The Effect of Biofertilizer of Azolla, Phosphate and Nitrogen Fertilizers on some Growth Traits of Rice

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Abstract
A field experiment was carried out during the summer season of 2019 in one of the fields of Al-Tahiniah village - Al-Mahaniyah district which is 36 km away from the Diwaniyah governorate center, to study the effect of azolla, phosphate and nitrogen fertilizers and the interactions between them on some growth traits of rice. The experiment was arranged as factorial experiment according to Complete Blocks Design (RCBD) at three replications. The experiment included three factors, the first factor included the application of azolla (Azolla pinnata L.) at 20 tons ha\(^{-1}\) and the control treatment (without application of azolla), while the second factor included application three levels of phosphate fertilizer (21, 42 and 84 kg P ha\(^{-1}\)) as well as the control treatment (without application of phosphate fertilizer), whereas the third factor included application two levels of nitrogen fertilizer (92 and 184 kg N ha\(^{-1}\)) as well as the control treatment (without application of nitrogen fertilizer). The results showed that the application of Azolla pinnata L. gave the highest means for the content of rice leaves of nitrogen and phosphorous, plant height and leaf content of chlorophyll with a significant increase 14.83, 30.30, 6.37 and 7.80% respectively. Also, the application of phosphate fertilizer at 42 kg P ha\(^{-1}\) was significantly superior and gave the highest results of the content of rice leaves of nitrogen and phosphorous, plant height and leaf content of chlorophyll with a significant increase 31.32, 12.38, 6.04 and 11.87% respectively. In addition, the application of nitrogen fertilizer at 184 kg N ha\(^{-1}\) had the highest results of the content of rice leaves of nitrogen and phosphorous, plant height and leaf content of chlorophyll with a significant increase 42.03, 16.22, 15.49 and 16.04% respectively, without significant difference with application of nitrogen fertilizer at 92 kg N ha\(^{-1}\). We can concluded that the application of Azolla pinnata L. reduced the amounts of phosphate and nitrogen fertilizer by 50% of recommended amounts and led to improve the growth traits of rice.

1. Introduction
The food problem has become more complicated as a result of the widening gap between agricultural production, especially cereal crops, and the large increase in the population of these countries, accompanied by low productivity per unit area due to adopting non-scientific methods in agriculture, which requires better use of agricultural lands and a scientific approach in cultivating crops including rice [1]. The nutrient management system in agricultural soils is one of the most important factors affecting the growth and productivity of rice because the soil is one of the important natural constituents in agriculture and a source of natural wealth, and the extent of its quality plays an important role in rice cultivation, however, agricultural intensification associated with the addition of high levels of mineral fertilizers affect negatively on the physical, chemical and biological properties of soil [2].

As it is known, nitrogen and phosphorus are among the elements by which soil fertility can be judged, and they are among the major elements of the plant as a result of the physiological roles that each element performs within the plant tissue. The nitrogen use excessive led to loss it from the soil through volatilization and leaching with rain water and abundant irrigation [3]. As well as the unexamined additions of phosphate fertilizers as a result of exposure of phosphorus in Iraqi soils to precipitation as complex compounds and transformation of phosphate fertilizers that are well-soluble in water to less soluble and ready to plant and adsorption on the surfaces of clay minerals led to the imbalance of the readiness of the elements in the soil and the negative impact of that on its fertile state [4]. Therefore, to reduce these effects and the problems resulting from them must take into account the indicators of environmental management safety by reducing pollutants and replacing chemical fertilization or part of it with biological fertilizers such as azolla when growing crops, especially rice [5].

Azolla pinnata L. is a fern plant belonging to the Azollaceae family, and it is widespread in different regions of the world. These plants are distinguished by their ability to exploit a wide range of water and double their biomass every two to three days [6], and that the blue-green algae symbiotic With Azolla stabilizes nitrogen at a higher rate than legumes, and also stimulates the activity of micro-organisms in the soil [7], as well as being a rich source of nutrients necessary for plants, which will positively affect the fertility of the soil and increase the readiness of nutrients for the plant [8]. As a result of the characteristics of azolla, research has tended to cultivate it as a plant accompanying rice to reduce environmental pollution, maintain biological balance, improve soil properties and increase its content of organic matter, as well as increase the readiness of a large part of the important nutrients for plants, which will positively affect plant growth and development and...
improve its economic yield and quality [5]. Due to the limited information available, this study was carried out in order to find out the effect of azolla, phosphate and nitrogen fertilizers and the interactions between them on some growth traits of rice.

