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Effect of wood vinegar on vegetative growth and nutrient uptake in two citrus rootstocks

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ABSTRACT

Purpose: It is believed that wood vinegar (WV) can improve soil nutrient availability and uptake, thereby improving plant growth and development. In this study we investigate the effect of WV on the availability of macro- and micro elements in the soil and the uptake, translocation and efficiency of these elements in seedlings of sour orange (SO) and Mexican lime (ML) as well as on plant growth. **Research method:** The applied WV (1 and 2%) (v/v) was added to the irrigation water at intervals of 3, 6, 9 and 12 weeks after planting. **Findings:** The results showed that the use of WV at both concentrations reduced the phosphorous (P) and potassium (K) concentration in the leaves of ML, reduced the percentage of calcium (Ca) uptake and efficiency of copper (Cu) in SO and increased the iron (Fe) in ML root (1150 to 1320 mg kg^{-1} DW). Although 1% WV increased soil availability of Ca, sodium (Na), zinc (Zn) and manganese (Mn) and thus decreased root K/Na and Ca/Na, WV 2% improved Mn and K availability but decreased Ca in the soil solution. Application of 1 and 2% WV reduced root dry weight by 16.1 and 12.9% in SO seedling, respectively and in ML seedlings 2% WV reduced total chlorophyll and leaf greenness. **Research limitations:** No limitations were found. **Originality/Value:** The results showed that although the addition of WV to the soil can reduce the pH and thereby increase the availability of some elements such as K and Mn, the increase in EC prevents the effective absorption and translocation of elements and thus plant growth such as root dry weight and greenness.

INTRODUCTION

Annually, 298788 tons of citrus fruits are produced in six southern provinces and three northern coastal provinces of Iran in an area of 5613130 hectares (Ahmadi et al., 2021). In the citrus production industry, various rootstocks are used and they are effective on characteristics (more than 20 horticultural characteristics) such as resistance to pests and soil diseases, early ripening of the crop (Davies & Albrigo, 1999), external and internal fruit quality (Aguilar-Hernández et al., 2020; Castle et al., 2016), depth of the root and tree growth (Albrigo et al., 2019). The citrus rootstocks have significant differences in physiological and morphological characteristics of the root system (Eissenstat & Achor, 1999) and then absorption of nutrients (Romero et al., 2006). Different ability of citrus rootstocks to absorb the elements from the soil and translocation those into shoot has been proven in many other previous studies (Albrigo et al., 2019; Romero et al., 2006). These differences can affect the water and mineral uptake efficiency through changes in root distribution, root growth and carbohydrate distribution (Pedrero et al., 2015). On the other hand, in many citrus production areas in Iran, soils have mineral imbalances due to high pH and alkalinity and in many cases, despite the addition of large amounts of micro and macro elements in the form of chemical fertilizers, trees suffer from disorders caused by deficiency or toxicity of these elements. Although Mexican lime (*Citrus aurantifolia*) are sensitive to chilling and Tristiza virus but is highly used by citrus growers, especially in the southern regions of Iran, due to its high percentage of seed germination and high transplant ability. Sour orange (*Citrus aurantium* L.) is a medium-sized rootstock with a deep root system in which the root density is low and the ability to produce secondary roots is moderate and despite having a positive effect on fruit yield and quality, it is sensitive to soils salinity, soil nematodes and especially Tristiza disease (Davies & Albrigo, 1999). In recent years, the use of some compounds such as humic acid, wood vinegar (WV) and biofertilizers has been increased with the aim of increasing the availability of elements in the soil (Azarmi-Atajan et al., 2023; Fani, 2023; Ziatabar Ahmadi et al., 2024). Wood vinegar (pyroligneous acid) is one of the main liquid by-products that obtained from the condensed vapors generated during the biomass pyrolysis and consisted of many complex organic components and compounds (Hou et al., 2018). Wood vinegar is composed mainly of water (80-90%) and more than 200 organic compounds such as acids, alcohols, phenols, aldehydes and esters (10-20%) but the main component of it is acetic acid (Aguirre et al., 2020). Its composition depends on the type of biomass used, the moisture content of feedstock and pyrolysis process used (Fagernäs et al., 2012; Martín et al., 2017; Mathew & Zakaria, 2015; Wang et al., 2010) and used in various areas such as food additive, anti-inflammatory agent, anti-fungal drug, pest control agent (Grewal et al., 2018) and as weed killing agent (Aguirre et al., 2020). Agriculture is one of the most important application fields of WV and it can be used as plant fortifier (Jothityangkoon et al., 2008; Mu et al., 2004) and soil amendments (Mahmud et al., 2016; Zulkarami et al., 2011). There is also evidence that WV application improve soil microbial conditions (Koç et al., 2019; Steiner et al., 2008), enhancing soil enzyme activity (Lashari et al., 2013), nutrient availability (Jeong et al., 2015), nutrient uptake (Pan et al., 2017), reduce the concentrations of metal ions in the soil and prevent their uptake by plants (Theapparat et al., 2015), immobilize metal contaminants such as nickel, zinc (Zn) and copper (Cu) in compost solid wastes and charcoal (Zhu et al., 2021a), has a beneficial effect on leaching of soluble salts, decrease the soil pH resulting in the improvement of crop productivity in saline soils (Lashari et al., 2013), affect the germination and growth of crop seed (Ling-jie et al., 2014; Pan et al., 2010) and promote the nitrogen utilization efficiency (Jianming, 2003; Tsuzuki et al., 2000). Wood vinegar has been shown to contain different levels of macro-elements such as K, P, Ca and mico-elements such as Fe, Mn, Cu, boron, Zn and molybdenum and some other elements such as aluminum, cadmium, arsenic, Na, lead and chromium (Zulkarami et al., 2011). Most of the previous syudies focused on the effect of WV on the growth of annual and herbaceous plants and had conflicting results on their growth. On the other hand, the effect of WV on the availability of soil elements, especially in alkaline soils with high percentage of lime and calcium, has not been investigated. It is supposed that WV application to soil may affect availability, uptake and translocation of nutrients from calcareous soils and the citrus rootstocks may vary in their reaction to the soil changes by WV. Therefore, the aim of this study was to investigate the effect of WV on soil availability and plant uptake, translocation and consumption of nutrients as well as vegetative growth of two common citrus rootstock seedlings in Iran.

MATERIALS AND METHODS

Preparation of the soil, plant culture and design of treatments

The pots (30 pots) were filled with 7 kg of surface soil (0-30 cm depth) collected from a field in the College of Agriculture and Natural Resources of Darab. The soil was classified as Coarse-loamy, carbonatic, hyperthermic Typic Haplustepts according to the USDA Soil Taxonomy. A soil sample (prior to use) was airdried, sieved (< 2 mm) and then particle size distribution, calcium carbonate equivalent, organic carbon, soil pH, electrical conductivity (EC) and cation exchange capacity (CEC) as well as the concentration of nitrogen (N), phosphorus (P), potassium (K), iron (Fe), magnesium (Mn), Zn and Cu were determined using standard soil analysis methods (Table 1). One-year-old seedling of a sour orange (SO) or Mexican lime (ML) was planted in each pot (5 replicates). Pots were irrigated to reach field capacity moisture content (approximately 50% of saturation percentage) and soil moisture remained nearly constant with pots weighting. The WV was prepared from a biochar production factory (Fasl5 Company) in Jannatshahr Rigone, Darab city which used fruit tree wood as raw material. The WV (1 and 2%) (v/v) required for each treatment and pot was diluted with water and added to the pots four times at intervals of 3, 6, 9 and 12 weeks after planting. The plants were placed in greenhouse conditions (30°C during the day and 22°C at night) for the first three months of the experiment and in the outdoor shed for the last month.

