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Mitigation of Carbonate-Induced Stress on Biochemical Properties of Soil and *Vicia faba* Growth Using Bio- and Organic Fertilizers

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THIS STUDY was carried out during the winter season of 2023/2024 as a pot experiment in a greenhouse to investigate the harmful effect of artificial calcium carbonate stress (S0: 1.90%, S1: 4.35%, S2: 6.81% and S3: 11.71%) as well as the efficiency of compost (C0: 0.00, C1: 0.75 and C2: 1.50%) and biofertilizers (B1: ARC-SWERI-501 *Rhizobium leguminosarum* sv. *viciae* and B2: ICARDA-441 *Rhizobium leguminosarum* sv. *viciae*) on soil chemical properties, faba bean (*Vicia faba* L. cv. 843) growth, nutrient uptake, biological activity and nodulation.The presence of increasing $CaCO_3$ concentrations had significant negative effects on all studied traits. Raising the level of compost additive decreased soil pH and significantly increased EC**,** OM and the availability of N, P and K compared to the control (C0) and the C2 treatment recorded the highest values (2.025 dS m^{-1}, 0.684%, 30.11 mg kg^{-1}, 4.781 mg kg^{-1} and 187.14 mg kg^{-1},respectively). Biofertilizers, especially B1, significantly enhanced soil biochemical properties and nutrient .The application of compost and biofertilizers together appeared a significant improvement in higher enzymatic activities (dehydrogenase, phosphatase and nitrogenase), nodules number, shoot dry weight, and macronutrient uptake. This highlights the value of integrated soil fertility management practices through organic and microbial amendments to reduce carbonate-induced stress to increase faba bean performance in the studied soils.

Keywords: Compost; Biofertilizer; Rhizobium; Calcium carbonate; Enzymatic activity; Nutrient uptake.

Calcareous soils occupy 30% of the earth's surface and are mostly found in arid or semi-arid regions where leaching is minimal (Wahba et al. 2019). Calcareous soils are characterized by having large amounts of free lime, and their amount of $CaCO_3$ can range from negligible levels to 95% (Taalab, et al. 2019). The distribution of calcareous soils in Egypt is about 0.65 m feddan (Hassan, 2012) and this area represented approximately 25-30% of the total (Abou Hussien et al., 2021). The high levels of $CaCO_3$ in calcareous soils, which can reach more than 15% (Abou Hussien et al., 2023; Nada et al., 2023) results in many constraints, such as fast water infiltration into soil, weak soil structure, poor water holding capacity of soil, low content of clay (Aboukila et al., 2018; Krouma 2023) and organic matter content in soils and also low cation exchange capacity (El-Hady and Abo-Sedera, 2006).The pH which, rises within the range of 7.6–8.4 (Abou Hussien et al., 2020) and $CaCO_3$ content are the most critical chemical problems in calcareous soils (Alghamdi et al., 2023). These parameters have a profound effect on nutrient availability such as phosphorus (P), nitrogen (N) and micronutrients (Alghamdi et al., 2023; Bolna et al., 2023), in addition to decreasing soil microbial activity and soil fertility, which in turn limits plant growth and production (Wedepohl, 1995).Tackling these problems needs the integrated soil management practices including application of organic compost and biofertilizers. Biofertilizers and composted organic amendments have to be practiced to minimize the adverse effects of chemical fertilizers. For example, rhizobial inoculants could be a low-cost and environmentally friendly alternative, providing soil fertility resulting from biological nitrogen fixation. In particular, biofertilizers were demonstrated to reduce the volume of chemical inputs and mitigate harmful effects on the environment by improving soil health and

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microbial activity, and thereby making crop production systems more sustainable (Alghamdi, 2007; Farag and Afiah, 2012 and Daylam et al., 2023). In addition to biofertilizers, organic materials like compost were improved soil structure, water holding capacity, and nutrient availability (particularly in nutrient poor soils such as sandy and calcareous soils). Compost amendments also foster and stimulate microbial diversity while augmenting the rhizosphere environment, which is conducive for nutrient cycling and nitrogen fixation. In short, these processes are essential in enhancing plant growth and resilience particularly under the strenuous conditions of arid and semi-arid zones (Ouedraogo et al., 2017; Dzokom, 2023 and Dzokom et al. 2024). Additionally, consideration must be given to the role of compost on soil enzymes, such as nitrogenase, phosphatase, and dehydrogenase, which promotes the turnover of organic matter, the nutrient cycling process and improves soil ecology (Kieloaho et al. 2016).

Legumes are important crops for human sustenance and are a major source of protein, mainly in many developing nations. Faba bean (Vicia faba L.) is arguably one of the most usable food crops in Egypt, all segments of the Egyptian population depend on faba beans as a part of their diet. It is a valuable source of protein, with a 28% protein content, and high content in essential minerals, such as manganese (Mn), iron (Fe) and phosphorus (P). Faba beans are grown worldwide on approximately 2.94 million ha, and in 2020, Egypt achieved an output of nearly 124590 t obtained from 37439 ha (Al-Suwaina et al. 2022 and Dhull et al. 2022). Its Cultivation of in calcareous sandy soils presents considerable agricultural difficulties due to nutrient immobilization (Sediqui et al. 2024) and low levels of microbial activity (Lehto, 1994). As such, determining how compost and biofertilizers can interact with calcium carbonate concentrations to influence the properties of some soils, and consequently, the enzymatic activities and growth of plants, could improve practices of soil management.

So the aim of this study was carried out to evaluate the combined and individual effect of biofertilizer inoculation with Rhizobium strains of faba bean (*Vicia faba* L.) plants which namely B1: ARC-SWERI-501 *Rhizobium leguminosarium sembiovar vicia* and B2: ICARDA-441 *Rhizobioum leguminosarium sembiovar vicia*) and compost application on properties of sandy soil that cultivated faba bean under carbonate stress, plant growth and nutrients (N, P and K) uptake and enzyme activity , contributing to enhancement of sustainable soil management systems.

2. Materials and Methods

2.1. Soil Location and soil sampling

Surface (0-30 cm) soil samples were collected randomly from different locations of Kafour El-Raml, Qewaisna, Menofia Governorate, Egypt (30° 32' 26.0" N, 31° 11' 18.7" E).The collected samples air-dried, ground, good mixed and sieved using a 2 mm sieve. A small portion of the fine soil (2 mm) was taken and analyzed for some physical and chemical properties and the content of available nutrients using scientific procedures described by **Cottenie et al. (1982), Page et al. (1982) and Klute (1986).** The obtained data are recorded in Table (1).

2.2. Prepration of applicated fertilizers

The used fertilizers mentioned in Fig (1) were prepared as follow:

2.2.1. Compost

The compost prepared from some organic residues (maize stalk"MS" and animal farmyard manure "FYM") as described by Abou Hussien et al (2016), The used compost was analyzed for its main properties using the methods described by Page et al (1982) The found data are shown in Table (2).

Table (1): Physical and chemical properties of the studied soil.

a-Soil physical properties				
Particle size distribution			Texture class	Field capacity (%)
Sand	Silt	Clay		
88.59%	4.8%	6.61%	Sandy	19.68

b-Soil chemical properties			
pH in 1:2.5 (soil:water) Susp.	EC Soil paste extract (dS m^{-1})	OM, %	$CaCO_3$ (%)
7.6	1.45	0.45	1.9

Soluble ions (mmol L^{-1})							
Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}
3.6	3.2	2.9	4.8	0	4.3	3.8	6.4

Soil content of available nutrients (mg kg^{-1})					
N	P	K	Fe	Mn	Zn
20.7	3.1	155.46	3.8	3.1	0.84

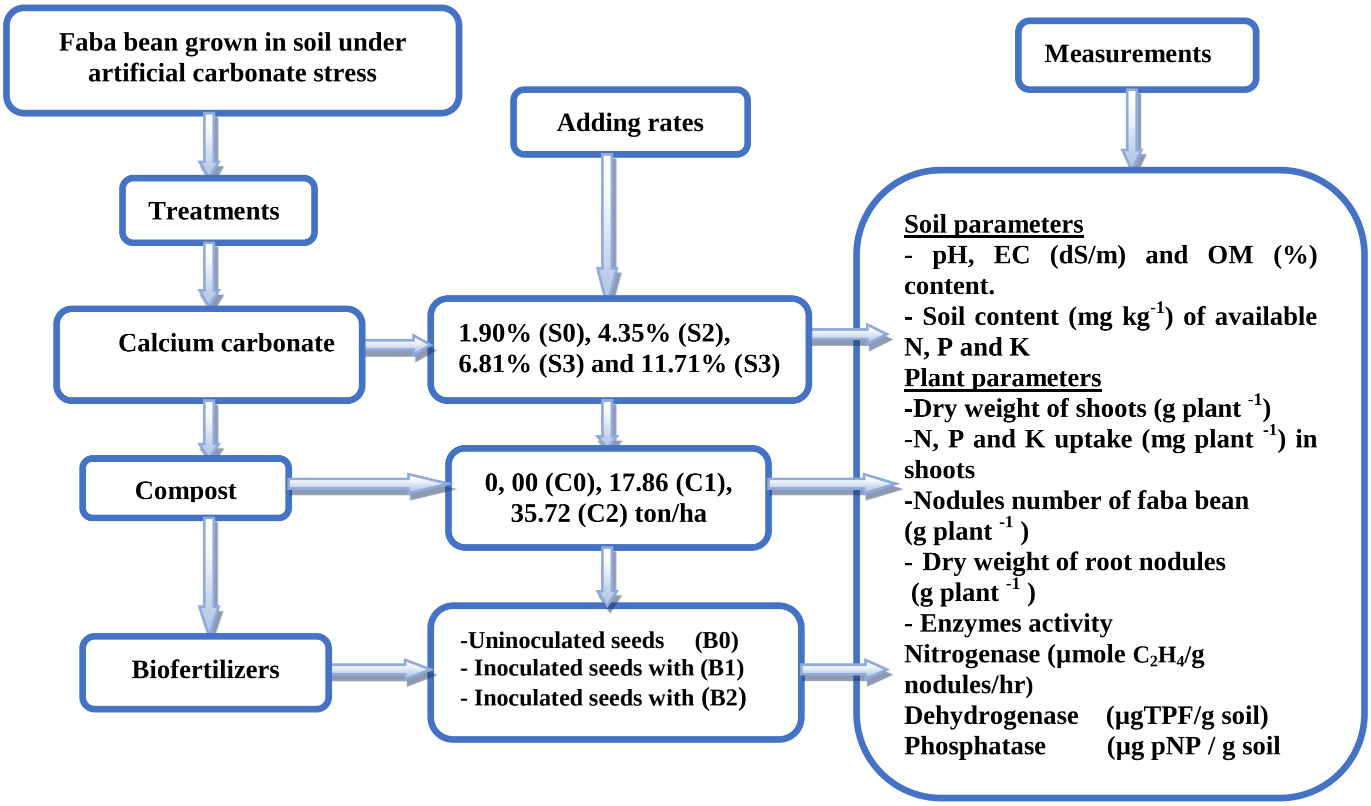

Fig. 1. Used treatments: calcium carbonate (S), compost (C) and biofertilizers i.e. B1 (ARC-SWERI-501, *Rhizobium leguminosarium sembiovar vicia*) and B2 (ICARDA-441 *Rhizobium leguminosarium sembiovar vicia*) and the measured parameters in this study.

