Effect of biological, organic and mineral fertilization on the concentrations of some macro and micro nutrients in cauliflower plant (*Brassica oleracea* L.)

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Abstract. An experimental study was conducted at the University of Anbar in 2019, with a randomized complete block design RCBBD with three replicates to study the effects of some fertilizers in concentration of some minerals in cauliflower. A bio-fertilizer of Azobacter chroococcum (P1), Pseudomonas fluorescents (P2) and mixture of P1 and P2 (P3). Organic fertilizers were of two levels, O1 and O2, where manual fertilizers are O, 50% and 100% of recommended dose. Results showed that fertilizers increased concentration of each of N, P, K and Fe in the vegetative part of cauliflower, besides the significant effect of P. Mineral and organic fertilizers increased same minerals in cauliflower vegetative tissue. However, the 50% and 100% of recommended mineral fertilizer were not significantly different.

Key words: *Brassica oleracea*. Azobacter, Pseudomonas.

1. Introduction

Cauliflower (*Brassica oleracea* L.) is one of the cool-season crops that belongs to the cruciferous family of vegetables. It is popular and widely consumed due to its high nutritional value and bioactive compounds such as glucosinolates and carotenoids (Avato and Argentieri 2015). Cauliflower is a good source of vitamins such as B1, B2, B3, B5, B6, C, E and K, in addition to dietary fiber and folic acid, omega-3 fatty acids, potassium, phosphorus, iron, magnesium, manganese and proteins (Florkiewicz et al. 2014). Moreover, it also rich with antioxidants and antioxidant enzymes such as catalase, superoxide dismutase and peroxidase, which are found in vegetables (Manchali et al. 2012). Recent studies has proven that daily eating of cauliflower has many benefits for human health such as increasing the resistance of the body against specific types of cancers, cardiovascular and degenerative diseases, immune dysfunction, and age-related macular degeneration (Fuentes et al. 2015). The rapid growth of world population governs a rising demand for water and food production (Nizami et al. 2017), which result in increased the consumption of the use of the non-renewable resources. The economy today takes a linear shape, with abundant amounts of spoilable produce. The uncontrolled consumption of materials exhausts their global resources relatively rapid, that pushes up their price. Food production can be increased by expanding cultivable areas or by increasing fertilizer doses used. However, the use of mineral fertilizers influences biogeochemical cycles of nutrients, particularly N and P, which significantly influence eutrophication. Phosphorus becomes wide spread in the environment while its non-renewable deposits become insufficient (Svanbäck et al. 2019). Consequently, effective and environmentally-friendly fertilization system should be strongly adopted. Eventually, reaching the population to 10.4 billion in 2067 in Africa or Asia (Britt et al. 2018), that will exceed the ability of the planet to feed the people. The demand for crops will have increased by 100–110% by 2050 compare with 2005(Tilman et al. 2011). Little information is known about how the combined application of long-term organic and mineral fertilizer. Despite the productivity of soil is primarily caused by the high levels of nutrients available to crops, soil microorganisms also have a major effect on soil quality and, accordingly, govern soil productivity. Recent studies have reported that combining organic and mineral
fertilizers increased the number and size of fruits, fruit content of vitamins C, in leaves, and leaves mineral such as N, P, K, Fe, Mn, and Zn (Mathur 2010). Moreover, the fertilizer efficiency of organic factors is more lasting when compared with inorganic fertilizers. Therefore, combining mineral and bio-fertilizer could save mineral fertilizers compare to recommend in addition to a good quality product. Also this could increase yield up to 30%. Some studies have reported that applying bio-fertilizers leading to release P and fixing N consequently give better results by improving the plant yield and quality as found on wheat (Ramos et al. 2002). When adding organic fertilizers this will improve microbial, chemical and physical properties of the soil. Therefore, to keep soil in this level of activities, organic fertilizer should be added. On the other hand, (Roghanian et al. 2012) stated that applying the compost in levels of 15-60 t/ha led to good changes in soil chemical and physical properties. Therefore, this study aimed to find out the beneficial effects of using Azotobacter chroococcum, and Pseudomonas fluorescents beside N, P and K fertilizers on cauliflower growth and nutrients content.