Determination of plant traits

At the end of the experimental period (120 days after planting), the greenness index of the developed mature leaves (leaves 4 and 5 from the tip of the shoot) was determined by SPAD-502 (Minolta, Japan), leaf surface area was determined for 10 leaves of each seedling using a leaf area meter (Delta-T Devices, England) using WinDIAS3 software and then the chlorophyll concentration was determined in two fully developed leaves. For this purpose, 50 mg of fresh sample was macerated with 1 mL of methanol, vortexed for 20 seconds and centrifuged (16870 rcf) for 4 min at 4°C. The supernatant was separated and 1 mL of methanol was added to the solid again, and the vortexing and centrifugation steps were repeated as in the previous step, and the liquid phase was separated again and its absorbance was measured at two wavelengths of 652 and 665 nm using a microplate spectrophotometer (Synergy 2, BioTek, Winooski, USA) (Warren, 2008). The length of the main branch of each seedling (plant height) was recorded with a ruler. The fresh weight of the roots, stems and leaves were measured and then the samples were placed in an oven at 70°C for 48 h and then their dry weight was measured.

Table 1. Some properties of the wood vinegar used.

Property	pH	EC ($dS\ m^{-1}$)	Organic carbon (%)	N (%)	P (%)	K (%)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)
	3.54	5.15	10.1	0.05	trace	0.01	40.4	trace	5.5	2.8

Determination of nutrient concentration in soil, roots, stem and leaves

At the end of the experiment, the roots, stems and leaves of the plants were harvested separately, rinsed with deionized water and oven-dried at 70°C for three days. The dried samples were powdered using an electric mill and after ashing at 550°C and acid dissolution, the P concentration in the extracts was determined using the yellow color method (Jackson, 2005), as well as the concentrations of calcium (Ca), magnesium (Mg) and sodium (Na) was determined using a flame photometer (ELE, UK) and Fe, Mn, Zn and Cu were determined using an atomic absorption spectrophotometer (AAS; PG 990, PG Instruments Ltd. UK).

After removing the roots from the pots, the soil was dried and the characteristics of each treatment were determined (Table 2). To calculate the uptake and translocation percentage of each element, the root nutrient content was divided by the soil nutrient content or the content of that element in the shoot by its root content. To calculate the efficiency of the element, the total dry weight of the plant was divided by the total amount of elements accumulated in it (Gourley et al., 1994; Yan et al., 2019).

Statistical analysis

A factorial experiment in a completely randomized design was conducted under greenhouse and shed conditions. Treatments included seedling of two rootstocks (sour orange and Mexican lime) and WV application: without WV (WV_0), 1% WV ($WV_{1\%}$) and 2% WV ($WV_{2\%}$). Each treatment included five replicates and each replicate included one pot containing one seedling. Statistical analysis of the data was performed using SAS software for Windows V9 (SAS Institute Inc., Cary, NC, USA). Differences between means were detected by the least significant differences (LSD) test at a significance level of 5% and graphs created using Microsoft Office Excel 2016 software.

RESULTS

Soil properties

$WV_{1\%}$ treatment had no significant effect on soil pH and EC, while $WV_{2\%}$ treatment significantly decreased and increased them from 7.86 to 7.76 and 0.40 to 0.45 $dS\ m^{-1}$ respectively (Table 2). The use of WV had no effect on the availability of P, Fe and Cu in the soil (Table 2). Although soil K availability was not affected by using 1% WV, it was significantly increased (2.6%) by 2% WV. Treatment with $WV_{1\%}$ and $WV_{2\%}$ increased and decreased soil soluble Ca concentration, respectively. Although $WV_{1\%}$ treatment increased the concentration of Na and Zn in the soil, $WV_{2\%}$ treatment had no effect on the concentration of these elements. Addition of WV at concentrations of 1 and 2% increased soil Mn availability by 12 and 15.7%, respectively (Table 2).

Table 2. Changes in soil properties after the application of wood vinegar (WV).

			Macro nutrient concentration (g kg^{-1})				Micro nutrient concentration (mg kg^{-1})			
Treatment	EC (dS/m)	pH	P	K	Ca	Na	Fe	Mn	Zn	Cu
WV_0	0.40^b	7.86^a	4.73^a	0.22^b	0.20^b	0.13^b	3.48^a	5.69^c	1.56^b	1.09^a
$WV_{1\%}$	0.41^b	7.79^{ab}	4.87^a	0.23^{ab}	0.23^a	0.14^a	3.59^a	6.47^b	1.70^a	1.10^a
$WV_{2\%}$	0.45^a	7.76^b	4.96^a	0.23^a	0.19^c	0.12^b	3.54^a	6.75^a	1.57^b	1.09^a

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

Table 3. Means comparison of wood vinegar (WV) effects on leaf nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings.

	Leaf nutrient concentration									
	P (%)		K (%)		Ca (%)		K/Ca		Cu (mg kg^{-1} DW)	
Treatment	SO	ML	SO	ML	SO	ML	SO	ML	SO	ML
WV_0	0.26^d	0.41^a	1.89^{bc}	2.05^a	2.43^a	2.31^b	0.78^{bc}	0.89^a	9.25^{bc}	10.34^{ab}
$WV_{1\%}$	0.27^d	0.31^{bc}	1.85^{bc}	1.82^c	2.40^a	2.41^a	0.77^{bc}	0.76^c	10.35^{ab}	7.67^d
$WV_{2\%}$	0.29^{cd}	0.35^b	1.85^{bc}	1.94^b	2.29^b	2.19^c	0.80^b	0.88^a	11.42^a	8.49^{cd}

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

Plant macro nutrient concentration

The results showed that the use of WV had no effect on the P concentration in SO seedlings (in any organ) and only at a concentration of 2%, reduced the efficiency of this element. In ML seedlings, although the use of both WV levels (1 and 2%) reduced the P concentration in the leaves by 23 and 14%, respectively it had no effect on this element in the roots and stems (Table 3). In general, the P concentration of leaf (Table 3) and root (0.19 vs. 0.25%) and its translocation efficiency were higher in ML seedlings than in SO seedlings (Fig. 4A).

The K concentrations in roots and stems were different in two rootstock seedlings but the use of WV had no effect on it. Although the use of WV did not change the K concentration of SO leaves, the use of 1 and 2% WV in ML seedlings reduced the K concentration of leaves by 11.2 and 56.5%, respectively (Table 6). The results showed that the concentration of K in the root (Fig. 3B), stem (Fig. 2A) and the percentage of K uptake were higher in ML seedlings than in SO, but the translocation efficiency and efficiency of this element in SO was higher than that of ML seedlings (Fig. 4).

Although the leaf Ca concentration of SO did not change with the use of 1% WV, it was significantly reduced by the addition of 2% WV (Table 3). In ML seedlings, the use of 1 and 2% WV increased and decreased leaf Ca concentration, respectively (Table 3). In SO, root Ca concentration decreased by 1% of WV application, while it had no effect on ML root Ca concentration. Regardless of the effect of WV, the Ca concentration in the ML root was significantly lower than that of SO (Table 5). The use of WV did not affect Ca uptake percentage in ML seedlings, but at concentrations of 1 and 2%, it reduced this trait by 26.7 and 11.9% in SO seedlings, respectively (Table 6). Regardless of the WV effect, the percentage of Ca uptake in SO was about 69% higher than that in ML seedlings, while the translocation efficiency and Ca efficiency in ML were higher than that in SO seedlings (Fig. 4A, 4B). The use of 1 and 2% WV in SO reduced the percentage of Ca uptake and the use of 1% WV reduced the concentration of this element in the roots, while it had no effect in ML (Table 6).

