

Fruit juices acts as biocatalyst in efficient synthesis of potentially bioactive imidazoles

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Abstract

An efficient and eco-friendly itinerary for the synthesis of imidazole derivatives (**3a-3h**) from reaction between substituted aldehydes (**1a-1h**), benzil (**2a**) and ammonium acetate (**2b**) in presence of *Citrus limon* L. juice, *Vitis vinifera* L. juice and *Cocos nucifera* L. juice has been carried out. The purity of compounds was confirmed by melting point and thin layer chromatography. All synthesized compounds (**3a-3h**) were characterized by ¹HNMR, FTIR and CHN spectral techniques and tested for *in vitro* herbicidal activity against *Raphanus sativus* L. (Radish seeds). The compounds (**3a-3h**) were also evaluated for their antifungal activity against *Rhizoctonia solani* and *Colletotrichum gloeosporioides* by poisoned food techniques method. Antibacterial activity was also determined against *Erwinia cartovora* and *Xanthomonas citri* by inhibition zone method. From activity data, it was observed that compounds **3f** and **3c** were found most active against *Raphanus sativus* L. (root) and *Raphanus sativus* L. (shoot) respectively. Compound **3d** was found most active against *Rhizoctonia solani* and *Colletotrichum gloeosporioides* fungus at highest concentration. Compound **3b** has shown maximum inhibition zone i.e. 2.10-7.10 mm against *Erwinia cartovora* at 2000 µg/mL concentration. Maximum *Xanthomonas citrii* growth was inhibited by compounds **3c** showing inhibition zone 1.00-5.00 mm at highest concentration.

Introduction

Heterocyclic compounds specially containing nitrogen atoms have been the major molecules in organic chemistry because of their extraordinary activities, particularly their anticancer activities (**1-3**). One-pot synthesis of **medicinally** important heterocycles under environmentally-friendly conditions has remained one of the crucial topics in organic as well as medicinal chemistry (**4**). Imidazole is a **key** heterocyclic moiety widespread in many bioactive compounds as well as in synthetic drugs (**5**). **Imidazoles fused with other heterocycles such as benzothiazole and benzimidazole are found in core of various drugs as well as natural products.** Imidazoles have a broad range of biological activities such as anticancer, antifungal, antiviral, antibacterial, antitubercular, anti-parasitic, antihistaminic, anti-inflammatory, anti-neuropathic, anti-obesity and antihypertensive (**6-15**). In 1858, imidazole was first synthesized by Heinrich Debus from glyoxal and formaldehyde, but the % yield in this method was quite low (**16**). Later in 1977, Van Leusen synthesized imidazole using a three component reaction with aldimines and tosylmethyl isocyanide, but the yield was low (**17**). In 1996, Zhang *et al.* reported the synthesis of imidazoles *via* Ugi four component reactions of arylglyoxals, primary amines, carboxylic acids and isocyanide (**18**). In 2003, a novel one-pot synthesis of tetra substituted imidazoles under solvent-free conditions and microwave irradiation was reported by Balalaie *et al.* (**19**). Wolkenberg *et al.* also reported facile and efficient method for synthesis of imidazoles from 1,2-diketones and aldehydes in the presence of NH₄OAc (**20**). However, most of these methods have some pitfall such as use of hazardous solvents, strong acidic or basic conditions, expensive procedure, laborious work-up process and longer reaction time. Thus we turned our efforts to develop a green method for the synthesis of substituted imidazoles. Recently, fruit juices used as biocatalyst in synthesis of heterocyclic compounds because of their eco-friendly character, non-hazardous, readily available and cost effective. Therefore, in this paper we reported one-pot three components reaction between substituted aldehydes, benzil and ammonium acetate in presence of *Citrus limon* L. juice, *Vitis vinifera* L. juice and *Cocos nucifera* L. juice for clean and

facile synthesis of substituted imidazoles. Moderate to excellent yields, inexpensive catalyst, easy work-up and mild reaction conditions are some beauties of present methodology.

Results And Discussion

In present study the synthesis of imidazole derivatives (**3a-3h**) were reported by one-pot three components reaction between substituted aldehydes *viz.* 2-Hydroxybenzaldehyde (**1a**), 4-Methoxybenzaldehyde (**1b**), 4-Chlorobenzaldehyde (**1c**), 4-Bromobenzaldehyde (**1d**), 3-Hydroxybenzaldehyde (**1e**), 4-Methylbenzaldehyde (**1f**), 3-Nitrobenzaldehyde (**1g**), 4-Hydroxy-3-methoxybenzaldehyde (**1h**), benzil (**2a**) and ammonium acetate (**2b**) in the presence of *Citrus limon* L. juice, *Vitis vinifera* L. juice, *Cocos nucifera* L. juice respectively (**Scheme 1**). The progress of reaction was monitored by thin layer chromatography using Hexane: Ethyl acetate (80:20, v/v) as an eluent.

To standardize the reaction condition, we started our study with the 3-components reaction of 3,4-Dimethoxybenzaldehyde (20 mmol), benzil (20 mmol) and ammonium acetate (40 mmol) in presence of *Citrus limon* L. juice (**Table 1, Entry 1-4**). It was observed that when the amount *Citrus limon* L. juice was 4.0 mL in reaction mixture then yield of product was 87% and time of completion of reaction was more i.e. 2.5 h (**Table 1, Entry 1**). But when the amount of *Citrus limon* L. juice was increased from 4.0 mL to 8.0 mL, then yield of product was also good i.e. 86% and time of completion of reaction was reduced (**Table 1, Entry 3**). Through the result of initial possibility and optimization study under consideration, we move our attention toward explored the same model reaction in presence of *Vitis vinifera* L. juice, *Cocos nucifera* L. juice respectively. It was found out that excellent yield of product i.e. 87% and 89% were obtained respectively when the concentration of *Vitis vinifera* L. juice, *Cocos nucifera* L. juice was 8.0 mL in reaction mixture and time of completion of reaction was also less (**Table 1, Entry 3**). The physical data of this study are presented in **Table 2**. After completion of the reaction, the solid products was collected by simple filtration and then recrystallized in methanol to afford pure imidazole derivatives (**3a-3h**). All the synthesized imidazole derivatives were shown in **Figure 1**. The structure of synthesized compounds was confirmed by ¹H NMR, FTIR, CHN analysis as well as comparison of their melting points with those of reported compounds. The compound *viz.* 2-(4-methoxyphenyl)-4,5-diphenyl-1*H*imidazole (**3b**) displayed IR absorptions at 3317.3, 3063.3., 1592.9, 1210.5 and 1447.6 cm⁻¹ indicating the presence of NH, C=CH, C=C aromatic, OCH₃ and C=N respectively and melting point 229-230°C. From spectral study it was found that compound *viz.* 2-(4-bromophenyl)-4,5-diphenyl-1*H*imidazole (**3d**) showed ¹H NMR spectrum in DMSO-*d*₆, displayed multiplet at 7.54-7.93 δ integrating for proton of aryl group, singlet at 13.09 δ integrating for one proton of NH group and melting point 194-196°C. The compound *viz.* 3-(4,5-diphenyl-1*H*imidazol-2-yl) (**3e**) phenol displayed IR absorptions at 3188.9, 3316.0, 3063.1, 1592.8 and 1449.0 cm⁻¹ indicating the presence of NH, OH, C=CH, C=C aromatic and C=N respectively and melting point 254-255°C. The compound *viz.* 4,5-diphenyl-2-(*p*-tolyl)-1*H*imidazole (**3f**) showed ¹H NMR spectrum in CDCl₃, displayed multiplet at 7.12-7.98 δ integrating for proton of aryl group, singlet at 9.89 δ integrating for one proton of NH group and melting point 233-235°C. The compound (**3f**) also displayed IR absorptions at 3316.3, 3063.2, 1593.0 and 1448.6 cm⁻¹ indicating the presence of NH, C=CH, C=C aromatic and C=N respectively. The compound *viz.* 2-(3-nitrophenyl)-4,5-diphenyl-1*H*imidazole (**3g**) showed ¹H NMR spectrum in CDCl₃, displayed a singlet at 10.05 δ integrating for one proton of NH group, multiplet at 7.43-8.39

δ integrating for proton of aryl group, singlet at 8.62 δ integrating for one proton of =CH group, doublet at 8.14 δ integrating for proton of aryl group and melting point 197-198°C. The compound *viz.* 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-2-methoxyphenol (**3h**) displayed IR absorptions at 3183.8, 3315.0, 3063.5, 1592.7, 1210.2 and 1430.4 cm^{-1} indicating the presence of NH, OH, C=CH, C=C aromatic, OCH₃ and C=N respectively and melting point 200-202°C. To show the beauty of current protocol, the previous protocols and their yields for the synthesis are summarized in **Table 3**. We observed that *Citrus limon* L. juice, *Vitis vinifera* L. juice, *Cocos nucifera* L. juice catalyst gives the best catalytic activity in terms of product yield and reaction time as compared to other catalysts in literature. Therefore the present procedure for synthesis of imidazole derivatives is considered as sustainable and eco-friendly protocol. The possible mechanism for the formation of substituted imidazoles is shown in **Scheme 2**. It involves condensation of dicarbonyl compound such as benzil with an aldehyde in presence of ammonium acetate which is good source of ammonia. The aryl aldehyde and benzil are first activated by acid catalyst by nucleophilic attack on carbonyl groups. Other side the catalyst converts ammonium acetate to ammonia, which forms an intermediate with activated aldehyde. This intermediate reacted with activated benzil and then cyclization takes place to form substituted imidazoles.