Table (2): Chemical properties and nutrients content in the experimental Compost.

pH (1:5) compost: water	EC (1:10) compost. : water extract dSm^{-1}	Organic matter (%)	Organic carbon (%)	Total N (%)	C/N (%)
7.51	1.86	45.71	26.51	1.23	21.55

Available content of nutrients (mg kg^{-1})					
N	P	K	Fe	Mn	Zn
895	715	2010	410	499	55.5

The pile of organic residues was designed with an area of 2 m^2 and a height of 1 m from mixture of the prepared maize stalks and FYM (as organic activators) at ratio of 1.86:1 respectively by weight (Abou Hussen et al., 2016). The organic waste inside the pile was arranged in 5 layers of equal height to create good conditions. A activator mixture containing 65 g of $Ca(H_2PO_4)_2$, 200 of $CaCO_3$, 6.4 g of urea (46% nitrogen) and 2000 ml of fresh fertile soil and water suspension (1:5 ratio) was added on each layer. Then, the pile content was properly moistened to reach about 50 – 60 % of its water holding capacity. To enhance the aerobic decomposition process, the piles were turned upside down every 15 days. Additional water was sprayed during the turning process to keep moisture content of the pile at almost 60 % of water holding capacity. The composting process continued until C/N ratio of the composted materials reached up to 21.55/1 (about 3 months). Representative sample of Compost was taken air dried, ground and analyzed for chemical properties as well as the content of some macro- and micronutrients as described by Page et al.(1982).The obtained data are listed in Table (2).

2.2.2. Prepared biofertilizers

The prepared and characterized fine soil (Table, 1) Was treated by $CaCO_3$ 99% (<100 µm, Egyptian Jordanian Co. for Calcium Carbonate) with homogenous mixing at different rates i.e. 0.0, 2.5, 5 and 10% to become the final percentage of total carbonate (original + added) in form of calcium carbonate as follows: 1.90% (S0), 4.35% (S1), 6.81% (S2) and 11.71% (S3) respectively. 108 pots (30 cm in diameter and 40 cm in depth) were brought and 27 pots were allocated for each calcium carbonate treatment. Each pot was filled with 5 kg of prepared soil so we had 4 main groups i.e. S0, S1, S2 and S3 (27 pots/main group). Then all pots were fertilized by 240 kg of ordinary super phosphate (15.3% P_2O_5) ha^{-1} as P fertilizer (0.5 g super phosphate pot^{-1}) and good mixed with the potted soil. The pots of each main group were divided into three sub groups (9 pots / subgroup) representing the application rates of compost treatment .i.e. 0.00 (C0), 0.75(C1) and 1.50 %(C2) which good mixed with the potted soil. Then the pots of each sub group were divided into three sub sub groups (three pots / sub sub group) according to inoculate with biofertilizers .i.e., without B0, with B1, and with B2 Consequently, every sub-sub group included 3 replicates leading to a total number of 108 experimental units. Before sowing directly, the inoculation process was carried out by covering the seeds with a layer of 16% gum arabic solution as a sticking agent. The used bacterial inoculation is homogeneously mixed with the gum-coated seeds at a rate 0.6 kg inoculums/100kg seeds. To implement the experimental units for the sub sub groups, the following was done: the seeds of faba been were divided into three equal weights, where the first uninoculated (B0) while the second and the third were inoculated with B1 and B2 respectively as mentioned before that. Therefore, the studied treatments were arrangement within the experimental units in split split plot design with three replicates in three ways. Then each pot was planted by four faba bean seeds.

The moisture content was kept at soil field capacity (19.68%). Irrigation process was repeated every five days. After 15 days of planting, the plants of each pot were thinned to two plants. At 20 days after planting, the basic fertilization regime carried out according to standard practices set by the Ministry of Agriculture and Soil Reclamation (MASR). All pots were fertilized by ammonium nitrate (33 % N) and potassium sulphate (48 % K_2O) at rates of 60 and 120 kgha-1 (0.125 and 0.250 g pot-1) respectively. As well as other equal dose of both N and K fertilizers was added at 40 old days. All applications of N and K fertilizers were added with irrigation water.

2.4. Soil and Plant analyses

At 60 old days, Plants of each treatment were taken whole and then gently washed with tap water to remove soil particles. The root nodules of each plant were separated to determine nitrogenase activity in fresh nodul according to Hardy et al (1973). The detached root nodules and shoots of each plant were air dried and oven-dried at 70^{o}C for 48 hrs to estimate the dry weights. The dried shoots of plants for each pot were ground, kept and taken for determine essential nutrients contents (N, P and K). 0.5 g of dried shoots for each pot were digested with a mixture of $HClO_4$ and H_2SO_4 (at 1: 3, v/v, respectively) as described by Chapman and Prat (1961) to determine the concentrations of N, P and K , The blue color method of Jackson (1973) was used to analyses the samples for P spectrophotometrically (JENWAY 6405 UV/VIS). As shown by Jackson (1973), the K^+ was measured via flame photometry (Gallenkamp Co., London, UK.). Kjeldahl digestion (Ningbo Medical Instruments Co., Ningbo, China) was used to determine the N content using the techniques outlined in Page et al. (1982). After harvest, soil sample was taken from each pot and analyzed for some chemical properties and the content of available essential nutrients (N, P and K) mg/kg using the methods described by **Cottenie et al. (1982)** and Page et al. (1982). Soil samples were collected from each pot to measure their contents of organic matter according to Walkley and Black method as outlined in Sparks et al. (2020), soil pH by pH meter (model Consory C3210) in soil : water suspension prepared at 1:2.5 and EC using EC meter in soil paste extract (model WTW inolab Cond 720) as mention by Page et al. (1982).

At the same harvest day, soil sample from root zone of each pot was taken separately and kept in the frigid, where it is used to assay calorimetrically dehydrogenase and phosphatase enzymes activity according to Page et al. (1982) and Tabatabai (1982), respectively.

2.5. Statistical analyses

The split-split plot ANOVA (Snedecor and Cochran, 1989) with three replicates was used to evaluate impact of studied treatments on soil chemical properties, faba bean (*Vicia faba* L. cv. 843) growth, nutrient uptake, biological activity and nodulation. The Duncan's Multiple Range test (Duncan, 1955) was employed to compare the differences among treatment means to identify which are significantly different. Significant difference was determined at 5% level of probability.

3. Results

3.1. Soil chemical properties.

The interaction between carbonate amounts in soil, organic fertilizer rates, and types of biofertilizers and their individual effects on pH, EC, OM and soil content of available macronutrients are displayed in Tables (3) and Fig.(2, 3 and 4).

Soil pH

Figure (2) shows that Increasing in carbonate rates from 1.90% (S0) to 11.71% (S3), caused rising in soil pH for all treatments. However, compost and biofertilizers counter-balance this effect. The lowest pH values were recorded for the highest rate of compost (C2, 1.5%) which was inoculated with biofertilizer strain B2, on contrary soil pH at treatment of S3 C0 B0 recorded the highest value. These values suggest that organic and biofertilizer treatments provide a buffering effect on high carbonate soil alkalinity.

Electrical conductivity (EC)

Figure (3) and Table (3) illustrate that soil electrical conductivity significantly increased with increasing the concentration of carbonate and compost application rates, and non-significantly with biofertilizer inoculation. The highest EC value (2.23 dS m^{-1}) was recorded at the highest carbonate level (S3), highest compost rate (C2), and with B2 inoculation. The individual effect of carbonate content showed a significant increase in EC from 1.859 dS m^{-1} (S0) to 2.100 dS m^{-1} (S3). According to compost addition increased EC significantly from 1.903 dS m^{-1} (C0) to 2.025 dS m^{-1} (C2). Although the main effect of biofertilizer on EC was not statistically significant ($p > 0.05$), the trend showed slightly higher EC with B2 treatment (1.99 dS m^{-1}) compared to B0 (1.93 dS m^{-1}).

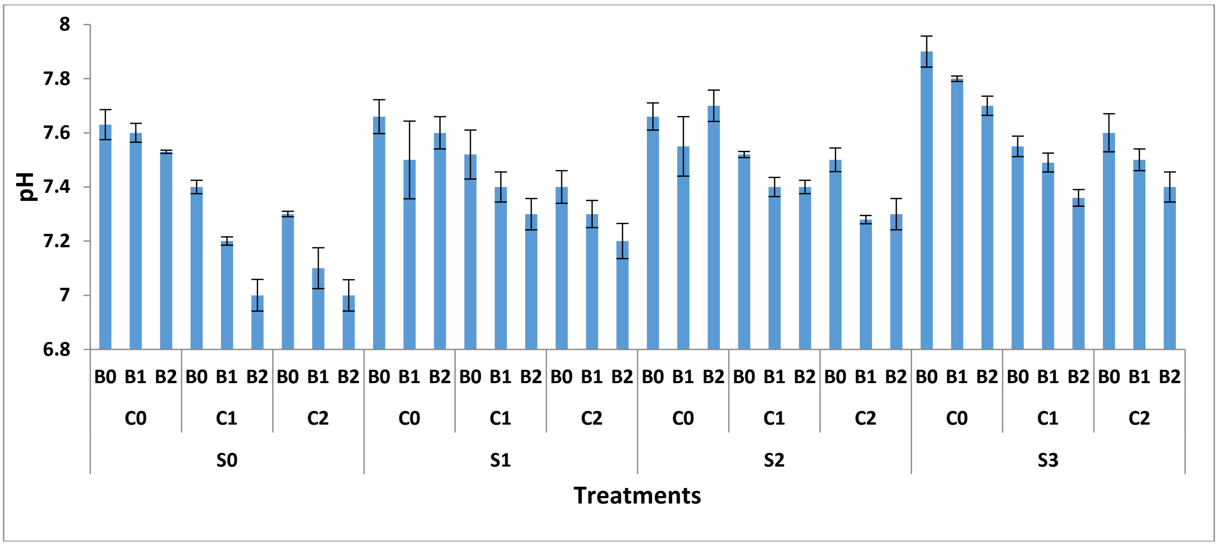

Fig. (2). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on values of soil pH.