In SO seedlings, none of the WV levels and in ML seedlings, $WV_{2\%}$ treatment had no effect on the Na concentration in the root and only 1% WV in ML seedlings increased the concentration of this element in the roots in comparison to control (Table 5). Regardless of the effect of WV, the Na concentration of leaves (Fig. 1B) and stems (Fig. 2C) of SO seedlings was 24.5 and 15.7% higher than that of ML, respectively. In both rootstocks, application of 2% WV reduced the Na concentration in the stem (Fig. 2C). The uptake percentage of Na varied between two rootstocks and its efficiency also varied between rootstocks and WV levels. The results showed that ML seedlings had higher Na efficiency than SO (0.88 vs. 0.74) and in both plants, 1% WV caused a significant decrease in Na efficiency (Fig. 4B).

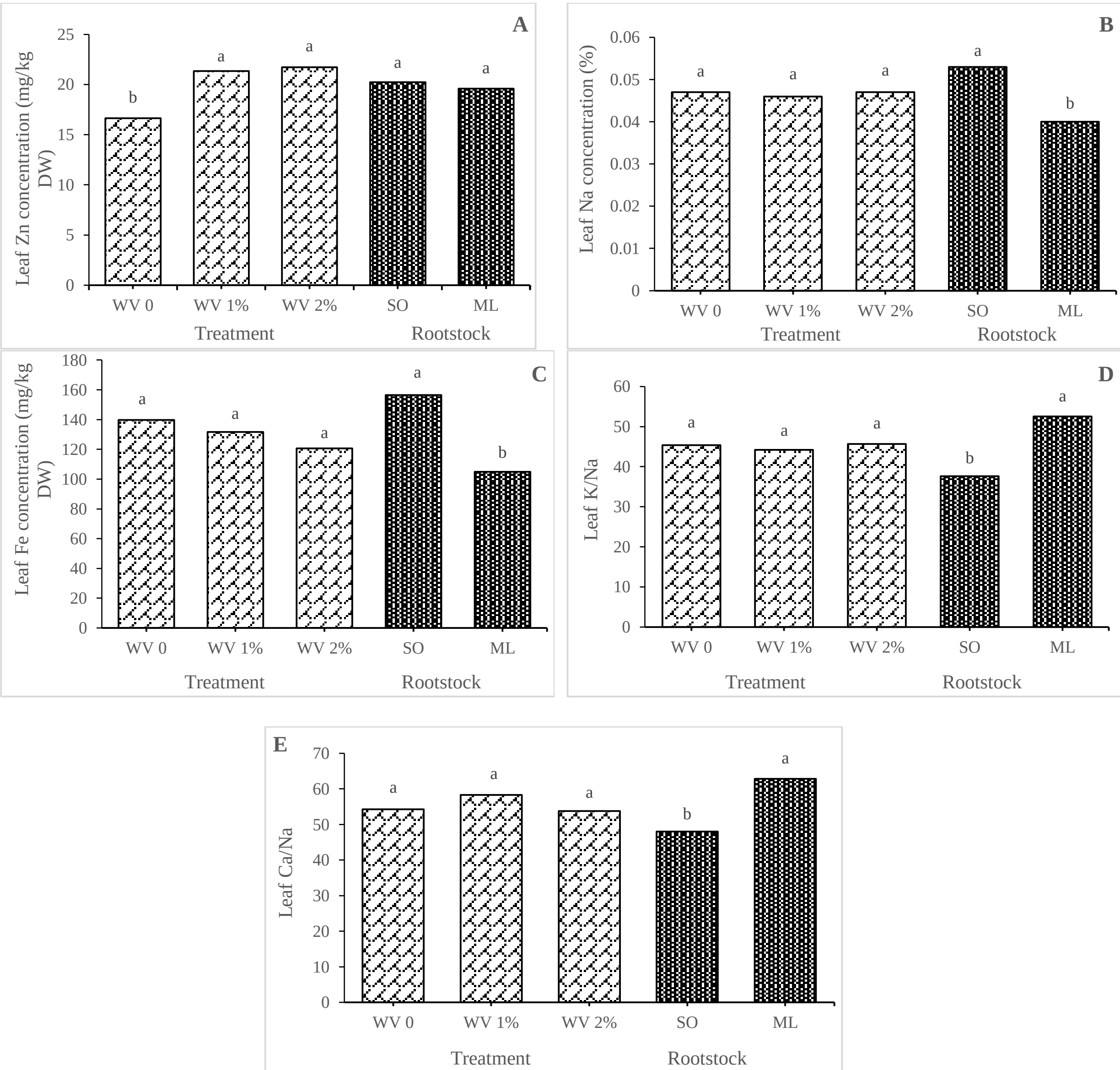

Fig. 1. Effects of wood vinegar (WV) and rootstock on leaf nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings. A: Zn, B: Na, C: Fe, D: K/Na, and E: Ca/Na. Different letters above columns represent significant differences at p<0.05 and columns with the same letters represent non-significant difference.

Table 4. Means comparison of wood vinegar (WV) effects on stem nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings.

	Stem nutrient concentration					
	Mn (mg kg^{-1} DW)		Zn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Treatment	SO	ML	SO	ML	SO	ML
WV_0	5.53^{b}	6.31^{a}	11.03^{b}	15.37^{a}	4.69^{ab}	5.21^{a}
$WV_{1\%}$	5.94^{ab}	5.42^{b}	15.01^{a}	13.38^{ab}	5.49^{a}	3.71^{c}
$WV_{2\%}$	5.73^{ab}	6.28^{a}	13.51^{ab}	13.83^{a}	5.35^{a}	3.89^{bc}

