Original Research Article

Effect of Different Soil Amendments and Biofertilizers on *Pisum sativum* L. in Sandy Soil

ABSTRACT

Two successive field experiments were conducted out at Ismailia Agricultural Research Center Station experimental farm (Latitude 30º 35′ 41.901″ N and Longitude 32º 16′ 45. 843″E) to study the effect of cyanobacterial to investigate the influence of several biofertilizers (*Anabaena variabilis*, *Azollae*), some soil amendments (feldspar, bentonite, zeolite) and their combines on pea plant (*Pisum sativum* L.) type Super 2, during the winter seasons of 2022/2023 and 2023/2024. The results demonstrated that all treatments increased all growth parameters, although mixing treatments outperformed single treatments. The treatment *Azollae* mixed with feldspar soil amendments produced the most significant growth characteristics, including plant height, plant dry weight, total chlorophyll, number of leaves, number of pods, pod length, pod diameter, number of seeds on pod, pod fresh weight, pod dry weight,100 seeds fresh weight ,100 seeds dry weight and total green pod yield (tons fed^{-1}). It also has the greatest NPK content and uptake in plant and grains as well as carbohydrates, proteins contents in grain. Following that, *Azollae* was combined with each of zeolite or bentonite, and *Anabaena variabilis* was mixed with each soil amendments in both seasons. Soil analysis follows the same pattern as plant analysis, with treatments of *Azollae* mixed with each of the soil amendments yielding the highest significant values of nitrogen, phosphorus and potassium (NPK) and dehydrogenase enzyme activity in soil in both seasons, followed by *Anabaena variabilis* mixed with each soil amendments. Single treatments of *Azollae* and *Anabaena variabilis* resulted in higher plant and soil analysis than single soil amendments feldspar, bentonite, and zeolite, while the control treatment yielded the lowest results in both seasons. Therefore, the recommendation from this study is that employing a single soil conditioner with *Azollae*, or *Anabaena variabilis* as a biofertilizer increased pea plants more than a single application of either.

Keywords: *Pea plant (Pisum sativum), Anabeana varibilis Azollae, Soil amendments, Bentonite, Feldspar, Zeolite.*

1. INTRODUCTION

In Egypt, the pea plant (*Pisum sativum* L.) is one of the most important export and cash crop. Therefore, potential methods must be investigated as well as methods for enhancing productivity and yield of pea (Galal et al., 2021). The continued application of chemical fertilizers not only pollutes the environment while also inhibiting microbiological activity and soil organic matter (Pahalvi et al., 2021). Bio fertilizers offer beneficial microorganisms to agricultural production in terms of nutrient availability, particularly N and P; as a result, they are beneficial for overall plant growth and are referred to as plant growth-promoting rhizobacteria (PGPR) (Basu et al., 2021). Plant growth promotion via PGPR inoculation is mediated by their effects on important metabolic processes such as nitrogen fixation, phosphate solubilization, and plant growth hormone synthesis (Vocciante et al., 2022).

Anabaena varibilis is one member of cyanobacteria, which are free-living, photosynthetic prokaryotes with both single and filamentous forms, these prokaryotes can be found in fresh, marine, and terrestrial settings (Darnajoux,2022). These plants had both vegetative and specialized heterocyst cells for photosynthesis and nitrogen fixation (Kuraganti et al.,2020). Cyanobacteria such as *Anabaena* *varibilis*, *Azollae*,, *Acutodesmus dimorphus*, *Nostoc* sp. and *Chlorella vulgaris* have been used as biofertilizers to boost crop development (Ammar et al., 2022). Cyanobacteria are an excellent biofertilizer due to their ability to fix atmospheric nitrogen, secrete secondary metabolites, improve soil structure, fertility, and crop yield (Ramakrishnan et al.,2023).

Azollae is the name given to the symbiotic interaction between *Anabaena azollae* and cyanobacteria, which provides resource complementarities between the two sections and the associated plants (Pratte and Thiel,2021). Cyanobacteria gain shade next to *Azolla* , fronds in this symbiotic relationship, and *Anabaena Azollae* benefits from the nitrogen (N) fixed by bacteria. The *Azollae*,- Cyanobacteria symbiosis, referred to simply as "*Azollae*" has the ability to accumulate a high amount of fixed nitrogen, making it a potential biofertilizer for a variety of crops, including rice (*Oryza sativa* L.) (Roy et al., 2020), soybeans (Glycine max) (Sholkamy et al., 2015), and wheat (*T. aestivum*) (Kollah et al.,2016). Incorporating *Azollae*, into the soil before or after planting improves soil fertility and reduces the demand for synthetic fertilizer (Kimani et al.,2020). Several researchers have observed considerable yield increases due to *Azollae* (Yao et al.,2018). *Azollae*, unlike other biofertilizers, has been reported to have numerous other benefits in rice fields, including the recovery of a Ca, Mg, P, Fe, S and K, as well as a wider range of macro and micronutrients, than rice alone (Tang et al., 2018).

Soil amendments formed from waste products can be converted into pellets that immediately hydrate and interact with the soil-water matrix, slowly releasing vital nutrients to the soil due to the mineralization of organic nutrients and organic matter (O.M). This enhances the soil's physicochemical and biological qualities, allowing for more sustainable agriculture (Usharani et al., 2019). Bentonite is type of natural clay composed of the montomorillonite mineral that belongs to smectite group (2:1 minerals) which has a high cation exchange capacity (CEC) and water holding capability (Kumari and Mohan,2021). It can be used as a natural soil supplement that releases water and nutrients for plant uptake during dry spells while storing a substantial amount of water and nutrients during rainfall events (Mi et al., 2021). Feldspars ($KAlSi_3O_8$) are the essential constituents of rocks that are weathered into secondary minerals, according to the environmental conditions. Feldspars are often considered as relatively stable, the main source of clay minerals are phyllosilicates like smectite or vermiculite, feldspars are transformed completely into kaolinite and/or gibbsite, and iron oxides (Oyebanjo et al., 2020). Zeolites are crystalline hydrated aluminosilicate minerals made from inter linked tetrahedral of alumina (AlO_4) and silica (SiO4) (Cataldo et al., 2021). Zeolites have been used as slow-release fertilizers, (Soltys et al., 2020).

Therefore, this experiment was done to investigate the effect of some soil amendments (bentonite, feldspar and zeolite) in the presence of *Anabaena variabilis* and *Azollae* as a biofertilizer on pea crop growth and productivity in sandy soil during two winter seasons of 2022/2023 and 2023/2024.

