Egypt. J. Soil Sci. **Vol. 62,** No. 3, pp. 293 - 309 (2022)

Egyptian Journal of Soil Science

http://ejss.journals.ekb.eg/

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Performance of *Priestia megaterium* Azolla Extract and Compost as P-activators for The Cultivation of Faba Bean in Calcareous Soil

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Two consecutive factorial experiments were carried out during the two winter seasons 2020/2021 and 2021/2022 to study the application of *Priestia megaterium*in oculant, Azolla extract or compost as P-activators to reduce the inorganic phosphorus (Pi) fertilizer forthe potential cultivation of faba bean in calcareous soils. The treatments were applied in a split-plot design to compare the full dose or half dose of Pi, individually and in combination with P-activator. The effect of applied treatments was estimated in terms of the rhizosphere biology, shoot and root metrics, nodulation status, photosynthetic pigments, nutrients uptake as well as yield and yield components. Biologically, total microbial count and phosphate dissolvers recorded increases in their numbers in response to half dose of Pi coapplied with each of P-activators, especially where they had been received compost. Coaddition of *P. megaterium,* Azolla extract and compost revealed a significant improvement in vegetative growth in terms of plant height, root surface area, nodulation status and total chlorophyll. At post-harvest, estimated overall performance indicated variable enhancements for yield and yield components in response to P-activators with superiority of compost coaddition with *P. megaterium* and Azolla extract under half dose of phosphorus fertilizer to relatively emulate that attained by full dose. Accordingly, a possible reduction in P-fertilizers can be achieved using *P. megaterium,* Azolla extract with compost as P-activators in calcareous soil for faba bean cultivation.

Keywords: Groundwater; Sodicity Indices; Magnesium Hazard; Total Hardness; EC-Na% Wilcox and Riverside Diagrams; Hydrogeochemical Classification.

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Received: 21/7/2022; Accepted: 30/8/2022
DOI: 10.21608/EJSS.2022.157189.1527

Introduction

Faba bean (*Vicia faba* L.) belongs to Fabaceae family, which represents a resource of nutritious food and fodder in addition to sustaining natural resources and maintaining ecological balance (Meena et al., 2018). Faba bean has great importance for Egyptians because most of their protein intake comes from a vegetable origin, mostly egumes, leading to a high consumption rate of faba bean with a reduction in self-sufficiency followed by a decline in food security(Abdel Maaboud et al., 2019). Such reductions could be attributed to the intense competition between faba bean and other strategic winter season crops such as wheat and clover on the limited cultivated land in Nile valley and Delta. Accordingly, Egypt is highly ranked as an importer, with 309,355 tons or 40.48% of total global imports (Dhull et al., 2021). Faba bean plants act as a bio-factory for nitrogen by fixing 130 to 160 kg N/ha, making it one of the best crops applied as green manure (Singh et al., 2013). It is known that legume plants exceed the requirements of P over non-nodulating ones for metabolic pathways of energy transfer that proceed during nodule functioning (Sulieman and Schulze 2010).

Among the macronutrients, phosphorus is of substantial importance after nitrogen because of its role in biomolecule synthesis, energy pathways, photosynthesis, respiration and regulation of many enzymes making its availability critical for crop production. Unluckily, non-renewable resources and a considerable reactivity of P ions relative to various soil constituents cause forced retention of most soil-P, making it less mobile and not as much toward plants in most soil conditions and ecosystems of agriculture worldwide (Raghothama 1999). Estimated available-P makes up about 1% of soils' total-P(Kucey 1983). More than80% Pifrom appliedfertilizer turns out to be unattainable for plant uptake as a result of sorption, precipitation by reaction with Al^{3+} and Fe^{3+}(in acidic soils) and Ca^{2+}(in calcareous soils) (Asomaning 2020) or microbial immobilization (Yadav and Verma 2012). So, frequently adding fertilizer-P at "normal" rates may not result in optimal productivity of crops cultivated in soils of arid and semi-arid regions due to P-deficient, especially in alkaline and calcareous soil (Habib 2021) owing to the formation of less soluble calcium phosphate minerals(Hopkins and Ellsworth 2005). Other threats are the low organic matter, less cation exchange capacity, unavailability of many nutrients, soil crusting, and its effect on emergent seedlings (Wahba et al., 2019). Egypt holds around 0.27 million hectares of calcareous soil, representing about 0.27% of the total area (Taalab et al., 2019), dominating desert lands in the northern coastal zone and Sinai (Wassif and Wassif 2021). From the point of view of sustainability, the intensive implementation of Pi fertilization to sustain crop requirements causes environmental threats, including "eutrophication" resulting from P losses from soils through increased runoff, erosion, and leaching into water (Pietrzak et al., 2020). This linkage between harsh conditions of calcareous soils and P fertilization problems has motivated the researchers to think of alternative strategies for cultivating crops under such issues in environmental ecofriendly sound (Ibarra-Galeana et al., 2017). The concept "P-activators" used to indicate various methods were classified into three types: 1) bio-inoculants and bio-fertilizers; 2) organic matter and/or 3) zeolites and other materials, which intend to accelerate and strengthen soil P transformations to plant-available forms in soil solution (Zhu et al., 2018).

Bioinoculant or biofertilizers were based on harnessing the innate ability of rhizosphere microbes to liberate Pi from recalcitrant structures, as considered by Gaafar et al., (2021) and Ganzour et al., (2020)under calcareous soil conditions to diminish the use of conventional P-fertilizers. Among rhizosphere bacteria, the bacillus genus, such as *B. subtilis*, *B. cereus*, *B. thuringiensis*, *B. pumilus*, *B. megaterium*, etc., could reveal different mechanisms to enhance plant growth by increasing the availability of thenutrient (Meena et al., 2016). Genus *Priestia megaterium*, formerly known as *Bacillus megaterium* (Gupta et al., 2020), possesses the power to release a high concentration of phosphorus because it can transform insoluble P into soluble forms by secretion of acids and phosphatase (Zhu, et al. 2018).

Azolla is a small floating aquatic nitrogen-fixing fern with distinctive composition. It represents a modern approach as a pteridophyte of agronomic significance, particularly in Egypt, because it is beneficial as a source of several crucial nutrients and bioactive compounds (Rashad 2021). The symbiosis between nitrogen-fixing, blue-green algae and Anabaena empowered botanists and agriculturists interested in eco-friendly sustainable agriculture for years to apply Azolla as a reputable source of organic matter and nitrogen for crops (Yadav et al., 2014). The common forms for introducing Azolla as a biofertilizer include wet or dried fern biomass for manuring cultivated soil (Rabie et al., 2020) or Azolla extract sprayed directly as a foliar (Altai et al., 2019)or integrated ingredient with organic fertilizers such as compost (Seleiman et al., 2022) or compost tea (Abou Hussien et al., 2021).