Table 1: Model reaction of 3,4-Dimethoxybenzaldehyde (20 mmol), benzil (20 mmol) and ammonium acetate (20 mmol) using *Citrus limon* L. juice, *Vitis vinifera* L. juice and *Cocos nucifera* L. juice as catalyst

| Entry | Catalyst Concentration (mL) | Method A | | Method B | | Method C | |
|-------|-----------------------------|------------|-----------|----------|-----------|----------|-----------|
| | | Time (h) | Yield (%) | Time (h) | Yield (%) | Time (h) | Yield (%) |
| 1 | 4.0 | 2.5 | 87 | 2.5 | 62 | 9 | 43 |
| 2 | 6.0 | 1.8 | 79 | 2.0 | 79 | 8 | 52 |
| 3 | 8.0 | 1.5 | 86 | 1 | 87 | 5 | 89 |
| 4 | 10.0 | 1.0 | 60 | 40 min | 50 | 6 | 80 |

Table 2: Physical data of substituted imidazole derivatives (**3a-3h**)

| S. No | Product | Ar | Method A | | Method B | | Method C | | m.p. (°C) |
|-------|-----------|----------------------------|----------|-----------|----------|-----------|----------|-----------|---------------------------------------|
| | | | Time (h) | Yield (%) | Time (h) | Yield (%) | Time (h) | Yield (%) | |
| 1 | 3a | 2-OHPh | 4 | 92 | 3 | 86 | 1 | 88 | 119-120 |
| 2 | 3b | 4-OCH ₃ Ph | 2 | 81 | 5 | 83 | 1 | 85 | 229-230 |
| 3 | 3c | 4-ClPh | 5 | 79 | 4 | 92 | 7 | 89 | 229-230; (Lit. 228-230) (21) |
| 4 | 3d | 4-BrPh | 3.5 | 80 | 1.5 | 79 | 2 | 85 | 194-196 |
| 5 | 3e | 3-OHPh | 9 | 80 | 8 | 81 | 5 | 80 | 254-255 |
| 6 | 3f | 4-CH ₃ Ph | 3 | 83 | 4.5 | 80 | 4 | 80 | 233-235 |
| 7 | 3g | 3-NO ₂ Ph | 7 | 88 | 2 | 78 | 3 | 78 | 197-198 (Lit. 198-200) (22) |
| 8 | 3h | 4-OH-3-OCH ₃ Ph | 7 | 88 | 3.5 | 81 | 5 | 89 | 200-202 |

Table 3: Comparison of the results of the present methods for the synthesis of imidazoles with other catalysts in the literature (**3a-3h**)

| S.No. | Catalyst | Solvent | Temperature (°C) | Time (h) | Yield (%) | References |
|-------|--|-------------------|------------------|----------|-----------|--------------|
| 1 | InCl ₃ .3H ₂ O | Methanol | 25-30 | 8 | 76 | (23) |
| 2 | NiCl ₂ .6H ₂ O/L-proline | Ethanol | 75-80 | 1.5 | 89 | (24) |
| 3 | L-proline | Methanol | 60-64 | 9 | 87 | (25) |
| 4 | KH ₂ PO ₄ | Methanol | 25-30 | 1 | 89 | (26) |
| 5 | Zr(acac) ₄ | Ethanol | 75-80 | 2 | 95 | (27) |
| 6 | DABCO | <i>t</i> -Butanol | 60-65 | 12 | 92 | (28) |
| 7 | CAN | Methanol | - | 10 | 75 | (29) |
| 8 | NiCoFe ₂ O ₄ | Ethanol | 75-80 | 20 | 95 | (30) |
| 9 | <i>Citrus limon</i> L. juice | Solvent-free | RT | 1.5 | 86 | Present work |
| 10 | <i>Vitis vinifera</i> L. juice | Solvent-free | RT | 1.0 | 87 | Present work |
| 11 | <i>Cocos nucifera</i> L. juice | Solvent-free | RT | 5.0 | 89 | Present work |

Table 4: Herbicidal activity of substituted imidazoles (3a-3h)

| Compounds | Growth Inhibition (%) | | | | | | | |
|-----------|----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|-----------------------------|-----------------------------|
| | Root | | | | Shoot | | | |
| | 50 ($\mu\text{g/mL}$) | 100 ($\mu\text{g/mL}$) | 150 ($\mu\text{g/mL}$) | 200 ($\mu\text{g/mL}$) | 50 ($\mu\text{g/mL}$) | 100 ($\mu\text{g/mL}$) | 150 ($\mu\text{g/mL}$) | 200 ($\mu\text{g/mL}$) |
| 3a | 25.00 \pm 0.81 | 50.00 \pm 0.86 | 66.60 \pm 1.41 | 83.33 \pm 0.99 | 47.50 \pm 0.98 | 56.25 \pm 1.07 | 71.25 \pm 1.01 | 81.25 \pm 1.05 |
| 3b | 64.60 \pm 1.06 | 75.38 \pm 1.95 | 85.36 \pm 1.51 | 92.66 \pm 1.16 | 36.95 \pm 1.03 | 51.36 \pm 1.05 | 64.80 \pm 1.12 | 84.02 \pm 0.30 |
| 3c | 16.60 \pm 0.63 | 33.33 \pm 1.00 | 58.33 \pm 0.93 | 83.33 \pm 1.52 | 37.50 \pm 1.53 | 52.50 \pm 0.93 | 68.75 \pm 1.71 | 87.50 \pm 0.91 |
| 3d | 50.00 \pm 0.92 | 66.60 \pm 0.38 | 80.00 \pm 0.83 | 90.00 \pm 0.83 | 38.46 \pm 0.51 | 53.80 \pm 1.01 | 66.15 \pm 0.64 | 83.07 \pm 0.85 |
| 3e | 28.13 \pm 1.02 | 49.17 \pm 0.34 | 65.63 \pm 0.94 | 84.12 \pm 0.95 | 40.25 \pm 0.91 | 51.36 \pm 1.57 | 67.63 \pm 1.10 | 80.36 \pm 0.99 |
| 3f | 66.60 \pm 0.86 | 76.60 \pm 0.99 | 86.60 \pm 0.93 | 93.33 \pm 0.98 | 47.69 \pm 1.01 | 61.50 \pm 1.00 | 72.30 \pm 1.00 | 84.61 \pm 1.12 |
| 3g | 33.33 \pm 0.93 | 50.00 \pm 1.44 | 75.00 \pm 0.78 | 91.66 \pm 1.57 | 56.25 \pm 1.52 | 68.75 \pm 1.66 | 81.20 \pm 1.00 | 86.25 \pm 1.01 |
| 3h | 48.66 \pm 1.01 | 61.36 \pm 1.00 | 78.12 \pm 1.00 | 89.66 \pm 1.16 | 46.32 \pm 0.96 | 58.96 \pm 1.89 | 71.30 \pm 1.01 | 83.66 \pm 1.13 |

All values are mean \pm S.D.

Table 5: Antifungal activity of substituted imidazoles (**3a-3h**)

| Compounds | Growth inhibition (%) | | | | | | | |
|-----------|---|-----------------|-----------------|-----------------|---|-----------------|-----------------|-----------------|
| | Fungi | | | | | | | |
| | <i>Rhizoctonia solani</i> (conc.) µg/mL | | | | <i>Colletotrichum gloeosporioides</i> (conc.) µg/mL | | | |
| | 250 | 500 | 1000 | 2000 | 250 | 500 | 1000 | 2000 |
| 3a | 22.85 ± 1.44 | 57.14 ± 1.55 | 65.71 ± 0.73 | 77.14 ± 0.97 | a | a | a | a |
| 3b | 20.78 ± 0.78 | 51.67 ± 1.03 | 63.29 ± 0.20 | 76.91 ± 1.39 | 22.80 ± 1.23 | 42.49 ± 1.12 | 64.87 ± 1.43 | 81.13 ± 0.99 |
| 3c | 21.21 ± 0.62 | 39.39 ± 0.77 | 63.63 ± 1.35 | 78.78 ± 0.77 | a | a | a | a |
| 3d | 23.69 ± 1.16 | 42.22 ± 1.21 | 68.17 ± 0.14 | 82.69 ± 0.65 | 21.36 ± 1.00 | 46.66 ± 0.41 | 67.59 ± 0.47 | 83.54 ± 1.09 |
| 3e | a | a | a | a | 10.69 ± 0.97 | 23.54 ± 0.48 | 37.28 ± 2.14 | 66.39 ± 0.51 |
| 3f | a | a | 23.69 ± 0.67 | 38.25 ± 0.51 | a | a | a | a |
| 3g | a | a | a | a | a | a | 11.69 ± 1.21 | 30.82 ± 0.58 |
| 3h | 25.54 ± 1.43 | 45.38 ± 2.77 | 67.58 ± 0.70 | 80.15 ± 2.23 | 18.90 ± 0.50 | 38.69 ± 0.51 | 59.34 ± 0.99 | 77.82 ± 1.46 |

All values are mean ± S.D.

a: No Growth inhibition

Table 6: Antibacterial activity of substituted imidazoles (**3a-3h**)

| Compounds | Inhibition Zone (mm) | | | | | | | |
|-----------|--|----------------|----------------|----------------|--|----------------|----------------|----------------|
| | Bacteria | | | | | | | |
| | <i>Erwinia cartovora</i> (conc.) µg/mL | | | | <i>Xanthomonas citri</i> (conc.) µg/mL | | | |
| | 250 | 500 | 1000 | 2000 | 250 | 500 | 1000 | 2000 |
| 3a | 0.90 ± 0.07 | 1.10 ± 0.10 | 1.80 ± 0.07 | 2.20 ± 0.32 | a | a | a | a |
| 3b | 2.20 ± 0.25 | 3.00 ± 0.40 | 5.00 ± 0.63 | 7.10 ± 0.50 | 2.50 ± 0.20 | 3.00 ± 0.30 | 3.70 ± 0.30 | 4.40 ± 0.30 |
| 3c | 2.00 ± 0.27 | 3.00 ± 0.25 | 3.50 ± 0.26 | 4.00 ± 0.45 | 1.00 ± 0.18 | 3.00 ± 0.47 | 4.50 ± 0.36 | 5.00 ± 0.81 |
| 3d | 0.20 ± 0.04 | 0.40 ± 0.05 | 0.70 ± 0.07 | 1.00 ± 0.15 | 0.10 ± 0.01 | 0.40 ± 0.02 | 0.70 ± 0.10 | 1.10 ± 0.20 |
| 3e | 1.00 ± 0.15 | 1.20 ± 0.15 | 2.00 ± 0.25 | 3.00 ± 0.37 | a | a | a | 0.40 ± 0.02 |
| 3f | a | a | a | 0.60 ± 0.07 | a | a | 1.70 ± 0.07 | 2.30 ± 0.15 |
| 3g | a | 1.00 ± 0.15 | 2.00 ± 0.42 | 5.00 ± 0.55 | 1.10 ± 0.22 | 1.50 ± 0.09 | 1.80 ± 0.07 | 2.30 ± 0.20 |
| 3h | 1.50 ± 0.26 | 2.00 ± 0.20 | 3.00 ± 0.45 | 4.00 ± 0.47 | 0.60 ± 0.07 | 0.90 ± 0.12 | 1.60 ± 0.05 | 2.00 ± 0.15 |

All values are mean ± S.D.

a: No

inhibition zone

Herbicidal Activity

All compounds (**3a-3h**) were tested for herbicidal activity against *Raphanus sativus* L. at 200, 150, 100 and 50 µg/mL concentrations as shown in Table 4. Results were shown in the form of primary screening. All compounds were diluted to 1000 µg/mL concentration as a stock solution. Herbicidal activities of compounds were evaluated against *Raphanus sativus* L. by inhibitory effect of compounds on the growth of weed roots and shoots. The percentage of inhibition growth was calculated from mean differences between treated and control. From the herbicidal activity results, we observed that compound **3f** was exhibited maximum percentage growth inhibition i.e. 93.33 against *Raphanus sativus* L. (root) whereas compound **3c** was exhibited maximum percentage growth inhibition i.e. 87.50 against *Raphanus sativus* L. (shoot) respectively at 200 µg/mL concentrations. The compounds **3f** and **3c** showed broad-spectrum herbicidal activity because of presence of methyl and chloro substitution at phenyl ring. The box plot and graphical representation of herbicidal activity of all compounds against *Raphanus sativus* L. were shown in Fig 2, Fig 3, Fig 4 and Fig 5.

