

Multiplication of native endomycorrhizae isolated from arid soils on organic substrates in wheat plants (*Triticum aestivum*)

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Abstract

Purpose Organic residues of coffee pulp, sugarcane bagasse and mature bovine manure are a source of organic matter and nutrients for the multiplication of endomycorrhizae consortia. Therefore, the purpose of this research is to multiply the AMFs in such substrates to decrease soil and water pollution.

Method A pot experiment under greenhouse conditions was conducted in order to evaluate the influence of agricultural residues (C2-GEC, C3-PAR, C12-PRO, C14-ZAR) with different genera of endomycorrhizae isolated from semi-arid soils, 75 days after the crop was established. Agronomic characteristics and mineral content of N, K, Ca, Mg, and Fe in root and shoot were evaluated in wheat (*Triticum aestivum*).

Results Multiplication of endomycorrhizae was influenced by the residue type. Greater production of spores was observed in the coffee pulp, followed by the sugarcane bagasse, where a higher colonization was obtained in combination of C2-GEC and C3-PAR consortia. This consortia combination also was one of those that have increased the content of N, K, Ca, Mg, and Fe in roots and shoots of wheat.

Conclusion Combination of native endomycorrhiza substrates and consortia provides an alternative tool that benefits the physiology and nutrition of the plant to be used in sustainable agricultural production systems.

Keywords Coffee pulp, Sugarcane bagasse, Bovine manure, Mycorrhizal fungi, Organic waste

Introduction

The current world population demands the production of quality and more sustainable food with less damage to

the ecosystem. Nowadays, fertilizers and pesticides are used at a high level in the intensive production systems of plants. The symbiotic arbuscular mycorrhizal fungi (AMF) is an inexpensive and nondestructive method for achieving a high crop yield. It is based on the establishment of a sustainable agricultural system with low inputs, where mycorrhizal fungi can efficiently collect and use low-level soil nutrients and have a great impact on the functioning and stability of any ecosystem (Maggirwar et al. 2017).

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AMFs or endomycorrhizae are important components of soil microbiology that can be exploited to improve crop development and contribute to the establishment of more sustainable agriculture, this approach would reduce, or even eliminate, the need for chemical fertilizers and pesticides in organic agriculture (Spatafora et al. 2016; Benami et al. 2020).

This is possible because plants colonized by AMF exploit larger volumes of soil and have a higher absorption of nutrients, as well as a greater tolerance to drought, saline stress, and heavy metals. They also provide additional benefits by acting as plant growth promoting agents and as a biological control against phytopathogens (Zhou et al. 2020).

Agricultural food production generates a large amount of biomass that is not used, for example, stubble, husks, straws, and pruning remains, which are often burned in the open air since it is a fast and less expensive practice. However, this process provokes an adverse environmental cost reflected in an important source of greenhouse gas emissions such as carbon dioxide, in soil and water (Zeidabadi et al. 2018).

A possible sustainable alternative is to reuse these wastes as a fungi's substrate for multiplication. Fungi play an important role in the decomposition of organic waste and contribute significantly to the optimal bioconversion of agricultural waste to be reused (Ravindran et al. 2018). The most used AMF multiplication method is with the substrate-soil combination with a trap culture because it is less artificial and more profitable and capable of producing large amounts of highly efficient inoculants in the mycorrhizal colonization of plant roots (Selvakumar et al. 2018). Microbial pathways promote growth through the following mechanisms: nitrogen fixation, phosphate solubilization, iron uptake, modulation of the level of phytohormones, excretion of antibiotics, enzymes, and siderophores (Martínez et al. 2018). In the

multiplication of endomycorrhizae, a good substrate or carrier must have moisture retention capacity, easy processing, free of granular forming materials, easy to sterilize, adequate adhesion to the seeds, low cost, and adequate availability. In addition, it must guarantee the survival of the microorganisms used (Barazetti et al. 2019; Mujica-Pérez 2020). Some substrate components for the production of endomycorrhizae are perlite, peat, inorganic clay, zeolite, vermiculite, and sand (Mukhongo et al. 2016; Mahanty et al. 2017); whereas, others combine inorganic products with organic waste (Rodrigues and Rodrigues 2017), and only organic materials such as bagasse, waste, and sugarcane ash, rice straw, chickpea husk, among others (Cifuentes et al. 2013; Schlemper and Stürmer 2014; Sharma et al. 2015; Kadian et al. 2018; Kadian et al. 2019). The spore density and colonization of the roots in host plants are largely determined by the compatibility of the endomycorrhizae with the host plants, environmental factors, and the interactions between the endomycorrhizae and the chemical compounds produced by these plants. (Sarah and Ibrar 2016; Vital-Vilchis et al. 2020). According to the above, the objective of this research was to determine the influence of type of residue in the multiplication of native endomycorrhizae of semi-arid soils on agronomic parameters and mineral content in wheat plants (*Triticum aestivum*).