2. Materials and Methods
A pots experiment was conducted during September 2019 on the farm of the College of Agriculture, University of Anbar, Iraq. The treatments were randomly arranged according to the randomized complete block design (RCBD) with three replications. The mineral fertilizers were as the following: Urea (46% N) was the source of nitrogen (N), applied with application rate 300kg N/ha in split applications. The first dose was at planting, while the second was after 28 days, and the third after 68 days after cauliflower head formation. While the phosphor (P) fertilizer was added as DAP 21% P with application rate 120kg/ha, once two weeks before transplanting. The Potassium (K) as potassium sulfate 42% K applied with application rate 120 k/ha, added 10 days before transplanting. Organic fertilizer (Orgift) was obtained from local market. Two bacterial isolates; Azotobacter and Pseudomonas were obtained from the department of soil Science, University of Anbar, loaded on nutrient media with density 10^6/ml. The treatments were as:

1- Organic fertilizer, symbolized as O0, and O1 (Orgift) 40g/100 m.
2- Mineral fertilizers, O, 50% and 100% of application rate, symbolized as M1, M2 and M3 for each of N.P.K.
3- Bio-fertilizers, symbolized as the control Po, Pseudomonas P1, Azotobacter P2, then P3=P1+P2.

The nitrogen and potassium were determined according to (Black et al. 1965), while phosphorus was determined according to Olsen method that reported by (Page 1982). Fe and Zn were determined according to Tandon (1999). The soil samples were collected to a 30cm depth from the soil surface. Some chemical and physical characteristics of soil under study are listed in Table 1. Seeds of snowball cauliflower were germinated in July, then transplanted into the pots on mid-September. Each pot filled with a 20 kg of soil, mixed with organic matter and mineral fertilizers as recommended by (Al-Ajil 1984). The soil was wetted to the field capacity limit based on w/w (weight of soil/ weight of water) method, the bacteria previously mentioned were added and mixed with the soil. Two plants were planted in each pot.

3. Results and Discussion:
3.1. Nitrogen content:
The results listed in Table (2) indicate the effect of organic, biological and mineral fertilization on the concentration of nitrogen in the plant tissues, where the evidence showed a significant difference resulted from adding organic fertilizer, which led to increase in the concentration of nitrogen in the cauliflower plant reached to 4.04% compared to the control treatment that gave 2.50%. Similarly, adding the mineral fertilizer also showed a significant increasing in the concentration of nitrogen in the plant tissues either in the presence or absence of organic matter, this increasing took an ascending pattern with the application rate of mineral fertilizer i.e., 0.50 and 100% that gave 2.82, 3.47 and 3.51%, respectively (Table 2). Same way, the addition of the biological fertilizer inoculated with a mixture of azotobacter and sedomonas gave the highest concentration of nitrogen in the plant reached 3.48%,
followed by the fertilizer inoculated with the azotobacter bacteria reached 3.33% and the fertilizer inoculated with the bacterium sedomonas at a rate of 3.25%, while the control treatment gave the lowest concentration of nitrogen in the plant with a rate of 3.03%. In terms of interaction between the study treatments, the observed results listed in table (2) shows that there are no significant differences for the interaction effect between the addition of organic fertilizer and the addition of mineral fertilizers on the concentration of nitrogen in the plant (M × O=N.S) moreover, no significant interaction was observed with the addition of organic matter and the addition of biological fertilizers (P × O). In addition, there was a significant interaction between the addition of mineral fertilizers and biological fertilizers (P × M = 0.229). Meanwhile there was a significant effect of triple interaction (P × M × O = 0.324), representing by adding organic fertilizer, bio-fertilizer and mineral fertilizer together. The combined treatment of Pseudomonas and Azotobacter gave higher N content. This could be attributed to release of organic acids such as; Isovaleric, Isobutyric, Succinic, and Lactic acid, which have a positive effect in dissolving minerals, so the N absorbed by plant was increased (Gauri et al. 2012), and also for decreasing pH of rhizosphere which promotes fixation by bacteria (Khalil 2011).

