

Article

Trichoderma-Enriched Vermicompost Extracts Reduces Nematode Biotic Stress in Tomato and Bell Pepper Crops

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Abstract: Root-knot nematode (RKN) is a serious threat to crops worldwide due to the difficulty in controlling it and the limited eco-friendly alternatives to deal with the biotic stress it causes. In the present work, water-extractable fractions obtained from vermicompost (WSFv), vermicompost enriched with *Trichoderma asperellum* (WSFta) and *T. virens* (WSFtv) were tested as biotechnological tools to reduce the impacts of RKN on gas exchange, water use efficiency (WUE) and nutrient concentration in tomato and bell pepper plants. The plants were infected with 5000 eggs and eventual J2 of RKN and then treated with the water-extractable fractions for seven weeks. It was observed that the addition of WSFta, WSFtv and WSFv increased the CO₂ assimilation, stomatal conductance and WUE in the tomato plants. In the bell pepper plants, WSFta, WSFtv, WSFv increased the stomatal conductance, while WUE was higher in the treatment with WSFtv. In fact, the parameters associated with the gas exchange were usually higher in the bell pepper than in the tomato plants. Overall, higher contents of N, Mg, B and Mn were detected when the extracts were applied in both bell pepper and tomato plants. The application of the water-extractable fractions, inoculated or not with *Trichoderma*, attenuates the RKN damage on the gas exchange parameters and successfully enhanced the nutrient concentration in the infected tomato and bell pepper plants, showing that it could be an important and promising tool for reducing the damage caused by this pathogen. We suggest that both the tomato and pepper plants can cope with the dilemma between growth and stress response via stomata regulation that are modulated by the WSF and *Trichoderma*.

Keywords: *Meloidogyne incognita*; plant biostimulants; biological control; stress tolerance

1. Introduction

Tomato (*Solanum lycopersicum* L.) and bell pepper (*Capsicum annuum* L.) are important crops usually cultivated in a long-term and continuous monoculture which results in severe disease cycles. Nematodes are one of the most damaging pests, and their effects on agriculture cause an estimate economic loss of 100 billion dollars yearly [1]. Root-knot nematodes (RKN) (e.g., *Meloidogyne incognita*) are endoparasites that invade the roots of the plant in the zone of elongation and migrate to the vascular cylinder, where they exhibit the formation of galls, which deform the vascular tissue [2,3]. In the infection process, the water flow and nutrient concentration of the host are damaged, impairing growth and productivity [4,5]. Several studies have shown that RKN can damage photosynthesis and

overall plant growth. Strajnar et al. [6] reported a reduction between 60 and 70% in the photosynthetic rate in tomato plants due to the infestation of *M. ethiopica*, while *M. incognita* reduced the chlorophyll content in cotton leaves [7]. Reduction in the photosynthesis rates in tomato plants might occur only two days after RKN infestation [6] and, since the nutrient concentration is a process related to the water flow and dependent of the energy from the photosynthesis, RKN can also affect the nutrition of the plant [8]. According to these authors, infected tomato plants reduced their phosphorus uptake, while infected chickpea and mung bean plants showed decreased levels of nitrogen, phosphorus and potassium [9]. Certain strategies such as covering crops, soil flooding and solarization have been used to overcome the adverse effects of RKN [10,11], but these strategies are insufficient. Another possible approach is the fumigation of the soil using chemical pesticides. Products such as methyl bromide are efficient in controlling RKN [12], but their negative impact on both the environment and on human health have led many countries to ban bromide pesticides [13].

The use of biological agents (e.g., living microorganisms or products from their metabolism) has emerged as an eco-friendly alternative for controlling RKN or activating a defense system that results in plant tolerance. Disease-controlling microorganisms can excrete toxic substances to pathogens, to parasites and to their eggs and/or larvae [14]. Fungal antagonists such as *Trichoderma* have notable application against RKN [15]. Plant bio stimulants are natural substances used to spur the defenses of the plant against RKN.

Humic materials are among the most effective groups of plant bio stimulants, affecting enzyme activity, protein metabolism, photosynthesis and tolerance against biotic and abiotic stress [16–18]. Vermicompost are renewable sources of humic materials that are able to trigger the priming stimulus, resulting in abiotic stress resistance in plants [19]. Water-soluble fraction (WSF) from these bioresources stimulates plants such as tomato [20,21], *Callophyllum brasiliense* [22], lettuce [23], bell pepper [24] and maize [25,26]. It can modulate the development of the root, the primary and secondary metabolisms, and the balance in hormones and reactive oxygen, resulting in enhanced nutritional efficiency and disease suppression [17,23,27,28]. We have recently shown that WSF enriched with *Trichoderma* boosts the yield of tomato and bell pepper plants infected with RKN [29]. A 10-fold increase in yield for tomato plants was observed when water-extractable fraction of vermicomposts enriched with *T. virens* was applied when compared to the control. Regarding biocontrol of RKN, the same treatment was able to reduce the number of J2 eggs by 62% on bell pepper and 46% on tomato plants, respectively, when compared to the control. This study examined the potential physiological mechanisms related to the stress alleviation of *M. incognita* in infected tomato and bell pepper plants using WSF extracted from vermicompost of cow manure (WSFv), vermicompost enriched with *Trichoderma asperellum* (WSFta) or with *T. virens* (WSFtv). The present study tested two hypotheses: (i) the effect of the application of the water-extractable fractions from vermicompost is shown by the enhancement of nutrient concentration; and (ii) the addition of *Trichoderma* mitigates the damage of the plant growth infected-tomato and bell pepper plants by nematodes.

