MICROBIAL BIOMASS CARBON AND NUTRIENT AVAILABILITY AS INFLUENCED BY MYCORRHIZAE AND IRON-ETHYLENEDIAMINE TETRAACETIC ACID

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Nowadays, the scientific community is focusing on alternative environmentally friendly management approaches. Mycorrhizae has a lot of potential as an agrochemical solution. These effective eco-friendly parameters are gaining traction in soil sustainability and agricultural production restoration. The interaction of mycorrhizae with iron-ethylenediamine tetraacetic acid (Fe–EDTA) is, nevertheless, unknown. The effects of four treatments (control, mycorrhizae, foliar spray of iron, and mycorrhizae + foliar spray of iron) (C, M, F, and M+F) on soil respiration, microbial biomass carbon (MBC), metabolic quotient (qCO_2), infection rate, nutrients content, root weight and eggplant yield (*Solanum melongena* L.*)* were investigated. M had the highest soil respiration (233.20 mg CO_2-C g^{-1} day), infection rate (72 %), MBC (107.58 µg C g^{-1} soil), and microbial quotient values (8.52 %). Despite this, F had no effect on phosphorus content, MBC, or chlorophyll rate when compared to control. More specifically, the infection rate, qCO_2, microbial biomass, roots weight, nitrogen and phosphorus contents, but not iron content, were all associated to fruit yields. These findings showed that chelated iron application alone affected soil respiration but had no positive effects on MBC, whereas it affected MBC and qCO_2 when combined with mycorrhizal fungi, confirming a lower stress on soil microbes and implications for the accumulation of soil organic carbon in soils.

mycorrhizal fungi, MBC, qCO_2, soil respiration, chelated Fe (Fe–EDTA), eggplant yield

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INTRODUCTION

Iron (Fe) insufficiency is a widespread problem that limits plant production. Fe is essential for plant development and production (Colombo et al., 2014; Majeed et al., 2020). Fe can catalyze 140 enzymes, demonstrating that it is an appropriate cofactor for biochemical reactions (Brittenham, 1994; Kroh, Pilon, 2020). Subsequently, Fe develops chlorophyll, thylakoid synthesis, and chloroplast formation (Miller et al., 1995; Izadi et al., 2020). Higher soil pH in arid land (Al-Maliki et al., 2018; Al-Maliki, Bresam, 2020) might constrain Fe absorption by plant roots and restricts the availability of Fe to plant (Wiedenhoeft, 2006; Peleg et al., 2008). Foliar feeding is a low-cost, fast and easy approach with a high absorption rate that could be used instead of soil treatments to alleviate Fe deficiency in crop plants. Alvarez-Fernandez et al., (2004) studied the effect of foliar $FeSO_4$ on deficient pear trees, and it was concluded that foliar fertilization did not completely cure Fe deficiency, and that a supplemental strategy, such as Fe (III) chelate addition to the soil was required. The efficiency of foliar spray of Fe for enhancing plant crop is not consistent and could depend on Fe stability, Fe movement into leaf cuticle, mobility and translocation into leaf tissue (Schonherr et al., 2005; Fernandez et al., 2006, Rodriguez-Lucena et al., 2010; Malhotra et al., 2020). The major indications of iron-deficiency are yellow leaf and green veins net (Jones, 2020). Inoculation of mycorrhizal fungi can correct chlorophyll concentrations in the plant, substituting the deficiency of Fe in the yellowish leaf which implies that the uptake and translocation of iron have been accelerated (Wang et al., 2007; Gui et al., 2020). It was shown that soil inoculation with various types of microbes increased the Fe content in wheat, white lupin, and cucumber plants (Zhang et al., 2009; Zhao et al., 2014). Accordingly, soil microbes might have influenced the status of Fe in the rhizospheric zone. Meanwhile, the concentration of bio-available Fe in a solution

can be reduced due to uptake by roots and microbes (Marschner et al., 2011). Consequently, Fe deficiency could lead to limited plant growth (Majeed et al., 2020). Mycorrhizae have been well studied to increase the absorption of phosphorus (P) using phosphatase enzyme (Dubey, Fulekar, 2011; Qiu et al., 2019) leading to a promoted plant growth. Besides, the mycorrhizae excrete glomalin which participates in soil fertility (Lopez-Merino et al., 2015) and soil aggregate formation (Rillig, 2004; Al-Maliki, Al-Masoudi, 2018; Al-Maliki, Bresam, 2020). Similarly, mycorrhizal fungi have been associated with an increase nutrient uptake and are thought to be a crucial component in the removal of heavy metals from soils due to their symbiotic relationships (Shi et al., 2020; Al-Maliki and Al-Shamry., 2022). However, iron uptake by mycorrhizal fungi is less understood. Plant–microbial interactions were demonstrated with Fe acquisition by Robin et al. (2007) who noticed that the inoculation of *Pseudomonas* with transgenic tobacco, which accumulates more Fe, is a central strategy to prevent Fe from depletion. Nevertheless, concrete evidence has not been specifically identified to inform that microbes and their associations with plants could promote Fe acquisition.

