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ORIGINAL PAPER

Inoculation with *Trichoderma harzianum* and *Azospirillum brasilense* increases nutrition and yield of hydroponic lettuce

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Abstract
The use of beneficial fungi and bacteria stimulate plant growth and serve to improve yield and food quality in a sustainable manner. The electrical conductivity of nutrients solution is closely linked to better nutrition of vegetable plants in a hydroponic system. Therefore, objectives of current study were to evaluate the effect of isolated and combined inoculation with *Azospirillum brasilense* and *Trichoderma harzianum* under two electrical conductivities on growth, nutrition, and yield of lettuce in hydroponic cultivation. The experiment was designed in a strip-plot block with five replications in a 4×2 factorial scheme. The treatments were consisted of four microbial inoculations (without, *A. brasilense, T. harzianum* and co-inoculation) and electrical conductivities (1.2 and 1.4 dS m^{-1}). Inoculation with *A. brasilense* and *T. harzianum* increased lettuce root growth by 47% and 20%, respectively. The single inoculation of *T. harzianum* provided higher fresh leaves yield (24%) at electrical conductivity of 1.2 dS m^{-1}, while single inoculation with *A. brasilense* increased fresh leaves yield by 17% at electrical conductivity 1.4 dS m^{-1}. The lowest shoot NO_3^- accumulation (40%) was observed with inoculation of *A. brasilense* and highest (28%) with inoculation *T. harzianum* in both electrical conductivities. Inoculation with *A. brasilense* increased leaf accumulation of K, P, Ca, Mg, Fe, Mn, Cu, and Zn, which are essential for human nutrition and being recommended to improve yield of lettuce plants in hydroponics. It is recommended to use EC 1.4 dS m^{-1} of the nutrients solution to improve accumulation of K, Mn, Cu, and Zn, regardless of inoculations for biofortification of lettuce with application of fertilizers.

Keywords Plant growth-promoting bacteria · Plant growth-promoting fungi · Co-inoculation · Leaf nitrate · Food biofortification

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Introduction

Lettuce is one the most cultivated and consumed leafy vegetable in the world for having desirable culinary characteristics "in natura" in the form of salads (Dalastra et al. 2020a). Americana lettuce is fresh cut and minimally processed, highly requested for home, fast food, and catering consumption vegetables (FAOSTAT 2021). Lettuce has been recognized as an important functional food for being a rich source of vitamins, minerals, and biologically active compounds such as photosynthetic pigments and phenols which increase its utilization human food (Artés and Allende 2014).

The water recirculation system in nutrient film technique (NFT) in hydroponic is serving an effective strategy to save water consumption as compared to soil production system (Carvalho et al. 2018) and contribute to world sustainability goals by increasing water use efficiency to overcome water crisis (Das et al. 2021; Kappel et al. 2021; Singh et al. 2021). In current scenario, hydroponic system is an alternative way of growing vegetables with balanced nutrients supply via nutrients solution that enabled productivity gains, versatility, precocity, and most importantly post-harvest improvements. In addition, hydroponic cultivation allows lettuce to be grown throughout the year, even in periods of adverse temperatures (Dalastra et al. 2020b).

The higher nutrients' concentration (electrical conductivity) in a nutrients' solution can increase yield and precocity of hydroponic lettuce with reduced water consumption (Dalastra et al. 2020b). However, increase in the electrical conductivity (EC) of nutrient solution leads to a higher use of fertilizers and leaf nitrate (NO_3^-) concentration in lettuce which are harmful to human health (Tabaglio et al. 2020). Improper nutrient solution management such as using too high or too low nutrient concentrations and imbalanced ions composition can inhibit plant growth by causing nutrient-induced toxicity or deficiency (Nozzi et al. 2018; Kumar et al. 2019; Laik et al. 2021).

The use of beneficial microorganisms is an emerging technology to improve nutrients acquisition in vegetables plants. Inoculation of *Azospirillum brasilense* is related to the activity of nitrate reductase to reduce nitric N into nitrate in leaves (Pereira-Defilippi et al. 2017). It also contributed to improve N use efficiency with a reduction of fertilizers' application by 25% (Fukami et al. 2016; Galindo et al. 2020). In addition, the fungus *Trichoderma* is widely used to control phytopathogens (Mathys et al. 2012) as well as modifying root architecture system by stimulating auxin signaling (Garnica-Vergara et al. 2015). It increases enzymatic activity, production of secondary metabolics, nutrients' acquisition by roots, and nutrients' use efficiency in plants (Malmerca et al. 2015). The use of these microorganisms can improve fertilizers use efficiency and reduce their consumption in hydroponic cultivation. The use of *Azospirillum brasilense* and *Trichoderma harzianum* have little studied as growth promoters while reducing fertilizers application in Americana lettuce under NFT-hydroponic cultivation. Therefore, it was hypothesized that these microorganisms would increase plant yield and nutrient acquisition, and reduce fertilizer consumption in lettuce hydroponic cultivation. Therefore, the objective of study was to verify beneficial effects of microorganisms and electrical conductivity on lettuce plant growth and nutrition in a hydroponic system.

Materials and methods

Characterization and experimental conduct

The trail on hydroponic lettuce NFT cultivation was developed under protected shed with 30% shading at São Paulo State University (UNESP), Ilha Solteira-SP, Brazil under geographic coordinates (20°25′07″ S 51°20′ 31″ W) and an altitude of 376 m. Experiment was conducted from 1st June to 20th July, 2021 with the duration of 15 days in seedling production and 31 days in hydroponic cultivation until harvest. The weather data were collected from the outdoor area of greenhouse from an automatic meteorological station of UNESP (Fig. 1).

The experimental units in NFT system were installed on individual benches with a 10% slope and 6 m in length. The cultivation channels were made of PVC with a rectangular section of 8 cm wide and 4 cm high and upper perforations to accommodate plants at every 25 cm interval. Each bench consisted of 6 cultivation channels, 20 cm apart with an individual pumping system and a reservoir of 300 L with a flow rate of 1L per minute and an exposure period to nutrients solution with continuous flow.