2. MATERIALS AND METHODS

The current investigation on pea plant (*Pisum sativum* L.), type Super 2, was conducted during two successive winters in 2022/2023 and 2023/2024 at the Ismailia Agricultural Research Station (ARC), Ismailia Governorate, Egypt (30º 35′ 41.901″ N and 32º 16′ 45. 843″ E), in a field experiment on sandy soil. Soil was analyzed according to (Jackson , 1973). to investigate their physical and chemical characteristics shown in Table (1). *Anabaena variabilis* and *Azollae* were supplied by ARC., Soils, water, Environ. Inst., Agric. Microbiol. Dept. and soil amendments (bentonite, feldspar and zeolite) were purchased from private company, they were added to the soils before seed sowing. During soil preparation, recommended doses of potassium and phosphorous fertilization were added as potassium sulphate (48% K_2SO_4) and monocalcium super phosphate (15.5% P_2O_5), and nitrogen as ammonium nitrate (33.5% kg N fed^{-1}). The experiment included 12 treatments, each with three repetitions and the design of experiment was completely randomized design, by concentration of soil amendments bentonite, feldspar, and zeolite (500Kg fed^{-1}), while two biofertilizers *Anabaena variabilis* and *Azollae*at a dose of (250g fed^{-1}). Treatments of the experiment were as following:
Control (without application), *Anabaena variabilis* (*A. variabilis*), *Azollae* , bentonite (B), feldspar (F), zeolite (Z), *A. variabilis* + B, *A. variabilis* + F, *A. variabilis* + Z, *Azollae* + B , *Azollae* + F and *Azollae* +Z, each treatment was planted in three lines (2.8m^2) for each treatment. All Treatments were added before planting, *A. variabilis* and *Azollae*, were added another time after 30 days of planting.

Table (1). Physical and chemical properties of experimental of used soil and Soil amendments.

	Soil	Bentonite	Feldspar	Zeolite
		Particle size distribution		
Sand	95.35	4.7	70.5	92.0
Silt	2.35	30.5	10.6	5.2
Clay	2.30	64.8	18.9	2.8
Texture	sand	clay	Sandy loam	Sand
		Some chemical properties		
pH1:2.5	8.14	7.2	7.8	8.1
Ec dS m^{-1}	1.03	8.7	1.4	1.4
Sp %	10	190	42	15
Ca^{+2} (meq l^{-1})	2.92	27.5	4.5	4.5
Mg^{+2} (meq $^{-1}$l)	2.18	22.5	3.5	2
Na^{+} (meq l^{-1})	4.03	34.6	6.1	4.7
K^{+} (meq l^{-1})	0.9	3.75	0.75	0.35
HCO_3^- (meq $^{-1}$l)	1.47	4.55	1.5	1.0
Cl^- (meq l^{-1})	5.5	67.5	10	8.5
SO4 $^{-2}$ (meq $^{-1}$l)	3.06	16.2	3.35	2.05

After 45 days of plants samples were collected from each treatment to investigate the effect of these treatments on pea plants as following:

PLANT ANALYSIS

Growth and seed yield characters

1- Plant height (cm)
2- Plant dry weight(g)
3-Total chlorophyll
4- No of leaves
5- No. of pods plant^{-1}
6- Pod length(cm)
7- Pod diameter (cm)
8- No. of seeds pod
9- Pod fresh weight(g)
10 – Pod dry weight(g)
11-100 seeds fresh weight
12-100 seeds dry weight
13- Total green pod yield (ton fed^{-1})

Total Chlorophyll

Chlorophyl were extracted from 1 g fresh young leaves in a dimethylformamide (DMF) soln. Overnight at 4℃ to estimate the mass of chlorophyll total per leaf. At wavelengths of 663, 470, and 647 nm, the pigments were calculated using Moran's equation and a spectrophotometer Beckman Du 7400 (Moran et al.,1982).

Nitrogen, potassium and phosphorus contents in plant and grains

Macronutrient N P K content was measured and averaged across two seasons. Plant harvesting samples, including grain and leaves, were oven-dried at 70°C for 48 hours. They were then digested with a 1:1 mixture of sulfuric (H_2SO_4) and perchloric acid ($HClO_4$). Plant nutrients were measured in aliquots using the Kjeldahl method for nitrogen and phosphorus was determined by spectrophotometer with stannous chloride reduced molybdo-phosphoric blue color method. potassium was measured by flame photometers (Jackson, 1973).

Carbohydrates and Protein percentage

To determine carbohydrate, 10 ml of 80% ethanol was used to ground fresh seeds (1g), then the extract was diluted with water to a final level of 10 ml. Concentrated H_2 O_4 (96%) was added to 5ml test tubes, briskly shaken, and incubated at room temperature for 40 minutes. Each tube was filled with approximately 1 ml of 5% phenol, and a spectrophotometer measurement at 490 nm was recorded (Dubios et al. 1956). The total protein percentage in dry grains was determined using micro-Kjeldated method according to A.O.A.C. (1995). The total protein percent was calculated by the multiplication of nitrogen values by 6.25.

Soil analysis

NPK content in soil

Soil samples were collected from the of pea plants to determine available N, P, and K using Jackson (1973) method.

Dehydrogenase activity (DHA)

Soil biological activity of rhizosphere of pea plants was determined in soil samples collected from pea rhizosphere in terms of dehydrogenase activity (DHA) in soil determined according to Casida (1964).

Statistical Analysis

The experimental data obtained were subjected to analysis of variance (ANOVA) and Co-State software (CoStat V. 2005) to calculate LSD at a significant level (P = 0.05), as stated by (Gomez and Gomez ,1984).

Results

The experiment's findings showed that the biofertilizers *A. variabilis, Azollae*, soil amendments bentonite (B), feldspar (F) and zeolite (Z) improved all growth parameters; however, the results of their mixture (*A. variabilis* + B, *A. variabilis* + F, *A. variabilis* + Z, *Azollae* + B, *Azollae* + F and *Azollae* + Z) were higher than those of single applications and the control treatment. Additionally, the results of the second season slightly higher than the first season. Some growth parameters reported in Table (2) such as, plant height (cm), dry weight, total chlorophyll content in leaves, number of leaves). The maximum plant height (70.3 cm) was observed by the B+*A. variabilis* treatment, followed by *Azollae* + B (67 cm), with no significant differences between treatments in the second season. Dry weight in the second seasons were also higher than the first season, while the treatment of B+ *Azollae*, Z+*A. variabilis*, and F+ *A. variabilis* reported the highest dry weight (28.07, 27.42, and 27.3 g) respectively. Total chlorophyll improved in the second season compared to the first, the treatment of F+ *Azollae* recorded the highest chlorophyll content (44.48 mgg^{-1}) with non-significant difference between Z+ *Azollae*(43.45 mgg^{-1}) and Z+ *A. variabilis* (42.70 mg g^{-1}). As a consequence, as plant height and chlorophyll increased, so did the number of leaves, maximum number leaves (24.3 and 24) of was recorded by *Azollae* + F and Z+ *Azollae treatment*, then, (23.67, 23) reported by B+ *Azollae* in both seasons and finally the control treatment recorded the lowest plant height, dry weight, and number of pods in both seasons.

