Comparative Efficacy of Potassium Salts Against Soil-borne and Air-borne Fungi and Their Ability to Suppress Tomato Wilt and Fruit Rots

Jabnoun-Khiareddine H1, Abdallah R2, El-Mohamedy R1, Abdel-Kareem F1, Gueddes-Chahed M1, Hajlaoui A1 and Daami-Remadi M1

1UR13AGR09-Integrated Horticultural Production in the Tunisian Centre-East, Regional Center of Research on Horticulture and Organic Agriculture, University of Sousse, Tunisia
2National Agronomic Institute of Tunisia, 1082 Tunis Mahrajene, University of Carthage, Tunisia
3Department of Plant Pathology, National Research Center, Dokki, Giza, Egypt

Abstract

Potassium sorbate (PS), potassium bicarbonate (PB) and dipotassium hydrogenphosphate (DPHP) were assessed for their antifungal activity against Fusarium oxysporum f. sp. lycopersici (FOL). F. oxysporum f. sp. radicis-lycopersici (FORL), F. solani, Verticillium dahliae (VD), Rhizoctonia solani, Colletotrichum coccodes, Pythium aphanidermatum, Sclerotinia sclerotiorum, Botrytis cinerea and Alternaria solani. They were screened for their ability to suppress Verticillium and Fusarium wilts, and Fusarium Crown and Root Rot (FCRR), for their effects on tomato growth, and for their potential control of Botrytis, Alternaria, Rhizoctonia and Anthracnose fruit rots. PS (0.25-1.5%), DPHP (0.1-0.6 M) and PB (0.1-0.6 M) inhibited fungal growth in a concentration-dependent manner, with the greatest inhibition achieved using the highest concentrations. Inter specific variations in sensitivity were detected with P. aphanidermatum, S. sclerotiorum and B. cinerea being the most sensitive to all salts. Single treatments using PS (0.25%), PB (50 mM) and DPHP (50 mM) resulted in varied degree of protection against wilts. PS led to 50, 78.26 and 65% lower wilt severity as respectively compared to VD-, FOL- and FORL-inoculated controls. PS had significantly increased plant height, root and aerial part fresh weights by 20.61, 30.76 and 33.02%, respectively, compared to FORL-inoculated plants and had improved root fresh weight by 42.18 and 32.87% compared to FOL-and VD-inoculated plants, respectively. PB-based treatment led to 60.86 and 30% lower Fusarium wilt and FCRR severity but did not suppress Verticillium wilt. DPHP suppressed only Fusarium wilt by 65.21%. Used as fruit treatment, DPHP and PS significantly decreased Botrytis, Alternaria, Rhizoctonia and Anthracnose fruit rots by 46.68 and 30.81%, 14.04 and 15.74%, 20 and 31.67%, and 19.17 and 25.24%, respectively, compared to inoculated and untreated controls. PB-based treatment resulted in 12.83% significantly lower Rhizoctonia fruit rot. These results showed that PS may be used as potential abiotic agent for successfully controlling fungal tomato diseases.

Keywords: Antifungal activity; Fruit rot; Potassium salts; Tomato pathogens; Wilt severity

Introduction

Fusarium wilt caused by Fusarium oxysporum f. sp. Lycopersici (FOL), Fusarium Crown and Root Rot (FCRR) incited by Fusarium oxysporum f. sp. radicis-lycopersici (FORL) and Verticillium wilt caused by Verticillium dahliae (VD) are among the most devastating tomato vascular diseases in Tunisia [1,2]. These soil-borne pathogens are economically important wilting pathogens in both greenhouse and open field-grown tomatoes in warm producing areas. In fact, yield losses up to 50 and 90% due to Verticillium wilt and FCRR disease, respectively, have been reported on severely infected tomato cultivars [1-3].

Management of these pathogens is difficult due not only to their endogenous growth, but also to the multi-years longevity of their resting structures in the soil (chlamydospores and microsclerotia) and to the inefficacy of fungicides once they reach the xylem [4-7]. Moreover, in Tunisia, tomato is regarded as one of the most important crops in terms of both value and cropped areas [8] and its continuous cropping leads to a buildup of fungal resting structures in the soil, resulting in substantial disease problems as well as the emergence of new races of these pathogens that overcome plant resistance [9-11]. For instance, the emergence of the race 2 of V. dahliae in Tunisia was recently reported and was shown to be involved in the outbreak of Verticillium wilt symptoms on resistant tomato cultivars [12]. That is why strategies developed for controlling these tomato diseases over years, such as cultural practices, chemical treatments, use of resistant cultivars, grafting, soil solarisation, biological control, etc., were often short lived and did not give satisfactory results. These strategies are either inefficient in reducing soil inoculum levels or harmful to the environment and human health [13].

In addition, as in Tunisia tomato is grown mainly in monoculture, wilt diseases are often associated with root rots and stem decay diseases caused by F. solani, Rhizoctonia solani, Colletotrichum coccodes, Pythium aphanidermatum and Sclerotinia sclerotiorum, which are reportedly responsible for severe growth reduction and yield losses. Moreover, the incidence of grey mold (Botrytis cinerea) and early blight (Alternaria solani), which are among the most important diseases of tomato aerial parts both in greenhouse or open field crops leading to lower quantity and quality of fruit yields, is in constant increase [14,15].

The increasingly important economic impact of these pathogens together with the absence of curative treatments justifies increased...
attention to search for alternative methods for tomato diseases’ management. An interesting alternative to chemical control involves the use of the antimicrobial properties of some organic and inorganic salts, generally used in food processing and preservation, to control plant diseases [16-18].

