

Synthesis, Physicochemical and Microbial Evaluation of 4-Bromophenylazo-2-Naphtholazo-4¹-Chlorobenzene (A Novel Disazo Dye)

Odilora, C. A; Orakwue, F. C.; Iloh, E. O; Okwuego, P. O.

Department of Pure and Industrial Chemistry,
Chukwuemeka Odumegwu Ojukwu University, Uli. Anambra State. Nigeria

ABSTRACT

Azo dyes are the most important synthetic colourants which have been widely used in textile, paper manufacturing and printing. A novel disazo dye, 4-Bromophenylazo-2-naphtholazo-4¹-chlorobenzene was synthesized and characterized using UV-Visible, FTIR spectrophotometric and elemental analytical tools. The result of the elemental analysis was found to be in conformity with the molecular formula of the dye. The UV-Visible spectra indicated that the dye absorbed at visible region of 479.0 nm, characteristics of intensely coloured dyes. The FTIR spectral study showed absorption bands conforming to the various functional groups in the dye. The colour imparted by the dye on the tested fibres using Gray Scale for colour change, indicated excellent lightfastness, excellent heatfastness, and poor washfastness except nylon which showed fair washfastness. The toxicological testing indicated that the dye has no effect on the DNA of *E.coli*, and *A.niger* but at high concentration, it denatured human DNA. The dye does not have adverse effect on the growth of *zea mays* used for growth profile evaluation. Microbial toxicity of the synthesized dye on *E.coli*, *A.niger* and *Bacillus cereus* showed that all the strains are moderately resistant to the dye.

KEYWORDS: Dye, 4-Bromoaniline, environment, fastness, toxicology, DNA.

INTRODUCTION

Across the world, in different industries like textile, leather, paper, food, *etcetera*, commercial dyes find huge applications. Synthetic organic colourants are often made from petroleum compounds which are carbon based. Most of the synthetic pigments except carbon black are not stable and wear away at the time of application (Alessia, *et al*, 2017). Synthesis of dyes had witnessed increase in a bid to obtain dyes with better fastness properties. Modern colourants are synthetic in nature, and majority of the commercial dyes belong to azo dyes. Depending on the chemical features, these dyes fall into several categories defined by the features for which they have affinity or by the methods by which they are applied (Fernandez, 2004). Several researchers have synthesized disperse dyes with modified molecular structures aimed at enhancing properties like colour fastness, intensity and strength on polyesters. These new disperse dyes include dyes with long conjugation of naphthalene

How to cite this paper: Odilora, C. A | Orakwue, F. C. | Iloh, E. O | Okwuego, P. O. "Synthesis, Physicochemical and Microbial Evaluation of 4-Bromophenylazo-2-Naphtholazo-4¹-Chlorobenzene (A Novel Disazo Dye)" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-10 | Issue-4, August 2026, pp.29-36, URL: www.ijtsrd.com/papers/ijtsrd141901.pdf



IJTSRD141901

Copyright © 2026 by author (s) and International Journal of Trend in Scientific Research and Development Journal. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0) (<http://creativecommons.org/licenses/by/4.0>)



ring (Mabhai *et al*, 2022), pyrazole-based dyes (Omar *et al*, 2023). The growing demand for dyes of high fastness ability, effective and of low environmental impact which would ensure that a synthesized dye achieves its intended goal gave rise to this study.

Materials and Methods

Materials

All the reagents used were of the purest grades and were used as supplied unless stated otherwise. The microorganisms were isolates from Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State. Nigeria.

Methods

Preparation of 2-Naphthol solution

Solution of 2-naphthol was prepared by dissolving 14.4 g of the compound in 150.0 cm³ of 2 M solution of sodium hydroxide.

Preparation of sodium nitrite solution

A 50 cm³ of 2 M solution of sodium nitrite was prepared by dissolving 6.9 g of sodium nitrite in 50 cm³ of distilled water..

First diazotization

A 22.0 cm³ of concentrated hydrochloric acid was mixed with 100.0 cm³ of distilled water. The solution was used to dissolve 17.2 g of 4-chloroaniline and the solution formed was cooled to 0 °C. Then, cold 50.0 cm³ of the prepared sodium nitrite solution was slowly added to the solution of 4-chloroaniline with stirring for 10 minutes, maintaining the temperature to 0 °C to give a 4-chlorobenzenediazonium salt solution.. Thereafter, the solution of the prepared 2-naphthol was coupled with the diazonium salt solution by mixing the solutions slowly with stirring for 30 minutes. A red dye was obtained, filtered, washed with distilled water, dried and then recrystallized in acetone.