Antimicrobial Evaluation

Antifungal activity

All synthesized compounds (**3a-3h**) were screened for their fungicidal activity against 2 fungal strains viz. *Rhizoctonia solani* and *Colletotrichum gloeosporioides* by poisoned food technique method. DMSO was used as negative control against fungal strains. The result of antibacterial activity of tested compounds is shown in Table 5. Most of synthesized compounds possess a moderate to good activity against *R. solani* and *C. gloeosporioides* respectively. Compounds **3e** and **3g** showed no antifungal activity at all concentrations against *R. solani*. Compound **3f** has shown no growth inhibition upto 500 µg/mL concentrations against *R. solani*. Compound **3f** exhibited 23.69 and 38.25 % growth inhibition against *R. solani* fungus at 1000 µg/mL and 2000 µg/mL concentrations respectively. Compounds **3a**, **3c** and **3f** showed no antifungal activity at all concentrations against *C. gloeosporioides*. Compound **3g** has shown no growth inhibition at lower concentrations. Compound **3g** exhibited 11.69 and 30.82 % growth inhibition against *C. gloeosporioides* fungus at 1000 µg/mL and 2000 µg/mL concentrations respectively. From antifungal activity results, we concluded that compound **3d** was shown to most promising against *R. solani* and *C. gloeosporioides*. This result may be due to substitution of bromo group on phenyl ring. The box plot and graphical representation of antifungal activity of all compounds against *Rhizoctonia solani* and *Colletotrichum gloeosporioides* were shown in Fig 6, Fig 7, Fig 8 and Fig 9.

Antibacterial activity

The propitious antifungal activity of synthesized compounds (**3a-3h**) has inspired authors to test further for antibacterial activity. All synthesized compounds (**3a-3h**) were tested for their *in vitro* antibacterial activity against two bacterial strains *Erwinia cartovora* and *Xanthomonas citri* by inhibition zone method using DMSO as negative control. The results of antibacterial activity of synthesized compounds were shown in Table 6. Compound **3f** has shown no inhibition zone at lower concentrations. Compound **3f** exhibited 0.60 mm inhibition zone against *Erwinia cartovora* at 2000 µg/mL concentration. Compound **3g** has shown no inhibition zone at 250 µg/mL concentration. Compound **3g** exhibited 1.00 mm, 2.00 mm and 5.00 mm inhibition zone against *Erwinia cartovora* at 500, 1000 and 2000 µg/mL concentrations respectively. Compound **3a** has shown no inhibition zone at all the concentrations against *Xanthomonas citri*. Compound **3e** has shown no inhibition zone at lower concentrations. Compound **3e** exhibited 0.40 mm inhibition zone against *Xanthomonas citri* at 2000 µg/mL concentration. Compound **3f** has shown no inhibition zone at lower concentrations. Compound **3f** exhibited 1.70 mm, 2.30 mm inhibition zone against *Xanthomonas citri* at 1000 and 2000 µg/mL concentrations respectively. Maximum *Erwinia cartovora* growth was inhibited by compounds **3b** showing inhibition zone 2.20-7.10 mm. Maximum *Xanthomonas citri* growth was inhibited by compounds **3c** showing inhibition zone 1.00-5.00 mm. This inhibition may be due to presence of methoxy and chloro groups on phenyl groups. The box plot and graphical representation of antibacterial activity of all compounds against *Erwinia cartovora* and *Xanthomonas citri* were shown in Fig 10, Fig 11, Fig 12 and Fig 13.

Experimental

All reagents used were of analytical grade. Melting points were determined on Ganson electric melting point apparatus and are uncorrected. The progress of the reaction was monitored *via* thin-layer chromatography. The ¹H NMR spectra were recorded on "Brucker Ac 400 F" (400 MHz) nuclear magnetic resonance spectrometer. All chemical shifts are given as δ values (ppm) with reference to tetramethylsilane as an internal standard. The peak patterns are indicated as follows: s = singlet, d = doublet, t = triplet, m = multiplet and brs = broad singlet. Infrared spectra (4000-350 cm⁻¹) of the synthesized compounds were recorded in KBr pellets on Perkin Elmer FT-IR-R2X spectrophotometer and frequency was recorded in cm⁻¹. Elemental analysis was performed using ThermoFinnigan CHN elemental analyser. Coupling constant (*J*) values are expressed in Hz.

Bio evaluation

Herbicidal Activity

Solutions of 50 $\mu\text{g}/\text{mL}$, 100 $\mu\text{g}/\text{mL}$, 150 $\mu\text{g}/\text{mL}$ and 200 $\mu\text{g}/\text{mL}$ of the test compounds in DMSO were prepared. Agar powder (5g) was put into boiling distilled water (1L) until it dissolved, and then cooled down to 40-50°C. The solution (2mL) containing test compounds and melting agar (18mL) was mixed and this mixture was added to a Petridish with 4.5 cm diameter. The agar plate without test compound was used as an untreated control. Then 15 seeds of *Raphanus Sativus* L. (Radish) were put on the surface of the agar plate. The Petridishes were covered with glass lids, and the cultivation conditions were kept at 25 \pm 1 °C and 12 hours in light and 12 hours in dark alternating for seven days. Seven days later, the root lengths and shoot lengths of *Raphanus sativus* L. were measured. The growth inhibitory rate related to untreated control was determined by given formula (31).

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Antifungal activity

All synthesized compounds (3a-3h) were tested for their antifungal activity against *Rhizoctonia solani* and *Colletotrichum gloeosporioides* respectively. Fungal species were grown in laboratory on Potato dextrose agar (PDA) media. The antifungal activity was determined by poisoned food technique method (32). The required amount of synthesized compounds dissolved in 1 mL of DMSO was incorporated aseptically into 99 mL aliquots of sterilized potato dextrose agar cooled at 45° C after brief shaking. Each lot of medium was poured into Petri dishes and allowed to solidify. DMSO was used as negative control. Each dish was inoculated centrally with a 5 mm mycelial disc cut from the periphery of 2-3 days old fungal colonies. Inoculated Petri plates were incubated in the dark 25 \pm 2°C for 48-72 h and colony diameters were measured periodically till the control dishes were nearly completely covered with fungus growth. All observations were made in triplicate. The degree of inhibition of growth was calculated from the mean differences between treatments and the control as percentage of latter by using the formula.

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Treated}}{\text{Control}} \times 100$$

Control = mycelial growth in control dish

Treated = mycelial growth in treated dish

Antibacterial activity

Bacterial species *Erwinia cartovora* and *Xanthomonas citri* were grown on Luria-Bertani medium in laboratory. Antibacterial activity was evaluating using inhibition zone method **(33)**. 250, 500, 1000 and 2000 µg/ mL concentrations of synthesized compounds were prepared from the stock solution by taking appropriate amount and diluting with DMSO. DMSO was used as negative control. The circular paper discs of 10 mm diameter were prepared from Whatman's Filter paper No. 1. The disc were kept in Petri plate and autoclaved at 15 lbs pressure 20 minutes. Two paper discs were used for each concentration of the synthesized compounds. The excess of solution absorbed by paper discs was removed by holding them vertically by sterile forecep. Such soaked discs were transferred aseptically to Petri plates containing media and bacterial suspension spread over the surface. The Petri plates were kept in an incubator at 25±2°C overnight and then examined for inhibition zone at these different concentrations of compounds. The experiment was performed in triplicate and activity was determined on the basis of inhibition zone (in mm).

Statistical Analysis

The experiments were performed in triplicates for each treatment and the mean value were recorded and expressed as mean ± S.D. The descriptive statistics in form of box-and-whisker diagram were also presented in this paper. The spacing between the different parts of the box indicates the degree of dispersion and skewness in the data. **This is convenient way of visually displaying the data distribution through their quartiles.**

Composition and preparation of green catalyst

General procedure for extraction of *Citrus limon* L. juice: The main component of lemon juice are moisture (85%), carbohydrate (11.2%), citric acid (5-7%), protein (1%), ascorbic acid or vitamin-C (0.5%), fat (0.9%), minerals (0.3%), fibers (1.6%) and some other organic acids. Due to presence of citric and ascorbic acids (Vitamin C) in lemon juice, it acts as acid catalyst in organic synthesis. Fresh lemon was cut using knife and then pieces were pressed in a fruit juicer to get the juice extract. Then the juice was filtered through cotton and then through whatman filter paper no 1 to remove solid material, to get clear juice which was used as a catalyst **(34)**.

Method for preparation of *Vitis vinifera* L. juice: The main constituents of grape juice are water (70-80%), sugar content (150 to 250 g/L) and organic acids viz. citric, malic and tartaric acids found in grapes. Fresh grapes were purchased from the local market. Then washed thoroughly under running tap water followed by rinsing thrice with distilled water. Grapes were squeezed and juice were strained initially through a muslin cloth then passed through whatman filter paper No. 1 **(35)**.

Method of preparation of *Cocos nucifera* L. juice:___*Cocos nucifera* is a member of family Arecaceae (palm family). Its juice is also called coconut water and used for the treatment of high blood pressure, hypertension and diarrhea related dehydration. The main ingredients per 100 g of coconut juice of *Cocos nucifera* are water

(94.99 g), carbohydrates (3.71 g), protein (0.72 g), fat (0.2 g), ascorbic acid (2.4 mg) and pantothenic acid (0.043 mg). Coconut juice also contains many natural occurring bioactive enzymes such as acid phosphatase, catalase, dehydrogenase, diastase, peroxidase, RNA-polymerase etc. Due to presence of ascorbic acid and pantothenic acid, coconut juice is weakly acidic. The coconut juice was obtained by perforating the fruit with a knife. The coconut juice was filtered using filter paper whatman no 1 for the elimination of residues to get clear juice which used as a catalyst **(36)**.

General method for the preparation of substituted imidazole derivatives (3a-3h)

By *Citrus limon* L. juice (Method A)

Substituted aldehydes (20 mmol) **(1a-1h)** was taken in clean round-bottom flask. Then 20 mmol of benzil **(2a)**, 40 mmol of ammonium acetate **(2b)** and 8 mL of *Citrus limon* L. juice were added and mixture was stirred at room temperature. The completion of reaction was monitored by thin layer chromatography. The solid was separated out, then filtered and washed with ice cold water to get the products **(3a-3h)**, which was further recrystallized with methanol. All compounds **(3a-3h)** were characterized by ¹H NMR, FTIR and CHN spectroscopy.

By *Vitis vinifera* L. juice (Method B)

The mixture of substituted aldehydes (20 mmol) **(1a-1h)**, 20 mmol benzil **(2a)**, 40 mmol ammonium acetate **(2b)** and 8 mL of *Vitis vinifera* L. juice was stirred at room temperature. The completion of reaction was monitored by thin layer chromatography. The solid was separated out, then filtered and washed with ice cold water to get the products **(3a-3h)**, which was further recrystallized with methanol. All compounds **(3a-3h)** were characterized by ¹H NMR, FTIR and CHN spectroscopy.

