Synthesis of 4-phenylazo-1-naphthol and its antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici*

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Abstract

This study is based on the synthesis of 4-phenylazo-1-naphthol ($C_{16}H_{12}N_2O$) as an azo coupled dye through a coupling reaction of phenyl diazonium salt and α- naphthol in ice-cold chilled water. Azo coupling involved an electrophilic substitution reaction of phenyl diazonium cation with α- naphtholate ion, the coupling partner. The 4-phenylazo-1-naphthol was characterized through fourier transform infrared spectroscopy (FTIR). Antifungal activity of 4-phenyl-azo-1-naphthol was checked against *Fusarium oxysporum* f. sp. *lycopersici* (FOL), a soil-borne fungal pathogen causing wilt disease in tomato. Eight concentrations ranging from 0.78 to 100 mg mL^{-1} were tested against the fungus. None of the concentration suppressed the fungal growth. Instead, all the concentrations variably enhanced the fungal biomass over control by 8–28%. This study concludes that the synthesized compound did not possess antifungal potential against FOL.

Keywords: Antifungal agent, Azo dye, Fusarium wilt, Synthesis

Introduction

Fusarium species are ubiquitous in nature and cause soil-borne diseases of great economic importance in horticultural and food crops worldwide (Bodah, 2017; Javaid *et al.,* 2018; Akhtar *et al.,* 2020). In addition to the losses caused by *Fusarium* species, some of them are capable of producing mycotoxins in food and agricultural commodities (Gautier *et al.,* 2020). *F. oxysporum* f. sp. *lycopersici* is one of the most prevalent fungal pathogens that induce vascular wilt of tomatoes (Khurshid *et al.,* 2014; Devi *et al.,* 2022). It is a major soil-borne systemic disease occurring throughout tomato-growing areas and severely affects crops (Kamali *et al.,* 2022). In the absence of a host, FOL can survive indefinitely in infected soils as dormant propagules. The presence of host roots triggers the chlamydospore's germination and infection hyphae adhere to the root surface for penetration. Later on, it infects vascular tissues and inhibits the plant xylem vessels, resulting in vessel clogging, and the appearance of wilt-like symptoms (Srinivas *et al.,* 2019). It displays a unique pathway of infection where fungal mycelium invades intercellularly root cortical cells and enters inside xylem vessels. The pathogen produces microconidia within these vessels and is transported to upper vessels eventually causing the host plant's death (Maurya *et al.,* 2019).

Several disease management strategies such as crop rotation, resistant cultivars, cultural techniques, and biological and chemical control are available against FOL (Javaid and Bashir, 2015; Khurshid *et al.,* 2017; Maurya *et al.,* 2019). Among them, chemical control is an effective measure of controlling FOL (Akram *et al.,* 2018). The application of fungicides namely benzimidazoles, fuberidazole, thiophante, benomyl, carbendazim, thiabendazole, propiconazole and prochloraz have provided substantial control of Fusarium wilt, root rot and crown rot of tomatoes (Nel *et al.,* 2007). Mohamed *et al.* (1980) reported that the application of Vitavax-captan or thiuram as fungicidal treatment is effective in controlling the growth of the pathogen. Copper oxychloride and metamidoxime have also been found effective against FOL as new products (Nedelcu and Alexandri, 1995). In addition, azoxystrobin, iprobenfos, tebuconazole, streptomycin, fentin hydroxide, carbendazim, strobilurin, epoxiconazole, myclobutanil, prochloraz, fosetyl-al, flumorph, bromuconazole, benlate, fludioxonil and pyrimethanil have been reported very effective against FOL (Akram *et al.,* 2018). However, there is need to explore more and more compounds against *Fusarium* species.

Azo dyes are organic compounds containing R−N=N−R′ group where R and R′ are generally aryl groups. Prior to the synthesis of Mauvaine in 1856, the first synthetic organic dye (Filarowski, 2010), roots and leaves of some plant species were used to dye the textile (Tsemeugne *et al.,* 2017). Nowadays, almost all the molecules used in dyeing industry are synthetic, mostly azo dyes (Bae and Freeman, 2007; Rathod and Thakr, 2013). In addition, azo dyes are also known for their pharmaceutical and antimicrobial properties (Sopbué *et al.,* 2013; Gaffer *et al.,* 2016). Therefore, the present investigation was carried out to synthesize 4-phenylazo-1-naphthol and evaluation of its antifungal potential against *F. oxysporum* f. sp. *lycopersici*.