Second diazotization

The second diazotization was done by dissolving 3.6 g of 4-bromoaniline in a mixture of 100.0 cm³ distilled water and 22.0 cm³ concentrated hydrochloric acid and the solution cooled to 0 °C. Then, a 50 cm³ of cold 2 M sodium nitrite solution prepared by dissolving 6.9 g of sodium nitrite in 50 cm³ of distilled water was slowly added to the 4-bromoaniline solution with stirring for 10 minutes, maintaining the temperature at 0 °C. This resulted to the formation of 4-bromobenzenediazonium salt. Sodium hydroxide solution prepared by dissolving 1.5 g of sodium hydroxide pellets in 38 cm³ of distilled water was used to dissolve 7.0 g of the monoazo dye prepared previously.

This was coupled to the 4-bromobenzene diazonium salt solution over 30 minutes with continuous stirring. The dye obtained was filtered with Whatman No. 11cm filter paper, washed with distilled water, and dried in free air for 24 hours.

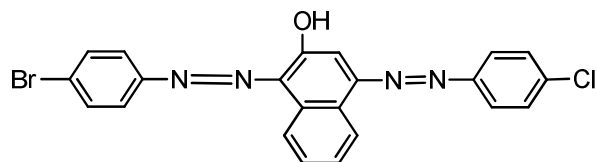


Fig 1: 4-Bromophenylazo-2-naphtholazo-4-chlorobenzene

Chromatographic Analysis And Percentage Yield Determination.

The purity of the prepared dye sample was checked by Thin Layer Chromatography on a rectangular silica gel TLC plate (3.5 cm x 10 cm) using 3-methoxy ethane/water/acetone solvent mixture (5:2:3 ratio) as the mobile solvent, and the retardation factor

(R_f) value was calculated by the mathematical expression,

$$R_f = \frac{\text{Distance moved by the sample}}{\text{Distance moved by the solvent}}$$

The percentage yield was determined using the relationship

$$\% \text{ yield} = \frac{MR}{MMR} \div \frac{MP}{MMP} \times \frac{100}{1}$$

Where MR = mass of the reactant
MMR = molar mass of the reactant
MP = mass of the product
MMP = molar mass of the product

Physical Measurement

Melting point of the dye was determined using Perkin Elmer DSC 8500 melting point apparatus. The elements present were detected using Thermo Jarrel Ash IRIS Inductively Coupled Plasma Optical Emission Spectrometer.

UV-Visible absorption spectra of the dye scanned from 900nm to 200nm using 10.0 cm³ methanol, was obtained on Jenway model 6850 spectrophotometer.

Infrared (IR) spectra of the compound, was recorded on a Perkin Elmer 100FTIR spectrophotometer.

Dyeing of fabrics

The pieces of Doctor Flannel worsted wool, fine worsted wool, 100% cotton, polyester, acrylic fibre and nylon, (each 2 x 3 cm) were respectively immersed in methanol-dye solution contained in a clean 250 cm³ beaker and continuously stirred gently with a glass rod for 50 minutes. It was thereafter allowed to stand for 24 hours with occasional swirling of the beaker. Then, the pieces of dyed fabrics were carefully removed from the beaker, rinsed with enough distilled water, placed on a white cardboard paper and allowed to dry in a free air for 24 hours (Odilora and Omatseye, 2004; Mohammed; Bello, 2010 and Odilora *et al* 2025).

Heatfastness Evaluation

Heatfastness evaluation was carried out using the method described by Ukponmwen *et al* (2006). The dyed fabrics were placed on a white background flat surface. They were heat-pressed with an electric pressing iron at a medium temperature of 60 °C for 30 seconds.

Lightfastness Evaluation

Lightfastness evaluation was done using the method described by Odilora *et al* (2001). The dyed fabrics were firmly affixed to a white cardboard paper with office pin and exposed to hot sunlight for 2 hours. Thereafter, the exposed dyed fabrics were compared with the unexposed fabrics (control samples), to see if any colour change has occurred.

Washfastness Evaluation

Washfastness test was conducted as described by Odilora and Ogaga (2006); Odilora *et al* (2025) “Good Mama” detergent solution was prepared by dissolving 0.5 g of the detergent in 30cm³ of distilled water in a 250 cm³ beakers at a temperature of 45 °C separately. The dyed fabrics were immersed in the solutions respectively and vigorously stirred continuously for 5 minutes with a glass rod. The washed fabrics were then carefully removed from the detergent solution, rinsed with sufficient water, placed in a white cardboard paper and left to dry in free air for 24 hours.