By *Cocos nucifera* L. juice (Method C)

A mixture of substituted aldehydes (20 mmol) **(1a-1h)**, 20 mmol benzil **(2a)**, 40 mmol ammonium acetate **(2b)** and 8 mL of *Cocos nucifera* L. juice was stirred at room temperature. Completion of reaction was confirmed by thin layer chromatography (TLC). The solid was separated out, then filtered and washed with ice cold water to get the products **(3a-3h)**, which was further recrystallized with methanol. All compounds **(3a-3h)** were characterized by ¹H NMR, FTIR and CHN spectroscopy.

All the imidazole derivatives **(3a-3h)** were prepared according to Method A, B and C.

Characterization data of selected compounds

2-(4-methoxyphenyl)-4, 5-diphenyl-1H-imidazole (3b): IR (ν_{\max} cm⁻¹) (neat): 3317.3 (NH); 3063.3 (C=CH); 1592.9 (C=C, aromatic); 1210.5 (OCH₃); 1447.6 (C=N)

2-(4-chlorophenyl)-4, 5-diphenyl-1H-imidazole (3c): Elemental Analysis found: C, 76.24; H, 4.57; Cl, 10.72; N, 8.47; Required: C, 74.32; H, 4.50

2-(4-bromophenyl)-4, 5-diphenyl-1Himidazole (3d): ^1H NMR (400 MHz, DMSO- d_6): δ 7.54-7.58 (m, J = 8 Hz, 2H, Ar-H); 7.59-7.63 (m, J = 8 Hz, 1H, Ar-H); 7.69-7.80 (m, J = 8 Hz, 2H, Ar-H); 7.87-7.93 (m, J = 8 Hz, 2H, Ar-H); 13.09 (s, 1H, NH)

3-(4, 5-diphenyl-1Himidazol-2-yl) phenol (3e): IR (ν_{max} cm^{-1}) (neat): 3188.9 (NH); 3316.0 (OH); 3063.1 (C=CH); 1592.8 (C=C, aromatic); 1449.0 (C=N)

4, 5-diphenyl-2-(p-tolyl)-1Himidazole (3f): ^1H NMR (400 MHz, CDCl_3): δ 7.12-7.98 (m, J = 8 Hz, Ar-H); 9.89 (s, 1H, NH); IR (ν_{max} cm^{-1}) (neat): 3316.3 (NH); 3063.2 (C=CH); 1593.0 (C=C, aromatic); 1448.6 (C=N)

2-(3-nitrophenyl)-4, 5-diphenyl-1Himidazole (3g): ^1H NMR (400 MHz, CDCl_3): δ 7.43-7.93 (m, J = 8 Hz, Ar-H); 8.14 (d, J = 8 Hz, 1H, Ar-H); 8.39 (d, J = 8 Hz, 1H, Ar-H); 8.62 (s, 1H, =CH), 10.05 (s, 1H, NH)

4-(4, 5-diphenyl-1Himidazol-2-yl)-2-methoxyphenol (3h): IR (ν_{max} cm^{-1}) (neat): 3183.8 (NH); 3315.0 (OH); 3063.5 (C=CH); 1592.7 (C=C, aromatic); 1210.2 (OCH₃); 1430.4 (C=N)

Conclusions

An efficient, simple and solvent-free methodology has been developed for synthesis of biologically active substituted imidazole derivatives (**3a-3h**). The present method offers much superiority such as simple and efficient catalytic system, simple work-up, no use of toxic solvents, cheap and products were obtained in good to excellent yields. All compounds (**3a-3h**) were also evaluated for their bio efficacy in terms of herbicidal activity against *Raphanus sativus* L. (Radish) seeds, antifungal activity against *Rhizoctonia solani* & *Colletotrichum gloeosporioides* and antibacterial activity against *Erwinia cartovora* and *Xanthomonas citri*. Based on activity data, it can be concluded that some of synthesized compounds possessed good activity due to substitution of methyl, chloro, bromo and methoxy groups on phenyl ring.

Declarations

Conflicts of interest

Authors declare that there is no conflict of interest regarding the publication of this paper.

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Figures

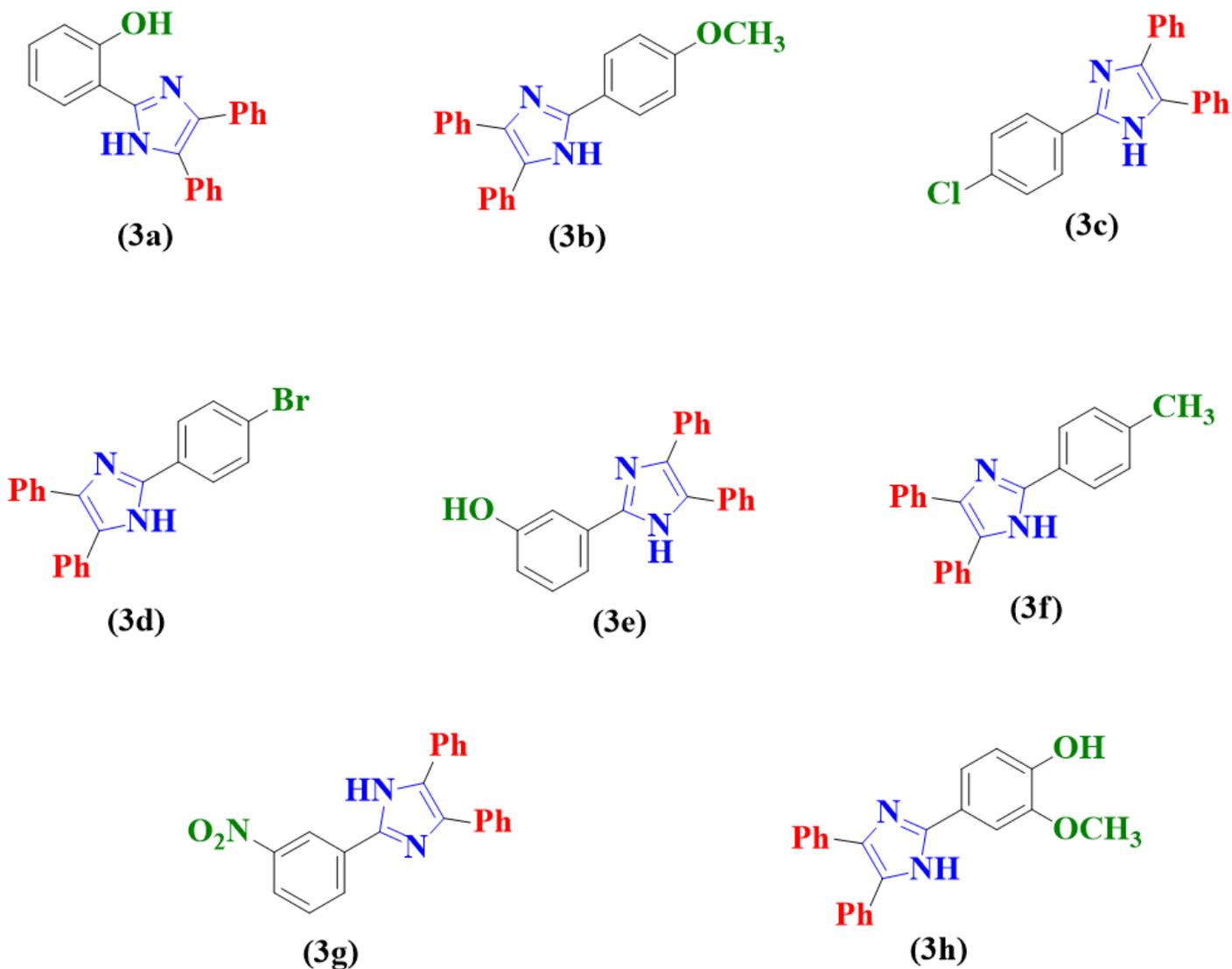


Figure 1

Substituted imidazole derivatives (3a-3h)

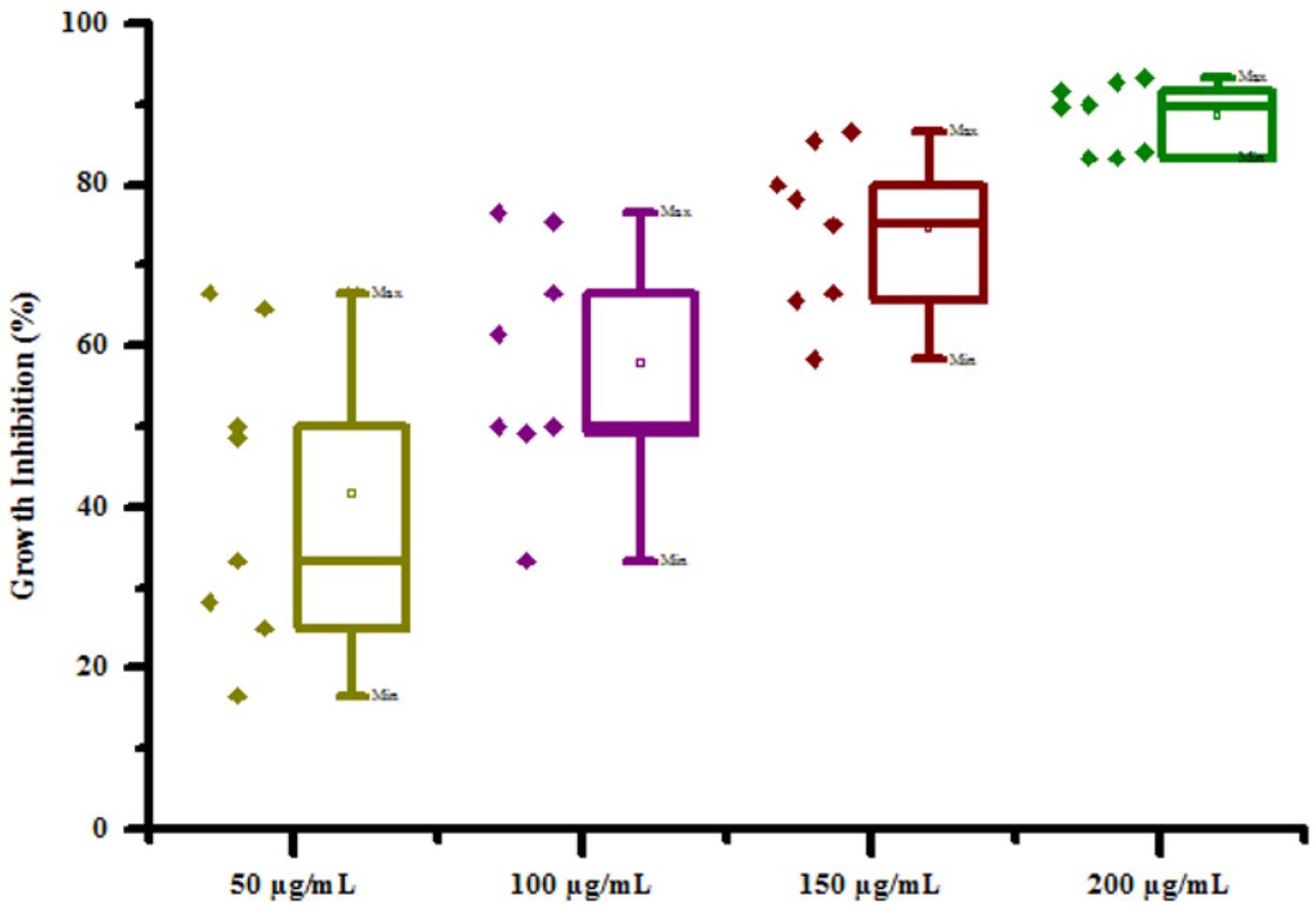


Figure 2

Box plot of substituted imidazoles (3a-3h) against *Raphanus sativus* L. (root)