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

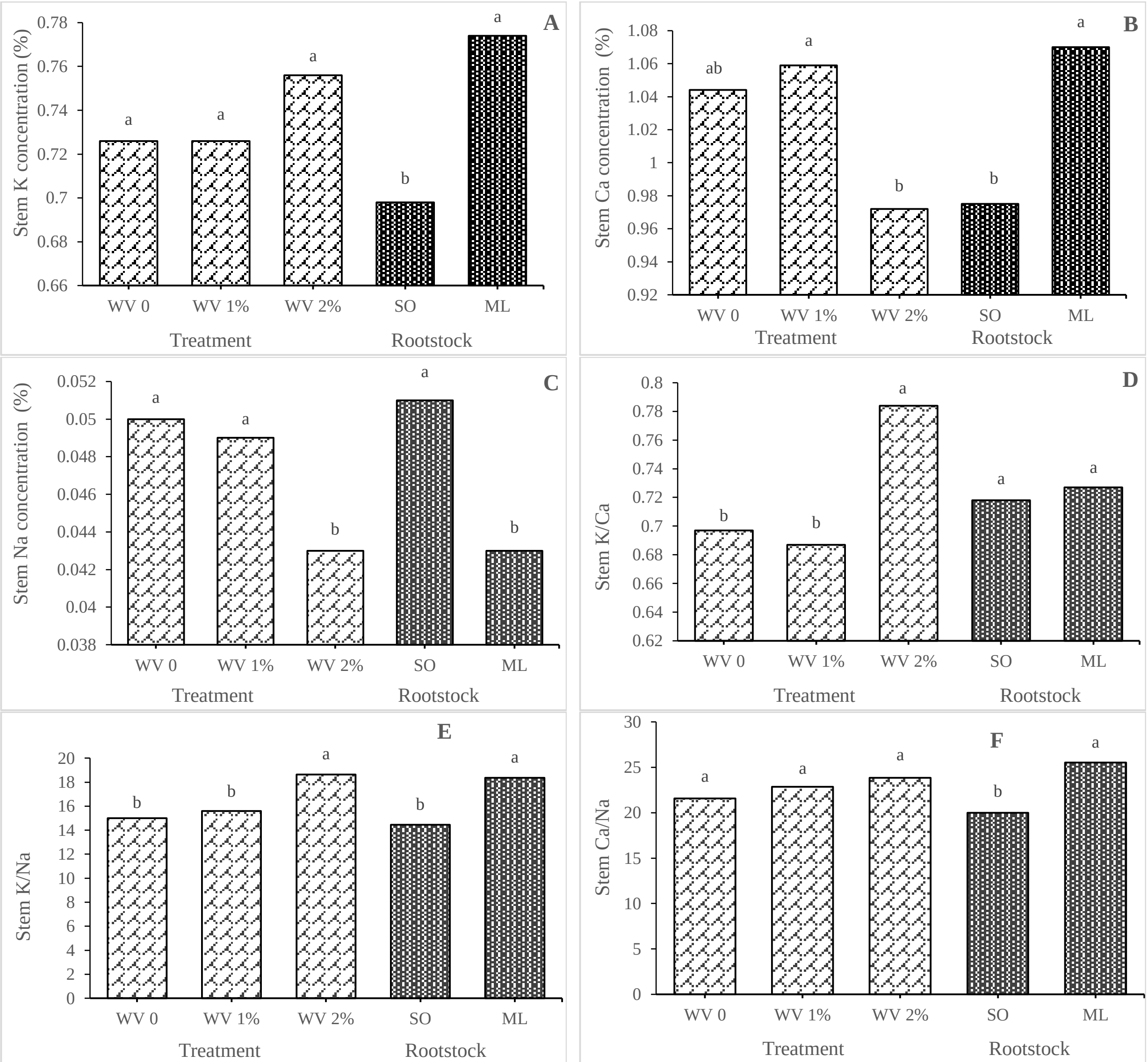

Fig. 2. Effects of wood vinegar (WV) and rootstock on stem nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings. A: K, B: Ca, C: Na, D: K/Ca, E: K/Na, and F: Ca/Na. Different letters above columns represent significant differences at $p<0.05$ and columns with the same letters represent non-significant difference.

The addition of WV had no effect on the K/Ca in the leaves of SO seedlings, but in ML seedlings, $WV_{1\%}$ treatment resulted in a significant reduction in this ratio due to the reduction in both K and Ca concentrations (Table 3). Although the use of 1% WV had no effect on K/Ca in the stem, the use of 2% WV resulted in a significant increase (11%) of this ratio in both rootstocks (Fig 2D). Overall, the K/Ca value in the ML root (1.58) was higher than that in SO (0.41).

In roots, stems and leaves of ML seedlings, K/Na was 46.4, 21.4 and 28.3% higher than that of SO seedlings (Fig. 3D, 2E, 1D). Although the use of 1% WV had no effect on the K/Na ratio in the stems of both rootstocks, the $WV_{2\%}$ treatment increased this ratio by 19.5% in both rootstocks and in the roots of both rootstocks only $WV_{1\%}$ treatment decreased this ratio. In the roots of ML seedlings, the ratio of Ca/Na was 51% lower than that in SO seedlings, but this ratio in the stems and leaves of ML seedlings was 21.6% and 23.6% higher respectively in SO seedlings (Fig. 2F, 1E). In the roots of both rootstocks, application of 1% WV significantly reduced the ratio of Ca/Na, but $WV_{2\%}$ treatment had no effect on this ratio (Fig. 3E).

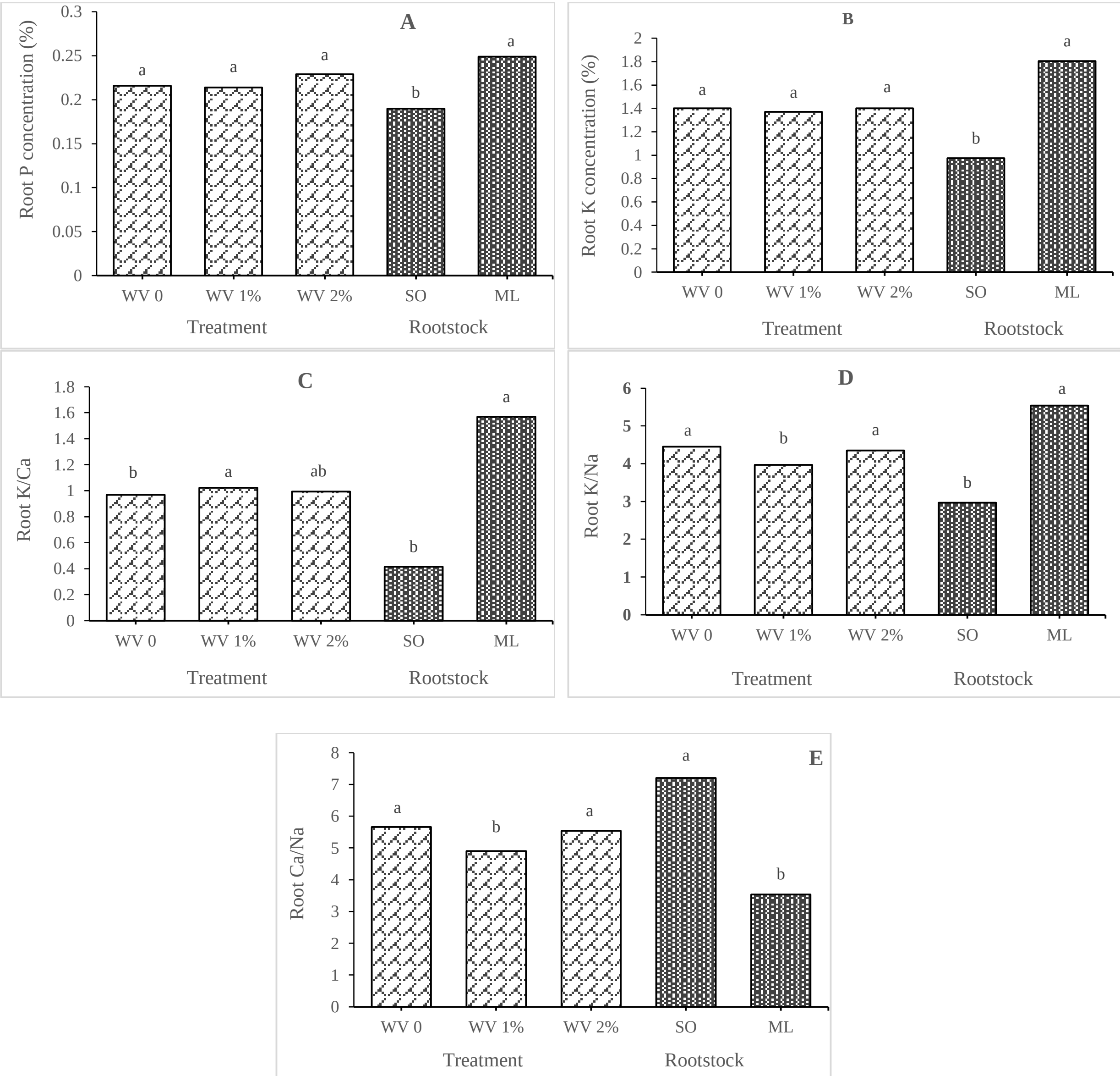

Fig. 3. Effects of wood vinegar (WV) and rootstock on root nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings. A: P, B: K, C: K/Ca, D: K/Na, and E: Ca/Na. Different letters above columns represent significant differences at p<0.05 and columns with the same letters represent non-significant difference.

Table 5. Means comparison of wood vinegar (WV) effects on root nutrient concentration in sour orange (SO) and Mexican lime (ML) seedlings.