The lettuce cultivar Angelina, vigorous plants with excellent skirt formation, closed heads, compact and uniform in open field and hydroponics, moderate level of resistance to bacteriosis, intense and bright green leaves as well as safe in climatic fluctuations (high adaptation to tropical growing conditions) was used to develop seedlings nursery. The seedlings' nursery was developed in phenolic foam in 15 days which were then transplanted into permanent benches of NTF hydroponic system where they remained for 31 days until harvest. The nutrients' solution was composed of concentrated Hidrogood Fert Nacional fertilizers with nutrients concentrations of (ppm): 0.01 of N, 0.009 of P, 0.028 of K, 0.0043 of S, 0.0033 of Mg, 0.00006 of B, 0.00001 of Cu, 0.00109 of Fe, 0.00007 of Mo, 0.00005 of Mn and 0.00002 of Zn; also used Calcium Nitrate fertilizer with nutrients concentrations of 0.0155 ppm of N and 0.0265 ppm of Ca

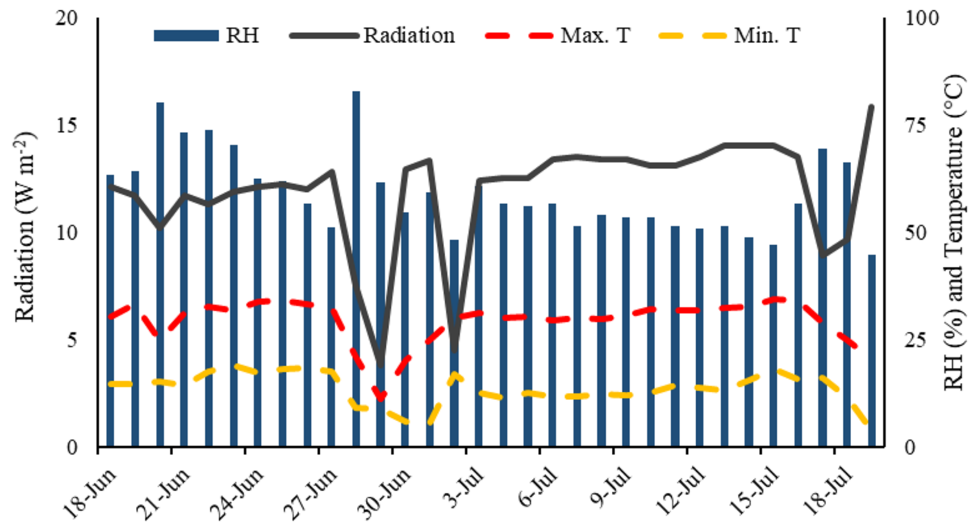

Fig. 1 Relative humidity (RH), maximum (Max. T), and minimum temperature (Min. T) and radiation during experiment conduction

along with Hidrogood Fert Ferro EDDHA fertilizer with Fe 0.006 ppm concentrations. To reach an electrical conductivity of 1.4 dS m^{-1}, the nutrients solution was added with an amount of 0.622 g L^{-1} of Hidrogood Fert Nacional water, 0.462 g L^{-1} of calcium nitrate and 0.028 g L^{-1} of Hidrogood Fert Ferro EDDHA. To reach an electrical conductivity of 1.2 dS m^{-1}, the nutrients solution was added with an amount of 0.533 g L^{-1} of Hidrogood Fert Nacional water, 0.396 g L^{-1} of calcium nitrate and 0.024 g L^{-1} of Hidrogood Fert Ferro EDDHA. Measurement and correction of conductivity and pH were performed daily in the morning. The EC was readjusted as determined for each cultivation bench with replacement of fertilizers if necessary and pH was maintained between 5.5 and 6.5, using phosphoric acid (85%) for pH above 6.5 and sodium hydroxide (25%) for pH below 5.5 (Fig. 2).

Treatments and experimental design

The experiment was designed in a strip-plot blocks in a 4 × 2 factorial scheme with 5 replications. Each experimental unit was represented by 8 plants. The first factor (vertical strips) was composed of foliar inoculation of microorganisms [control- without inoculation, *Azospirillum brasilense* at a dose of 300 mL of inoculant in spray volume of 300 L ha^{-1} from strains Ab-V5 and Ab-V6 with guaranteed of 2×10^8 colony-forming units (CFU) ml^{-1}], *Trichoderma harzianum* at a dose of 500 mL of inoculant in spray volume of 300 L ha^{-1}, strains ESALQ-1306 with guaranteed of 2×10^9 CFU ml^{-1}, and co-inoculation of both microorganisms). The inoculation treatments were carried out in the morning at a temperature of 21 °C with a relative humidity of 80% using an 18 L costal sprayer after 10 days after seedlings transplantation. The second factor (horizontal strips) was two electrical conductivities of the nutrient solution (1.2 dS m^{-1} used for lettuce in tropical regions of Brazil and 1.4 dS m^{-1} improve leaves yield without causing damage). Each cultivation bench made a block where foliar spray was applied with each electrical conductivity.

Assessments

The harvest was carried out 31 days after transplanting. At the time of harvest, fresh matters of root and shoots, length of shoots and roots were quantified, and number of leaves per plant, volume of plant and root using volumetric cylinder and leaf chlorophyll index (ClorofiLOG®—model CFL—1030 Falker) were evaluated. The material was dried in a forced air incubator at 60 °C for 72 h to obtain dry matters of root, shoot and total of plants. Crop yield was calculated via equation (yield in kg m^{-2} = shoot fresh weight in kg × plant population m^{-2} (19.5 plants m^{-2}).

After drying, plant materials was grinded in a Wiley mill and determined according to the methodology of Malavolta et al. (1997) for the concentrations of N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn in lettuce shoots while N concentration in shoots and roots. The determination of nitrate and ammonium concentration in leaves and roots was carried out according to the methodology Cataldo et al. (1975). The nutrients' accumulation in shoot and roots of lettuce was calculated from dry matter and nutrient concentrations of each sample via equation (accumulation in g $plant^{-1}$ or mg $plant^{-1}$ = dry weight in kg $plant^{-1}$ × concentration of nutrients in g kg^{-1} or mg kg^{-1}).

