Solvent-free Rice Husk Mediated Efficient Approach for Synthesis of Novel Imidazoles and their *In vitro* Bio evaluation

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Abstract

Abstract

An efficient and facile route for the synthesis of substituted imidazoles **(10a-10g)** has been developed by reacting substituted aldehydes (1–7), benzil (8) and ammonium acetate (9) in presence of solid supported acid catalyst *viz*. Rice Husk Ash.SO_3H (RHA.SO_3H) at room temperature. The purity of compounds was confirmed by melting point and thin layer chromatography. After the completion of reaction the crude product was recrystallized with ethylacetate to give pure samples **(10a-10g)** in good yield. All synthesized compounds **(10a-10g)** were characterized by ^{1}HNMR and FTIR spectral techniques. The solid acid catalyst has been characterized by SEM, TEM and X-ray diffraction method. All synthesized compounds were tested *in vitro* herbicidal activity against *Raphanus sativus* L. seeds. The compounds (**10a-10g**) were also screened for their antifungal activity against *Rhizoctonia solani* and *Aspergillus niger* by poisoned food technique method. From bio evaluation data, it was found that **compounds 10c and 10a were** most active against *Raphanus sativus* L. seeds. Compound 4-(4,5-diphenyl-1*H*-imidazol-2-yl)phenol **(10g)** was found most active against *R. solani* fungus at highest concentration. Compound 2-(4,5-diphenyl-1*H*-imidazol-2-yl)phenol **(10f)** has shown maximum percentage inhibition i.e. 71.89 against *A. niger* at 200 µg/mL concentration.

Introduction

Developing world demands for new bioactive compounds in the field of agriculture and pharmaceuticals because food and health are two very crucial issues for increasing population. At the same time safe and environmentally benign synthesis of novel heterocyclic compounds, impose significant claim to the organic chemist. Use of biocatalyst enlightened several avenues in organic synthesis to make it an indispensible and lucrative era. Lately, one-pot synthesis of potentially bioactive heterocyclic moieties under environmentally friendly conditions has remained one of important topics in organic as well as in medicinal chemistry. Among the multitude of nitrogen-containing heterocycles compounds, imidazole is a key heterocyclic molecule prevalent in many bioactive compounds as well in synthetic drugs (1). A number of methods have been reported for preparation of imidazoles including the condensation reaction of dicarbonyl compounds with substituted aldehydes and ammonium acetate under strong acidic conditions with high temperatures. However, in most of these methods they either employed a metal catalyst or long reaction time, tedious workup, expensive reagents and low atom economy (2). Thus we turned our attention to develop an eco-friendly method for synthesis of substituted imidazoles under solvent-free conditions without generating any hazardous by-products. Keeping in view the biological potential of imidazole nucleus, the present research work was carried out for synthesis of substituted imidazoles in presence of solid supported recyclable acid catalyst *viz*. Rice Husk Ash.SO_3H at room temperature. Metal free, short reaction time, excellent yields, mild reaction condition, simple work-up, high atom economy, cost effectiveness and no need of column chromatography are some beauties of this present methodology. All the products **(10a-10g)** of reactions were isolated in pure form by recrystallized with ethylacetate and no chromatographic techniques were required. All the synthesized compounds **(10a-10g)** were fully characterized *via* ^{1}HNMR and FTIR spectral data and evaluated for *in vitro* herbicidal activity against *Raphanus sativus* L. (Radish) seeds. The compounds (**10a-**

10g) were also evaluated for their antifungal activity against *Rhizoctonia solani* and *Aspergillus niger* by poisoned food technique method.

Experimental

All chemicals and solvent used were of analytical grade. Melting points were determined on Ganson electric melting point apparatus and are uncorrected. The completion of the reaction was monitored by (TLC) thin layer chromatography. The characterization of synthesized biocatalyst was done on JSM-6100 Scanning Electron Microscope SEM, Transmission Electron Microscope TEM and X-Ray diffraction methods. Infrared spectra of the synthesized compounds were recorded in KBr pellets on Perkin Elmer FTIR-R 2X spectrophotometer and frequency was expressed in cm^{-1}. The ^{1}HNMR spectra were recorded in $CDCl_3$ or $(CD_3)_2SO$ using tetra methyl silane (TMS) as internal reference on "Brucker Ac 400 F"(400 MHz) nuclear magnetic resonance spectrometer. The chemical shifts values were quoted in delta, while *J* value in Hz and are compatible with the assigned structures. The following abbreviations correlate with the multiplicity of NMR signals: s = singlet, d = doublet, t = triplet, m = multiplet and brs = broad singlet.

Synthesis of Rice Husk Ash.SO_3H (RHA.SO_3H)

The biocatalyst was prepared by sorption of sulphuric acid (1 mL) on the rice husk ash (30 g). The mixture was heated for 3 h at 100ºC to give RHA.SO_3H (*3*). The synthesized biocatalyst has been shown in Fig 1.

Transmission Electron microscope

TEM images of RHA.SO_3H **(**Fig 2, Fig 3, Fig 4 and Fig 5**)** reveals that particles of Rice Husk were uniformely sorpted by sulphuric acid. Fig 2 showed rice husk Ash.SO_3H particles at 100 nm, Fig 3, Fig 4 presented the particles at 50 nm respectively and Fig 5 showed the particles at 20 nm. A low and high magnification of TEM images of RHA.SO_3H aggregated uniformely and found in 50-20 nm.

X-Ray diffraction analysis

XRD analysis of the biocatalyst was done with the help of X-ray diffractometer with a Cu (K_α) radiation source (K_α= 1.5406) for confirm the phase transformation of RHA. The XRD scans were recorded from 10º-80º 2 with minimum step size omega: 0.001. Phase analysis was performed by comparing the d-values and intensity ratios of the main fundamental peaks with data available in the data book published by the Joint Committee of Powder Diffraction Standards (1974) (*4*). At low temperature, it was found that nature of RHA was amorphous and crystallization arisen when temperature was raised (*5*). Fig 6 1f clearly shows amorphous form of untreated rice husk (d-spacing 4.0217 Aº) but Fig 7 shows crystalline form (d-spacing 3.4982 Aº) of Rice husk Ash.SO_3H. The difference in d-spacing confirms amorphous to crystalline phase transformation.