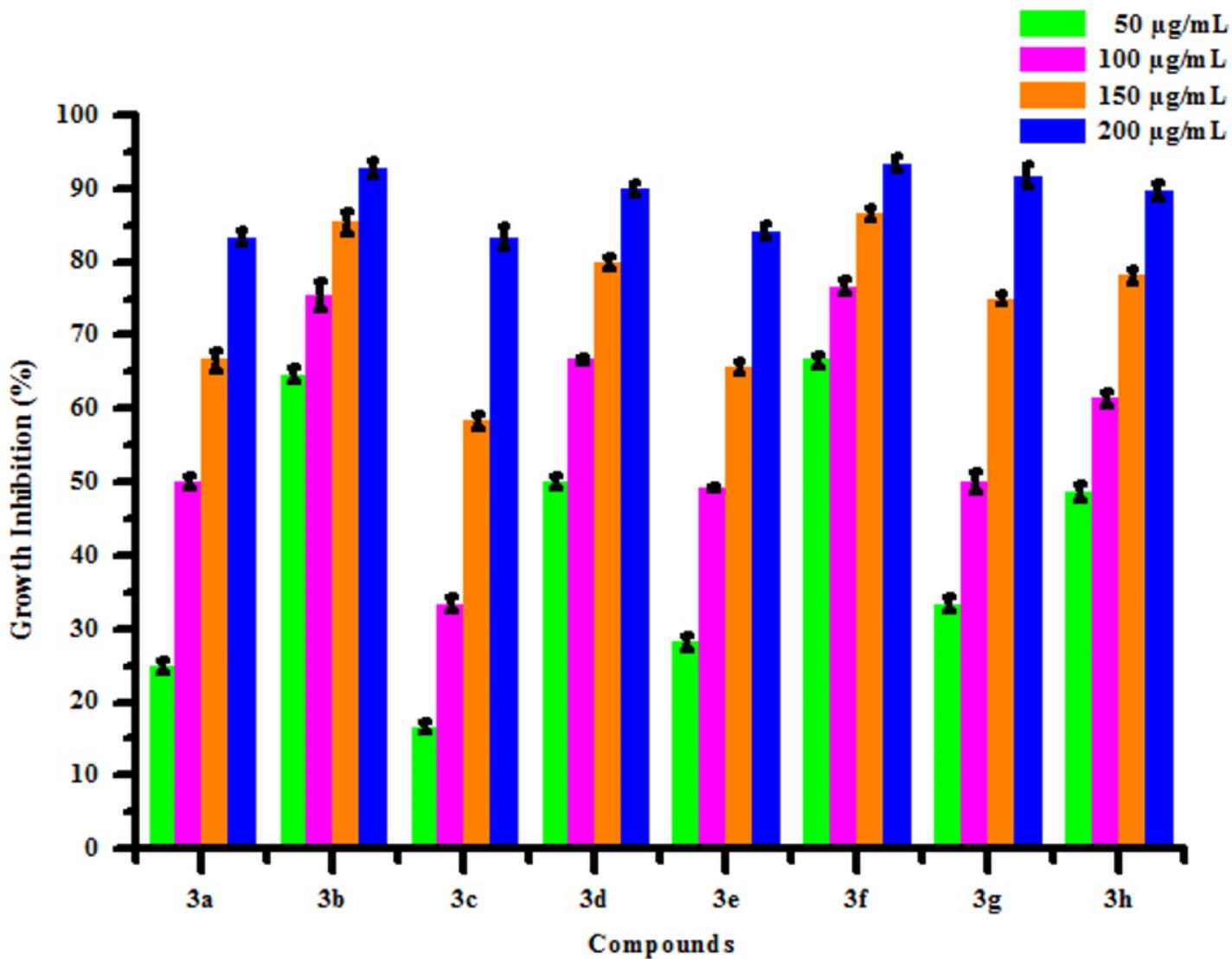


Figure 3

Herbicidal activity of substituted imidazoles (3a-3h) against *Raphanus sativus* L. (root)

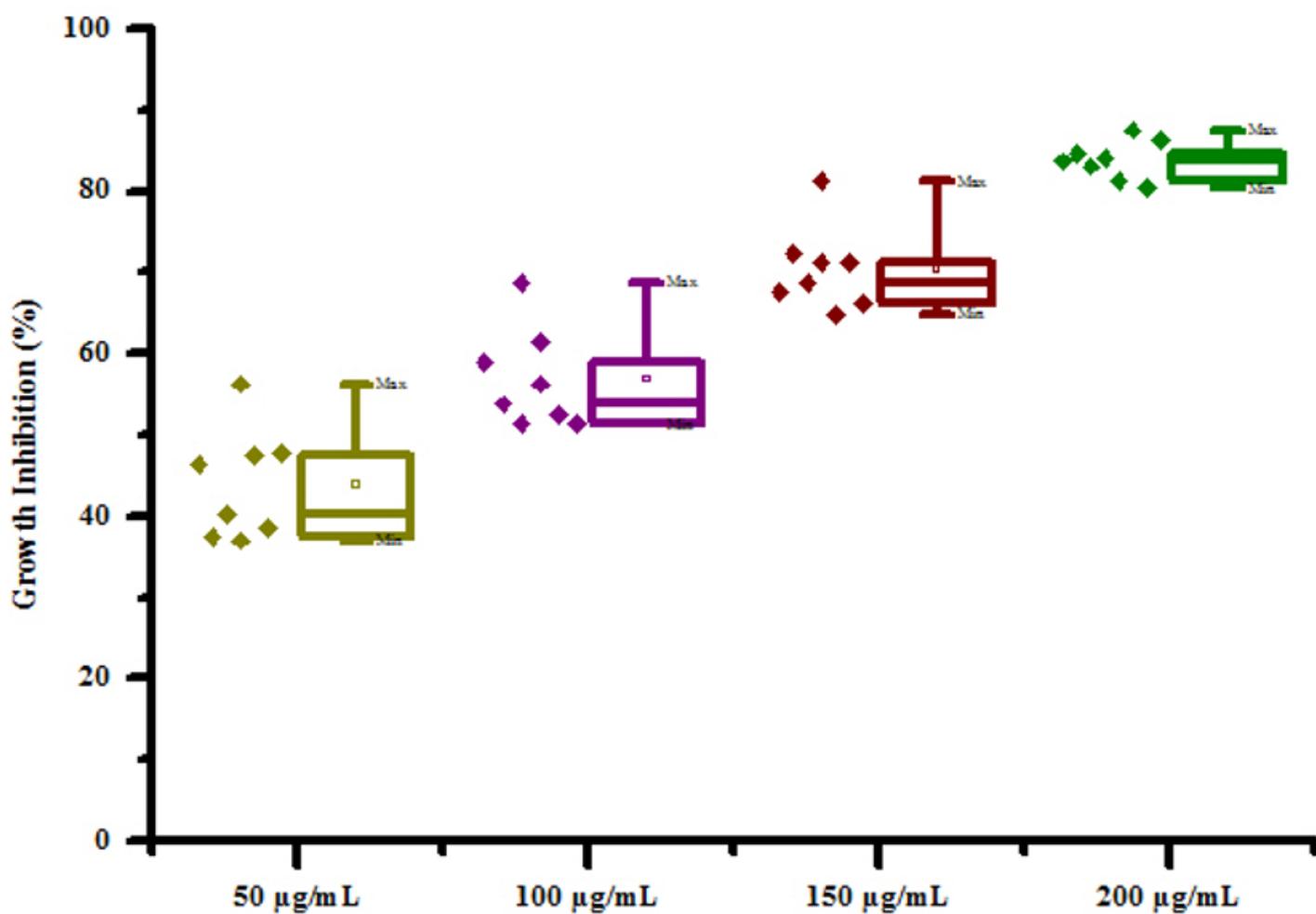


Figure 4

Box plot of substituted imidazoles (3a-3h) against *Raphanus sativus* L. (shoot)

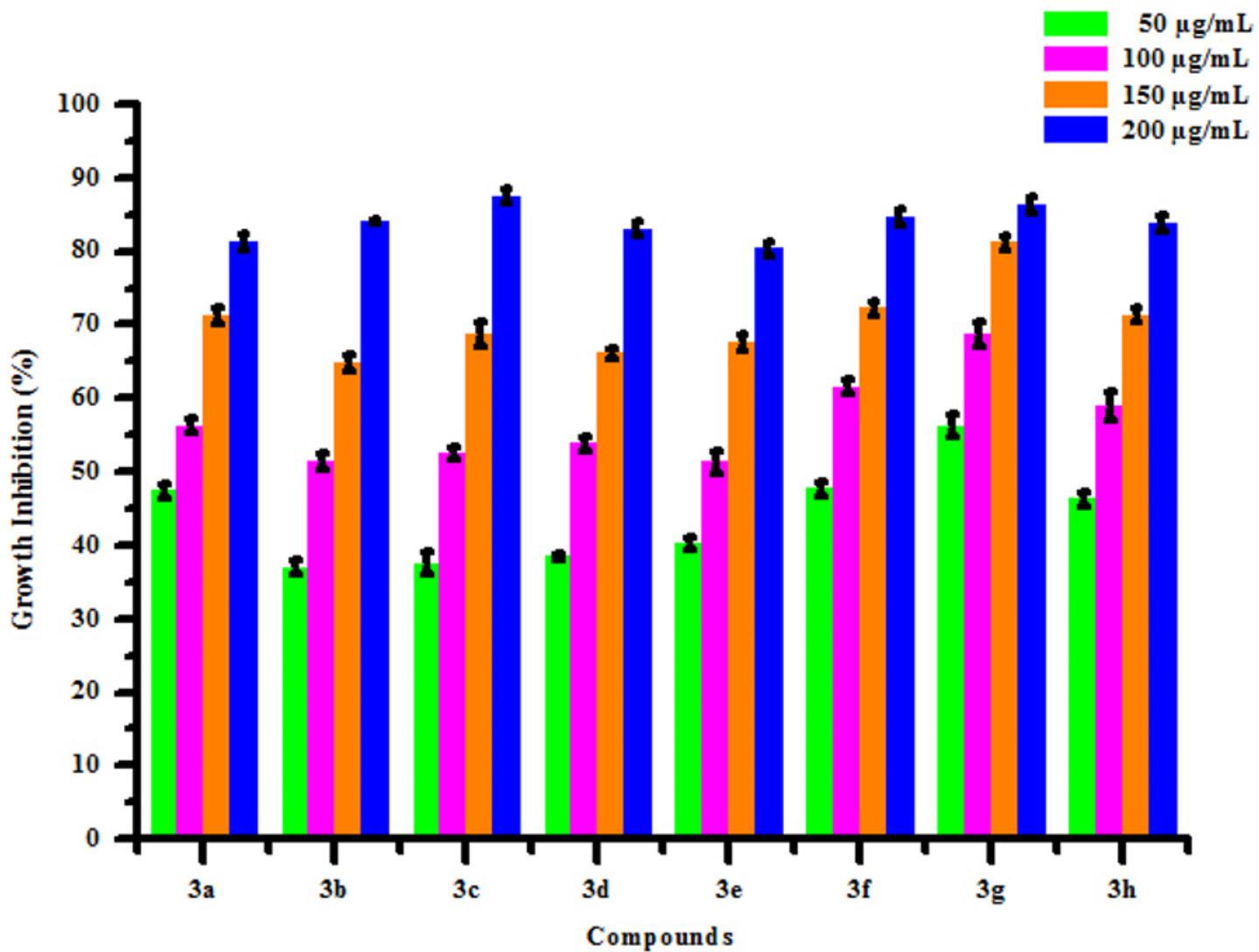


Figure 5

Herbicidal activity of substituted imidazoles (3a-3h) against *Raphanus sativus* L. (shoot)