Compost or composted organic material is a valuable resource of bio-transformed matter, including low molecular weight organic acids, humic acids and lignin; it has also been stated for promising aspects acting to lessen P fixation by soils (Hosseinpur et al., 2011; Verma 2013) and consequently applied as considerable P-activator (Zhu, et al. 2018). The review by Guppy et al., (2005) reported several output products due to decomposition of organic matter act to compete P for soil sorption sites resulting in raised P in soil solution. So, the current study research in a variable influence of *P. meg*aterium and/or Azolla extract with or without compost under lesser P-fertilizer comparing with full dose of mineral phosphorus to cultivate faba bean in calcareous soil.

1. Materials and Methods

1.1. Location and soil of experiment

Two field experiments were performed on calcareous soilat Nubaria Agricultural Research Station, El-Behira Governorate, Egypt, located in the North-Western of Egypt at latitude 30°31'04.0"N; longitude 30°14'19.0"E&mean altitude 29m above sea level, during 2020/2021 and 2021/2022winter seasons. Before sowing, the soil was sampled at a depth of 0 –20 cm; air-dried; ground; sieved through a 2 mm sieve for physical and chemical analysis according to the methods described Page et al., (1982). Some physical and chemical properties of analyzed soil are outlined in Table 1.

TABLE 1. Physical and chemical properties of the soil

Property	Season 2020/2021	Season 2021/2022
Mechanical analysis:		
Sand %	50.28	50.35
Silt %	24.51	22.93
Clay %	25.21	26.72
Texture grade	Sandy Loam	Sandy Loam
$CaCO_3$ (%)	20.3	19.6
pH (1:2.5 water extract)	8.10	8.58
EC (dS m^{-1}, at 25°C)	1.95	1.67
(%) Organic matter	0.36	0.41
(%) Total-N	0.025	0.031
Available-P(mg kg^{-1})	4.01	4.22
Available-K(mg kg^{-1})	114.38	115.17

1.2. Compost

The compost was obtained from Agricultural Microbiology Research Department, Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt. Some physical, chemical and biological properties of the used compost were assayed as described by Page, et al. (1982), and the data are illustrated in Table 2. The values of compost traits likely indicate the maturity of the used compost.

TABLE 2. Some physical, chemical, and biological properties of the used compost

Property	Value
Bulk density (kg/m^3)	437.4
pH (1:10)	7.13
EC (dS/m at 25°C)	4.96
Organic matter %	43.65
Total nitrogen %	1.63
C/N Ratio	15.57
Total soluble-N (mg/kg)	625
Available phosphorus (%)	0.53
Available potassium (%)	1.42
Seed germination test of cress seeds (%)	96

1.3. Bioinoculant preparation

Both bacteria, namely *Rhizobium leguminosarum* bv. Viciae (mixture of ARC 221 and ICARDA 441)and *P. megaterium* (Pm) were supplied by Biofertilizer Production Unit, Agric. Microbiol. Dep., SWERI, ARC, Giza, Egypt. Each of Rhizobia or *P. megaterium* were individually rejuvenated and cultured on Yeast Mannitol or Nutrient broth, respectively (Atlas 2005). Mother cultures were incubated at 28°C on a rotary shaker until the early log phase had been developed to about 10^9 cells per ml of culture.

1.4. Azolla Extract preparation (AE)

Azolla (*A. pinnata*) was kindly provided by Biofertilizer Production Unit, Agric. Microbiol. Dep., SWERI, ARC, Giza, Egypt. Azolla was sampled for analysis according to AOAC (2005). Some characters of Azollaare presented in Table 3.

TABLE 3. Characters of Azolla

Property	Value
Dry matter %	92.17
Organic matter %	82.97
Ash (%)	17.03
Nitrogen (%)	3.64
Phosphorus (%)	0.47
Potassium (%)	0.59
Crude protein (%)	22.75
Soluble sugar (%)	3.6

Azolla extract (AE) was prepared according to the procedure of Bindhu (2013). One kg of fresh Azolla had been boiled for 30 min in one liter of distilled water, cooled at room temperature, filtered through a sheet of cheesecloth and kept in the refrigerator as crude-AE until following usage.

1.5. Field experiment layout and sampling

Two factorial experiments were consecutively carried out during the two winter seasons 2020/2021 and 2021/2022onthe 11^{th} and 10^{th} of November, respectively, to research the application of *P. megaterium* inoculant or Azolla extract with or without compost to reduce the P-fertilizer for potential cultivation of faba bean on calcareous soils. The experiments were designed in a split-plot design with four replicates assigning compost at main plots and the following treatments at subplots: 1) control treatment which received476 kg ha^{-1}calcium superphosphate (15.5% P_2O_5) as a full dose; 2) half dose of superphosphate (238 kg ha^{-1}); 3)AE+ a half dose of superphosphate; 4) Pm+ a half dose of superphosphate, or 5) AE + Pm + a half dose of superphosphate.

All farming and agronomic practices were managed according to the Egyptian Ministry of Agriculture and Land Reclamation (EMALR) recommendations for cultivating faba bean on newly reclaimed soils, taking in mind the stated treatments. Prior 15 days of sowing, the surface layer of plots was mixed with superphosphate and compost. Directly before sowing, the seeds of faba bean (*Vicia faba* L., Giza 716) were inoculated with *R. leguminosarum* and*P. Megaterium* using gamma radiated vermiculite as a carrier taking in mind the stated treatments. At ten days after sowing, all treatments received an activation dose (47.6kg N ha^{-1}) as232.2kg ha^{-1} of ammonium sulfate (20.5% N).For AE application, crude-AE was diluted to 60% by addition of 120 ml of crude-AE to 200 L of tap water drenched by hand sprayer at 476 litersha^{-1}repeated three times after 15, 30 and 45 days from sowing as recommended by Altai, et al. (2019).After 35 days from sowing,119 kg ha^{-1}potassium sulfate (48% K_2O) was added.

After 60 DAS, samples consisting of five whole plants with their rhizosphere soil were randomly uprooted from each plot at the vegetative stage. Each plant was separated into rhizosphere soil, shootand root materials to measure some biological, biometrical or physiological characteristics. After 120 days of planting, the crop of each plot was harvested above 5 cm of the soil surface to quantify yield and yield components at the harvest stage.