	Root nutrient concentration					
	Ca (%)		Na (%)		Fe (mg kg^{-1} DW)	
Treatment	SO	ML	SO	ML	SO	ML
WV_0	2.44^a	1.17^c	0.33^b	0.31^b	1960.0^a	1150.0^d
$WV_{1\%}$	2.17^b	1.12^c	0.33^b	0.36^a	2050.0^a	1300.0^c
$WV_{2\%}$	2.45^a	1.15^c	0.33^b	0.32^b	1700.0^b	1320.0^c
	Mn (mg kg^{-1} DW)		Zn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Treatment	SO	ML	SO	ML	SO	ML
WV_0	113.2^b	80.8^c	31.3^c	40.2^b	18.6^e	42.8^b
$WV_{1\%}$	134.3^a	83.8^c	31.8^c	51.5^a	23.7^d	36.4^c
$WV_{2\%}$	113.5^b	108.3^b	37.2^{bc}	58.7^a	24.1^d	59.8^a

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

Table 6. Means comparison of wood vinegar (WV) effects on uptake percentage, translocation efficiency and nutrient efficiency in sour orange (SO) and Mexican lime (ML) seedlings.

		Ca (%)		Mn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Uptake percentage	Treatment	SO	ML	SO	ML	SO	ML
	WV_0	0.55^a	0.15^d	0.90^a	0.36^c	0.75^c	1.02^b
	$WV_{1\%}$	0.41^c	0.14^d	0.90^a	0.36^c	0.96^{bc}	0.89^{bc}
	$WV_{2\%}$	0.49^b	0.16^d	0.61^b	0.41^c	0.87^{bc}	1.29^a
		Zn (mg kg^{-1} DW)		Mn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Translocation efficiency	Treatment	SO	ML	SO	ML	SO	ML
	WV_0	51.32^{ab}	53.77^{ab}	36.42^{dc}	53.50^a	47.99^a	34.77^b
	$WV_{1\%}$	56.88^a	48.53^{bc}	32.84^d	49.76^a	44.48^a	31.00^b
	$WV_{2\%}$	55.27^a	47.10^c	39.68^c	45.38^b	48.34^a	23.69^c
		P (%)		Mn (mg kg^{-1} DW)		Cu (mg kg^{-1} DW)	
Nutrient efficiency	Treatment	SO	ML	SO	ML	SO	ML
	WV_0	0.46^a	0.36^d	18.40^b	22.55^a	92.24^a	59.37^c
	$WV_{1\%}$	0.46^a	0.39^{bc}	15.78^c	23.09^a	75.67^b	72.98^b
	$WV_{2\%}$	0.42^b	0.38^{cd}	17.98b^c	20.14^b	72.78^b	51.07^c

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

Plant micro nutrient concentration

In SO seedlings, $WV_{2\%}$ treatment significantly reduced the concentration of root Fe, while in ML seedlings; the use of both concentrations of WV increased the concentration of this element (Table 5). Although the efficiency and translocation efficiency of Fe were higher in ML than in SO, but due to the lower Fe uptake in ML than SO (Fig. 4B), finally the concentration of Fe in the leaves of ML seedlings (104.8 mg/kg) was lower than that of SO seedlings (156.5 mg/kg).

In SO seedlings, the concentration of Mn in the stem was not affected by WV, but in ML seedlings, the use of 1% WV reduced the concentration, and 2% WV had no effect on it (Table 4). Root Mn concentration increased by 1% WV in SO seedlings and by 15.7 and 25.5% in ML seedlings using 2% WV, respectively, compared to the control treatment (Table 5). Regardless of the effect of WV, the percentage of Mn uptake was higher in SO seedlings than in ML seedlings, but increasing the translocation efficiency of this element in ML resulted in a higher efficiency of Mn in ML than in SO seedlings (Table 6). Although the use of 1% WV in SO and 2% in ML increased the Mn concentration in the roots (Table 5), it did not increase due to the ineffectiveness of WV on Mn translocation in SO and its reduction in

ML (Table 6), only the Mn efficiency of SO and ML in $WV_{1\%}$ and $WV_{2\%}$ decreased and the Mn concentration in the leaves also did not change.

Addition of WV to the soil increased the Zn concentration in the leaves of both rootstocks (Fig. 1A). Although before the addition of WV to the soil (control treatment), the Zn concentration was higher in the ML stem than in the SO stem (15.4 vs. 11.0), only the use of 1% WV increased the concentration of this element in the SO stem and the addition of WV had no effect on the Zn concentration in the stem of ML (Table 4). The addition of WV to the soil did not change the Zn concentration in the SO root, but in ML, the addition of 1 and 2% WV increased the Zn concentration in the root by 22.11 and 31.54%, respectively (Table 5). The percentage of Zn uptake was not affected by the type of rootstock and the concentration of WV, and on the other hand, the translocation efficiency of this element decreased by $WV_{2\%}$ treatment only in ML. The efficiency of Zn in SO seedlings (54.04%) was higher than that of ML (39.9%) and the use of 1 and 2% WV reduced the efficiency of this element in both rootstocks by 15.51 and 20.97%, respectively (Fig. 4C).

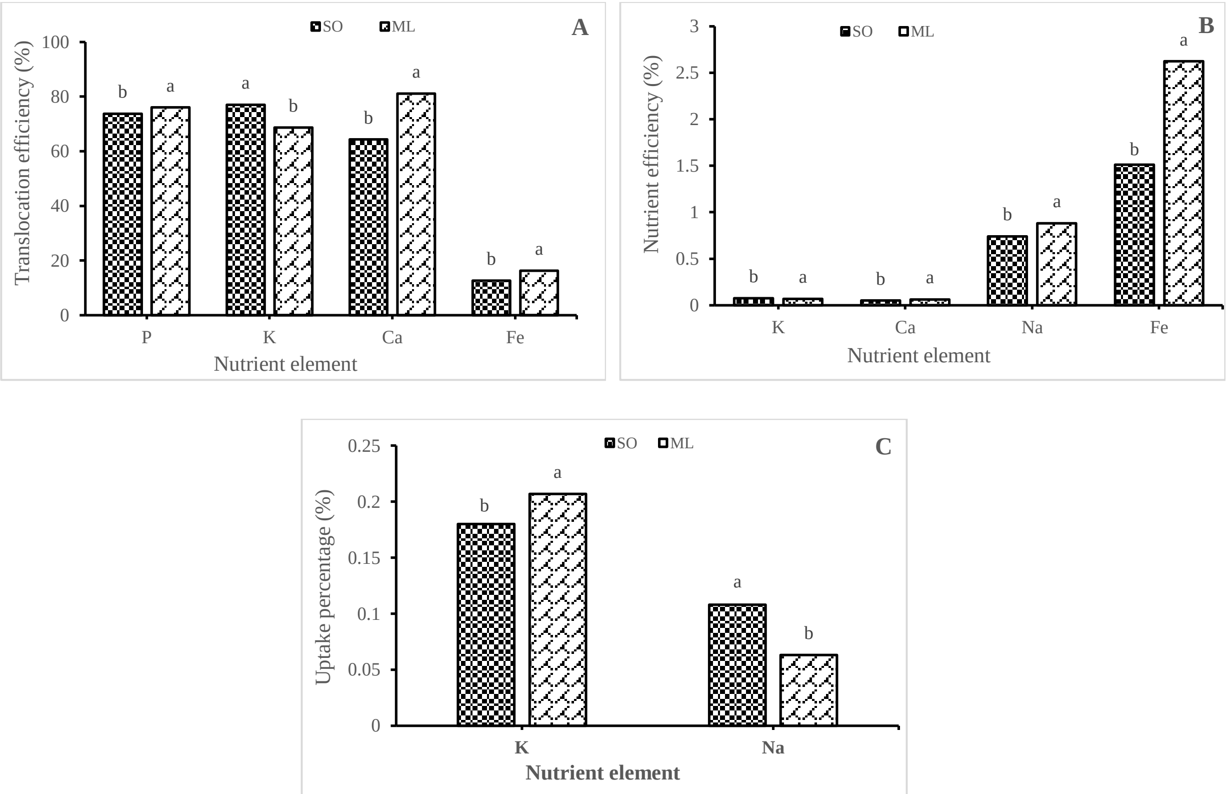

Fig. 4. Comparison of translocation efficiency (A), nutrient efficiency (B), and uptake percentage (C) in sour orange (SO) and Mexican lime (ML) seedlings. Different letters above columns represent significant differences at $p<0.05$ and columns with the same letters represent non-significant difference.