Methods and materials

Arbuscular Mycorrhizal fungi

The AMFs used in this study belong to the repository of the Microbiology Laboratory, Department of Horticulture, UAAAN, which are native consortia identified at different localities and wild hosts in semi-arid soils (Table 1) in Coahuila, Mexico (Fig. 1).

Table 1 Description of consortiums and sampling sites in semi-arid zones of Coahuila

Keycode	HMA Genus	Wild Host	Altitude (masl)	Soil textura
C2-GEC	<i>Glomus sp.</i> , <i>sp. 2.</i> , <i>sp. 3</i> ; <i>Claroideoglomus sp.</i> , <i>Acaulospora sp.</i> , <i>sp. 2</i> y <i>Gigaspora sp.</i>	<i>Fouquieria splendens</i>	1185	Sandy loam
C3-PAR	<i>Acaulospora sp. 1</i> , <i>sp. 2</i> , <i>sp. 3</i> , <i>sp. 4.</i> , y <i>Glomus sp.</i>	<i>Agave lechuguilla</i>	1 468	Sandy loam
C12-PRO	<i>Acaulospora sp. 1</i> , <i>sp. 2</i> , <i>sp. 3.</i> , y <i>Glomus sp. 1</i>	<i>Vachellia farnesiana</i>	447	Clay loam
C14-ZAR	<i>Acaulospora sp. 1</i> , <i>sp. 2</i> , <i>sp. 3</i> , <i>sp. 4</i> y <i>sp. 5.</i>	<i>Opuntia sp.</i>	354	Clayey