For rhizosphere soils, the total count was performed using a spread plating method on soil extract agar. The microbial counts were estimated as a colony-forming unit (CFU), per gram of soil, after incubation for 10 or 14 days at 28°C for total microbial or phosphate dissolvers count. Halo zones formed by the action of phosphate dissolvers were estimated on modified Bunt and Rovira's agar medium using the method of Louw and Webley (1958). Phosphomonoesters and dehydrogenase enzymes were assayed calorimetrically using *p*-nitrophenyl phosphate disodium hexahydrate (PNP) and 2,3,5-triphenyl tetrazolium chloride (TTC), as chromogenic substrates, in the rhizosphere according to procedures of Tabatabai and Bremner (1969) and Casida et al., (1964), respectively.

To estimate root surface area, ml equivalent of 0.01N NaOH was used to quantify the absorbed amount of 1N HCl on roots(Carley and Watson 1966). Total leaf chlorophyll was extracted using 80% acetone and calculated according toLichtenthaler (1987).Nutrient concentrations were measured in the wet digested material using concentrated sulphuric acid and perchloric acid as a catalyst. Total nitrogen was determined using the micro-Kjeldahl method while phosphorus was determined spectrophotometrically using ammonium molybdate and stannus chloride reagents, as stated by Page, et al. (1982).The protein was estimated by multiplying N% by 6.25,according to Mariotti et al., (2008).

1.6. Statistical analysis

The obtained data were statistically analyzed as advocated by Petersen (1994). Duncan’s Multiple Range test (DMRT) at 0.05 significant level was used as a post hoc test to measure specific differences between means. Manipulation and statistical analysis of data were processed using CoStat version 6.451 software.

2. Results and Discussion

2.1. Rhizosphere characteristics

The rhizosphere biological characters of faba bean cultivated on calcareous soil inoculated with*P. Megaterium* or treated with Azolla extract with or without compost under lesser P-fertilizer were represented by the total count of aerobic bacteria and phosphate dissolver microorganisms as well as related activities through quantification of dehydrogenase and phosphomonoesters enzymes. The data of total microbial and phosphate dissolvers counts are depicted in Figure 1. The application of compost as organic matter extremely raised the microbial count by 24.78 and 20.08 % and phosphate dissolvers by 42.48 and 25.09 % over non-applied compost treatments in 1^{st} and 2^{nd} seasons, respectively. Fertilization with a half dose of phosphorus combined with Azolla extract and *P. megaterium* into compost manured soil maximumly stimulated the proliferation of phosphate dissolvers to achieve 26.25 X 10^4 and 34.02 X 10^4 CFU/g soil during 1st and 2nd seasons, respectively. The fertilization with the full dose of P-fertilizer in the absence of compost hindered the reproduction of both bacteria and phosphate dissolvers. Under current treatments on calcareous soil, the rhizosphere of faba bean harbored about 0.26-0.37% of total counted bacteria (Figure 2). With compost addition under half dose of P-fertilizer, individual inoculation with *P. megaterium* or application of Azolla extract increased the ratio of phosphate dissolvers to total bacteria to record 0.341or 0.336%, respectively. The increment was maximized when they were applied together (0.372%) to exceed the double dose of P-fertilizer (0.313%). The simultaneous increase in both microorganisms' classes due to compost addition may indicate the enhancement influence of organic matter toward phosphate dissolvers allowing other microbial communities to capture the solubilized P and other released nutrients and vis versa for more growth. On another view, Kucey (1983) suggested a possible shortage of carbon may result in a lack of significant relationship between phosphate solubilizers and available-P because they solubilize P constitutively, not inducible or that microbial numbers reflect native-P status, not present P status. According to Kucey (1983), despite in the phosphate-solubilizing bacteria and fungi constitute about 0.5 and 0.1% of total bacterial and fungal populations, respectively, the fungi solubilize an average of ten times more P than the bacteria. The phosphate sources, including mineral, organic or combination, could explain 39.1% and 45.77% of the bacteria and fungi variability, respectively (Gumiere et al., 2019). Also, phosphorus levels in soil, including excess or deficient levels, were identified to induce shifts in soil microbial communities (Ducousso-Detrez et al., 2022). So, our results are consistent with Zainuddin et al., (2022) for the integration of biofertilizer with a low rate of chemical fertilizer, but inconsistent with Emam et al., (2020) because the highest CFU counts of phosphate solubilizing bacteria in maize rhizosphere treated with the mineral fertilizers.