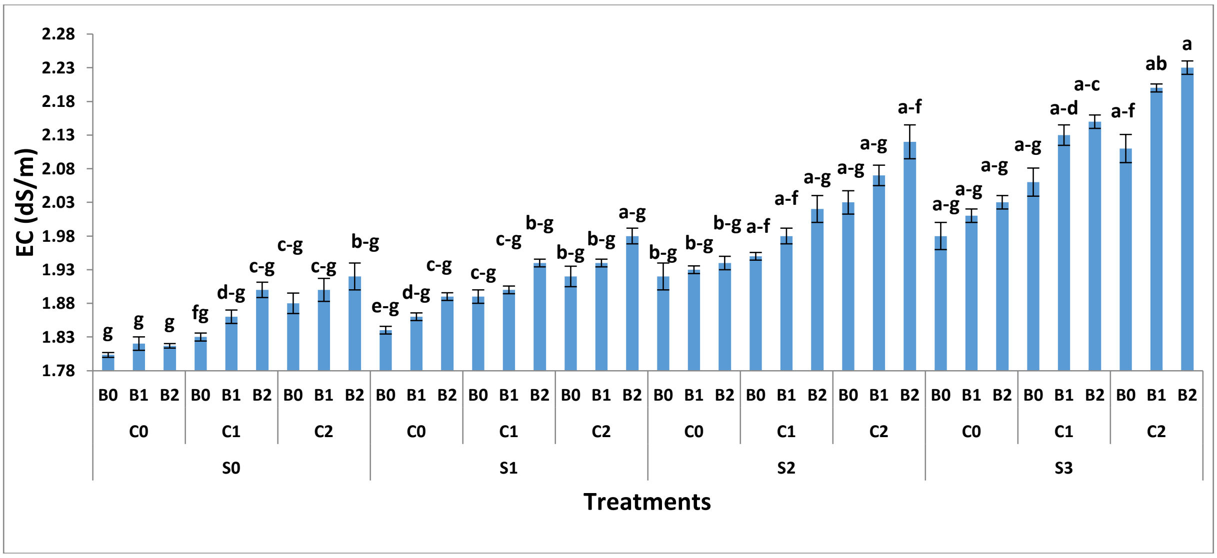

Fig. (3). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on values of soil EC (dSm^{-1}).

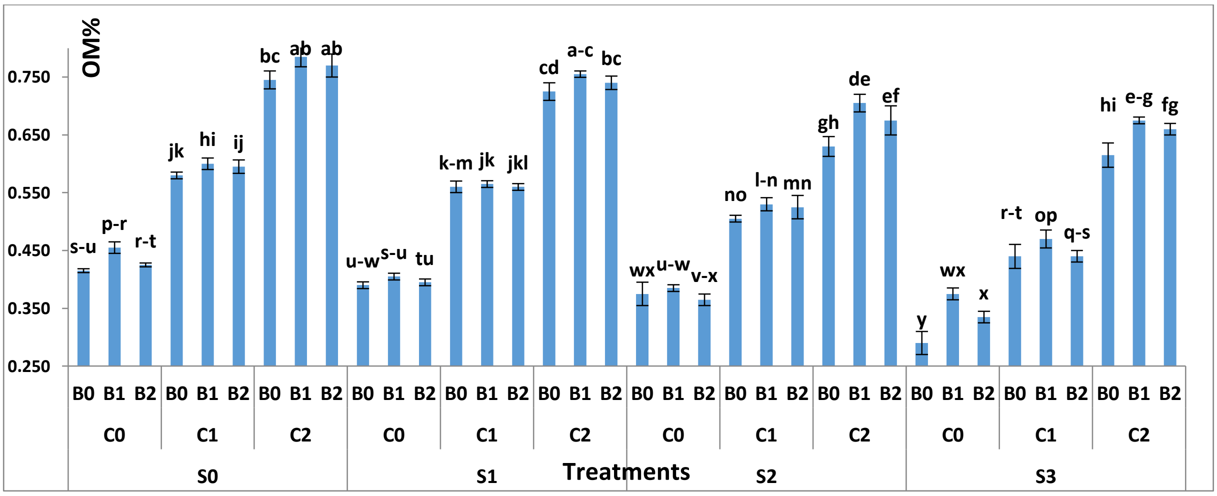

Fig. (4). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on values of soil OM (%).

Table (3). Individual effect of treatments (total carbonate levels, compost rates and biofertilizers types) on soil EC and OM

	Treatments	EC ($dS\ m^{-1}$)	OM (%)
S	S0	1.859 c	0.564 a
	S1	1.907 bc	0.567 a
	S2	1.996 b	0.520 b
	S3	2.100 a	0.478 c
	LSD at 0.05	0.0947	0.0110
C	C0	1.903 b	0.383 c
	C1	1.966 ab	0.529 b
	C2	2.025 a	0.684 a
	LSD at 0.05	0.0892	0.0176

	B0	1.93 a	0.498 c
B	**B1**	1.97 a	0.558 a
	B2	1.99 a	0.542 b
	LSD at 0.05	0.1006	0.0095
	S×C	**ns**	*****
	S×B	**ns**	*****
	C×B	**ns**	*****
	S×C×B	**ns**	*****

S = calcium carbonate, S0 = 1.9% calcium carbonate, S1= 4.35% calcium carbonate,S2= 6.81% calcium carbonate S3 = 11.71% calcium carbonate , C=compost rate, C0= 0.00 ton of compost/ha, C1= 17.86 ton of compost/ha, C2= 35.72 ton of compost/ha, B = bio-fertilizer type, B0= uninoculated seeds ,B1= inoculated seeds with ARC-SWERI-501, *Rhizobium leguminosarum sv. viciae* ,B2= inoculated seeds with ICARDA-441 *Rhizobium leguminosarum sv. viciae* , LSD0.05 = The least significant difference between treatments (at 5% level of probability), * and ns : significant and not significant at 0.05 level of probability.

Organic matter (OM)

The soil content of Soil organic matter was significantly influenced by all studied factors and their interactions (Table 3 and Fig.4). Increasing the carbonate level was associated with a reduction in OM, decreasing from 0.564% (S0) to 0.478% (S3). Regarding, compost application rate caused significantly increased in soil content of OM from 0.383% (C0) to 0.684% (C2). Also, inoculation with B1 and B2 strains significantly enhanced OM compared to the uninoculated control, with B1 achieving the highest OM value (0.558%). The highest OM content (0.78%) was observed under treatment of (S0C2B1), indicating the beneficial effect of organic and biological amendments under carbonate stress.

Available content of N, P, and K in the Soil

As presented in Table (4) and figures (5, 6 and 7), an increase in calcium carbonate content resulted in a significant decrease in the available concentrations of N, P, and K in the soils. The highest values were recorded with the lowest content of carbonate (S0). While nitrogen, phosphorus, and potassium reached maximum values of 30.11, 4.781, and 187.14 mg kg^{-1}, respectively. These values progressively declined to 24.92, 3.605, and 174.09 mg kg^{-1} at the highest carbonate level (S3). Compost application rate significantly increased the soil available content of nutrients under carbonate stress. The 1.5% compost (C2) resulted in the highest nutrient availability (29.56, 4.538, and 185.01 mg kg^{-1} for N, P and K, respectively), compared with the control treatment (C0), which had the lowest values (25.34, 3.946, and 177.01 mg kg^{-1} for N, P, and K, respectively). Biofertilizer inoculation had a significant effect on soil nutrient content. Strain B1 (*ARC-SWERI-501*) led to the greatest increase in available nutrients (30.09 mg of N kg^{-1}, 4.367 mg of P kg^{-1}, and 184.80 mg of K kg^{-1}), followed by strain B2 (*ICARDA-441*). The uninoculated control (B0) consistently showed the lowest nutrient levels (24.71, 3.993, and 177.87 mg kg^{-1}, respectively). According to figures (5, 6 and 7), the combined application of 1.5% compost and B1 (C2B1) achieved the highest soil available content of nutrients (N, P and k) under carbonate stress, particularly under low to moderate carbonate conditions. For example, under S0, available content of N, P, and K reached 36.36, 5.18, and 196.50 mg kg^{-1}, respectively. Although most interaction effects were statistically non-significant, a significant interaction was observed between compost and biofertilizer treatments (C×B) for potassium availability. Table. (4) confirmed these trends, showing that available content of nutrients decreased with increasing carbonate levels but was markedly improved with compost and biofertilizer treatments, especially the C2B1 combination.

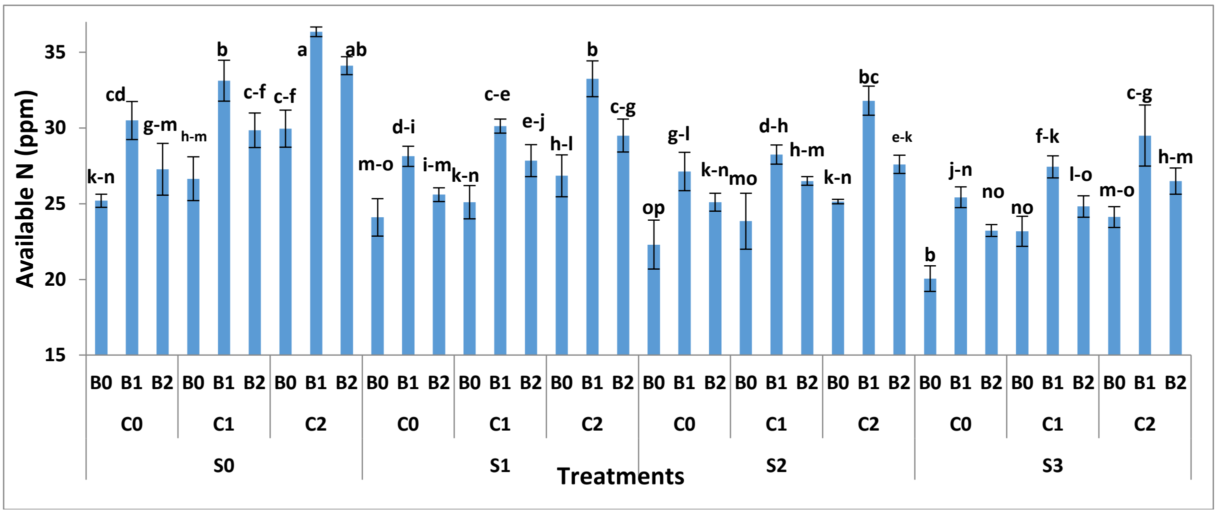

Fig. (5). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on available soil content of N (ppm).