masl= meters above sea level

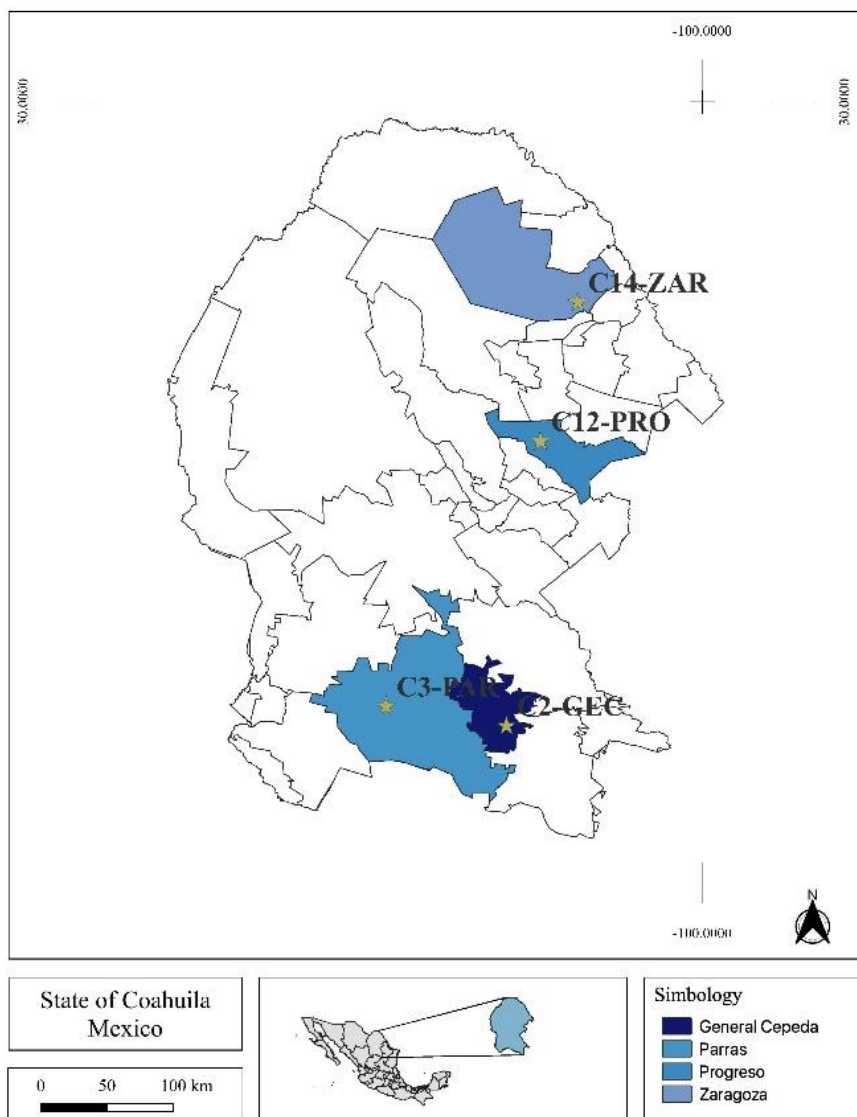


Fig. 1 Sampling sites in soils of Coahuila, Mexico

Establishment of the experiment

This research was conducted in a greenhouse with an average temperature of 27°C and relative humidity of 35% at Universidad Autónoma Agraria Antonio Narro (UAAAN), Saltillo, Coahuila, Mexico (25° 35' 63" North latitude, 101° 03' 49" West longitude, altitude of 1581 m). Polystyrene pots with one-kilogram capacity were used and filled with the following substrates: bovine manure (EB), coffee pulp (PC), and sugarcane bagasse (BC), mixed with sand (1:1 w/w) previously sterilized. (3 times) in an autoclave at 121°C for 15 minutes. Wheat seeds (*Triticum aestivum*) var. "Pelón Colorado" were sowed in each pot, which was inoculated directly with 50 g of soil (20 ± 3 spores) from each native consortium (Gerdemann and Nicolson, 1963) in each pot containing the mixed substrate and soil. The experiment was maintained under greenhouse conditions for 75 days, the water was applied to the plants manually of 0.25 to 0.75 L, too was applied a Steiner nutrient solution (1984), with phosphorus reduced to 75% during vegetative growth every third day.

Determined variables

After 75 days of growth, the following parameters were evaluated: plant height (AP), stem diameter (DT), root length (LR), fresh aerial biomass (BF) and root fresh (RF). Samples were dried in an oven for 48 h at 75°C and weighed to obtain the aerial biomass (BS) and dry root (RS).

AMF estimation

Colonization percentage was performed in triplicate in bean roots (Phillips and Hayman 1970), which were cut into 1 cm pieces and placed in test tubes. Then, 10 ml of KOH at 10% was added and placed in a water bath for 5 min at 70°C, adding H₂O₂ at 3% (1 min) and acidifying

with HCl at 2% (5 min). The roots were washed with distilled water between each reagent added and finally stained with Trypan Blue Solution (Thermo Fisher Scientific, Waltham, MA, USA) at 0.05% (10 min water bath or 24 h at room temperature). Subsequently, color was eliminated, and the roots were placed in a lactoglycerol solution. Once stained, they were placed on lamellae, using lactoglycerol, to be observed under an optical microscope at 40X, and recording mycorrhizal fungal structures (arbuscules, vesicles or hyphae) in cortical cells and root segments. Three fields of each segment were observed applying McGonigle et al. (1990) formula.

Substrates characteristics and mineral content

At the beginning of the experiment, the pH and electrical conductivity of substrates (EC) were determined using a potentiometer-conductivimeter (Hanna HI 98129, Woonsocket, RI, USA.) an aqueous medium of the substrate (1:2 v/v for pH and 1:5 v/v for CE). The N content in shoot and root samples in each plant substrate was determined by the micro Kjeldahl distillation (Kjeldahl, Novatech, Avante Tecnología) technique (Nelson and Sommers 1980). The P was determined using the Olsen et al. method (1954) using acid digestion in HNO₃ and HClO₄ (Murphy and Riley 1962) in a UV-visible spectrophotometer (Biomate 5, Thermo Electron Scientific Madison). The content of K, Ca, Mg and Fe were measured using the technique of Beatty and Kerber (1993) by acid digestion in an atomic absorption spectrum (Xplor AA dual, GBC Scientific Equipment). The substrate characteristics are shown in Table 2.