2. Material and Methods

A field experiment was carried out during the summer season of 2019 in one of the fields of Al-Tahiniyah village - Al-Mahanawiya district which is 36 km away from the Diwaniyah governorate center in a soil as shows their physical and chemical properties in Table 1, to study the effect of azolla, phosphate and nitrogen fertilizers and the interactions between them on some growth traits of rice. The experiment was arrangement as factorial experiment according to Randomized Complete Blocks Design (RCBD) at three replications. The experiment included three factors, the first factor included the application of azolla (*Azolla pinnata* L.) at 20 tons ha$^{-1}$ as shows their chemical properties in Table 2 and the control treatment (without application of azolla), while the second factor included application three levels of phosphate fertilizer (21, 48 and 84 kg P ha$^{-1}$) as well as the control treatment (without application of phosphate fertilizer), whereas the third factor included application two levels of nitrogen fertilizer (92 and 184 kg N ha$^{-1}$) as well as the control treatment (without application of nitrogen fertilizer).

### Table 1. Physical and chemical soil properties.

| Trait         | Value | Unit       |
|---------------|-------|------------|
| Sand          | 190.2 | g Kg$^{-1}$Soil |
| Loam         | 459.5 | g Kg$^{-1}$Soil |
| Clay         | 350.3 | g Kg$^{-1}$Soil |
| EC$^{-}$       | 2.92  | ds m$^{-1}$ |
| pH           | 7.53  | -----      |
| CEC          | 19.06 | cmole Kg$^{-1}$ Soil |
| O.M          | 0.96  | %          |
| Available N  | 19.83 | mg Kg$^{-1}$ Soil |
| Available P  | 3.56  | mg Kg$^{-1}$ Soil |
| Available K  | 118.61| mmole L$^{-1}$ |
| Ca$^{2+}$     | 12.67 | mg L$^{-1}$ |
| Mg$^{2+}$     | 10.41 | mg L$^{-1}$ |
| Na$^{+}$      | 20.04 | mg L$^{-1}$ |
| Cl$^{-}$      | 28.42 | mmole L$^{-1}$ |
| SO$_4^{2-}$   | 13.19 | mmole L$^{-1}$ |
| HCO$_3^{-}$   | 11.08 | mmole L$^{-1}$ |
| CO$_3^{2-}$   | Nil   | -----      |
| Available Fe | 0.4320| mg Kg$^{-1}$ Soil |
| Available Mn | 0.3537| mg Kg$^{-1}$ Soil |

### Table 2. Chemical properties of *Azolla pinnata* L.

| Trait           | Value | Unit       |
|-----------------|-------|------------|
| Crude Lipids    | 3.41  |            |
| Crude Protein   | 24.86 |            |
| Sugars          | 3.17  |            |
| Carbohydrates   | 6.05  |            |
| Fiber           | 10.96 | %          |
| Chlorophyll     | 0.33  |            |
| Ash             | 12.28 |            |
| Calcium         | 1.51  |            |
| Phosphorous     | 1.39  |            |
| Potassium       | 2.56  |            |
| Iron            | 1534.04|          |
| Zinc            | 312.59| mg L$^{-1}$ |
| Manganese       | 2327.47|            |
| Boron           | 27.62 | mg L$^{-1}$ |
| Copper          | 8.04  | mg L$^{-1}$ |
| Cobalt          | 7.84  | mg L$^{-1}$ |
Soil management especially plowing were carried out as required, the net area of sub plot was (3 m long x 5 m width) 15 m² which contained 15 lines, 0.20 m apart. A distance of 1 m was left between the experimental units and 2 m between the replicates. The seeds of rice (Dusht cv.) were sown on 17th July 2019 at a seeding rate 120 Kg ha⁻¹ [9]. At anthesis stage, the content of of rice leaves of nitrogen according to Jackson (10), phosphorous according to Page et al., (11), potassium according to Walinga et al., (12) and leaf content of chlorophyll (SPAD) were estimated, while at the harvest time the plant height was measured. The recorded data were statistically analyzed according to the analysis of variance at p > 0.05 by using the Gnestat software. The least significant difference (LSD) at the level of 0.05 probability was employed to compare the differences among treatment means [13].