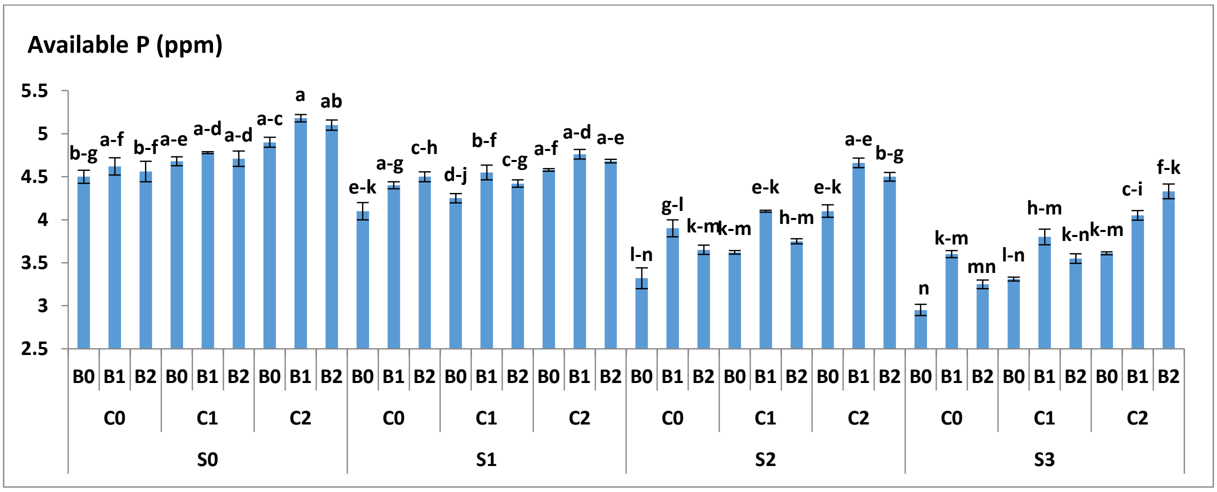

Fig. (6). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on available soil content of P (ppm).

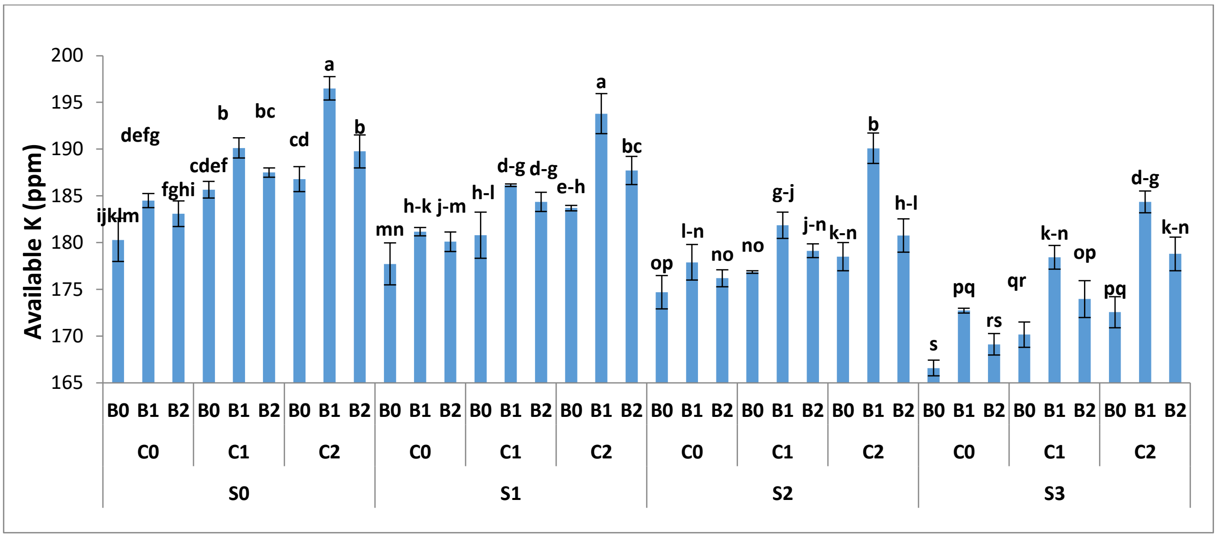

Fig. (7). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on available soil content of K (ppm).

Table (4). Individual effect of treatments (total carbonate levels, compost rates and biofertilizers types) on available soil content of N, P and K.

Treatments		Available content (ppm) of macronutrients		
		N	**P**	**K**
S	**S0**	30.11 a	4.781 a	187.14 a
	S1	27.83 b	4.471 b	183.95 b
	S2	26.41 c	3.956 c	179.56 c
	S3	24.92 d	3.605 d	174.09 d
LSD at 0.05		0.8634	0.2029	0.986
C	**C0**	25.34 c	3.946 c	177.01 c
	C1	27.06 b	4.126 b	181.25 b
	C2	29.56 a	4.538 a	185.01 a
LSD at 0.05		0.5896	0.1757	1.1535
B	**B0**	24.71 c	3.993 b	177.87 c
	B1	30.09 a	4.367 a	184.80 a
	B2	27.16 b	4.250 a	180.88 b
LSD at 0.05		1.3207	0.1757	1.3398
	S×C	**ns**	**ns**	**Ns**
	S×B	**ns**	**ns**	**Ns**
	C×B	**ns**	**ns**	*****
	S×C×B	**ns**	**ns**	**Ns**

S = calcium carbonate, S0 = 1.9% calcium carbonate, S1= 4.35% calcium carbonate,S2= 6.81% calcium carbonate S3 = 11.71% calcium carbonate , C=compost rate, C0= 0.00 ton of compost/ha, C1= 17.86 ton of compost/ha, C2= 35.72 ton of compost/ha, B = bio-fertilizer type, B0= uninoculated seeds ,B1= inoculated seeds with ARC-SWERI-501, *Rhizobium leguminosarum* sv. *viciae* ,B2= inoculated seeds with ICARDA-441 *Rhizobium leguminosarum sv. viciae* , LSD0.05 = The least significant difference between treatments (at 5% level of probability), * and ns : significant and not significant at 0.05 level of probability.

3.2. Biological Activity

Tables (5) and Figures (8, 9 and 10) present the effects of $CaCO_3$ levels, compost rates, and types of biofertilizer on enzymatic activities. Both the individual and interactive effects of these treatments influenced nitrogenase activity in faba bean nodules, as well as dehydrogenase and phosphatase activities in rhizosphere of sandy soil.

Nitrogenase Activity

Nitrogenase activity, an important biological measure of nitrogen fixation, was affected by individual treatments and the compost × biofertilizer (C × B) interaction. The low carbonate concentration (S0) showed the highest activity, with a significant decrease in activity as a result of increased carbonate content in the soil as shown in Fig.(8). The S0 treatment had the highest level of nitrogenase (2.08 µmol C_2H_4/g nodules/hour) followed closely by S3 (1.90 µmol C_2H_4/g nodules/hour). Likewise, compost application was positively related to nitrogenase activity. The highest value (2.15 µmol C_2H_4/g nodules/hr) under the C2 (1.5%) treatment was significantly greater than the control (C0) with a nitrogenase value of 1.84 µmol C_2H_4/g nodules/hr.

Regarding biofertilizer treatments, B1: *Rhizobium leguminosarum* sv. *viciae* (ARC-SWERI-501) resulted in the highest nitrogenase activity (2.77 µmol C_2H_4/g nodules/hr), followed by B2 : *Rhizobium leguminosarum* sv. *viciae* (ICARDA-441) at 2.57 µmol C_2H_4/g nodules/hr, while the uninoculated control (B0) had the lowest activity (0.62 µmol C_2H_4/g nodules/hr). Although not all combined treatments showed statistically significant differences, a clear trend was evident.

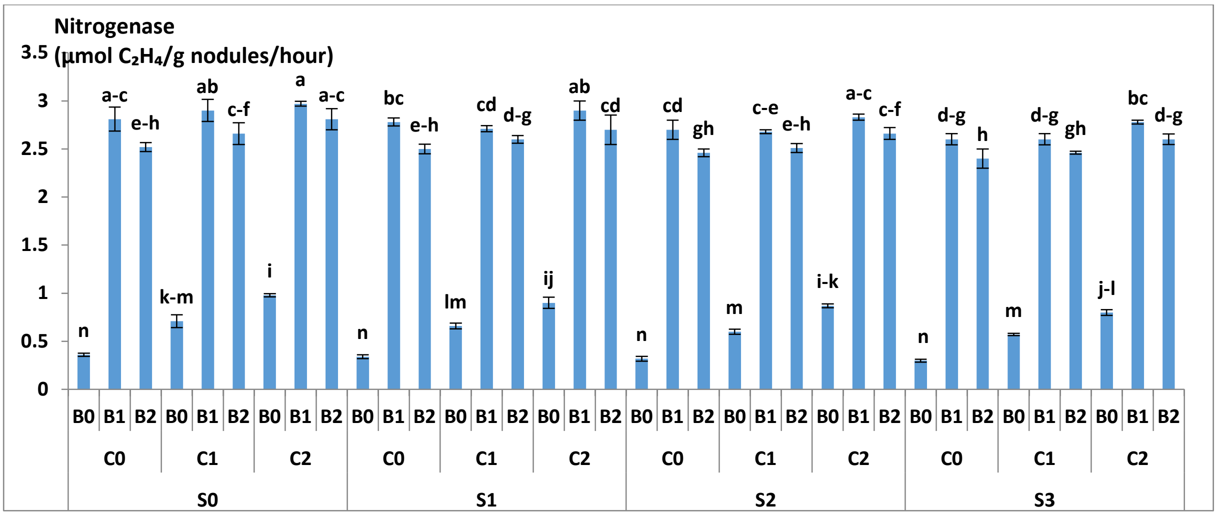

Fig. (8). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on Nitrogenase activity (µmol C_2H_4/g nodules/hr).

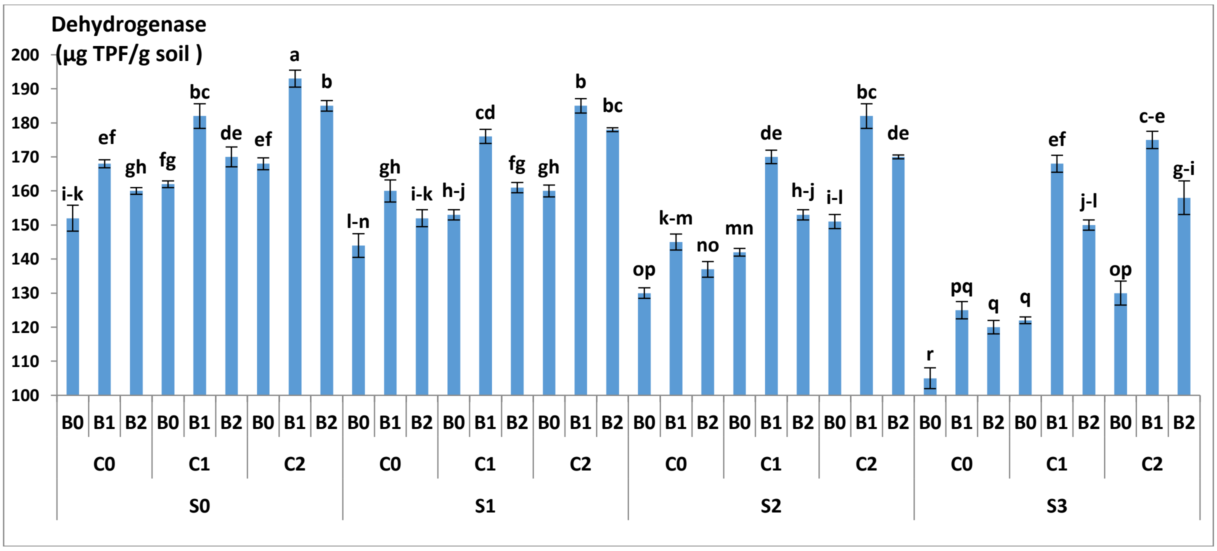

Fig. (9). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on Dehydrogenase activity (µg TPF/g soil).