Arbuscular mycorrhizal fungi (AMF) affect soil microbial biomass by modifying root exudation following root colonization (Raiesi, Ghollarata, 2006; Zarea et al., 2009; Xiao et al., 2019) thereby leading to improvements in soil properties and plant yield. Microbial biomass carbon (MBC) is an important aspect of soil biological activity and biochemical processes as they are responsible for plant developments, soil organic matter decomposition (SOM) and nutrient cycling (Sparling, 1997; Dick et al., 1997; Saurabh et al., 2020). Besides, the metabolic quotient (qCO_2) is another beneficial parameter reflecting the enhancements in soil quality. It is the respiration rate per unit microbial biomass C and is used as an indicator for the ecophysiological conditions of soil microbes (Anderson, Domsch, 1993; Abd El-Azeim et al., 2020). It is a sensitive measure of the soil carbon quality and could effectively reflect the effect of *AM* fungi and Fe on the behaviour of soil microbes. Antisari et al. (2013) studied the effects of Fe_3O_4 on qCO_2 and found that qCO_2 was raised in soils showing microbial stress. A decrease in qCO_2 in soil following the addition of organic wastes was interpreted as an increase in carbon utilisation efficiency. (Anderson, Domsch, 1993). Soil toxicity, soil type, organic residues, and the presence of earthworms are all possible influences on metabolic quotient (Scullion, Malik, 2000; Al-Maliki et al., 2017, 2021) as well as mycorrhizal inoculation (Lermen et al., 2015). Mycorrhizal inoculation, for example, has recently been shown to lower the metabolic quotient, implying the existence of non-stressful soil bacteria in polluted soil (Lermen et al., 2015). Fe compounds might cause a biochemical and metabolic alteration in leaves and roots leading to more exudates inducing soil microbes. Nevertheless, higher concentrations of Fe might affect metabolic quotient and the consequences for the soil–plant interactions leading to more stress to soil microbial activity (Antisari et al., 2013). Despite enormous studies released on qCO_2 (Bastida et al., 2008; Anderson, Domsch, 2010), little has been recognized about how qCO_2 is influenced by AM fungi and foliar spray of Fe through the plant growth process.

As far as the eggplant is concerned, there has been evidence that the growth of eggplants and potato crops was improved by mycorrhizae and foliar spray of Fe (Ortas, 2003; Al-Jobori, Al-Hadithy, 2014; Al-Zabee, Al-Maliki, 2019). However, the involvements of mycorrhizal fungi and foliar spray of Fe in developing soil–plant–microbes relationships have still been unclear. The proposed hypothesis of this research is that the application of mycorrhizal fungi and foliar spray of Fe might enhance soil microbes, nutrients content and plant productivity. As a result, the goal of this study was to see if changing plant Fe status would affect plant-microbial communities and increase MBC, metabolic quotient, infection rate, nutrient availability, root density, and plant production in arid land soils.

MATERIAL AND METHODS

Experimental site

The experiment was carried out in Babylon City (Almhanawiya locality), (32°50′82.17″N, 44°34′39.12″E) during the 2019 cultivation season. The soil texture was clay loam (38 % clay, 25 % sand and 37 % silt). The soil pH and electric conductivity were 6.9 and 3.9 ds m^{-1}, respectively.

The field was ploughed as a preparation for the cultivation process. The field was previously cultivated with wheat plant. The area of the experimental unit was 4 m^2, with four rows. The length of the rows was 1.1 m and the distance between each row was 0.5 m. Seeds were sown on 2/2/2019 in containers in sandy soil and peatmoss at a ratio of 2 : 1 as well as 10 g of mycorrhizal fungi inoculums which had infected roots, soil and spores. The spores of mycorrhiza fungi (*Glomus mosseae*) were proved by *wet sieving* and *decanting* procedures (Gerdemann, Nicolson, 1963). One gram of the inoculant consisted of 42 mycorrhizal spores.

All seedlings were transferred into the field after 6 weeks, when the plants had achieved the proper size (3–4 genuine leaves). M*ycorrhizal* fungal *inoculums (10 g)*, which had infected roots, soil and spores, were again mixed with the seedlings roots in the cultivation process. Ten seedlings of eggplant (Barcelona)

were planted in each experimental unit and the upper third of the rows and on one side only. Plants were separated by 0.4 m.