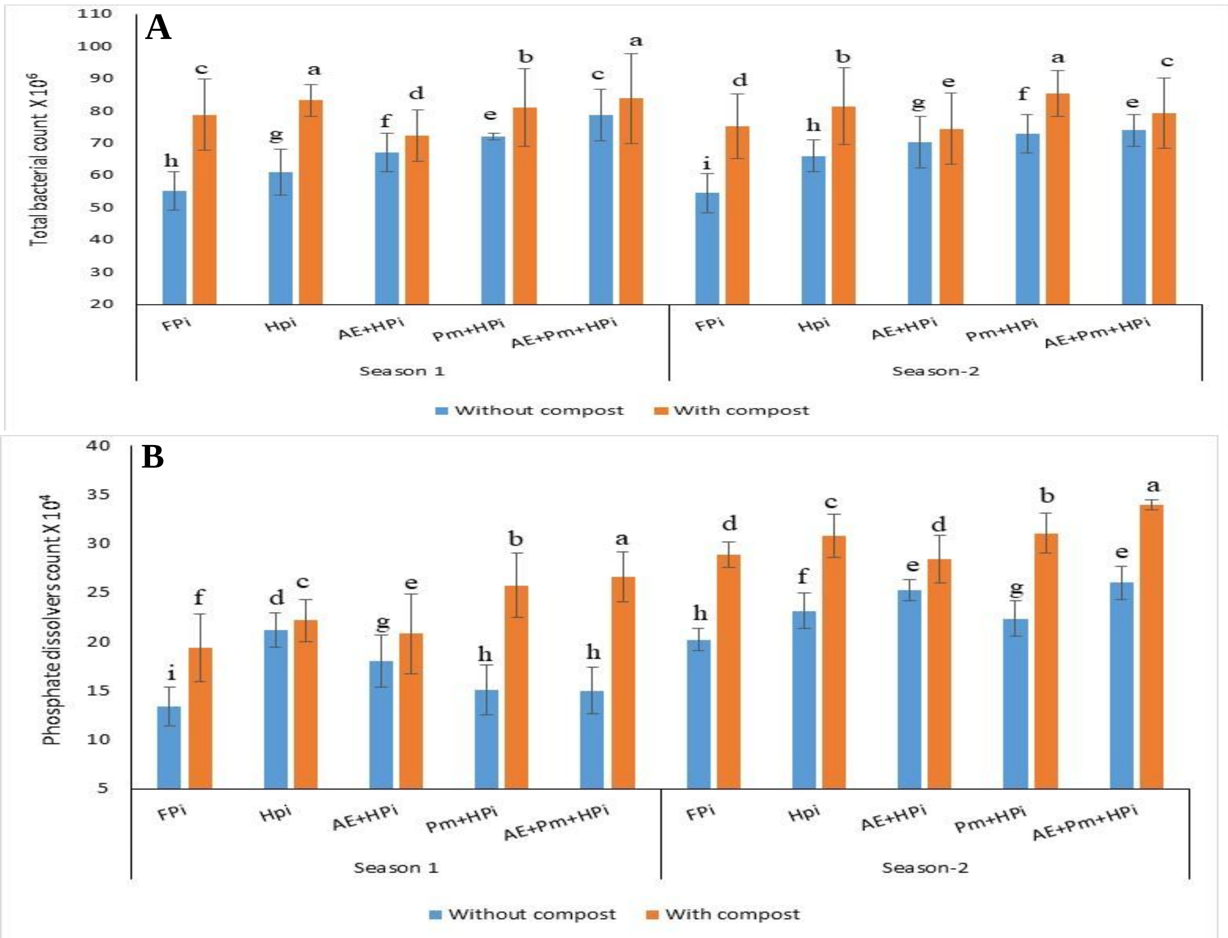

Fig. 1. Total count of rhizosphere bacteria (A) and phosphate dissolvers (B) as affected by different P-activators under less P-fertilizer on calcareous soil. FPi=full dose of P-fertilizers; HPi=half dose of P-fertilizer; AE= Azolla extract; Pm= *P. megaterium*.
*Non-significant difference between means of treatments was indicated with similar letters.

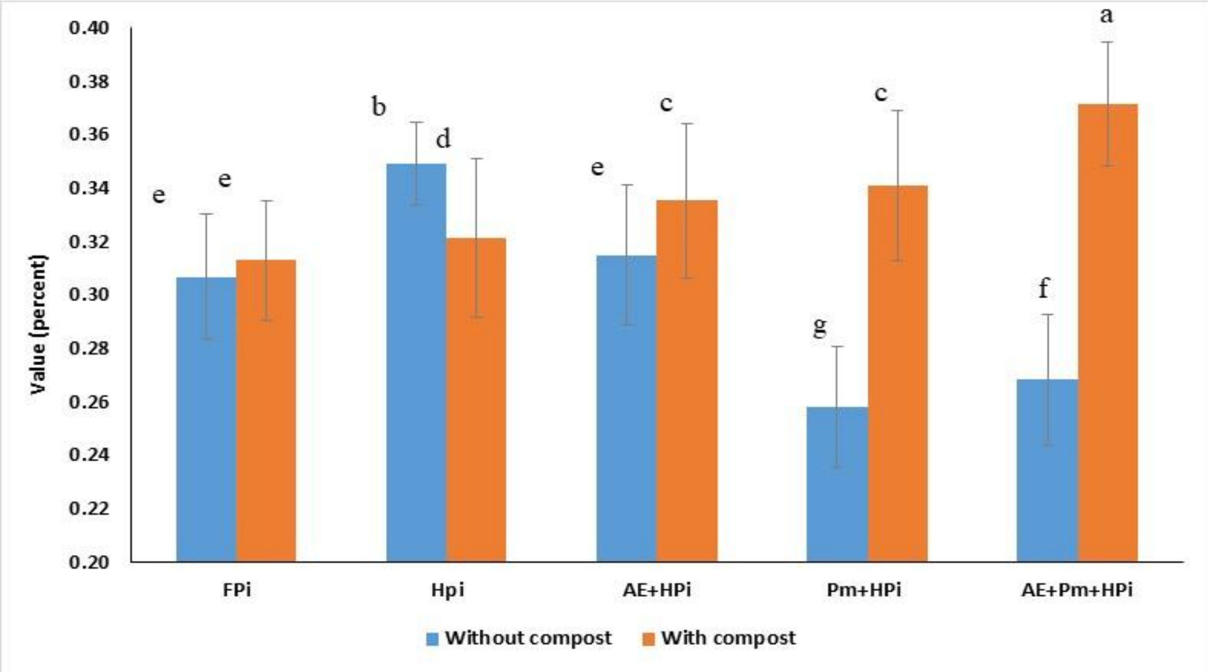

Fig. 2. The estimated percent of phosphate dissolvers in the rhizosphere of faba bean cultivated on calcareous soil under stated treatments. FPi=full dose of P-fertilizers; HPi=half dose of P-fertilizer; AE= Azolla extract; Pm= *P. megaterium*.

Regarding certain assayed enzymes, the data in Table 4 reveal that the impact of organic matter had been reflected on the overall activity of microorganisms and, specifically, on the solubilization of organic phosphorus as indicated by jumping in the values of dehydrogenases and phosphatases, respectively due to compost addition. The increase in dehydrogenase activity does not exceed 4.6 and 2.77% for the two growing seasons. In comparison, the per cent increases ranged from 107.46 – 125.37% for acid phosphatase and 66.21-91.33% for alkaline phosphatase during growing seasons. The reduction in mineral P-fertilizer hindered the overall activities below the recommended dose, but the application of Azolla extract and *P. megaterium* superbly raised such reduction by 7.62 and 7.76% over the recommended dose during the 1st and 2nd seasons, respectively. Dehydrogenases are exoenzymes that intervene in most soil processes by sharing in metabolic pathways of soil microorganisms giving a comprehensive idea about the potential of soil to harbour biochemical activities that are crucial to soil fertility (Das and Varma 2011). The presence of compost as organic matter contains readily metabolizable compounds that potentially act as energy sources for microorganisms along with the possibility of having stabilized enzymes leading to a rise in the activity of dehydrogenase enzyme (Bueis et al., 2018), especially under the current situation of calcareous soil. The jumping in enzyme activities may indicate the easily available nutrients, organic matter and growth factors associated with Azolla extract as well as successful colonization of *P. megaterium* within the faba bean rhizosphere. Such findings are in harmony with the results of Joergensen et al., (2010), in which a higher dehydrogenase activity is always found with organic fertilization than with balanced NPK fertilization.