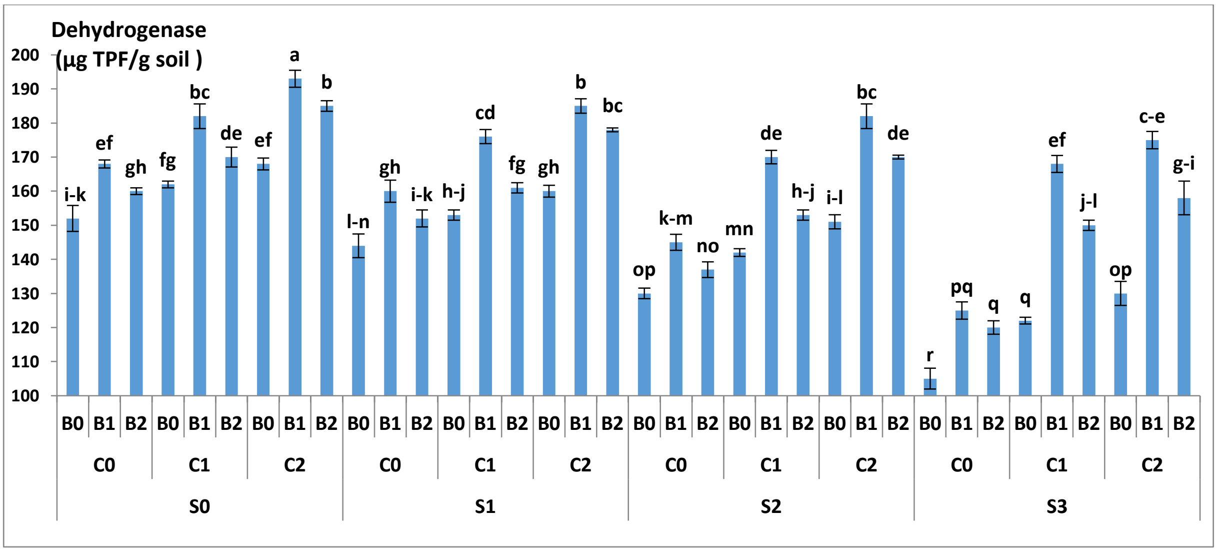

Fig. (10). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on Phosphatase activity (µg pNP/g soil/hr).

Table. (5). Individual effect of treatments (total carbonate levels, compost rates and biofertilizers types) on some enzymes activity.

	Treatments	Nitrogenase (C_2H_4/g nodules/hr)	Dehydrogenase (µg TPF/g soil)	Phosphatase (pNP/g soil/hr)
S	S0	2.08 a	171.11 a	73.00 a
	S1	2.01 b	163.22 b	68.22 b
	S2	1.96 bc	153.33 c	62.66 c
	S3	1.90 c	139.22 d	58.00 d
	LSD at 0.05	0.0583	2.5803	1.41669
C	C0	1.84 c	141.50 c	57.58 c
	C1	1.97 b	159.08 b	65.50 b
	C2	2.15 a	169.58 a	73.33 a
	LSD at 0.05	0.0689	1.9779	1.1371
B	B0	0.62 c	143.25 c	54.75 c
	B1	2.77 a	169.08 a	76.58 a
	B2	2.57 b	157.83 b	68.08 b
	LSD at 0.05	0.0177	2.61311	2.098
	S×C	ns	*	*
	S×B	ns	*	ns
	C×B	*	*	*
	S×C×B	ns	ns	ns

S = calcium carbonate, S0 = 1.9% calcium carbonate, S1= 4.35% calcium carbonate,S2= 6.81% calcium carbonate S3 = 11.71% calcium carbonate , C=compost rate, C0= 0.00 ton of compost/ha, C1= 17.86 ton of compost/ha, C2= 35.72 ton of compost/ha, B = bio-fertilizer type, B0= uninoculated seeds ,B1= inoculated seeds with ARC-SWERI-501, *Rhizobium leguminosarum sv. viciae* ,B2= inoculated seeds with ICARDA-441 *Rhizobium leguminosarum sv. viciae* , LSD0.05 = The least significant difference between treatments (at 5% level of probability), * and ns : significant and not significant at 0.05 level of probability.

The best nitrogenase activity (2.97 µmol C_2H_4/g nodules/hr) was recorded in the S0C2B1 combination, which had the lowest carbonate level, the highest compost rate, and inoculation with strain B1. The lowest activity (0.30 µmol C_2H_4/g nodules/hr) was observed in treatment of S3C0B0, which had the highest carbonate content, without compost and biofertilizer.

Dehydrogenase Activity

Dehydrogenase activity (DA), an indicator of microbial oxidative capacity, was significantly affected by $CaCO_3$ levels, compost rates, and biofertilizer treatments (Fig. 9). Activity decreased consistently with increasing carbonate concentrations. For example, S0 exhibited the highest value (171.11 µg TPF/g soil), whereas S3 had the lowest (139.22 µg TPF/g soil) as shown in Table (5). The application of compost increased dehydrogenase activities. The C2 treatment had dehydrogenase activity of 169.58 µg TPF/g soil, compared to 141.50 µg TPF/g soil for the control (C0). In terms of soil dehydrogenase activity for the biofertilizer treatments, strain B1 (169.08 µg TPF/g soil) had the highest soil dehydrogenase activity, followed by strain B2 while the uninoculated control (B0) had the lowest (143.25 µg TPF/g soil). The compost × biofertilizer (C × B) interaction indicative that the addition of compost has the potential to increase the positive effects of Rhizobium strains, especially under conditions where the potential for carbonate stress was low.

Phosphatase Activity

Phosphatase activity was important in the process of phosphorus mineralization, and similar to nitrogenase and dehydrogenase activities, it followed a specific trend. Table (5) showed that, the raised carbonate levels decreased phosphatase activity considerably. S0 had the highest total value (73.00 µg pNP/g soil/hr), while S3 had the lowest (58.00 µg pNP/g soil/hr). Compost application significantly increased phosphatase activity. The C2 treatment had the highest value (73.33 µg pNP/g soil/hr) compared to the control (C0). Biofertilizer treatment B1 recorded the highest phosphatase activity (76.58 µg pNP/g soil/hr), followed by B2 (68.08 µg pNP/g soil/hr). The control (B0) recorded the lowest value (54.75 µg pNP/g soil/hr), with all differences being statistically significant. The combined application of compost and biofertilizer (C × B) significantly enhanced phosphatase activity, suggesting a synergistic role in which compost boosts microbial activity and phosphorus availability when paired with effective Rhizobium strains. The overall trend shown in Table (5) and Figures (8, 9 and 10) reveals a clear decrease in enzymatic activities as carbonate levels increased. Furthermore, the positive effects of compost and biofertilizer (specifically organism B1) were greater under less carbonate stress. These results noted suggest a synergistic relationship - with compost improving microbial activity, and biofertilizers enhancing nitrogen fixation.

3.3. Number of Nodules and Shoot Dry Weight

The nodule number and shoot dry weight of faba bean plants were affected by total carbonate levels, compost rates and biofertilizer strains, either individually or interacting, as show in Table (6) and Figures (11 and 12).

Number of Nodules

Nodule formation, a key indicator of effective nitrogen fixation, was significantly affected by all individual and interaction effects, as shown in Table (6). According to the individual treatment effects in Table 6, the highest number of nodules (83.89 nodules/plant) was recorded at the lowest carbonate level (S0), while the lowest number (47.77 nodules /plant) occurred under the highest carbonate stress (S3). Compost application significantly improved nodule formation. The highest number of nodules was determined on plants treated with C2 (85.58 nodules/plant), followed by C1 (69.50 nodules/plant). The lowest number of nodules measured from the control (C0) (52.08 nodules/plant). In regards to biofertilizer treatments, strain B1 produced the greatest amount of nodulation (75.25 nodules/plant) with a significant difference to B2 (69.83 nodules/plant) and uninoculated control B0 (62.08 nodules/plant). Fig. (11) revealed that, the combined treatment S0C2B1 produced the highest number of nodules (112 nodules/plant), while the treatment with the lowest amount of nodules (30 nodules/plant) was under the S3C0B0 treatment, in which the plants were under the highest carbonate stress without compost or inoculation (Fig.11).

Shoot Dry Weight

Shoot dry weight (g/plant), an important indicator of plant growth and biomass accumulation, was significantly influenced by both individual factors and all interaction levels. Individually (Table 6), plants grown under the S0 treatment exhibited the highest average shoot dry weight (5.59 g/plant), which was significantly greater than that of plants under S3 (4.86 g/plant). Compost application significantly enhanced shoot biomass. The highest weight was recorded in the C2 treatment (5.71 g/plant), followed by C1 (5.27 g/plant), while the control (C0) recorded the lowest value (4.75 g/plant). Concerning the effects of biofertilizer, the strain B1 had the highest performance, producing; a dry weight of 5.56 g/plant, whilst the B2 strain produced a dry weight of 5.32 g and the uninoculated control B0 only produced 4.85 g. The maximum shoot biomass value recorded under the S0C2B1 treatment was 6.50 g/plant, whilst the lowest value of 4.22 g/plant was recorded in the S3C0B0 treatment (Fig. 12). These results clearly indicate the negative impact of increased carbonate content and the positive interactive effects of compost and biofertilizer on both nodulation and shoot dry weight of faba bean. Table (6) illustrate the decline in nodulation and shoot biomass with increasing carbonate levels. The beneficial effects of compost and biofertilizer (especially strain B1) were most pronounced under carbonate stress. This study reveals a synergistic interaction where compost improves the root environment and nutrient availability, while Rhizobium inoculation enhances nitrogen fixation. Together, these treatments significantly improve plant growth and performance under carbonate stress conditions.