The experiment was laid out as a randomized complete block design (RCBD) with 8 replications and 4 treatments (control, mycorrhizae, foliar feeding of Fe and mycorrhizae + foliar feeding of Fe) (C, M, F, and M+F), respectively. The 6% Fe chelate (FeEDTA) was added at the rate of 40 mg l^{-1}. The targeted plants were sprayed early in the morning until getting wet, using a 10-litre manual sprinkler. Iron was splashed at two stages, following two weeks and one month of transplanting.

Eggplants were continuously harvested every two weeks (totally six times) during the period of April 15, 2019 to July 15, 2019. At the end of the field experiment, samples were taken from the rhizosphere area which is close to roots to measure soil respiration, MBC, qCO_2, microbial metabolic quotient, infection rate, roots weight, nutrients content in leaves (N, P, Fe), chlorophyll rate and total yield.

Measurements

Soil respiration was measured by the Alkali trap method (Anderson, 1983). A flask with 10 g of soil was used to measure the CO_2 flux in the soil. The flask contained a trap solution (5 ml, 1M NaOH) suspended by a wire at about 0.05 m from the soil. The alkali trap was placed in a flask for three different periods (10, 20 and 30 days). Besides, a $BaCl_2$ (2.0 ml) of 30% (w/v) was mixed with the samples before titration so that CO_3 could be precipitated as $BaCO_3$. The total CO_2 flux was estimated then by titration with HCl_2 (0.5M) to determine the final CO_2 flux. The flask for the control treatment did not contain soil to correct the amount of CO_2 in the environment. Soil microbial biomass C was measured based on Horwath et al. (1996), chloroform fumigation incubation (CFI) by determining the CO_2 mineralized in 10-day incubation of unfumigated and fumigated samples and then the unfumigated data was subtracted from the fumigated data. For soils fumigation, 10 g fresh soil was taken from each plot and inserted inside 50 ml beakers. Chloroform was situated in a 50 ml beaker inside the desiccator. In a laboratory hood, boiled chloroform was placed in a sealed desiccator for around 30 seconds. The boiling process was repeated 4 times. Samples were incubated for 24 h in the chloroform desiccator. At the end of the incubation period, the desiccator was air vacuumed for 3 days until the smell of chloroform removed. All samples were then placed in sealed jars. Evolved CO_2 was measured based on the above method of soil respiration and according to equations given below:

CO_2 mineralized (Meq CO_2) in 10-day incubation of unfumigated soil at 25 °C (µg C g^{-1} soil per day):

$$\text{Meq } CO_2 = \text{Meq NaOH} - \text{Meq HCl} \quad (1)$$

$$CO_2 \text{ (mg)} = \text{Meq } CO_2 \times \text{equivalent weight of } CO_2 \quad (2)$$

The equivalent weight of CO_2 is 22 g $mole^{-1}$.

CO_2 mineralized in 10-day incubation of fumigated soil at 25 °C (µg C g^{-1} soil per day) was also estimated using the equations (1) and (2).

$$\text{Microbial biomass C (µg C } g^{-1} \text{ soil)} = (0.71\ CO_2F - 0.23\ CO_2C)/k_C \quad (3)$$

The k_C (constant) value at 25 °C is 0.45 (Jenkinson, Powlson, 1976; Howarth et al., 1996; Dalal, 1998), while 0.71 and 0.23 are constant parameters.

Metabolic quotient (qCO_2) (Mg CO_2-C g^{-1} µg C g^{-1} soil) and microbial quotient (%) were calculated based on Dalal (1998) and Al-Maliki et al. (2017) according to the following equations:

$$\text{Metabolic quotient} = (\text{microbial respiration})/(\text{microbial biomass carbon}) \quad (4)$$

$$\text{Microbial quotient} = (\text{microbial biomass carbon})/(\text{total organic carbon}) \quad (5)$$