3. Results and Discussion

3.1. Leaves content of nitrogen (%)

The results at the Table 3 show that the application of azolla had significant effect on the leaves content of nitrogen and gave the highest mean (1.451%) compared without application of azolla which gave the lowest mean (1.193%). The reason of an increasing may be due to the role of azolla application in increasing the nitrogen content of the soil solution and an increasing its absorption by the roots and its transfer to the vegetative parts of the plant. These results are in agreement with Yadav et al., [14] who indicated that the that application of azolla to the soil contributes to providing the crop with the elements necessary for growth including nitrogen. The results at the Table 3 indicate that the leaves content of nitrogen was significantly affected by phosphorous fertilizer levels (0, 21, 42 and 84 Kg P ha⁻¹), the application of 42 Kg P ha⁻¹ had the highest mean (1.476%) compared with others especially control treatment which had the lowest mean (1.124%). Also, the results at the Table 3 show that there are significant difference among nitrogen fertilizer levels (0, 92 and 184 Kg N ha⁻¹) in the leaves content of nitrogen, the application of 184 Kg N ha⁻¹ recorded the highest mean (1.470%) with non-significant difference with 92 Kg N ha⁻¹ (1.461%) while the control treatment recorded the lowest mean (1.035%). The interaction between application of azolla and phosphorous fertilizer levels had significant effect on the leaves content of nitrogen (Table 3), the application of azolla with 42 Kg P ha⁻¹ had the highest mean (1.712%) whereas the control treatment (without azolla + 0 Kg P ha⁻¹) had the lowest mean (1.021%). Also, The interaction between application of azolla and nitrogen fertilizer levels had significant effect on the leaves content of nitrogen (Table 3), the application of azolla with 92 Kg N ha⁻¹ gave the highest mean (1.734%) while the control treatment (without azolla + 0 Kg N ha⁻¹) gave the lowest mean (1.017%). The interaction between application of phosphorous and nitrogen fertilizer levels had significant effect on the leaves content of nitrogen (Table 3), the application of phosphorous at level 42 Kg P ha⁻¹ and nitrogen fertilizer at level 184 Kg N ha⁻¹ had the highest mean (1.732%) with non-significant difference with 42 Kg P ha⁻¹ + 92 Kg N ha⁻¹ (1.642%) and 84 Kg P ha⁻¹ + 92 Kg N ha⁻¹ (1.578%) whereas the control treatment (0 Kg P ha⁻¹ + 0 Kg N ha⁻¹) had the lowest mean (0.912%). The results at the Table 3 indicated that the interaction between three factors had significant effect on the leaves content of nitrogen, the application of azolla with 42 Kg P ha⁻¹ and 92 Kg N ha⁻¹ and application of azolla with 42 Kg P ha⁻¹ and 184 Kg N ha⁻¹ gave the best results (2.057 and 1.997%) respectively, while the control treatment gave the lowest mean (0.840%).

Table 3. Effect of Azolla fertilizer and phosphorous and nitrogen fertilizer on the leaves content of nitrogen (%).

| Bio fertilizer (BF) | Phosphorous fertilizer levels (Kg P ha⁻¹) | Nitrogen fertilizer levels (Kg N ha⁻¹) | BF x P |
|--------------------|------------------------------------------|--------------------------------------|--------|
|                    | 0                                        | 92                                   | 184    |
| Without Azolla     | 0.840                                    | 1.067                                | 1.157  |
|                    | 0.963                                    | 1.123                                | 1.240  |
|                    | 1.023                                    | 1.227                                | 1.467  |
|                    | 1.243                                    | 1.337                                | 1.633  |
|                    | 0.983                                    | 1.410                                | 1.290  |
| With Azolla        | 1.030                                    | 1.650                                | 1.540  |
|                    | 1.083                                    | 2.057                                | 1.997  |
|                    | 1.110                                    | 1.820                                | 1.440  |
| LSD 0.05           | 0.213                                    | 0.123                                |        |