Statistical analysis

The study was established under a completely randomized experimental design with a factorial arrangement A

x B, where factors A are three substrates, and factor B are four consortia; each treatment was replicated four times, one plant per pot as an experimental unit. Analysis of variance one way and Tukey's mean comparison test ($P \leq 0.05$) were performed with SAS 9.2 software for Windows (SAS Institute Inc.).

Results and discussion

Substrate characteristics

Table 2 shows the chemical properties and mineral content of substrates. The pH is slightly alkaline with statis-

tical difference among them, being this component responsible for the availability and absorption of nutrients in the form of anions and cations by plants (Neina 2019). The EC was higher in the coffee pulp substrate; although, it did not exceed 2 dS/m, m, being an admissible value for wheat production (Miransari and Smith 2019). The content of N and Ca was not significant. The BC substrate showed the highest content in P and K; while the PC substrate was higher for Fe and EB for Mg content. Salinas et al. (2020) indicate that using organic substrates with a greater content of minerals increases the biomass in plants considerably.

Table 2 Chemical properties and mineral content of the evaluated substrates

Substrate	pH (H ₂ O)	EC dS/m	N %	P mg/kg	K g/kg	Ca	Mg	Fe mg/kg
Bovine manure	7.75a	1.16b	0.67a	21.12b	2.36b	3.41a	3.26a	6.13b
Coffee pulp	7.67ab	1.48a	0.59a	5.93c	2.82b	3.94a	2.99ab	12.07a
Sugarcane bagasse	7.37b	1.13b	0.51a	26.25a	7.19a	4.18a	2.58b	6.01b

Each value is the mean of four replicates. Values with same letter within a column are not significantly different (Tukey ($P \leq 0.05$))

Wheat parameters

The interaction between substrates and consortia was significant ($P \leq 0.05$) in the agronomic parameters evaluated (Table 3). The coffee pulp substrate (PC) resulted with the highest values in all variables, while the bovine manure substrate stands out in length and root dry weight. The cane bagasse substrate stands out in plant height and root length. Regarding C2-GEC consortia, in combination with EB, it is superior in root length, combined with BC it is superior in plant height, stem diameter, root length, and fresh biomass, while in PC it is superior in all variables. Also, with waste sugarcane compost mixed with soil up to 50–60 % of the tomato plant's height and weight had their maximum value (Cifuentes et al. 2013). The addition of organic onion residues compost obtained positive effects on the fresh weight of the

lettuce plant, recommending a dose of 8 Kg/m², which can replace urea as a chemical fertilizer (Pellejero et al. 2017), In addition, when combining onion residues and mature bovine manure, a positive effect on tomato plants and fruits equal to chemical fertilization is obtained (Pellejero et al. 2021). Kadian et al. (2019) indicate that the inoculation of *Glomus mosseae* using rice straw as a dry substrate and composted in different quantities, increases the morphological parameters in wheat plants, and Ingrassia et al. (2019) report similar results with the inoculation of AMF, observing an increase in the production of aerial and underground biomass of 4% and 11.3%, respectively, in *T. durum*. The positive effect on wheat plants inoculated with different native consortia is clear evidence of the beneficial mechanisms of these rhizospheric fungi, which have an important role in the synthesis of phytohormones which promote plant

growth and radical development (Shao et al. 2018, Zhang et al. 2019). An increase in plant growth can be explained by the ability of AMF to facilitate the absorption of water and nutrients from the environment at its root where it develops and by modifying morphology (Chen et al. 2016). On the other hand, the use of sub-

strates with adequate chemical characteristics, inoculated with native AMF, promotes greater root development and exploration of hyphae in the medium; moreover, the supply of water and nutrients increased in plant biomass and leaf area in wheat and corn plants (Coccina et al. 2019; Al-Maliki et al. 2021).

