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The effect of organic amendments on soil microbial activity and yield of faba bean (*Vicia faba*) inoculated with *Rhizobium leguminosarum*

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ABSTRACT

A two year (2021 and 2022) field experiment was conducted to study the effect of organic amendments on soil microbial activity and yield of faba bean (*Vicia faba* L.) inoculated with *Rhizobium leguminosarum* at Savadkuh in the north of Iran as a factorial experiment based on a randomized complete block design with 3 replications. The treatments included seed inoculation at two levels (inoculation with *Rhizobium leguminosarum* bv. *Viciae* and non-inoculation) and fertilization at 5 levels [cattle manure (CM) @30 tonnes/ha, vermicompost of manure (VM) @20 tonnes/ha, vermicompost of *Azolla* (VA) @20 tonnes/ha, chemical fertilization based on soil analysis (NP), and control]. Present study found that the soil microbial biomass carbon (C) increased over time until 120 days after emergence (DAE). The maximum microbial biomass-C (441 mg/g soil) and bacterial count (545 × 10^5 cfu/ml) were obtained from the inoculated VA treatment. The highest yields of pods (14400 kg/ha) and seeds (1266 kg/ha) were obtained when the use of vermicompost was accompanied by inoculation of *R. leguminosarum*, so that in plants without inoculation, the use of vermicompost increased the yield of pods compared to the NP by 34% and compared to CM, it increased by 44%, but this increase was 39% and 19% for inoculated plants compared to NP and CM, respectively. Although the seeds of vermicomposted plants showed lower iron (Fe) content, they showed higher nitrogen (N), phosphorus (P), copper (Cu) and zinc (Zn) content than other plants. In total, the results showed a significant improvement in soil microbial activity, absorption of nutrients and bean yield by using vermicompost (especially VA) under *R. leguminosarum* inoculation. Therefore, in the low-fertile soils of Iran, the combined use of high-quality vermicompost together with *R. leguminosarum* can strengthen soil microbial activity and bean production and be a suitable alternative to chemical fertilizers.

Keywords: Faba bean, Microbial biomass, Pod yield, Rhizobium, Vermicompost

After cereals, legumes are the main source of food for humans, especially for protein supply. Faba bean (*Vicia faba* L.) is an important legume that is consumed as a rich source of P, Mn, and Fe with 25 to 38% protein content in developing countries. The cultivation area of faba beans amounts to about 2.94 million ha in the world (Dhull *et al.* 2022). Iran ranks 12th globally by producing over 46,000 tonnes of faba beans from an area of 36,000 hectare (ha), but its mean yield of 1278 kg/ha is lower than the mean global yield (Sheikh *et al.* 2022). An important factor in the low yield of crops in Iran's agricultural lands is the low-fertility of the soil and the lack of elements required by the plant. To compensate for this shortage, farmers use chemical fertilizers indiscriminately. This practice not only increases the cost of production, but also has negative effects on the microbial condition of the soil and the environment (Daylam *et al.* 2023).

The presence of organic matter in soil is a proper indicator of soil fertility, which is the result of the interaction of physical, chemical, and biological processes (Ghadimi *et al.* 2021). Organic matter is important for better soil fertility and structure, and overall soil health (Badawy *et al.* 2023). The superiority of vermicompost among organic fertilizers has been established for the long-term preservation of soil fertility (Hemati *et al.* 2022). Vermicompost production is an anaerobic process that is performed by a certain group of earthworms (*Eisenia fetida*) with the help of some soil-borne microorganisms, especially bacteria and actinomyces that contain easily available nutrients in the form of nitrates, exchangeable P, K, Fe, soluble Mg, and so on (Ozdemir and Turp 2023).

