Biochemical Characterization, Phytotoxic Effect and Antimicrobial Activity against Some Phytopathogens of New Gemifloxacin Schiff Base Metal Complexes

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String of Fe(III), Cu(II), Zn(II) and Zr(IV) complexes were synthesized with tetradeionate amino Schiff base ligand derived by condensation of ethylene diamine with gemifloxacin. The novel Schiff base (4E,4′E)-4,4′-(ethane-1,2-diyldiazenylidene)bis[7-{(4Z)-3-(aminomethyl)-4-(methoxyimino)pyrrolidin-1-yl}-1-cyclopropyl-6-fluoro-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid] (GMFX-en) and its metal complexes were identified and confirmed by elemental analyses, FT-IR, UV/VIS, 1H-NMR spectra, magnetic susceptibility, conductometric measurements and thermal analyses. The FT-IR spectral data showed the chelation behavior of GMFX-en toward the metal ions through oxygen of carboxylate group and nitrogen of azomethine group. In the light of all spectral data, these complexes presumably have octahedral geometry configurations. Thermal analysis specified that the decaying of the metal complexes exist in two or three steps with the final residue metal oxides. Antimicrobial activity of the new prepared metal complexes was screened against some common phytopathogens and their mode of action has been also discussed. The potential phytotoxic effectiveness of the new complexes was furthermore inspected on two commonly experimental plants. The complexes showed significant antimicrobial and phytotoxic effects against the majority of tested phytopathogens and the two tested plants, respectively. The potential antimicrobial activity of the complexes proved their possibility to be used successfully in agropharmacutical industry to control many serious phytopathogens. The phytotoxicity of the studied complexes also indicated their possibility as potential bio-based herbicides alternatives to weed control in crop fields.

Keywords: gemifloxacin Schiff base, metal complexes, antimicrobial activity, phytotoxicity, chelation theory, phytopathogens.

Introduction

Schiff bases can be effortlessly produced by the intensification reaction of primary amines together with carbonyl compounds. Schiff base ligands have been substantially studied in coordination chemistry due to their ease of synthesis, effectively steric, electronic features, and good solubility in many solvents. The presence of azomethine group in Schiff base ligands performs a paramount role in their antimicrobial activities. Schiff base ligand is capable of coordinating with metal ions and stabilize them in various oxidation state and increased their antimicrobial activity depending on dipole moment, solubility, their enzymatic action, and cell permeability. Schiff base complexes have been utilized as model for biological procedures and as a catalytic for oxidation and polymerization of organic compounds. Support information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202100365
base may be bi, di, tri or polydentate chelates together with metal ions through nitrogen and oxygen donor atoms formulation steady complexes.\[7–13\]

Gemifloxacin (GMFX) is an artificial wide ranging antimicrobial compound versus both Gram-positive and negative bacteria.\[14\] GMFX implicates an effective antibacterial activity against respiratory tract pathogens.\[14\] GMFX can be interrogated with aliphatic or aromatic amines supply a Schiff base.\[14,15\] In the literature, an attention has been paid to fluoroquinolones Schiff base complexes with transition metals.\[16\] On the other hand, the heavy metals and antibiotics could produce polluting molecules and induce phytotoxicity for crops. So, it is important to evaluate the phytotoxic effect of these new synthesized complexes for determining the possibility for their using in agropharmaceutical industry.\[17\]

In view of the above consideration, the goal of this study was to evaluate the impact of certain elements, such as Fe(III), Cu(II), Zn(II) and Zr(IV) on the efficiency of gemifloxacin in new form as GMFX-en (Figure 1). The compounds were investigated by elemental analysis, molar conductance measurements, FT-IR, UV/VIS, \(^1\)H-NMR and thermal analyses. Whereas the antimicrobial activity was assessed towards phytopathogenic fungi: Monilinia fructicola (G. Winter) Honey, Penicillium digitatum (Pers.) Sacc., and Colletotrichum acutatum J.H. Simmonds and three bacterial species: Clavibacter michiganensis corrig. (Smith) Davis et al., Xanthomonas campestris Pammel (Dowson) and Bacillus megaterium (Pammel) Dowson. Phytotoxicity assay was performed against Solanum lycopersicum L. (tomato) and Lepidium sativum L. (cress).