**Table 1.** Some characteristics of the soil of the experiment.

| Parameter                  | Measuring unit | Value |
|---------------------------|----------------|-------|
| EC_e                      | dSm⁻¹          | 2.75  |
| pH                        | -              | 7.84  |
| O.M                       | gm km⁻¹        | 8.6   |
| Lime                      | gm km⁻¹        | 165   |
| Gypsum                    | gm km⁻¹        | 3.5   |
| Bulk density              | Meca gram m⁻³  | 1.32  |
| Parts size distribution   |                |       |
| Sand                      |                |       |
| Silt                      |                | 555   |
| Clay                      |                | 143   |
| texture                   |                |       |
| Available Nitrogen        | mg Kg⁻¹        | 82.0  |
| Microbial density         | ⁶*cfu gm Soil  | 3.2   |
| Available Phosphorous     | mg Kg⁻¹        | 12.3  |
| Available Potassium       | mg Kg⁻¹        | 143.0 |
| CEC                       | c mole c kg⁻¹ soil | 50.25 |
| Ions dissolved            | Mmol l⁻¹       |       |
| Ca⁺⁺                      |                | 16.5  |
| Mg⁺⁺                      |                | 7.61  |
| K⁺                        |                | 5.70  |
| Na⁺                       |                | 9.8   |
| SO₄²⁻                     |                | 7.45  |
| Cl⁻                       |                | 8.44  |
| HCO₃⁻                     |                | 4.54  |
| CO₃⁻                      |                | -     |
### Table 2. Effect of bio-fertilizer, mineral fertilizers and organic fertilizers on % N content in cauliflower

| P.G.P.R | O.M1 | O.M2 | AVERAGE |
|---------|------|------|---------|
|         | M    | M    |         |
|         | M1   | M2   | M3   | M1   | M2   | M3   |         |
| P0      | 1.8  | 2.3  | 2.7  | 3.3  | 3.78 | 4.30 | 3.03    |
| P1      | 1.9  | 2.80 | 2.9  | 3.6  | 4.1  | 4.2  | 3.25    |
| P2      | 2.2  | 2.9  | 2.5  | 3.6  | 4.5  | 4.3  | 3.33    |
| P3      | 2.3  | 2.9  | 2.9  | 3.9  | 4.55 | 4.35 | 3.48    |
| Average | 2.05 | 2.72 | 2.75 | 3.6  | 4.23 | 4.28 |         |

### Phosphorus content

The results listed in Table (3) showed that there were significant differences for adding organic fertilizer to increase the concentration of phosphorus in cauliflower plant, where the phosphorus concentration 0.77% when adding organic fertilizer compared to the control treatment, which gave 0.56%. Likewise, the results of the statistical analysis showed that the mineral fertilizer addition also gave significant differences in the concentration of phosphorus in the plant, regardless in presence or absence organic matter, as well as the phosphorus in the plant increase with increasing levels of mineral fertilizer addition 0%, 50%, 100% of Fertilizer recommendation for cauliflower at rates of 0.61, 0.69, and 0.70%, respectively. Significant differences were observed when using biological fertilizers inoculated with Azotobacter and Pseudomonas bacteria at a rate of 0.71%, while the control treatment gave the lowest concentration of phosphorus in the plant with a rate of 0.54%. In the same direction, the interaction between the treatments used in the study, shown in Table (3), demonstrated presence of significant differences for the interfering effect between adding organic and mineral fertilizers on nitrogen concentration in the plant (= M × O) as well as the presence of significant interference when adding organic matter and biological fertilizers (P × O = 0.058). In addition, there was no significant interaction between mineral and biological fertilizers (P × M). Similarly, the significant was not observed in the combination of the organic matter, mineral and bio-fertilizer (P × M × O) together.