In leguminous plants like beans, faba beans, soybean, and chickpea, the symbiotic rhizobium bacteria that are established on the host plant's roots can fix atmospheric nitrogen and make it available to the plant (Allito *et al.* 2021). These bacteria are not only capable of fixing N_2 symbiotically but they can also be involved in dissolving nutrients like P, K, and Fe and produce phytohormones, vitamins, and siderophores (Ucar 2021). Allito *et al.* (2021) documented the positive effects of *R. leguminosarum* strains

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on increasing the growth and yield of faba beans in addition to 40–80 kg/ha saving in N application. Additionally, the importance of vermicompost in agriculture is related to its effect on the improvement of soil quality and its potential for the replacement of chemical fertilizers (Rasouli *et al.* 2022). Contradictory results have been reported about the effect of the combined application of rhizobial bacteria with organic and chemical fertilizers on some leguminous plants, but there are minor reports on the faba bean plant. Therefore, we studied the effect of *R. leguminosarum* bacteria and different organic fertilizers on the population and carbon biomass of soil microbes and nutrient absorption as well as grain yield of faba bean.

MATERIALS AND METHODS

Field and treatments: The present study was carried out at an experimental farm in Savadkuh, Mazandaran (48°20' E, 38°19' N, 460 m amsl) during 2021 and 2022. The study was a factorial experiment based on a randomized complete block design with 3 replications. The first factor was the faba bean symbiotic *Rhizobium leguminosarum* bv. *Viciae* at two levels (inoculation with the R-Etli strain and non-inoculation) and the second factor was organic fertilizers at five levels including cattle manure (CM) @30 tonnes/ha; vermicompost manure (VM) @20 tonnes/ha; vermicompost of *Azolla* (VA) @20 tonnes/ha; chemical fertilization based on soil analysis (NP); and control. The faba bean seeds used in the research were the Barakat cultivar. The *R. leguminosarum* was provided by the Soil and Water Research Institute, Iran. Each experimental plot was composed of six 5 m long sowing rows with an inter-row spacing of 40 cm and on-row spacing of 25 cm. After the plots were prepared, the organic fertilizers were distributed as per the sowing map and were completely mixed with the soil. To inoculate the seeds, the bacteria were added to the seeds (7 g of inoculants containing 10^7 alive cells/g). After the seeds were inoculated and dried, they were sown manually at a depth of 5 cm.

Preparation of vermicomposts: Paddy residues (rice straw and rice husk) were dried and chopped before use in the experiment. Cattle manure (CM) was obtained from a dairy unit in Rasht. Fresh *Azolla* was collected from paddy fields after harvesting the rice. After preparing the materials for the production of vermicompost of manure + rice straw mixture (VM), rice straw (one–fourth of the weight of manure) was added to manure and mixed, but for the production of vermicompost of manure + *Azolla* mixture (VA), *Azolla* (one-tenth of the weight of manure) was added to the manure and mixed. Vermicomposting was carried out in a vermireactor containing a very active population of the earthworm *Eisenia andrei*. The physicochemical traits of cattle manure and its vermicomposts are given in Table 1.

Microbial biomass-C and count of soil: The soil rhizosphere was sampled at five steps including 60 (T_1); 80 (T_2); 100 (T_3); 120 (T_4) and; 140 (T_5) days after emergence (DAE). Soil microbial biomass-C was detected by chloroform fumigation extraction method with fumigation at atmospheric pressure. The total population of the bacteria was measured using plate cultures and directly counting the bacteria (Kelly *et al.* 1999).

Yield and nutrient analysis: At the physiological maturity step, 1 m^2 was harvested, and grain number, pod number, and pod and grain yields were determined. To determine the total dry weight, the whole plant was oven-dried at 75°C for 72 h and weighed. In addition, the contents of N and P in the grains were detected by the Kjeldahl method and the vanado-molybdo colorimetric method, respectively (Jackson 1962), and the Fe, Zn and Cu contents of the grains were estimated by atomic absorption spectrometry (Shimadzu AA–6300, Japan) (Jones 1972).

Statistical analysis: Data were subjected to the analysis of variance (ANOVA) for statistical analysis. The significance of the treatment effect was determined by the magnitude of F–value ($P \leq 0.05$). When the F–test revealed the significance of the treatments, the means were separately compared using the LSMEANS method with LSD adjustment at P = 0.05. The statistical analysis of the results was performed by the general linear model (GLM) in the SAS (Ver. 9.2) software suite.