Furthermore, dehydrogenase activity is usually low in soils with high amounts of NPK fertilization because these enzymes are highly sensitive to the inhibitory effects associated with high mineral fertilization (Wolińska and Stępniewska 2012).

Applying phosphorus-based fertilizers has been shown to lead to seasonal variations in microbial activity as well as soil microbial biomass meanwhile long-term P-deficiency fertilization can significantly decrease dehydrogenase activity together with soil microbial biomass and bacterial diversity (Dincă et al., 2022). The ability of phosphatases to persist in soils for a long time through binding onto the soil humic and clay may give one possible explanation for these extremely high levels of enzyme activity due to their release during activity assays (Sinsabaugh 1994). The negative feedback mechanism suggested by Olander and Vitousek (2000) could explain the negative effect of a high dose of P on both phosphatase enzymes. According to the negative feedback, low nutrient supply induces enzymes toward mineralization against higher nutrients and suppresses enzymes toward ceasing mineralization, creating a primary way by which plants and microbes regulate the mineralization in response to nutrient supply.

2.2. Growth attributes of faba bean

Some morphological traits were measured for faba bean plants cultivated on calcareous soil after 60 DAS to assay the influence of the inoculation with P. megaterium and/ or Azolla extract in the presence or absence of compost under P-deficient conditions. The obtained results represented in Table 5 reveal a significant improvement due to compost application in the plant height, shoot dry weight, root surface area, and nodulation status during two grown seasons. The increased percentage for plant height, shoot dry weight and nodules dry weight in compost manured soil was recorded 2.44 - 3.84%, 15.35 - 16.54% and 19.21 - 8.81% over unmanured soil in 1st and 2nd seasons, respectively.

TABLE 4. Some enzymatic activities in the rhizosphere of faba bean plants as a result of *P. meg*aterium and/or Azolla extract with or without compost application under full and half dose of P-fertilizer in calcareous soil

Parameters / Treatments		Dehydrogenase (µg TPF/g/24 h)	Acid Phosphatase (mg PNP/g DW soil/hr)	Alkaline Phosphatase (mg PNP/g DW soil/hr)	Dehydrogenase (µg TPF/g/24 hr)	Acid Phosphatase (mg PNP/g DW soil/hr)	Alkaline Phosphatase (mg PNP/g DW soil/hr)
		Season 1			Season 2		
Organic matter treatments (Org.F)							
	Without compost	242.48b	14.89b	23.08b	258.38b	16.21b	26.01b
	With compost	253.44a	33.56a	44.16a	265.58a	33.63a	43.23a
Phosphorus treatments (PF)							
	Full dose of Pi	248.10b	20.80b	32.30b	260.15b	21.54d	31.54b
	Half dose of Pi (HPi)	232.00d	25.29a	32.79ab	255.00c	24.97c	33.97ab
	Azolla Extract (AE)+ HPi	242.21c	22.28b	31.91b	251.30c	22.86d	33.36b
	P. megaterium (Pm)+ HPi	250.50b	25.43a	34.80ab	263.10b	26.73b	35.73ab
	AE + Pm + HPi	267.00a	27.33a	36.33a	280.35a	28.52a	38.52a
Org.F X PF							
Without compost	Full dose of Pi	244.00de	9.41f	22.41c	261.80c	11.22f	21.22c
	HPi	230.00g	16.17e	21.17c	256.00de	16.16de	24.16c
	AE + HPi	237.42efg	13.75e	23.00c	248.10f	14.27e	25.27bc
	Pm + HPi	241.00def	15.09e	23.83c	256.00de	18.38d	27.38bc
	AE + Pm + HPi	260.00b	20.01d	25.01c	270.00b	21.01c	32.01b
With compost	Full dose of Pi	252.20bc	32.18bc	42.18ab	258.50cd	31.85b	41.85a
	HPi	234.00fg	34.40ab	44.40ab	254.00e	33.77ab	43.77a
	AE + HPi	247.00cd	30.81c	40.81b	254.50e	31.45b	41.45a
	Pm + HPi	260.00b	35.76a	45.76ab	270.20b	35.07a	44.07a
	AE + Pm + HPi	274.00a	34.65ab	47.65a	290.70a	36.02a	45.02a

*Non-significant difference between means of treatments was indicated with similar letters.

However, such a significant improvement did not include the branch number or root dry weight of grown plants. Despite vigorous was caused for plants by a full dose of P-fertilizer, the co-application of *P. megaterium*, Azolla extract and compost under reduction in P-fertilizer simultaneously enhanced plant height (100.30 and 101.31 cm), shoot dry weight (26.05 and 28.83 g plant-1), root surface area (122.50 and 139.18 ml NaOH), nodules number (39.67 and 48.33 nodule plant^{-1}) as well as nodule dry weight (493.30 and 588.70 g plant^{-1}) during growing seasons.

Increments may cause the tallest plants in cell elongation and multiplication as a result of enhanced nutrient uptake beside plant growth-promoting substances such as phytohormones and cytokinin-like substances resourced from compost (Miezah et al., 2008); Azolla extract (Maswada et al., 2021) and/or *P. megaterium*(Zhu, et al. 2018). The boosted shoot dry weight may be owed to a variety of direct and indirect mechanisms such as the production of growth-promoting substances and solubilization of minerals such as P, as previously stated by Korir et al., (2017). The positive action of *P. megaterium* on root morphology was previously proven by López-Bucio et al., (2007) as an inhibition in primary-root growth followed by better reformate of lateral-root number, growth and length, leading to modulating root architecture via auxin-and-ethylene independent mechanisms. Enhancement of nodulation may have resulted from boosting survival and colonization of rhizobium directed by compost as a soil conditioner for calcareous soil (Bezabeh et al., 2021); Azolla extracts as an organic matter enclosing growth factors(Bindhu 2013) or synergistic effects of co-inoculated *P. megaterium* as PGPR (Korir, et al. 2017). Along with enhanced roots, stimulating survival and growth of rhizobia in the soil allows the developing plant-root system to have a greater chance of being colonized by more rhizobia to exceed the number of potential colonization sites and more release available photosynthates from the healthy host. The obtained results are in harmony with Elkoca et al., (2007). On the other hand, phosphorous deficiency act to diminish photosynthates supply to the nodule causing reduce less bacterial growth and the total population of legume-nodulating microorganisms (Korir, et al. 2017).