| Bio fertilizer (BF) | Nitrogen fertilizer levels (Kg N ha⁻¹) | BF Means |
|--------------------|--------------------------------------|----------|
|                    | 0                                    | 92       | 184     |
| Without Azolla     | 1.018                                | 1.188    | 1.374   |
| With Azolla        | 1.052                                | 1.734    | 1.567   |
| LSD 0.05           | 0.107                                | 0.062    |         |

| Phosphorous fertilizer levels (Kg P ha⁻¹) | Nitrogen fertilizer levels (Kg N ha⁻¹) | P Means |
|------------------------------------------|--------------------------------------|---------|
|                                            | 0                                    |         |
|                                            | 92                                   | 1.937   |
|                                            | 184                                  |         |

...
3.2. Leaves content of phosphorous (%)

Table 4 reveals that the application of azolla had significant effect on the soil content of phosphorous and gave the highest mean (0.349%) compared without application of azolla which gave the lowest mean (0.304%). The reason of an increasing may be due to the role of zolla application in increasing the soil content of phosphorus due to its content of the elements necessary for plant growth and development (Table 2). These results are in agreement with Yadav et al., [14] who indicated that the application of azolla to the soil contributes to providing the crop with the elements necessary for growth as a result of its role in increasing the numbers and effectiveness of the microorganisms responsible for mineralization of organic matter in the soil. Also, these results are in agreement with Setiawati et al., [15] who reported that the application of azolla to the soil led to a significant increase in the phosphorus content of rice leaves. The results at the Table 4 indicate that the leaves content of phosphorous was significantly affected by phosphorous fertilizer levels, the application of 42 Kg P ha⁻¹ had the highest mean (0.345%) compared with control treatment which had the lowest mean (0.307%). These results are in agreement with Sharma et al., [16] who indicated that application of phosphate fertilizer was significantly increased the phosphorous content of rice leaves. The results at the Table 4 show that the nitrogen fertilizer levels were significant effect on the leaves content of phosphorous, the application of 184 Kg N ha⁻¹ recorded the highest mean (0.344%) with non-significant difference with 92 Kg N ha⁻¹ (0.339%) whereas the control treatment recorded the lowest mean (0.296%).

The increase in the nitrogen and phosphorous content of rice leaves as a result of appropriate levels application of phosphate and nitrogen fertilizers may be attributed to the role of application of these fertilizers in improving the physical, chemical and biological characteristics of the soil, which may have been positively reflected in increase in the soil solution content of plant nutrients, and then increasing its readiness in the root zone (rhizosphere) as well as increasing the growth of the root system and its propagation capacity, which led to an increase in the absorption capacity of the roots and raising the efficiency of their absorption of nutrients and their transfer to different plant tissues including leaves, and then increasing their content of these elements [17].

Table 4. Effect of Azolla fertilizer and phosphorous and nitrogen fertilizer on the leaves content of phosphorous (%).

| Bio fertilizer (BF) | Phosphorous fertilizer levels (Kg P ha⁻¹) | Nitrogen fertilizer levels (Kg N ha⁻¹) | BF x P |
|--------------------|------------------------------------------|--------------------------------------|--------|
|                    | 0                                       | 92                                   | 184    |
| Without Azolla     | 0.912                                   | 1.238                                | 1.223  |
|                    | 21                                      | 1.387                                | 1.390  |
|                    | 42                                      | 1.642                                | 1.732  |
|                    | 84                                      | 1.578                                | 1.537  |
| LSD 0.05           | 0.151                                   | 0.087                                |        |
| With Azolla        | 1.053                                   | 1.642                                | 1.732  |
|                    | 1.177                                   | 1.578                                | 1.537  |
| LSD 0.05           | 1.035                                   | 1.461                                | 1.470  |
| LSD 0.05           | 0.075                                   |                                     |        |

| Bio fertilizer (BF) | Nitrogen fertilizer levels (Kg N ha⁻¹) | BF Means |
|--------------------|--------------------------------------|----------|
| Without Azolla     | 4.176                                | 4.924    |
|                    | 4.595                                | 5.139    |
| With Azolla        | 4.241                                | 5.083    |
|                    | 5.134                                | 5.678    |
| LSD 0.05           | 4.176                                | 4.924    |