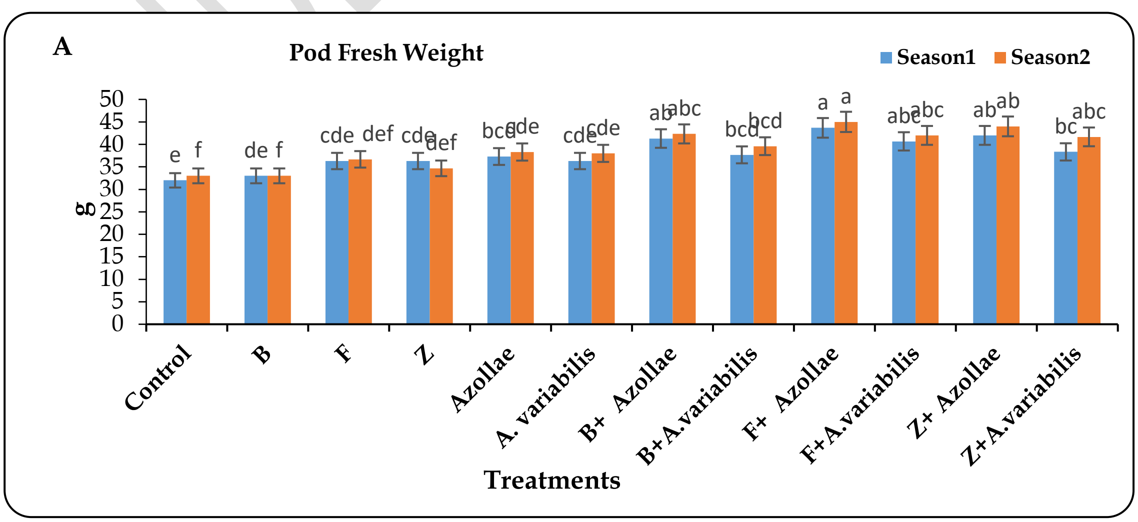
Table (2). Effect of soil amendments and biofertilizers on plant height, dry weight, total Chlorophyll and No. of leaves per plant of pea plant during both winter seasons.

	Plant height (cm)		Plant dry weight (g)		Total Chlorophyll (mgg^{1})		No of leaves plant $^{-1}$	
	Season1	Season 2	Season1	Season 2	Season1	Season 2	Season1	Season 2
Control	50.30(h)	51.70(e)	12.76(f)	6.30(d)	6.30(d)	24.22 (h)	14.00(f)	15.6(e)
B	54.67(g)	57.70(d)	15.09(e)	8.30(cd)	8.30(cd)	25.76(g)	14.30(f)	15.6(e)
F	57.70(ef)	57.70(d)	16.82(d)	11.00(bcd)	11.00(bcd)	29.46 (f)	15.00(ef)	16.3(e)
Z	55.00(fg)	55.79(d)	16.26(d)	10.00(bcd)	10.00(bcd)	25.76(g)	15.00(ef)	15.67(e)
Azollae	61.00 (cd)	64.00(bc)	18.70(c)	12.00(abc)	12.00(abc)	33.60(cd)	15.30(ef)	19(cd)
A. variabilis	59.33(de)	61.70 (c)	18.62(c)	12.00(abc)	12.00(abc)	31.67 (e)	17.00(c)	17.3(de)
B+ Azollae	63.00(bc)	66.30 (b)	25.77(a)	13.33(ab)	13.33(ab)	37.12(c)	23.00(ab)	23.67(a)
B+A.variabilis	61.66(bcd)	62.30 (c)	19.70(c)	12.00(abc)	12.00(abc)	40.15(b)	19.30(cd)	20.67(bc)
F+ Azollae	66.00(a)	70.30(a)	26.74(a)	14 .00 (a)	14 .00 (a)	44.48 (a)	24.00(a)	24.00(a)
F+A.variabilis	61.33(bcd)	65.70 (b)	23.05(b)	13.33(ab)	13.33(ab)	42.70 (a)	21.30(bc)	22.00(ab)
Z+ Azollae	64.00 (ab)	66.30 (b)	25.77(a)	13.33(ab)	13.33(ab)	43.45 (a)	24.00(a)	24.30(a)
Z+A. variabilis	62.00(bcd)	65.00 (b)	22.86(b)	13.33(ab)	13.33(ab)	35.36(cd)	20.00(c)	21.00(bc)
L.S.D.(0.05)	2.75	3.24	1.09	3.73	3.73	1.83	2.49	2.60

Table (3) and Figure (1 A and B) reported pod characteristics such as number of pods, pod length (cm), pod diameter (cm), and seed number in pod, pod fresh weight and dry weight with the highest number of pods (18.3) in the F+ *Azollae* treatment, followed by (16.3) in the Z+ *Azollae* and F+ *A. variabilis* treatments, with a low significant difference between the number of pods in other mixed treatments, while single applications of *Azollae* and *A. variabilis* reported (15) and (14.6) pods, respectively. In comparison to single treatments, pod length, diameter, and number of seeds increased when soil amendments were added in conjunction with each biofertilizer. In the second season, F+ *Azollae* had the largest pod length, diameter, and number of seeds (12.67 cm, 2.00 cm, and 8.67 seed), while Z+ *Azolla* treatment showed non-significant differences (12.33, 2 cm, and 8.66 seed), and B+ *Azolla* and F+*A. variabilis* treatments showed low significant differences in the second season. Although pod fresh and dry weight increased in the second season compared to the first, the highest pod fresh and dry weight was recorded in both seasons when soil amendments and biofertilizers were present, so when the length and diameter of the pod increased, its fresh and dry weight increased accordingly in both seasons, and all mixed treatments reported higher pod fresh and dry weight than single and control treatments. F+ *Azollae* treatment recorded (45.00, 12.67g) pod fresh and dry weight respectively, with slight significant difference recorded by Z+ *Azollae* (44, 12.33 g) and B+ *Azollae* (42.33, 12.33g) in the second season. The number of seeds in pods developed with pod length, so the largest seed number was 8.67 seeds, followed by 8.66 seeds in the Z+ Azollae treatment, however other combined treatments improved the seed number in pods compared to the single and control treatments.

Table (3). Effect of soil amendments and biofertilizers on pods characteristics of pea plant during both winter seasons.

	No. of pods Plant $^{-1}$		Pod length (cm)		Pod diameter(cm		Seeds No pod^{-1}	
	Season1	Season 2	Season1	Season 2	Season1	Season 2	Season1	Season 2
Control	22.80 (j)	24.22 (h)	7.66(f)	7.67(e)	0.77(f)	0.80(g)	6.33(d)	7(c)
B	23.43(i)	25.76(g)	7.67(f)	8.67(de)	0.86(f)	1.00(f)	7.00(cd)	7.33(bc)
F	23.73(h)	29.46 (f)	8.67(def)	9.33(cde)	1.17(e)	1.3(e)	7.3(bcd)	7.67(abc)
Z	28.60 (d)	25.76(g(	8.33(ef)	8.67(de)	0.87(f)	1.00(f)	7.00(cd)	7.33(bc)
Azollae	30.48 (f)	33.60(cd)	8.67(def)	9.67(cd)	1.53(d)	1.73(c)	8.00(bcd)	8.30(ab)
A. variabilis	26.10 (g)	31.67 (e)	8.67(def)	9.33(cde)	1.27(e)	1.53(d)	7.30(bcd)	8.33(ab)
B+ Azollae	33.34(d)	37.12(c)	10.67(ab)	12.33(ab)	1.87(ab)	1.93(ab)	8.33(ab)	8.33(ab)
B+A. variabilis	36.17 (c)	40.15(b)	9.33(cde)	10.67(bc)	1.63(cd)	1.76(bc)	8.00(abc)	8.30(ab)
F+ Azollae	40.39 (a)	44.48 (a)	11.33(a)	12.67(a)	1.97(a)	2.00(a)	8.67(a)	8.67(a)
F+A. variabilis	38.99 (b)	42.70 (a)	10.33(abc)	12.00(ab)	1.76(bc)	1.93(bc)	8.30(ab)	8.30(ab)
Z+ Azollae	40.28(ab)	43.45 (a)	11.00(a)	12.33(ab)	1.93(ab)	2.00(a)	8.66(a)	8.66(a)
Z+A. variabilis	31 .44(ef)	35.36(cd)	9.67(bcd)	11.00(abc)	1.67(cd)	1.8(bc)	8.00(abc)	8.30(ab)
L.S.D.(0.05)	1.5	1.83	1.26	1.84	0.19	0.19	1.22	1.01



