Synthesis of 1-phenylazo-2-naphthol and evaluation of its fungicidal potential against *Sclerotium rolfsii*

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Abstract

Many azo compounds are known for their antifungal properties. In the present study, 1-phenylazo-2-naphthol ($C_{16}H_{12}N_2O$) was synthesized as an azo-coupled dye through coupling reaction of phenyl diazonium salt and β- naphthol in ice-cold chilled water. The azo coupling preferably occurs at ortho position of the same ring since the charge density gets reinforced at this position. The characterization of the compound was performed through FTIR. The antifungal activity was tested against a highly problematic soil-borne plant pathogen, *Sclerotium rolfsii* Sacc. The compound was dissolved in dimethyl sulfoxide (DMSO) and mixed with malt extract broth to prepare 6 mL of 100 mg mL^{-1} growth medium. Its concentrations in the range of 0.78 to 100 mg mL^{-1} were tested against the fungal pathogen. None of the concentration was found antifungal. In contrast, all the concentrations increased the fungal biomass to variable extents ranging from 0 to 29%. This study concludes that the synthesized compound 1-phenylazo-2-naphthol does not possess antifungal property against *S. rolfsii*.

Keywords: Antifungal, Azo dye, 1-Phenylazo-2-naphthol, *Sclerotium rolfsii.*

Introduction

Sclerotium rolfsii Sacc. is a destructive plant pathogen worldwide that infects more than 500 plant species including nuts, chili, sunflower, maize, soybean, brinjal, cucumber, chickpea, tomato and beans (Bosamia *et al.,* 2020; Ali *et al.,* 2020; Jabeen *et al.*, 2021). It is responsible for substantial yield losses by causing root rot, stem blight, foot rot, damping off, seedling blight, stem rot, sclerotium wilt and collar rot disease in valuable crops (Sun *et al.*, 2020; Sharf *et al.*, 2021). At the initial stages, the observed disease symptoms in susceptible plants include yellowing, which is soon followed by wilting and plant death (Nazerian and Ashnaei, 2021). It produces white mycelia on the infected plant stem and characteristically exhibits small, uniform-sized, tan to brown sclerotial bodies on the stem epidermis near the soil line (Cer and Morca, 2020). It grows well at temperature range between 25–30 °C and at intermediate soil moisture levels (Tarafdar *et al.*, 2018). Keeping this in view, there is a strong need to develop fast and reliable management strategies.

The efforts to control *S. rolfsii* have inadequate success due to the pathogen's prolific growth, wide host range and the aptitude to yield a huge number of sclerotia (Murthy *et al.*, 2018). So far, it can be managed through integrated (Ali *et al.*, 2020), biological (Javaid *et al.,* 2020; 2021), botanical (Javaid and Khan, 2016; Khan *et al.,* 2020) and chemical approaches (Ram *et al.,* 2020). However, out of these, synthetic products are considered to be the most effective (Khan and Javaid, 2015). Butt *et al.* (2021) reported that metalaxyl+mencozeb fungicide significantly repressed the mycelial and sclerotial growth of *S. rolfsii.* Nine fungicides *viz.* propiconazole, mycobutanil, hexaconazole, mancozeb, captan, copper oxychloride, vinclozoline, thiophenate methyl and carbendazim have also been found effective against *S. rolfsii* (Kumar *et al.,* 2018). However, there is need to explore more synthetic compounds against this pathogen.

Aromatic azo molecules are significant class of compounds being used as dyes, pharmaceuticals, food additives, textiles, indicators, cosmetics and pigments (Al-Rubaie and Mhessn, 2012; Tsemeugne *et al.,* 2017). These compounds are prepared through diazotization and coupling involving reaction of aromatic amines and NaNO2/HCl to prepare a diazonium salt, subsequently coupling with electron-rich aromatics (Kyei *et al.,* 2020). Chemical compounds showing antimicrobial properties may have an azo linkage. Many azo compounds are

known for their antifungal (against *Candida albicans*) and antibacterial (against *Staphylococcus aureus, S. pyrogenes, Enterococcus faecalis, Pseudomonas aeruginosa, Salmonella typhi* and *Escherichia coli*) properties (Adu *et al.,* 2020). The antimicrobial potential of these compounds depends on the type of moiety to which the azo portion of the compound is linked (Shaki *et al.,* 2015; Kyei *et al.,* 2020). This study was conducted to synthesize and evaluate the antifungal potential of 1-phenylazo-2-naphthol against *S. rolfsii*.

Materials and Methods

Aniline (93.13 g mol^{-1}, pure yellowish liquid, b.p. 184 °C, flash point 158 °F), β-naphthol (m.p. 121 to 123 ºC, ≥ 99.99%), sodium nitrite (≥ 99.99%), hydrochloric acid and sodium hydroxide were purchased from Central Chemicals. All reagents were of analytical grade and used without further treatment.