2. Materials and Methods

2.1. Vermicompost Production and *Trichoderma* Strains Inoculation

Dairy cow manure and *Eisenia fetida* worms (80 worms/dm³) were used to produce three different vermicomposts in polyethylene containers (310 dm³). They were defined in this study as vermicompost without *Trichoderma* inoculation, vermicompost inoculated with *T. asperellum* and vermicompost inoculated with *T. virens*. For this purpose, *T. asperellum* (Tr266B) and *T. virens* (F1d5c1) strains were grown in a potato dextrose agar medium using a rotary shaker operating at 120 rpm (5 days, 25 °C). Next, the fungal suspensions were adjusted to 1×10^6 spores mL⁻¹ with the aid of a Neubauer chamber and an optical microscope and 2 L were spread separately on each container, 15 and 30 days after the beginning of the vermicomposting process. For the control treatment, the same volume of deionized water (2 L) was spread. The moisture of the material was controlled

weekly and kept at 60–70%, using distilled water over 120 days of the process, when the material was used for extraction and application in plants.

2.2. Growth of Bell Pepper and Tomato Plants

Tomato (cv. Santa Cruz Kada Gigante) and bell pepper (cv. Magali R), both susceptible to *M. incognita* race 1, were grown in a greenhouse at the Embrapa Hortaliças research center, DF, Brazil. For this purpose, 15 day-old tomato and bell pepper seedlings grown on peat moss and perlite substrate (Carolina Soil, RS, Brazil) were transplanted to 5 L plastic pots filled with sterilized samples of an Oxisoil with the following characteristics: pH: 6.0, P: 25.7 mg dm⁻³, K: 1.9 mmolc dm⁻³, Ca: 19.1 mmolc dm⁻³, Mg: 11.3 mmolc dm⁻³ and C: 30.2 g dm⁻³. The plants were irrigated (one dripper per pot, 25 L h⁻¹ dripper, Netafim, Israel) to maintain optimal levels of water in the soil, which was determined by soil sensors (Irrigas[®], Hidrosense, SP, Brazil). The plants were properly monitored from the transplant till 70 days for the harvesting.

2.3. *M. Incognita* Inoculation

Female root-knot nematodes were collected from tomato roots in the experiment area of Embrapa Hortaliças, identified by isoenzyme standard [30] and then subjected to perineal cutting to identify the species (*M. incognita* race) [31]. Subsequently, the nematodes were multiplied on Rutgers tomato plants. *Meloidogyne* race was identified by the differentiating host test [32]. Approximately 45 days after inoculation, the eggs and juveniles of the second stage (J2) were extracted from the root systems of the plants and quantified under a microscope stereoscope for the immediate inoculation of the experiments, using suspensions adjusted to deliver 5000 eggs and potential J2s per plant. The inoculation occurred right after the plants were transplanted. The plastic pots containing the RKN inoculated bell pepper and tomato plants were placed on a previously disinfected elevated bench; the greenhouse was divided in two in order to avoid cross-contamination between the experiments.

2.4. Water-Soluble Fraction Obtained from the Vermicomposts

After 120 days of vermicomposting, fresh samples were obtained, separated and immediately processed in the laboratory. The different WSFs were obtained by mixing the vermicomposts with distilled water in a 1:5 solid-liquid ratio (v:v). This ratio was achieved after preliminary testing of five different solid-liquid ratios (data not shown). The materials were shaken mechanically for four hours, followed by siphoning and immediate application of 200 mL pot⁻¹ next to the plant's root zones. A control treatment using the same volume of distilled water was also conducted. The plants were treated weekly and at the same rate for seven weeks, starting after the transplanting. The chemical characterization of the same WSFs was published previously by dos Santos Pereira et al. [29].

2.5. Gas Exchange Measurements

Gas exchange in the plants was evaluated 50 days after transplanting and RKN inoculation. The following parameters were determined using a portable photosynthesis analyzer for infrared radiation (LICOR Li-6400XT, Lincoln, NE, USA): Photosynthetic rate (A), stomatal conductance (gs), internal CO₂ concentration (Ci) and transpiration rate (E). The water use efficiency (WUE) was calculated using the A/E relationship, while the average carboxylation capacity (ACi) was calculated by A/Ci. The measurements were performed in the third full and healthy expanded leaf exposed to sunlight, between 8 and 10 am. The concentration of CO₂ artificially injected into the assimilation chamber was fixed at 400 μmol mol⁻¹. The evaluations were made under 300 μmol m⁻² s⁻¹ of photosynthetically active flux density for solanaceous crops [33].

2.6. Nutrient Concentration on Plants Tissues

The plants were harvested 70 days after transplanting, oven-dried at 65 °C until constant weight (Ethik 410-TD, São Paulo, Brazil) and passed through a knife mill (Solab, São Paulo, Brazil). The material underwent a digestion process in a microwave oven (Mars Xpress CEM, Matthews, NC, USA), using concentrated HNO₃ and HCl. The microwave digestion conditions were: 10 min of the ramp (heating), temperature maintenance at 170 °C (for 15 min) with 1600 W of power and cooling for 20 min until room temperature. Subsequently, the samples were transferred to polypropylene tubes, where the final volume of 50 mL was completed with deionized water for the quantification of nutrients through plasma atomic emission spectrometry (Shimadzu ICPE-9000 OES, Kyoto, Japan). The levels of N-total were obtained by concentrated H₂SO₄ digestion, and the extract was analyzed by the Kjeldahl method.