General procedure for synthesis of substituted imidazoles (10a-10g)

A mixture of the substituted aldehydes (0.1 mmol) **(1-7)**, benzil (0.1 mmol) **(8)** and ammonium acetate (0.2 mmol) **(9)** and Rice husk ash.SO_3H (0.50 g) were added in 50 mL round bottom flask and stirred at room

temperature **(Scheme 1)**. The progress of the reaction was monitored by Thin Layer Chromatography (TLC). The product was dried and recrystallized with ethylacetate to isolate the pure product **(10a-10g)**. Further all the synthesized compounds has been analysed by ^{1}HNMR and FTIR spectral techniques. The reaction was found to complete within 40 min to give product in quantitative yield **(Table 1, Entry 3)**. Various derivatives of imidazoles are shown in **Table 3**, and the speciality of the catalyst in comparison of others is shown in **Table 4**.

Bioevaluation

Bioevaluation

Test for Herbicidal activities

Solutions of 50 µg/mL, 100 µg/mL, 150 µg/mL and 200 µg/mL of the **test** compounds in DMSO were prepared. Agar powder (5gm) was put into boiling distilled water (1L) until it dissolved, and then cooled down to 40-50 ºC. The solution (2 mL) containing test compounds and melting agar (18 mL) was mixed and this mixture was added to a Petridish with 4.5cm diameter. The agar plate without test compound was used as an untreated control. The 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±1 ºC 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 (*6*).

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

Antifungal activity

Amongst the several methods available, poisoned food technique (*7*) which is the most common was used for testing antifungal activity. The test fungus was grown on Potato dextrose agar medium. 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^{o} C after brief shaking. Each lot of medium was poured into Petri dishes and allowed to solidify. 1 mL DMSO in media was taken as 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±2^{o}C for 48-72 h and colony diameters were measured periodically till the control dishes were nearly completely covered with fungus growth. Three replicates were used for each concentration of a chemical together with three dishes containing only the solvent and no toxicant. 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

Results And Discussion

The synthesis of substituted imidazole derivatives (Scheme-1), and then recrystallized from ethylacetate to furnish **(10a)** as intermediate compound in quantitative yield **(Table 3, Entry 1)**. Inspired by this result concentration of catalyst was optimized through the above reaction by using different concentrations 0.12, 0.25, 0.50, 0.75, 1.0, 1.12 and 1.25 g of RHA.SO_3H at room temperature **(Table 1, Entry 1-7)**, for 40 min to give the desired products **(10a)**. Reusability was evaluated without loss of activity as shown in **Table 2** and the reaction procedure was performed in absence of catalyst at the same conditions, a low yield is obtained which shows the value of the prescribed catalyst. The possible mechanism for synthesis of imidazoles (Scheme 2) depicted below. It involves condensation of dicarbonyl compound such as benzil with an aldehyde in presence of ammonium acetate which is good source of ammonia. Preasumably 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 4 indicates the comparison of the activity of different catalysts by considering the yield of the reaction. We observed that Rice Husk Ash. SO_3H best give catalytic activity in terms of product yield, solvent and reaction time compared to other catalysts in the literature such as $InCl_3.3H_2O$, Clay, $FeCl_2$, $ZrCl_4$, L-proline, $[H\text{-}Bim]BF_4$ and $NiCl_2.6H_2O/Al_2O_3$. Rice husk is an easily available and inexpensive catalyst, which makes this method green and mild. In addition, above catalyst is a renewable catalyst which follows one of the green chemistry principles regarding the maximum yield of renewable resources.

Table 1: Screening of Rice Husk Ash: SO_3H for synthesis of compound (**10a)** at room temperature

Entry	Amount of Catalyst (g)	Time (h)	Yield (%)
1	0.12	1.4	70
2	0.25	1.2	75
3	**0.50**	**0.40**	**87**
4	0.75	0.30	82
5	1.00	0.30	84
6	1.12	0.25	84
7	1.25	0.25	82

Table 2: Reusability of catalyst (Rice Husk Ash: SO_3H)

Reuse Cycle	Fresh	First	Second	Third
Time (h)	40	40	55	60
Yield (%)	87	87	82	81

Table 4: Comparison of the results of the present methods used for synthesis of imidazoles with the reported methods

S.No.	Catalyst	Solvent	Temperature (ºC)	Time (h)	Yield (%)	References
1	$InCl_3.3H_2O$	Methanol	RT	8.2	76	(*8*)
2	Clay	Solvent free	60	1.0	89	(*9*)
3	$ZrCl_4$	Ethanol	RT	2.0	74	(*10*)
4	L-proline	Methanol	60	9.0	85	(*11*)
5	$[H\text{-}Bim]BF_4$	-	100	1.0	82	(*12*)
6	$NiCl_2.6H_2O/Al_2O_3$	Ethanol	60	1.5	90	(*13*)
7	**RiceHusk Ash.SO_3H**	**Solvent Free**	**RT**	**0.40**	**87**	**Present work**

Table 5: Herbicidal activity of substituted imidazoles **(10a-10g)**

Compounds	Growth Inhibition (%)							
	Root				Shoot			
	50 (µg/mL)	100 (µg/mL)	150 (µg/mL)	200 (µg/mL)	50 (µg/mL)	100 (µg/mL)	150 (µg/mL)	200 (µg/mL)
10a	41.05	58.93	68.01	81.00	47.98	51.05	67.06	89.11
10b	43.00	61.73	84.03	89.04	52.87	64.00	73.12	88.31
10c	37.91	52.74	63.91	93.00	34.16	51.04	78.00	87.00
10d	31.00	47.03	61.98	84.07	26.03	53.94	73.01	89.00
10e	a	a	a	a	a	a	a	a
10f	a	a	a	a	a	a	a	a
10g	7.65	19.87	46.02	75.79	17.48	38.01	59.00	64.62

a: no growth inhibition

Table 6: Antifungal activity of substituted imidazoles **(10a-10g)**

Compounds	Growth inhibition (%)							
	Fungi							
	Rhizoctonia solani				*Aspergillus niger*			
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL
10a	40.00	49.55	58.61	70.00	42.10	51.52	62.00	71.12
10b	42.01	51.10	60.01	70.08	40.21	48.64	58.72	69.61
10c	a	a	26.00	49.00	38.11	47.00	58.64	70.72
10d	30.05	39.78	48.56	59.61	39.88	49.55	60.02	71.75
10e	35.08	46.00	55.12	65.88	a	a	a	a
10f	39.56	48.61	57.85	68.72	41.00	52.85	61.00	71.89
10g	41.56	52.00	61.12	71.01	39.79	49.85	58.12	70.12

a: no growth inhibition

Characterization data of some selected compounds

2-(4-methoxyphenyl)-4,5-diphenyl-1H-imidazoles (10a): yellow solid. m.p: 229-230 ºC; ^{1}H NMR (400 Hz, $CDCl_3$): δ 3.88 (s, 3H, **OCH_3**), 7.09-7.37 (m, **Ar-H**); 7.54-8.87 (m, **ArH**); 11.76 (s, 1H, **NH**); IR (v_{max} cm^{-1}) (neat): 3297 (**NH**), 3062 (**C=CH**), 1587 (**C=C**, aromatic), 1183 (**OCH_3**)