2.3. Photosynthetic pigments

The photosynthesis of faba bean plants was assayed in terms of total chlorophyll pigment as tabulated in Table 6. Plants that received compost manifested a significant impact on the photosynthetic pigments of faba bean, caused increments in total chlorophyll by 5.22 and 4.42%, 1st and 2nd seasons, respectively, meanwhile, a slight variation between various P-activators, under half of P as well as a full dose of P, on the content of chlorophyll.

TABLE 5. Some morphological traits of faba bean plants as a result of *P. megaterium* and/or Azolla extract with or without compost application under full and half dose of P-fertilizer in calcareous soil

Parameters / Treatments		Plant height (cm)	Branches (No. plant⁻¹)	Shoot dry weight (g plant⁻¹)	Root dry wt. (g plant⁻¹)	Root Surface area (ml NaOH plant⁻¹)	Nodule number (No. plant⁻¹)	Nodule dry wt. (mg plant⁻¹)	Plant height (cm)	Branches (No. plant⁻¹)	Shoot dry weight (g plant⁻¹)	Root dry wt. (g plant⁻¹)	Root Surface area (ml NaOH plant⁻¹)	Nodule number (No. plant⁻¹)	Nodule dry wt (mg plant⁻¹)
		Season 1							Season 2						
Organic matter treatments (Org.F)															
Without compost		94.18b	3.22a	20.07b	3.48a	83.67b	27.73b	628.46b	93.76b	3.19a	22.73b	3.35a	84.67b	27.80b	678.80b
With compost		96.48a	3.42a	23.15a	3.22a	91.00a	45.73a	749.17a	97.36a	3.39a	26.49a	3.64a	104.34a	49.80a	738.63a
Phosphorus treatments (PF)															
Full dose of Pi		93.50d	3.20ab	25.53a	2.93b	95.00b	37.50c	652.00c	93.35c	3.16ab	27.96a	3.17a	107.50a	43.17a	661.00d
Half dose of Pi (HPi)		89.15c	2.77b	17.00c	3.60ab	86.66c	33.83d	616.65	88.70d	2.77b	19.77c	3.70a	91.25c	33.83c	650.00e
Azolla extract (AE)+ HPi		96.50b	3.50a	19.50bc	3.87a	77.08e	33.50d	716.00b	97.05b	3.45ab	23.62b	3.73a	81.25e	34.33c	786.00a
P. megaterium (Pm)+ HPi		98.55ab	3.69a	21.98ab	2.98b	82.09d	40.17a	866.77a	99.10ab	3.65a	25.03ab	3.53a	87.91d	39.67b	782.57b
AE + Pm + HPi		98.95a	3.46a	24.04a	3.37ab	95.84a	38.67b	592.65e	99.60a	3.46ab	26.68ab	3.35a	104.59b	43.00a	664.00c
Org.F X PF															
Without compost	Full dose of Pi	92.40	3.03ab	25.00ab	2.40c	93.33c	19.33i	531.00h	91.40c	3.11a	26.79ab	3.67ab	95.00d	23.00g	552.00j
	HPi	87.40f	2.70b	15.03d	3.80ab	93.33c	28.33g	497.00i	86.50d	2.73a	18.65d	3.50ab	95.00d	26.33f	583.70i
	AE + HPi	95.20bcd	3.33ab	17.98cd	4.47a	78.33f	20.67h	586.30g	95.20b	3.21a	20.89cd	3.33ab	83.33f	20.33h	703.00g
	Pm + HPi	98.30ab	3.68a	20.32bcd	3.33abc	84.18d	32.67f	836.00c	97.80ab	3.60a	22.79bcd	3.00ab	80.00g	31.67e	816.00c
	AE + Pm + HPi	97.60abc	3.37ab	22.04abc	3.40abc	69.18h	37.67e	692.00f	97.90ab	3.31a	24.53abc	3.27ab	70.00i	37.67d	739.30e
With compost	Full dose of Pi	94.60cd	3.37ab	26.06a	3.47abc	96.68b	55.67a	773.00d	95.30b	3.20a	29.14a	2.67b	120.00b	63.33a	770.00d
	HPi	90.90e	2.84ab	18.98cd	3.40abc	80.00e	39.33d	736.30e	90.90c	2.80a	20.89cd	3.90a	87.50e	41.33c	716.30f
	AE + HPi	97.80abc	3.66a	21.02abc	3.27abc	75.83g	46.33c	845.70b	98.90ab	3.68a	26.36ab	4.13a	79.18h	48.33b	869.00a
	Pm + HPi	98.79a	3.70a	23.65abc	2.63bc	80.00e	47.67b	897.53a	100.40a	3.69a	27.27ab	4.07a	95.83c	47.67b	749.13b
	AE + Pm + HPi	100.30a	3.54ab	26.05ab	3.33abc	122.50a	39.67d	493.30l	101.30a	3.60a	28.83a	3.43	139.18a	48.33b	588.70h

TABLE 6. Total chlorophylls of faba been plants as a result of *P. megaterium* and/or Azolla extract with or without compost application under full and half dose of P-fertilizer in calcareous soil

	Parameters / Treatments	Total chlorophyll (mg g^{-1} FW) Season 1	Season 2
		Organic matter treatments (Org.F)	
	Without compost	1.15b	1.13b
	With compost	1.21a	1.18a
		Phosphorus treatments (PF)	
	Full dose of Pi	1.17c	1.15d
	Half dose of Pi (HPi)	1.10d	0.99e
	Azolla extract (AE)+ HPi	1.20b	1.20c
	P. megaterium (Pm)+ HPi	1.22a	1.22a
	AE + Pm + HPi	1.20b	1.20a
		Org.F X PF	
Without compost	Full dose of Pi	1.15d	1.12d
	HPi	1.02e	0.96f
	AE + HPi	1.18c	1.20b
	Pm + HPi	1.20b	1.21ab
	AE + Pm + HPi	1.18c	1.17c
With compost	Full dose of Pi	1.18c	1.18c
	HPi	1.18c	1.03e
	AE + HPi	1.22a	1.21b
	Pm + HPi	1.24a	1.24a
	AE + Pm + HPi	1.21ab	1.23a

Nevertheless, *P. megaterium* inoculation treatmentshowed a significant increase in the chlorophyll content especially in combination with compost. Furthermore, the co-inoculation with *P. megaterium* and addition of Azolla extract under a reduced dose of P enhanced the physiological status of plants in the absence of compost and maximized it with the presence of compost to almost emulating a double dose of P-fertilizer for indicated measures. Many mechanisms are excreted by *P. megaterium* to enhance photosynthesis in the plant, including phytohormones production, which play an effective role in the expression of photosynthesis-related genes and PSII damage repair mechanism imperatively considering these changes in the levels ofhormones interlinked with improved photosynthesis efficacy as previously stated by Akram et al., (2019). The simultaneous action of compost and *P. megaterium* to deliver nutrients and growth regulators for plants potentially succeeded to raised chlorophyll contents along with the stimulative influence of Azolla extract that was previously verified for maize plants sprayed with Azolla extract under nitrogen and water deficiencies (Maswada, et al. 2021).