Statistical and correlation analysis

The data of all variables presented normal distribution and homogeneous variances (Shapiro–Wilk test), submitted to analysis of variance. The significance of the mean squares

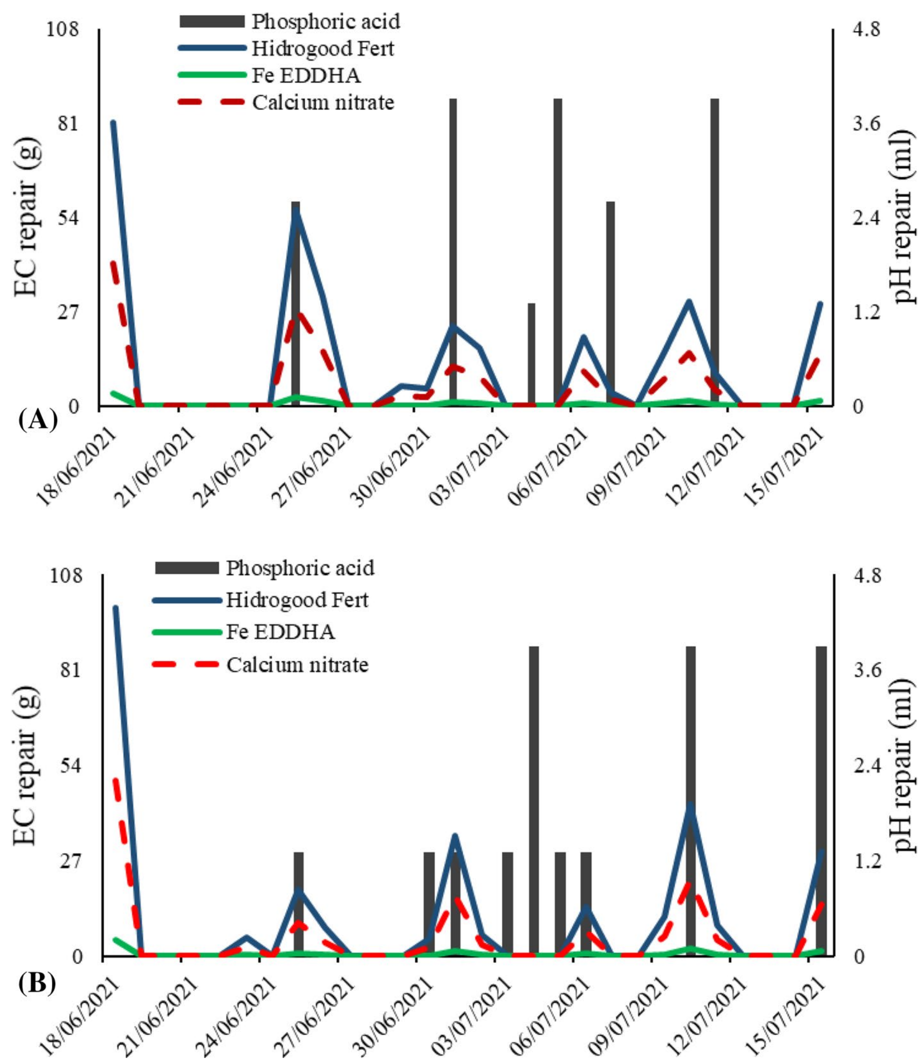

Fig. 2 pH and electrical conductivity (EC) in hydroponic NFT system with EC 1.2 dS m^{-1} (**A**), with EC 1.4 dS m^{-1} (**B**) in the period of the experiment

was tested by Fisher's test (F test) at 5% probability level. The means of inoculations were different by Tukey test at 5% probability level. The means of electrical conductivity level were different by F test at 5% probability level. The corrplot package of R software was used to evaluate relationship among lettuce plants, productive components, and fruit yield and nutrients accumulation parameters (R Core Team 2015).

Results

Lettuce production in hydroponic system

There was a significant interaction ($p < 0.01$) between electrical conductivity (EC) and inoculations for leaf number (LN), shoot fresh matter (SFM), root fresh matter (RFM), leaf yield (YIELD) and root volume (RV), and ($p < 0.05$) for root length (RL) (Fig. 3). There was no significant interaction ($p > 0.05$) for leaf chlorophyll index (LCI), root dry matter (RDM), and shoot dry matter (SDM). Inoculation with *A. brasilense* increased LCI in relation to other inoculations, and all inoculations had higher LCI in relation to non-inoculated. The LCI of lettuce was statistically not different with the effect of EC. There was no effect of inoculations EC on RDM and SDM; however, inoculation with *A. brasilense* and co-inoculation provided highest RDM and SDM in hydroponic lettuce plants (Table 1).

Inoculation with *A. brasilense* in EC 1.4 dS m^{-1} was observed with greater LN, SFM and YIELD as compared to others treatments, while inoculation with *T. harzianum, A. brasilense* and co-inoculation in EC 1.2 dS m^{-1} increased LN as compared without inoculation. Inoculation with *T. harzianum* and co-inoculation increased SFM and YIELD as compared to others inoculations (Fig. 3A, B and F). The highest RFM and RV were verified at higher nutrients' solution concentration (1.4 dS m^{-1}) in all treatments except co-inoculation. The highest RFM and RV at EC 1.4 dS m^{-1} was verified with inoculation of *A. brasilense* as compared other inoculations (Fig. 3C, D). The