2-(4-chlorophenyl)-4,5-diphenyl-1H-imidazoles (10b): pale yellow solid. m.p: 229-230 ºC; ^{1}H NMR (400 Hz, $CDCl_3$): δ 7.61-7.85 (m, **Ar-H**); 7.43-8.81 (m, **Ar-H**); 11.62 (s, 1H, **NH**) IR (v_{max} cm^{-1}) (neat): 3320 (**NH**), 3064 (**C=CH**), 1590 (**C=C**, aromatic), 1450 (**C=N**), 751(**C-Cl**)

2-(4-methylphenyl)-4,5-diphenyl-1H-imidazoles (10c): pale yellow solid. m.p: 233-235 ºC; ^{1}H NMR (400 Hz, $CDCl_3$): δ 2.52 (s, 3H, **CH_3**); 7.28-7.85(d, **4H**); 8.09-8.64(m, **Ar-10H**); 11.62(s, 1H, **NH**); IR (v_{max} cm^{-1}) (neat): 3321 (**NH**), 3057 (**C=CH**), 1589 (**C=C**, aromatic), 1452 (**C=N**)

***2-(2-chlorophenyl)-4,5-diphenyl -1H-imidazoles (10d)*:** pale yellow solid. m.p: 195-197 ºC; ^{1}H NMR (400 Hz, $CDCl_3$): δ 6.83-7.61 (m, 4H, **Ar-H**); 8.40-8.86 (m, **Ar-H**); 11.00(s, 1H, **NH**) IR (v_{max} cm^{-1}) (neat): 3318 (**NH**), 3066 (**C=CH**), 1592 (**C=C**, aromatic), 1449 (**C=N**), 753 (**C-Cl**)

Herbicidal assay

All synthesized compounds **(10a-10g)** were tested for herbicidal activities against *Raphanus sativus* L. at various concentrations 200, 150, 100 and 50 µg/mL as shown in **Table 5** and Figs 8-9. Results were recorded in the form of primary screening. Synthesized compounds were diluted to 1000 µg/mL concentration as a

stock solution. Herbicidal activities of synthesized compounds were evaluated against *Raphanus sativus* L. by inhibitory effect of the compounds on the growth of weed roots and shoots. The percentage of inhibition of growth was calculated from the mean differences between treated and control. From the herbicidal activity results, we identified that compound 10c was exhibited maximum percentage growth inhibition i.e. 93.00 against *Raphanus sativus* L. (root) whereas compound 10a was exhibited maximum percentage growth inhibition i.e. 89.11 against *Raphanus sativus* L. (shoot) respectively at 200 µg/mL concentration. The compound 10e and 10f show no growth inhibition at all the concentration.

Antifungal activity

All synthesized compounds (**10a-10g**) were tested for their *in vitro* antifungal activity against *Rhizoctonia solani* and *Aspergillus niger***.** The percentage growth inhibition of compounds against *R. Solani* and *A.niger* **was** presented in Table 5. From the fungicidal activity results, we concluded that compounds **10g** and **10f** was found to be most likely against *R. solani* and *A. niger* respectively. The growth inhibition may be attributed to substitution of hydroxy group on phenyl ring. The graphical representation of antifungal activity of all synthesized compounds **(10a-10g)** against *Rhizoctonia solani* and *Aspergillus niger* were shown in Figs 10-11.

Conclusions

We have reported an efficient and eco-friendly one-pot multicomponent synthesis of substituted imidazoles by reaction of substituted aldehydes, benzil and ammonium acetate in presence of $RHA.SO_3H$ in very reliable yields. The current procedure is very efficient, safe and environmental friendly. In addition, the catalyst could be reused for at least three runs without loss of catalytic activities. All synthesized compounds **(10a-10g)** were fully characterized by ^{1}HNMR and FTIR spectral techniques. We also tested the herbicidal activity of synthesized compounds **(10a-10g)** against *Raphanus sativus* L. (Radish) seeds, fungicidal activity against *R. solani*, *A. niger*. Based on activity data, we concluded that strong electron donating groups at the phenyl ring exhibit an excellent activity profile as compared to electron withdrawing group.

Declarations

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Author Contributions

Conceptualization: Susheel Gulati, Rajvir Singh, Suman Sangwan, Kamla Malik

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Formal analysis: Susheel Gulati, Rajvir Singh, Suman Sangwan, Jyoti Punia

Investigation: Susheel Gulati, Rajvir Singh, Suman Sangwan

Methodology: Susheel Gulati, Rajvir Singh, Suman Sangwan

Supervision: Suman Sangwan, Rajvir Singh

Validation: Susheel Gulati, Rajvir Singh, Suman Sangwan, Suprita Rana

Writing-original draft: Suman Sangwan, Susheel Gulati

Writing-review & editing: Susheel Gulati, Rajvir Singh, Suman Sangwan

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Availability of data and materials: The datasets and samples of the compounds used during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: Authors declare no conflict of interest.

Abbreviations

NMR Nuclear Magnetic Resonance

FTIR Fourier transform infra-red

SEM Scanning electron microscope

TEM Transmission Electron microscope

RHA Rice Husk Ash

TLC Thin layer chromatography

XRD X-Ray diffraction

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Table 3

Table 3 is available as a download in the supplementary files section.