Several organic and inorganic salts have been widely used for suppression of fungal pathogens on various crops (open field and protected) responsible for various diseases on different plant organs and growth stages [19]. Importantly, these salts have a broad spectrum of antimicrobial activity with low mammalian toxicity, possess biocompatibility [16,17], and are generally recognized as safe (GRAS). Thus, they are widely exploited in food industry as preservatives, pH regulators and antimicrobial agents [17].

Potassium bicarbonates and carbonates have been demonstrated to inhibit the growth of several fungal pathogens infecting vegetables and ornamental crops at the post-harvest stage [17,20-22]. In the same manner, foliage spays of sodium or potassium bicarbonates led to decreased powdery mildew severity (Leveillula taurica) in pepper plants [23]. These salts were eventually registered as pesticide ingredients in the USA and as a horticultural fungicide in the UK [19,24] and they exhibit antifungal properties when applied as foliar sprays at concentrations between 5 and 20 g/l [19]. Multiple mechanisms are possibly involved in the registered antifungal effect of bicarbonate salts, such as pH elevation on the leaf surface [25] and collapse of fungal cell walls due to K⁺ imbalance or dehydration of fungal spores [26]. In addition to its potential to reduce the environmental impact of synthetic fungicides, potassium bicarbonate (PB) has lower risk of fungal resistance development due to its multiple modes of action, lower cost and shorter application to harvest interval [26].

Dipotassium hydrogen phosphate (DPHP) is among the most studied phosphate salts for their antifungal properties [19]. Reuveni et al., [27] described phosphates as ideal candidates for fungal disease management as they are fast absorbed by the plant, they have high mobility within tissues and have low cost nutrient source. Furthermore, phosphates exhibit antifungal activity through the induction of systemic acquired resistance; thus, providing long-lasting plant protection and are also effective against many fungal pathogens [19].

Potassium sorbate (PS) is a weak acid exhibiting antimicrobial as well as antifungal, activity [28,29]. PS was among the most effective compounds used for the control of orange molds caused by fungi responsible for wilts, crown and root rots, stem decay and ornamental crops at the post-harvest phase [17,20-22]. In the same manner, foliage sprays of sodium or potassium bicarbonates led to decreased powdery mildew severity (Leveillula taurica) in pepper plants [23]. These salts were eventually registered as pesticide ingredients in the USA and as a horticultural fungicide in the UK [19,24] and they exhibit antifungal properties when applied as foliar sprays at concentrations between 5 and 20 g/l [19]. Multiple mechanisms are possibly involved in the registered antifungal effect of bicarbonate salts, such as pH elevation on the leaf surface [25] and collapse of fungal cell walls due to K⁺ imbalance or dehydration of fungal spores [26]. In addition to its potential to reduce the environmental impact of synthetic fungicides, potassium bicarbonate (PB) has lower risk of fungal resistance development due to its multiple modes of action, lower cost and shorter application to harvest interval [26].

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Materials and Method

Plant material

Tomato seeds (cv. Rio Grande) were superficially disinfected by immersion in absolute ethanol for 2 min, followed by extensive rinsing in sterile distilled water. Seeds were sown in alveolus plates filled with previously sterilized peat. Seedlings were grown in a growth chamber at 24-26°C with 12-h photoperiod and 70% relative humidity. They were watered daily and fertilized once a week with a standard nutrient solution according to Pharand et al. [32]. Experiments were performed using 30 days-old tomato plants.

Fruit material

Tomato (cv. Sahel) fruits were harvested at the firm-mature red stage and sorted based on size and the absence of physical injuries or apparent disease infection. Before treatments, fruits were surface-disinfected with 2% sodium hypochlorite for 3 min, then rinsed with sterile distilled water, and air-dried.

Fungal pathogens

The fungal pathogens used in this study were: FOL, FORL, F. solani, VD, C. coccodes, R. solani, P. apriandermatum, S. sclerotiorum, B. cinerea, and A. solani. These pathogens were isolated from roots, crowns, stems, leaves and fruits of diseased tomato plants and were held in the fungal culture collection of the laboratory of Phytopathology in the Regional Center of Research on Horticulture and Organic Agriculture of Chott Mariem-Tunisia. Cultures of each fungus were maintained on Potato Dextrose Agar (PDA; Difco, Detroit, MI, USA) and were stored in PDA slants at 5°C until further use.

Liquid cultures used for substrate inoculation were prepared on Potato Dextrose Broth (PDB) and incubated at 25°C under continuous shaking at 150 rpm during 4 to 5 days. Concentration of the conidial suspension used was adjusted to 10⁷ conidia/ml using a Malassez haemocytometer.

Potassium salts tested

Potassium salts used in this study and their respective chemical characteristics are given in Table 1. PS was tested at concentrations of 0.25, 0.5, 1, and 1.5% (v/v) while DPHP and PB were tested at 0.1, 0.4, and 0.6 M.

In vitro antifungal activity of potassium salts against tomato pathogens

The inhibitory effect of potassium salts on the mycelial growth of tomato pathogens was evaluated on PDA medium supplemented with streptomycin sulfate (300 mg/l). The desired quantities of the tested salts were added to autoclaved and molten PDA to achieve the targeted concentrations tested. For each compound, a 10 ml aliquot of amended PDA was aseptically poured into a Petri plate (9 cm in diameter), with an unamended PDA dish used as a control. Three agar plugs (6 mm in diameter) cut from 7-day-old fungal cultures were plated at 2 cm from the edge of the plate and equidistantly spaced from each other by 3 cm. Plates were sealed with parafilm and incubated in the dark at 24°C for 4 days. At the end of the incubation period, mycelial growth was measured with a ruler as a linear distance in millimeters from the edge of the plate to the center of the colony.