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Table 3. Effect of bio-fertilizer, mineral fertilizers and organic fertilizers on % P content in cauliflower

| P.G.P.R     | O.M1 | O.M2 | AVERAGE |
|-------------|------|------|---------|
|             | M    | M    |         |
|             | M1   | M2   | M3      | M1   | M2   | M3      |
| P0          | 0.31 | 0.41 | 0.51    | 0.60 | 0.71 | 0.72    | 0.54   |
| P1          | 0.53 | 0.61 | 0.62    | 0.82 | 0.84 | 0.81    | 0.70   |
| P2          | 0.50 | 0.69 | 0.66    | 0.77 | 0.80 | 0.79    | 0.71   |
| P3          | 0.59 | 0.66 | 0.71    | 0.79 | 0.81 | 0.81    | 0.72   |
| Average     | 0.48 | 0.59 | 0.62    | 0.74 | 0.79 | 0.78    |         |

| P.G.P.R | O.M  | AVERAGE |
|---------|------|---------|
|         | M    |         |
|         | M1   | M2     | M3     |
| P0      | 0.41 | 0.67   | 0.54   |
| P1      | 0.58 | 0.82   | 0.70   |
| P2      | 0.61 | 0.78   | 0.71   |
| P3      | 0.65 | 0.80   | 0.72   |
| Average | 0.56 | 0.77   |         |

| P.G.P.R | M     |         |
|---------|-------|---------|
|         | M1   | M2     | M3     |
| P0      | 0.45 | 0.56   | 0.61   |
| P1      | 0.67 | 0.72   | 0.71   |
| P2      | 0.63 | 0.74   | 0.72   |
| P3      | 0.69 | 0.73   | 0.76   |
| Average | 0.61 | 0.69   | 0.70   |

LSD(0.05) 0.029=O 0.035=M 0.041 =P 0.050 =M×O 0.058 =P×O  M×P=N.S P×M×O=N.S

3.3. Potassium content

Table (4) presents the effect of mineral, organic and biological fertilization on the concentration of potassium in the plant, which took the same direction of phosphorous and nitrogen, where the results of the statistical analysis showed the presence of significant differences when adding the organic fertilizer led to increase the potassium concentration of cauliflower plants, which reached its concentration by adding organic fertilizer at a rate of 2.51% compared to not adding organic fertilizer, which averaged 1.27%. In the same direction, the results of the statistical analysis showed that the addition of mineral fertilizer also showed significant differences in the potassium concentration in the plant, either in presence or absent of organic matter, as well as the increase in potassium in plants, especially at the level of addition 50% of the fertilizer recommendation for cauliflower, whose rate was 2.09. While it reached a rate of 1.91 and 1.67% for each of the 100% addition level and treatment without the organic matter addition. In the same context, the results of the potassium concentration in the plant, showed the presence of significant differences when using biological fertilizers, where the addition of the biological fertilizer inoculated with a mixture of azotobacter and pseudomonas gave the highest concentration of potassium in the plant at a rate of 1.99%, while the rate of phosphorus was close to when addition of both the fertilizer inoculated with azotobacter bacteria and the fertilizer inoculated with the bacterium sedomonas at a rate of 1.92%, while the control treatment gave the lowest concentration of potassium in the plant with a rate of 1.73%. Regarding the role of interaction between the treatments under study, the results of the statistical analysis shown in Table (4) took the same way that there were no significant differences for the interaction effect between organic and mineral fertilizers addition on the potassium concentration in the plant (M × O=N.S) As well as there is no significant interaction when adding the organic matter and biological fertilizers (P × O). In addition, there was no significant interaction between mineral and biological fertilizers addition (M × P). As well
as the absence of a significant effect of triple interference (P × M × O), adding organic matter, bio-fertilizer and mineral fertilizer together (Mengel et al. 2001).