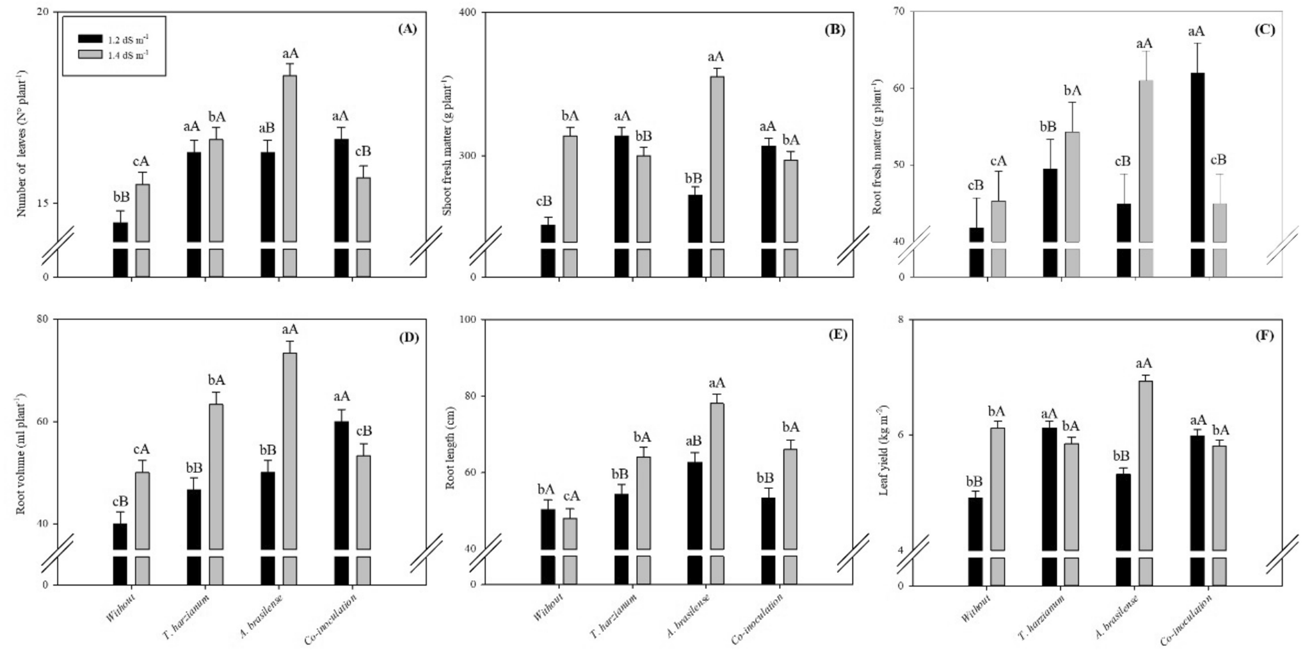

Fig. 3 Interaction between foliar inoculation and EC rates on lettuce root fresh matter (**A**), root length (**B**), root volume (**C**), leaf number (**D**), shoot fresh matter (**E**), and yield fresh matter (**F**). Uppercase letters indicate difference between EC of nutrition solution, and lowercase letters indicate differences between inoculations. Error bars indicate the standard deviation of the mean. Tukey (5%) = F value

Table 1 Shoot length (SL), leaf area (LA), leaf number (LN), shoot fresh matter (SFM), shoot length (SL), root volume (RV), leaf chlorophyll index (LCI), shoot dry matter (SDM), root dry matter (RDM), and leaf yield (YIELD) of lettuce as influenced by different incubation and EC levels

F.V	LN	LCI	SFM	RFM	RDM	SDM	YIELD	RV	RL
	n° plant	–	g plant^{-1}				kg m^{-2}	ml	cm
Inoculations									
Without	16.0	31.3 c	282.8	43.6	4.0 b	9.2 b	5.5	45.0	49.2
T. harzianum	16.5	36.5 b	307.0	51.9	4.0 b	9.8 b	6.0	55.0	59.2
A. brasilense	17.3	40.3 a	313.8	53.0	5.2 a	12.2 a	6.1	61.7	70.3
Co-inoculation	16.2	34.1 b	302.1	53.5	4.8 a	11.2 a	5.9	56.7	59.7
EC									
1.2 dS m^{-1}	16.0	35.0 A	286.3	49.6	4.4 A	14.1 A	5.6	49.2	55.2
1.4 dS m^{-1}	17.0	36.1 A	316.6	51.4	4.6 A	15.3 A	6.2	60.0	64.0
Probability									
Block	0.612	0.466	0.416	0.680	0.099	0.111	0.406	0.393	0.260
EC	0.010**	0.282^{ns}	0.000**	0.34^{ns}	0.289^{ns}	0.554^{ns}	0.000**	0.000**	0.000**
INO	0.090^{ns}	0.000**	0.001**	0.006**	0.000**	0.000**	0.001**	0.000**	0.000**
EC*INO	0.008**	0.317^{ns}	0.000**	0.000**	0.156^{ns}	0.777^{ns}	0.000**	0.000**	0.018*
CV (%)	5.42	6.98	8.35	8.92	8.22	6.36	6.34	7.48	7.36

CV coefficient of variation, *EC* electrical conductivities, *INO* inoculations

*Significant at 5% of probability by the F test

**Significant at 1%

RL was highest with inoculation of *T. harzianum, A. brasilense*, and co-inoculation at 1.4 dS m^{-1} as compared to 1.2 dS m^{-1}. In addition, inoculation with *A. brasilense* increased RL in relation to other inoculations in both ECs (Fig. 3E).

Shoot nutrients' accumulation

There was a significant interaction ($p < 0.01$) between EC and inoculations for shoot ammonium accumulation (NH_4^+-Shoot), nitrate (NO_3^--Shoot), nitrogen (N-Shoot), potassium (K-Shoot), and calcium (Ca-Shoot). In addition, zinc (Zn-Shoot) and manganese (Mn-Shoot) were significant at ($p < 0.05$), while phosphorus (P-Shoot), magnesium (Mg-Shoot), copper (Cu-Shoot), and iron (Fe-Shoot) were not significant ($p > 0.05$). Inoculation with *A. brasilense* increased P-Shoot and Cu-Shoot in relation to other inoculations. There was no effect of EC on P-Shoot; however, EC 1.4 dS m^{-1} increased Cu-Shoot as compared to EC 1.2 dS m^{-1} (Table 2).

The higher NH_4^+-Shoot was observed with inoculation of *T. harzianum* in EC 1.2 dS m^{-1} and inoculation with *A. brasilense* in EC 1.4 dS m^{-1} as compared to others in the same EC (Fig. 4A). The lower NO_3^--Shoot was noted with inoculation of *A. brasilense* in both EC as compared to other inoculations (Fig. 4B). The higher N-Shoot was observed with inoculation of *A. brasilense* in EC 1.2 dS m^{-1} as compared to other inoculations. The highest N-Shoot was noted with inoculation of *A. brasilense* and co-inoculation in EC 1.4 dS m^{-1} as compared to other inoculations (Fig. 4C). Inoculation with *A. brasilense* was observed with higher concentration of K-Shoot, Ca-Shoot, Zn-Shoot, and Mn-Shoot regardless of nutrients' solution concentration (Fig. 4D–G).

Root nitrogen accumulation

There was a significant ($p < 0.01$) interaction between EC and inoculations for shoot ammonium accumulation (NH_4^+-Root) and nitrate (NO_3^--Root), and significant ($p < 0.05$) for nitrogen (N-Root) (Table 3).