Table 3 Effect of different substrates and consortia on agronomic parameters in *Triticum aestivum* plants

Substrate	Consortia	Plant height (cm)	Stem diameter (mm)	Root length (cm)	Fresh biomass (g)	Dry biomass (g)	Fresh root (g)	Dry root (g)
Bovine manure	C2-GEC	32.7de	2.6d	20.7a	2.5d	1.31cd	2.5c	0.7f
	C3-PAR	31.7de	2.6d	19.5ab	2.7cd	1.6cd	3.5c	1.5cd
	C12-PRO	35.1bc	3.5cd	16.5bc	4.1bc	1.0de	3.4c	2.4ab
Coffee pulp	C14-ZAR	31.1e	4.5bc	18.2ab	4.0bc	1.6cd	5.5b	1.9bc
	C2-GEC	42.2a	6.4a	19.5ab	8.1a	4.5a	8.1a	2.7a
	C3-PAR	40.2ab	5.5ab	18.5ab	5.2b	1.1de	5.7b	0.9de
Sugar-cane Bagasse	C12-PRO	39.7ab	5.2ab	15.2d	2.7cd	1.0de	4.0bc	0.9ef
	C14-ZAR	42.2a	5.1ab	15.5cd	4.7bc	0.9e	4.2bc	0.9ef
	C2-GEC	42.2a	5.1ab	20.2ab	6.1ab	2.7b	3.5c	1.9bc
C.V. (%)	C3-PAR	34.5cd	4.6bc	16.5bc	5.2b	1.9bc	4.1bc	1.7bc
	C12-PRO	41.2ab	4.0bc	17.0ab	5.0b	2.1bc	3.2c	0.8ef
	C14-ZAR	38.1ab	4.5bc	16.2bc	4.2bc	1.79bc	2.5c	1.2cd
Tukey (P<0.05)	S	***	***	*	***	***	***	*
	C	**	Ns	***	**	***	**	**
	SxC	*	**	Ns	***	***	***	***

Each value mean of four replicates, * indicates the level of significance at ($P \leq 0.05$). Values followed by the same letter (s) within a column are not significantly different from each (Tukey ($P \leq 0.05$)).

Estimation AMF

Colonization in wheat roots reached 47% with the C2-GEC and C3-PAR consortia in combination with the cane bagasse substrate (Fig. 2A); while, the highest spore production was observed with the C2-GEC consortium in combination with the coffee pulp substrate,

resulting in an average of 199 spores in 100 g of sample (Fig. 2B).

These effects are supported by the data from Yang et al. (2018) who, when using compost, found greater root colonization and spore density in soybean plants inoculated with AMF. Kadian et al. (2018), used chickpea peel as substrate and detected greater root colonization

and number of AMF spores in sorghum, wheat, and barley plants.

The use of organic substrates efficiently promotes the growth of plants and the multiplication of AM fungi; in

addition, the members of the Gramineae family have a rapidly developing fibrous root system, which makes them ideal trap plants for the massive production of AMF (Tanwar et al. 2013).