**Results and Discussion**

The new prepared metal complexes of Fe(III), Cu(II), Zn(II) and Zr(IV) with GMFX-en are stable, colored, and non-hygroscopic in nature. The complexes were solvable in dimethylformamide and dimethyl sulfoxide. The exquisite physical features and distinctive data of the synthesized ligand and its metal complexes were measured (Table 1). The results of the chemical analysis showed that all the complexes were air stable at the room temperature. At room temperature, the molar conductance of GMFX-en in the free state is \(17.50 \, \Omega^{-1} \, \text{mol}^{-1} \, \text{cm}^2\), and the same data for metal complexes has been reported to range from 100.82 to 270.69 \(\Omega^{-1} \, \text{mol}^{-1} \, \text{cm}^2\). Conductance statistics demonstrate that the metal complexes were electrolyte in contrast with GMFX-en.\[16,18\]

**FT-IR Spectral Studies**

Table 2 lists the positions of the significant FT-IR bands of Schiff base and its metal complexes. FT-IR of the complexes (Figure S1) is compared with those of GMFX-en orderly to mark the spot of assortment that possibly implicated in chelation. The GMFX-en spectrum revealed that the bands were obscured specialized for the bands of NH\(_2\) group for ethylene diamine and C=O of pyridone group of GMFX. Registered a strong band at 1634 cm\(^{-1}\) refers to the C=N and it is determined to condensation of the amino group with a pyridone group, as a result of the formation of Schiff base linkage.\[19\] Complexes spectra involved bands expand from 3428–3433 cm\(^{-1}\) generated from the vibration of O–H signalizing the subsistence of water molecules in all complexes.\[13\] The influence of oxygen of carboxylate group with metal ions has been registered by the obscurity of the band at 1725 cm\(^{-1}\)

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![Figure 1](https://example.com/figure1.png)

**Figure 1.** Structure of GMFX-en (4\(E,E\),4\(^{\prime}\)E)-4,4\(^{\prime}\)-(ethane-1,2-diyldiazanylylidene)bis[7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)pyrrolidin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid).
Table 1. Elemental analysis and physico-analytical data for GMFX-en and its metal complexes.

| Compounds | M.Wt. | Yield% | M.p./C | Color      | Found (calc.)% | Λ (M.F.) cm⁻¹ mol⁻¹ | ζ (M.F.) cm⁻¹ | ν (M.F.) cm⁻¹ |
|-----------|-------|--------|--------|------------|----------------|---------------------|---------------|--------------|
| GMFX-en   | 802.762 | 81.00  | 340    | Yellowish-white | 56.70           | 5.40                | 20.80         |
| Complex A | 1127.107 | 77.20  | 300    | Dark-red   | 40.31           | 5.42                | 14.72         |
| Complex B | 1052.218 | 80.10  | 220    | Brown      | 41.80           | 5.25                | 15.36         |
| Complex C | 1029.142 | 75.36  | 210    | Light-brown | 40.40           | 5.12                | 16.26         |
| Complex D | 1088.886 | 72.69  | 300    | Dark-brown | 41.80           | 5.10                | 16.35         |

Electronic Spectra and Magnetic Studies

The assignments of the spotted electronic absorption bands of the GMFX-en and its metal complexes, magnetic data, and molar absorptivity (ε) of the established complexes are indexed in Table 3. Actually, the occurrences of diverse bands in the electronic spectra of GMFX-en display bands at 237, 268 and 345 nm (Figure 3) that may be referred to π-π* and n-π* transitions, respectively. The electronic spectrum of the complex A exhibits an L→M (C.T) at 450 nm. This complex presumably has octahedral configuration, and geometry is confirmed by measured magnetic moment (H_eff = 1.80 B.M.). For complex B electronic spectrum exhibits an L→M (C.T) charge transfer band at 480 nm. The band detected at 580 nm for copper complex may be referred to 2B₁g→2E₉ transition. The Cu(II) complex’s magnetic moment (1.70 B.M.) is very similar to the spin value (1.73 B.M.) predicted for one unpaired electron, indicating an octahedral geometry. The electronic spectrum of complexes C and D exhibit an M→L (C.T) charge transfer bands at 445 and 424 nm. In the light of these results, these complexes presumably have octahedral configurations.