The AM fungal infection rate was assessed based on Kormanik et al. (1980). The eggplant roots were exposed to water to remove soil particles. The roots were divided into fractions (0.01 m) and then applied to 10% (w/v) (KOH) in flasks and incubated for 15 min at 90 °C. Roots also were filtered by a 10% (w/v) (H_2O_2) for 1 min. The roots were then rinsed in 10% hydrochloric acid (w/v) for 3 min. Moreover, all roots were stained with acid fuchsin dye for 10–15 min at 90 °C. A 4X optical microscope Carl Zeiss producer in Germany was used to estimate the percentage of infection. Total N and P were estimated by the Kjeldahl method by employing an automated colorimetry and a Technicon auto-analyzer (Technicon Instruments Corp) (Parkinson, Allen, 1975). The chlorophyll content was determined by a chlorophyll meter (*SPAD*-502; Konica Minolta, *Japan*) (Dwyer et al., 1991). The readings of 10 chlorophyll leaf samples were taken for each experimental unit by a portable chlorophyll meter. Plant roots were washed to remove any attached soil particles and then rinsed with deionized water. Roots were then situated into paper bags and oven-dried at 60 °C for 3 days. The total dry weight of roots was noted. Regarding Fe concentrations, the leaf was washed by detergent (0.1%) (Mistol, Henkel) which can get rid of any contamination, and then ultimately tap water was used to clean any remaining materials. Additionally, leaves were digested by using a di-acid mixture of ($HNO_{3:}$ $HClO_4$) (Chapman, Pratt, 1961) to estimate the Fe concentrations using an Atomic Absorption Spectrophotometer ZA3000 Series ZA3000 series soil (japan) (Khan, Cornfield, 1968). The total yield of eggplant was calculated by dividing the weight of eggplant in each experimental unit to the area of the experimental unit and then the outcomes multiplied by 10 000 m^2 to obtain the total yield in a hectare.

Table 1. Effect of treatments (C (control), F (iron), M (mycorrhiza), M+F (mycorrhiza + iron)) and incubation period (10, 20, and 30 days) on soil respiration (mg CO_2 g^{-1} soil). Values are means ± SE; means with a common letter superscript do not differ significantly (P < 0.05)

Treatments	Incubation 0–10 days	Incubation 10–20 days	Incubation 20–30 days	Means
C	204.67 ±2.60 [a]	207.0 ±12.3 [a]	103.00 ±3.51 [a]	171.6 ±17.6 [a]
F	220.00 ±5.77 [a]	233.50 ±4.25 [a]	151.33 ±5.93 [b]	201.6 ±13.0 [b]
M	206.00 ± 7.0[a]	298.3 ±13.0 [b]	195.3 ±10.1 [c]	233.2 ± 17.1[c]
M+F	240.67 ±5.81 [c]	261.00 ±4.93 [c]	138.33 ±3.76 [b]	213.3 ± 19.1[b]
Means	217.83 ±4.97 [a]	250.0 ±11.0 [b]	147.0 ± 10.3[c]	

Statistical analysis

Two-way analysis of variance (ANOVA) was performed to analyze soil respiration using two factors (treatments and time). Four treatments (control, mycorrhiza, foliar spray of Fe and mycrorrhiza + foliar spray of Fe) were prepared and replicated for eight times. Results for the microbial biomass carbon, microbial quotient, metabolic quotient, infection rate, N, P, chlorophyll percentage, roots weight, iron content and total yield were analyzed by one-way ANOVA. Means differences were calculated by Tukey's significance difference (HSD) test with a significance level of $P < 0.05$.

RESULTS

The response of mycorrhizal fungi and foliar spray of Fe to soil respiration

Overall, there was a marked increase in soil respiration in M, F and M+F treatments as compared to the untreated soils (Table 1). The percentage increases in soil respiration were 36, 23 and 29 % in M, F and M+F treatments, respectively. The highest percentage increase was recorded when the soil was inoculated by mycorrhizal fungi alone; soil respiration significantly ($P < 0.05$) increased, by up to 36 % over the control treatments. Besides, soil respiration fluctuated over time. The highest significant increase was at day 20 and then a greater decline at day 30 was observed. More importantly, there was an interaction ($P < 0.001$) plot between treatments and incubation confirming that there was a profound fluctuation in soil respiration throughout the time. For instance, at day 10, soil respiration was higher in M+F than in the other treatments, but this scenario was not consistent as M treatment raised clearly soil respiration later at day 20 and day 30 over other treatments (Fig.1).

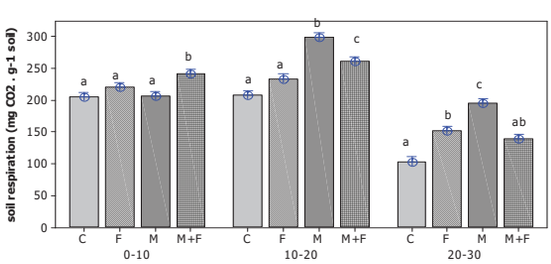

Fig. 1. Interaction (P < 0.001) plot between treatments (C (control), F (iron), M (mycorrhiza), M+F (mycorrhiza + iron)) and incubation period (0–10 days, 10–20 days, and 20–30 days) for respiration mg CO2–C g_1 soil . day. Different superscript letters represent statistical difference (P < 0.05)

The effect of mycorrhizal fungi and foliar spray of Fe on microbial biomass, metabolic quotient, infection rate, chlorophyll rate, nutrients availability, roots and plant yield

The highest significant increase in microbial biomass carbon (MBC) was in M treatment (Table 2). However, Fe neither significantly increased MBC, nor lowered metabolic quotient. Metabolic quotient showed significant decreases in all treatments over control except for the Fe treatment which did not differ significantly from the control treatment. A greater decrease in metabolic quotient was when AM fungi were applied alone. A combination of mycorrhizal fungi with Fe significantly improved MBC and metabolic quotient.