Materials and Methods

Analytical grade aniline (93.13 g mol^{-1}, pure yellowish liquid, b.p. 184 °C, flash point 158 °F), α-naphthol (m.p. 95 to 96 ºC, ≥ 99.99% pure), sodium nitrite (≥ 99.99% pure), hydrochloric acid and sodium hydroxide were procured from central chemicals and used without further treatment.

Synthesis of 4-phenylazo-1-naphthol

Five grams of aniline were dissolved in 16 mL of conc. HCl and the same amount of water in a small beaker. The diazotization step was carried out by adding a solution of 4 g of sodium nitrite in 20 mL of water. Thereafter, a solution of 7.8 g of 1-naphthol in 45 mL of 10% sodium hydroxide was prepared in a beaker. The solution was cooled to 5 °C by immersing in an ice bath ice. The 1-naphthol solution was vigorously stirred followed by slow addition of cold diazonium salt solution developing a dark brown color which later on converted to dark brown crystals of 2-phenylzo 1-naphthol. The mixture was allowed to stand in an ice bath for 30 min with occasional stirring. Filtered the mixture through a funnel, spread it on filter paper with a glass rod, let it dry in indirect sunlight, then put the powdery granules in a vial.

Antifungal activity

To prepare a stock solution of 100 mg mL^{-1} of the synthesized compound, 0.6 g were dissolved in 0.5 mL dimethyl sulfoxide (DMSO) and raised the volume to 6 mL by adding autoclaved malt extract broth (MEB). It was serially double diluted to prepare lower concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 mg mL^{-1}. A similar series of concentrations of control treatments were prepared with the same amount of DMSO but without the compound. To each test tube (5-mL), 1.0 mL of the growth media of different concentrations was poured. Culture of *F. oxysporum* f. sp. *lycopersici* was obtained from Biofertilizers and Biopesticides Lab., Punjab University Lahore (Fig. 1A). and inoculated with 20 µL of *S. rolfsii* was added. Each treatment was replicated three times. Tubes were incubated at 28 °C for 7 days. Thereafter, fungal biomass was collected, dried and weighed (Javaid and Samad, 2012).

Statistical analysis

All the data were analyzed by ANOVA followed by application of LSD test at 5% level of significance using Statistix 8.1.

Results and Discussion

Physical characteristics of the synthesized compound

Fig. 1 B shows the physical appearance of synthesized azo dye 4-phenyl-azo-1-naphthol. The product appeared as crystalline solid with sharp melting point around 165 ºC.

FTIR spectra of the synthesized compound

The azo coupling preferably occurs at the para position of the same ring since the charge density gets reinforced at the para position. It involves an electrophilic substitution reaction of phenyl diazonium cation with the coupling partner α-naphtholate ion. Fig. 2 describes the proposed route for the synthesis of 4-phenylazo-1-naphthol while Fig. 3 displays the FTIR spectrum of the prepared 4-phenylazo-1-naphthol. The broad peak at 3550-3215 cm^{-1} shows the presence of H-bonded hydroxyl (-OH) groups, peaks at 2900–2950 cm^{-1} correspond to sp2 hybridized methine (-CH) groups of Ph-CH. A sharp peak at the 1510-1500 cm^{-1} shows the azo group (-N=N-) in 4-phenylazo-1-naphthol. Benzene ring was confirmed by the presence of different peaks around 1620-1680 cm^{-1}. The (C-O-C) stretching vibrations were around 1016 cm^{-1}. The characteristic peak 1450–1400 cm^{-1} shows the bending frequency of –NH- group under the influence of azo coupling groups. The main characteristic peaks and their assignments are given in Table 1.

Antifungal activity of the synthesized compound

The synthesized azo dye compound, 4-phenyl-azo-1-naphthol did not show antifungal activity against *S. rolfsii.* Instead, all the concentrations of this compound variably increased fungal biomass by 8–28% over control (Fig. 4). Kovac *et al.* (2014) reported that azo compounds are useful and perform different mechanisms of action. These compounds are importance due to their versatile biological activities and functions. In contrast to the findings of the present study, many azo dye compounds have been reported as antifungal in nature (Raghavendra and Kumar, 2015). By contrast, some studies also show that non-effectiveness of azo compounds against fungal growth. Prashantha *et al.* (2021) worked on novel derivatives of azo dyes (6–9) that were prepared by amino-methylbenzoic acid diazotization and tested them against *Fusarium oxysporum*. The findings were in accordance with present studies where dyes