| Phosphorous fertilizer levels (Kg P ha⁻¹) | Nitrogen fertilizer levels (Kg N ha⁻¹) | P Means |
|----------------------------------------|--------------------------------------|----------|
| 0                                      | 92                                   | 184      |
| 4.176                                  | 4.924                                | 5.695    |
| 4.595                                  | 5.139                                | 5.678    |
| 4.241                                  | 5.083                                | 5.678    |
| 5.134                                  | 5.678                                | 5.678    |
| 4.176                                  | 4.924                                | 5.695    |
| 4.595                                  | 5.139                                | 5.678    |
| 4.241                                  | 5.083                                | 5.678    |
| 5.134                                  | 5.678                                | 5.678    |
| 4.176                                  | 4.924                                | 5.695    |
| 4.595                                  | 5.139                                | 5.678    |
| 4.241                                  | 5.083                                | 5.678    |
| 5.134                                  | 5.678                                | 5.678    |
| 4.176                                  | 4.924                                | 5.695    |
| 4.595                                  | 5.139                                | 5.678    |
| 4.241                                  | 5.083                                | 5.678    |
| 5.134                                  | 5.678                                | 5.678    |
| 4.176                                  | 4.924                                | 5.695    |
| 4.595                                  | 5.139                                | 5.678    |
| 4.241                                  | 5.083                                | 5.678    |
| 5.134                                  | 5.678                                | 5.678    |
| 4.176                                  | 4.924                                | 5.695    |
| 4.595                                  | 5.139                                | 5.678    |
| 4.241                                  | 5.083                                | 5.678    |
| 5.134                                  | 5.678                                | 5.678    |
The interaction between application of azolla and phosphorous fertilizer levels had significant effect on leaves content of phosphorous (Table 4), the application of azolla with 42 Kg P ha\(^{-1}\) had the highest mean (0.384%) whereas the control treatment (without azolla + 0 Kg P ha\(^{-1}\)) had the lowest mean (0.283%). Also, The interaction between application of azolla and nitrogen fertilizer levels had significant effect on the leaves content of phosphorous (Table 4), the application of azolla with 92 Kg N ha\(^{-1}\) gave the highest mean (0.379%) while the control treatment (without azolla + 0 Kg N ha\(^{-1}\)) gave the lowest mean (0.271%). The interaction between application of phosphorous and nitrogen fertilizer levels had significant effect on the leaves content of phosphorous (Table 4), the application of phosphorous at level 42 Kg P ha\(^{-1}\) and nitrogen fertilizer at level 92 Kg N ha\(^{-1}\) had the highest mean (0.364%) compared with others especially control treatment (0 Kg P ha\(^{-1}\) + 0 Kg N ha\(^{-1}\)) which had the lowest mean (0.272%).

The results at the Table 4 indicate that the interaction between three factors had significant effect on the leaves content of phosphorous, the application of azolla with 42 Kg P ha\(^{-1}\) and 92 Kg N ha\(^{-1}\) gave the best result (0.428%) while the control treatment gave the lowest mean (0.234%). The results showed that without application of Azolla pinnata L. the rice plants response was more pronounced to the high levels of phosphate and nitrogen fertilizers, but the application of Azolla pinnata L reduced the added amounts of phosphate and nitrogen fertilizers at 50% for each of them, which illustrates the integrated role of the three factors in improving soil properties and then increasing its fertility, reducing the added amounts of chemical fertilizers as well as improving the growth of the rice root system and increasing its ability to benefit of the available nutrients, their absorption and transfer to the leaves, and an increasing their nitrogen and phosphorous content.

### 3.3. Leaves content of potassium (%)

The results at the Table 5 showed that no significant effect of azolla application, phosphorous and nitrogen levels and the interactions between studied factors on the leaves content of potassium.