RESULTS AND DISCUSSION

Microbial biomass-C in the plant rhizosphere: The measurement of soil microbial biomass-C (Fig 1) showed that it increased over time in both years although decrease was observed in this trait after the T_4 stage in most treatments. In non-inoculated conditions, the soil microbial biomass-C was 38–450 mg/g in the first year and 45–330 mg/g in the second year, but they increased to 67–490 and 76–550 mg/g in the inoculated conditions, respectively. So, the inoculated treatments had higher biomass-C than the non–inoculated treatments in both years. Even in the treatment of non–inoculated B0, the biomass-C content increased from 14 in T_1 to 161 in T_4 whereas it increased from 23 in T_1 to 255 in T_4 in the inoculated one. The organic

Table 1 The physicochemical features and nutrient status of organic treatments

Parameter	Ash	OC	N	P	K	OM	C:N	Cu	Fe	Mn	Zn	*p*H	EC
			(g/kg)			(%)			(mg/kg)				(dS/m)
CM	197	402	16.8	9.4	10.4	412	24.4	142	215.4	109	184	8.22	1.08
VA	311	304	32.4	17.2	14.8	293	9.31	171	542.3	307	291	6.83	1.44
VM	441	318	18.25	4.15	13.7	316	16.5	175	416.6	248	355	7.55	1.126

CM, Cattle manure; VA, Vermicompost of Azolla; VM, Vermicompost of manure.

Table 2 Comparison of means for the interactive effect of amendments and inoculation with *R. leguminosarum* on measured traits

Year	*R. leguminosarum*	Amendment	Pod (No./m^2)	Grain (No./m^2)	Pod yield (kg/ha)	Grain yield (kg/ha)	Biological yield (kg/ha)	Grain N (%)	Grain P (mg/g)
2021		VM	28.46b	121.6b	8010b	960a	2660b	2.31a	300.0a
		VA	31.42a	160.3a	9150a	953a	2950a	2.58a	275.5b
	Non-inoculation	CM	27.76b	114.3b	7290c	843c	2550b	1.64b	212.2d
		NP	24.53c	116.2b	6920cd	901b	2530b	1.84b	225.5c
		Control	25.04c	101.0b	6600d	816c	2260c	0.46c	212.2d
		VM	39.79a	181a	13770a	1196a	2500a	1.38b	319.9a
		VA	36.66b	165b	13440ab	1203a	2150b	2.67a	296.6b
	Inoculation	CM	36.15b	159b	13200ab	1180a	2110b	0.73d	243.3c
		NP	33.03bc	149bc	12960bc	1086b	1700c	0.85c	300.0b
		Control	30.12c	135c	12400c	896c	1770c	0.83c	219.9d
2022		VM	29.33b	125.6a	8550a	1030ab	2400a	2.23a	293.3a
		VA	35.50a	127.3b	8590a	1050a	1890b	2.47a	286.6a
	Non-inoculation	CM	28.56b	122.3b	8490a	990ab	1500c	1.84a	191.1c
		NP	29.23b	126.3c	7480b	970ab	1330d	2.09a	242.2b
		Control	26.10c	106.6c	6520c	910b	1150e	0.79b	225.5b
		VM	46.31a	205.6a	15030a	1250ab	3100a	1.38b	351.1a
		VA	44.86a	21.3a	14640a	1330a	3060a	2.67a	312.2b
	Inoculation	CM	44.53a	192.6b	11970b	1190bc	2950a	1.17bc	250.0d
		NP	39.63b	180.6c	13140b	1190bc	2380b	0.73c	291.1c
		Control	36.46c	175.6c	11260b	1030c	2320b	0.83c	212.2e

Similar letter(s) in each column shows insignificance of the difference based on the LSD test. VM, vermicompost of manure; VA, vermicompost of *Azolla*; CM, cattle manure; NP, chemical fertilization.

treatments, especially the inoculated ones, outperformed the control and NP treatments at the T_4 and T_5 stages although non-inoculated NP at T_3 in the second year (Fig 1C) and inoculated NP at T_2 in both years (Fig 1B and 1D) exhibited higher biomass-C than some organic treatments (Fig 1A–D). Biomass-C was low at the early growth stages due to lower temperature and there was no significant difference between the treatments (Yadav *et al.* 2021). The interesting point is the higher biomass-C of the chemical fertilizer treatment versus the organic treatments at the T_2 and T_3 stages, which may be related to the faster microbial propagation of the bacteria by the chemical fertilizers and the low rate of the microbial decomposition of the organic matter and the application of nutrient compounds in the plant rhizosphere at these stages.