Inoculation with *T. harzianum* and co-inoculation were noted with greatest NH_4^+-Root in lettuce plants at EC 1.4 dS m^{-1} as compared to other inoculations. The greatest NH_4^+-Root was verified within co-inoculation at EC 1.2 dS m^{-1} as compared to other inoculations (Fig. 5A). The lowest NO_3^--Root was verified with inoculation of *Trichoderma* and co-inoculation in EC 1.4 dS m^{-1} in relation to other inoculations. The lower NO_3^--Root was observed with co-inoculation and without inoculation at 1.2 dS m^{-1}. The single inoculation of *A. brasilense* and *T. harzianum* were observed with greater NO_3^--Root accumulation at EC 1.2 dS m^{-1}, while single inoculation with *A. brasilense* and without inoculation under EC 1.4 dS m^{-1} (Fig. 5B).

Correlations

There was a positive and significant correlation between RL and RV and all variables except NH_4-Shoot, NO_3-Shoot, Ca-Shoot, Mg-Shoot, Fe-Shoot, Cu-Shoot, Mn-Shoot, Zn-Shoot, and NO_3-Root. SFM, LN and YIELD had a positive and significant correlation between RL, RV, RDM, LCI, N-Shoot, P-Shoot, K-Shoot, Fe-Shoot, NH_4-Root, and N-Root and negative with NH_4-Shoot and NO_3-Root. LCI had positive and significant correlation between RFM, RV, RL, LN, SFM, YIELD, SDM, RDM, N-Shoot, and P-Shoot, and negative with NO_3-Shoot. NH_4-Shoot had a negative

Table 2 Shoot ammonium accumulation (NH_4^+), nitrate ($NO_3.^-$), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), cooper (Cu), manganese (Mn), and iron (Fe) of lettuce as influenced by different incubation and EC levels

F.V	NH_4^+ mg m^{-2}—	NO_3^-	N g m^{-2}-	P	K	Ca	Mg	Zn mg m^{-2}	Cu	Mn	Fe
Inoculations											
Without	11.00	59.06	0.39	0.09 c	0.56	0.18	0.06 a	1.12	0.20 c	0.91	3.51 a
T. harzianum	14.40	66.01	0.46	0.12 bc	0.64	0.20	0.07 a	1.31	0.18 c	1.08	4.79 a
A. brasilense	12.98	33.10	0.56	0.16 a	0.93	0.29	0.09 a	2.14	0.52 a	1.82	8.18 a
Co-inoculation	10.32	55.71	0.52	0.14 b	0.74	0.21	0.07 a	1.37	0.44 b	1.20	5.13 a
EC											
1.2 dS m^{-1}	13.49	51.79	0.47	0.13 A	0.70	0.21	0.07 a	1.41	0.24 B	1.06	5.24 a
1.4 dS m^{-1}	10.85	55.15	0.49	0.13 A	0.74	0.22	0.07 a	1.56	0.43 A	1.44	5.56 a
Probability											
Block	0.223	0.130	0.230	0.818	0.064	0.760	0.801	0.844	0.314	0.845	0.980
EC	0.022*	0.721 ns	0.000**	0.409 ns	0.000**	0.000**	0.642 ns	0.543 ns	0.000**	0.456**	0.730 ns
INO	0.054 ns	0.004**	0.000**	0.000**	0.001**	0.000**	0.276 ns	0.012**	0.012**	0.014**	0.104 ns
EC*INO	0.024**	0.042*	0.000**	0.134 ns	0.000**	0.000**	0.500 ns	0.050*	0.322 ns	0.048*	0.730 ns
CV (%)	10.80	14.93	6.14	8.68	8.86	6.34	15.39	15.17	13.27	18.42	19.88

Uppercase letters indicate differences between EC of nutrient solution and lowercase letters indicate differences among inoculation by Tukey test