The results showed that in SO seedlings, the application of 2% WV increased the Cu concentration in the leaves by 0.19%, but in ML seedlings, the addition of 1 and 2% WV reduced the concentration of this element in the leaves by 25.8 and 17.9%, respectively (Table 3). Addition of WV to the soil did not affect the concentration of stem Cu in SO seedlings, but in ML seedlings, both concentrations of WV resulted in a significant decrease in stem Cu concentration (Table 4). The concentration of root Cu in SO seedlings increased due to the application of both WV concentrations, but in ML seedlings, the use of 1% WV decreased the concentration (42.8 vs. 36.4) and 2% WV increased the concentration (42.8 vs. 59.8) of this element in the root (Table 5). Although WV use had no effect on Cu uptake in SO seedlings,

$WV_{2\%}$ treatment in ML increased Cu uptake (Table 6). The translocation efficiency of Cu in SO was not affected by the use of WV, while in ML, the use of 2% WV reduced this feature (Table 6). The efficiency of Cu in SO decreased in both WV concentrations, but in ML, $WV_{1\%}$ increase the efficiency of this element by 18.6% (Table 6).

Changes in physiological and morphological traits

Although in SO seedlings, the leaf chlorophyll concentration and leaf greenness showed no changes by the use of WV, but in ML seedlings, 2% WV reduced leaf greenness and total chlorophyll compared to the control treatment (Table 7). Application of 2% WV reduced the root dry weight by 16.1% and 12.9% compared to the control treatment but did not effect on shoot and total dry weight of SO seedlings (Table 7). Of course, the results showed that the WV application had no effect on plant height; shoot fresh and dry weight, root-to-shoot ratio and chlorophyll a and b concentrations in the seedlings.

Table 7. Means comparison of the effects of wood vinegar (WV) and rootstock interaction on some morphological features in sour orange (SO) and Mexican lime (ML) seedlings.

Treatment	SPAD		Total chlorophyll (mg/g FW)		Root dry weight (g)		Shoot dry weight (g)		Total dry weight (g)	
	SO	ML	SO	ML	SO	ML	SO	ML	SO	ML
WV_0	62.66[ab]	62.40[ab]	18.4[b]	26.4[a]	3.10[a]	1.82[c]	6.92[a]	5.34[b]	9.98[a]	7.17[c]
$WV_{1\%}$	64.69[a]	59.95[b]	22.1[ab]	26.0[a]	2.60[b]	1.90[c]	6.25[ab]	5.65[b]	9.19[a]	7.65[bc]
$WV_{2\%}$	64.37[a]	52.65[c]	21.0[ab]	19.6[b]	2.70[b]	1.71[c]	6.26[ab]	5.19[b]	8.89[ab]	6.90[c]

Means followed by a similar letter in the same column indicates non-significant difference (n=4).

DISCUSSION

In this study the use of 1% WV increased the concentration of K, Ca, Na, Mn and Zn and 2% WV increased the concentration of K and Mn in the soil. Increasing the Ca, K and Na in the soil by the use of WV may be due to the dissolution of soil salts such as calcium carbonate (Najafi-Ghiri et al., 2022) but increasing the concentration of Mn and Zn can be due to the effect of WV on soil pH and prevent the stabilization of these elements on soil particles and thus increase their availability (Yamato et al., 2006). It has been shown that the use of WV in saline soils can cause leaching of soluble salts, reduction of pH and thus improve the performance of plants in these soils (Lashari et al., 2013) and in soils contaminated with heavy metals, by helping to stabilize this metals on soil particles, can remediate these soils (Theapparat et al., 2015).

In SO seedlings, the main reason for the reduction of leaf P, K and Ca by WV application, was the reduction in the uptake of these elements by the roots, while in ML seedlings; it is due to the increase in leaf area and the dilution effect. In confirmation of the results, it was reported that the addition of WV to the root environment reduced the absorption of K and P in lettuce (Chen et al., 2016) and K and Mg in cicer (*Cicer arietinum* L.) (Fedeli et al., 2022). Although WV increases the availability of some nutrients by reducing the pH of the root environment, the presence of various organic compounds in its composition can lead to the formation of complexes with some ions and reduce their availability (Pan et al., 2017). Also it has been shown that in basil (*Ocimum basilicum*) and cucumber (*Cucumis sativus*), the use of pine WV, although increased the leaf N and had no effect on the leaf K concentration, but significantly reduced the P, Mg, Fe and Ca (Abdolahipour & Haghighi, 2019). The cell walls and especially middle lamella consist of polygalactrunic acid and their carboxylic groups (R-COO) act as cation exchangers in this site. Plant species differ considerably in their root cation exchange capacity (CEC) and the changes in rhizosphere conditions such as pH effect on root CEC (Marschner, 2011). The change in the content of Ca and K in the root by adding

WV can be due to the change in the CEC of the root tissue caused by changes in the pH of the root environment.

The use of WV did not affect the availability of Fe in the soil and Fe uptake percentage in both rootstocks was reduced by adding 2% of WV. It is believed that Fe has a high affinity with some compounds such as organic acids (Marschner, 2011). On the other hand, WV contains large amounts of organic acids and phenolic compounds, and probably the binding of Fe with these compounds has reduced its availability to plant roots.

Although the application of WV increased the Mn in the roots of both rootstocks, but due to the inhibition of the translocation, the concentration of this element in the leaves did not change. The behavior of both seedlings was very similar to Mn, so that there was no difference between two rootstocks in terms of Mn nutritional traits except the percentage of uptake. In confirmation of these results, it is stated that regardless of the species or cultivar and environmental conditions, the Mn critical concentration in the developed leaves of different plants is the same (10 to 20 mg kg^{-1} dry weight) (Marschner, 2011).

Application of WV increased the leaf Zn concentration in both rootstocks and in both levels but the Zn efficiency in both rootstocks decreased with WV application. Low availability of Zn in calcareous soils (with high pH) is mainly due to the adsorption of Zn on clay or $CaCO_3$, rather than from the formation of sparingly soluble $Zn(OH)_2$ or $ZnCO_3$ (Rengel, 2015; Trehan & Sekhon, 1977). In addition, Zn uptake and translocation to the shoot are inhibited by high concentrations of bicarbonate and HCO_3^- (Marschner, 2011). It seems that WV by decreasing the pH of the root environment and preventing the stabilization of ions on soil particles could increase Zn and Mn availability and increase it in the roots of ML, SO stems and leaf of both rootstocks. The pattern of changes in leaf Cu concentration due to the use of WV in two rootstocks was the opposite, so that it increased in SO and decreased in ML seedlings. The increase in Cu concentration of SO leaves due to the use of WV was due to the increased uptake of this element. However, in ML seedlings, reduction in leaf Cu concentration was mainly due to the decrease in translocation and stem Cu concentration. Contrary to the obtained results in current study, it has been observed that adding WV to animal waste and charcoal can stabilize heavy elements such as nickel, Zn and Cu on their particles and thus reduce the concentration of these elements in soil solution (Zhu et al., 2021a). The results of some studies have shown that the use of WV can increase the vegetative growth of the plant sometimes by 70 to 80% (Jeong et al., 2015). For example, the use of WV increased leaf area, number of fruits and plant dry weight (Abdolahipour, 2019) and the area and volume of roots (Burnette, 2010) in cucumber (*Cucumis sativa*) seedlings. Also, it increased leaf area index, number of pods per plant, plant height and dry weight in rapeseed (*Brassica napus* L.) (Zhu et al., 2021b). The acids in WV increase the concentration of protons (H^+) in leaf tissue and changing the pH of leaf cell sap increases the activity of these cells and plant growth (Zhu et al., 2021b). In the present study, the use of WV with 2% concentration increased leaf area and decreased root dry weight of both rootstocks and did not affect other morphological traits. Due to the presence of some growth inhibitor compounds such as phenol and chrysol in the composition of WV, researchers believe that WV can in some cases reduce plant growth (Jeong et al., 2015; Pan et al., 2017). The use of WV has a positive effect on plant growth if the concentration of all compounds in it is appropriate and balanced (Zhu et al., 2021b).