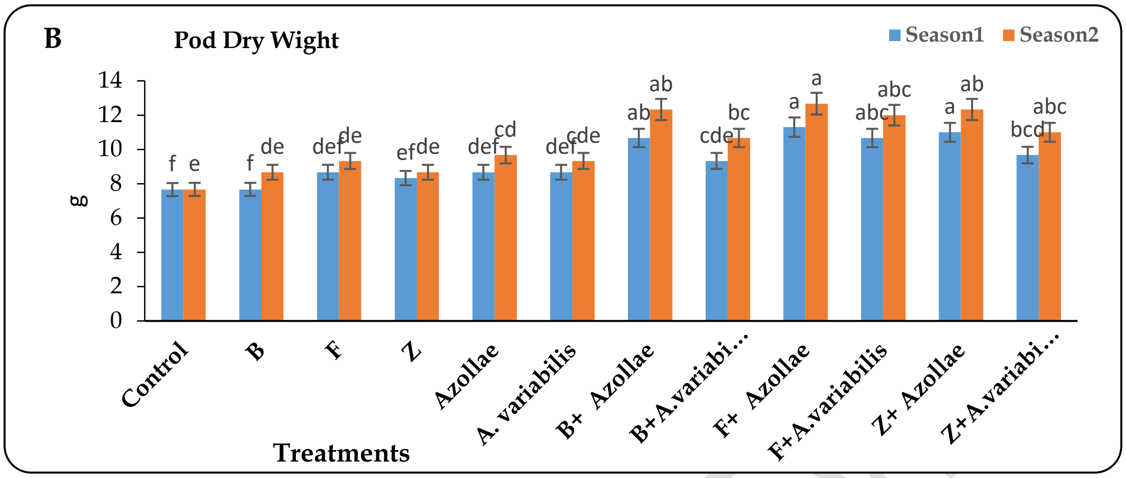


Fig (1) Effect of soil amendments and biofertilizers on (A) pod fresh and (B) dry weight of pea plant during both winter seasons.

Data in Table (4) showed the weight of fresh and dried 100 seeds, as well as the yield of pea plants, as a result of applying soil amendments and biofertilizers to pea plants within both winter seasons. The F+ *Azollae* and Z+ *Azollae* treatments had the highest fresh and dry weight (59.67 and 58.67 g, respectively), but their dry weight (14.67 and 14.33 g) was lower. Additionally, the same treatments had the highest total green yield in both seasons (1.73 and 1.97 (t fed^{-1}), and 1.70 and 1.97 (t fed^{-1}), respectively. In contrast, solo treatments yielded less 100 seeds fresh and dry, as well as total green yield, than combination treatments, but the control treatment yielded minimum data at all.

	100 fresh seeds weight (g)		100 dry seeds weight (g)		Total green pod yield (t fed^{-1})	
	Season1	Season 2	Season1	Season 2	Season1	Season 2
Control	45.33(e)	52.33(c)	10.67(e)	11.00(e)	1.30(d)	1.43(e)
B	47.33(de)	54.67(bc)	11.33(de)	12.33(d)	1.37(cd)	1.47(de)
F	49.33(bcd)	56.00(ab	12.00(cde)	13.33(c)	1.43(bcd)	1.633(cde)
Z	49.33(bcd)	55.66(abc)	12.00(de)	12.33(d)	1.40(cd)	1.53(cde)
Azollae	50.67(bcd)	57.00(abc)	12.67(bcd)	13.67(bc)	1.43(bcd)	1.67(bcd)
A. variabilis	50.30(cde)	56.33(abc)	12.33(bcd)	13.67(bc)	1.43(bcd)	1.67(bcd)
B+ Azollae	55.30(ab)	58.67(ab)	13.67(ab)	14.33(ab)	1.63(ab)	1.87(ab)
B+A. variabilis	52.34(bcd)	57.67(ab)	13.00(abc)	14.00(abc)	1.47(bcd)	1.70(bc)
F+ Azollae	58.00(a)	59.67(a)	14.00(a)	14.67(a)	1.73(a)	1.97(a)
F+A. variabilis	54.33(abc)	58.00(ab)	13.00(abc)	14.00(abc)	1.57(abc)	1.73(bc)
Z+ Azollae	56(ab	58.67(ab)	13.67(ab)	14.33(ab)	1.70(a)	1.97(a)
Z+A. variabilis	54.33(abc)	58.00(ab)	13.00(abc)	14(abc)	1.47(bvd)	1.70(bc)
L.S.D.(0.05)	7.155	4.26	1.64	0.93	0.23	0.20

Table (4). Effect of soil amendments and biofertilizers on pods yield of pea plant during both winter seasons.

Tables (5and 6) and figures (2and3) showed the contents of nitrogen (N), phosphorus (P), and potassium (K) in pea plant and grain and its uptake in plant and grains, in the winters of 2022/2023 and 2023/2024, respectively. A similar pattern was observed in NPK content and uptake in grains and plant , with the highest content of N, P, and K recorded by combining *Azollae* and *A. variabilis* with soil amendments and the increased in the second season rather than the first, whereas the highest percentages were F+ *Azollae* (5.14, 2.25, 0.79) in plant and (5.34, 2.21, 0.87) in grains, whereas their uptake were (5.74, 2.25,0.86 ton fed^{-1}) in plant and (3.03, 1.26, 0.49 ton fed^{-1})in grains respectively, with minimal significant differences between Z+ *Azollae* and (5.12 , 2.17, 0.75) in plant and (5.27, 2.18, 0.87) in grains , their uptake were (5.40, 2.30,0.80 ton fed^{-1}) in plant and (2.72, 1.24,0.49 ton fed^{-1}) in grains respectively and B+ *Azollae* (4.76, 2.10, 0.84) in grains and (5.04, 2.16, 0.66) in leaves with uptake (5.30,2.27,0.70 ton fed^{-1})in plants and (2.68,1.190.49 ton fed^{-1}) in grains respectively, in the second season. While the single treatments had lower N P K content and uptake than the mixed treatments, they were higher than the control treatment in both seasons.