CV coefficient of variation, *EC* electrical conductivities, *INO* inoculations

*Significant at 5% of probability by the F test

**Significant at 1%

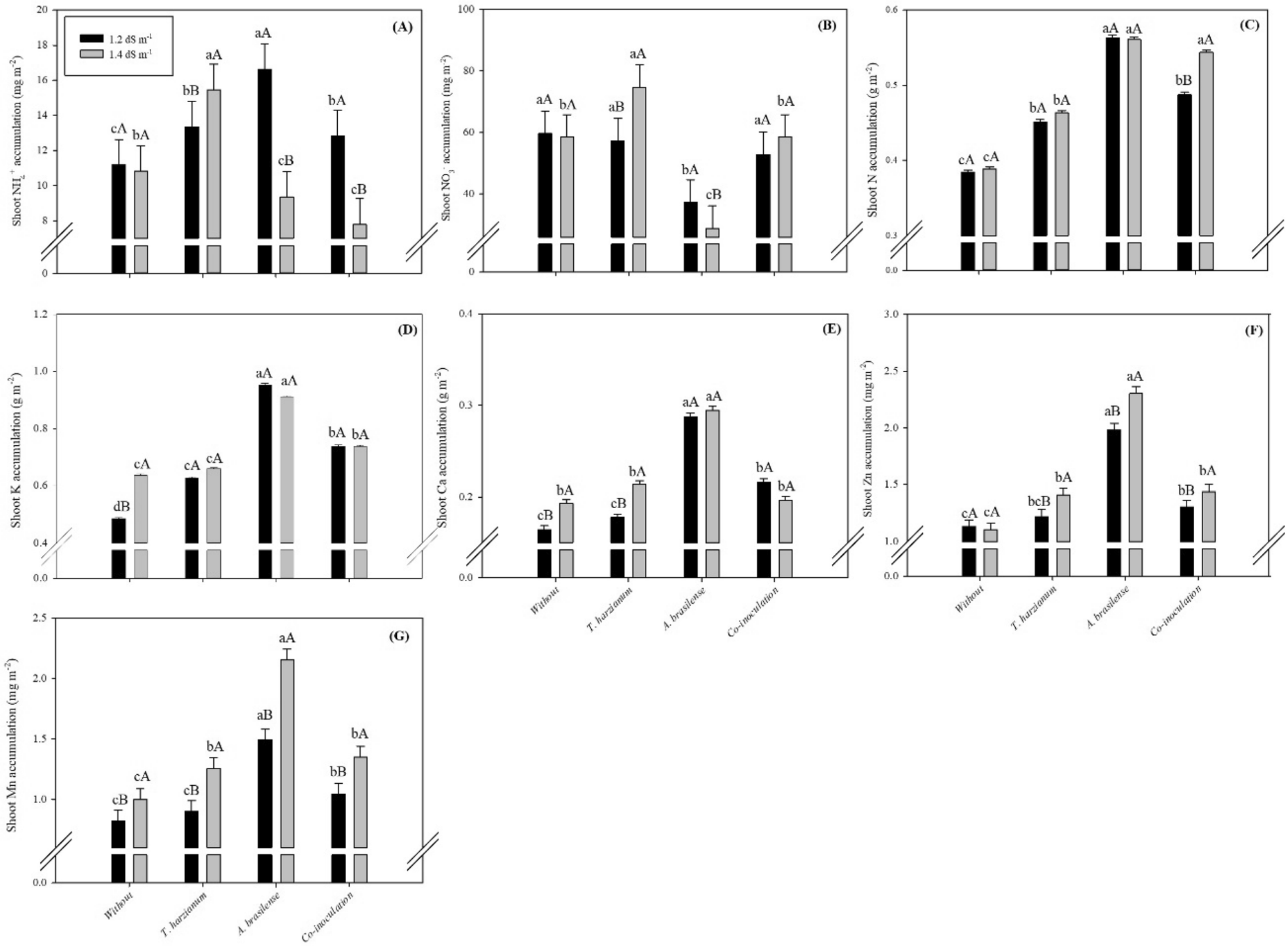

Fig. 4 Interaction between foliar inoculation and EC rates on lettuce shoot ammonium accumulation (**A**), nitrate (**B**), nitrogen (**C**), potassium (**D**), calcium (**E**), zinc (**F**), and manganese (**G**). Uppercase letters indicate difference between EC of nutrition solution, and lowercase letters indicate differences between inoculations. Error bars indicate the standard deviation of the mean

and significant correlation with RFM, RV, RL, LN, SFM, YIELD, N-Shoot, K-Shoot, N-Root, and NH_4-Root, and positive with Ca-Shoot, Cu-Shoot, and Mn-Shoot. There was negative significant correlation of NO_3-Shoot with RFM, RL, RV, LN, SFM, YIELD, N-Shoot, P-Shoot, Ca-Shoot, Mg-Shoot, Cu-Shoot, Fe-Shoot, Mn-Shoot, Zn-Shoot, and N-Root. N-Shoot had a positive and significant correlation with RFM, RL, RV, LN, SFM, YIELD, SDM, RDM, LCI, P-Shoot, K-Shoot, Fe-Shoot, Zn-Shoot, NH_4-Root, and N-Root, and negative with NO_3-Shoot and NO_3-Root (Fig. 6).

Discussion

The isolated inoculation of *T. harzianum* and *A. brasilense* provided greater growth, mass accumulation, and root volume of lettuce plants under EC 1.4 dS m^{-1}, while inoculation with *A. brasilense* at EC 1.4 dS m^{-1} favored shoot fresh matter and leaf yield. The co-inoculation at EC of 1.2 dS m^{-1} increased RFM and RV, while co-inoculation and single inoculation of *T. Harzianum* at EC of 1.2 dS m^{-1} had higher SFM and YIELD of lettuce (Fig. 3). Inoculation with *A. brasilense* increased SDM, RDM and LCI in relation to other inoculations (Table 1). Crop yield is easily altered by interaction between microorganisms and plants (Emmett et al. 2017). The balance interaction of plant and microorganisms contributes to better plant growth and development. Plant-associated microbial communities play a significant role in plant growth, plant nutrition, and carbon cycle (Vacheron et al. 2015). Plant growth is stimulated by a wide range of mechanisms and performance of microorganisms such as secretion of indole-3-acetic acid (IAA), ethylene, cytokinins, and gibberellins that all contribute to greater yield (Meza et al. 2015). Inoculation with *Azospirillum brasilense* increased leaf chlorophyll content and greater photosynthetic efficiency in several crops that favors greater plant growth and dry matter accumulation in plant tissues (Alvarez et al.

Table 3 Root ammonium accumulation (NH_4^+-Root), nitrate ($NO_3.^-$-Root), and nitrogen (N-Root) of lettuce as influenced by different incubation and EC levels

F.V	NH_4^+ mg m^{-2}	NO_3^-	N g m^{-2}
Inoculations			
Without	136.7	60.5	0.15
T. harzianum	95.4	43.6	0.15
A. brasilense	85.5	30.9	0.20
Co-inoculation	110.3	29.1	0.20
EC			
1.2 dS m^{-1}	86.2	38.6	0.16
1.4 dS m^{-1}	127.8	43.5	0.19
Probability			
Block	0.877	0.718	0.244
EC	0.001^{**}	0.000^{**}	0.067^{ns}
INO	0.041^{*}	0.001^{**}	0.019^{*}
EC*INO	0.050^{*}	0.000^{**}	0.050^{*}
CV (%)	19.58	18.68	12.87

Uppercase letters indicate differences between EC of nutrient solution and lowercase letters indicate differences among inoculation by Tukey test

CV coefficient of variation, *EC* electrical conductivities, *INO* inoculations

*Significant at 5% of probability by the F test

**Significant at 1%

2019; Galindo et al. 2019). Foliar inoculation with *A. brasilense* offers the highest concentration of chlorophyll a, chlorophyll b, and carotenoids in leaves (Bulegon et al. 2016). Foliar inoculation of *A. brasilense* and *T. harzianum* showed better performance and rapid responses in plants with short cycle. Inoculation with *Trichoderma* increased shoot and root growth with greater crop yield (Chirino-Valle et al. 2016; Jamal Uddin et al. 2016). The *Trichoderma* has the ability to stimulate and increase production of auxins in primary and secondary roots as well as increased shoots growth in relation to higher chlorophyll content (Nieto-Jacobo et al. 2017).