Soil bacterial count: The bacterial count was significantly affected by the interaction of bacteria × organic treatments. The comparison of means showed that the organic fertilizers increased the bacterial population in the non–inoculated plants by 22–32% and in the inoculated plants by 11–38% versus the control. The highest bacterial population was related to the treatment of VA in the inoculated plants (Fig 2). The high microbial activity of the soil can accelerate the soil nutrient cycle and help plant growth (Rasouli *et al.* 2022). There are reports showing the increase in soil microbial population and biomass-C by manure vermicompost (Ghadimi *et al.* 2021) and vermicompost of residuals (Basdemir *et al.* 2022), compared to chemical fertilizers.

Grain yield and yield components: The pod number, grain number, and pod and grain yield were significantly influenced by the interaction of year × bacteria × organic treatment. The comparison of means for the number of pods revealed that the treated plants by organic fertilizers significantly outperformed the control in both years in both non-inoculated (9.82–20.32% increase versus the control in the first year and 8.66–26.54% increase in the second year) and inoculated conditions (17.42–30.04% increase in the first and 35.31–48.67% increase in the second year). These plants produced 11.4–36.9% and 13.5–15.7% more grains number than the control in the non-inoculated conditions and 15.6–25.24% and 34.1–49.5% more grains in the first and second years in the inoculated conditions, respectively. They were superior to the chemical fertilizer (NP) treatment in both inoculation conditions. The highest number of grains in both years (205.6 and 181 grains/m^2