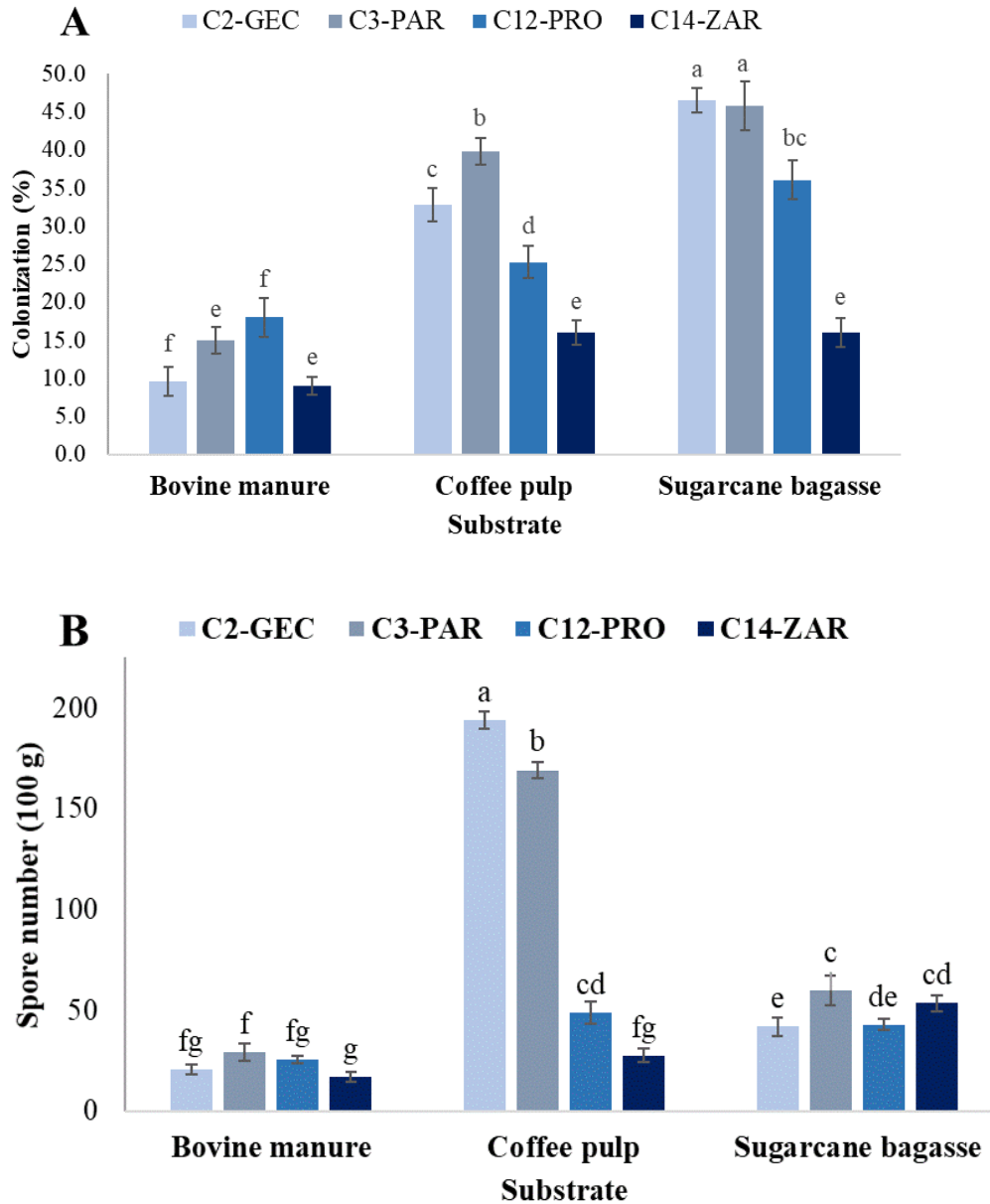


Fig. 2 Effect of different substrates and consortia evaluated in: **A.** Wheat root colonization; and **B.** Number of spores (100 g) sample

Root colonization with AMF is directly proportional to the root growth of the host plant; since the use of organic waste as a substrate increases the absorption of nutrients,

improves the root system, increases the spore population, and promotes plant growth (Chaiyasen et al. 2017). Therefore, an inoculum production system of AMF has

quality when, in addition to stimulating sporulation, it provides high efficiency (Coelho et al. 2014).

Mineral content in wheat

The absorption of minerals in roots and shoots in wheat plants was dispersed with significant differences between substrates and consortia. The consortium C2-GEC with substrate BC provoked the highest radical and aerial P content (Fig. 3B); likewise, the same effect was observed in N when inoculated in PC and BC (Fig. 3A), being statistically equal to the C3-PAR consortium. The latter treatment was also higher in K and Mg content when combined with EB substrate (Fig. 3C and 3E). Ca content increased with the same consortium in combination with EB and PC substrates (Fig. 3D). The highest content of Mg and Fe was observed in C3-PAR consortium tissue samples (Figs. 3E, F). These results allow the proposal that the type of AMF consortium plays an important role in the absorption of minerals in plants, especially wheat.

The application of different types of AMF and substrates in wheat plants showed favorable efficiency in the absorption of mineral nutrients. This effect may be attributed to the fact that the microorganisms reduce the distance between the cations and the plant, a condition that allows to capture macro and micronutrients for plant growth (Raklami et al. 2019; De Souza Campos et al. 2021).

The absorption of water and nutrients in plants inoculated with native endomycorrhizae is carried out through their fungal structures, altering the morphology the root and making the process of absorption more efficient (Bitterlich et al. 2018; Nyoman-Rai et al. 2020).

A high transfer of N influenced by AMF, from the substrate to the plants, stimulates the synthesis of amino acids and proteins, causing increases in the physiological

activity of the host plants; while the low content of elements in the root is attributed to a transfer from the root to the aerial part of the plant (Rollon et al. 2017).

In contrast to this investigation, Lazarevic et al. (2018), when evaluating the application of AMF in winter wheat, found an increase in the Ca content in shoots, but not in the Mg content, nor in the micronutrient content. The enrichment of beneficial microorganisms in organic substrates or compost found in our study is supported by the report of Andrade et al. (2021), who increased the mineral content in wheat and corn leaves by using a formulate containing AMF produced in brachiaria as a host plant.