Potassium salts tested in vitro

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical formula</th>
<th>Molecular weight (g/mol)</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium sorbate (PS)</td>
<td>C₇H₁₂KO₇</td>
<td>150.22</td>
<td>Loba Chemie, (Mumbai, India)</td>
</tr>
<tr>
<td>Potassium bicarbonate (PB)</td>
<td>K₂HPO₄</td>
<td>174.20</td>
<td>Suvchem, (Maharashtra, India)</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate (DPHP)</td>
<td>K₂HPO₄</td>
<td>100.12</td>
<td>Loba Chemie, (Mumbai, India)</td>
</tr>
</tbody>
</table>

Table 1: Potassium salts tested in this study.

25°C for varying times depending on growth rate of each organism and time necessary for reaching the edge of the plate. This was as briefly as 1 day for fast-growing organisms like *P. aphanidermatum* and as long as 7 days for slower growing ones like VD. Colony diameters were measured at two perpendicular points and the mean was determined. The mycelial growth inhibition percentage was calculated according to the formula of Tiru M et al., as follows: $I\% = \frac{[(C_2 - C_1)]}{C_2} \times 100$ with C2: Mean pathogen colony diameter in control plates and C1: Mean pathogen colony diameter in presence of tested compounds [33]. The experiment was repeated twice, with three replicates per individual treatment.

Statistical analyses were performed, for each salt tested, following a completely randomized factorial design where pathogens and salts' concentrations were the two fixed factors. Six replicates were used per individual treatment and means were separated using Fisher's protected LSD or Duncan's Multiple Range tests (at $P \leq 0.05$). The whole experiment was repeated twice but only the data of one essay is presented in the present study. Statistical analyses were performed using SPSS software version 16.

**Effect of potassium salts on tomato wilt severity under growth chamber conditions**

PS (0.25%, v/v), PB (50 mM) and DPHP (50 mM) were tested *in vivo*, as foliar spray, for controlling Verticillium wilt, Fusarium wilt and FCRR. These salts' concentrations were chosen as the optimal ones for *in vivo* experiments without any significant phytotoxic effects [34].

At 30 days-old, tomato seedlings (cv. Rio Grande) were sprayed with 50 ml of each salt aqueous solution using a hand sprayer. This quantity was sufficient to ensure complete coverage of all plant surfaces. Plants sprayed with distilled water only served as untreated controls. Five days post-treatment, seedlings were carefully removed from alveolus to maintain a high relative humidity (about 95%) and stored at 25°C. Disease symptoms were evaluated by determining the average lesion diameter 5 days post-inoculation by *P. aphanidermatum* and *C. coccodes* challenge. Each individual treatment was repeated thrice using 5 fruits per replication.

**Results**

In *vitro* evaluation of the antifungal activity of potassium salts against tomato pathogens

For each salt tested, ANOVA analysis revealed a significant (at $P \leq 0.01$) variation in the average fungal colony diameter depending on tomato pathogens tested and potassium salts' concentrations used.

Data shown in Table 2 indicated that in PS-amended PDA, mycelial growth inhibition of all pathogens varied in a concentration-dependent manner with 100% inhibition recorded at concentrations above 0.25%. Linear growth of tested fungi decreased significantly with the increase of PS concentrations. In fact, as compared to the untreated control, inhibition of mycelial growth (combined data of all fungi tested) ranged from 23.26 to 100% using PS at 0.25% compared to 51.92-100% and 57.84-100%, achieved using this salt at 0.5 and 1% respectively. However, at the highest concentration, 1.5%, PS had completely suppressed pathogens' radial growth.

Tomato pathogens exhibited variable responses when grown on PDA amended with different PS concentrations (Table 2). In fact, increasing PS concentration from 0.25 to 1% resulted in 25.75-69.03% and 23.69-57.84% decrease in *F. solani* and *C. coccodes* mycelial growth, respectively. However, this salt had totally suppressed the *in vitro* growth of *P. aphanidermatum*, *S. sclerotiorum* and *B. cinerea* at the lowest concentration used (0.25%). *F. solani*, *R. solani*, *C. coccodes* and *VD* were inhibited by 26.80, 53.67, 23.26 and 60.91%, respectively, using PS at 0.25% and were totally suppressed using this salt at 0.5%. *A. solani* radial growth decreased by 22.87 and 73.26% using PS at 0.25 and 0.5%, respectively, and was completely suppressed at higher concentrations (1 and 1.5%).

As given in Table 3, PI inhibited mycelial growth of all pathogens grown in amended-PDA medium in a concentration-dependent manner and the greater inhibitory effect was recorded at the highest concentrations used. In fact, this salt reduced pathogens’ growth (combined data of all fungi tested) by 0.39-100% at 0.1 M compared to 7.09-100% and 55.04-100% decrease achieved using PB at 0.4 and 0.6 M, respectively.

When grown on PDA medium amended with PB at 0.1 to 0.6 M, mycelial growth of *FOL*, *FORL* and *F. solani* was inhibited from 3.36%-77.99%, 7.41%-62.55%, and 2.29%-47.71%, respectively, compared to 21.85%-89.28%, 31.40%-55.04% and 8.12%-57.36% decrease, compared to the untreated controls, noted on *R. solani*, *C. coccodes* and *VD* treated cultures, respectively. For *A. solani*, these inhibition
Effect of potassium salts on verticillium and fusarium wilts severity under growth chamber conditions

The three potassium salts, applied as foliar spray 5 days before single inoculation with three soil-borne fungi (VD, FOL and FORL), was evaluated for their effects on wilt severity and plant growth in single inoculation with three soil-borne fungi (VD, FOL and FORL), and on tomato pathogens' mycelial growth (combined data of all fungi tested). In fact, tomato pathogens' mycelial growth (combined data of all fungi tested) was decreased significantly with the increase of DPHP concentrations. In fact, tomato pathogens' mycelial growth (combined data of all fungi tested) was decreased significantly with the increase of DPHP concentrations.