6 and 8 exhibited moderate antifungal activities, whereas dyes 7 and 9 were not effective. Similarly, some new azo dyes were synthesized by trichlorotriazine moieties and pyrazole and their antimicrobial potential was evaluated against *Candida albicans* (Rizk *et al.,* 2015). One series of dyes significantly inhibited the mycelial growth of *C. albicans* while the rest of tested compounds were not effective against the fungal pathogen. Likewise, Singh *et al.* (2014) prepared a series of novel azo dyes and evaluated them against *C. albicans, Aspergillus flavus* and *A. niger*. Among the tested compounds only two were found effective against the tested fungal species. The same findings were given by Kumar *et al.* (2013) where only one compound showed antifungal potential while rest of the dyes were not antifungal in nature.

Conclusion

This study concludes that the synthesized compound 4-phenylazo-1-naphthol did not show antifungal activity against *S. rolfsii.* Further studies are suggested to check antifungal activity of this compound against other phytopathogens. In addition, derivatives of this compound can be prepared to enhance its antifungal properties.

Table 1: The main characteristic IR absorption peaks in 4-phenylazo-1-naphthol and their assignments.

4-phenylazo-1-naphthol	**Assignments**
b 3600-3250 cm^{-1}	*v*O-H stretching
m 3050-2850 cm^{-1}	*vC*-H stretching (sp^2 and $sp^{3)}$
m 1614 cm^{-1}	*v*C-C stretching
vs 1549 cm^{-1}	*v*CC + *v*CH naphthol
m 1478-1531 cm^{-1}	*v*CC +*v*CH +*v*CN
b 1406 cm^{-1}	*v*CO + *v*CH + *v*CN +*v*CC
teath like 1478-1531 cm^{-1}	aromatic *v*C=C
s 1215 cm^{-1}	bending C-N
s 1504-1530 cm^{-1}	*v*N=N stretching
s 1000-1015 cm^{-1}	*v*C-O stretching
s 757 cm^{-1}	*w*CH naphthol

Abbreviations: vs: very strong, w: weak and m: medium.

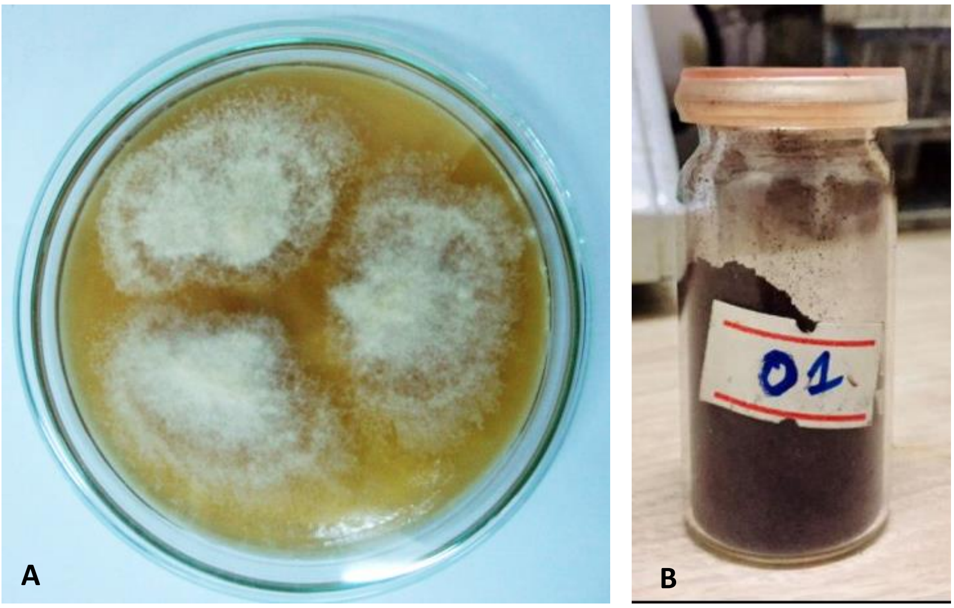

Fig. 1: Pure culture of *Fusarium oxysporum* f. sp. *lycopersici* (**A**) and synthesized azo dye 4-phenyl-azo-1-naphthol (**B**).