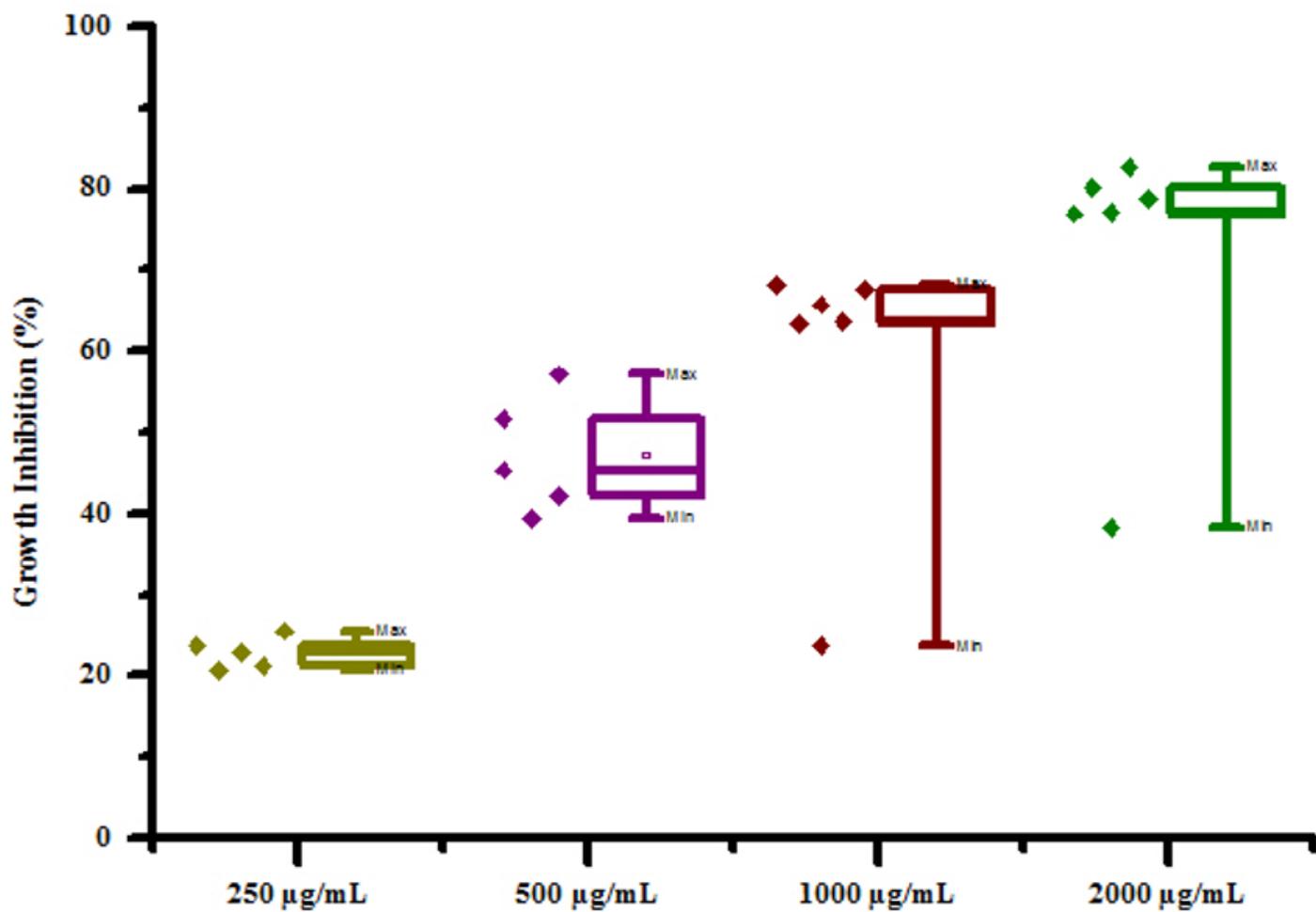


Figure 6

Box plot of substituted imidazoles (3a-3h) against *Rhizoctonia solani*

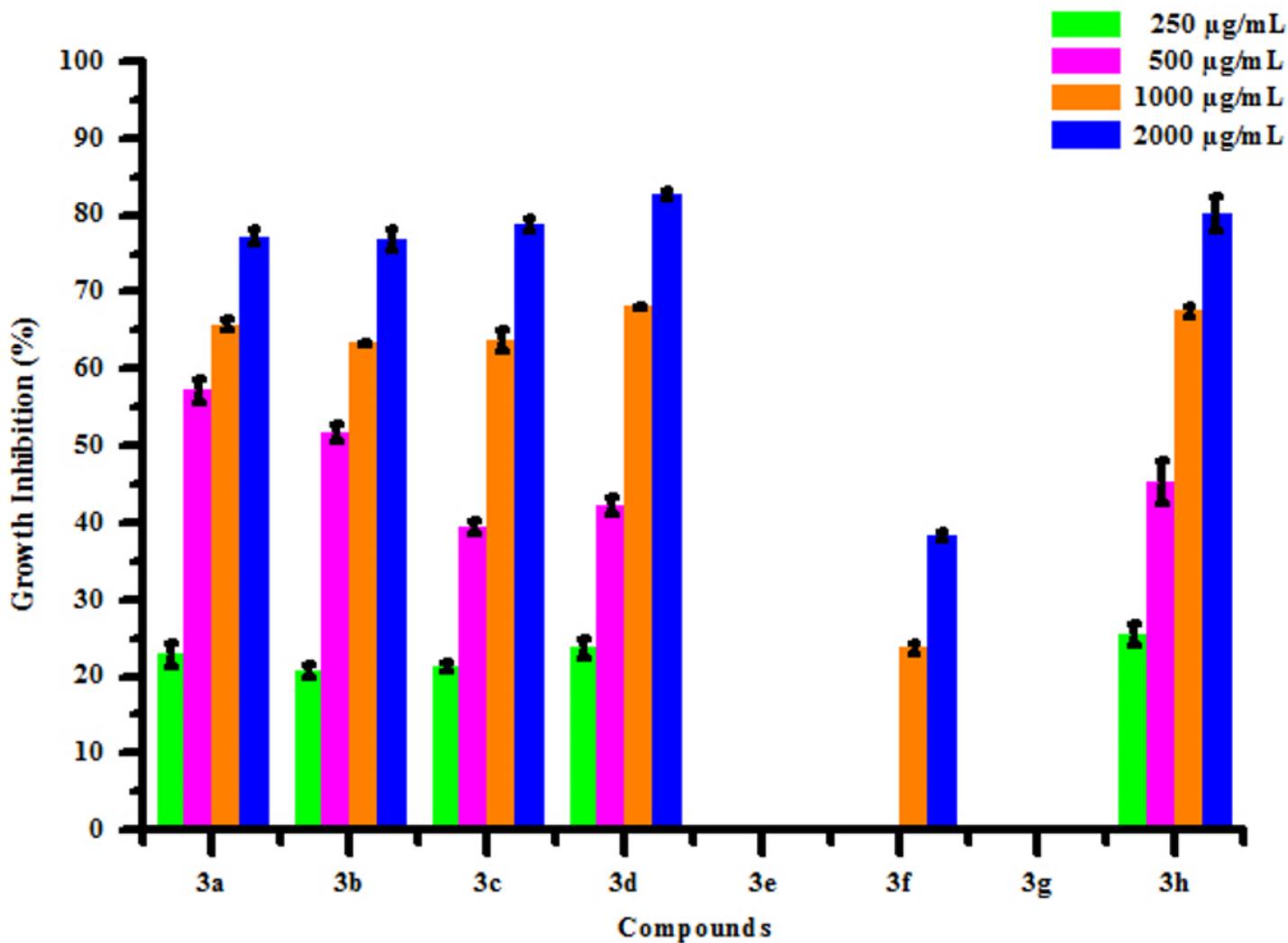


Figure 7

Antifungal activity of substituted imidazoles (3a-3h) against *Rhizoctonia solani*