Changes in the ratio of mineral elements in two studied rootstocks showed that SO seedlings tend to absorb and accumulate Ca in their roots, while ML seedlings accumulate more K in their roots. The reason for this difference can be attributed to the difference in the apoplastic space and the root CEC of two plants (Marschner, 2011), or the difference in their leaf surface and, as a result, their transpiration rates.

The effect of 1% WV on reducing the K/Na and Ca/Na in the roots of both rootstocks can be due to the increase in the availability of Na in the soil and increased its absorption by the roots of both plants (Table 2). Contrary to these results, some researchers emphasize the effect of WV on reducing the adverse effects of salinity on plants (Jayasankaran et al., 2022) and consider this effect to be due to the effect of WV on reducing osmotic stress and stimulating the plant to absorb water, thereby reducing the toxicity of Na and Cl ions (Theerakulpisut et al., 2017).

CONCLUSION

Without considering the effects of WV, the two rootstocks used in this research were different from each other in terms of uptake percentage and especially translocation and efficiency of mineral elements and this should be considered in practice. Addition of WV to the soil decreased the pH but did not significantly change the availability of elements such as P, Fe and Cu, increased the availability of K, Mn and Zn, and increased its EC. The effect of WV on the concentration of mineral elements and their ratio in leaves and roots of plants was different depending on the rootstock type. Although the application of WV had no effect on P, K and K/Ca in the leaves and Zn in the root of SO, it caused a reduction in P, K and increase in the leaf K/Ca, Zn and Fe in the roots of ML seedlings. On the other hand, the application of WV did not have a significant effect on the vegetative growth of the seedlings. It is suggested to investigate the effect of higher concentrations and other WV sources on citrus rootstock seedlings, but application of higher WV concentrations will likely to further reduce soil pH and increase the availability of some elements, thereby increasing soil EC and inhibitor compounds and the formation of complexes may prevent plant growth. The responses of two rootstocks to the application of WV were different and this should be considered in practice.

Conflict of interest

The authors declare that they have no conflict of interest.

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REFERENCES

Abdolahipour, B., & Haghighi, M. (2019). The effects of pine wood vinegar on the germination, growth and photosynthetic characteristics of cucumber. *Iran Agricultural Research, 38*(2), 83-90. https://doi.org/10.22099/iar.2020.31134.1305

Ahmadi, K., Gholizadeh, H., Ebadzadeh, H. R., Hosseinpur, R., Hatami, F., Abdeshah, H., Rezaei, M. M., Kazemifard, R., & Fazli-Estabragh, M. (2021). Yearbook of agricultural statistics, Vol. 3, Fruits. Ministry of Agriculture, Office of Statistics and Information Technology, Iran. (in Persian).

Aguilar-Hernández, M. G., Sánchez-Rodríguez, L., Hernández F., Forner-Giner M. Á., Pastor-Pérez J. J., & Legua, P. (2020). Influence of new citrus rootstocks on lemon quality. *Agronomy, 10*(7), 974. https://doi.org/10.3390/agronomy10070974

Aguirre, J. L., Baena, J., & Martín, M.T. (2020). Composition, ageing and herbicidal properties of wood vinegar obtained through fast biomass pyrolysis. *Energies 13*(10), 2418. https://doi.org/10.3390/en13102418

Albrigo, L. G., Stelinski L. L., & Timmer, L.W. (2019). Citrus. Boston, CABI Press.

Azarmi-Atajan, F., Sayyari-Zohan, M. H., & Mirzaei, A. (2023). Evaluation of the growth and status of some nutrients in pistachio seedlings treated with phosphorus under different levels of irrigation water salinity. *Journal of Horticulture and Postharvest Research*, *6*(3), 261-270.

https://doi.org/10.22077/jhpr.2023.6271.1314

Burnette, R. (2010). An introduction to wood vinegar. ECHO Asia Notes, 7, 1-6.

Castle, W., Bowman, K., Grosser, J., Futch, S. & Graham, J. (2016). Florida citrus rootstock selection guide. IFAS Publication SP248.

Chen, J., Wu, J., Si, H., & Lin, K. (2016). Effects of adding wood vinegar to nutrient solution on the growth, photosynthesis, and absorption of mineral elements of hydroponic lettuce. *Journal of Plant Nutrition, 39*(4), 456-462. https://doi.org/10.1080/01904167.2014.992539.

Davies, F. S., & Albrigo, L.G. (1999). Citrus. Acribia, Wallingford. CABI Press.

Eissenstat, D. & Achor, D. (1999). Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. *The New Phytologist*, *141*(2), 309-321. https://doi.org/10.1046/j.1469-137.1999.00342.x

Fagernäs, L., Kuoppala, E., Tiilikkala, K., & Oasmaa, A. (2012). Chemical composition of birch wood slow pyrolysis products. *Energy & Fuels, 26*(2), 1275-1283. https://doi.org/10.1021/ef2018836

Fani, E. (2023). Response of some physiological and biochemical traits of Pursalane (*Portulaca oleracea*) to silica fertilizer under salinity stress. *Journal of Horticulture and Postharvest Research*, *6*(2), 157-168. https://doi.org/10.22077/jhpr.2023.5689.1291

Fedeli, R., Vannini, A., & Celletti, S. (2022). Foliar application of wood distillate boosts plant yield and nutritional parameters of chickpea. *Annals of Applied Biology, 182*(1), 57-62. https://doi.org/10.1111/aab.12794

Gourley, C., Allan, D., & Russelle, M. (1994). Plant nutrient efficiency: A comparison of definitions and suggested improvement. *Plant and Soil*, *158*, 29-37. https://doi.org/10.1007/BF00007914

Grewal, A., Abbey, L., & Gunupuru, L. R. (2018). Production, prospects and potential application of pyroligneous acid in agriculture. *Journal of Analytical and Applied Pyrolysis*, *135*, 152-159. https://doi.org/10.1016/j.jaap.2018.09.008

Hou, X., Qiu, L., Luo, S., Kang, K., Zhu, M., & Yao, Y. (2018). Chemical constituents and antimicrobial activity of wood vinegars at different pyrolysis temperature ranges obtained from Eucommia ulmoides Olivers branches. *RSC Advances, 8*(71), 40941-40949. https://doi.org/10.1039/C8RA07491G

Jackson, M. L. (2005). Soil chemical analysis: Advanced course. UW-Madison Libraries parallel press.

Jayasankaran, P., Vigneshwari, R., Renugadevi, J., & Sritharan, N. (2022). Standardisation of seed priming treatments with Pyroligneous acid (PA) to enhance seed germination and seedling growth under saline stress in Greengram (*Vigna radiata* L.). *The Pharma Innovation Journal, 11*(7), 2676-2681.

Jeong, K. W., Kim, B. S., & Ultra, Jr. V.U. (2015). Effects of rhizosphere microorganisms and wood vinegar mixtures on rice growth and soil properties. *Korean Journal of Crop Science*, *60*, 355-365. http://doi.org/10.7740/kjcs.2015.60.3.355

Jianming, Z. (2003). Effect of the solution of wood vinegar on yield and nitrogen-utilization of rice. *Journal of Anhui Agricultural Sciences, 31*(4), 542-543.