2.4. Nutrients uptake

Nutrients uptake was quantified based on nitrogen (N), phosphorus (P) and potassium (K) in seeds, the obtained datais presented in Table 7. Compost addition significantly boosted N-uptake by 12.23 and 14.37%, P-uptake by 25.81 and 27.42% as well as K-uptake by 26.62 and 27.37% over non-received ones in 1^{st} and 2^{nd} seasons, respectively. A notable enhancement in N, P and K uptake in response to co-inoculation with *P. megaterium* and Azolla extract addition because they not only act as phosphate dissolvers but also as plant growth-promoting agents for improving root architecture to capture the nutrients from the soil solution as well as improve nodulation status for optimum nitrogen fixation resulting in higher nutrients content.

TABLE 7. Nutrients uptake of faba bean plants as a result of *P. megaterium* and/or Azolla extract with or without compost application under full and half dose of P-fertilizer in calcareous soil

	Parameters / Treatments	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})	K uptake (kg ha^{-1})	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})	K uptake (kg ha^{-1})
		Season 1			Season 2		
		Organic matter treatments (Org.F)					
	Without compost	81.17b	4.92b	13.15b	85.33b	5.07b	12.68
	With compost	91.10a	6.19a	16.65a	97.59a	6.46a	16.15
		Phosphorus treatments (PF)					
	Full dose of Pi	100.47a	7.08a	17.85a	111.34a	6.98a	17.45a
	Half dose of Pi (HPi)	71.44e	4.69c	12.10c	71.77e	5.03c	12.59c
	Azolla extract (AE)+ HPi	76.76d	4.74c	13.52bc	82.58d	5.04c	12.59c
	P. megaterium (Pm)+ HPi	86.63c	5.44b	14.60b	91.09c	5.79b	14.46b
	AE + Pm + HPi	95.40b	5.83b	16.42a	100.51b	5.99b	14.96b
		Org.F X PF					
Without compost	Full dose of Pi	95.17b	6.94ab	17.67a	109.11a	6.06c	15.15cd
	HPi	65.76f	4.09d	9.23d	62.34g	4.93d	12.31e
	AE + HPi	75.34e	4.12d	11.29cd	77.18f	4.71d	11.79e
	Pm + HPi	82.14d	4.63cd	12.58c	85.25de	4.71d	11.79e
	AE + Pm + HPi	87.45c	4.80cd	14.99b	92.76bc	4.94d	12.35e
With compost	Full dose of Pi	105.76a	7.21a	18.03a	113.56a	7.90a	19.75a
	HPi	77.12e	5.29c	14.98b	81.20ef	5.14d	12.86e
	AE + HPi	78.18de	5.36c	15.75ab	87.98cd	5.36cd	13.40de
	Pm + HPi	91.12bc	6.25b	16.62ab	96.93b	6.86b	17.14bc
	AE + Pm + HPi	103.34a	6.86ab	17.86a	108.26a	7.03b	17.58b

*Non-significant difference between means of treatments was indicated with similar letters.

Furthermore, the applied compost holds organic-P and inorganic compounds that could be converted to available forms for the plant by stimulating the action of Azolla extract for microbial activities leading to higher nutrient uptake. Such findings align with that of Khosravi et al., (2017) for imperative usage of biological fertilizers instead of chemical P fertilizers to reduce costs and environmental hazards. On the other hand, the addition of mineral phosphorus without an efficient P-activator, even in considerable amounts, tends to become less available to plants quickly, as previously achieved by Bezabeh, et al. (2021) for faba bean grown on calcareous soil.

Yield and yield components

Overall performance of different P-activators across two seasons for several pods per plant, the weight of 100 seeds, seed yield and seed content of protein was portrayed in Figure 3 to evaluate stated treatments on yield and yield components of faba bean. Despite the reduction in productivity of fab bean plants due to reducing the dose of P fertilizer to half, an individual application of P-activators relatively enhanced yield and yield components with superiority of *P. megaterium* followed by compost followed by Azolla extract, respectively but still less than a double dose of P-fertilizer. However, maximum productivity was achieved in response to the coaddition of all P-activators using the half dose of P-fertilizer to emulate what was attained by double dose. On the other hand, the responsibility for the performance of different treatments was varied depending on the yield component to outcome 12.67, 10.62, 11.25 and 10.40% of pods, 100 seeds wt., seed yield and content of seed protein, respectively, due to coaddition of all P-activators under half dose of p-fertilizer. The overall performance was estimated based on the values tabulated in Table 8, representing yield and some yield components of faba bean grown on calcareous soil using a full dose of P against a half dose with an application of P-activators viz. *P. megaterium* as P-dissolver and Azolla extract or compost as organic amendments. Except for seed protein percentage, all yield components significantly increased due to compost addition by 19.00-20.17%, 4.83-4.14% and 16.43-17.62% for the number of pods per plant, the weight of 100 seeds and seed yield, respectively, during two seasons. Furthermore, the effect of either an absolute full dose of P-fertilizer or an absolute half dose or combined with *P. megaterium* and/or Azolla extract was varied depending on the presence or absence of compost. The mentioned increases may be due to the improvement in vegetative growth and nutrient uptake by the action of *P. megaterium,* compost or Azolla extract as P-activator beside other integrated plant growth-promoting factors.