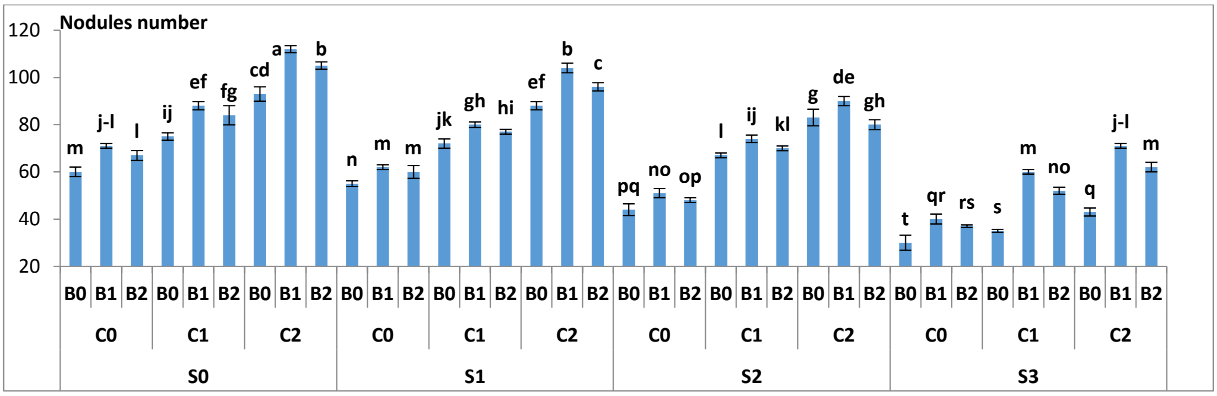

Fig. (11). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on Nodules number of faba bean rootss as mean values.

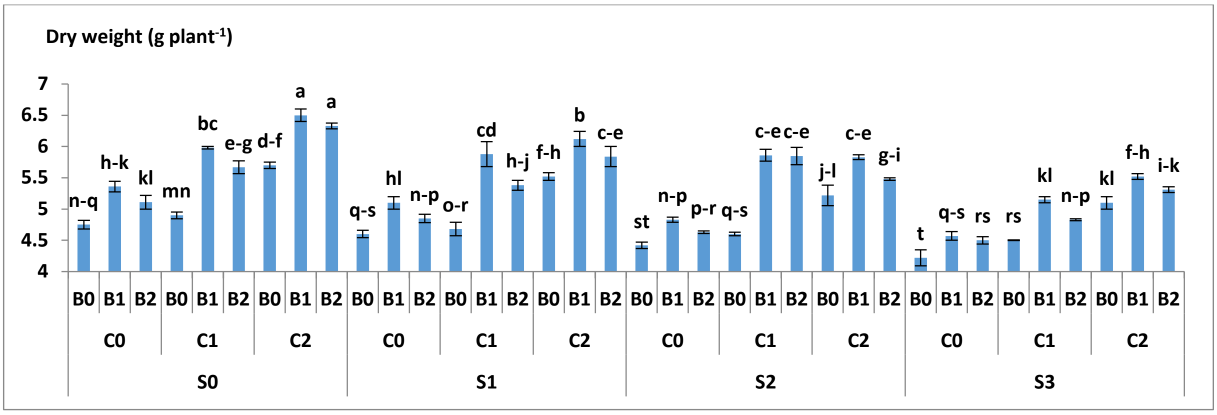

Fig. (12). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on dry weights of faba bean shoots (g plant $^{-1}$) as mean values.

Table (6). Individual effect of treatments (total carbonate levels, compost rates and biofertilizers types) on Nodules number and dry weights of faba bean shoots (g plant $^{-1}$) as mean values.

Treatments		Nodules Number	Dry weight (g plant $^{-1}$)
S	S0	83.89 a	5.59 a
	S1	77.11 b	5.33 b
	S2	67.44 c	5.19 c
	S3	47.77 d	4.86 c
LSD at 0.05		1.51	0.07
C	C0	52.08 c	4.75 c
	C1	69.50 b	5.27 b
	C2	85.58 a	5.71 a
LSD at 0.05		0.96	0.062
B	B0	62.08 c	4.85 c
	B1	75.25 a	5.56 a
	B2	69.83 b	5.32 b
LSD at 0.05		1.76	0.061
	S×C	*	*
	S×B	*	*
	C×B	*	*
	S×C×B	*	*

S = calcium carbonate, S0 = 1.9% calcium carbonate, S1= 4.35% calcium carbonate,S2= 6.81% calcium carbonate S3 = 11.71% calcium carbonate , C=compost rate, C0= 0.00 ton of compost/ha, C1= 17.86 ton of compost/ha, C2= 35.72 ton of compost/ha, B = bio-fertilizer type, B0= uninoculated seeds ,B1= inoculated seeds with ARC-SWERI-501, *Rhizobium leguminosarum sv. viciae* ,B2= inoculated seeds with ICARDA-441 *Rhizobium leguminosarum sv. viciae* , LSD0.05 = The least significant difference between treatments (at 5% level of probability), * and ns : significant and not significant at 0.05 level of probability.

3.4. Macronutrients (N, P and K) uptake

Table (7) in conjunction with Fig. (13, 14 and 15) demonstrate the individual and interactive effects of total carbonate levels, compost rates, and biofertilizer strains on nitrogen (N), phosphorus (P), and potassium (K) uptake by faba bean plants. Statistical analysis indicated that nutrient uptake was significantly influenced by the main treatments and specific interaction levels, particularly for nitrogen.

Nitrogen (N) Uptake

Nitrogen uptake was significantly affected by carbonate levels, compost rates, and biofertilizer types, as well as by all interaction combinations (S × C, S × B, C × B, and S × C × B) as shown in Table (7).The results of Table 7 showed that the maximum N uptake was obtained at the lowest level of carbonate (S0), with 216.72 mg/plant, and then declined with the increase in carbonate stress (In S3, N uptake was 178.02 mg/ plant). The maximum N uptake of 216.72 mg/plant was observed at the lowest carbonate level (S0) and decreased with increasing carbonate stress, declining to 178.02 mg/plant at S3 (Table 7). In addition, N uptake was significantly influenced by the application of compost. The mean value was highest in the C2 treatment (213.92 mg/plant), followed by C1 (196.08 mg/plant) and lowest in C0 (179.74 mg/plant). Among the biofertilizers, the strain B1 had the highest total N uptake (210.48 mg/plant), which was significantly different from B2 (196.72 mg/plant) and the control B0 (182.53 mg/plant).Fig.(13) reveals that, the best synergistic effect was found in S0C2B1, where the maximum nitrogen uptake (283.80 mg/plant) was observed. In contrast, the lowest uptake (147.06 mg/plant) was recorded in S3C0B0, indicating the aggravated adverse effect of high carbonate without organic and microbial amendments.

Phosphorus (P) Uptake

Phosphorus uptake was significantly influenced by the individual effects of compost and biofertilizer, but less so by interactions (Table 7 and Fig. 14). The values of P uptake was highest at S0 (24.44 mg/plant), and decreased significantly under S2 and S3 (21.28 and 19.84 mg/plant, respectively). Compost treatment C2 led to the greatest P uptake (25.33 mg/plant), outperforming both C1 (21.81 mg/plant) and C0 (19.31 mg/plant). Biofertilizer B1 also showed the most favorable results (23.94 mg/plant), significantly higher than B0 (20.33 mg/plant). Although the overall S × C and S × B interactions were not statistically significant, the B1 × C2 combination under S0 still recorded the maximum value (30.40 mg/plant).These findings reflect the important role of compost and effective Rhizobium strains in improving phosphorus availability and uptake under calcareous conditions.

Potassium (K) Uptake

Potassium uptake patterns were similar as for N and P and demonstrated significant individual effects and interaction effects (most notability C × B) (Table 7 and Fig.15).Table 7 showed that K uptake was highest in S0 at 173.86 mg/plant, steadily decreasing to 144.26 mg/plant in S3. As with P uptake, application of compost significantly improved K uptake with C2 highest (180.20 mg/plant) followed by C1 and C0. For biofertilizers, B1 had the highest value of K uptake at 170.40 mg/plant, followed by B2 (160.2 mg/plant), and B0 (146.80 mg/plant). The greatest K uptake (211.2 mg/plant) was from S0C2B1, and the lowest value (115.2 mg/plant) was from S3C0B0, which again demonstrated the negative effects of high carbonate stress, while also showing the positive effects of organic and microbial soil amendments. Figures 13 to 15 depicted decreasing N, P and K uptake as carbonate levels were increased. The benefits of compost and the use of biofertilizer, and especially the B1 strain biofertilizer, were much greater in the low carbonate level. Compost likely increases nutrient availability and biofertilizers improved nutrient uptake, especially nitrogen fixation and uptake by the root system. The results from this study support an integrated use of organics and microbes as a potential strategy to improve nutrient uptake in calcareous sandy soils.

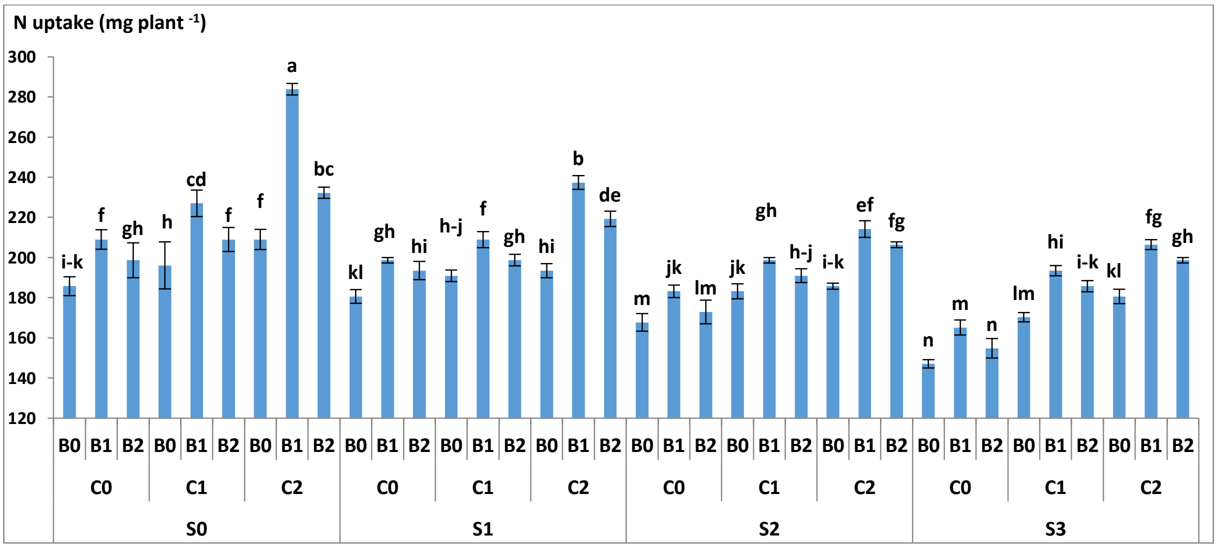

Fig (13). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on N uptake (mg plant $^{-1}$) .