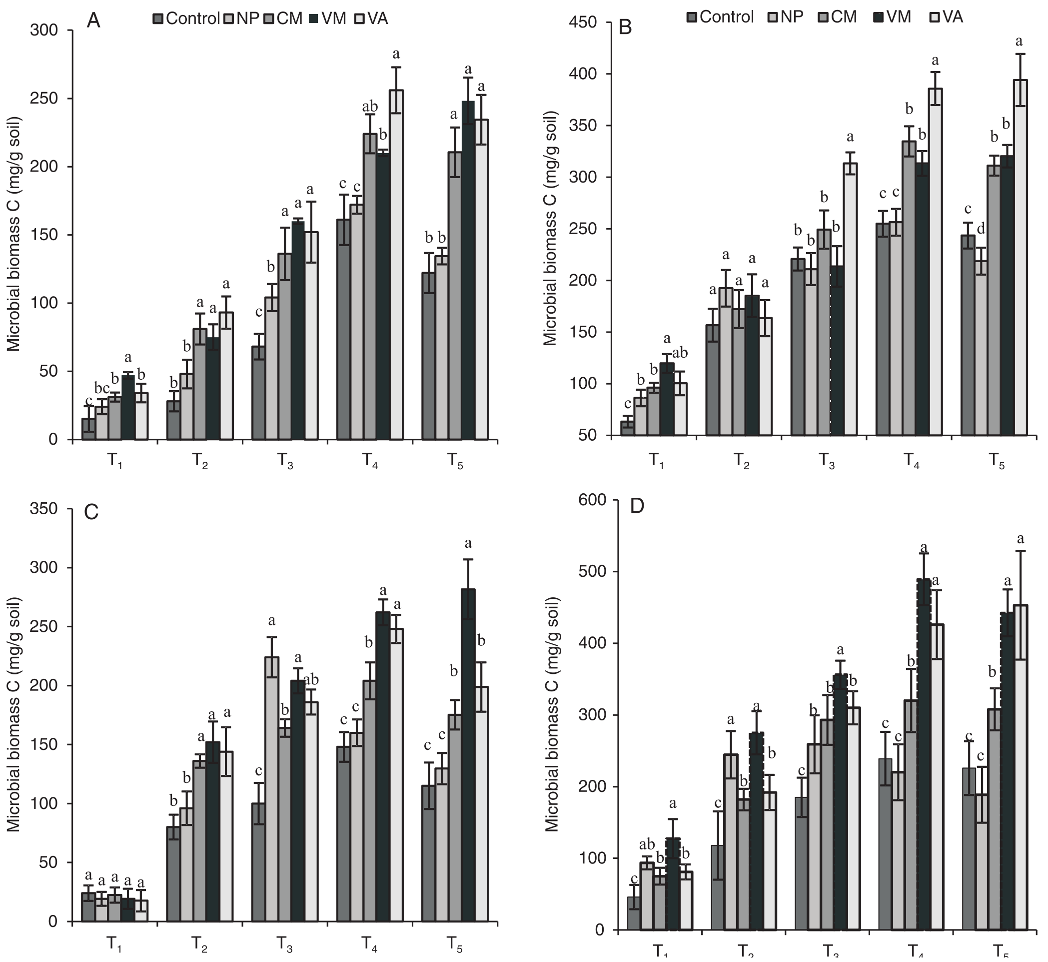

Fig 1 Mean value of the effect of organic amendment on changes in soil microbial biomass carbon during plant growth in the 2021 (A and B) and 2022 years (C and D). Parts A and C represent non-inoculation and parts B and D represent inoculation by *Rhizobium leguminazorom* (545 × 10^5 cfu/ml). Treatment details are given under Materials and Methods.

in the first and second years, respectively) was observed in the plants treated with the VA (Table 2). According to the comparison of mean biological yield, the MV increased this trait by 29.2% (first year) and 52.3% (second year) in the non-inoculated conditions and by 33.5% (first year) and 25.1% (second year) in the inoculated conditions versus the control. Compared to the control, the VA enhanced biological yield by 17.6% (first year) and 36.3% (second year) in the inoculated conditions and by 40.7% (first year) and 23.9% (second year) in the inoculated conditions (Table 2). Based on the comparison of means, the pod yield was the highest in both years when the inoculated plants were treated with the manure vermicompost (1377 and 1503 g/m^2 in the first and second years, respectively) and *Azolla* (1344 and 1453 g/m^2 in the first and second years, respectively). They were better than the control by 32.9% and 30.2% in the first year and 55.4% in the second year, respectively (Table 2). The comparison of means for the grain yield showed that the organic treatments increased this trait versus the control by 7.13–30.98% in the first year and 7.83–27.86% in the second year in the non-inoculated conditions and by 19.88–36.13% in the first year and 45.229–51.10% in the second year in the inoculated conditions. The maximum grain yield of the inoculated plants in both years was related to those treated with VM (119 and 125 g/m^2 in the first and second years, respectively) and VA (120 and 133 g/m^2 in the first and second years, respectively) (Table 2). This finding is consistent with the reports of Dahal and Ghosh (2022), and Lokhande *et al.* (2023). Atakli *et al.* (2022) reported that vermicompost increased pod number, grain number, and plant weight by producing humic acid and facilitating nutrient uptake by plants. Furthermore, the grain yield of faba beans is a function of pod number and grain number per pod. So, any factor that increases these two traits will increase grain and pod yields (Lokhande *et al.* 2023). The highest pod and grain yields were obtained from inoculation + vermicompost application, which supports the results of Ghadimi *et al.* (2021). It can be concluded that the chemical and physical properties of vermicompost (Basdemir *et al.* 2022) may increase N accumulation in plants by increasing nutrient retention capacity, growth-regulating hormones, and the activity of microorganisms (Ghadimi *et al.* 2021). The number of auxiliary branches, the number of pods, and the number of grains increase with the increase in photosynthesis and growth rates (Lokhande *et al.* 2023).