Synthesis of 1-phenylazo-2-naphthol

Aniline (5 g) was dissolved in concentrated hydrochloric acid (16 mL) and 16 mL of water was added in a small conical flask. Diazotization step was carried out by adding of a solution of 4 g of $NaNO_3$ in 20 mL of water. A solution of 2-naphthol was prepared by dissolving 7.8 g of the compound in 45 mL of NaOH (10%) solution. The solution was cooled to 5 °C by placing in an ice bath. The solution was stirred continuously along with slow addition of cold diazonium salt solution resulting in appearance of a redish color that developed to reddish crystals of 1-phenylazo-2-naphthol. The mixture was filtered, airdried in the shade and the powdery granules were kept in a vial (Tanvir *et al.,* 2021).

Antifungal activity

The stock solution of 100 mg mL^{-1} concentration was prepared by dissolving 0.6 g of the synthesized compound was dissolved in 0.5 mL DMSO followed by addition of 5.5 mL autoclaved malt extract broth (MEB). This stock solution was used to prepare lower concentrations viz. 50, 25, 12.50, 6.25, 3.12, 1.56 and 0.78 mg mL^{-1} concentrations by addition of MEB. For comparison, a series of control treatments with the same amount of DMSO in different concentrations was prepared. Experiment was carried out in 5-mL volume test tubes, each contained 1 mL growth media. A suspension of *S. rolfsii* (50 µL) was added to each test tube and the tubes were incubated at 28 °C. Experiment was conducted in triplicate.

Statistical analysis

The statistical analysis of the data was performed by ANOVA followed by application of LSD test (P≤5%) using Statistix 8.1 software.

Results and Discussion

Physical characteristics of the synthesized compound

Fig. 1B represents the physical appearance of synthesized azo dye 1-phenylazo-2-naphthol. The product appeared as red crystalline solid with sharp melting point around 178 ºC.

FTIR analysis of 1-phenylazo-2-naphthol

The azo coupling possibly occurs at ortho position of the same ring because the charge density gets reinforced at this position. The FTIR spectrum of the synthesized 1-phenylazo-2-naphthol and the proposed route of its preparation are presentended in Fig. 2 and 3, respectively. The broad peak at 3550-3215 cm^{-1} shows the presence of H-bonded hydroxyl (-OH) groups, whereas the peaks at 2900–2950 cm^{-1} correspond to sp2 hybridized methine (-CH) groups of Ph-CH. Sharp peak at the 1510-1500 cm^{-1} indicates the azo group (-N=N-) in 1-phenylazo-2-naphthol. The confirmation of benzene ring was done by the presence of different peaks around 1620–1680 cm^{-1} (C=C-H). The typical peak 1450–1400 cm^{-1} displays the bending frequency of –NH-group under the influence of azo coupling groups.

Antifungal activity

None of the concentrations of the synthesized compound 1-phenylazo-2-naphthol showed antifungal activity against *S. rolfsii.* In contrast to that, these concentrations of the compounds variably increased fungal biomass by 0–29% over control (Fig. 3). Diab *et al.* (2015) found that azo dye derivatives and their metal ion complexes had pronounced antimicrobial activities. Moreover, these have chelating properties that are involved in metal transport across the fungal or bacterial membranes and if atoms production has interfered then these can notably boost the growth of microbes. In lines with the findings of the present study, El-Sonbati *et al.* (2017) reported that azo dyes prepared from Ni (II) complex had no antifungal potential against *Alternaria alternata* and *Fusarium oxysporum.* Likewise, naphthol-based azo dyes were synthesized and tested against many fungal and bacterial pathogens (Çanakc and Serin, 2020). The findings showed that M1 had no adverse effect on growth of the tested fungi namely *Candida albicans, Saccharomyces cerevisiae* and *Kluyveromyces fragilis.* However, M2 exhibited the least antifungal potential in contrast to ampicillin which was used for comparison analysis while the other compounds showed moderate antifungal activities. In the case of bacterial pathogens such as *Escherichia coli, Klebsiella pneumoniae, Bacillus cereus, Bacillus megaterium, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus brevis,* a variable to no bactericidal effect was noted with the synthesized compounds. Similarly, Patel and Patel (2018) also

worked on novel azo dyes and evaluated their antifungal activities against *Aspergillus clavatus, A. niger* and *C. albicans*. The findings revealed that only two compounds were effective against *C. albicans*, whereas the rest of all derivatives showed very poor efficacy against *A. clavatus* and *A. niger*. The antifungal activities of azo dyes and their derivatives showed distinct behavior towards the different pathogenic fungal strains. This might be the effect of resistance mechanism involved in pathogenic fungal species.