2.7. Statistical Analysis

Each experiment with RKN-inoculated tomato and bell pepper plants included the four previously described treatments with six replicates, in a completely randomized design. The analysis of variance and a Fisher's Least Significant Differences test (one-way ANOVA, $p < 0.05$) were determined using the R statistical software version 3.6.1.

3. Results

3.1. Gas Exchange Measurements

3.1.1. Photosynthetic Rate (A)

The tomato plants (Figure 1A) were affected by the treatments, with the highest average for *A* observed for WSFta, followed by WSFtv and WSFv. These values were respectively 90, 30 and 17% higher ($p < 0.05$) when compared to the control. There was no significant difference for *A* between the treatments for the bell pepper plants (Figure 1B), which showed a general average of 14.5 $\mu\text{mol CO}_2\text{ m}^{-2}\text{ s}^{-1}$.

3.1.2. Stomatal Conductance (gs)

With the exception of the tomato plants in the WSFv treatment, the *gs* values were stimulated in all treatments that received WSF, when compared to the control plants (Figure 1C,D). For the tomato plants, the WSFta treatment was 357% higher than the control. The WSFtv treatment showed a smaller value than the WSFta one, in the order of 0.12 $\text{mol H}_2\text{O m}^{-2}\text{ s}^{-1}$, but it was still higher than the control plants. For the bell pepper plants, WSFta, WSFv and WSFtv treatments did not differ between them but were higher than the control. The values observed in the WSFta treatment was 122% higher ($p < 0.05$) than the control, while WSFv and WSFtv were 105 and 94% higher than the control.

3.1.3. Internal CO₂ Concentration (Ci) and Transpiration Rate (E)

Internal CO₂ concentration decreased due to the addition of the different WSF in tomato plants (Figure 1E). The plants of the control showed an average of 292.01 $\mu\text{mol CO}_2\text{ mol}^{-1}$, while WSFtv, WSFta and WSFv treatments presented values of 230.18, 199.22 and 199.34 $\mu\text{mol CO}_2\text{ mol}^{-1}$, respectively. These decreases were in the order of 20 and 32% compared to control. For the bell pepper plants (Figure 1F), no differences between the treatments were observed, and a general average of 298.20 $\mu\text{mol CO}_2\text{ mol}^{-1}$ was recorded. The tomato plants had their *E* drastically reduced in 57, 68 and 73% for WSFtv, WSFv and WSFta, when compared to the control (Figure 1G). However, similarly to *Ci*, no differences were observed between the bell pepper plants (Figure 1H).