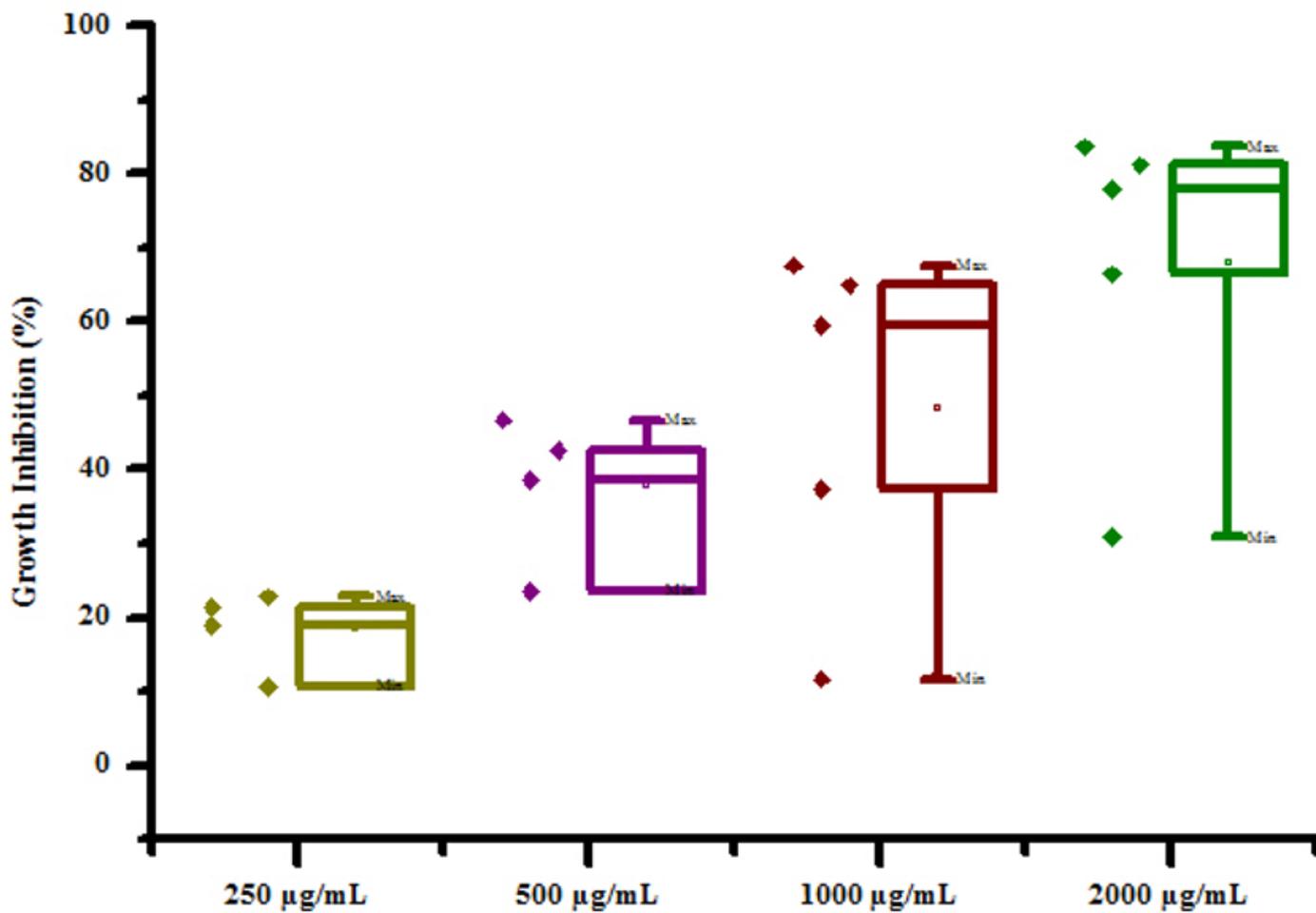


Figure 8

Box plot of substituted imidazoles (3a-3h) against *Colletotrichum gloeosporioides*

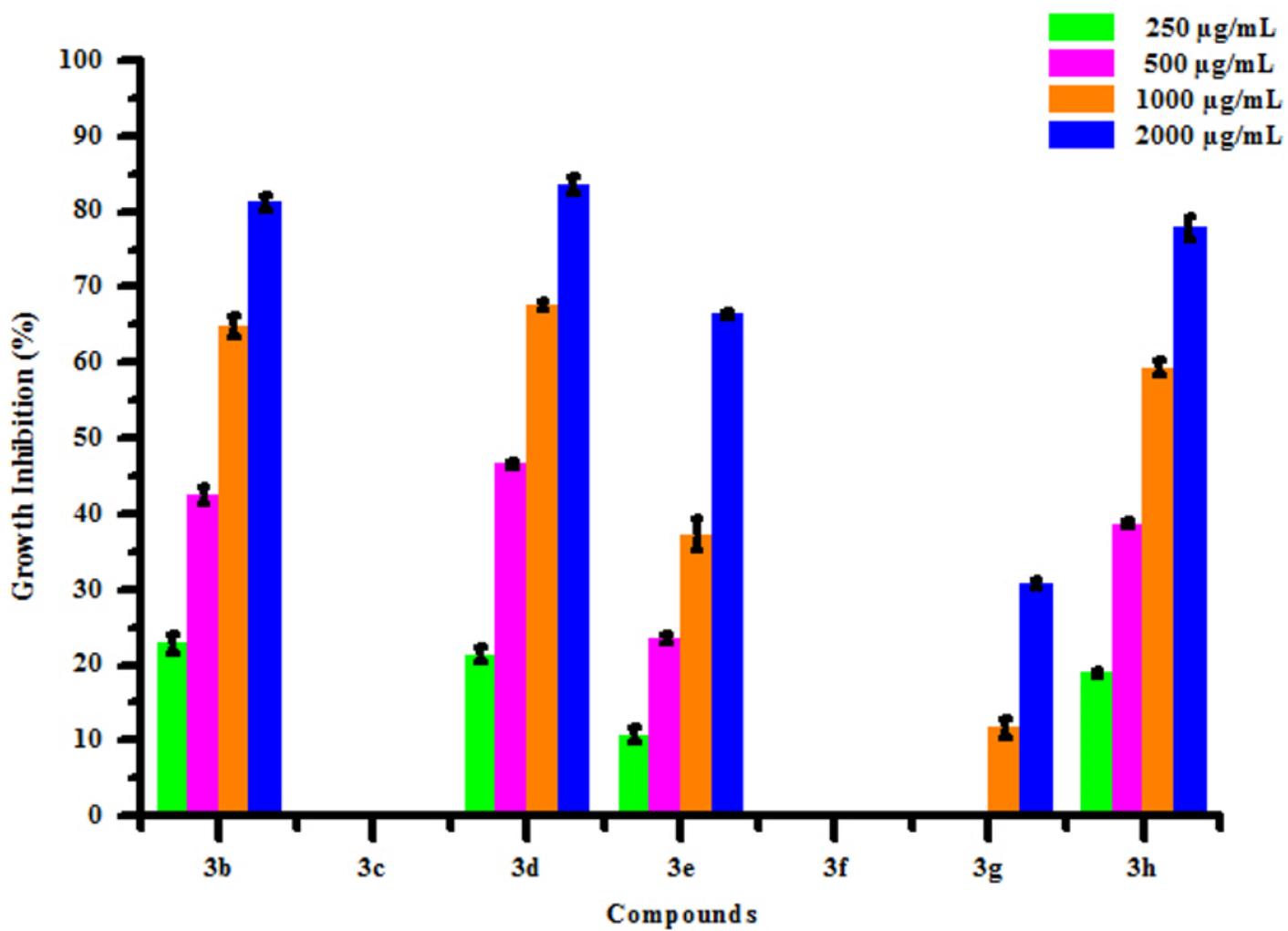


Figure 9

Antifungal activity of substituted imidazoles (3a-3h) against *Colletotrichum gloeosporioides*