There were noticeable improvements in infection rate after the application of Fe foliar spray and mycorrhizal inoculation as compared with the control treatment (Table 2). The highest increase was at AM fungi inoculation alone, although the result was not significantly ($P < 0.05$) different from M+F treatment. There was also a maximum decent increase in chlorophyll rate at AM fungi treatment as compared to the control treatment. However, foliar spray of Fe on its own did not increase chlorophyll rate over control. As soon as Fe was combined with AM fungi, a significant ($P < 0.05$) enhancement in chlorophyll rate was observed suggesting that the application of foliar spray of Fe alone was not adequate to develop chlorophyll rate.

Table 2. Effect of treatments (C (control), F (iron), M (mycorrhiza), M+F (mycorrhiza + iron)) on microbial biomass carbon (MBC), metabolic quotient (qCO_2), and microbial quotient (Mq), infection rate, chlorophyll, nitrogen in leaf, phosphorus, Fe, fruit number, fruit yield and dry weight of roots. Values are means ± SE. Means with a common letter superscript do not differ significantly ($P < 0.05$)

Parameters	Treatments			
	C	F	M	M+F
MBC (µg C g^{-1} soil)	65.79 ± 2.77^{a}	72.17 ± 3.66^{a}	107.58 ± 1.65^{c}	93.64 ± 0.92^{b}
qCO_2 (Mg CO_2-C g^{-1}- µg C g^{-1} soil)	3.11 ± 0.09^{a}	3.06 ± 0.19^{a}	1.91 ± 0.06^{c}	2.57 ± 0.08^{b}
Mq (%)	5.85 ± 0.32^{a}	6.22 ± 0.38^{a}	8.52 ± 0.39^{b}	6.72 ± 0.20^{a}
Chlorophyll (spad)	52.33 ± 1.45^{a}	55.66 ± 0.88^{a,c}	62.33 ± 1.20^{b}	57.33 ± 0.66^{c}
Infection rate (%)	14.33 ± 2.19^{a}	25.00 ± 1.73^{b}	74.00 ± 2.08^{c}	65.33 ± 1.76^{c}
N (%)	1.28 ± 0.11^{a}	2.94 ± 0.08^{b}	2.32 ± 0.12^{c}	3.34 ± 0.14^{b}
P (%)	0.57 ± 0.01^{a}	0.64 ± 0.03^{ab}	0.72 ± 0.02^{bc}	0.78 ± 0.02^{c}
Fe (mg kg^{-1})	73.33 ± 3.33^{a}	114.20 ± 3.13^{b}	91.20 ± 1.73^{c}	130.00 ± 2.89^{d}
Fruit number	54 ± 2.08^{a}	70 ± 0.66^{b}	78 ± 1.15^{c}	68 ± 0.33^{b}
Roots dry weight (g)	2.23 ± 0.12^{a}	2.90 ± 0.05^{b}	4.16 ± 0.08^{c}	3.90 ± 0.05^{c}
Fruit yield (t ha^{-1})	12.77 ± 0.56^{a}	18.88 ± 0.55^{bd}	25.00 ± 0.97^{c}	21.00 ± 0.57^{d}

The N, P and Fe contents were significantly ($P < 0.05$) higher in M+F treatment than in the other treatments (Table 2). Notwithstanding, the P content in soil following iron sprays was not different from the control treatment and this result was consistent with a non-significant raise in the chlorophyll content. A higher increase in fruit number and yield was observed following the inoculation of mycorrhizae if compared to control treatment (Table 2). Foliar spray of Fe increased fruit number and yield. Total yield was not statistically ($P < 0.05$) different in F and M+F treatments whereas it seemed that M alone outperformed these treatments in enhancing total yield. We have proved that the plant leaves had higher Fe content in the F treatment over the M treatment.