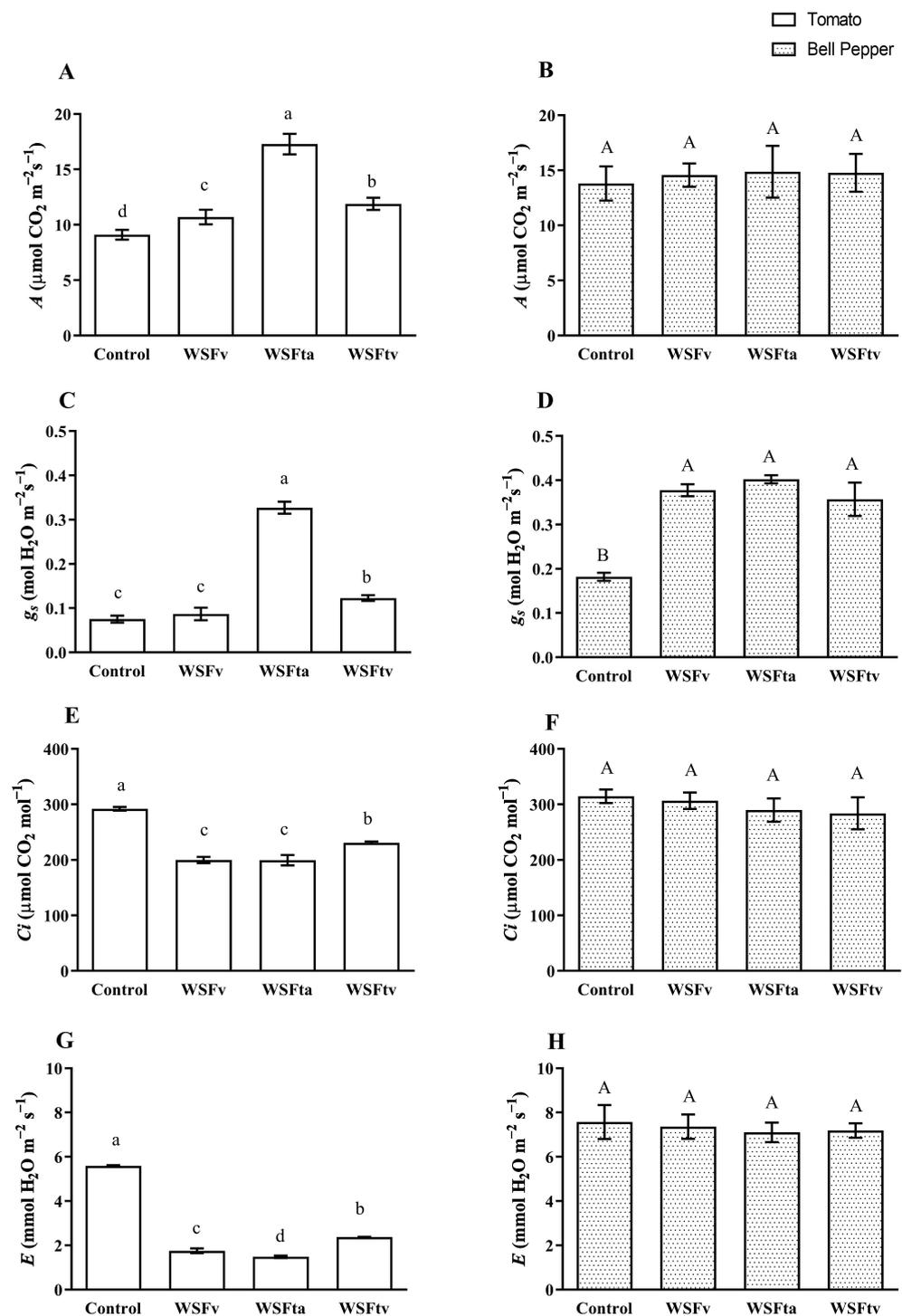


Figure 1. Photosynthetic rate (A) (A,B), stomatal conductance (g_s) (C,D), internal CO_2 concentration (C_i) (E,F) and leaf transpiration (E) (G,H) in *Meloidogyne incognita* infected tomato and bell pepper plants treated with water-soluble fraction from vermicompost enriched or not with *Trichoderma asperellum* and *T. virens*. Lowercase letters and upper letters represent (LSD, $p < 0.05$) the effects of treatments for tomato plants and bell peppers plants, respectively. Bars represents means \pm standard deviations.

3.1.4. Water Use Efficiency (WUE) and Carboxylation Capacity (AC_i)

The tomato plants that received WSF significantly increased the WUE when compared to the control plants (Figure 2A). The WSFta treatment reached a value of $11.43 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, which represented an increase of 525% when compared to the control

($1.83 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$). The values for the WSFv and WSFtv treatments were 225 and 169% higher than the control, respectively. For the bell pepper plants, the WSFtv treatment showed the highest value of $2.06 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, 24% higher than the control ($1.66 \mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) (Figure 2B). The WSFv and WSFta treatments showed no significant difference from the control.

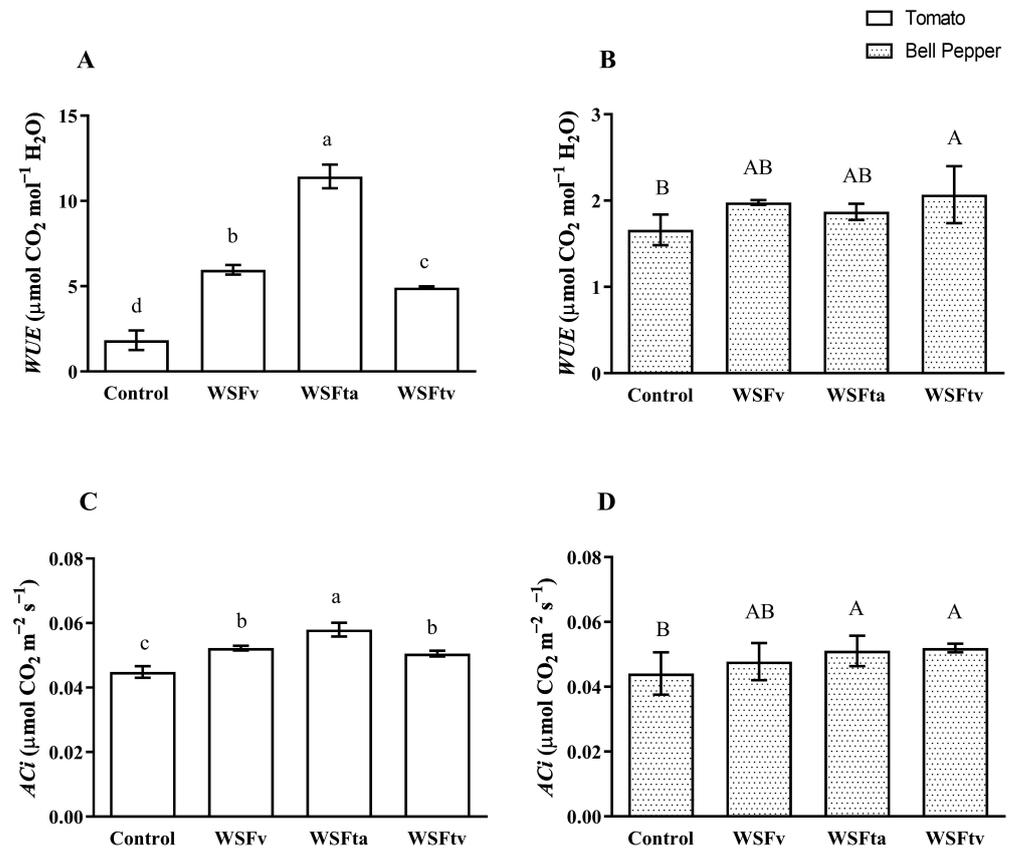


Figure 2. Water use efficiency (WUE) (A,B) and carboxylation capacity (ACi) (C,D) in *Meloidogyne incognita* infected tomato and bell pepper plants treated with water-soluble fraction from vermicompost enriched or not with *Trichoderma asperellum* and *T. virens*. Lowercase letters and upper letters represent (LSD, $p < 0.05$) the effects of treatments for tomato plants and bell peppers plants, respectively. Bars represent means \pm standard deviation.