#### Table 5. Effect of Azolla fertilizer and phosphorous and nitrogen fertilizer on the leaves content of potassium (%).

| Bio fertilizer (BF) | Phosphorous fertilizer levels (Kg P ha\(^{-1}\)) | Nitrogen fertilizer levels (Kg N ha\(^{-1}\)) | BF x P |
|--------------------|-----------------------------------------------|---------------------------------------------|--------|
|                    | 0                                             | 92                                          | 184    |
| Without Azolla     | 0.837                                         | 0.821                                       | 0.817  | 0.825 |
|                    | 0.827                                         | 0.818                                       | 0.812  | 0.819 |
|                    | 0.820                                         | 0.813                                       | 0.809  | 0.814 |
|                    | 0.814                                         | 0.806                                       | 0.803  | 0.808 |
|                    | 0.822                                         | 0.817                                       | 0.807  | 0.815 |
| With Azolla        | 0.816                                         | 0.808                                       | 0.800  | 0.808 |
|                    | 0.806                                         | 0.799                                       | 0.798  | 0.801 |
|                    | 0.801                                         | 0.796                                       | 0.793  | 0.797 |
| LSD 0.05           | N.S                                           | N.S                                         | N.S    | N.S   |
| BF x N             |                                               |                                             |        |
| Bio fertilizer (BF)| Nitrogen fertilizer levels (Kg N ha\(^{-1}\)) | BF Means                                |        |
|                    | 0                                             | 92                                          | 184    |
| Without Azolla     | 0.825                                         | 0.814                                       | 0.810  | 0.816 |
| With Azolla        | 0.811                                         | 0.805                                       | 0.800  | 0.805 |
| LSD 0.05           | N.S                                           | N.S                                         | N.S    | N.S   |
| P x N              |                                               |                                             |        |
| Phosphorous fertilizer levels (Kg P ha\(^{-1}\)) | Nitrogen fertilizer levels (Kg N ha\(^{-1}\)) | P Means                                |        |
|                    | 0                                             | 92                                          | 184    |
|                    | 0.830                                         | 0.819                                       | 0.812  | 0.820 |
|                    | 0.821                                         | 0.813                                       | 0.806  | 0.813 |
|                    | 0.813                                         | 0.806                                       | 0.803  | 0.807 |
|                    | 0.808                                         | 0.801                                       | 0.798  | 0.802 |
The results at the Table 6 show that the application of azolla had significant effect on the plant height and gave the highest mean (90.16 cm) compared without application of azolla which gave the lowest mean (86.09 cm). The reason of an increasing may be due to the azolla content of the necessary elements of plant growth and development (Table 2) which led to activating the processes of cell division and elongation and stimulating the vegetative growth, including plant height. The results at the Table 6 indicate that the plant height was significantly affected by phosphorous fertilizer levels (0, 21, 42 and 84 Kg P ha\(^{-1}\)), the application of 42 Kg P ha\(^{-1}\) had the highest mean (90.78 cm) compared with others especially control treatment which had the lowest mean (85.61 cm). The positive effect of phosphorous fertilizer when application in appropriate amounts may be due to its role in stimulating the spread of roots and increasing the absorption of nutrients from the soil solution and their accumulation in plant tissue as well as its direct role in the formation of energy-rich compounds such as ATP, GTP, CTP and UTP, which are necessary in the formation of phospholipids and in the formation of accompaniments enzymatic activity that accompanies the assimilation of carbohydrates, which leads to stimulation of vegetative growth and increasing plant height [18]. These results are in agreement with Sharma et al., [16] and Massawe and Mrema [19] who indicated that the application of phosphorous fertilizer led to significant increase plant height of rice. Also, the results at the Table 6 show that there are significant difference among nitrogen fertilizer levels (0, 92 and 184 Kg N ha\(^{-1}\)) in the plant height, the application of 184 Kg N ha\(^{-1}\) had the highest mean (92.39 cm) with non-significant difference with 92 Kg N ha\(^{-1}\) (92.00 cm) while the control treatment recorded the lowest mean (80.00 cm). The reason of the increasing could be due to the role of nitrogen in the synthesis of amino acids, which are the cornerstone for the formation of proteins, synthesis of nucleic acids, as well as its entry into the formation of enzymatic conjugates such as NAD, NADP and FDA which are cofactors for enzymes as well as in composition of energy compounds [20] which led to increasing plant height. These results are in agreement with Pramanik and Bera [21], Islam et al., [22] and Gewaily et al., [23] who indicated that the plant height of rice was significantly increase by application of nitrogen fertilizer. The interaction between application of azolla and phosphorous fertilizer levels had significant effect on the plant height (Table 6), the application of azolla with 42 Kg P ha\(^{-1}\) had the highest mean (94.39 cm) whereas the control treatment (without azolla + 0 Kg P ha\(^{-1}\)) had the lowest mean (83.24 cm). Also, The interaction between application of azolla and nitrogen fertilizer levels had significant effect on plant height (Table 6), the application of azolla with 92 Kg N ha\(^{-1}\) gave the highest mean (95.16 cm) while the control treatment (without azolla + 0 Kg N ha\(^{-1}\)) gave the lowest mean (78.25 cm). The interaction between application of phosphorous and nitrogen fertilizer levels had significant effect on the plant height (Table 6), the application of phosphorous at level 42 Kg P ha\(^{-1}\) and nitrogen fertilizer at level 92 Kg N ha\(^{-1}\) gave the highest mean (95.33 cm) with non-significant difference with 42 Kg P ha\(^{-1}\) + 184 Kg N ha\(^{-1}\) (95.15 cm) whereas the control treatment (0 Kg P ha\(^{-1}\) + 0 Kg N ha\(^{-1}\)) had the lowest mean (77.02 cm). The results at the Table 6 indicate that the interaction between three factors had significant effect on the plant height, the application of azolla with 42 Kg P ha\(^{-1}\) and 92 Kg N ha\(^{-1}\) and application of azolla with 42 Kg P ha\(^{-1}\) and 184 Kg N ha\(^{-1}\) gave the best results (100.60 and 99.50 cm) respectively, while the control treatment gave the lowest mean (74.27 cm).