Correlation coefficient between soil respiration, microbial biomass carbon, metabolic quotient and infection rate, plant roots and some plant parameters

There were closest relationships between infection rate, metabolic quotient qCO_2, plant roots, MBC, and fruit yield (Table 3). We suggest that MBC, roots and microbial quotient are more useful indicators for developing plant growth. In terms of the metabolic quotient, it was correlated significantly ($P < 0.05$) negatively to all parameters addressed. There was a strong pertinent relationship between MBC and P content, suggesting that MBC is an important driver in decomposing organic materials and releasing nutrients. Interestingly, correlations were found between N, P and fruit yield ($r = 0.59$, P-value = 0.01), ($r = 0.70$, P-value = 0.01), respectively, whereas the Fe content did not correlate to fruit yield.

DISCUSSION

The effect of mycorrhizal fungi and Fe foliar spray on soil respiration

To the best of our comprehension, this is the first study to monitor soil respiration in response to the foliar spray of Fe and mycorrhizae in arid lands. We

Table 3. Correlation coefficient between soil respiration (SR), microbial biomass (microbial biomass carbon; MBC), metabolic quotient (qCO_2), infection rate (IR), plant roots dry weight (PR) and some plant parameters

	qCO_2	SR	MBC	PR	IR
Infection rate %	0.79**	0.29	0.96***	0.96***	
Chlorophyll	0.69*	0.09	0.87**	0.79**	0.83***
Nitrogen (%)	0.82***	0.72**	0.40	0.58*	0.32
Phosphorus (%)	0.80***	0.20	0.90***	0.82***	0.82**
Fruit number	0.86***	0.23	0.93**	0.79**	0.74**
Fruit yield (t ha^{-1})	0.89***	0.34	0.95***	0.92***	0.88**

Significance level: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

hypothesized that mycorrhizae and Fe could support soil microbes in the soil. This hypothesis seems to be achieved as obvious enhancements in soil respiration were noted. Based on our results, there was a higher density of roots, mycorrhizal infection rate and microbial biomass which can stimulate soil microbial activity by providing roots with exudates and carbon. F increased soil respiration significantly (by 23 %) and the tangible reason behind that is that F plays a vital role in supporting the plant growth and roots density which can inevitably improve their exudates system leading to an ameliorated soil microbial activity. Fe is a certain cofactor of forming enzymes which participate in meristematic plant developments and chlorophyll formation (Kosegarten et al., 1998; Vigani, 2012; Kroh, Pilon, 2020). These findings support the concept that Fe somehow regulates the microbial rhizosphere community (Lehmann, Rillig, 2015), perhaps by changing the carbon flow into roots.

Besides, it is well known that Fe significantly impacts the activation of enzymes that are capable of minimizing the plant cells damage, as well as its engagement in the metabolism process, chlorophyll synthesis and photosynthesis (Fageria et al., 2010). These benefits of Fe element could encourage roots system excretions and microbial activity improvements. However, in the present study, these benefits of Fe were temporary and then the mycorrhizal fungi inoculation was progressively more effective in raising soil respiration over the 20 and 30 days of the incubation periods. Based on our data, there was a quite interesting quantity of roots in M treatment which undoubtedly supported soil respiration for longer periods. Furthermore, the slow decomposition of mycorrhizal fungal substances might prove that these substances were not readily degraded at day 10 but exhibited further decomposition thereafter at days 20 and 30 due to more accessible materials in soil stimulating soil microbes. Further research into the role of the interaction between Fe and mycorrhizae in mediating the plant–microbial interactions over time is needed.

The effect of mycorrhizal fungi and Fe foliar spray on microbial biomass and metabolic quotient, infection rate and chlorophyll rate, nutrients availability, roots and plant yield

Mycorrhizae and its combination with Fe have been proved to rise MBC. The precise reason is that there was a quite interesting increase in soil respiration and roots growth to which more encouragements to microbial biomass in soil occurred by providing mucilages and roots secretions as such. Moreover, AM fungi have a concrete role in developing MBC by employing glomalin and hyphal walls which encouraged MBC content in soil. Glomalin, amino acid, and sugar of mycorrhizal fungi can stimulate soil microbial biomass (Toljander et al., 2008; Parihar et al., 2020). Soil microbial biomass forms less than 5 % of organic matter in soil representing a labile source of C, N, P, and S. Also, it is an agent of nutrient transformation and soil formation (Lavelle, Spain, 2001). Nevertheless, the sole application of Fe did not enhance MBC and metabolic quotient which is opposite to our expectations. It is likely, on one hand, a result of the non-improved P content and chlorophyll rate after Fe amendments. On the other hand, the mobility of Fe through plant leaves and roots might not be adequate to boost microbial biomass in the soil leading to lower amounts of MBC. If the iron is correctly moved into the leaf tissue, a perfect outcome of foliar spraying would be seen. Additionally, a good result of MBC depends also on how stable is Fe in leaf tissue. The greater the movement of Fe from leaves into roots and soils, the greater the influence on soil microorganisms. A study focused on the amounts of Fe in roots and soil needs to be conducted to monitor its effect on MBC after moving from plant leaves.