1H-NMR Spectra

The 1H-NMR spectra of the ligand and its complexes were registered to emphasize the binding of the Schiff base to the metal ions (Table S1, Figure 4). The complexes did not detect the signal at 11 ppm (COOH) in the GMFX-en spectrum, asserting that the ligand was deprotonated and chelated with metal ions. Furthermore, owing to the existence of water molecules in the complexes, the 1H-NMR spectra for...
complexes indicate a novel peak in the range 4.20–4.55 ppm. Matching the GMFX-en Schiff base's key peaks to their complexes, the spectra of complexes with the chemical change induced by Schiff base binding to the metal ion contain all of the peaks of the free ligand.\[^{39-41}\]

**Thermal Analyses (TG and DTG) and Thermodynamic Parameters**

The stoichiometry of the resultant volatile decaying components as well as the properties of the complexes was studied employing thermal analyses. The TG decay stages with the temperature maximum and weight loss for the complexes are indexed in Table 4 and presented graphically in Figure 5. TG data for GMFX-en manifested one stage of decaying with temperature maxima 171, 338 and 532 °C corresponding to the loss of \(18\text{C}_2\text{H}_2 + 2\text{HF} + 2\text{NH}_3 + 6\text{NO} + 2\text{N}_2\) with total weight loss amounted to 96.90 % (calc. 97.02 %). The TG curve of complex A exhibited two stages from 25 to 132 °C and 132 to 680 °C. The initial stage of decay, with a maximum temperature of 69 °C with a mass loss of 11.20 % (calc. 11.18 %) may be assigned to the removal of seven non-absorbed coordinated water molecules. The last step showed mass loss of 76.18 % (calc. 76.41 %), leaving the residue observed over 670 °C. The thermogram of complex B showed three stages in the extent 25 to 137 °C, 137 to 211 °C and 211 to 650 °C. The first step due to the loss of 5H\(_2\)O lattice water with mass loses 8.64 % (calc. 8.26 %). The second step from 137 up to 211 °C display loss of 10 C\(_2\)H\(_2\) + 2HF + H\(_2\)S + 4NO\(_2\) + 2NH\(_3\) + H\(_2\) species corresponding to 51.02 % (calc. 51.73 %) leaving at the back the residue observed over 998 °C analogous to CuO \(+ 5\) C related to mass loss of 13.00 % (calc. 12.82 %). The complex C with in the temperature range of 35 to 1000 °C, the complex started decomposition at 35 °C in three distinct degradation phases. Within the temperature range of 35 to 116 °C, the first step of decomposition corresponded to the loss of 3H\(_2\)O lattice water, resulting in a weight loss of 5.52 %, which agrees well with the calculated value of 5.25 %. The loss of Schiff base ligand molecules and coordinated water molecules were assigned to the second and third stages of decaying, which happened between 116 and 650 °C. It represented an 82.57 % weight loss, which is in good agreement with the measured estimate of 83.35 %, leaving at the back the
residue observed over 999 °C analogous to ZnO + 3 C related to mass loss of 12.00 % (calc. 11.40 %). Lastly, the thermogram of complex D showed two steps in the range 31 – 125 °C and 125 – 660 °C. The first step due to loss of 5H2O uncoordinated water molecules showed mass loss of 8.23 % (calc. 8.26 %). The second step showed loss of ligand molecules and coordinated water molecules corresponding to 76.77 % (calc. 76.01 %), leaving at the back the residue observed over 660 °C analogous to ZrO2 + 4C related to mass loss of 15.00 % (calc. 15.72 %).

