearic acid</th>
<th>Oleic acid</th>
<th>Linoleic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replication</td>
<td>3</td>
<td>2559.099ns</td>
<td>2868.72ns</td>
<td>0.02ns</td>
<td>81.800ns</td>
<td>0.009ns</td>
<td>185.58ns</td>
<td>0.003ns</td>
</tr>
<tr>
<td>Treatment</td>
<td>7</td>
<td>16454.371**</td>
<td>3435229.64**</td>
<td>2.795***</td>
<td>9902.187***</td>
<td>9.042***</td>
<td>23506.7***</td>
<td>0.147ns</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>39030.370</td>
<td>44657.71</td>
<td>0.092</td>
<td>1259.751</td>
<td>0.165</td>
<td>3121.4</td>
<td>0.074</td>
</tr>
<tr>
<td>CV(%)</td>
<td></td>
<td>9.94</td>
<td>2.56</td>
<td>1.83</td>
<td>10.68</td>
<td>1.47</td>
<td>10.19</td>
<td>13.21</td>
</tr>
</tbody>
</table>

n.s. ** non significant, significant at 1 and 5% probability level respectively.

Table 2. Mean comparison of effects of different treatments of inoculation of seed with growth enhancer bacteria on Quantitative traits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grain yield (kg ha(^{-1}))</th>
<th>Biological yield (kg ha(^{-1}))</th>
<th>Protein yield (kg ha(^{-1}))</th>
<th>Oil yield (kg ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1643.4d</td>
<td>6682.9e</td>
<td>250.13d</td>
<td>424.20d</td>
</tr>
<tr>
<td>Azotobacter chroococcum</td>
<td>1921.7bcd</td>
<td>7883.5c</td>
<td>317.40bc</td>
<td>497.95cd</td>
</tr>
<tr>
<td>Azospirillum brazilians</td>
<td>2000.0abc</td>
<td>8271.5b</td>
<td>334.20bc</td>
<td>525.33bc</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>1779.4abc</td>
<td>7401.5d</td>
<td>280.60cd</td>
<td>501.68cd</td>
</tr>
<tr>
<td>Azotobacter + Azospirillum</td>
<td>2137.0ab</td>
<td>9370.9a</td>
<td>368.45ab</td>
<td>569.55bc</td>
</tr>
<tr>
<td>Azotobacter + Pseudomonas</td>
<td>2039.3abc</td>
<td>8403.9b</td>
<td>343.55b</td>
<td>587.10abc</td>
</tr>
<tr>
<td>Azospirillum + Pseudomonas</td>
<td>2080.7abc</td>
<td>8596.9b</td>
<td>356.65ab</td>
<td>606.78abc</td>
</tr>
<tr>
<td>Azotobacter + Azospirillum + Pseudomonas</td>
<td>2284.3a</td>
<td>9390.5a</td>
<td>407.48a</td>
<td>671.28a</td>
</tr>
</tbody>
</table>

In each column, the difference between two means which have one common letter is not significant at 5% probability level.

Table 3. Mean comparison of effects of different treatments of inoculation of seed with growth enhancer bacteria on Qualitive traits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein percentage</th>
<th>Oil percentage</th>
<th>Stearic acid (%)</th>
<th>Palmitic acid (%)</th>
<th>Oleic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.20f</td>
<td>25.82d</td>
<td>1.66b</td>
<td>62.25h</td>
<td>14.20f</td>
</tr>
<tr>
<td>Azotobacter chroococcum</td>
<td>16.50d</td>
<td>25.93d</td>
<td>1.93ab</td>
<td>66.43g</td>
<td>16.29e</td>
</tr>
<tr>
<td>Azospirillum brazilians</td>
<td>16.70cd</td>
<td>26.27cd</td>
<td>2.03ab</td>
<td>67.22f</td>
<td>16.57e</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>15.77e</td>
<td>28.17b</td>
<td>2.10a</td>
<td>71.30d</td>
<td>17.87d</td>
</tr>
<tr>
<td>Azotobacter + Azospirillum</td>
<td>17.25b</td>
<td>26.66c</td>
<td>2.14a</td>
<td>68.21e</td>
<td>16.49e</td>
</tr>
<tr>
<td>Azotobacter + Pseudomonas</td>
<td>16.85bcd</td>
<td>28.76ab</td>
<td>2.16a</td>
<td>72.69c</td>
<td>18.65c</td>
</tr>
<tr>
<td>Azospirillum + Pseudomonas</td>
<td>17.12bc</td>
<td>29.17a</td>
<td>2.19a</td>
<td>73.43b</td>
<td>19.13b</td>
</tr>
<tr>
<td>Azotobacter + Azospirillum + Pseudomonas</td>
<td>17.83a</td>
<td>29.36a</td>
<td>2.28a</td>
<td>75.26a</td>
<td>20.40a</td>
</tr>
</tbody>
</table>

In each column, the difference between two means which have one common letter is not significant at 5% probability level.
REFERENCES