The tomato plants had their ACi significantly affected ($p < 0.05$) by the different WSF (Figure 2C), and the WSFta treatment was 30% higher than the control. The WSFv and WSFtv treatments were about 18% higher than the control. In the bell pepper plants (Figure 2D), the WSFtv and WSFta treatments were 16% higher when compared to the control. The WSFv treatment did not differ from the control.

3.2. Nutrient Uptake

3.2.1. Macronutrients

The content of N was higher ($p < 0.05$) in the tomato plants that received WSF (Figure 3A). The WSFv, WSFta and WSFtv treatments respectively showed increases in the order of 46, 37 and 35% when compared to the control plants. In bell pepper plants, the content of N was 64 and 61% higher than the control. Neither the tomato nor the bell pepper plants had their p content changed by the treatments (Figure 3B). Similarly, the content of K and S were not affected in the bell pepper plants (Figure 3C,D), while Ca was not affected in the tomato plants (Figure 3E).

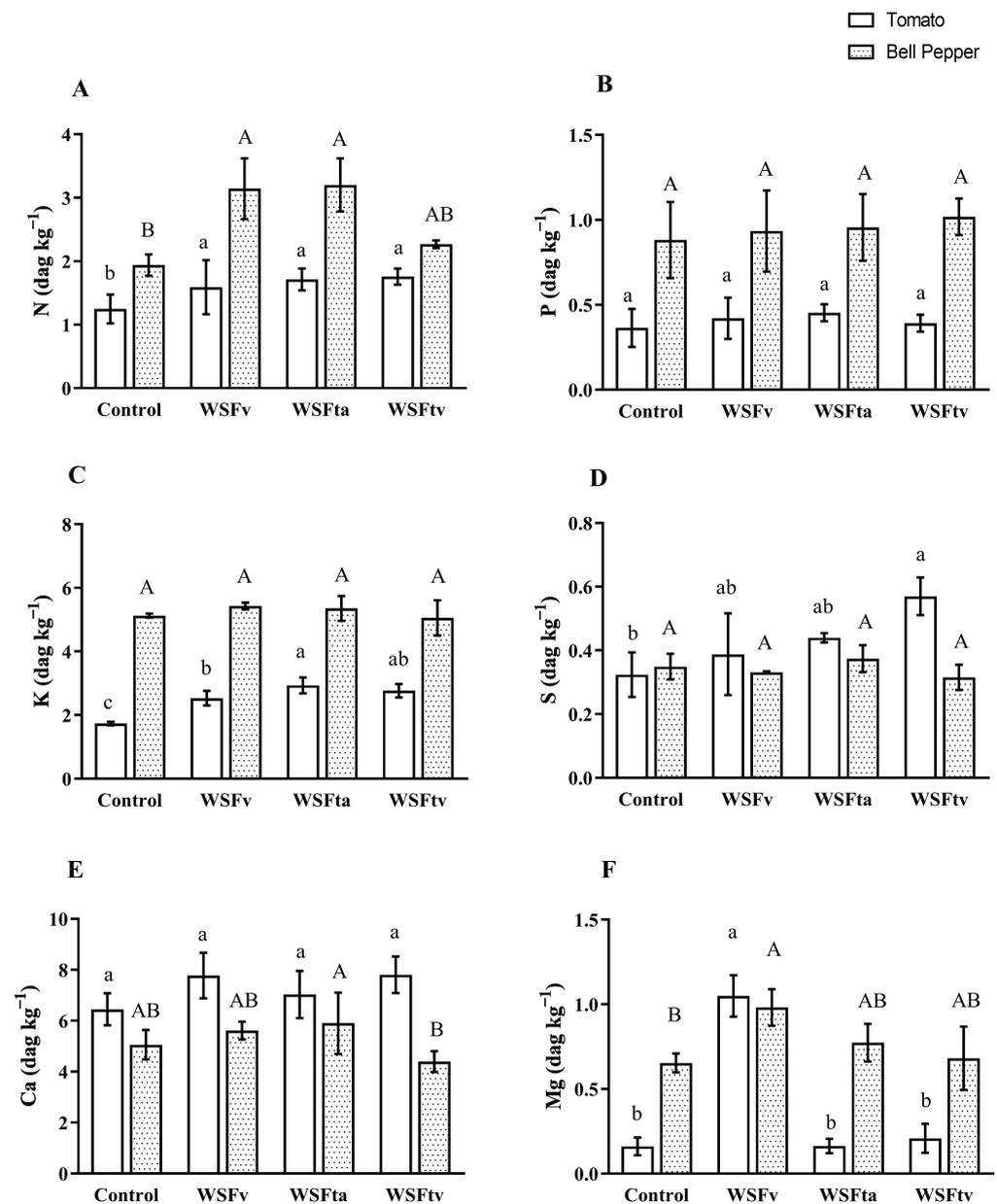


Figure 3. Macronutrient content (A): Nitrogen, (B): Phosphorus, (C): Potassium, (D): Sulfur, (E): Calcium and (F): Magnesium) in *Meloidogyne incognita* infected tomato and bell pepper plants treated with water-soluble fraction from vermicompost enriched or not with *Trichoderma asperellum* and *T. virens*. Lowercase letters and upper letters represent (LSD, $p < 0.05$) the effects of treatments for tomato plants and bell peppers plants, respectively. Bars represent means \pm standard deviation.