Conclusion

In general, the combination of both (C2-GEC and C3-PAR) and organic residues (coffee pulp and sugarcane bagasse) substrates increases the plant biomass, and the absorption of nutrients by root and shoot from in the wheat crop. In addition, it is an alternative tool that benefits the physiology and nutrition of the plant to be used in sustainable agricultural production systems.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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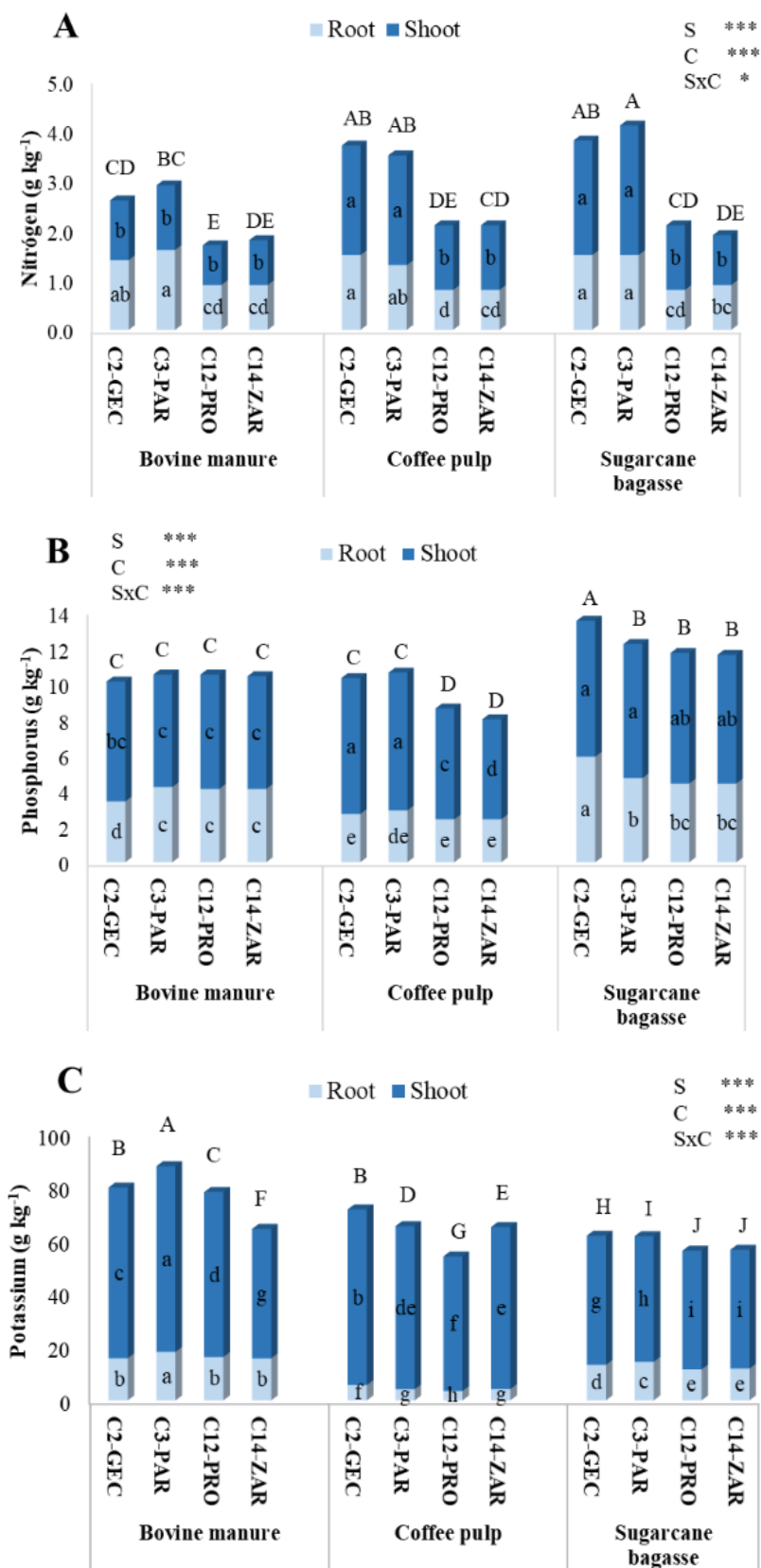