Gray Scale Assessment

Analysis of the results of the fastness tests was done using the American Association of Textile Chemists and Colorists (A.A.T.C.C) Gray Scale for Colour Change. The fastness-tested dyed fabrics were compared with the control samples (corresponding to the untested dyed fabrics) to determine if any colour change(s) had occurred and to what extent if it did occur. The Gray Scale is graded 1-5 where 1 stands for very poor result and 5 for excellent result (A.A.T.C.C (1994) ; Ukponmwen *et al*, (1999)

Microbial Evaluation

The microbial evaluation was done by genotoxicity testing, seed germination bioassay and beneficial bacterial toxicity.

Genomic DNA extraction

Genomic DNA of *E.coli*, *Aspergillus niger* and human blood were extracted using Quick-DNA™ Fungal/Bacterial Miniprep Kit according to recommended protocol (Zymo Research).

About 50-100 mg (wet weight) pure fungi cells that had resuspended in up to 200 µL of water was added to a ZR bashing bead lysis tube (0.1 mm and 0.5 mm). Seven hundred and fifty (750 µL) bashing bead™ buffer was added to the tube and vortexed at maximum speed for ≥ 15 min. ZR Bashing Bead™ Lysis tube (0.1 and 0.5 mm) was centrifuged in a microcentrifuge at 10,000 g for 1min. Thereafter, 400µL of the supernatant liquid was added to a zymo-Spin™ III-F filter in a collection tube and centrifuged at 8000 g for 1 min. About 1,200 µL of genomic lysis buffer was added to the filtrate in the collection tube after which 800µL of the mixture was transferred to a zymo-spin™ IIC column in a collection tube and centrifuged at 10,000 g for 1min. The flow through was discarded from the collection tube and the step repeated. About 200 µL DNA pre-washed buffer was added to the Zymo-Spin™ IIC column in a new collection tube and centrifuged at 10,000 x g for 1 min. About 500 µL DNA wash buffer was as well added to the zymo-spin™ IIC column and

centrifuged at 10,000 g for 1 min. The zymo-Spin™ IIC column was then transferred to a clean 1.5 mL microcentrifuge tube and 60 µL DNA elution buffer was added directly to the column matrix. Centrifugation was finally done at 10,000 g for 30 sec to elute the DNA.

Protocol for Bacterial Extraction

Genomic DNA was extracted using Quick-DNA™ Miniprep Plus Kit (Zymo Research), according to recommended protocol.

Each sample 200 µl was added to a microcentrifuge tube. BioFluid and Cell Buffer (200 µl) and 20 µl of proteinase K were added to it and mixed thoroughly using a vortex for 10-15 seconds and the tube containing the mixture incubated at 55 °C for ten minutes on a heating block. One volume Genomic Binding Buffer (i.e. 420 µl) was added to the digested sample and mixed thoroughly with a vortex mixer for 10-15 seconds. The mixture was then transferred to a Zymo-spin™ IIC-XL Column in a new Collection Tube and centrifuged at 12000 g for 1 minute. The collection tube was discarded with the flow through. DNA Pre-Wash Buffer (400 µl) was added to the spin column in a new Collection Tube and centrifuged at 12,000 g for 1 minute. The spin column was then transferred to clean microcentrifuge tube. DNA Elution Buffer (50 µl) was added directly on the matrix and incubated for 5 minutes at room temperature, and then centrifuged at maximum speed for 1 minute to elute the DNA. The eluted DNA was stored at ≤ -20 °C for future use.

Human DNA Extraction

Genomic DNA was extracted using QIAamp^R Blood Mini Kit (QIAGEN), according to recommended protocol.

Protocol

QIAGEN Proteinase(20 µl) was added into a 1.5 cm³ microcentrifuge tube. The sample (200 µl) and 200 µl of Buffer AL were added to it and mixed thoroughly using a votex for 10-15 seconds and then incubated the tube at 56 °C for 10 minutes on a heating block. The 1.5 cm³ microcentrifuge tube was centrifuged to remove drops of the mixture from the lid. Ethanol (96-100%) (200 µl) was added and mixed thoroughly by votex. Tube was also centrifuged to remove drops from the lid. The mixture was then transferred to a QIAamp Mini spin Column in a 2 cm³ Collection Tube and centrifuged at 6000 x g(8000 rpm) for 1 minute. The collection tube was discarded with the flow. Buffer AW2 500 µl was added to the spin column in a new Collection Tube and centrifuged at full speed for 1 minute (This eliminates the chance of possible Buffer AW2 carryover). The spin column was then transferred to a clean 1.5 cm³

microcentrifuge. Buffer AE(200 μ l) was added directly on the matrix and incubated for 1 minute below room temperature (15-25 $^{\circ}$ C), then centrifuged at 6,000 x g(8000 rpm) for 1 minute to elute the DNA. The eluted DNA was stored at \leq -20 $^{\circ}$ C for future use.