To date, more attention has been given to evaluating the effects of mycorrhizae on the microbial community while no studies have focused on the effect of mycorrhizae and Fe combination on MBC and metabolic quotient which shows how much stressed are soil microbes and the implications on C accumulation. A greater decrease in metabolic quotient was by AM fungi applied alone. To justify these phenomena, it seems that the lower estimate of the metabolic quotient is a result of using the energy of microbes efficiently or they were under less stress (high-quality soil). This study suggests that AM fungi are of great importance for arid land ecosystems since they can decrease microbial stress. Many studies reported that fungi are more efficient than the bacterial population at assimilating C, which leads to lower metabolic quotients (Adu, Oades, 1978; Sakamoto, Oba, 1994; Zhao et al., 2020). The metabolic quotient is a vital parameter for microbial efficiency (Sakamoto, Oba, 1994). Lower metabolic quotients may be an indicator of less stressed microbial populations (Insam, Domsch, 1988; Al-Maliki, Scullion, 2013) and the potential for soil organic matter stabilization. The most significant increase in microbial quotient recorded in M suggests that there was an increase in soil carbon sequestration capacity and thereby the soil organic carbon was perfectly accumulated. The microbial quotient is the ratio of microbial biomass to soil organic C and it can signalize how efficient is the soil organic matter that is utilized by microbial community (Pankhurst et al., 2002; Sun et al., 2020). It can be a sensitive indicator of the soil carbon quality. These data are in line with Srivastava, Singh (1989) who noticed that a higher soil microbial quotient can enhance C accumulation in the soil.

Noticeable improvements in infection rate after combining Fe foliar spray with mycorrhizal inocu-

lation were shown. The highest increase was when AM fungi solely inoculated the soil, and the reason might be attributed to the enhancements in the soil respiration which played a role in organic matter decomposition leading to more nutrients and enormous metabolic production in soil encouraging roots growth to meet fungal hyphal lengths and the outcome for increased mycorrhizal symbiotic relationships in soil. A considerable increase in chlorophyll rate in AM fungi treatment is probably due to the higher microbial activity and mycelium network in soil causing higher nutrients uptake resulting in promoted chlorophyll synthesis. These results are consistent with Kullu et al. (2020) and Morte et al. (2000) who noted an increase in chlorophyll rate after inoculation of AM fungi. We postulated that foliar spray of Fe was capable of enhancing the chlorophyll rate in the plant as such. However, the opposite was true, foliar spray of Fe alone did not increase chlorophyll rate over control. Our current study was not in line with Alvarez-Fernandez et al. (2004) who found that $FeSO_4$ application to pear trees caused increased chlorophyll concentrations. Even though, once Fe was combined with AM fungi, a significant enhancement in chlorophyll rate was observed suggesting that the application of foliar spray of Fe alone was not adequate to develop chlorophyll rate. The possible convenient reason is that the soil conditions, as a priority, have to be improved physically, chemically and biologically by mycorrhizal secretions to facilitate colonization of mycorrhizal fungi with plant roots and consequences on nutrients availability and chlorophyll promotion.

Our findings gave a more detailed explanation of how Fe is relevant to plant-microbial interactions to encourage more sustainable agriculture. N, P, and Fe contents were significantly higher in M+F treatment over the other treatments. The justification for this episode is that the combination of F and M might have interesting benefits for nutrient contents in the soil since there was an improvement in soil respiration in this treatment which could consequently have increased the organic matter decomposition and nutrients available to plant roots. The other possibility are the noticeable raises in MBC and microbial quotient which played an obvious strategic function in maintaining soil pH and carbon accumulation inducing nutrients releases from soil to plant roots. Additionally, soil microbes can generate or improve Fe solubility by producing organic acids or siderophores causing a higher nutrients uptake.

We hypothesized that foliar spray of Fe might increase P content in the plant. Notwithstanding, P content in leaves following iron sprays was not different from the control treatment and this result was consistent with a non-significant rise in chlorophyll content. The unambiguous reason could be attributed to the lack of chlorophyll development in the plant which might affect plant metabolism and photosynthesis (Wiedenhoeft, 2006; Fageria et al., 2009; Altuntas et al., 2020) resulting in weak contributions of plant roots to nutrient uptake. Fe in the soil is closely linked to the P availability, especially when it appears to form highly insoluble iron–phosphates mineral chemical complexes (Borggaard et al., 1990). Thus, it is convenient to see that P mobilization mechanisms can interlink with the availability of Fe in plants. Additional studies into the relationship between P and Fe could better explain how soil mycorrhizae could aid to the uptake of both these elements.