The kinetic factors such as (ΔH*), (E*), (G*), and (S*) (Table 5) were graphically tested using the Coats-Redfern and Horowitz-Metzger models equations (Figure S2).[42,43] Decomposition steps of activation energies were found to be in the range of 61.44 – 229.49 kJ mol⁻¹.[44,45] The positive sign of G* for complexes demonstrated that the final residue’s free energy was higher than that of the initial compounds, implying that all decomposition steps were non-spontaneous. For the subsequent decomposition stages of a given complex, the values of the activation, ΔG*, increased significantly. This can be explained by the fact that substantially rising the values of TΔS* from one stage to the next overrides the values of ΔH*. Negative ΔS* values for the degradation process, on the other hand, suggested that the activated complex was more ordered than the reactants or that the reaction was sluggish.[46,47]

**Figure 2.** Structures of complexes A, B, C and D. Complex A: M = Fe(III), n = 3 (FeC38H62F2N12O15Cl3). Complex B: M = Cu(II), n = 2 (CuC38H54F2N12O9S). Complex C: M = Zn(II), n = 2 (ZnC38H54F2N12O9Cl2). Complex D: M = Zr(IV), n = 2 (ZrC38H56F2N12O13Cl2).

**Antimicrobial Activity**

**Bactericidal Effect**

The results of antibacterial activity of the compounds were represented in (Figure 6) where all tested treatments showed antibacterial effects against all tested bacteria especially at the higher tested concentrations. The highest antibacterial activity against *C. michiganensis* was spotted in the case of GMFX-en and complex B at 250 ppm (Figure 4). Regarding *B. megaterium* and *X. campestris* the highest substantial effect was observed in the case of GMFX-en at 250 ppm followed by the complex C at 250 ppm. On the other hand, complex D at 100 ppm showed the lowest effect against *B. megaterium* and the same complex did not show any effect against *X. campestris* (Figure 6).

**Fungicidal Effect**

GMFX-en and its complexes were verified for fungicidal activity versus three serious phytopathogenic fungi. (*P. digitatum*, *C. acutatum* and *M. fructicola*) compared to the positive control Azoxystrobin (Table 6). Results were demonstrated as fungal growth inhibition percentage. All examined substances clarified antifungal effect in a dose dependent manner. The best antifungal effect against *P. digitatum* was observed in the case of the complexes A and B at 100 ppm. On the other hand, the lowest effect was observed in the case of GMFX-en at 50 ppm compared to Azoxystrobin. The highest activity against *C. acutatum* was obtained in the case of Azoxystrobin followed
by complex C and GMFX-en at 100 ppm. The lowest effect was obtained with GMFX-en 50 ppm. There is no observed activity in the case of the complexes A and B at both tested doses and the complex B 50 ppm (Table 6). Furthermore, the complexes A, B, C and D at 100 ppm displayed the highest activity against *M. fructicola*. The lowest substantial effect has been observed in the case of GMFX-en 50 ppm.

**Mode of Action**

The obtained microbicide effect of the studied compounds might be due to the chemical structures of the free ligand as well as the toxicity of the studied metal ions.\[^{48,49}\] The principle of cell permeability of the tested microorganisms clarified the enhanced antimicrobial activity of newly prepared metal chelates.\[^{50-52}\] The polarity of metal ions can be reduced in particular by partially sharing the positive charge with the parent ligand’s donor groups and as a result of orbital overlap with the ligand orbitals.\[^{53}\] On the other hand, the chelation process enhancing the delocalization of electrons above the chelate ring, which increase the lipophilicity of the central ions. Because of the increase in lipophilicity, the tested compounds were able to penetrate deeper into the microorganism’s cells.\[^{54,55}\]

**Phytotoxicity Effect**

GMFX-en and its metal complexes were found to be extremely phytotoxic to all of the plants examined, *S. lycopersicum* and *L. sativum*, at all tested concentrations. In contrast to the control treatment, the studied compounds have decreased the seeds germination and radical elongation for both tested plants.