Inoculation with *A. brasilense* provided greater NH_4^+-Shoot at EC 1.2 dS m^{-1}, while inoculation with *T. harzianum* provided higher NH_4^+-Shoot at EC 1.4 dS m^{-1} in lettuce plants (Fig. 4A, B). Inoculation with *A. brasilense* favored the reduction of NO_3^--Shoot at both EC in relation to other inoculations. Greater shoot N accumulation was noted with all inoculations that demonstrates greater use efficiency of this nutrient (Table 2). The highest NH_4^+-Root was observed with inoculation of *T. harzianum* and co-inoculation of both microorganisms at EC of 1.4 dS m^{-1}. This effect was verified with co-inoculation at EC of 1.2 dS m^{-1} in relation to other inoculations (Fig. 5A). The lower NO3-Root was noted with co-inoculation and without inoculation in EC 1.2 dS m^{-1} and with inoculation of *T. Harzianum* and co-inoculation at EC 1.4 dS m^{-1} in relation to other inoculations (Fig. 5B). The results for nitrate and ammonium did not agree with N uptake by plant, shoot, and root of lettuce. The inoculation with greatest accumulation demonstrates that cell wall nitrogen was broken down to easily loaded forms (Table 3).

The inoculation with *Azospirillum brasilense* stimulates nitrate reductase activity in plant leaves, thus reducing nitrate to nitrite (Pereira-Defilippi et al. 2017). Inoculation with *Trichoderma* increases nitrogen cycle, production of secondary metabolism, amino acids, nutrient acquisition, and nutrient use efficiency by plants (Malmierca et al. 2015). Nitrate reduction occurs in cytosol (through the activity of nitrate reductase) into nitrite that enters the plastids into roots or chloroplasts in leaves and reducing to ammonia (by the action of nitrite reductase) which is fixed via glutamate synthase/glutamine synthase into amino acids, glutamine, and glutamate which in turn serve as substrate for transamination reactions and for the production of amino acids used for protein ingestion (Yoneyama and Suzuki 2020).

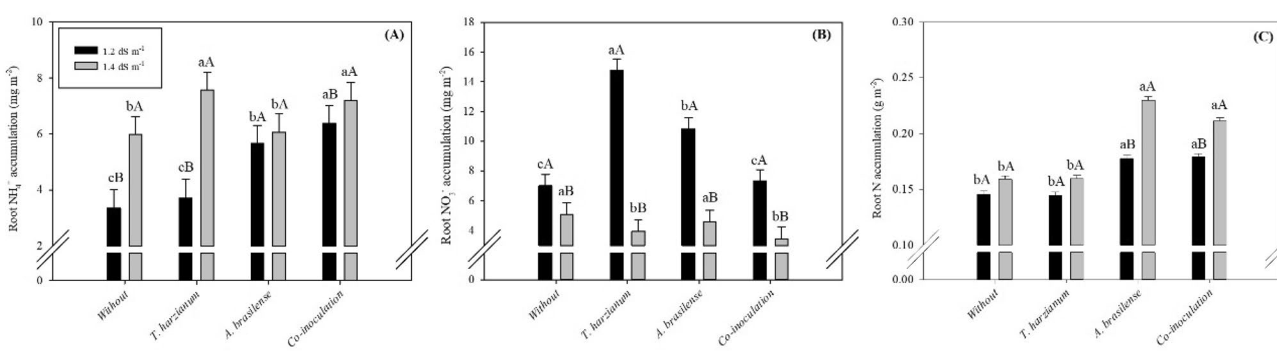

Fig. 5 Interaction between foliar inoculation and EC rates on lettuce root ammonium accumulation (**A**), nitrate (**B**), and nitrogen accumulation (**C**). Uppercase letters indicate difference between EC of nutrition solution, and lowercase letters indicate differences between inoculations. Error bars indicate the standard deviation of the mean

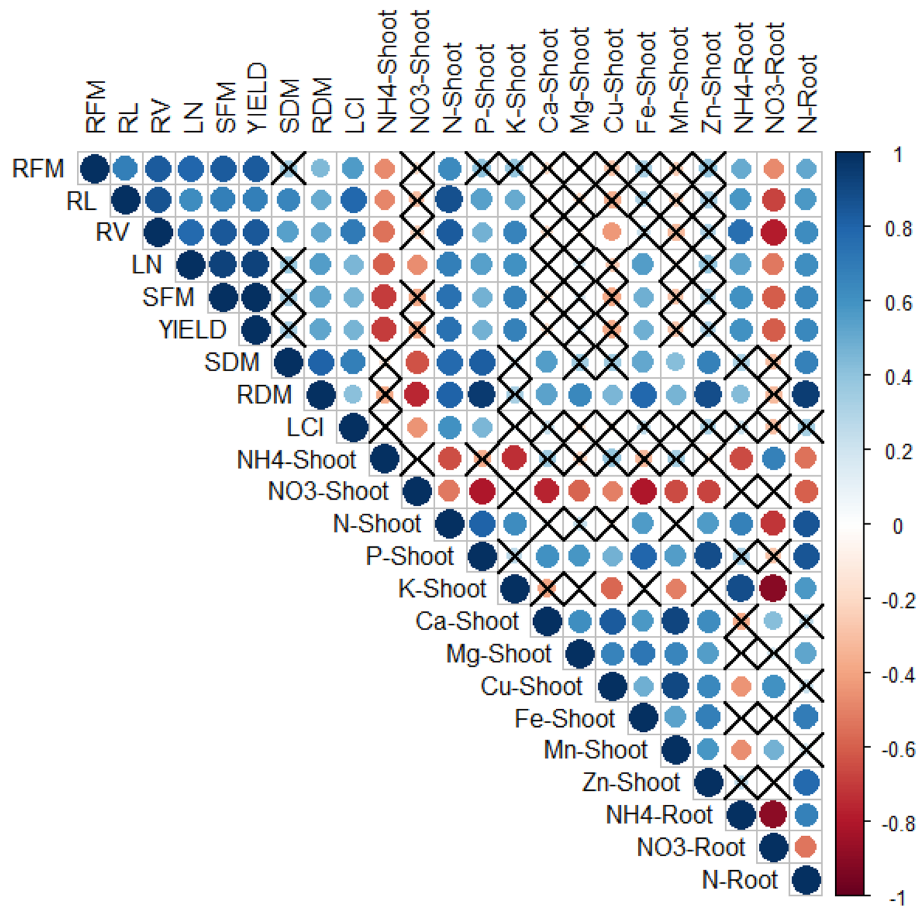

Fig. 6 Heatmap of the Pearson correlation coefficients obtained from variables analyzed in lettuce in response to EC rates and inoculations *A. brasilense* and *T. harzianum* and with co-inoculation. X indicates not significant correlation ($P < 0.05$). Root fresh matter (RFM), root length (RL), root volume (RV), leaf number (LN), shoot fresh matter (SFM), yield shoot fresh matter (YIELD), shoot dry matter (SDM), root dry matter (RDM), leaf chlorophyll index (LCI), shoot nutrients' accumulation on ammonium (NH4-Shoot), nitrate (NO3-Shoot), nitrogen (N-Shoot), phosphor (P-Shoot), potassium (K-Shoot), calcium (Ca-Shoot), magnesium (Mg-Shoot), copper (Cu-Shoot), iron (Fe-Shoot), manganese (Mn-Shoot), zinc (Zn-Shoot), root nutrients' accumulation on ammonium (NH4-Root), nitrate (NO3-Root), and nitrogen (N-Root)