Results presented in Table 4 showed clearly that in DPHP-amended PDA, pathogens' linear growth varied in a dose-related manner by decreasing significantly with the increase of DPHP concentrations. In fact, tomato pathogens' mycelial growth (combined data of all fungi tested) was reduced by 0.37%-60.71% at 0.1 M DPHP compared to 3.36%-100% and 23.26%-100% recorded using this salt at 0.4 and 0.6 M, respectively.

Moreover, when DPHP concentrations increased from 0.1 to 0.6 M, the inhibition of FOL, FORL and F. solani radial growth augmented from 0.37 to 36.57%, from 5.92 to 37.98%, and from 6.87% to 48.80%, respectively. However, for R. solani, C. coccodes and VD, these inhibition rates ranged between 2.49%-75.45%, 3.49%-23.26%, and 13.71%-42.64%, respectively. With the increase of DPHP concentrations, mycelial growth of R. solani was also reduced by 4.26%-78.68% compared to 6.38%-100%, 60.71%-100% and 3.03%-100% noted for S. sclerotiorum, B. cinerea and P. aphanidermatum, respectively. Thus, these three last pathogens were the most sensitive fungal species to DPHP as their radial growth was completely suppressed at 0.4 M.

Effect of potassium salts on verticillium and fusarium wilts severity under growth chamber conditions

The three potassium salts, applied as foliar spray 5 days before single inoculation with three soil-borne fungi (VD, FOL and FORL), was evaluated for their effects on wilt severity and plant growth in comparison to untreated and inoculated or non-controls.

Wilt severity

All pathogen-inoculated tomato plants showed 60 DPI typical wilt
Table 5: Effect of different Dipotassium hydrogenphosphorous (K₂HPO₄) concentrations on the in vitro mycelial growth of tomato fungal pathogens cultured at 25°C on PDA medium.

<table>
<thead>
<tr>
<th>Treatment / Pathogen</th>
<th>Disease index</th>
<th>0</th>
<th>0.1</th>
<th>0.4</th>
<th>0.6</th>
<th>Average per fungus tested&lt;sup&gt;ab&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium oxysporum f. sp. lycopersici</td>
<td>4.47</td>
<td>4.45</td>
<td>4.32</td>
<td>2.83</td>
<td>4.02 ab</td>
<td></td>
</tr>
<tr>
<td>F. oxysporum f. sp. radicis-lycopersici</td>
<td>4.78</td>
<td>4.50</td>
<td>4.28</td>
<td>2.97</td>
<td>4.13 a</td>
<td></td>
</tr>
<tr>
<td>F. solani</td>
<td>4.85</td>
<td>4.52</td>
<td>4.30</td>
<td>2.48</td>
<td>4.04 ab</td>
<td></td>
</tr>
<tr>
<td>Verticillium dahliae</td>
<td>3.28</td>
<td>2.83</td>
<td>2.50</td>
<td>1.88</td>
<td>2.62 e</td>
<td></td>
</tr>
<tr>
<td>Colletotrichum coccodes</td>
<td>4.30</td>
<td>4.15</td>
<td>3.50</td>
<td>3.30</td>
<td>3.81 bc</td>
<td></td>
</tr>
<tr>
<td>Rhizoctonia solani</td>
<td>4.82</td>
<td>4.70</td>
<td>4.27</td>
<td>1.18</td>
<td>3.74 c</td>
<td></td>
</tr>
<tr>
<td>Sclerotinia sclerotiorum</td>
<td>4.97</td>
<td>4.65</td>
<td>0</td>
<td>0</td>
<td>2.40 f</td>
<td></td>
</tr>
<tr>
<td>Pythium aphanidermatum</td>
<td>4.40</td>
<td>4.27</td>
<td>0</td>
<td>0</td>
<td>2.16 g</td>
<td></td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>4.67</td>
<td>1.83</td>
<td>0</td>
<td>0</td>
<td>1.62 h</td>
<td></td>
</tr>
<tr>
<td>Alternaria solani</td>
<td>4.30</td>
<td>4.12</td>
<td>2.80</td>
<td>0.92</td>
<td>3.03 d</td>
<td></td>
</tr>
<tr>
<td>Average per potassium phosphate concentration&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.48 a</td>
<td>4.00 b</td>
<td>2.59 c</td>
<td>1.55 d</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean mycelial growth per fungal pathogen for all potassium phosphate concentrations combined.
<sup>b</sup>Mean mycelial growth per potassium phosphate concentration tested for all fungal pathogens combined.
<sup>c</sup>For fungal pathogens and potassium phosphate concentrations tested, values (means) followed by the same letter are not significantly different according to Duncan's Multiple Range test at P ≤ 0.05.

Table 4: Effect of different potassium salt-based treatments on disease severity on tomato cv. Rio Grande plants inoculated with Verticillium dahliae (VD), Fusarium oxysporum f. sp. lycopersici (FOL) and F. oxysporum f. sp. radicis-lycopersici (FORL) noted 60 days post-inoculation under growth chamber conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease index</th>
<th>VD</th>
<th>FOL</th>
<th>FORL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium sorbate (PS)</td>
<td>1.6 b</td>
<td>1 bc</td>
<td>1.4 c</td>
<td></td>
</tr>
<tr>
<td>Potassium bicarbonate (PB)</td>
<td>2.8 ab</td>
<td>1.8 b</td>
<td>2.8 b</td>
<td></td>
</tr>
<tr>
<td>Dipotassium hydrophosphate (DPHP)</td>
<td>3 a</td>
<td>1.6 b</td>
<td>3 ab</td>
<td></td>
</tr>
<tr>
<td>Inoculated control</td>
<td>3.2 a</td>
<td>4.8 a</td>
<td>4 a</td>
<td></td>
</tr>
<tr>
<td>Uninfected control</td>
<td>0 c</td>
<td>0 c</td>
<td>0 d</td>
<td></td>
</tr>
</tbody>
</table>

For each pathogen tested, values (means) followed by the same letter are not significantly different according to Duncan’s Multiple Range test at P ≤ 0.05.