**Conclusions**

This study investigated the new trace metal complexes resulted from the interaction of some biological ions such as Fe(III), Cu(II) Zn(II) and Zr(IV) with GMFX-en which formed from the condensation reaction of GMFX with ethylene diamine. Results showed that the studied compounds have notable antimicrobial effect, in a dose-dependent manner, against all tested phytopathogens. The chemical structure of the free ligand GMFX-en and its toxic nature are both important factors in its promising antimicrobial activity of some trace elements. In addition, the examined compounds showed a significant phytotoxic effect on the two tested plants. The potential antimicrobial and
Figure 3. Electronic absorption spectra for GMFX-en and its metal complexes.
Figure 4. $^1$H-NMR spectra for GMFX-en and its metal complexes.
phytotoxic effects of the new metal complexes indicated that they could be used effectively in controlling both serious phytopathogens and harmful weeds. In addition, the new innovative complexes could minimize the risk of appearance new resistant strains of fungal, bacterial, or harmful weeds to the traditional chemical compounds.

**Experimental Section**

**Chemicals**

Whole chemicals utilized were of analytical reagent grade and applied with no additional purifying. GMFX, ethylene diamine (en), glacial acetic acid, absolute ethanol, FeCl₃, CuSO₄·5H₂O, ZnCl₂·2H₂O, ZrOCl₂·8H₂O K₂Cr₂O₇, H₂SO₄ and AgNO₃ were purchased from Obour Pharmaceutical Industrial Company and Aldrich Chemical Co. All glass were steeped overnight in chromic blend (Potassium dichromate + concentrated sulfuric acid) swill completely with bidistilled water and desiccated in an oven at 100°C.

**Preparation of GMFX-en Schiff Base (C₂₀H₄₄F₂N₁₂O₆)**

An ethanolic solution was formed by combining 30 mL gemifloxacin (2 mmol, 0.77 g) with 40 mL ethylenediamine (1 mmol, 0.07 mL) and refluxing it for 4 h in the existence of 1 mL glacial acetic acid. The subsequent combination was settling on a water bath, and then, the temperature was lowered to 0°C. Yellowish-white precipitate was collected out, washed multiple spells by way of ethyl alcohol and desiccated below vacuum over CaCl₂.

**Preparation of Metal Complexes**

The dark red [Fe(GMFX-en)(H₂O)₂]Cl₂·7H₂O complex A was prepared by blending 0.5 mmol (0.401 g) of GMFX-en in 30 mL absolute ethyl alcohol with 0.5 mmol (0.081 g) of FeCl₃ in 20 mL ethyl alcohol. The blend was refluxed for 3 h and the subsequent was collected out and desiccated under below vacuum over CaCl₂. The brown, light brown, dark brown solid complexes [Cu(GMFX-en)(H₂O)₂]SO₄·5H₂O complex B, [Zn(GMFX-en)(H₂O)₂]Cl₂·3H₂O complex C and [ZrO (GMFX-en)(H₂O)₂]Cl₂·5H₂O complex D were set in identical technique qualified above by utilizing CuSO₄·5H₂O, ZnCl₂·H₂O and ZrOCl₂·8H₂O, respectively.

**Instruments**

The Carbon, Hydrogen and Nitrogen were done elemental experiments done via a Perkin Elmer 2400 CHN elemental analyzer. The proportion of metal ions was gravimetrically measured by transforming solid compounds into metal oxide and using the atomic
absorption technique utilizing a spectrometer model PYE-UNICAM SP 1900 fitted with the corresponding lamp. FT-IR spectra in KBr discs were enrolled in the area from 4000–400 cm⁻¹ with FT-IR 460 PLUS Spectrophotometer.¹H-NMR spectra have been registered on Varian Mercury VX-300 NMR Spectrometer utilizing (D₆)DMSO as solvent. TG-DTG processes were conducted out under N₂ atmospheric conditions within the temperature range from ambient temperature to 1000°C using TGA-50H Shimadzu, the sample weight was precisely weighted in an aluminum crucible. The absorption spectra were registered as solutions in (D₆)