The value of 2.93 dag kg⁻¹ of K for the tomato plants under the WSFta treatment (Figure 3C) was 69% higher than the control (1.73 dag kg⁻¹). Yet, the values of 2.76 and 2.53 dag kg⁻¹ observed in the WSFtv and WSFv treatments were higher than the control, in the order of 60 and 46%, respectively. Still, considering the tomato plants, the WSFtv treatment resulted in a content of S of 0.57 dag kg⁻¹, a value 78% higher than the one observed in the control plants (0.32 dag kg⁻¹; Figure 3D). The tomato and bell pepper plants under the WSFv treatment showed the highest content of Mg, in the order of 1.05 and 0.98 dag kg⁻¹ (Figure 3F), respectively.

3.2.2. Micronutrients

In both crops, the content of Mo, Zn and Cu were not affected by the treatments (Figure 4A–C). The content of B and Fe in the bell pepper plants also did not change compared to the control. In the tomato plants, the WSFtv treatment showed the highest value for B, while WSFv presented the lowest value (Figure 4D). The first one was 184% higher than the control, while the second was 58% lower.

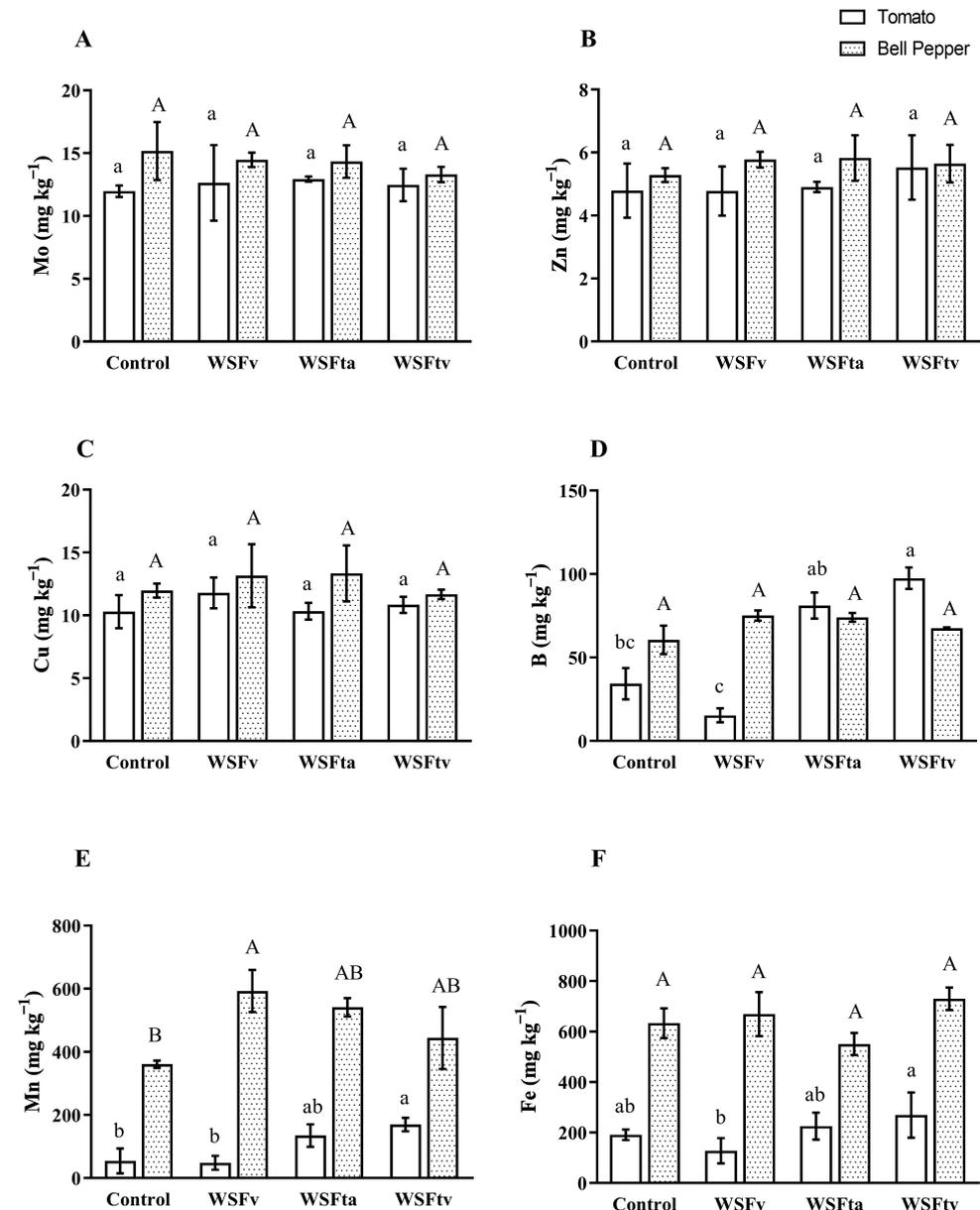


Figure 4. Micronutrient content (A): Molybdenum (Mo), (B): Zinc (Zn), (C): Copper (Cu), (D): Boron (B), (E): Manganese (Mn), and (F): Iron (Fe) in *Meloidogyne incognita* infected tomato and bell pepper plants treated with water-soluble fraction from vermicompost enriched or not with *Trichoderma asperellum* and *T. virens*. Lowercase letters and upper letters represent (LSD, $p < 0.05$) the effects of treatments for tomato plants and bell peppers plants, respectively. Bars represent means \pm standard deviation.