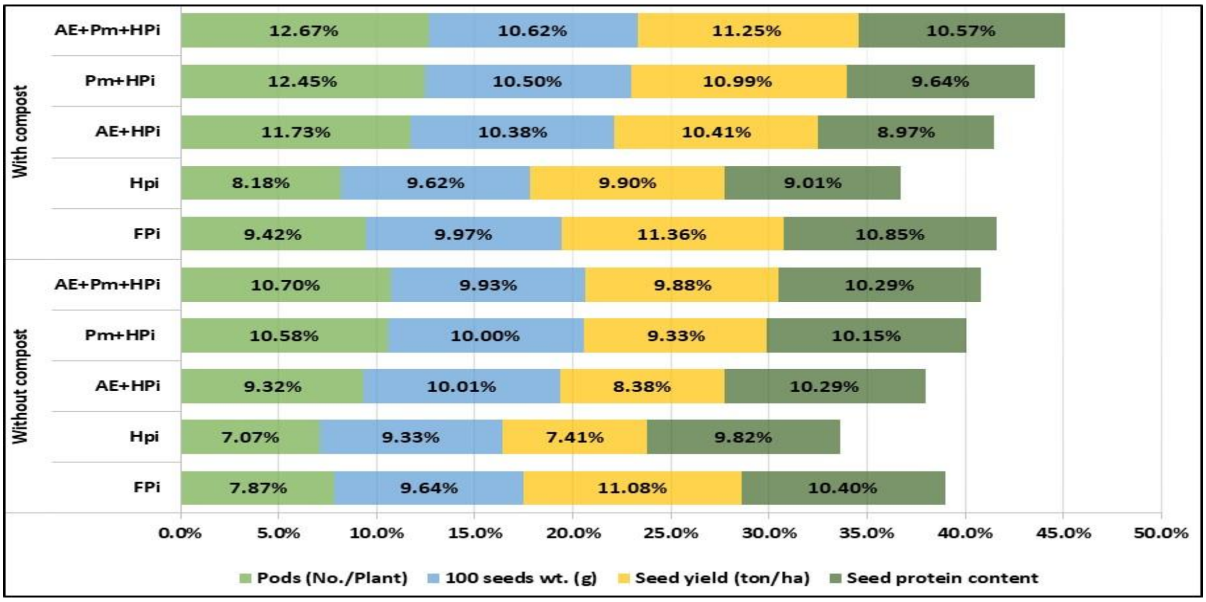

Fig. 3. Overall performance of different P-activators for yield and yield components of faba bean cultivated on calcareous soil

Table 8. Yield and some yield components of faba bean plants as a result of *P. megaterium* and/or Azolla extract with or without compost application under full and half dose of P-fertilizer in calcareous soil

Parameters / Treatments		Pods (No.plant^{-1})	100 seed weight (g plant^{-1})	Seeds yield(ton ha^{-1})	Seed protein %	Pods (No. plant^{-1})	100 seed weight (g plant^{-1})	Seeds yield (tonha^{-1})	Seed protein %
		Season 1				Season 2			
		Organic matter treatments (Org.F)							
	Without compost	14.00	77.80b	2.07b	24.61a	13.09b	78.32b	2.10b	25.46a
	With compost	16.66	81.56a	2.41a	23.52a	15.73a	81.56a	2.47a	24.68b
		Phosphorus treatments (PF)							
	Full dose of Pi	13.53c	78.01b	2.52a	24.98ab	12.19c	78.55c	2.56a	27.24a
	Half dose of Pi (HPi)	11.62d	75.80c	1.97d	22.91c	11.07d	75.46d	1.94d	23.35b
	Azollaextract (AE)+ HPi	16.36b	81.16a	2.10c	23.11bc	14.96b	81.55b	2.15c	24.22b
	P. megaterium (Pm)+ HPi	17.51a	81.55a	2.28b	23.94abc	16.74a	82.46a	2.32bc	24.65ab
	AE + Pm + HPi	17.66a	81.90a	2.35b	25.37a	17.10a	81.69b	2.43ab	25.90ab
		Org.F X PF							
Without compost	Full dose of Pi	12.25d	76.79d	2.48ab	24.05abc	11.15de	77.12e	2.53a	27.06a
	HPi	10.90e	74.18e	1.72c	24.05abc	10.13e	74.69f	1.63e	24.20ab
	AE + HPi	14.31c	79.12c	1.89bc	25.04ab	13.42c	80.61c	1.90d	25.52ab
	Pm + HPi	16.13b	79.68c	2.07abc	24.88ab	15.33b	79.99cd	2.15c	24.97ab
	AE + Pm + HPi	16.42b	79.22c	2.19abc	25.01ab	15.41b	79.21d	2.28bc	25.57ab
With compost	Full dose of Pi	14.80c	79.22c	2.55a	25.92a	13.22c	79.99cd	2.59a	27.41a
	HPi	12.33d	77.41d	2.22abc	21.77cd	12.01d	76.22e	2.26bc	22.49b
	AE + HPi	18.41a	83.19b	2.31abc	21.17d	16.49b	82.49b	2.40abc	22.92b
	Pm + HPi	18.88a	83.41ab	2.48ab	23.00bcd	18.15a	84.17a	2.49ab	24.34ab
	AE + Pm + HPi	18.90a	84.57a	2.51a	25.73a	18.78a	84.94a	2.58a	26.22ab

*Non-significant difference between means of treatments was indicated with similar letters.

Also, the sustaining action of compost may be indirectly on biological conditions of soil or directly for grown plants basically cultivated on calcareous soils. Among other functions, phosphorus plays a vital role in root development and nodulation, with legume crops which particularly have a higher demand for phosphorus to perform the metabolic activities connected to BNF in nodules and resource large requirements of energy in the form of ATP (Sulieman and Tran 2015). Our findings are consistent with Bezabeh, et al. (2021) and previously confirmed by Houassine et al., (2019) for faba bean and by Rotaru and Sinclair (2009) for soybean.

3. Conclusions

The stimulation effect of applied *P. megaterium,* Azolla extract and organic matter is a product of various impacts on total microbial count and phosphate dissolvers and their enzymatic activities indicates a potential success of P-activators on calcareous soil. Such impacts directed plants to enhance their vegetative growth, which ultimately incarnated quantitative and qualitative improvement of yield and yield components of crop along with the environment. Despite the positive action of Azolla extract, it was not satisfied with the individual impact on the plant growth as P-activator on calcareous soil to produce a higher yield compared to compost application or coinculation with *P. megaterium*. So, more research on Azolla extracts as either biofertilizer or P-activator is required in terms of cost, formulate and rates, other crops, or soil types.

Acknowledgement

Great gratitude from all authors toward Prof. Farid Sh. Badawi for his efforts in technical reviewing the manuscript of the research article and his appreciated advice.

Conflicts of Interest

The authors reported no potential conflict of interest.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

All authors have been shared in conceived, experiment design, assay and analysis, recording data, and writing and editing of the final manuscript.

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