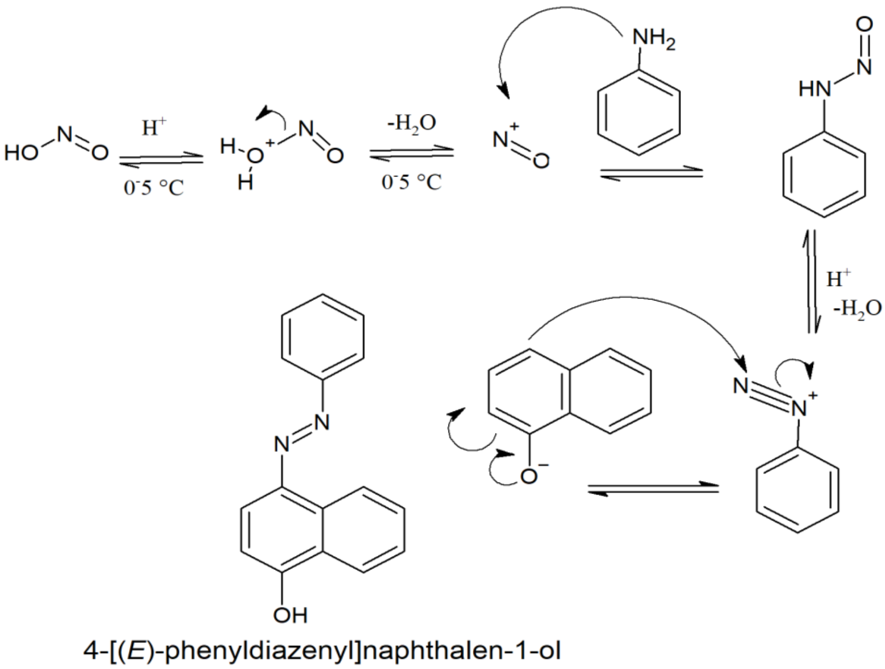

Fig. 2: Proposed route for the synthesis of 4-phenylazo-1-naphthol.

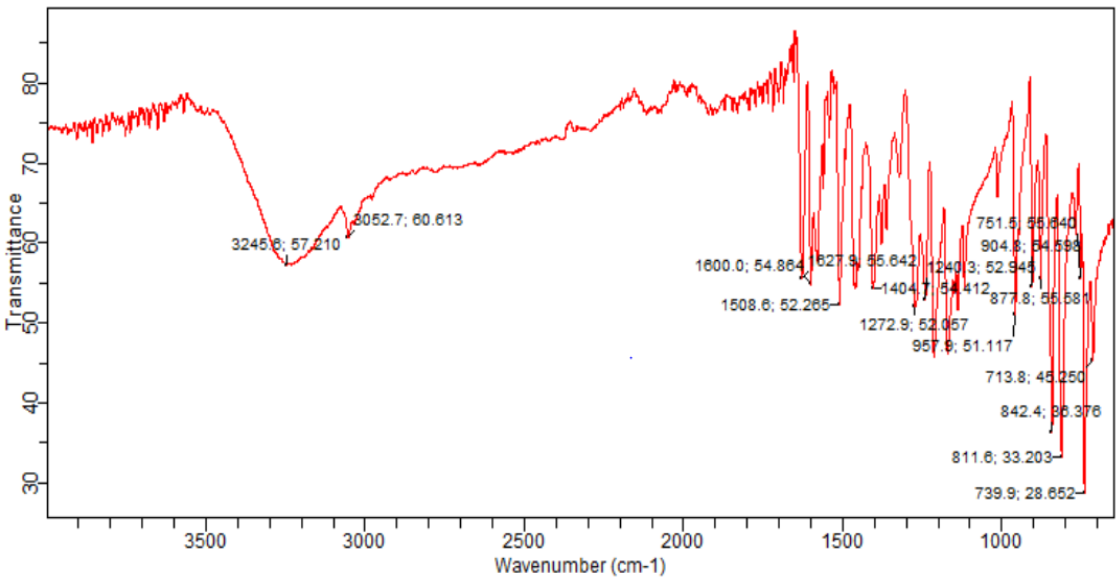

Fig. 3: FTIR spectrum of 4-phenylazo-1-naphthol.