Symptoms while uninoculated and untreated ones were symptomless. However, for each pathogen, wilt severity varied significantly (at P ≤ 0.05) depending on treatments tested (Table 5).

Verticillium wilt severity noted on PS-treated plants was significantly reduced by 50% compared to VD-inoculated and untreated control ones. Foliar spray with PB led to 12.5% reduction in wilt severity even statistically comparable to the VD-inoculated and untreated control and also similar to PS-based treatment. DPHP-based treatment did not provide any protection against Verticillium wilt severity which was comparable to that of VD-inoculated and untreated control.

PS-based treatment provided the highest protection against Fusarium wilt and led to 78.26% significantly lower disease severity, as compared to FOL-inoculated and untreated control and exhibited comparable effectiveness as the two other salts. PB and DPHP spray decreased Fusarium wilt severity by 60.86% and 65.21%, respectively, compared to FOL-inoculated and untreated control.

Plants were cultured on PDA medium and incubated at 25°C for 4 d. For fungal pathogens and potassium phosphate concentrations tested, values (means) followed by the same letter are not significantly different according to Duncan’s Multiple Range test at P ≤ 0.05.

Plant height

Plant height noted 60 DPI on PS-, PB- and DPHP-treated plants was significantly (at P ≤ 0.05) comparable to that noted on their respective VD- and FOL-inoculated or non-controls (Table 6). However, compared to FORL-inoculated plants, these treatments had significantly increased plant height by 20.61%, 19.55% and 9.5%, respectively. Plant heights noted on PS- and PB-treated plants were statistically similar and significantly higher than that of DPHP-treated plants but also comparable to that of the untreated and uninoculated control. Plant height noted on DPHP-treated plants was 14.16% lower than that of the untreated and uninoculated control.

Root fresh weight

Root fresh weight noted 60 DPI on tomato plants varied significantly (at P ≤ 0.05) depending on treatments tested. Indeed, data given in Table 7 showed that PS- and PB-based treatment had significantly increased root fresh weight by 32.87% and 27.94% compared to VD-inoculated plants and were statistically comparable to the untreated and uninoculated control. Root fresh weight noted on DPHP-treated plants was comparable to both controls.

Root fresh weight noted on PS-treated plants was significantly higher than that of FOL-inoculated plants and statistically comparable to that of the untreated and uninoculated control. This parameter noted on PB-treated plants was increased by 28.84%, even statistically insignificant, in comparison to FOL-inoculated and untreated control but was comparable to that noted on PS- and DPHP-
Effect of potassium salts tomato fruit rots severity

Results shown in Table 9 indicated that on each pathogen-inoculated tomato fruits, potassium salts had significantly \( \leq 0.05 \) reduced the average lesion diameter, 5 to 7 days after inoculation, compared to the inoculated and untreated controls. DPHP (0.4 M) followed by PS (0.25%, v/v) had significantly \( \leq 0.05 \) decreased grey mold severity by 46.68% and 30.81%, respectively, compared to \( B. \) cinerea-inoculated fruits. However, PB (0.4 M) decreased this parameter by 10.19%, even statistically insignificant, compared to \( B. \) cinerea-inoculated control.

A significant Anthracnose rot reduction, by 25.24% and 19.17% compared to \( C. \) coccodes-inoculated fruits, was also achieved 7 days after tomato treatment with PS followed by DPHP, respectively. However, PB-based treatment did not suppress Anthracnose rot where disease severity was statistically comparable to that noted on \( C. \) coccodes-inoculated control fruits.

PS- and DPHP-treated fruits showed decreased Alternaria rot severity, by 31.67% and 20%, compared to \( A. \) solani-inoculated fruits, while PB-treated fruits showed average lesion diameter statistically comparable to that of the inoculated control.

Discussion

The findings of the present investigation elucidated the comparative effectiveness of three potassium salts in inhibiting the \textit{in vitro} growth of wilt-, root-rot-, stem decay- and fruit rot-causing tomato pathogens and their relative ability to suppress Verticillium and Fusarium wilts and tomato fruit rots.

The current study highlighted the \textit{in vitro} antifungal activities of potassium salts against all tomato pathogens tested. Nevertheless, the efficiency of these abiotic agents differed depending on concentrations used and degree of sensitivity of targeted pathogens. For each potassium salt tested, the exact concentration required to inhibit fungal growth differed somewhat between tomato pathogens. These results are consistent with other studies demonstrating that suppression of microbial strains varies in response to different organic and inorganic salts Olivier et al. and Fagundes et al., \cite{17,35} show that the percentage of fungal inhibition in amended PDA medium was dependent on concentration used, and that the most significant decrease in mycelial growth was obtained with the highest concentration of salt tested.

Table 6: Effect of potassium salt-based treatments on height of tomato cv. Rio Grande plants inoculated with \textit{Verticillium dahliae} (VD), \textit{Fusarium oxysporum} f. sp. \textit{lycopersici} (FOL) and \textit{F. oxysporum} f. sp. \textit{radicis-lycopersici} (FORL) noted 60 days post-inoculation under growth chamber conditions.