Conclusion

This study concludes that the synthesized compound 1-phenylazo-2-naphthol does not possess antifungal potential against *S. rolfsii*. However, there is chance that if derivatives of this compound are prepared, they may have antifungal properties against this devastating soil-borne fungal pathogen.

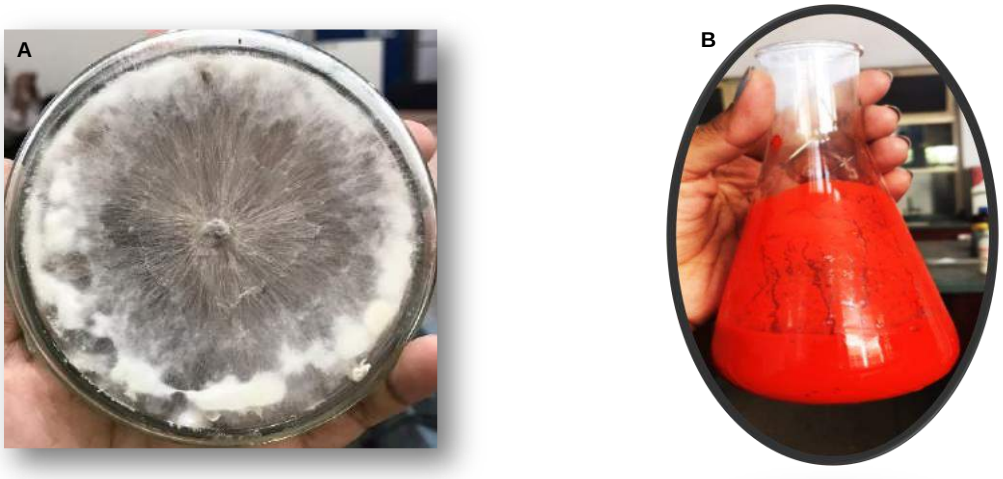

Fig. 1: Pure culture of *Sclerotium rolfsii* (**A**) and the synthesized azo dye 1-phenylazo-2-naphthol (**B**).

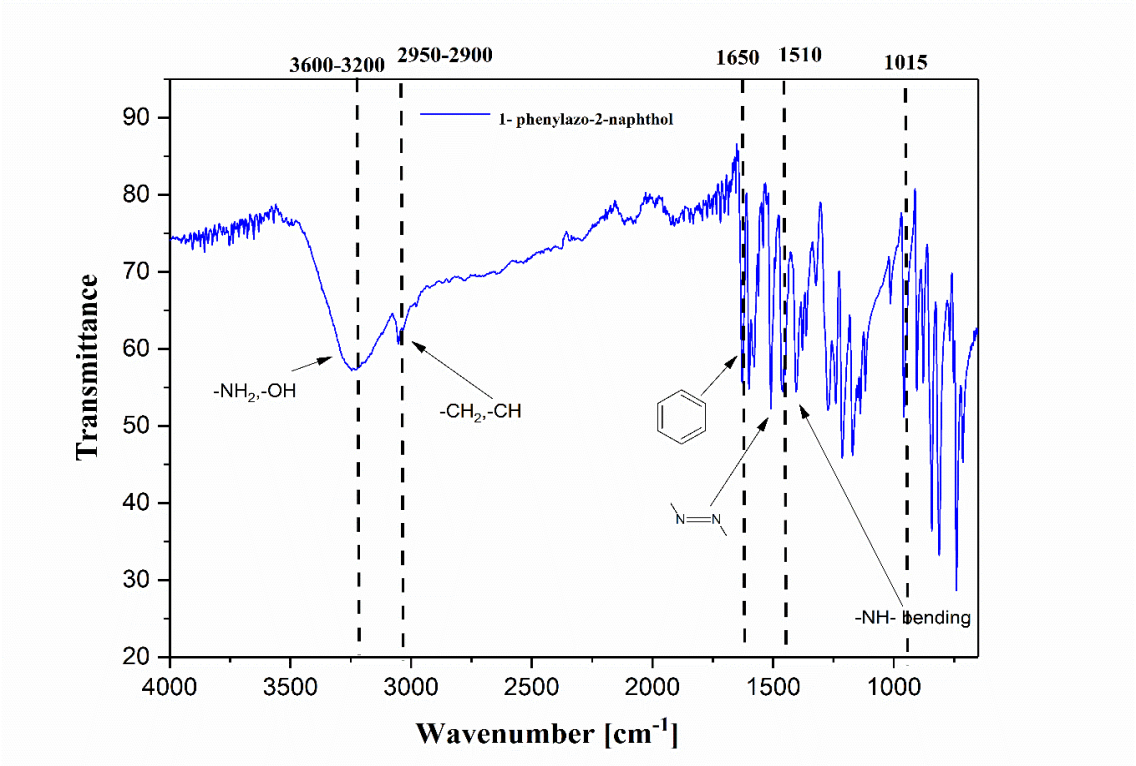

Fig. 2: FTIR spectrum of azo dye 1-phenylazo-2-naphthol.