Figure 5. TGA and DTG diagram for GMFX-en and its metal complexes.
| Compound    | Decomposition Range (K) | T, (K) | Method | Parameters | E* (KJ/mol) | A (s^-1) | ΔS* (KJ/molK) | ΔH* (KJ/mol) | ΔG* (KJ/mol) | R[a]   | SD[b]   |
|-------------|-------------------------|--------|--------|------------|-------------|-----------|---------------|--------------|--------------|--------|--------|
| GMFX-en     | 318–524                 | 448    | CR     |            | 76.43       | 2.42x10^9 | -0.0686        | 72.70         | 103.44       | 0.965  | 0.208  |
|             |                         |        | HM     |            | 87.35       | 1.33x10^8 | -0.0927        | 83.62         | 125.16       | 0.958  | 0.227  |
|             | 524–687                 | 623    | CR     |            | 142.64      | 1.08x10^13 | -0.0014        | 137.46        | 138.37       | 0.964  | 0.212  |
|             |                         |        | HM     |            | 170.89      | 1.88x10^12 | -0.0160        | 165.71        | 175.71       | 0.959  | 0.227  |
|             | 690–973                 | 810    | CR     |            | 134.71      | 1.73x10^6  | -0.1337        | 127.98        | 235.31       | 0.972  | 0.252  |
|             |                         |        | HM     |            | 141.87      | 1.88x10^6  | -0.1233        | 135.14        | 235.03       | 0.964  | 0.289  |
| Complex A   | 287–405                 | 342    | CR     |            | 62.07       | 2.17x10^9  | -0.0673        | 59.23         | 82.25        | 0.971  | 0.184  |
|             |                         |        | HM     |            | 61.44       | 2.55x10^7  | -0.1042        | 58.60         | 94.25        | 0.962  | 0.202  |
| Complex B   | 287–411                 | 351    | CR     |            | 76.53       | 4.58x10^8  | -0.0476        | 79.38         | 96.11        | 0.974  | 0.168  |
|             |                         |        | HM     |            | 82.30       | 2.36x10^10 | -0.0804        | 73.61         | 101.85       | 0.978  | 0.155  |
|             | 709–1273                | 762    | CR     |            | 229.49      | 4.27x10^13 | 0.0082         | 223.16        | 216.88       | 0.991  | 0.107  |
|             |                         |        | HM     |            | 253.54      | 1.23x10^18 | 0.0936         | 247.20        | 175.87       | 0.984  | 0.192  |
| Complex C   | 309–389                 | 366    | CR     |            | 70.39       | 2.91x10^6  | -0.12285       | 67.34         | 112.31       | 0.974  | 0.171  |
|             |                         |        | HM     |            | 78.70       | 2.00x10^9  | -0.06852       | 75.66         | 100.74       | 0.969  | 0.186  |
|             | 628–830                 | 744    | CR     |            | 112.49      | 1.16x10^8  | -0.0980        | 106.30        | 179.27       | 0.954  | 0.342  |
|             |                         |        | HM     |            | 181.18      | 3.43x10^10 | -0.0508        | 174.99        | 168.81       | 0.950  | 0.263  |
| Complex D   | 304–398                 | 351    | CR     |            | 70.39       | 3.16x10^6  | -0.1218        | 67.47         | 110.22       | 0.974  | 0.171  |
|             |                         |        | HM     |            | 72.38       | 6.97x10^8  | -0.0769        | 69.46         | 96.48        | 0.969  | 0.186  |
|             | 654–1273                | 740    | CR     |            | 243.17      | 5.22x10^16 | 0.06757        | 237.01        | 187.01       | 0.996  | 0.087  |
|             |                         |        | HM     |            | 296.47      | 9.16x10^18 | 0.11052        | 290.32        | 208.53       | 0.995  | 0.100  |

[a] correlation coefficients of the Arrhenius plots and [b] standard deviation.
DMSO. Room temperature magnetic susceptibility of the powdered samples was applied to the scientific magnetic balance of Sherwood utilizing Gouy at room temperature employing Hg[Co(CSN)₄] as a calibrant. Melting points have been transcribed on the Büchi apparatus. All tests were done with freshly formulated solutions at room temperature. The molar conductivity of the ligand solutions and their complexes at 1 × 10⁻³ M in dimethylformamide was registered at room temperature using CONSORT K410.

**Antimicrobial Investigation**

**Antibacterial Activity Assay**

*Tested bacteria.* The tested bacterial strains were *C. michiganensis*, *X. campestris* and *B. megaterium* and have been conserved as pure cultures in the collection of the School of Agricultural, Forestry, Food and Environmental Sciences (SAFE), University of Basilicata, Potenza, Italy.