<table>
<thead>
<tr>
<th>Treatment / Pathogen</th>
<th>Plant height (cm)</th>
<th>Plant height (cm)</th>
<th>Plant height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VD</td>
<td>FOL</td>
<td>FORL</td>
</tr>
<tr>
<td>Potassium sorbate (PS)</td>
<td>47.8 a</td>
<td>45.6 a</td>
<td>45.6 a</td>
</tr>
<tr>
<td>Potassium bicarbonate (PB)</td>
<td>46.2 a</td>
<td>47 a</td>
<td>45 a</td>
</tr>
<tr>
<td>Dipotassium hydrophosphate (DPHP)</td>
<td>46 a</td>
<td>51.4 a</td>
<td>40 b</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>44 a</td>
<td>45.2 a</td>
<td>36.2 c</td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>46.8 a</td>
<td>46.2 a</td>
<td>46.6 a</td>
</tr>
</tbody>
</table>

For each pathogen tested, values (means) followed by the same letter are not significantly different according to Duncan’s Multiple Range test at \( P \leq 0.05 \).

Table 7: Effect of potassium salt-based treatments on root fresh weight of tomato cv. Rio Grande plants inoculated with \textit{Verticillium dahliae} (VD), \textit{Fusarium oxysporum} f. sp. \textit{lycopersici} (FOL) and \textit{F. oxysporum} f. sp. \textit{radicis-lycopersici} (FORL) noted 60 days post-inoculation under growth chamber conditions.

<table>
<thead>
<tr>
<th>Treatment / Pathogen</th>
<th>Root fresh weight (g)</th>
<th>Root fresh weight (g)</th>
<th>Root fresh weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VD</td>
<td>FOL</td>
<td>FORL</td>
</tr>
<tr>
<td>Potassium sorbate (PS)</td>
<td>14.6 a</td>
<td>12.8 ab</td>
<td>10.4 ab</td>
</tr>
<tr>
<td>Potassium bicarbonate (PB)</td>
<td>13.6 a</td>
<td>10.4 abc</td>
<td>10.6 ab</td>
</tr>
<tr>
<td>Dipotassium hydrophosphate (DPHP)</td>
<td>10.4 bc</td>
<td>8.6 bc</td>
<td>9 bc</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>9.8 c</td>
<td>7.4 c</td>
<td>7.2 c</td>
</tr>
<tr>
<td>Uninoculated control</td>
<td>13.1 ab</td>
<td>13.2 a</td>
<td>13 a</td>
</tr>
</tbody>
</table>

For each pathogen tested, values (means) followed by the same letter are not significantly different according to Duncan’s Multiple Range test at \( P \leq 0.05 \).
In fact, the effectiveness of potassium salts in controlling various plant pathogens have been previously reported [18,36-40]. The mode of action of most salts is postulated to consist in a reduction of fungal fungal pressure that results in the collapse and shrinkage of fungal hyphae causing subsequent inhibition of mycelia growth and sporulation [23,41].

At concentrations ranging from 0.25% to 1.5%, PS followed by PB (0.1 to 0.6%) were found to be more efficient than DPHP (0.1 M to 0.6 M) in reducing mycelial growth of the ten tested fungi. In this sense, Mohamady et al. [42] reported the higher inhibitory effect of PS followed by potassium carbonate against radial growth of several tested fungi (FORL, FOL, F. solani, R. solani, Sclerotium rolfsii, Macrophomina phaseolina, Pythium spp. and Phytophthora spp.) which increased with the increase of concentrations and reached their maximum levels at 4% and 8%, respectively. Similarly, when studying the in vitro efficacy of eight food additives as possible alternatives to synthetic fungicides for the control of soil-borne pathogens, Arslan U et al. [31] find that the minimum PS inhibitory concentrations are 0.1% for F. oxysporum f. sp. melonis, 0.2% for M. phaseolina, 0.6% for R. solani, and 0.05% for S. sclerotiorum, whereas the minimum fungicidal concentrations were >2%, >2%, 0.8% and 0.1%, respectively. In the same sense, Mecettel et al. [18] showed that the anionic sorbate completely inhibit mycelial growth of F. sambucinum, the causal agent of potato dry rot, at 0.2 M, while for spore germination the ED50 was 0.56 mM. Sorbate has also shown toxicity on mycelium of Helminthosporium solani, an important potato pathogen and always ranked first in efficacy, since it was fungicidal at concentrations as low as 0.05 M [17]. Sorbate has also shown toxicity on mycelium of Helminthosporium solani, an important potato pathogen and always ranked first in terms of efficacy [17]. PS, which is the most commonly applied salt of sorbic acid, is reported to completely suppress growth of the banana pathogen Colletotrichum musae in vitro when used at 0.125% [43]. In this regards, Kader et al., [44] noticed that the reduction in mycelial growth of citrus and strawberry fruit decay pathogens (Geotrichum candidum, Penicillus digitatum, P. italicum and B. cinerea) is correlated to the gradual increase in PS concentrations with the highest inhibition observed at 4%. Yildirim et al., found that among food additives, PS shows strong inhibitory against mycelial growth of B. cinerea at rising doses [45]. At increasing concentrations (from 600 to 1600 μg/ml), this salt shows the strongest inhibition on mycelial growth and pycnidia and pycnidiospores production of Phomopsis viticola [46]. In the same sense, Mills et al., also reported that PS has significantly inhibited the growth of B. cinerea, F. solani var. coeruleum, Phytophthora erythroseptica and P. infestans infecting potato while [47,48] found that PS has completely suppressed V. dahliae growth at 0.25%.