Figures

Figure 1

Rice Husk and treated Rice Husk Ash

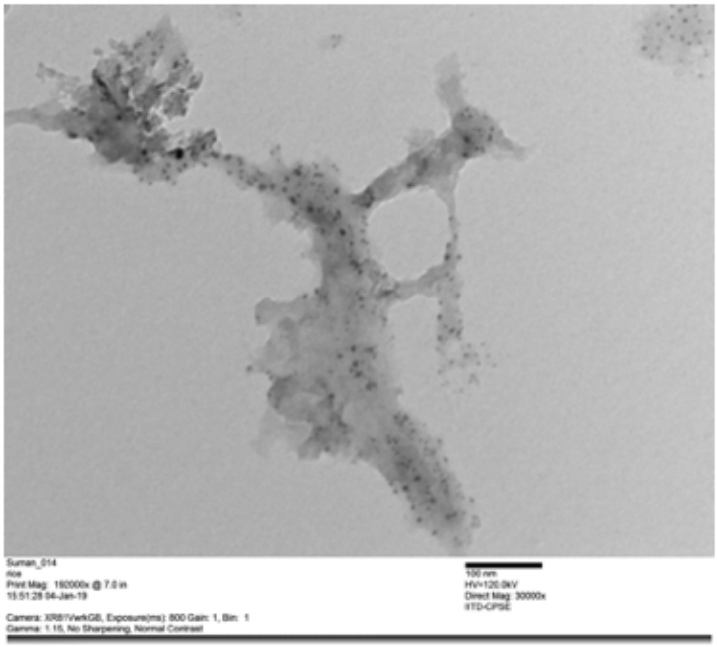

Figure 2

TEM images of RHA.SO3H

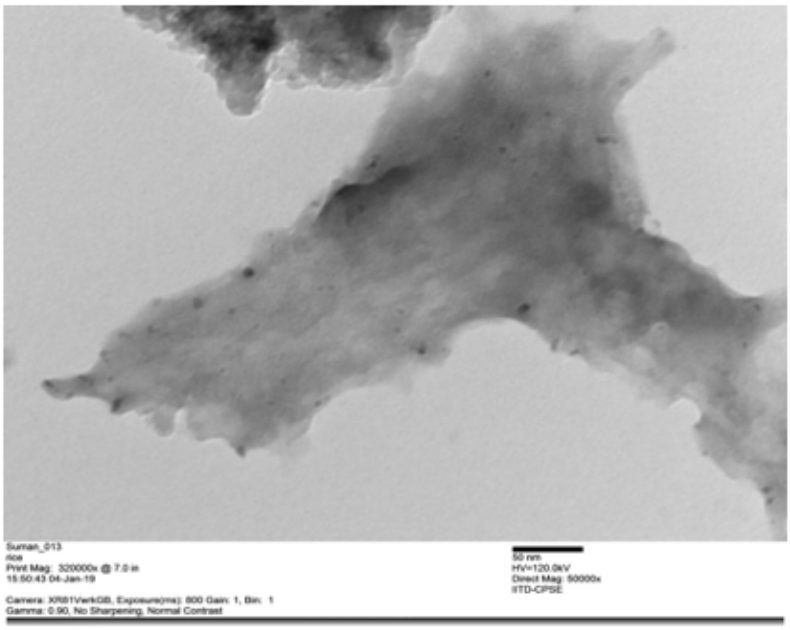

Figure 3

TEM images of RHA.SO3H

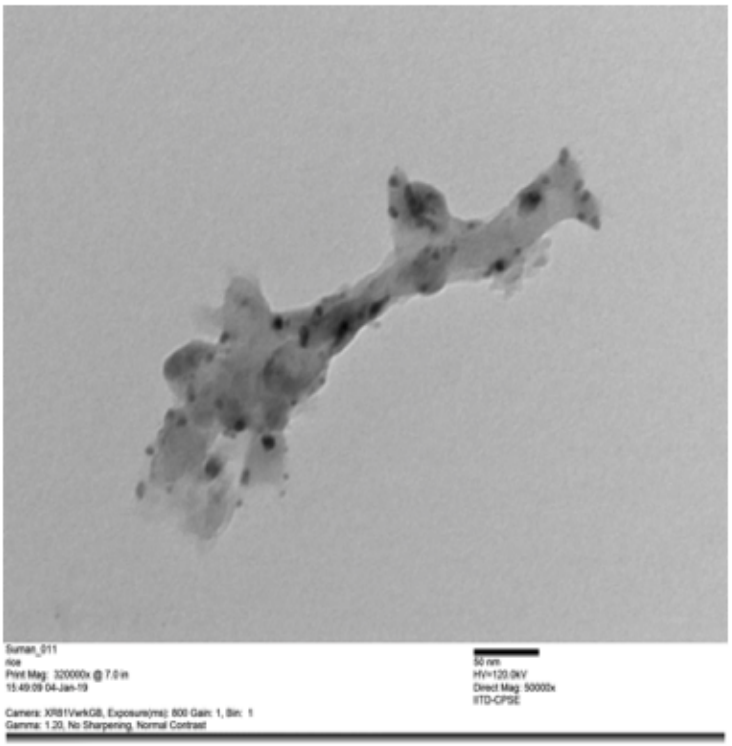

Figure 4