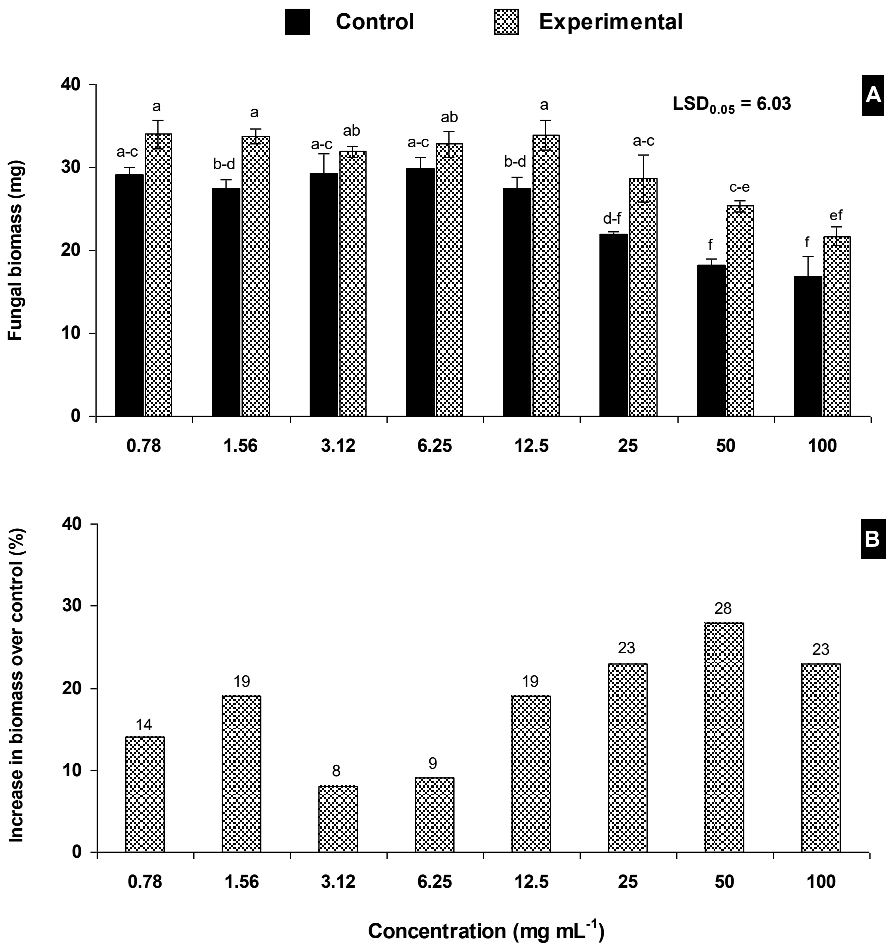

Fig. 4: Effect of different concentrations of azo dye on the growth of Fusarium oxysporum. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

References

Akhtar R, Javaid A, Qureshi MZ, 2020. Bioactive constituents of shoot extracts of *Sisymbrium irio* against *Fusarium oxysporum* f. sp. *cepae. Planta Daninha,* **38**: Article e020200961.

Akram S, Khan SM, Khan MF, Khan HU, Tariq A, Umar UU, Gill A, 2018. Antifungal activity of different systemic fungicides against *Fusarium oxysporum* f. sp. *lycopersici* associated with tomato wilt and emergence of resistance in the pathogen. *Pak. J. Phytopathol.,* **30**: 169-176.

Bae JS, Freeman HS, 2007. Aquatic toxicity evaluation of new direct dyes to the Daphnia magna. *Dyes Pigm.,* **73**: 81-85.

Bodah ET, 2017. Root rot diseases in plants: a review of common causal agents and management strategies. *Agric. Res. Technol.,* **5**: Article 555661.

Devi NO, Tombisana RKD, Debbarma M, Hajong M, Thokchom S, 2022. Effect of endophytic *Bacillus* and arbuscular mycorrhiza fungi (AMF) against Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. *Egypt. J. Biol. Pest Control,* **32**: 1-14.

Filarowski A, 2010. Perkin's mauve: the history of the chemistry. *Resonance,* **15**: 850-855.

Gaffer HE, Fouda MMG, Khalifa ME, 2016. Synthesis of some novel 2-amino-5-arylazothiazole disperse dyes for dyeing polyester fabrics and their antimicrobial activity. *Molecules,* **21**: 122-131.

Gautier C, Pinson-Gadais L, Richard-Forget F, 2020. Fusarium mycotoxins enniatins: An updated review of their occurrence, the producing *Fusarium* species, and the abiotic determinants of their accumulation in crop harvests. *J. Agric. Food Chem.,* **68**: 4788-4798.

Javaid A, Samad S. 2012. Screening of allelopathic trees for their antifungal potential against *Alternaria alternata* strains isolated from dying-back *Eucalyptus* spp. Nat. Prod. Res., 26: 1697-1702.

Javaid A, Bashir A (2015). Radish extracts as natural fungicides for management of *Fusarium* *oxysporum* f. sp. *lycopersici*, the cause of tomato wilt. *Pak. J. Bot.,* **47**: 321-324.