The principle mode of action of weak acid preservatives, such as sorbate, is believed to be the passage of the undissociated compound through the plasma membrane; once inside the cell in a higher pH environment, the acid dissociates causing an accumulation of protons and anions which cannot re-cross the plasma membrane [49]. In addition, sorbic acid and its salts are known for their ability to alter cell membrane and activity of several enzymes of Krebs cycle [50]. Moreover, sorbate effectively inhibits the growth of moulds, including mycotoxin-producing types, in culture media and in food products [51]. This salt inhibits the production of mycotoxins, including aflatoxins, patulin, citrinin, sterigmatocystin and ochratoxin [52-56].

At the highest dose tested (0.6 M), PB was more efficient in halting all pathogens’ growth in vitro. Previous studies reported that PB has significantly inhibited in vitro growth of various plant pathogens [17,31,41,47,57-60]. In this regard, Abdel et al., demonstrated that PB has significantly reduced the linear growth of A. solani, F. solani, F. oxysporum and Pythium sp [61]. In the same sense, Punja ZK noted that bicarbonate salt (PB) shows fungicidal effect and prevents sclerotial germination of S. rolfsii [57]. Zaker et al., also found that among four inorganic salts tested, PB performs highest antifungal activity against F. oxysporum, A. alternata and B. cinerea with the best inhibitory effect achieved using PB at 0.2 M. According to study, KHC03 is capable of significantly reducing the mycelial growth of F. oxysporum by greater than 95% in Czapek Dox broth at as low as 0.2% concentration. PB is also reported to inhibit the mycelial growth of F. solani by 46.14% after 7 days of culture in amended PDA [62,63]. Recently, Youssef et al., have tested the effect of a novel PB-based formulation and found from in vitro trials a complete inhibition of Penicillus digitatum, P. italicum, and P.
F. solani comparative evaluation of PS, PB and DPHP-foliar decays as well as foliar diseases and mainly fungal pathogens of tomato plants in Tunisia.

Our in vitro results highlighted the broad spectrum of antifungal activity of the three potassium salts tested. In fact, at a given concentration, single salt was able to inhibit the mycelial growth of fungal species belonging to eight different genera and also many species of the same genus. On the other hand, one pathogen could be inhibited by various salts. These results are consistent with many other reports [17,27,70-72]. In fact, these salts possess wide-spectrum antimicrobial properties and have been demonstrated to inhibit the growth of several fungal pathogens infecting stored fruits, vegetables, and ornamental species [17,19-22]. To our knowledge, this is the first report on the in vitro antifungal activity of potassium salts against major fungal pathogens of tomato plants in Tunisia.

The in vivo comparative evaluation of PS, PB and DPHP-foliar treatments’ ability to suppress Verticillium wilt, Fusarium wilt and FCRR severity clearly showed that these salts varied in their effectiveness against the three tomato diseases. In fact, as shown in the present study, PB-based treatment led to significant decrease in Fusarium wilt and FCRR diseases (by 60.86 and 30%) and failed to protect plants against Verticillium wilt at the concentration used. Bicarbonate salts, among which PB, have been extensively tested. According to the Environmental Protection Agency in USA (EPA), the use of PB as fungicide could offer a safe alternative to toxic conventional ones as it was not expected to be harmful for humans and the environment [19]. In addition, bicarbonate salts have shown successful management of various postharvest fruit decays as well as foliar diseases and mainly powdery mildew in diverse crops as they exhibit protectant in addition to eradicant activity. However, this is not the case in this study as FOL and FORL are two soil-borne pathogens and there was not any direct contact between these pathogens and PB salt. Hence, the present work gave additional information on the ability of PB to reduce the severity of two important tomato Fusarium diseases, under growth chamber conditions, although its mode of action needs to be more clarified.

DPHP was able to provide protection against only Fusarium wilt and led to 65.21% significantly lower disease severity, relative to FOL-inoculated and untreated control. In fact, Deliopoulos et al., reported that among the most studied phosphate salts for their antifungal properties is DPHP which manifests its antifungal activity by induction of systemic acquired resistance (SAR) which requires the signal molecule salicylic acid to be expressed [19,73]. In this regards, Biswas SK et al., found that foliar spray of 10 mM DPHP as inducer provides induced resistance against tomato Fusarium wilt, resulting in a decline in the disease incidence to 8.54% compared to 100% noted on FOL-inoculated plants at 15 DPI [74]. These results are also in line with Sarwar N et al., findings that showed that chickpea seed treatment with DPHP reduced Fusarium wilt disease by 30% [75]. In the same sense, Aleandri MP et al., found that plants grown from seeds treated with the abiotic inducer DPHP at 50 mM become more resistant to Monosporascus cannonballus [34]. In this regard, Sayed SA et al., reported the significant effects of DPHP in reducing the percentages of pre- and post-emergence damping-off caused by R. solani, F. solani and F. oxysporum compared to the control [76]. Furthermore, Lopez et al., found that DPHP reduces the severity of antracnose disease caused by C. gloeosporioides in cashew leaves [77]. The maximum reduction of the disease in detached leaves, without significant phytotoxic effects, is obtained with 50 mM DPHP, with an interval of at least 48 h between spray application and inoculation. Furthermore, Gottstein HD et al., found that dibasic and tribasic phosphate salts are able to induce systemic resistance to antracnose of cucumber caused by C. lagenarium under greenhouse conditions [78]. The acquired resistance in newly developed leaves remains effective till 5 weeks post-treatments. Hence, in the present study, the observed reduction in Fusarium wilt severity could be due to an induction of systemic acquired resistance.