Table (5). Effect of soil amendments and biofertizers on N, P, K contnt in pea plant during both winter seasons.

	N%		P%		K%	
	S1	S2	S1	S2	S1	S2
Control	2.39(f)	2.69(f)	0.60(g)	0.71(h)	0.33(f)	0.46(c)
B	2.55(ef)	3.14(e)	0.63(g)	0.81(gh)	0.34(f)	0.47(c)
F	2.86 (c)	3.78 (d)	0.74(ef)	0.92(f)	0.35(e)	0.49(c)
Z	2.76(de)	3.16(d)	0.65(fg)	0.83(fg)	0.41(e)	0.61(bc)
Azollae	3.16(c)	4.03 (d)	0.88(d)	1.45(d)	0.42(cd)	0.63(b)
A. variabilis	3.12(d)	4.02(d)	0.85(de)	1.09(e)	0.44(cd)	0.64(b)
B+ Azollae	3.84(a)	5.04 (ab)	1.10(bc)	2.16(a)	0.54(b)	0.66(b)
B+A. variabilis	3.26 (c)	4.37(c)	1.06 (c)	1.68(bc)	0.43(cde)	0.64(b)
F+ Azollae	3.87 (a)	5.14(a)	1.35(a)	2.25(a)	0.59(a)	0.79 (a)
F+A. variabilis	3.56 (b)	4.81(b)	1.09(c)	1.76(b)	0.45(c)	0.65(c)
Z+ Azollae	3.85(a)	5.12 (a)	1.22(b)	2.17(a)	0.55(b)	0.75(a)
Z+A. variabilis	3.51(b)	4.549(c)	1.05(c)	1.66(c)	0.53(b)	0.65(b)
L.S.D.(0.05)	0.22	0.25	0.11	0.094	0.03	0.04

Table (6). Effect of soil amendments and biofertizers on N, P, K content in grains of pea plant during both winter seasons.

	N%		P%		K%	
	Season1	Season 2	Season1	Season 2	Season1	Season 2
Control	2.43(f)	2.84(e)	0.59(e)	0.68(f)	0.3(d)	0.43(g)
B	2.55(ef)	3.24(de)	0.60(e)	0.85(e)	0.36(c)	0.46(fg)
F	2.80(cde)	3.46(d)	0.76(d)	0.95(e)	0.41(bc)	0.55(e)
Z	2.63(def)	3.47(d)	0.65(de)	0.86(e)	0.38 (c)	0.47(f)
Azollae	3.10 (bc)	4.65(bc)	0.90(c)	1.48(c)	0.44(b)	0.65(cd)
A. variabilis	2.87(bcd)	4.14 (c)	0.87(c)	1.17(d)	0.44 (c)	0.62(d)
B+ Azollae	3.63(a)	4.76(b)	1.18(ab)	2.10(a)	0.55(a)	0.84(b)
B+A. variabilis	3.14(b)	4.38(bc)	1.10(b)	1.69(b)	0.44(b)	0.65(c)
F+ Azollae	3.84 (a)	5.34(a)	1.23(a)	2.21(a)	0.56 (a)	0.87(a)
F+A. variabilis	3.55 (a)	4.69(b)	1.13(ab)	1.84(ab)	0.54(a)	0.81(b)
Z+ Azollae	3.78 (a)	5.27(a)	1.21(ab)	2.18(a)	0.55(a)	0.87(a)
Z+A. variabilis	3.53 (a)	4.36(bc)	1.13(ab)	1.67(b)	0.52(a)	0.66(c)
L.S.D.(0.05)	0.31	0.47	0.11	0.16	0.049	0.032

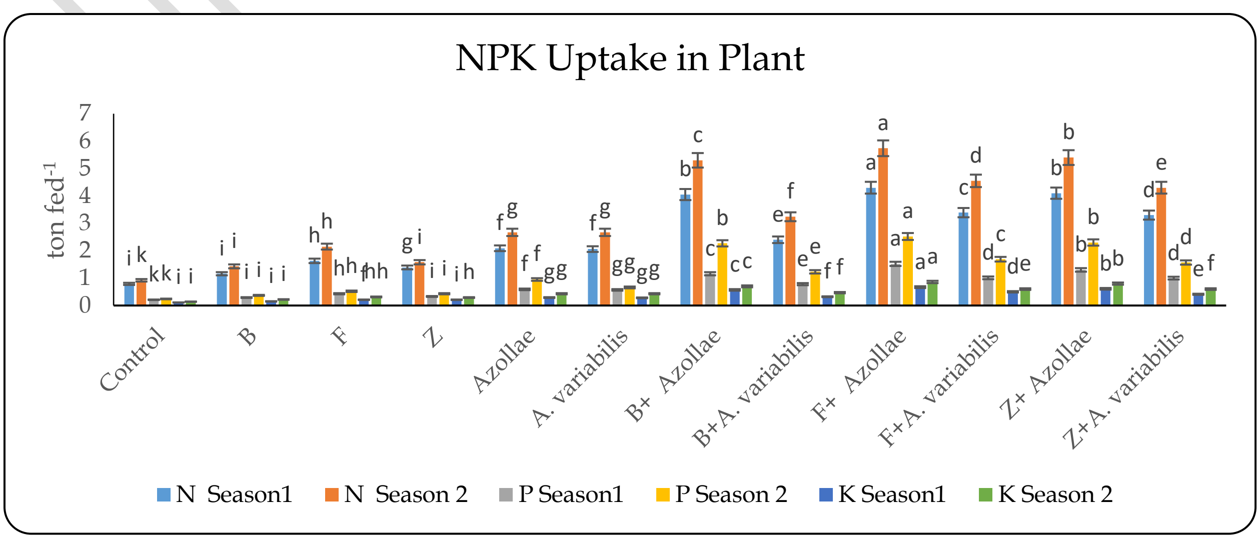


Fig. (2). Effect of soil amendments and biofertizers on N, P, K uptake in pea plant during both winter seasons.