P and N contents of grains: The comparison of means for the N content revealed that the organic and NP fertilizers significantly outperformed the control in the non-inoculated conditions in both years, but in the inoculated conditions, the plants treated with VA and VM were superior to the control significantly. Additionally, the grains of the plants treated with VA exhibited the highest N content in both years and both inoculation conditions although they did not differ from some treatments significantly. The comparison of means for the P content showed that the inoculated plants had higher grain P content than the control by 9.94–31.2% in the first

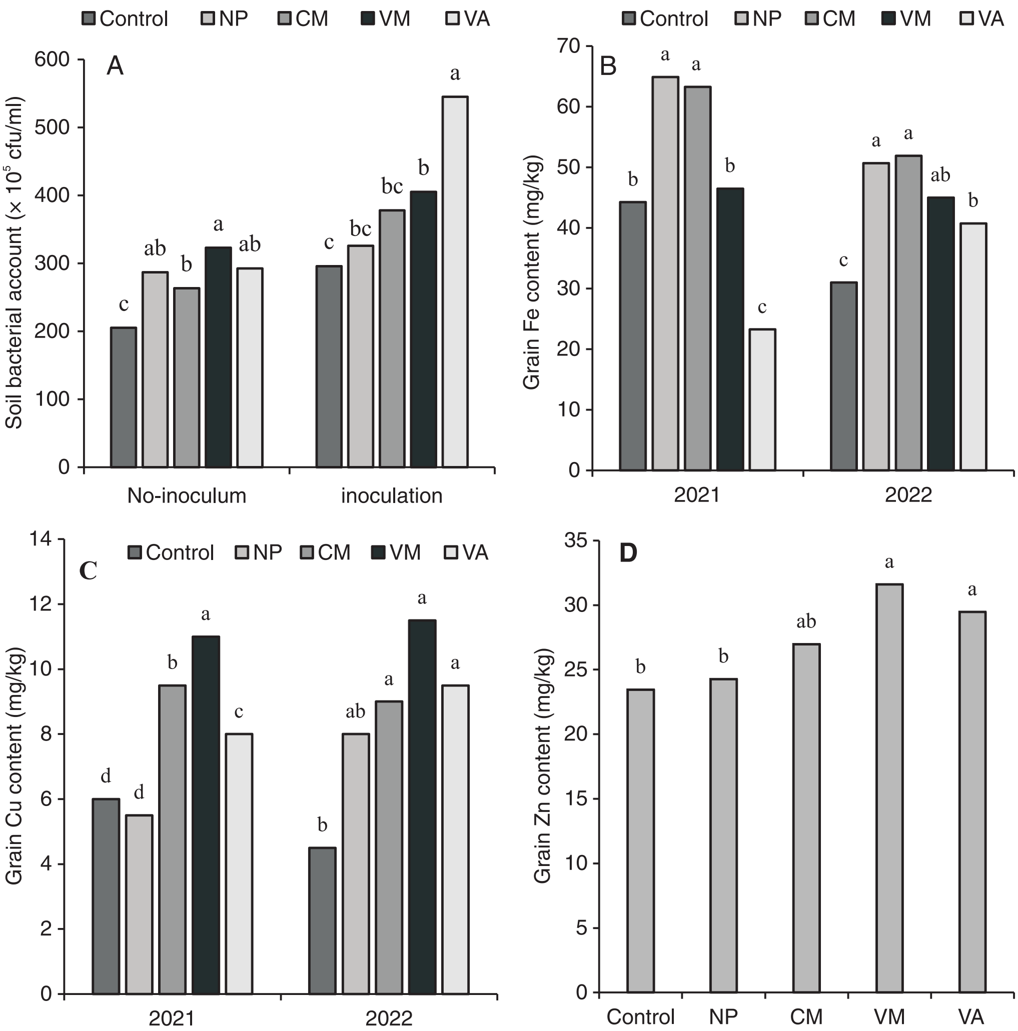

Fig 2 The comparison of means for the interaction effect of organic fertilizers × bacteria on the soil bacterial account (A), the interaction effect of year × organic fertilizers on Fe (B) and Cu (C), and the main effect of organic fertilizers on Zn (D). Similar letter(s) in each column shows the insignificance of the difference based on the LSD test at the P < 0.05 level. VM, vermicompost of manure; VA, vermicompost of *Azolla*; CM, cattle manure; NP, chemical fertilization.