### Table 6. Effect of Azolla fertilizer and phosphorous and nitrogen fertilizer on the plant height (cm).

| Bio fertilizer (BF) | Phosphorous fertilizer levels (Kg P ha\(^{-1}\)) | Nitrogen fertilizer levels (Kg N ha\(^{-1}\)) | BF x P |
|---------------------|---------------------------------------------|---------------------------------------------|--------|
|                     | 0                                          | 92                                          | 184    |
| Without Azolla      |                                             |                                             |        |
| 0                   | 74.27                                      | 86.07                                      | 89.40  |
| 21                  | 77.43                                      | 90.07                                      | 90.50  |
| 42                  | 80.63                                      | 90.07                                      | 90.80  |
| 84                  | 82.67                                      | 91.07                                      | 92.03  |
| 0                   | 79.77                                      | 92.70                                      | 91.47  |
| 21                  | 80.87                                      | 93.43                                      | 93.53  |
| 42                  | 83.07                                      | 100.60                                     | 94.39  |
| 84                  | 81.27                                      | 93.90                                      | 91.87  |
| LSD 0.05            | 1.48                                       | 0.85                                       |        |
| BF x N              |                                             |                                             |        |
| With Azolla         |                                             |                                             |        |
| 21                  | 80.87                                      | 93.43                                      | 93.53  |
| 42                  | 83.07                                      | 100.60                                     | 94.39  |
| 84                  | 81.27                                      | 93.90                                      | 91.87  |
| LSD 0.05            | 1.48                                       | 0.85                                       |        |