TEM images of RHA.SO3H

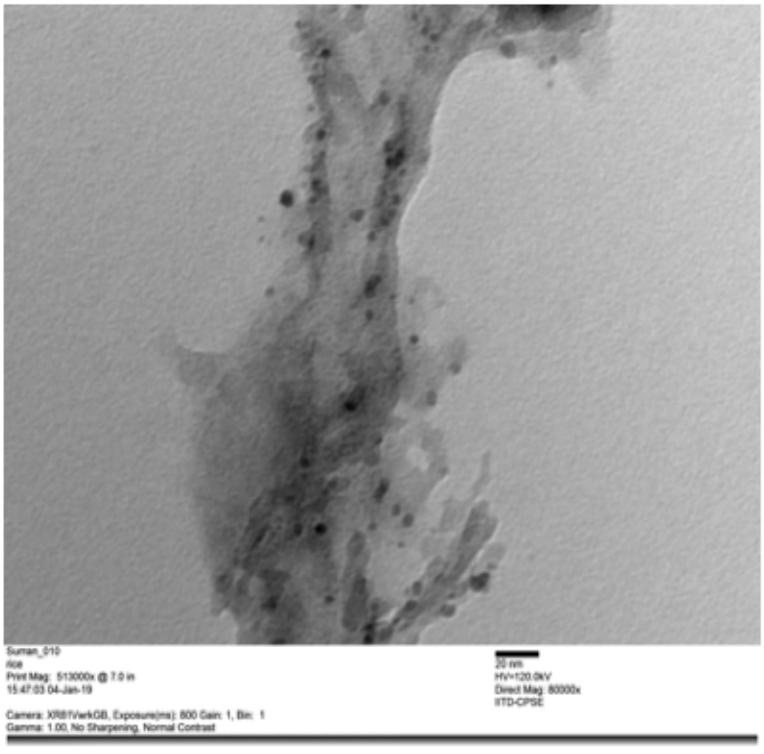

Figure 5

TEM images of RHA.SO3H

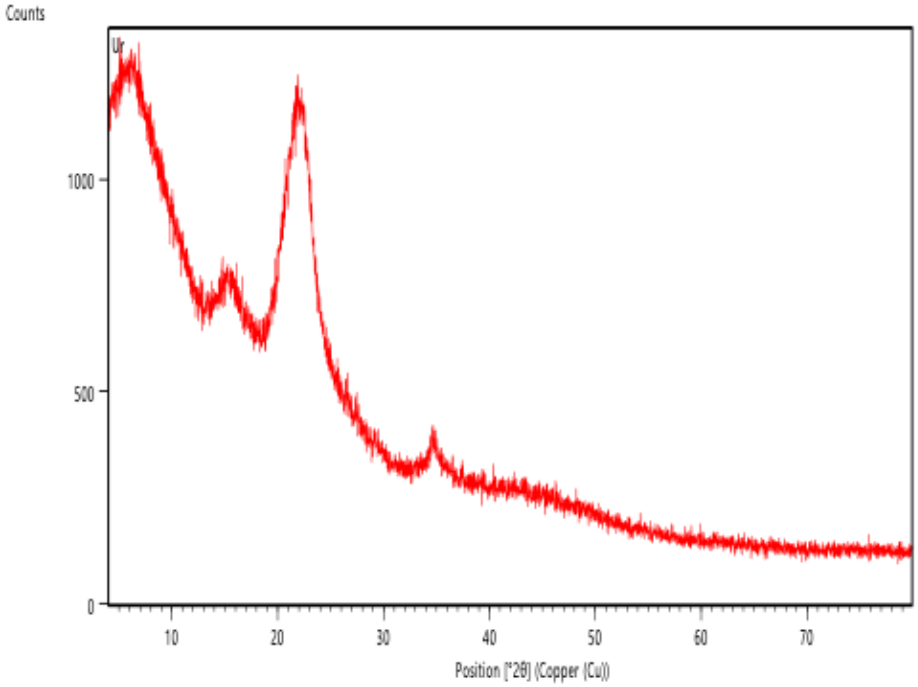

Figure 6

X-ray diffraction of Rice Husk

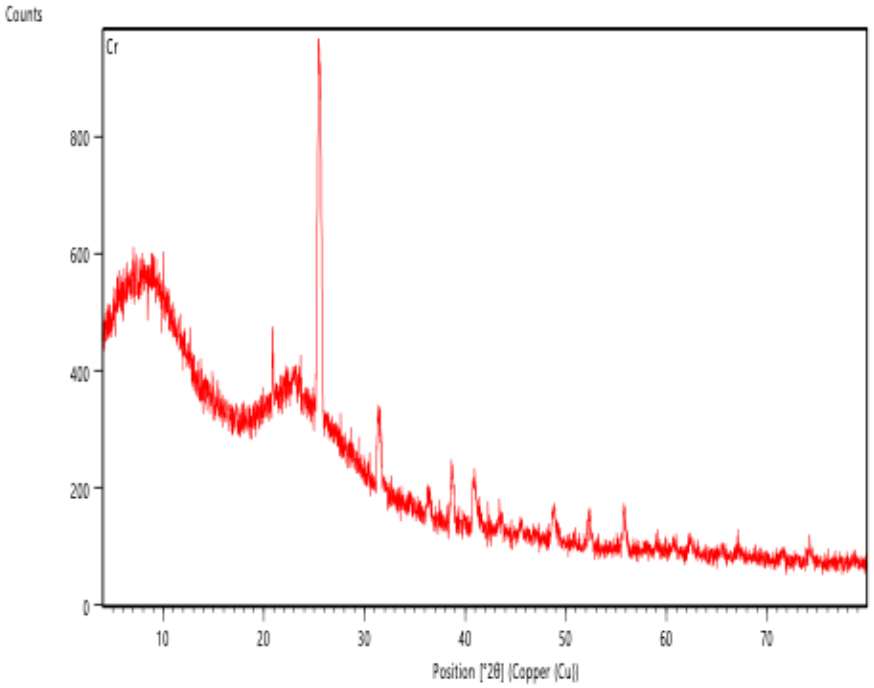

Figure 7

X-ray diffraction of RHA.SO3H