The tomato plants that received WSFtv showed 176.10 mg kg⁻¹ of Mn, while WSFta resulted in a value of 124.80 mg kg⁻¹ (Figure 4E). In the bell pepper plants, the Mn content of the WSFv treatment was 58% higher than the. The WSFta and WSFtv treatments were

statistically similar to those observed in the plants of the control treatment. Finally, the Fe content in the plants under the WSFtv treatment was 41% higher than the control plants (Figure 4F).

3.3. Effect of Adding the Different WSF

In addition to the individual evaluation of each treatment, the responses for the three different WSF were grouped and contrasted against the control treatment, and the results are presented in Tables 1–3. On average, the tomato plants that received WSF showed CO₂ assimilation 46% higher than the control (Table 1). In bell pepper plants, the effect was reduced, in the order of 7%. The gs values revealed that the plants under the WSF addition kept their stomata more open than the plants in the control with the exception of the WSFv treatment in tomato plants. On average, the tomato and bell pepper plants under WSF addition presented gs levels that were 125 and 111% higher than the control.

In the tomato plants, Ci and E were 28 and 67% higher in the control than the average of treatments under WSF addition. The bell pepper plants followed the same trend but to a lesser extent, resulting in 7 and 5% lower Ci and E in the plants under WSF addition. The WSF treatment showed averages 307 and 16% higher for WUE in the tomato and bell pepper plants, respectively, when compared to the control. The Aci values were also stimulated by the WSF, resulting in 20 and 14% more Aci for the tomato and bell pepper plants when compared to the control.

Except for P, S (Table 2) and Mo (Table 3), in the bell pepper plants, the other nutrients presented higher concentrations in the plants that received WSF. In the tomato plants, the addition of WSF resulted in values for N, K, Mg, S, B and Mn that were respectively 35, 58, 194, 47, 88 and 111% higher than the control. In the case of the bell pepper plants, increases in N (48%), Mg (25%), B (19%) and Mn (46%) were also observed.

Table 1. Photosynthetic rate (A), stomatal conductance (gs), Internal CO₂ concentration (Ci), transpiration rate (E), water use efficiency (WUE) and carboxylation capacity (Aci) in infested-RKN tomato and bell pepper plants treated with different water-soluble fractions (WSF) extracted from vermicomposts. Upwards pointing arrows indicate an increase and downwards pointing arrows indicate a decrease.

Crop		A	Gs	Ci	E	WUE	Aci
		$\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$	$\text{mol H}_2\text{O}$ $\text{m}^{-2} \text{s}^{-1}$	$\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$	$\text{mol H}_2\text{O}$ $\text{m}^{-2} \text{s}^{-1}$	$\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$	$\mu\text{mol CO}_2$ $\text{m}^{-2} \text{s}^{-1}$
Tomato	Control	9.10	0.08	292.23	5.59	1.83	0.044
	WSF	13.29	0.18	210.11	1.87	7.44	0.053
	Variation (%)	46 (↑)	125 (↑)	28 (↓)	67 (↓)	307 (↑)	20 (↑)
Bell pepper	Control	13.80	0.18	314.64	7.57	1.66	0.044
	WSF	14.74	0.38	293.45	7.22	1.92	0.050
	Variation (%)	7 (↑)	111 (↑)	7 (↓)	6 (↓)	16 (↑)	14 (↑)

Table 2. Content of macronutrients in infested-RKN tomato and bell pepper tissues treated with different water-soluble fractions (WSF) extracted from vermicomposts. Upwards pointing arrows indicate an increase and downwards pointing arrows indicate a decrease.

Crop		N	P	K	Ca	Mg	S
		dag kg^{-1}					
Tomato	Control	1.25	0.36	1.73	6.45	0.16	0.32
	WSF	1.69	0.42	2.74	7.54	0.47	0.47
	Variation (%)	35 (↑)	17 (↑)	58 (↑)	17 (↑)	194 (↑)	47 (↑)
Bell pepper	Control	1.94	0.99	5.13	5.06	0.65	0.34
	WSF	2.87	0.99	5.28	5.30	0.81	0.34
	Variation (%)	48 (↑)	0 (-)	3 (↑)	5 (↑)	25 (↑)	0 (-)

Table 3. Content of micronutrients in infested-RKN tomato and bell pepper tissues treated with different water-soluble fractions (WSF) extracted from vermicomposts. Upwards pointing arrows indicate an increase and downwards pointing arrows indicate a decrease.