A considerable increase in fruit number and yield was observed following the inoculation of mycorrhizae. A decent yield might be a result of the amelioration in soil microbial activity after mycorrhizal inoculation, causing more nutrients uptake and plant growth. Moreover, mycorrhizae produce phosphatase enzyme which converts the organic P to mineral P contributing to fruits growth (Smith, Read, 2008; Della Monica et al., 2020). Furthermore, mycorrhizae are capable of producing siderophores which chelate macro elements, increase their absorption and consequences for enhanced photosynthesis and plant growth (Al-Karaki, 2017). Foliar spray of Fe increased fruit number and yield as Fe plays vital roles in the growth and development of eggplant. There were clear increases in N content, soil respiration and roots weight in F treatments confirming the importance of such application in improving plant growth. Fe can participate in plant metabolism and enzymes formation (Brittenham, 1994) leading to proper growth of a plant. Total yield was not statistically different in F and M+F whereas it seemed to be that M alone outperformed these treatments in enhancing total yield. These data were not so far as our designed expectations despite the significant increase in yield over control. The mechanism responsible for rising eggplant yield in M treatment is that arbuscular mycorrhizae enhanced soil microbes, roots density, infection rate and chlorophyll and the implications for maximum plant production.

The correlation coefficient between soil respiration, microbial biomass carbon, metabolic quotient, infection rate, plant roots and some plant parameters

It is very important to realize the underlying relationships between soil microbial properties and plant development. We found closest relationships between infection rate (IR), metabolic quotient (qCO_2), plant roots (PR), microbial biomass carbon (MBC), and fruit yield, confirming that the yield was substantially affected by these parameters. Root colonization by fungi is a powerful aspect inducing nutrient uptake and productivity. Moreover, qCO_2 is also much more effective in promoting plant yield since it is considered a signal for organic matter accumulation and implications for the development of the plant yield. We suggest

that MBC, PR and microbial quotient are more useful indicators for developing plant growth. In terms of qCO_2, it was correlated negatively significantly to all parameters addressed, suggesting that the soil had efficient soil respiration causing low respiration, higher MBC, and C protection. Such underlying associations between these parameters might reflect a progressive development in soil quality and plant yield. There was a strong pertinent relationship between MBC and P content, suggesting that MBC is an important driver in decomposing organic materials and releasing nutrients. In other words, the microbial biomass could contain more particular microbes like fungi which have a role in converting the organic P to the mineral P. We discovered several remarkable correlations between plant yield and P and N elements in leaves, implying a strong association between key nutrients (N and P) and yield. Plant yield was most likely influenced by the availability of Fe in leaves.

CONCLUSION

The results showed that mycorrhizal fungi and their combinations influenced MBC, soil respiration, microbial quotient, and metabolic quotient (qCO_2) to a greater extent than the sole application of foliar spray of Fe, highlighting the importance of mycorrhizal fungi and their combinations for microbial mineralization processes, organic carbon stabilization, and nutrient cycling in arid land soils. Nonetheless, mycorrhizal fungus had no effect on microbial respiration at the start of the experiment, implying that mycorrhizal fungi degraded organic substances slowly at first but afterwards promoted decomposition. These findings have taken on new significance as the potential role of fungi in microbial activity and nutrient uptakes has become clearer over time.

Fruit yield was associated to infection rate, qCO_2, microbial biomass, roots, N and P content, but not iron content, implying that Fe plays only a minimal role in plant promotion. The rate of mycorrhizal infection was heavily dependent on the increase in soil microbial biomass and root growth. As a result, a stronger relationship between soil microbial biomass and infection rate was discovered, highlighting the importance of microbial biomass in increasing colonization percentages and plant production. In practice, however, foliar Fe input had a minimal influence on microbial biomass and soil microbial respiration when compared to mycorrhizal fungus treatment, which was due to a lack of microbial substances and nutrients from mycorrhizal fungi to form microbial biomass. Lower metabolic quotient (qCO_2) values in response to mycorrhizal fungal inoculation and foliar feeding can indicate a significant reduction in soil microbial stress, with positive implications for organic matter retention and productivity. The application of mycorrhizal fungus alone resulted in the highest value of eggplant yield, implying that mycorrhizae have used a promotional method to considerably boost plant yield. More research should be done to track Fe levels in plant roots and soils to see how roots and microorganisms affect plant Fe levels.

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