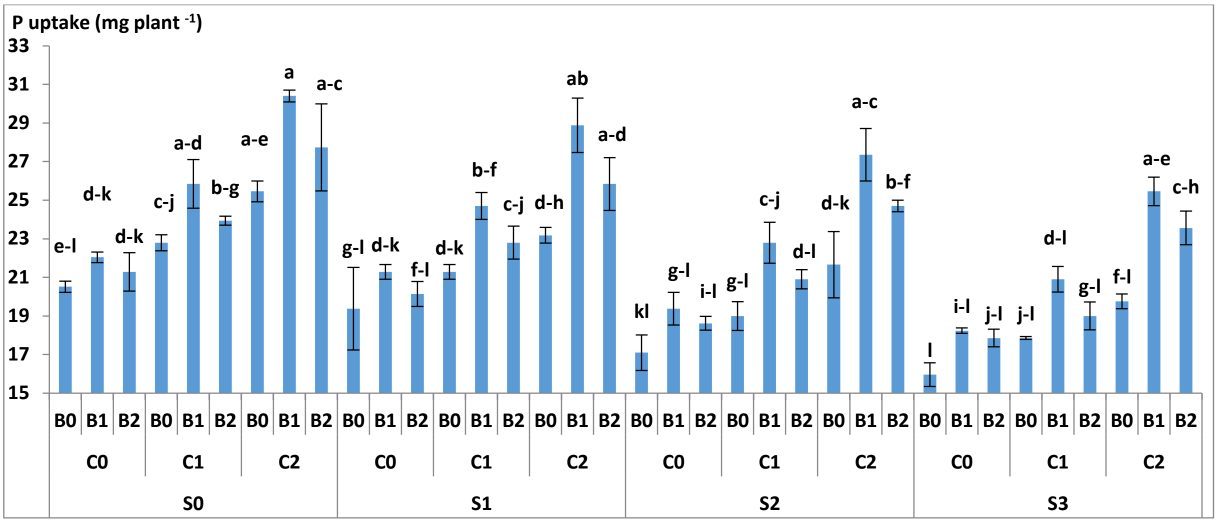

Fig (14). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on P uptake (mg plant $^{-1}$).

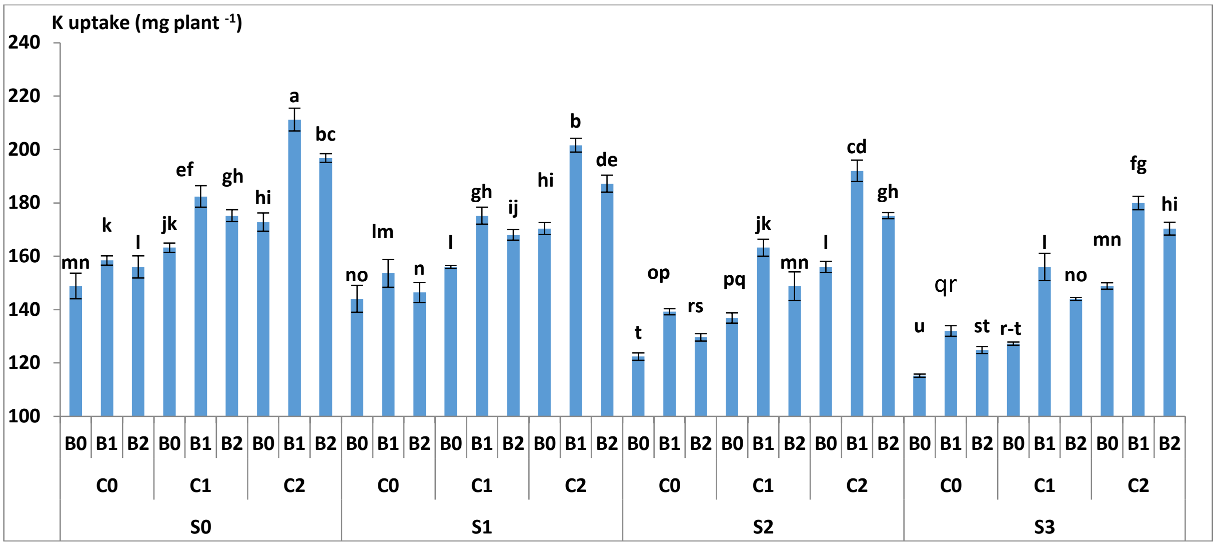

Fig (15). Interaction effect of treatments (total carbonate levels, compost rates and biofertilizers types) on K uptake (mg plant $^{-1}$).

Table (7). Individual effect of treatments (total carbonate levels, compost rates and biofertilizers types) on N, P and K. uptake.

Treatments		**N** (mg plant $^{-1}$)	**P** (mg plant $^{-1}$)	**K** (mg plant $^{-1}$)
S	**S0**	216.72 a	24.44 a	173.86 a
	S1	202.38 b	23.05 a	166.93 b
	S2	189.20 c	21.28 b	151.46 c
	S3	178.02 d	19.84 b	144.26
LSD at 0.05		2.61	1.68	2.26
C	**C0**	179.74 c	19.31 c	139.20 c
	C1	196.08 b	21.81 b	158.00 b
	C2	213.92 a	25.33 a	180.20 a
LSD at 0.05		2.79	1.58	1.75
B	**B0**	182.53 c	20.33 b	146.80 c
	B1	210.48 a	23.94 a	170.40 a

B2	196.72 b	22.19 ab	160.20 b
LSD at 0.05	2.8	2.65	3.93
S×C	*****	**ns**	**ns**
S×B	*****	**ns**	**ns**
CXB	*****	**ns**	*****
S×C×B	*****	**ns**	**ns**

S = calcium carbonate, S0 = 1.9% calcium carbonate, S1= 4.35% calcium carbonate,S2= 6.81% calcium carbonate S3 = 11.71% calcium carbonate , C=compost rate, C0= 0.00 ton of compost/ha, C1= 17.86 ton of compost/ha, C2= 35.72 ton of compost/ha, B = bio-fertilizer type, B0= uninoculated seeds ,B1= inoculated seeds with ARC-SWERI-501, *Rhizobium leguminosarum sv. viciae* ,B2= inoculated seeds with ICARDA-441 *Rhizobium leguminosarum sv. viciae* , LSD0.05 = The least significant difference between treatments (at 5% level of probability), * and ns : significant and not significant at 0.05 level of probability.

4. Discussion

4.1. Soil chemical properties

The decrease in soil pH followed by compost applications resulted from compost degradation (physically , chemically and biologically) and its decomposition products that have acidic effect as mentioned and discussed before that by Aiad (2024). As well as these acidic compounds resulted in dissolve carbonates compounds which associated by decrease in soil pH (Sweed 2012; Elgezari ,2016; Abou Hussien and Elbalawy, 2020 and Abou Hussien et al. 2021). The same reasons resulted in an increase in soil EC. In addition, in the four soils increase rate of added compost resulted in an increase in the soil content of OM, where with the same application rate of compost, the content of OM was decreased with the increase in the soil content of $CaCO_3$. The decrease in the OM content with the high $CaCO_3$ content may be explained based on the basic effect of $CaCO_3$ which dissolved more amounts of soil organic matter (Stevenson,1994). In this respect similar results were obtained by Sweed (2012) and Abou Hussien and Elbalawy (2020). Similarity, biofertilizers applications in B1 and B2 forms appeared a slight effect on soil pH , EC and OM ,where these effects were decreased at high content of $CaCO_3$ and these results El-Noamany (2014) reached similar results that showed lower efficiency of biofertilizers under calcareous soil conditions compared to clayey soil, which means that calcium carbonate content has a detrimental effect on the efficiency of biofertilizers. As shown from the obtained data in Table 4 and Fig. 5, 6 and 7, the soil content of available N, P and K decreased as a result of the increase in soil $CaCO_3$ content, which led to an increase in soil pH followed by an increase in the rate of N volatilization in the form of NH3. As well as the increase in soil $CaCO_3$ resulted in more fixation and precipitation rates of P in calcium form (Basak, 2006). Also, increasing $CaCO_3$ content resulted in a decrease of microbial activity in the soil (Abou Hussien and Elbalawy, 2020).

Compost applications to the soils having different contents of $CaCO_3$ resulted in a significant increase in these soils contents (mg k^{-1}) of available N, P and K. These results attributed to the high added amounts of the nutrients to the soil within compost applications (Nada et al. 2023). As well as compost applications reduced the soil pH and increased its content of OM which played a major role of bio-chemical activity in the soil followed by as increase in the nutrients (N, P and K) availability .In this respect under calcareous soil addition , Elgezari (2016) and Abou Hussien et al. (2017) found similar increase effect of compost applications on the increase in the soil content of available N, P and K. Recently , Nada et al (2023) and Aiad (2024) found similar increase in the soil content of available N, P and K as a result of compost applications under different soil condition.

In addition, biofertilizers applications to the soils having different content of $CaCO_3$ resulted in an increase of nutrients availability, where such applications led to an increase in the biological activity and OM content in the soil as well as decreased soil pH resulted from its acidic products . Similar results were obtained before that by El-Noamany (2014). Therefore to decrease the harmful effect of carbonates stress and increase the nutrients availability, both organic and biofertilizers must be applied in suitable forms.

4.2. Biological activity

In general as pointed before from the studies, biological activity and biochemical processes affected greatly by both physical and chemical properties and the system of soil management (Marie, 2019; El-Noamany, 2020; Xu et al. 2020 and Ma et al. 2024). Under this study conditions, increasing soil content of $CaCO_3$ or carbonates stress reduced the biological activity (enzymes activity and nodulation). The main reasons of such decreases were high values pH and the low soil content of available nutrients and OM. Results showed that soil carbonate levels, compost application, and biofertilizer inoculation have a significant effect on enzymatic activities associated with nitrogenase, dehydrogenase, and phosphatase. As $CaCO_3$ levels increased, observed activities of all three enzymes significantly decreased. These decreases may be due to decline of microbial efficiency and availability of essential nutrients under greater alkalinity In this regard, Pezzutto et al. (2002) and Ksouri et al. (2005) pointed out that Calcareous soil often contains excess of nutrients, however high pH and active calcium carbonate reduces its availability so nutrients deficiencies on plants are often observed in these soils. Adding compost significantly increased enzyme activities (nitrogenease, dehydrogenease and phosphatease) as a result of increasing soil organic carbon and nutrients availability and improving soil properties, which is reflected in microbial growth and production of enzymes. These are in the same line with previous studies confirming a positive impact of Compost as a vital role in stimulating soil microflora and enhancing the symbiosis between plant roots and nitrogen-fixing bacteria (Dozokom and Darnan, 2023). Also, Farid et al. (2023) found a significant increases in the total number of bacteria , fungi and actinomycetes in the soil treated by organic fertilizers (compost and biogas manure) . In addition, Tantawy et al. (2019) and El-Noamany et al. (2024) found a significant increases in the root nodules as a result from biofertilizers applications, where these application improve the growing environment in the root zone .Also, in this respect Abou Hussien and Elbalawy (2020) and Abou Hussien et al. (2020) found a significant increase on soil chemical properties and biological activity in both enzymes activity and nodulation in the soil treated by organic fertilization. The compost content of humic substances such as humic and fulvic acid has an effect that enhances the enzymatic activity of microbes and in this respect Afifi et al. (2017) pointed that humic acid and fulvic acid amendments enhanced nitrogenase activity in study on Response of faba bean plants to organic amendments on calcareous soil. In the same line, Masciandaro et al. (2002) found that, humic acid application improves plant physiological processes by enhancing macro and micronutrients availability in soil. As bio-fertilizer treatments (B1 and B2) had significant positive effect on all three measured enzymes compared to uninoculated controls. It was evident that strain B1 strongly promotes soil biochemical as shown in all estmated enzymes when compared to strain B2. Siczek and Lipiec (2016) indicated that Rhizobium inoculation led to an increase in a majority of enzymatic activities in the rhizosphere of faba bean during the vegetative stage. These enzyme activities are also related to increased availability and uptake of nutrients, especially P in plants (Šarapatka, 2002 and Dotaniya et al. 2014). These results are consistent with those of previous research which have demonstrated a positive effect of the symbiosis rhizobia on soil quality through enhancement in soil enzyme activities (Prasad et al. 2012; Othman and Mouradi et al. 2018). Also Ma et al. (2024) found that rhizobial treatment with Caucasian Clover under Field Conditions, improved soil physicochemical properties and enzyme activities, and positively affected soil microbial diversity and abundance. These changes enhanced soil fertility and optimized microbial community structure, promoting plant growth. The use of low levels of carbonate applied with high levels of compost and inoculation of B1 created the best conditions for enzymatic activity, indicating a synergism that will help to increase soil fertility and nutrient cycling within sandy soils under carbonate stress. The harmful effect of $CaCO_3$ on the biological activity may be improve with using of integrating sustainable soil management practices such compost and symbiosis rhizobia strains as a boifertilizer. Farrag and Bakr (2021) pointed out that the combination of acid producing bacteria and yeast with farmyard manure (FYM) was more effective than using FYM alone in improving of calcareous soil properties and enhancing wheat growth. The harmful effect of $CaCO_3$ on the biological activity may be improve with using of integrating sustainable soil management practices, Marie (2019) and El-Noamany (2020) found improve in soil chemical properties and increase in the biological activity as a result of mineral , organic and biofertilizers applications.