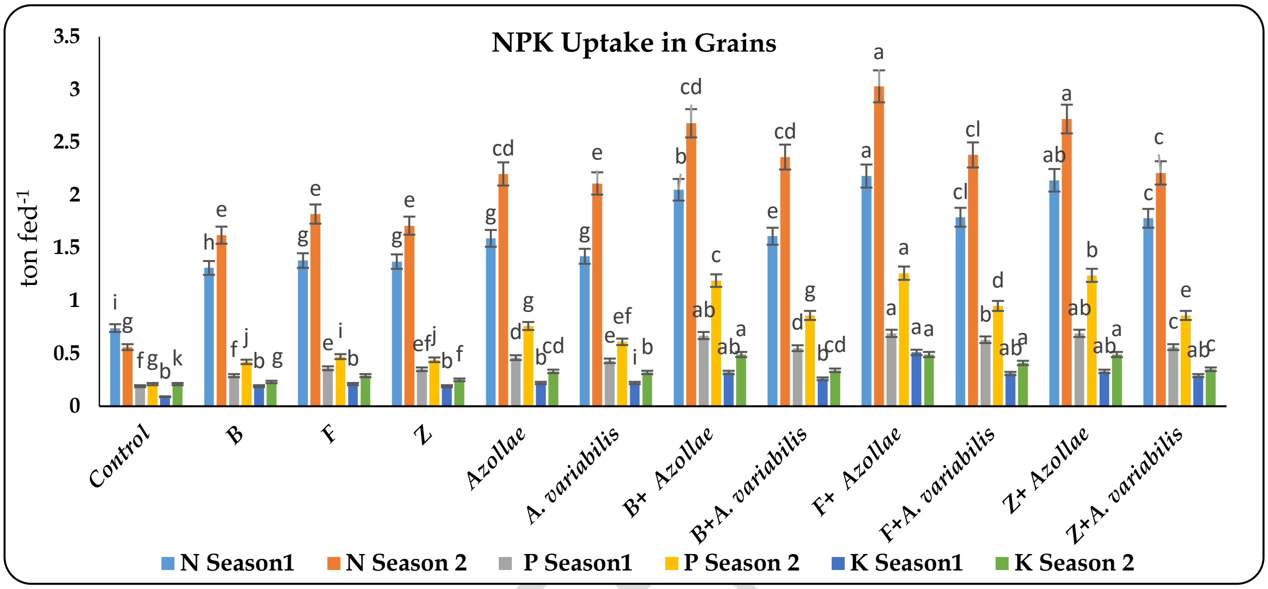


Fig (3). Effect of soil amendments and biofertizers on N, P, K uptake in grains of pea plant during both winter seasons.

Data in figures (4 and 5) demonstrated the impact of applying *Azollae* and *A. variabilis* as separate biofertilizers and in the presence of soil amendments on the percentages of carbohydrates and protein in grains of pea plants. The most significant carbohydrate and protein percentages (57.71, 33.37) and (57.38, 32.93) were reported by F+ *Azollae* and Z+ *Azollae* treatments in the second season. However, F+ *A. variabilis* (54.26% carbs and 28.52% protein) and Z+ *A. variabilis* (52.96% carbohydrates and 27.27% protein) recorded greater carbohydrates and protein percentages in grains in the second season compared to the first. In both seasons, the single application of *Azollae* and *A. variabili*, as well as soil amendments F, Z, and B, resulted in lower carbs and protein in pea plant grains, whereas the control treatment had the lowest carbohydrates and protein in pea plant grains.

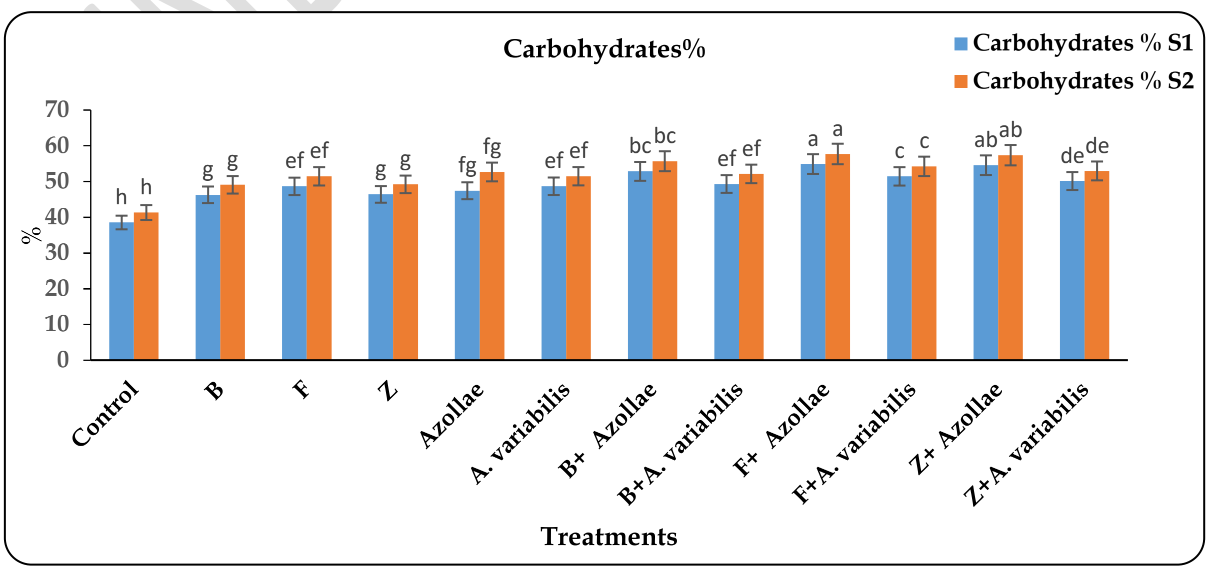


Fig. (4). Effect of soil amendments and biofertizers on carbohydrates percentage in grains of pea plant during both winter seasons.

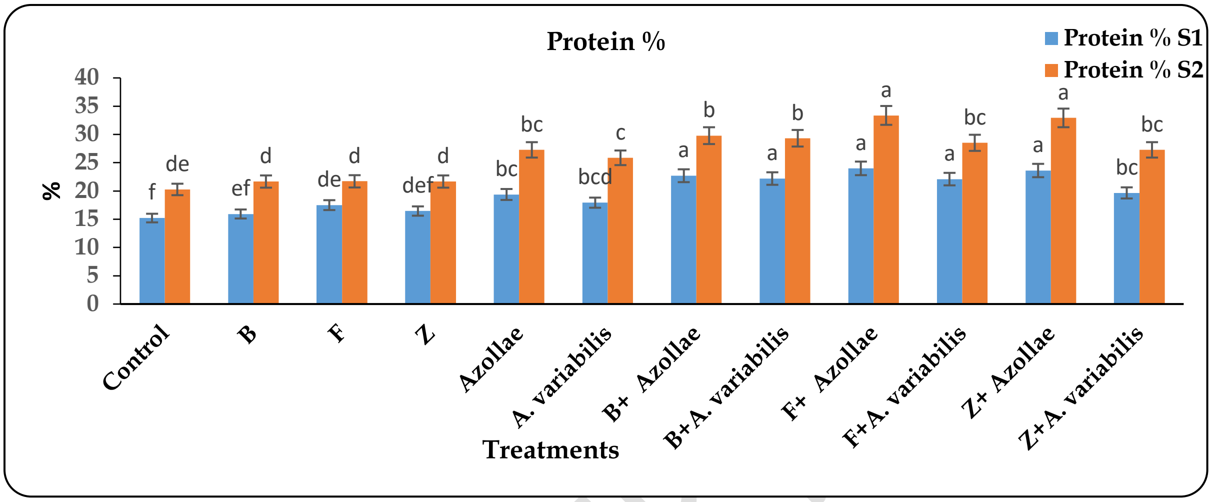


Fig. (5). Effect of biofertizers and soil amendments on protein percentage in grains of pea plant during both winter seasons.