Jothityangkoon, D., Koolachart, R., Wanapat, S., Wongkaew, S., & Jogloy, S. (2008). Using wood vinegar in enhancing peanut yield and in controlling the contamination of aflatoxin producing fungus. *International Crop Science, 4*, 253-253.

Koç, İ., Öğün, E., Namlı, A., Mendeş, M., Kutlu, E., & Yardım, E. (2019). The effects of wood vinegar on some soil microorganisms. *Applied Ecology and Environmental Research, 17*(2), 2437-2447. http://doi.org/10.15666/aeer/1702_24372447

Lashari, M. S., Liu, Y., & Li, L. (2013). Effects of amendment of biochar-manure compost in conjunction with pyroligneous solution on soil quality and wheat yield of a salt-stressed cropland from Central China Great Plain. *Field Crops Research, 144*, 113-118. https://doi.org/10.1016/j.fcr.2012.11.015

Ling-jie, X., Shuang-jian, Y., Min, D., Yuan-yuan, S., & Xiang-ge, D. (2014). Effects of bamboo vinegar on seeds germination and seedling growth of different flue-cured tobacco varieties. *Journal of Southern Agriculture*, *45*(10), 1764. http://doi.org/10.3969/j:issn.2095-1191.2014.10.1764

Mahmud, K. N., Yahayu, M., & Sarip, S. H. M. (2016). Evaluation on efficiency of pyroligneous acid from palm kernel shell as antifungal and solid pineapple biomass as antibacterial and plant growth promoter. *Sains Malaysiana*, *45*(10), 1423-1434.

Marschner, H. (2011). Marschner's mineral nutrition of higher plants. Academic Press.

Martín, M. T., Sanz, A. B., & Nozal, L. (2017). Microwave-assisted pyrolysis of Mediterranean forest biomass waste: bioproduct characterization. *Journal of Analytical and Applied Pyrolysis*, *127*, 278-285. https://doi.org/10.1016/j.jaap.2017.07.024

Mathew, S., & Zakaria, Z. A. (2015). Pyroligneous acid-the smoky acidic liquid from plant biomass. *Applied Microbiology and Biotechnology*, *99*, 611-622. http://doi.org/10.1007/s00253-014-6242-1

Mu, J., Uehara, T., & Furuno, T. (2004). Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants II: composition of moso bamboo vinegar at different collection temperature and its effects. *Journal of Wood Science, 50,* 470-476. https://doi.org/10.1007/s10086-003-0586-y

Najafi-Ghiri, M., Mirsoleimani, A., Boostani, H. R., & Amin, H. (2022). Influence of wood vinegar and potassium application on soil properties and Ca/k ratio in citrus rootstocks. *Journal of Soil Science and Plant Nutrition*, *22*, 334-344. https://doi.org/10.1007/s42729-021-006533

Pan, J-x., Cao, F-l., Wang, G-b., & Zhang, W-x. (2010). Effects of gingko wood vinegar on crops seed germination and seedling growth. *Seed*, *29*(2), 39-44.

Pan, X., Zhang, Y., Wang, X., & Liu, G. (2017, April). Effect of adding biochar with wood vinegar on the growth of cucumber. In *IOP Conference Series: Earth and Environmental Science* (p. 012149). IOP Publishing. https://doi.org/10.1088/1755-1315/61/1/012149.

Pedrero, F., Maestre-Valero, J., & Mounzer, O. (2015). Response of young 'Star Ruby'grapefruit trees to regulated deficit irrigation with saline reclaimed water. *Agricultural Water Management*, *158*, 51-60. https://doi.org/10.1016/j.agwat.2015.04.012.

Rengel, Z. (2015). Availability of Mn, Zn and Fe in the rhizosphere. *Journal of Soil Science and Plant Nutrition*, *15*(2), 397-409. http://dx.doi.org/10.4067/S0718-95162015005000036

Romero, P., Navarro, J., & Pérez-Pérez, J. (2006). Deficit irrigation and rootstock: their effects on water relations, vegetative development, yield, fruit quality and mineral nutrition of Clemenules mandarin. *Tree physiology*, *26*(12), 1537-1548. https://doi.org/10.1093/treephys/26.12.1537

Steiner, C., Das, K. C., Garcia, M., Förster, B., & Zech, W. (2008). Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia*, *51*(5-6), 359-366. https://doi.org/10.1016/j.pedobi.2007.08.002

Theapparat, Y., Chandumpai, A., Leelasuphakul, W., & Laemsak, N. (2015). Pyroligneous acids from carbonisation of wood and bamboo: their components and antifungal activity. *Journal of Tropical Forest Science*, 27, 517-526. http://www.jstor.org/stable/43596228

Theerakulpisut, P., Kanawapee, N., & Panwong, B. (2017). Seed priming alleviated salt stress effects on rice seedlings by improving Na^+/K^+ and maintaining membrane integrity. *International Journal of Plant Biology*, *7*(1), 6402. https://doi.org/10.4081/pb.2016.6402

Trehan, S., & Sekhon, G. (1977). Effect of clay, organic matter and $CaCO_3$ content on zinc adsorption by soils. *Plant and Soil*, *46*, 329-336. https://doi.org/10.1007/BF00010089

Tsuzuki, E., Morimitsu, T., & Matsui, T. (2000). Effect of chemical compounds in pyroligneous acid on root growth in rice plant. Report of the Kyushu Branch of the Crop Science Society of Japan, *66*, 15-16.

Wang, Z., Lin, W., Song, W., & Yao, J. (2010). Preliminary investigation on concentrating of acetol from wood vinegar. *Energy Conversion and Management*, *51*(2), 346-349. https://doi.org/10.1016/j.enconman.2009.09.031

Warren, C. (2008). Rapid measurement of chlorophylls with a microplate reader. *Journal of Plant Nutrition*, *31*(7), 1321-1332. https://doi.org/10.1080/01904160802135092

Yamato, M., Okimori, Y., Wibowo, I. F., Anshori, S., & Ogawa, M. (2006). Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. *Soil Science and Plant Nutrition*, *52*(4), 489-495. https://doi.org/10.1111/j.1747-0765.2006.00065.x

Yan, X-L., Wang, C., Ma, X., & Wu, P. (2019). Root morphology and seedling growth of three tree species in southern China in response to homogeneous and heterogeneous phosphorus supplies. *Trees, 33,* 1283-1297. https://doi.org/10.1007/s00468-019-01858-x

Zhu, K., Gu, S., & Liu, J., (2021a). Wood vinegar as a complex growth regulator promotes the growth, yield, and quality of rapeseed. *Agronomy, 11*(3), 510.

Zhu, J., Gao, W., Ge, L., Zhao, W., Zhang, G., & Niu, Y. (2021b). Immobilization properties and adsorption mechanism of nickel (II) in soil by biochar combined with humic acid-wood vinegar. *Ecotoxicology and Environmental Safety, 215,* 112159. http://doi.org/10.1016/j.ecoenv.2021.112159

Ziatabar Ahmadi, S. R., Seifi, E., Varasteh, F., & Akbarpour, V. (2024). Effect of biofertilizer inoculation on the growth and physiological traits of Red Angel and Wonderful pomegranate plantlets under salinity stress. *Journal of Horticulture and Postharvest Research*, *7*(2), 171-182. https://doi.org/10.22077/jhpr.2024.7168.1356

Zulkarami, B., Ashrafuzzaman, M., Husni, M., & Ismail, M. R. (2011). Effect of pyroligneous acid on growth, yield and quality improvement of rockmelon in soilless culture. *Australian Journal of Crop Science, 5*(12), 1508-1514.