Single application of PS resulted in 50,78.26, and 65% lower wilt severity, as respectively compared to VD-, FOL- and FORL-inoculated and untreated controls. In this regards, Mohamedy RSR et al., mentioned that in field trials, the highest reductions of root rot incidence and disease severity caused by F. solani, R. solani and S. rolfsii are recorded on tomato plants treated with PS used at 7.5% and 400 mM DPHP [79]. Indeed, disease incidence and severity are reduced by 65.4% and 62.5% in 2012, and by 63.2% and 53.8% in 2013 cropping seasons, respectively. In the same sense, Punja et al., reported that potassium carbonate, PS, and other salts are able to reduce black root rot caused by Chalara


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isolates from potato fields in Manitoba and in Petri dishes differ depending on fungal tomato and compounds tested in decreasing on orange and lemon, and reported that PS is among the most effective mold inhibitor, in significantly reducing the isolated spoilage efficient in reducing all tomato fruit rots. In this sense, Mehyar GF et al., control oranges post-harvest molds caused by of PS, sodium benzoate and sodium EDTA were reported to effectively postharvest decay of tomatoes and other stored fruits. The application A. solani wide-spectrum antimicrobial properties; their efficacy in controlling 4-7 g/kg body weight, equals 500 g for an adult human) [17]. very low levels of mammalian toxicity (For example, for PS, LD compounds have potential as food-borne pathogens [31]. In addition, these salts have potential as environmentally compatible, nontoxic postharvest fungicides and have pathogens as most studies are focused on the use of PS for soil-borne-disease management as most studies are focused on the use of this salt for the control of postharvest diseases of horticultural commodities or as food additives. The present study clearly indicates that PS could give sufficient control of three important tomato soil-borne diseases; nevertheless, more information on its antifungal activities needs to be elucidated.

Because most of the literature about potassium salts applications has focused on controlling foliar or postharvest diseases [19,23,80], there is little information on the effects of these salts on the promotion of tomato growth after challenge with soil-borne pathogens such as V. dahliae, FOL and FORL. Hence, important findings from our study revealed that foliar sprays of PS five days before pathogen challenge could enhance more than one tomato growth parameter in more than one pathosystem. In fact, this salt had significantly increased plant height, root and aerial part fresh weights by 20.61%, 30.76% and 33.02%, respectively, compared to FORL-inoculated plants and had also improved root fresh weight by 42.18% and 32.87% compared to FOL- and VD-inoculated plants, respectively. However, DPHP treatment had significantly augmented plant height by 9.5% as compared to FORL-inoculated plants, whereas, PB-foliar application resulted in 27.94% increase in root fresh weight relative to VD-inoculated plants. These findings are in line with Mohamedy et al., studies who revealed that among various chemical resistance inducers tested, PS and DPHP have positive effects on plant growth, yield and fruit quality of tomato plants grown under naturally field infested by root rot diseases caused by R. solani, F. solani and S. rolfsii, during two cropping seasons [79].

This study highlighted also the potential use of potassium salts for postharvest disease control of fresh tomatoes. In fact, our results indicated that DPHP and PS had significantly decreased grey mold, Rhizoctonia, Alternaria and Anthracnose fruit rots by 46.68 and 30.81%, 14.04 and 15.74%, 20 and 31.67% and 19.17 and 25.24% respectively, compared to B. cinerea-, R. solani-, C. coccodes- and A. solani-inoculated control fruits. However, application of PB to tomato fruits resulted in significant decrease of Rhizoctonia rot by 12.83%. In fact, different salts have been evaluated for the control of postharvest decay of tomatoes and other stored fruits. The application of PS, sodium benzoate and sodium EDTA were reported to effectively control oranges post-harvest molds caused by Penicillium digitatum and P. italicum [30,81].

In the present study, PS and DPHP were fund to be the most efficient in reducing all tomato fruit rots. In this sense, Mehyar GF et al., reported that Guam gum edible coating incorporated with PS is the most effective mold inhibitor, in significantly reducing the isolated spoilage molds for 20, 15, and 20 days of storage at 4°C on apples, cucumbers, and tomatoes, respectively [82]. In the same sense, Palou L et al., studied the inhibition effect of a wide range of food additives and GRAS substances alone and in mixtures against P. digitatum- and P. italicum-inoculated on orange and lemon, and reported that PS is among the most effective compounds tested in decreasing P. digitatum viable population by 70% to 80% [30]. In another study, PS exhibits the greatest antimicrobial activity out of 8 food additives tested to inhibit growth of several food-borne pathogens [31]. In addition, these salts have potential as environmentally compatible, nontoxic postharvest fungicides and have very low levels of mammalian toxicity (For example, for PS, LD₅₀ in rat: 4-7 g/kg body weight, equals 500 g for an adult human) [17]. However, PB was found to be ineffective against most tomato fruit rot pathogens even though biceranates were reported possess wide-spectrum antimicrobial properties; their efficacy in controlling postharvest fungal phytopathogens has been widely reported [17,20-22,35,37,41,80].

Overall, differences in effectiveness of potassium salts between in vitro and in fruit trials have been observed in this study. The direct inhibitory effect of the salts amended in PDA on the growth of B. cinerea or A. solani, C. coccodes and A. solani in Petri dishes differ considerably from the inhibitory ability of these salts to control these pathogens when applied to tomato fruits.

Conclusion

In this study, it was observed that potassium salts tested possess variable antifungal activity in vitro depending on fungal tomato pathogens tested and concentrations used. They were shown to have direct antifungal potential and also indirect effect by decreasing tomato wilt severity incited by FOL, FORL and VD and by enhancing growth. Based on these findings, it could be concluded that, when applied as foliar spray, PS used at 0.25% followed by PB applied at 50 mM may markedly suppress Verticillium wilt, Fusarium wilt, and Fusarium Crown and Root Rot severities and enhance tomato plant growth. Further studies are needed to more elucidate the exact mechanism deployed by these salts via different modes and timings of application. On fruits, PS followed by DPHP was more effective in reducing all pathogen-causing rots than PB. It can be concluded that inorganic salt treatments could be a very useful component of integrated disease management strategies.

References


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