Soil Analysis

The results of soil analysis followed the same pattern as plant analysis, with an increase in available NPK in the presence of biofertilizers *Azollaee* and *A. variabilis* as individual and in the combination of soil amendments than single soil conditioner treatments or control, however The second season produced better outcomes than the first season. Table (7) and figure (6) reported available NPK and dehydrogenase activity in the rhizosphere soil of pea plants throughout two winter seasons of 2022/2023 and 2023/2024. The highest available NPK contents were recorded by treatment of *F+ Azollae* (73.00, 17.56,74.99 mg kg^{-1}soil) respectively, followed by *Z+ Azollae* (72.67,16.86, 68.01 mg. kg^{-1}soil) respectively, in the second season. Soil microbial activity is evaluated using dehydrogenase enzyme activity (DHA), as an indicator of energy transfer. The maximum DHA activity was recorded in the presence of *Azollae* and *A. variabilis* mixed with soil amendments, as earlier studies revealed that the presence of biofertilizers enhanced the number of microorganisms in soil, hence improving energy transfer activity. The treatment of *F+ Azollae* ereported the greatest DHA activity (1.83 mg TPF g^{-1} dry rhizosphere soil^{-1} day^{-1}), with non-significant difference between *Z+ Azollae* and B+ *Azollae* (1.82 mg TPF g^{-1} dry rhizosphere soil^{-1} day^{-1}), then F+*A. variabilis* (1.81 mg TPF g^{-1} dry rhizosphere soil^{-1} day^{-1}) in the second season, however, the lowest DHA activity was recorded by control treatment in both seasons.

	N mg. kg^{-1}soil		P mg. kg^{-1}soil		K mg. kg^{-1}soil	
	Season1	Season 2	Season1	Season 2	Season1	Season 2
Control	42.00(f)	43.00(d)	7.54(f)	10.34(f)	34.20(h)	36.34(i)
B	43.33(f)	52.60(c)	8.83(e)	11.63(e)	40.05(g)	43.63(h)
F	51.33(e)	61.67(b)	9.09(e)	11.89(de)	46.19(f)	49.37(f)
Z	54.33(d)	58.67(cd)	8.84(e)	11.64(e)	45.31(f)	48.95(g)

Azollae	59.66(b)	63.33(b)	10.27(d)	13.08(d)	57.46(e)	61.69(de)
A. variabilis	58.33(c)	61.77(b)	9.29(de)	12.09(de)	55.78(e)	59.05(e)
B+ Azollae	64.33(a)	72.00(a)	13.6(b)	16.4(b)	75.69(a)	78.68(a)
B+A. variabilis	62.00(a)	69.67(b)	11.67(c)	14.4(c)	64.59(c)	67.7(c)
F+ Azollae	64.66(a)	73.00(a)	14.76(a)	17.56(a)	70.98(b)	74.93(b)
F+A. variabilis	64.33(a)	71.00(ab)	13.47(b)	16.27(b)	63.36(c)	66.45(cd)
Z+ Azollae	64.33(a)	72.67(a)	14.06(ab)	16.86(ab)	64.84(c)	68.01(c)
Z+A. variabilis	63.33(a)	71.00(a)	13.41(b)	16.21(b)	61.47(d)	64.13(de)
L.S.D.(0.05)	3.32	4.59	1.14	1.15	2.72	3.33

Table (7). Effect of soil amendments and biofertizers on NPK content in the rhizosphere soil of pea plant during both winter seasons .

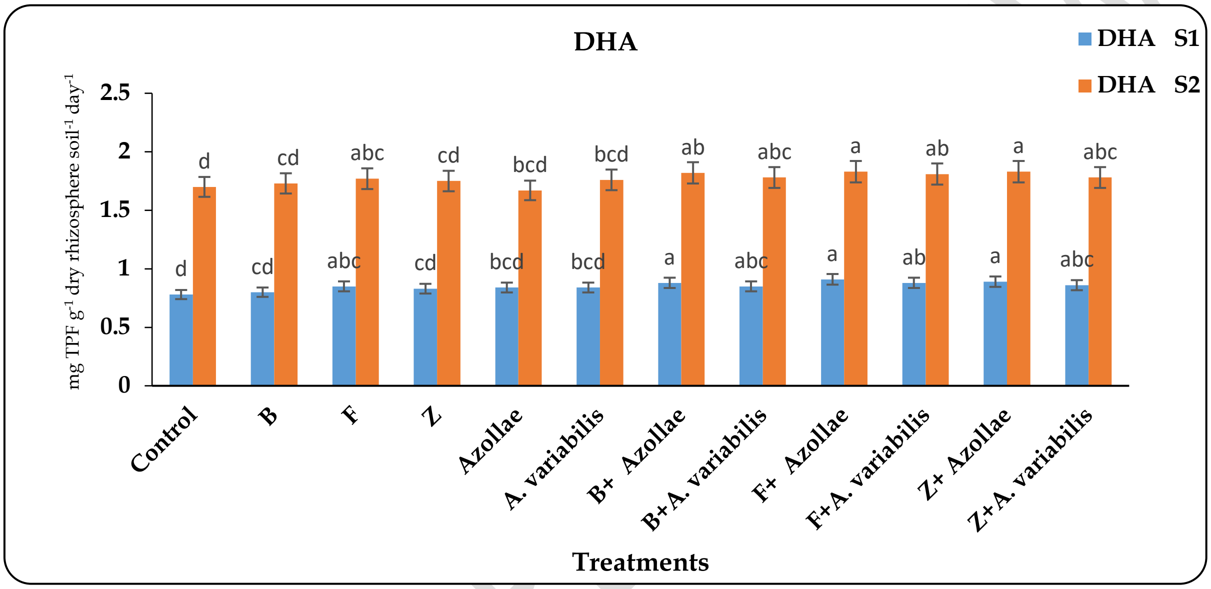


Fig (6). Effect of soil amendments and biofertizers on dehydrogenase activity in the rhizosphere soil of pea plant during both winter seasons.