year and 15.3–42.6% in the second year in all treatments, but the non-inoculated plants that were treated with CM and NP did not outperform the control (Table 2). The P content of vermicompost, which is gradually mineralized and becomes absorbable by plants, is effective in increasing P uptake by plants. Vermicompost contains higher quantities of nutrients, including N and other micronutrients and these nutrients are supplied to plants gradually (Ozdemir and Turp 2023). Owing to its high exchange capacity and oxidation potential, vermicompost contains elements that are readily available to plants, e.g. N in NO_3^- form (Tammam *et al.* 2023). P is also an immobile nutrient in the soil and is absorbed only when growing roots come in contact with organic and/or inorganic matter that contains an absorbable form of this nutrient. Faba beans can exert H^+ into the environment by N fixation. The acidification of the rhizosphere increases P solubility in soils with high *p*H, thereby increasing P uptake (Yadav *et al.* 2021).

Fe, Cu, and Zn contents of grain: The interaction effect of year × organic fertilizer on Fe and Cu, and the main effect of organic fertilizers on Zn were significant. The comparison of means of Fe content revealed that in both years, the plants treated with NP and CM outperformed the other treatments significantly. In the second year, all treated plants were significantly superior to the control plants. Comparing the treatments in the two years showed that NP was related to the highest grain Fe content in the first year (Fig 2). The comparison of means for Cu content displayed that the organic fertilizers increased this trait versus the control and NP in both years. The increase was 26.4–45.13% in the first year and 43.8–59.6% in the second year versus the control. The comparison of means showed that the grain Zn content increased by 13.3–28.8% by the organic treatments and 9.3–21.3% by the NP treatment versus the control. The highest grain Zn content was obtained from the plants treated with VM, showing insignificant differences from CM and VA (Fig 2). Among the fertilizer treatments, the VA and VM were related to the highest quantities of these elements. The advantage of vermicompost over other composts and organic fertilizers is the greater availability of nutrients (including N, P, K, Zn, Fe, Cu, and Mn), as well as the higher quality of its humic matter (Ucar 2021). Indeed, since organic fertilizers have high contents of organic matter and N, they improve the microbial activities of the soil, increase the availability of micronutrients to plants, and reduce soil nutrient loss (Rasouli *et al.* 2022). Consequently, soil nutrients increased in the treatments containing organic amendments versus chemical treatments (Amer *et al.* 2023) because vermicompost is a rich source of macro and micronutrients, vitamins, enzymes, and plant growth-promoting hormones. Since vermicompost can increase nutrient and water retention capacity, it can convert soil nutrients (Tammam *et al.* 2023), especially micronutrients like Cu, Fe, and Zn, into soluble forms that are available to plants by complexing them because it contains organic acids. It was observed that vermicompost increased Mn accumulation by accumulating Fe in the soil because there is a positive correlation between Mn and Fe. Mn and Fe availability in adequate quantities will improve the photosynthesis rate and plant growth (Basdemir *et al.* 2022).

Our study revealed that vermicomposting of plant and manure residues as organic fertilizer is an excellent way to increase the value of these residues. In both years, vermicompost, particularly *Azolla* vermicompost, increased the number of microorganisms in the soil, soil biomass–C, under the inoculation with *R. leguminosarum*, reflecting their positive effect on soil microbial activity. These changes improve the physical and biological properties of the soil and promote nutrient uptake by increasing their availability. The increase in the pod and grain yield and the grain N, P, Fe, Zn, and Cu contents reflect the positive effects of vermicomposts on the soil and plants. Additionally, a synergic effect was observed between *R. leguminosarum* and vermicomposts so that the highest grain yield in the first and second years, which were, on average, 5667 and 5081 kg/ha, respectively, were obtained from *Azolla* vermicompost + *R. leguminosarum*. It is suggested to survey the effect of vermicomposting on various organic materials that are rich and weak in nutrients, especially P and N, along with inoculation of plants or vermicompost enrichment with N_2-fixing bacteria in future studies.

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