LSD 0.05: 0.05 N.S N.S

N Means: 0.818 0.810 0.805

N.S
The results at the table 7 show that the application of azolla had significant effect on leaves content of chlorophyll and recorded the highest mean (22.69 SPAD) compared without azolla application which recorded the lowest mean (19.67 SPAD). The reason of an increasing may be due to the role of azolla in increasing leaves content of nitrogen (Table 3), Table 7 indicates that the application of phosphorous fertilizer levels was significantly effected on the leaves content of chlorophyll, the application of 84 Kg P ha\(^{-1}\) gave the highest mean (22.43 SPAD) while the control treatment gave the lowest mean (20.05 SPAD). The reason of an increasing may be due to the positive role of phosphorus when added in appropriate amounts in stimulating the growth of the root system and increasing the absorption of nutrients to plant such as nitrogen (Table 3), which plays an important role in the synthesis of photosynthetic pigments and then increasing leaves content of chlorophyll. Also, the results at the table 7 reveal that the leaves content of chlorophyll was significantly affected by nitrogen fertilizer levels, the application of 184 Kg N ha\(^{-1}\) recorded the highest mean (22.36 SPAD) with non-significant difference with 92 Kg N ha\(^{-1}\) (22.06 SPAD) while the control treatment recorded the lowest mean (19.27 SPAD). The reason may be attributed to the role of application of nitrogen fertilizer in increasing the plant content of nitrogen (Table 3), as nitrogen enters the formation of the porphyrin rings that are included in the formation of the basic chlorophylls for photosynthesis and respiration, as well as its entry into the formation of enzymatic accompaniments which are cofactors for enzymes [20]. These results are in agreement with Pramanik and Bera [21] and Islam et al., [22] who indicated that the application of nitrogen fertilizer led to significant increase the rise’s leaves content of chlorophyll.

**Table 7.** Effect of Azolla fertilizer and phosphorous and nitrogen fertilizers on the leaves content of chlorophyll (SPAD).

| Bio fertilizer (BF) | Phosphorous fertilizer levels (Kg P ha\(^{-1}\)) | Nitrogen fertilizer levels (Kg N ha\(^{-1}\)) | BF x P |
|--------------------|-----------------------------------------------|-----------------------------------------------|--------|
|                    | 0                                             | 92                                            | 184    |
| Without Azolla     |                                               |                                               |        |
| 0                  | 15.60                                         | 18.40                                         | 21.60  |
| 21                 | 17.37                                         | 18.77                                         | 22.27  |
| 42                 | 18.40                                         | 19.43                                         | 21.83  |
| 84                 | 19.60                                         | 21.37                                         | 22.53  |
| 0                  | 20.10                                         | 23.27                                         | 21.33  |
| With Azolla        |                                               |                                               |        |
| 0                  | 20.23                                         | 22.87                                         | 21.77  |
| 21                 | 22.23                                         | 27.83                                         | 24.87  |
| 42                 | 22.23                                         | 27.83                                         | 24.87  |
| 84                 | 20.60                                         | 24.57                                         | 22.67  |
| LSD 0.05           | 1.08                                          | 0.63                                          |        |

**Table 7.** Effect of Azolla fertilizer and phosphorous and nitrogen fertilizers on the leaves content of chlorophyll (SPAD).
The interaction between application of azolla and phosphorous fertilizer levels had significant effect on the leaves content of chlorophyll (Table 7), the application of azolla with 42 Kg P ha⁻¹ had the highest mean (24.98 SPAD) whereas the control treatment (without azolla + 0 Kg P ha⁻¹) had the lowest mean (18.53 SPAD). Also, The interaction between application of azolla and nitrogen fertilizer levels had significant effect on the leaves content of chlorophyll (Table 7), the application of azolla with 92 Kg N ha⁻¹ gave the highest mean (24.63 SPAD) while the control treatment (without azolla + 0 Kg N ha⁻¹) gave the lowest mean (17.74 SPAD). The interaction between application of phosphorous and nitrogen fertilizer levels had significant effect on the leaves content of chlorophyll (Table 7), the application of phosphorus at level 42 Kg P ha⁻¹ and nitrogen fertilizer at level 92 Kg N ha⁻¹ had the highest mean (23.63 SPAD) without significant differences with application of phosphorous at level 42 Kg P ha⁻¹ and nitrogen fertilizer at level 184 Kg N ha⁻¹ (23.35 SPAD) and application of phosphorous at level 84 Kg P ha⁻¹ and nitrogen fertilizer at level 92 Kg N ha⁻¹ (22.97 SPAD) while the control treatment (0 Kg P ha⁻¹ + 0 Kg N ha⁻¹) which had the lowest mean (17.85 SPAD). The results at the Table 7 indicate that the interaction between three factors had significant effect on the leaves content of chlorophyll, the application of azolla with 42 Kg P ha⁻¹ and 92 Kg N ha⁻¹ gave the best result (27.83 SPAD) while the control treatment gave the lowest mean (15.60 SPAD).

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