4.DISCUSSION

Azollae has potential as a biofertilizer for dry land vegetable crops, it is important to do an economic feasibility analysis before promoting its usage on farms (Thapa and Poudel,2023). *Azollae* promotes nitrogen fixation and nutritional enrichment in rice paddy fields. They break down lignin in the cell wall and release phenolic chemicals (Akhtar et al., 2021). In algal biofertilizer blue green algae (BGA) and some green algae are used as biofertilizer. Cyanobacteria are emerging microorganisms for sustainable agricultural development. They can solve nitrogen deficiency in plants, promote soil aeration, increase water retention, and provide vitamin B_{12} (57-60). *Nostoc linkia*, *Anabaena variabilis*, *Aulosira fertilisima*, *Calothrix* sp., *Tolypothrix* sp., and *Scytonema* sp. are the most effective nitrogen-fixing cyanobacteria found in rice growing areas. *Anabaena* and *Nostoc* thrive on dirt and rocks, capturing up to 20-25 kg of atmospheric nitrogen per hectare. *Anabaena* can fix 60 kg/ha/season of nitrogen and enhance soils with organic matter (Morsi et al.,2023). Natural soil amendments significantly increased accessible NPK levels in the soil. Using soil amendments such as zeolite, bentonite, and feldspar can enhance soil structure and nutrient content by minimizing leaching (El-Edfawy and Fanous,2022 and Eprikashvili et al., 2016). In this study the growth parameters were increased in the presence of *Azollae* and cyanobacteria associated with soil amendments (Tables 2, 3,4, and Fig.1), our results were matched with Kadhim and Alwan (2024), who demonstrated that, plant height and pods number rose considerably more with biofertilizers, this may be because N-fixers secrete specific organic acids and biochemical compounds that naturally promote plant growth (Mishra et al., 2014). These results were consistent with those of Ghazal et al. (2022) and Salem et al. (2023), who reported that the presence of soil amendments and cyanobacteria improves grains count and plant growth characteristics. Sarhan et al., (2016), reported that the application of feldspar improved the chemical contents of the NPK-studied seed in cotton growth and development, as well as K%, chlorophyll A, and/or B. This was achieved solely by making rock feldspar more soluble, which allowed plants to absorb more K. These findings are consistent with those of Merwad et al. (2016). The results of the study demonstrated that biofertilizers improve soil fertility, plant growth parameters, and yield by increasing NPK content and uptake in plants and grains (Tables 5&6 and Figs 2&3). In terms of how the presence of *Azollae* and *A. variabilis* cyanobacteria inoculation and soil amendments affect pea plant NPK uptake in grain plants, these findings were consistent with Salem et al. (2023) and, Zhang et al. (2020) who found that wheat plants treated with natural soil amendments (zeolite, bentonite, and feldspar) combined with cyanobacteria showed high trends in NPK uptake. According to Silva Filho et al. (2023), presence of NPK increased the production of total chlorophyll in plants significantly, NPK also had a significant effect on pea's photosynthetic pigments and antioxidant activity. Sarfraz et al. (2021) revealed that increasing NPK through roots, increased the total carbohydrates content and total soluble protein level in pea plant. The plant treated with biofertilizers may become physiologically more active, enabling it to manufacture the necessary amount of hormones or to accumulate sufficient food reserves, which could account for the plant's early maturity (Chakraborty and Akhtar, 2021). Usage of biofertilizers *Azollae* and *A. variabilis*, in combination with soil amendments, increased the percentages of carbs and protein in pea plants through both seasons (Figs 3& 4), the main aim of enhancing grain legume yield is to increase protein content using suitable agronomic modifications. In pulses, protein content is referred to as the economic yield. A complicated set of physiological and biochemical processes culminate in grain protein yield. This could be explained by the effective transfer of nitrogen from the vegetative portions to the growing seeds and the production of protein in the seeds from the reduced nitrogen molecules (Kumar et al. 2016). Carbohydrates and protein contents in grains increased in the presence of biofertilizers and soil amendments because grain protein concentration positively correlated with N-uptake and photosynthetic pigment, cyanobacterial inoculation improves growth, nitrogen fixation and yields in rice and wheat crops (Hamed et al., 2022). This could be attributed to improved root formation and increased nutrient uptake during crop growth (Reddy et al. 2014). As a result, greater plant development leads to higher translocation and accumulation of photosynthates in the pods, as shown Kumar et al. (2016) and Yogananda, Thimmegowda, and Shruthi (2020) conducted investigations on pea.

This study results revealed that the presence of biofertilizers *Azollae* and *A. variabilis* conjugated with soil amendments boosted NPK content in soil and dehydrogenase enzyme activity by increasing the number of microorganism groups in the rhizosphere soil (Table 7and Fig.6). Using of various integrated combinations over two consecutive years resulted in a considerable improvement in soil nutrient status due to the release of nutrients from unavailable to available forms (Sharma et al., 2023). Hu et al. (2021) in order to help dissolve K-bearing minerals and chelate silicon ions to add K to the soil's solution, a range of soil microbes can release organic acids that dissolve minerals demonstrated that tobacco dry weight and the uptake of both K and nitrogen (N) by tobacco seedlings increased significantly after the addition of combined biofertilizers and feldspar powder. Earlier investigations have demonstrated that biofertilizer inoculation can increase the concentration of accessible K in the soil and K uptake by crops such as maize (Abou-El-Seoud and AbdelMegeed, 2012), tobacco (Zhang and Kong, 2014), and tea (Pramanik et al., 2019). Previous research has shown that zeolites can improve soil characteristics, water and nutrient retention, crop productivity, and reduce heavy metal toxicity. Natural zeolites are effective soil improvers due to their high water and nutrient holding capacity (WHC), improved infiltration rate, saturated hydraulic conductivity, cation exchange capacity, and prevention of water loss from deep percolation (Mondal etal., 2021). Zeolites can serve as a fertilizer and chelating agent. Zeolites reduce nutrient release from organic and inorganic fertilisers, improving crop growth, pest management, pollution control, and productivity (Chen et al., 2017). Soil microbial activity improved in the presence of soil amendments because they were suppliers of minerals. Vašák et al. (2015) discovered that using mineral fertilizers in a balanced manner improves soil enzyme activity. The long-term use of high rates of mineral fertilizers dramatically reduces the soil pH and consequently influences the decline in microbial biomass and enzyme activity (Woli ´nska and epniewska, 2012). Soil microorganisms are crucial for energy and nutrient cycling, as well as regulating organic matter transformation (Sharma et al., 2023). Datta et al. (2020) found that co-application of bentonite and biofertilizers improved soil microbial characteristics, resulting in increased microbial biomass that benefits agriculture and the environment. According to Mi et al. (2017), adding bentonite to soil hydrodynamic parameters and millet crop performance in a semiarid environment dramatically boosted above-ground biomass and grain yield while improving water usage efficiency. The evaluation of soil dehydrogenase activity (DHA) revealed microbial activity as an indication of soil quality as well as for detecting the many elements that influence soil microbiological quality, such as the application of contaminants such as pesticides or excessive fertiliser use. Furthermore, in such context, the determination of dehydrogenase activity DHA in soil samples could potentially give us useful and large amount of information about biological characteristics of the soil because it is influenced by various other factors present in the soil environment, such as moisture, availability of oxygen, reduction-oxidation potential, pH, content of organic matter, the soil profile depth temperature, season of the year, and heavy metal contamination (Abd-elgwad, 2019).The comparatively high nutrient concentration and microbial population, which assisted in mobilising the unavailable source of nutrients in soil and thereby initiating the creation of the optimal nutrient supply across critical crop stages in addition to supplying other essential macro and micro-nutrients, were the reasons for the higher number of pods plant^{-1} in response to the integrated combination of nutrients (Meena et al., 2017).

5.CONCLUSION

In the present study, on integrated application of biofertilizers and soil amendments demonstrated substantial differences for all the growth, yield, yield attributing features, as well as biochemical attributes in pea plant. Among all treatments, *Azolla* combined with feldspar produced the most significant results, followed by *A. variabils* with feldspar; thus, the recommendation is to use an integrated combination of both biofertilizers with feldspar soil amendments to improve pea plant characteristics, which can be suggested as a cost-effective combination for achieving higher yield with greater quality on a sustainable basis.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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