The microorganisms used improved N acquisition from nutrients' solution as it increased use efficiency of N by 18% and even improve quality of lettuce by lowering leaf nitrate amount (44%) that will be consumed in natura, as excess consumption of nitrate consumed is toxic to human being. The NO_3^- ingested from food can be reduced to nitrite (NO_2^-) in digestive tract (Hathazi et al. 2018). Nitrite can also be combined with amines to form "nitrosamines", a substance characterized as carcinogenic and mutagenic (Robles 2014). The higher content of NO_3^- provides a bitter taste to vegetables, impairs palatability, purchase intent, texture, and overall flavor (Paulus et al. 2012).

There was a greater accumulation of P-Shoot, Ca-Shoot, Mg-Shoot, Fe-Shoot, and Zn-Shoot in hydroponic lettuce with inoculation of *A. brasilense* as compared to other inoculations. The co-inoculation of *A. brasilense* + *T. harzianum* provided greater root K accumulation as compared to other inoculations, while higher root Zn accumulation was observed with co-inoculation and isolated inoculation of *A. brasilense* in the lettuce (Table 2 and Fig. 4). Inoculation with *A. brasilense* increased concentrations of N, P, and K and their use efficiency in maize shoot (Marques et al. 2020). It also increased P and protein concentrations in tomato shoot seedlings (Mangmang et al. 2015). Foliar and/or seed inoculation with *A. brasilense* in wheat improved plant nutrition by increasing nutrients absorption as well as higher translocation of Ca and Mg lead to greater concentration of these nutrients in shoot (Besen et al. 2020; Boleta et al. 2020). Inoculation with *A. brasilense* provides greater use efficiency of fertilizers, improving plant nutrition (Galindo et al. 2021) that has consequently increased functioning of photosynthetic components. Photosynthetic CO_2 assimilation rates are favored by adequate K and Mg nutrition by increasing intracellular CO_2 levels and leaf photosynthetic activity (Singh et al. 2017).

The deficiency of K impairs plant photosynthesis due to critical role K in increasing leaf stomatal conductance and in guard cell regulation (Jákli et al. 2017). Plant growth and metabolism require translocation of carbohydrates from photosynthetically active tissues where sucrose loading is the major form of carbohydrate transportation into plants. The deficiency of K and Mg in phloem can impair carbon efficiency in plants, while the adequate supply of these two nutrients increases photo-protection (Trankner et al. 2018). The Ca^{2+}-binding proteins are chloroplast residents, but some are located in chloroplast membrane such as s-adenosylmethionine transporter (Stael et al. 2011). This Ca^{2+} acts as a cofactor for activation of redox enzymes of photosystem-II to regulate fructose-1, 6-bisphosphatase (FBPase), and sedoheptulose-1,7-bisphosphatase (SBPase) enzymes, which are the key factors of Calvin cycle in the pathway of carbon assimilation that occurs in chloroplast stroma (Wang et al. 2019).

Microorganisms can produce secondary metabolites that act as Fe and Zn carriers and facilitating nutrients' absorption by roots and consequently in the shoots (Goswami et al. 2016). Microbes-mediated biofortification is an effective ways to improve quality of food with less fertilizers application by following current environmental concern of the world (Jalal et al. 2022). Lettuce with high nutritional quality can eliminate supplementation of medicines due to its richness in vitamins A, B1, B2, C, iron, calcium, and minerals as well as amino acids and antioxidant characteristics that improve human diet and health (Santos et al. 2016).

Our study provided a new perspective on *Azospirillum brasilense* and *Trichoderma harzianum* inoculation and co-inoculation to increase yield, nutrient absorption, and agronomic biofortification of lettuce plants in hydroponic system. The positive effect of *A. brasilense* and *T. harzianum* on N, P, K, Mg, Ca, Mn, Cu, Zn, and Fe absorption by lettuce could improve human nutrition that lead to overcome drug supplementation for the deficiency of these nutrients. In addition, the positive effect of *A. brasilense* inoculation in reducing nitrate accumulation in shoot of lettuce was examined which in higher amount impairs palatability and flavor of lettuce leaves, while excessive consumption can cause health risks.

Conclusions

Inoculation with *Azospirillum brasilense* and *Trichoderma harzianum* favored root growth in lettuce plants. The inoculation with *T. harzianum* provided higher fresh leaves' yield in cultivation at an electrical conductivity of 1.2 dS m^{-1} which lead to reduction 15% in the use of fertilizers. In addition, fresh leaves' yield was increased with inoculation of *A. brasilense* at an electrical conductivity 1.4 dS m^{-1}.

The shoot NO_3^- accumulation in lettuce was lower with inoculation of *A. brasilense* at both electrical conductivities. Inoculation with *A. brasilense* at EC 1.2 dS m^{-1} and *T. harzianum* at EC 1.4 dS m^{-1} provided greater shoot NH_4^+ accumulation in lettuce. It did not affect shoot N accumulation in lettuce plants and resulted in highest shoot accumulations of this nutrient with inoculations and co-inoculations.

Inoculation with *A. brasilense* increased leaf accumulation of K, P, Ca, Mg, Fe, Mn, Cu, and Zn, all being nutrients for human nutrition and, therefore, lead to a biological biofortification. The EC 1.4 dS m^{-1} of nutrients' solution improved accumulation of K, Mn, Cu, and Zn without the effect of inoculations, thus resulting in biofortification with application of chemical fertilizers.

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Declarations

Conflict of interest 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.

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