Table 4. Effect of bio-fertilizer, mineral fertilizers and organic fertilizers on % k content in cauliflower

| P.G.P.R | O.M1 | O.M2 | AVERAGE |
|---------|------|------|---------|
|         | M1   | M2   | M3      | M1   | M2   | M3      |         |
| P0      | 1.10 | 1.30 | 1.20    | 1.90 | 2.60 | 2.30    | 1.73    |
| P1      | 1.15 | 1.41 | 1.23    | 2.20 | 2.90 | 2.70    | 1.93    |
| P2      | 1.19 | 1.44 | 1.25    | 2.34 | 2.80 | 2.50    | 1.92    |
| P3      | 1.22 | 1.48 | 1.30    | 2.31 | 2.83 | 2.81    | 1.99    |
| Average | 1.16 | 1.40 | 1.24    | 2.18 | 2.78 | 2.57    |         |

| P.G.P.R | O.M1 | O.M2 | AVERAGE |
|---------|------|------|---------|
|         | M1   | M2   | M3      |         |
| P0      | 1.20 | 2.26 |         | 1.73    |
| P1      | 1.26 | 2.60 |         | 1.93    |
| P2      | 1.29 | 2.54 |         | 1.92    |
| P3      | 1.33 | 2.65 |         | 1.99    |
| Average | 1.27 | 2.51 |         |         |

3.4. Iron content

The results listed in Table (5) present the effect of organic, biological and mineral fertilizer on the iron concentration in the plant, which indicated significant differences resulted from the addition of organic fertilizer led to an increase in the iron concentration in cauliflower plants at a rate of 131.46 mg. Kg⁻¹ compared to the control treatment, which gave 91.00 mg. Kg⁻¹. The results of the statistical analysis also showed that the addition of different levels of mineral fertilizer led to significant differences in the iron concentration in the plant, either in presence or absent of organic matter. Table (5) indicated that the addition of 50% of the fertilizer recommendation for cauliflower exceeded the level of full addition 100%, especially in the treatments with organic matter addition, which averaged 148.25 mg. Kg⁻¹, while the iron concentration reached 137.45 mg.kg⁻¹at the addition level of 100%. In addition to the convergence of levels 50% and 100% when no organic matter was added, with a rate of 92.85, 93.45 mg, kg⁻¹ for each of 50% and 100% respectively. Also the results indicated the effect of biological fertilization on the iron concentration in the plant, as it showed significant differences when using biological fertilizers. The fertilizer was inoculated with the azotobacter bacteria, at a rate of 116.10 mg, followed by pseudomonas 112.16 mg/kg. control treatment gave only 100.23 mg/kg. Combining bio-fertilizer with mineral fertilizer, organic with bio-fertilizers, and mineral with bio-fertilizers gave significant increases in K content. Meanwhile, second order interaction was also significant. There
significant increases probably due to more available humic and folic acid (Ge et al. 2000) and the effects of NPK fertilizers (Mahmood et al. 2013).

Table 5. Effect of bio, mineral and organic fertilizers on Fe (mg/kg) content in cauliflower

| P.G.P.R | O.M1 | O.M2 | AVERAGE |
|---------|------|------|---------|
|         | M    | M    |         |
|         | M1   | M2   | M3      | M1   | M2   | M3      | M1   | M2   | M3      |
| P0      | 78.4 | 80.6 | 90.5    | 101.2 | 130.7 | 120     | 100.23 |
| P1      | 88.6 | 96.1 | 94.3    | 107.5 | 155.9 | 130.6   | 112.16 |
| P2      | 89.2 | 98.2 | 93.9    | 112.5 | 152.7 | 150.1   | 116.10 |
| P3      | 90.6 | 96.5 | 95.1    | 113.6 | 153.7 | 149.1   | 116.43 |
| Average | 86.7 | 92.85| 93.45   | 101.2 | 148.25| 120     |         |

| P.G.P.R | O.M | AVERAGE |
|---------|-----|---------|
| P0      | 83.16 | 100.23 |
| P1      | 93.00 | 112.16 |
| P2      | 93.76 | 116.10 |
| P3      | 94.06 | 116.43 |
| Average | 91.00 | 131.46 |