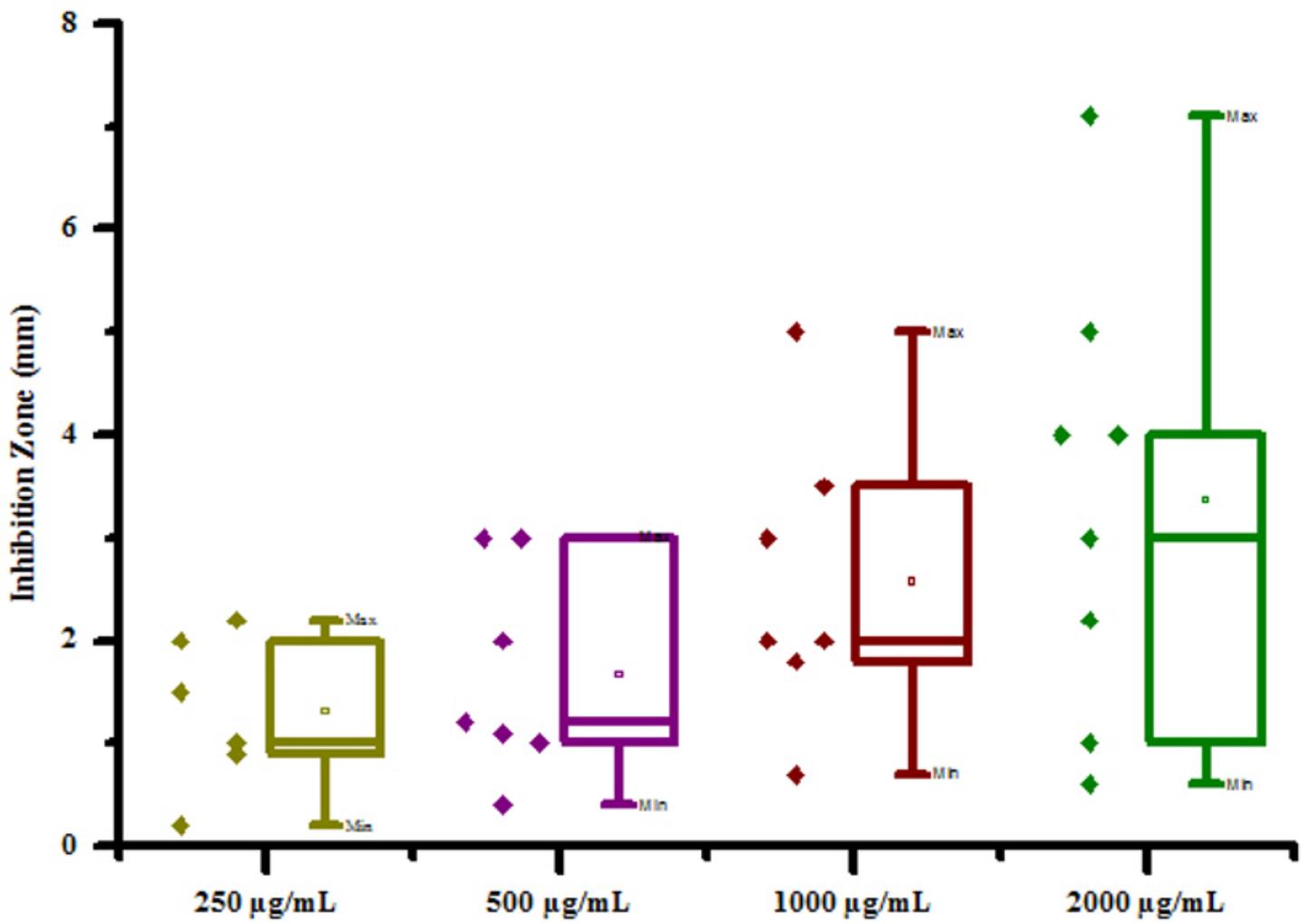


Figure 10

Box plot of substituted imidazoles (3a-3h) against *Erwinia cartovora*

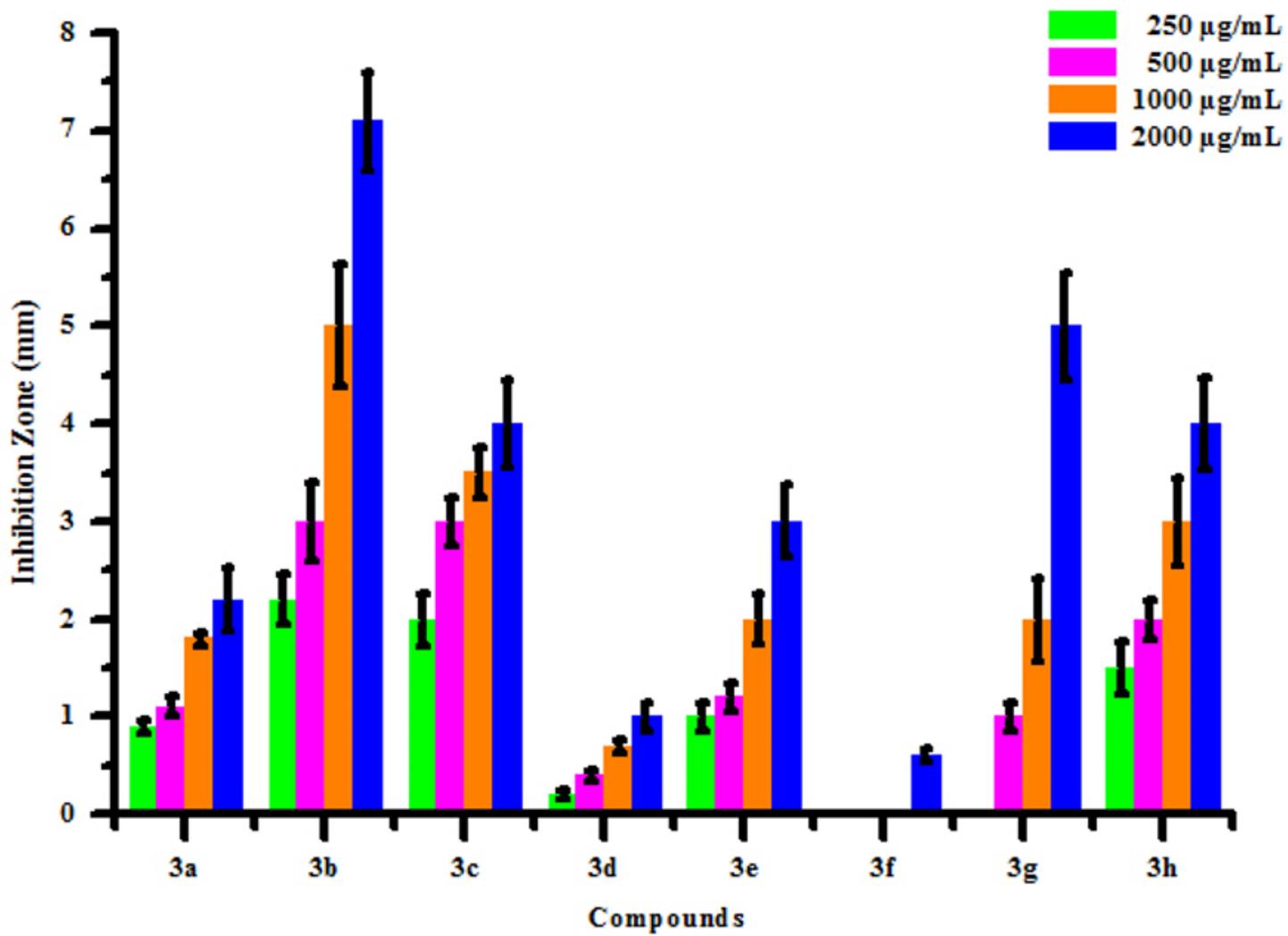


Figure 11

Antibacterial activity of substituted imidazoles (3a-3h) against *Erwinia cartovora*

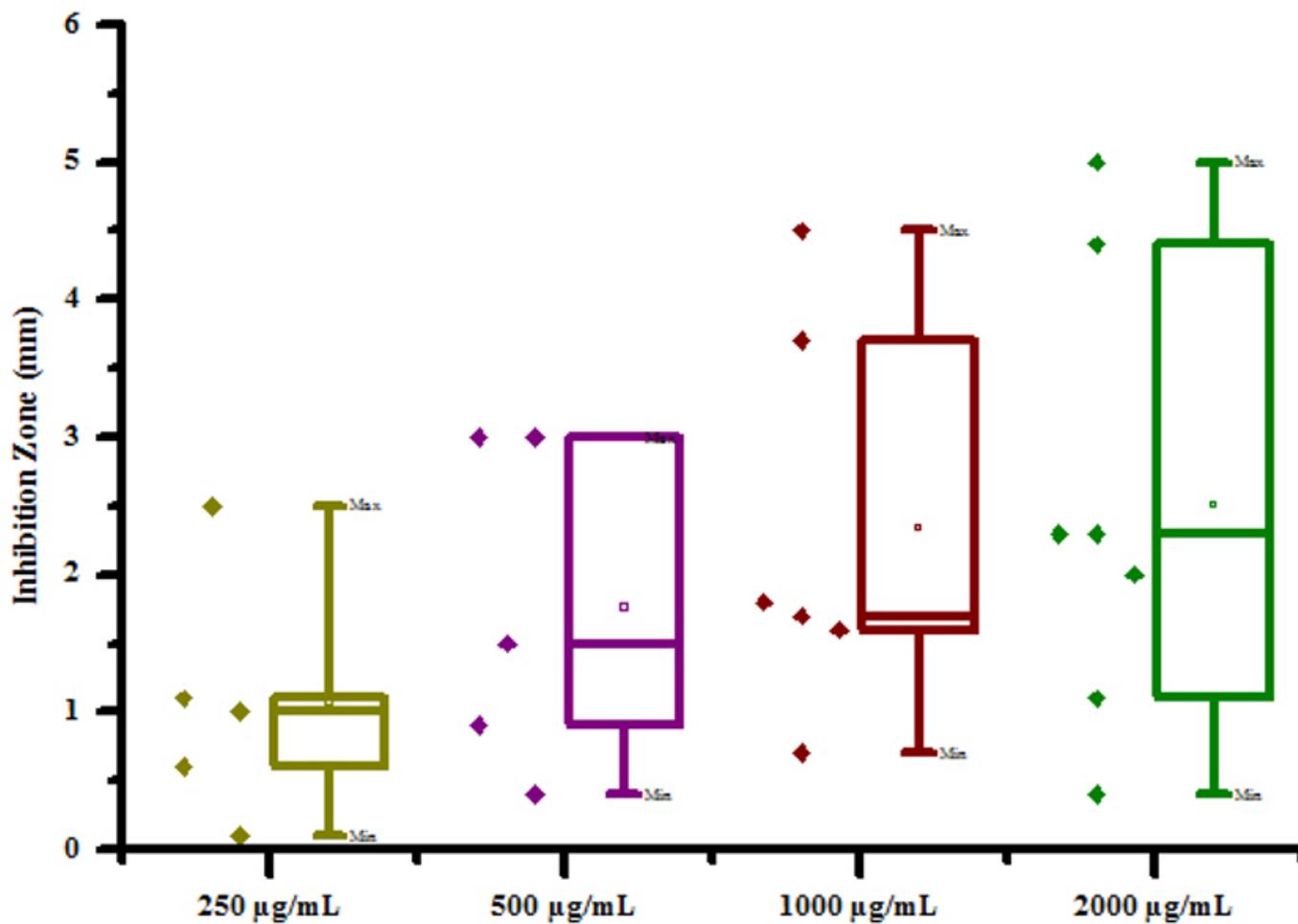


Figure 12

Box plot of substituted imidazoles (3a-3h) against *Xanthomonas citri*

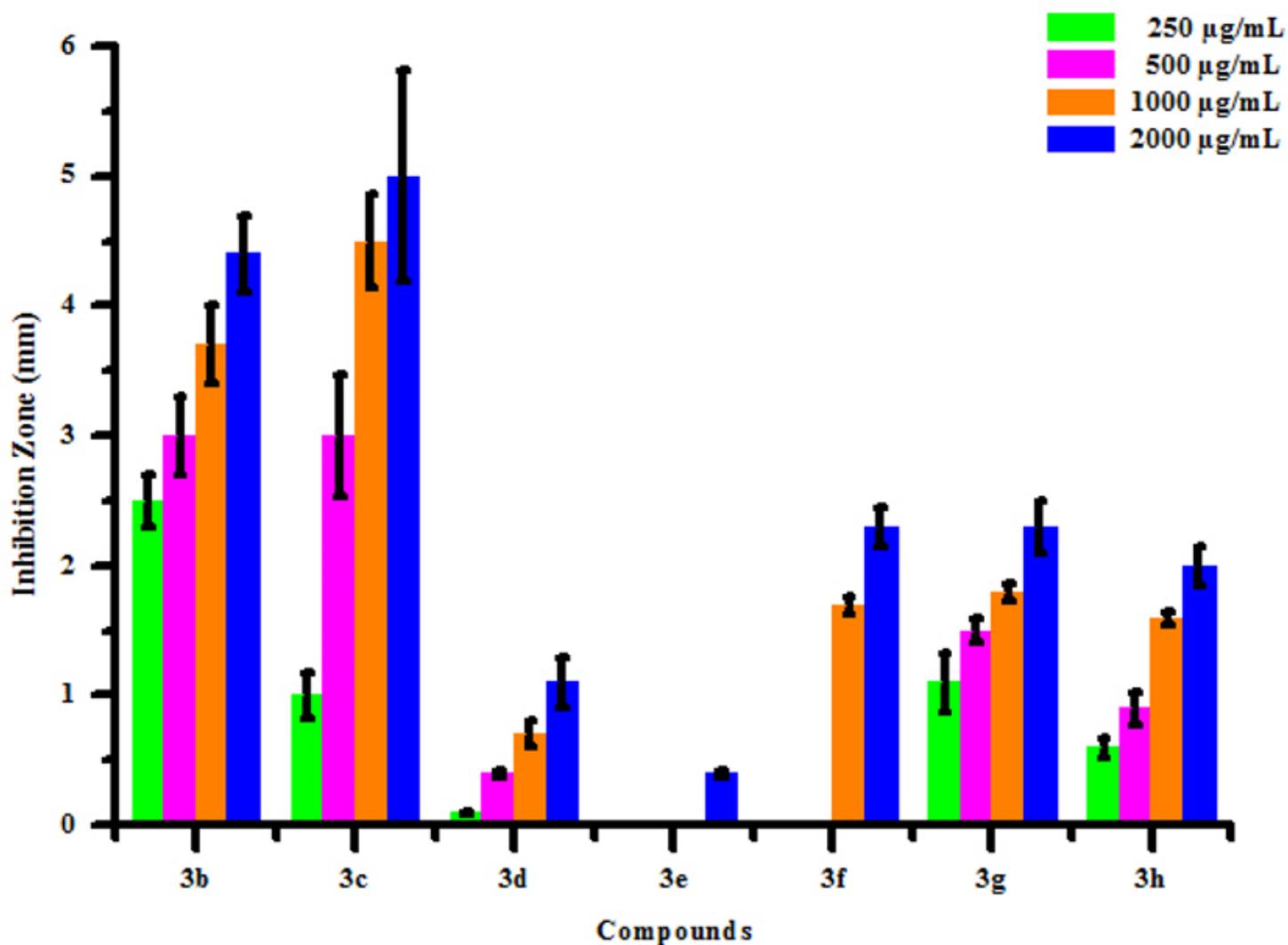


Figure 13

Antibacterial activity of substituted imidazoles (3a-3h) against *Xanthomonas citri*

Supplementary Files

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- [Scheme2.png](#)