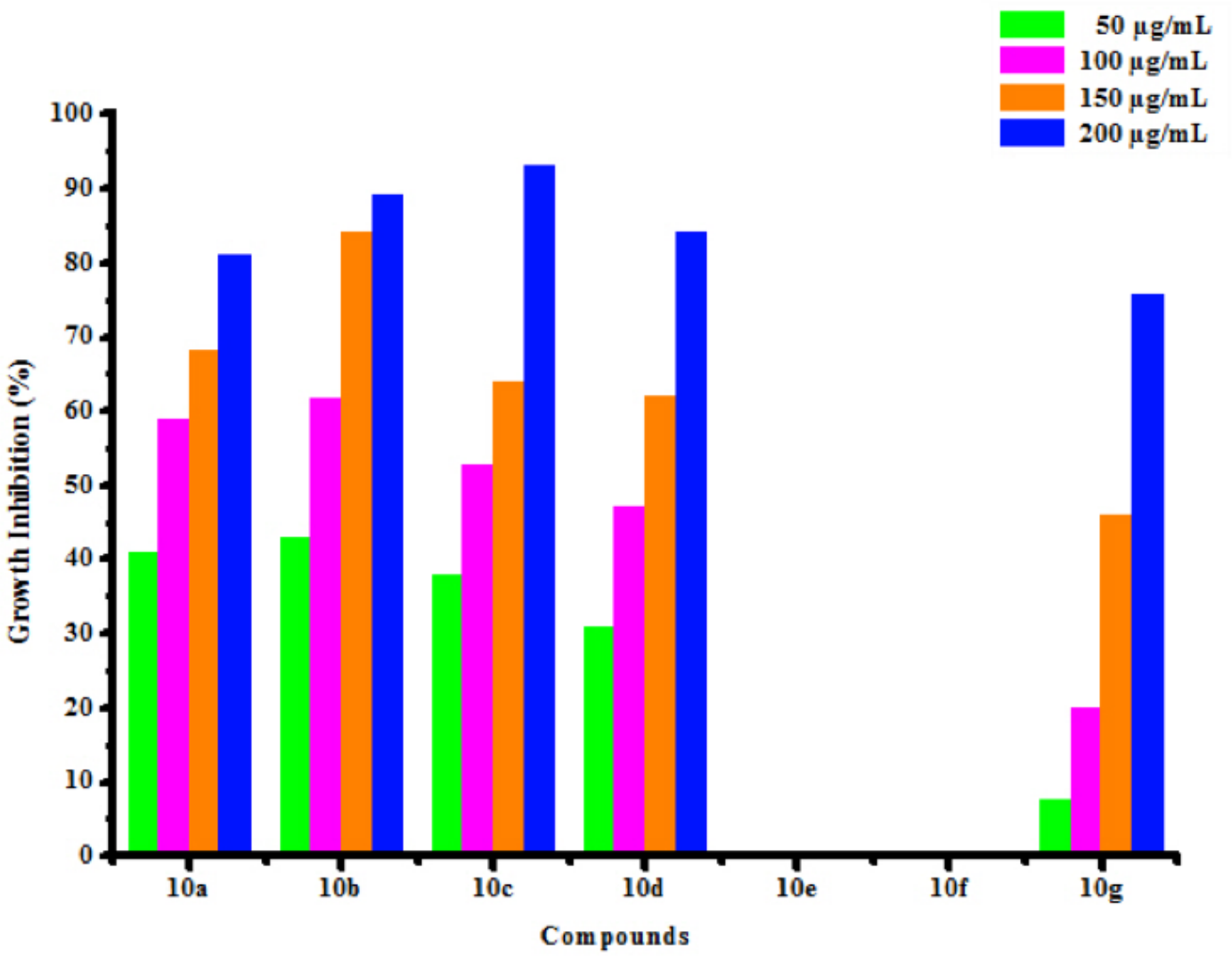

Figure 8

Herbicidal activity of synthesized compounds (10a-10g) against Raphanus sativus L. (root)

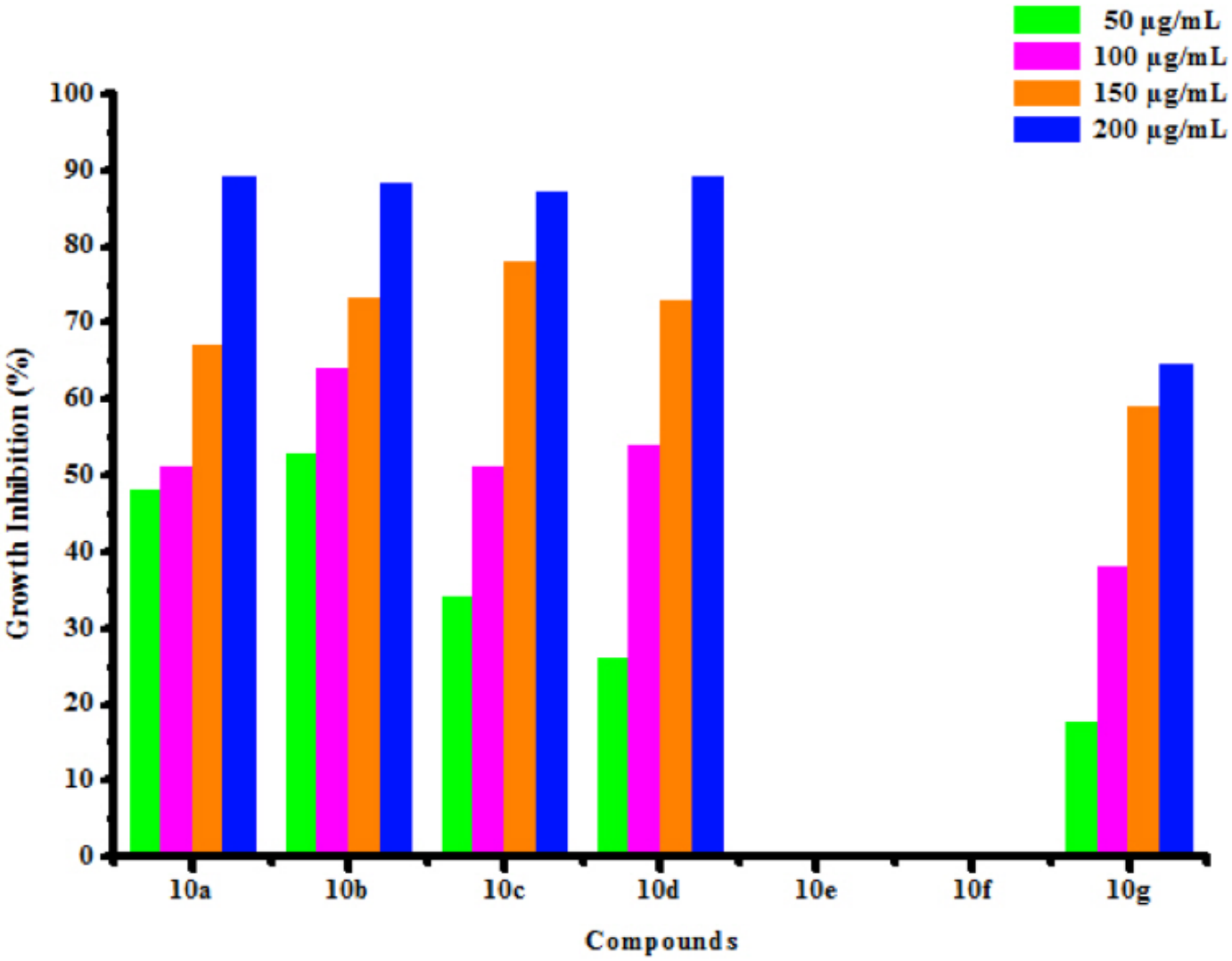

Figure 9

Herbicidal activity of synthesized compounds (10a-10g) against Raphanus sativus L. (shoot)