*Bactericidal assay.* The method of disc diffusion was executed.[⁵⁶] Every strain’s bacterial suspension was made in sterile Millipore H₂O, then inserted into soft agar (0.7 %) and regulated by UV-spectrophotometer (DAS s.r.l., Rome, Italy) to 10⁸ colony form units per milliliter (CFU/mL). In a Petri dish (Φ 9 cm) filled with 10 mL of king B nutrient media (KB), 4 mL of each tested bacterial suspension (10 %) was poured. Blank Discs (6 mm) (OXOID, Milan, Italy) were mounted on KB Petri and 20 μL of each suspension was transferred at concentrations: 100 and 250 ppm. Tetracycline (160 μg/mL) was utilized as control. The antimicrobial effect was determined after 24 h at 37 °C, by measuring the diameter of inhibition zones (mm). The experiment was carried out in triplicate with ± SDs.

**Antifungal Activity Assay**

*Tested fungi.* The tested phytopathogenic fungi were *M. fricticola*, *P. digitatum* and *C. acutatum*. All tested fungi were cultured on potato dextrose agar (PDA) and were previously identified using morphological and molecular methods. The amplicons were sequenced right away and matched to those in GenBank using Simple Local Alignment Search Tool program from 1990 (BLAST, USA). They were stored at 4 °C as pure cultures in the mycotheca of SAFE, University of Basilicata, Potenza, Italy.

*Fungicidal assay.* The fungicidal effectiveness of the studied compounds was determined by using incorporation assay,[⁵⁷] into PDA medium at two concentrations (50 and 100 ppm). Every Petri dish was inocu-
lated with a fungal disk (0.5 cm), from 96 h fresh culture. All plates were incubated at 22 °C for 96 h and the antifungal effect was estimated by measuring the diameter of the fungal mycelium (mm) compared to control (only PDA + Fungi). The percentage of mycelium growth inhibition (MGI) was calculated according to Zygařdo et al.,[58] (Formula 1) compared to Azoxystrobin (0.8 μL/mL):

\[
\text{MGI} \% = \frac{(\text{GC} - \text{GT})}{\text{GC}} \times 100
\]  

Where MGI is the percentage of mycelium growth inhibition, GC is the average diameter of fungal mycelium in PDA (control), and GT is the average diameter of fungal mycelium on the treated PDA dish.

**Phytotoxicity Assay**

The potential phytotoxic efficacy of the tested compounds was investigated on tomato and garden cress plants.[59,60] Seeds were sterilized for 1 min in 3% H2O2 then were immerged in Millipore H2O. Seeds were shaken gently for 2 h in dH2O (control) or the above-mentioned treatments. The tested concentrations were 2000, 1000, 500, 250 and 100 ppm. All seeds were placed in Petri dishes with filter paper (9 cm, Whatman No. 1). Two mL of dH2O or various compounds was added to each plate then sealed with Parafilm. All Petri dishes were incubated for 4 days in growth chamber at 24°C and 60% humidity. The radical length was calculated in cm and the germinated seeds were enumerated. The experiment was repeated three times, and the germination index (GI) was measured as following:

\[
\text{G.I.} \% = \frac{\text{SG.t} \times \text{RE.t}}{\text{SG.c} \times \text{RE.c}} \times 100
\]  

All values are expressed as the mean ± SDs. GI: germination index; SG.t: average germination (Treated seeds); RE.t: average radical elongation (treated seeds); SG.c: average germination (control); RE.c: average radical elongation (control). The statistical analysis was performed using SPSS software with post hoc Tukey B (P < 0.05).

**Data Availability Statement**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author Contribution Statement

A.A. Mohamed: Methodology, Formal analysis, Data curation, Investigation. Writing – review & editing. H.S. Elshafie: Methodology, Formal analysis, Investigation, Data curation, Writing – review & editing. S.A. Sadeek: Data curation, Writing – review & editing, Supervision. I. Camele: Data curation, Writing – review & editing, Supervision.

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