Crop		Mo	Zn	Cu	B	Mn	Fe
dag kg ⁻¹							
Tomato	Control	11.97	4.79	10.29	34.30	54.30	191.00
	WSF	12.68	5.07	10.98	64.60	117.71	207.63
	Variation (%)	6 (↑)	6 (↑)	7 (↑)	88 (↑)	111 (↑)	9 (↑)
Bell pepper	Control	15.17	5.28	11.97	60.53	360.67	633.33
	WSF	14.93	5.75	12.72	72.24	526.11	650.00
	Variation (%)	2 (↓)	9 (↑)	6 (↑)	19 (↑)	46 (↑)	3 (↑)

4. Discussion

RKN are one of the most damaging pathogens in agriculture. Generally, nematodes enter emerging host plant tissues below grounds, crowd up in a stem, invade shoots through buds and stomata and eventually destruct host-plant by feeding on plant tissues. The current control methods are insufficient and are harmful to the environmental and to the farmers' health. In this way, the water-extractable fractions of vermicomposts (WSF) have been reported as a biostimulant able to boost the formation of lateral roots root, cause acidification of the root's external medium and activates the plasma membrane H⁺ – ATPase [34,35]. When enriched with *Trichoderma* spp., the result is a WSF added with disease suppression capabilities [36,37]. Therefore, the development of efficient and eco-friendly products that control RKN and that activate the defense mechanisms of plants is crucial. In this study, the WSF extracted from vermicompost, vermicompost enriched with *T. asperellum* and *T. virens* were tested as potential biological tools to reduce the impacts of *M. incognita* on gas exchange, water use efficiency and nutrient concentration in infected tomato and bell pepper plants. Gas exchange parameters are directly related with photosynthesis and plant growth. We have showed that the parameters associated with the gas exchange are usually higher in the bell pepper than in the tomato plants as previously reported [38]. The stomatal conductance (gs) and transpiration (E) were also higher in the bell pepper plants when compared to the tomato plants. The tomato plants that were exposed to the treatments showed strongly reduced levels of E, which resulted in higher WUE and CO₂ assimilation.

The infected plants from the control treatment showed the lowest gs values, suggesting a closure of the stomata attributed to the RKN. It is a known fact that biotic and abiotic stress may increase the production of abscisic acid (ABA) and reactive oxygen species (ROS) raising a calcium wave in the cytosol, which results in stomata closing and concomitant reduction in water loss [34]. As previously described, RKN is established in the vascular cylinder, where galls are formed [2,3], which in itself affects the water flow in the plants [39].

We have observed that WSF and *Trichoderma* increased the stomatal conductance of plants in a similar manner with reported for cucumber plants treated with humic acids [40]. Since the treatments increased WUE in both the tomato and pepper plants, it appears that plants' dilemma between growth and stress response via stomata regulation is modulated by the WSF and *Trichoderma*. Especially in the case of the tomato plants, the regulation of stomatal opening was more efficient in the infected plants treated with WSFTa and WSFTv, resulting in higher CO₂ assimilation and fewer losses by leaf transpiration. As a result of this regulation, the values for WUE were higher in all the tomato plants that received any WSF when compared to the control. The mechanisms involved in this process are not clear but may be related to the presence of phytohormones in the WSF. Zhang et al. [41] detected cytokine in aqueous extracts of vermicomposts, whereas humic acids were more recently associated with the increases in ABA root concentration and root hydraulic conductivity [40].

Vermicompost humic acids modulate H₂O₂, nitric oxide, and both calcium and proton fluxes. It is also associated with root growth [25,42,43], affecting mineral nutrient concentration [44,45]. On the other hand, mineral deficiency is a common symptom in plants infected with RKN [8,9]. The disruption of the xylem in nematode galls or the presence of abnormally formed vessels interrupt water transport, decreasing the translocation of nutrients to the shoot in tomato plants [46,47], shifting the microbial dynamics and the supply of nutrients [48]. The addition of WSFta and WSFtv increased the content of N in both the tomato and bell pepper plants. The contrasting control plants with the WSF-average treatments showed an enhancement of nutrients content (N, Mg, B, and Mn). Humic materials activate the tricarboxylic acid cycle [19,49], assisting the ATP generation and plasma membrane H⁺ – ATPase activation [21,25], both essential to the nutrient concentration of the plant [50].

The target of rapamycin (TOR), a protein kinase, has emerged as a central coordinator of nutrient, energy and stress signaling networks [51] and it has been recently considered essential for nutrient concentration triggered by humic acids [19,52]. Since hormonal signaling is influenced by nutrient levels [53], and humic substances are endowed of hormonal activity, it is tempting to speculate that TOR might be related to the WSF ameliorate effects on RKN. Both WSF and *Trichoderma* appear to assist the plant by balancing both stress and growth signals. The infestation of nematodes causes the rhizospheric microbial communities to change [54]. Antagonistic strains such as *Trichoderma* may have a crucial role in the amelioration of the indigenous microflora of rhizosphere [54,55]. We have recently shown a reduction in the number of J2 eggs and the suppression of RKN reproduction in the bell pepper and tomato plants [29]. The *Trichoderma*-WSF combination probably prompts the defense mechanisms of the plants under biotic stress in *Solanaceae* via physiological mechanisms linked to photosynthesis and nutrient uptake.

5. Conclusions

The use of the WSF enriched or not with *Trichoderma* altered the plant ecophysiology of RKN-infected tomato and bell pepper plants by changing the WUE and the CO₂ assimilation. Adding the different WSF increased the nutrient concentration in RKN-stressed plants, unveiling the role of these biostimulants on plant nutrition under biotic stress. Thus, WSF has the potential of becoming an important and promising tool to reduce the damage caused by this pathogen in the evaluated crops.

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