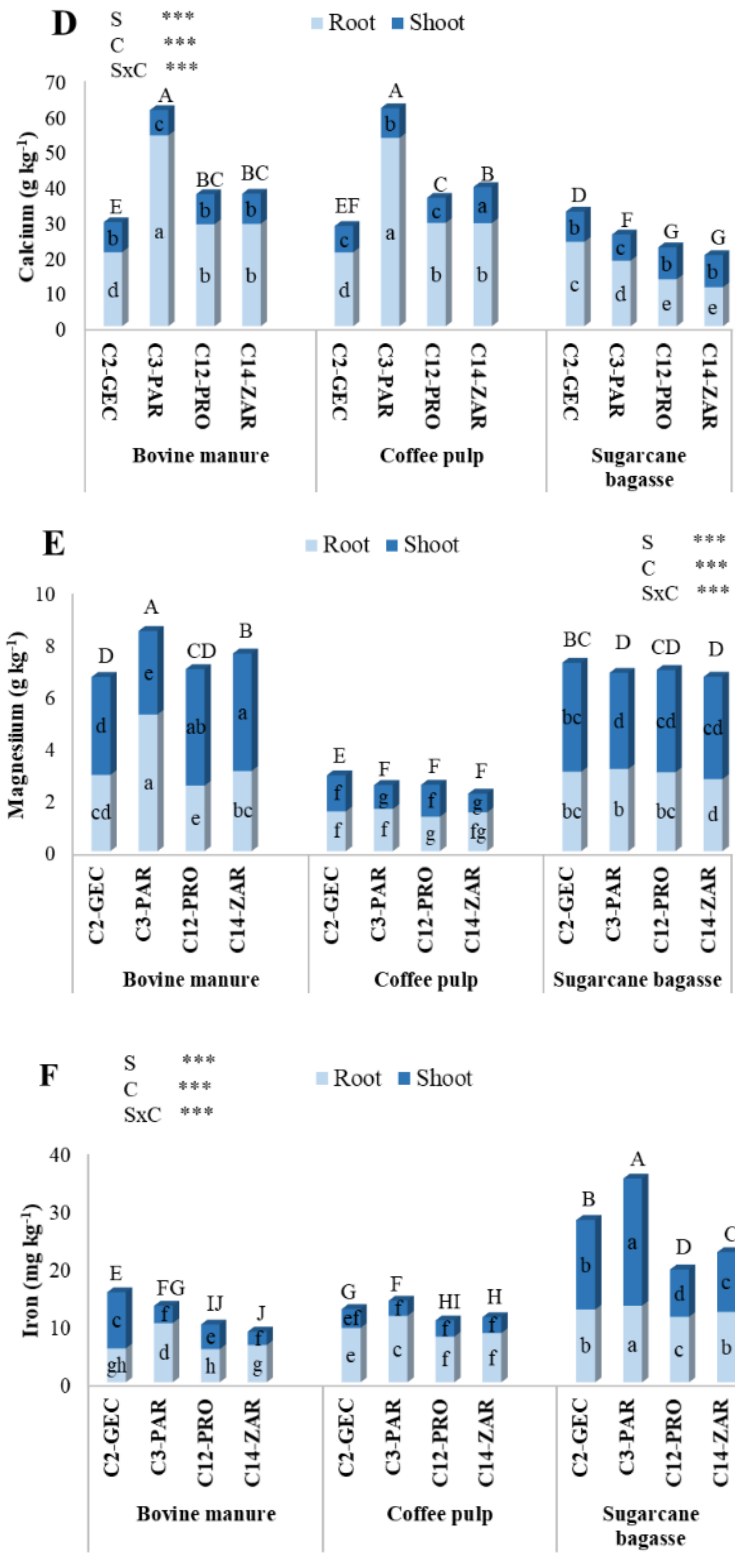


Fig. 3 Effect of different substrates and consortia evaluated on the mineral content in wheat roots and shoots: **A.** Nitrogen, **B.** Phosphorus, **C.** Potassium, **D.** Calcium, **E.** Magnesium y **F.** Iron



Continued Fig. 3 Effect of different substrates and consortia evaluated on the mineral content in wheat roots and shoots: **A.** Nitrogen, **B.** Phosphorus, **C.** Potassium, **D.** Calcium, **E.** Magnesium y **F.** Iron

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