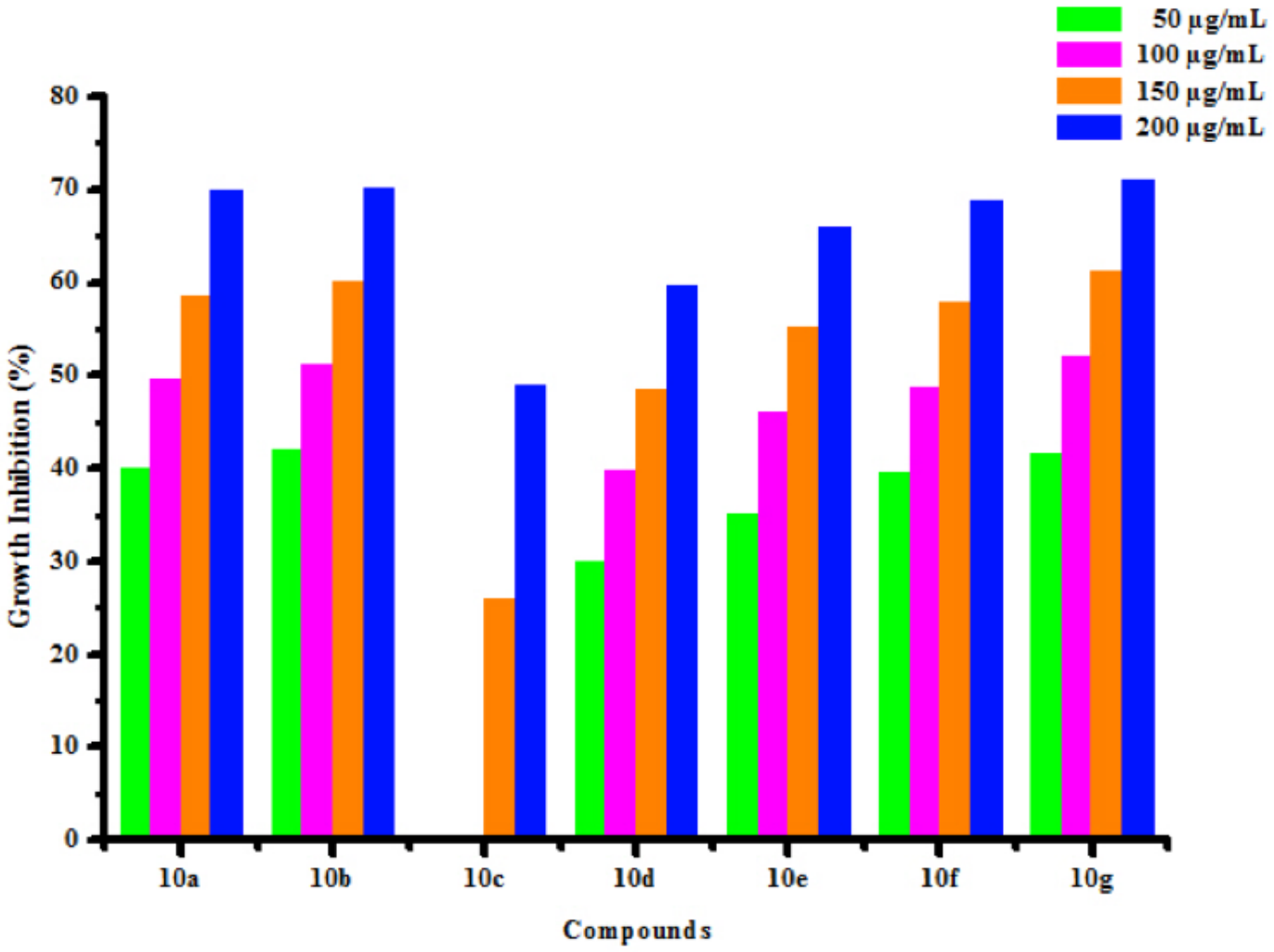

Figure 10

Antifungal activity of synthesized compounds (10a-10g) against Rhizoctonia solani