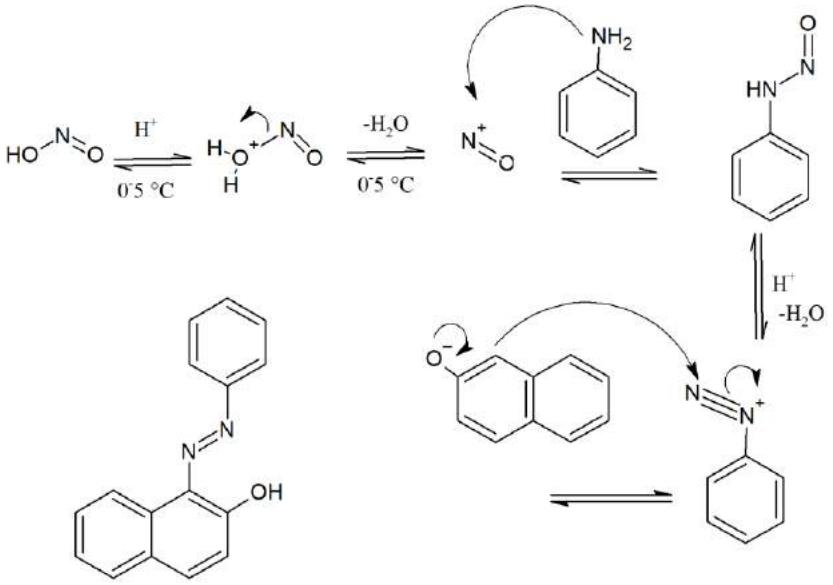

Fig. 3: Proposed route for the synthesis of azo dye 1-phenylazo-2-naphthol.

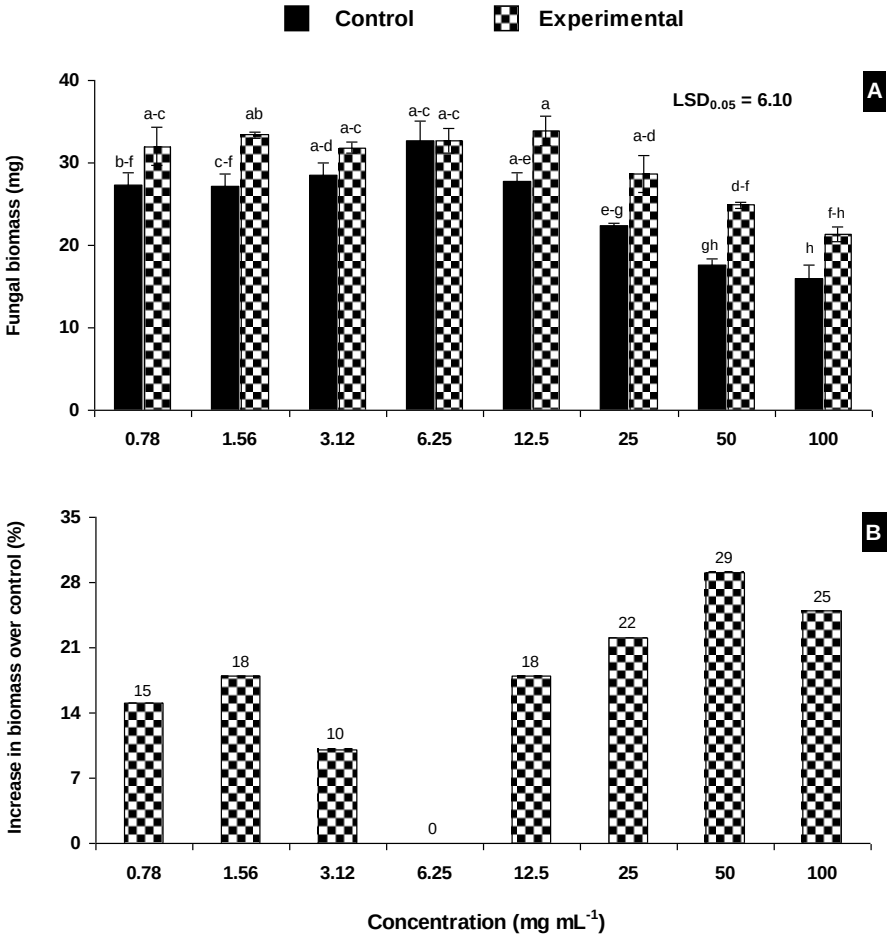

Fig. 4: Effect of different concentrations of azo dye on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

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