Javaid A, Latif U, Akhtar N, Ahmed D, Perveen S, 2018. Molecular characterization of *Fusarium moniliforme* and its management by methanolic extract of *Coronopus didymus. Pak. J. Bot.,* **50**: 2069-2075.

Kamali M, Guo D, Naeimi S, Ahmadi J, 2022. Perception of biocontrol potential of *Bacillus inaquosorum* KR2-7 against tomato Fusarium wilt through merging genome mining with chemical analysis. *Biology*, **11**: Article 137.

Khurshid S, Shoaib A, Javaid A, 2014. *In vitro* toxicity evaluation of culture filtrates of *Fusarium oxysporum* f. sp. *lycopersici* on growth and physiology of tomato under chromium (VI) stress. *J. Anim. Plant Sci.,* **24**: 1241-1245.

Khurshid S, Shoaib A, Javaid A, Akhtar F, Shafique M, Qaisar U, 2017. Management of Fusarium wilt of tomato by soil amendment with *Cenchrus pennisetiformis* under chromium stress. *Physiol. Mol. Plant Pathol.,* **97**: 58-68.

Kovac J, Gavaric N, Bucar F, Mozina S, 2014. Antimicrobial and resistance modulatory activity of Alpinia katsumadai seed phenolic extract, essential oil and post-distillation extract. *Food Technol. Biotechnol.,* **52**: 248-254.

Kumar CK, Keshavayya J, Rajesh T, Peethambar SK, Ali RS, 2013. Synthesis, characterization and antimicrobial activity of heterocyclic azodyes derived from thiadiazole. *Chem. Sci. Trans.,* **2**: 1346-1351.

Maurya S, Dubey S, Kumari R, Verma R, 2019. Management tactics for Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.): A review. *Management,* **4**: 1-7.

Mohamed HA, Ibrahim AN, Zaher EA, Abdelal HR, Omar SA, 1980. Fungicidal control of clover seedling diseases, and determination of Benlate and Vitavax/captan in plant organs. *Agric. Res. Rev.,* **58**: 79-90.

Nedelcu L, Alexandri AA, 1995. Synergistic effect between metamidoxime and copper oxychloride. *Probleme de Protectia Plantelor,* **23**: 13-21.

Nel B, Steinberg C, Labuschagne N, Viljoe A, 2007. Evaluation of fungicides and sterilants for potential application in the management of Fusarium wilt of banana. *Crop Prot.,* **26**: 697-705.

Prashantha AG, Ali RS, Keshavayya J, 2021. Synthesis, spectral characterization, DFT studies and antimicrobial activities of amino-methylbenzoic acid based azo dyes. *Inorg. Chem. Commun.,* **127**: Article 108392.

Rathod KM, Thakre NS, 2013. Synthesis and antimicrobial activity of azo compounds containing m-cresol moiety. *Chem. Sci. Trans.,* **2**: 25-28.

Rizk HF, Ibrahim SA, El-Borai MA, 2015. Synthesis, fastness properties, color assessment and antimicrobial activity of some azo reactive dyes having pyrazole moiety. *Dyes Pigm.,* **112**: 86-92.

Raghavendra KR, and Kumar KA, 2015. Synthesis and their antifungal, antihelmentic and dying properties of some novel azo dyes. *Int. J. Pharmaceut. Chem. Biol. Sci.,* **3**: 275-280.

Singh H, Sindhu J, Khurana JM, Sharma C, Aneja KR, 2014. Syntheses, biological evaluation and photophysical studies of novel 1, 2, 3-triazole linked azo dyes. *RSC Adv.,* **4**: 5915-5926.

Sopbué FE, Tsemeugne J, Tamokou J-D-D, Djintchui AN, Kuiaté J-R, Sondengam BL, 2013. Synthesis and antimicrobial activities of some novel thiophene containing azo compounds. *Heterocycl. Commun.,* **19**: 253-259.

Srinivas C, Devi DN, Murthy KN, Mohan CD, Lakshmeesha TR, Singh B, Srivastava RK, 2019. *Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity - A review. *Saudi J. Biol. Sci.,* **26**: 1315-1324.

Tsemeugne J, Fondjo ES, Tamokou JD, Tonle I, Kengne IC, Ngongang AD, Lacmata ST, Rohand T, Kuiate JR, Sondengam BL, 2017. Electrochemical behavior and in-vitro antimicrobial screening of some thienylazoaryls dyes. *BMC Chem.,* **11**: Article 119.