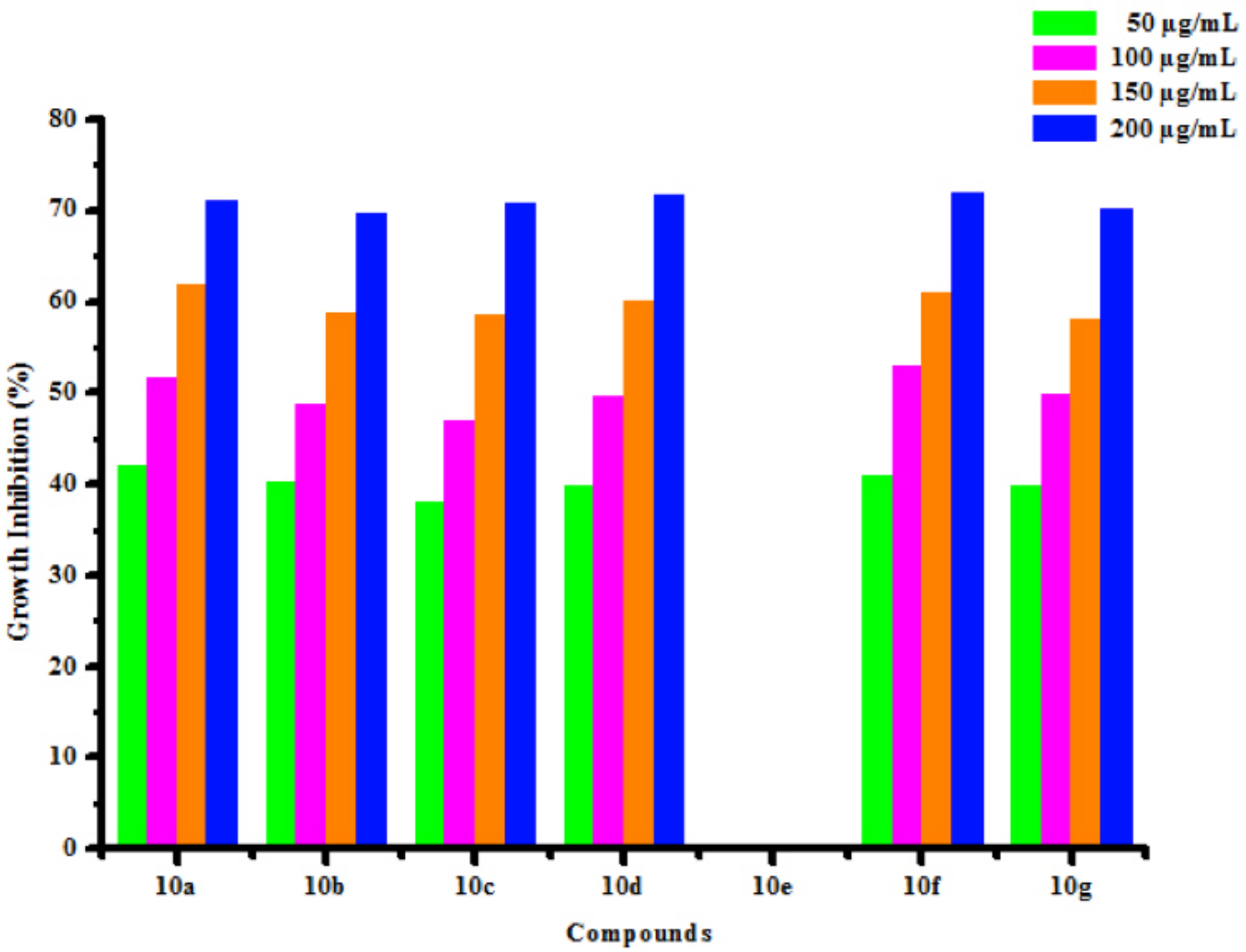

Figure 11

Antifungal activity of synthesized compounds (10a-10g) against Aspergillus niger

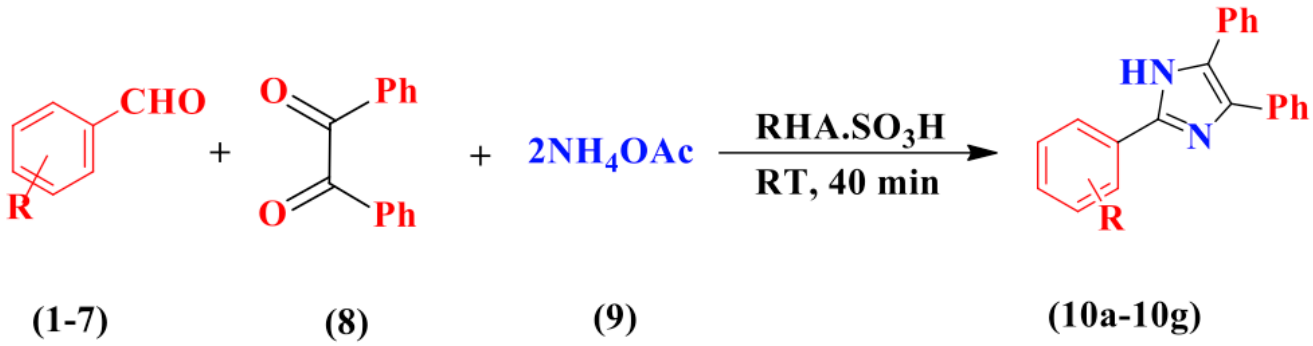

Figure 12

Scheme 1: Synthesis of substituted imidazoles (10a-10g)

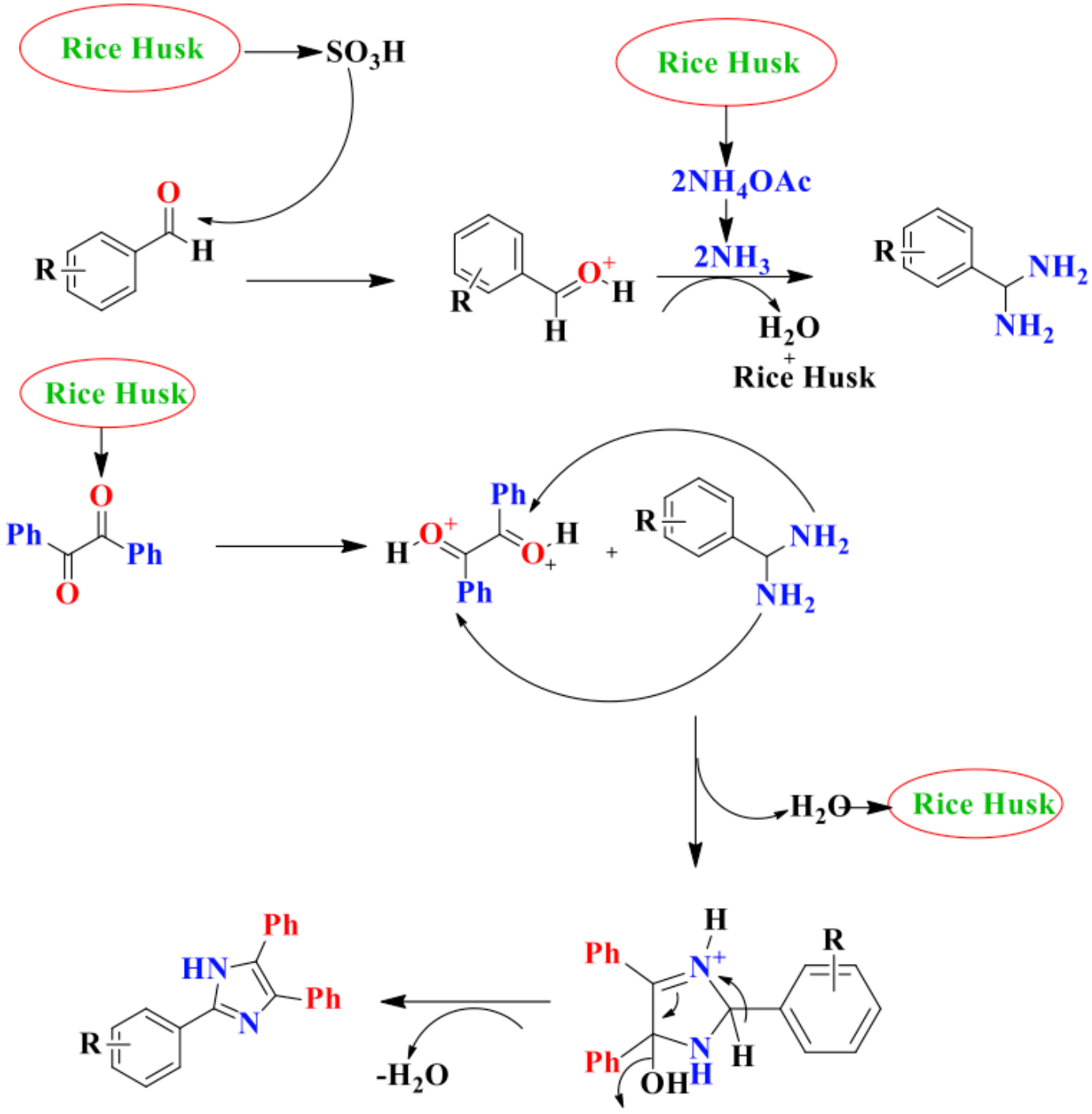

Figure 13

Scheme 2: Plausible mechanism for synthesis of substituted imidazoles (10a-10g)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table3.docx

- Onlinefloatimage1.png