4.3. Number of Nodules and shoot Dry Weight

It is apparent that shoot dry weight as well as nodule number in faba bean plants were significantly affected by calcium carbonate levels, compost application and biofertilizer inoculation, either individually or in combination. The observed tendencies supported our expectation that, calcium carbonate level in soil under the experiment conditions was detrimental to legume-rhizobia symbiosis, as well as growth of the plants; however, this harmful impact may be alleviated with organic (compost) and microbial (biofertilizer) applications. Organic matter in soil is a valuable parameter of soil fertility, which results from the associations of physical, chemical, and biological processes (Ghadimi et al. 2021). Organic matter leads to better soil fertility and structure, and soil vitality overall (Badawy et al. 2023). In this respect, Attia et al. (2023) found that fresh and dry weights of shoots and N and P uptake, as well as nodules numbers were significantly increased by compost application faba bean grown on a calcareous sandy soil to compared with control (without compost). Using of rhizobial treatment significantly increased the plant height and yield of Caucasian clover, improved soil physicochemical properties and enzyme activities, and had a positive effect on soil microbial diversity and abundance, creating conditions for improved soil fertility and the actors of the microbial community structure to promote plant growth (Ma et al. 2024). The number, fresh and dry weight of nodules could be increased due to the compost as a source of C, N, P and micronutrients for the native as well as exogenously inoculated rhizobia. Nitrogen in the presence of Rhizobium enhanced nodulation in legumes (Rashid et al. 1999). Plant height increased with the inoculation of Rhizobium

(Togay et al.Â 2008 and Ahmad et al. 2013) and Rhizobium plus N application (Khalil et al. 1989 and Rahman et al. 2002). Soumaya et al.(2016) studied growth and nutrient uptake of sulla(Hedysarum coronarium L.) affected by Rhizobium inoculation under calcareous soilconditions and reported that, Despite the edaphic conditions of Goubellat area witch were characterized with high pH (8.7) and active calcium carbonate (18%), nitrogen fertilization and Rhizobium inoculation showed a significant increase of growth parameters compared to the control. Our investigation appeared that,The synergistic effect of combining low carbonate levels, high compost rates, and effective Rhizobium inoculation was particularly evident. The S0C2B1 treatment yielded the highest nodule number (112 nodules/plant), while the combination of high carbonate without compost or inoculation (S3C0B0) resulted in the lowest count (30 nodules/plant). These findings underscore the value of integrated soil fertility management in overcoming the limitations of calcareous soils. The results of the previous study showed that the inoculants with rhizobium and compost increased the growth of peanut and number of root nodules (Malik et al. 2014).These results agree with those of Attia et al. (2023) who observed that Signification positive interactions were observed between compost application and the microbial seed inoculation treatment in number of nodules and their dry weight, root and shoot fresh and dry weight, N and P in the shoot, seed yield as well as N and P in seed and in straw.

4.4. Macronutrients (N, P and K) uptake

The present study examined the interactive and indivdual effects of compost application and/or Rhizobium-based biofertilizers on nutrients uptake in shoots of faba bean (Vicia faba L.) under carbonate stress in sandy soil. The results showed significant differences in macronutrients uptake (nitrogen, phosphorus, potassium) could be considered depending on experimental treatments compared with control. Excessive calcium carbonate in soil decreased nutrients uptake in faba bean plants. This finding is consistent with previous research which has shown that increases in calcium levels, along with soil pH, have a negative effect on nutrient availability and uptake for plants (Bavaresco et al. 2003 and Ksouri et al. 2005). High calcium concentrations in soil solution can form insoluble precipitates with several nutrients, resulting in limited bioavailability in excess of plants. In this regard, it was found that, Phosphorus fertilizers are fixed by $CaCO_3$ through adsorption and precipitation, resulting in an efficiency of less than 20 % (Vassilev and Vassileva, 2003) .The current research presented a decline in nitrogen uptake from 216.72 mg/plant at the lowest (S0), to 178.02 mg/plant at the highest carbonate concentration (S3). As shown from the obtained data, the soil content of available N, P and K were decreased as an increases in the soil content of $CaCO_3$ which resulted in an increase in soil pH followed by increase in the volatilization rate of N as NH_3 . As well as the increase in soil $CaCO_3$ resulted in more fixation and precipitation rates of P in calcium form (Basak, 2006). From the above, we find that, the increasing in soil pH which associate with the soil content of active calcium carbonate impedes the availability of essential nutrients, especially for plants, which have specialized nitrogen-fixing bacteria in their root nodules. Our findings are consistent with those of Abou Hussien and Elbalawy (2020) showed that, increasing in $CaCO_3$ content resulted in a decrease of microbial activity in the soil and nutrients uptake in plant. Adding compost to the soil considerably improved nutrient uptake in faba bean. Compost has long been identified as a means to improve soil fertility, nutrient retention, and beneficial microbial activity (Lakhdar et al. 2009; Castillejo and Castello, 2010 and Attia et al. 2023). In this study, compost had the most positive effects on nitrogen, phosphorus, and potassium uptake with the highest uptake at the C2 (1.5%) rate. Although compost improved uptake at the C1 (1.0%) rate, the treatment of C2 was significantly greater than the control treatment (C0). Compost serves as a source of supply, specifically for essential nutrients, especially nitrogen and phosphorus, by improving soil structure, the cation exchange capacity (CEC) of the soil, and microbial mineralization processes (Adu-Gyamfi et al. 2007; Ayuke et al. 2011 and Badawy et al. 2023). This are in harmony with the results reported by Attia et al. (2023) who found that N and P uptake, as well as nodules numbers were significantly increased by compost application faba bean grown on a calcareous sandy soil to compared with control (without compost). Biofertilizers, specifically Rhizobium species, were an important factor in enhancing nutrient uptake (nitrogen specifically) in faba bean. The strain ARC-SWERI-501 (B1) had higher nutrient uptake compared to the other Rhizobium strain (B2) and the non-inoculated control in all macronutrients. This strain has previously been reported to have better efficiency in nitrogen fixation than other strains, perhaps due to better adaptation to soil types under egyption conditions. The positive effect of Rhizobium inoculation on nutrient uptake, specifically nitrogen, agree with several studies that have reported the effect of Rhizobium on nutrient mobilization in legumes (Heggo and Barakah, 2004; Badawi et al. 2011; Afifi et al. 2017 and Ucar, 2021), particularly under sub-optimal soil conditions. In a previous study pointed out that, rhizobium inoculation had superior performance in the improvement of nutrients uptake by this crop. It also needs to be mentioned that application of Rhizobium bacteria as a source of N, P, K and other micronutrients may reduce the reliance on chemical fertilizers (Soumaya et al. 2016).The synergestic effect of S0C2B1, which produced the highest nutrient uptake, shows the potential for the use of biofertilizers along with organic amendments in nutrient management programs. Attia et al., 2023 showed that, combination effect of the compost application and microbial inoculants on growth, yield, N and P content of faba bean recorded significant positive interaction. Ultimately, the results of this study are useful for sustainable soil and yield management, particularly in areas of carbonate stress and poor soil fertility. The results reported here can be used by farmers and soil mangers to apply integrated organic–biofertilizer approach in their farming system. Application of compost at right rates (1.5% of soil weight or 25 ton ha^{-1}) not only improves soil structure, moisture holding capacity and nutrient availability but also minimizes use of expensive chemical fertilizers. Similarly, it is well established that inoculation of legume seeds with effective Rhizobium strains (e.g. B1: ARC-SWERI-501 *Rhizobium leguminosarum* sv. *viciae*) significantly increases biological nitrogen fixation (BNF) and soil biological activity as a whole, and thus enhances crop growth even in elevated carbonate environments.

5. Conclusion

The findings of the present study reveal that high levels of calcium carbonate in soil have a detrimental effect on some biochemical properties, availability of nutrients and enzymatic activity as well as growth of faba bean plants. The addition of compost, in combination with Rhizobium inoculation, has a positive synergistic effect. Compost improved soil organic matter content, reduced soil pH, and improved nutrient availability and Biofertilizers, particularly Rhizobium strain B1(ARC-SWERI-501 *Rhizobium leguminosarium sembiovar vicia*) helped to improve nutrient uptake and nodulation under moderate carbonate stress. The greatest improvements in soil fertility and plant performance occurred under the highest compost dose (1.50%), and with strain B1. A combined treatment of C2 + B1 is recommended, as the mixture improves faba bean plant performance under carbonate stress conditions by enhancing biological activity, nutrient uptake, and plant growth. The results in this study demonstrate that integrated use of organic and microbial management practices may alleviate the adverse effects with possibilities for sustainable legume production on soils under carbonate stress.

Declarations

Ethics approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors..

Consent for publication: All authors declare their consent for publication.

Competing interests: The authors declare that they have no conflict of interest in the publication.

Authors' contributions: This study was designed and implemented by authors, where all authors contributed in writing the manuscript, interpreting information present ed and have read and agreed to the version of the manuscript.

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