3.5. Zinc content

All the three fertilizers used gave significant increases in zinc content (Table 6). Organic fertilizer gave 77.72 mg/kg compared to the control which gave 67.69 mg/kg of zinc in cauliflower. The 50% does of mineral fertilizer gave higher content of zinc as compared to 100% of recommended mineral fertilizer Azotobacter and Pseudomonas gave high zinc content (76.7 mg/kg), the Pseudomonas (74.58 mg/kg) and Azotobacter (72.9 mg/kg), while the control treatment gave the lower value (66.65mg/kg). First order interactions of each of organic × mineral, organic with fertilizer, and mineral × bio-fertilizer were all significant in increasing zinc content. Same trend of significant was found with second order interaction. There was a significant effect of fertilizers on cauliflower zinc content, where the fertilizer could increase the nutrients uptake through the effects of auxins, gibberellins of cytokines (Mengel et al. 2001), and (Al-Shibani 2005). In terms of interaction, the interaction between the treatments used in the study showed that there are significant differences in the interaction effect between organic and mineral fertilizers addition to the concentration of zinc in the plant (0.188 = M × O) in addition to the presence of significant interaction when adding organic matter and Biofertilizers (P × O = 0.217) In addition, there was a significant interaction between the addition of mineral fertilizers and biological fertilizers (P × M = 0.266). In addition, there was a significant effect of triple interaction (P × M × O = 0.377) by adding organic matter, bio-fertilizer and mineral fertilizer together.
Table 6. Effect of bio-fertilizer, mineral fertilizers and organic fertilizers on Zn (mg/kg) content in cauliflower

| P.G.P.R | O.M1 | O.M2 | AVERAGE |
|---------|------|------|---------|
|         | M1   | M2   | M3      | M1   | M2   | M3   |   |
| P0      | 40.4 | 71.8 | 73.1    | 70.1 | 71.2 | 73.3 | 66.65 |
| P1      | 46.6 | 77.2 | 75.6    | 87.5 | 83.1 | 77.5 | 74.58 |
| P2      | 56.2 | 77.2 | 73.6    | 75.3 | 82.3 | 72.8 | 72.90 |
| P3      | 56.7 | 88.7 | 75.2    | 76.3 | 84.2 | 79.1 | 76.70 |
| Average | 49.9 | 78.7 | 74.3    | 77.3 | 80.2 | 75.6 |   |

| P.G.P.R | O.M | AVERAGE |
|---------|-----|---------|
|         | M   |         |
| P0      | 61.76 | 66.65    |
| P1      | 66.46 | 74.58    |
| P2      | 69.00 | 72.90    |
| P3      | 73.53 | 76.70    |
| Average | 67.69 | 77.72    |

LSD(0.05) O 0.133=M 0.154 =P 0.188=M×O 0.217 =P×O 0.266 M×P= 0.377P×M×O=

4. Conclusion
Application of Azotobacter + Pseudomonas + Organic fertilizers + 50% of recommended NPK fertilizers have increased the level of some macro and micro nutrients in cauliflower. Bio fertilizer of Azotobacter and Pseudomonas gave a significant increase in N content in cauliflower. Organic fertilizer, showed significant increases in P content. The three fertilizers used gave significant increases in K content in the cauliflower, in this context, the superiority was in the organic fertilizer treatment that gave 2.51% potassium content in the cauliflower. Similarly, the three fertilizers applied significantly increased iron in cauliflower and also the superiority was in the organic fertilizer treatment that gave 131.46 mg/kg of iron in cauliflower compared to the other treatments.

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