DNA Treatment with Synthesized Dye

The aliquots of the chemically synthesized dye at different concentrations (25 – 100 %) were added into the purified DNA sample, incubated at 37 $^{\circ}$ C for about 24 hours in order to determine the effect of the dye on the isolate.

Agarose gel electrophoresis

Two percent (2 %) agarose gel was prepared by dissolving 1.2 g of agarose in 60 mL of 1X TAE buffer. The mixture was heated to a clear solution using a microwave oven and allowed to cool to about 50 $^{\circ}$ C. About 3 μ L of ethidium bromide was added into the solution and mixed thoroughly. The agarose preparation was carefully poured into a gel tray with the gel comb in place and allowed to solidify. The tray was loaded into the gel tank and 1X TAE buffer was poured into the tank, making sure that the gel was properly submerged. The gel comb was carefully removed and 5 μ L of exposed DNA was mixed with 2 μ L of loading dye and loaded into the holes. The tank was connected to the power pack and set to run at 100 volts for 20 min. The bands were viewed using the gel documentation system (VILBER, GERMANY) (Uba, 2019).

Seed Germination Bioassay

The effect of synthesized dye at different concentrations was observed on *Zea mays* seed germination. The modified guideline for the testing of effluents on seeds of terrestrial plants according to the Organization for Economic Cooperation Development (OECD (1984) was used for this study. First, floatation method was adopted to determine the viability of the seeds. The seeds that sink represent the viable seed while the seeds that float represent

seeds that are nonviable. Surface sterilization of the viable seeds was then performed by immersing them into 70 % ethanol for 5 minutes to reduce contamination and finally washed thoroughly with water. Ten maize seedlings were placed in Petri dishes lined with tissue paper containing 20 mL of the filtered dye at different concentrations (25 – 100 %) while the Petri dishes lined with tissue paper containing 20 mL of sterile distilled water served as the control. The investigation of each concentration including the control was carried out in triplicates. The Petri dishes were covered with lids to prevent evaporation. The seeds were planted under room temperature for seven (7) days, after which the seed germination (%), shoot length (cm), root length (cm), relative root length percent and germination index (%) were measured and calculated (Gopinathan and Thirumarthy, 2012). The shoot length measured was taken from the base to the apical leaf of the plant using a transparent ruler; while the root length was also measured by the same procedure after it was harvested and carefully washed with distilled water.

Beneficial Bacterial Toxicity

The effect of synthesized dye at different concentrations was observed on selected important bacterial strains' growth by measuring zones of inhibition using disc diffusion technique. In this method, nutrient broth and potato dextrose broth media were used to develop isolate *E. coli*, *Bacillus cereus*, and *Aspergillus niger*. After incubation for 48 hr, the surface of Muller Hinton agar plate was seeded with 48 hr grown *E. coli*, *Bacillus cereus*, and *Aspergillus niger* test organisms. Sterile filter paper discs impregnated with different concentrations of the dye (25 -100 %) and sterile distilled water as control were kept on the seeded bacterial cells at equidistance and pressed lightly and kept at 30 $^{\circ}$ C for 48 h; observation for zone of inhibition was made (if any) (Rani *et al*, 2014; Akpuaka *et al* 2003)

RESULTS AND DISCUSSION

The synthesized dye has been investigated for its percentage yield, retardation factor, melting point, elemental composition, spectral analysis, fastness tests and microbial assay. The physicochemical data is recorded in table 1.

Table 1: Physicochemical data of the dye

| Colour | % yield | Rf value | Melting Point ($^{\circ}$ C) |
|--------|---------|----------|-------------------------------|
| Red | 92.52 | 0.82 | 307 |

The red-coloured dye gave high percentage yield. Only one spot was observed on the thin layer chromatographic plate, showing that the synthesized dye was pure.

The high melting point of the dye could be due to its high intermolecular interactions which gave rise to strong cohesive bonds. As a result, enormous energy is needed to cleave the bonds (Hari and Vithal, 2001). In addition, the high melting point could be due to other complex factors such as polarity, size, geometry and others.

Table 2 shows the elemental makeup of the dye

| Carbon | | Nitrogen | | Oxygen | | Bromine | | Chlorine | | Hydrogen | | Total % |
|------------|--------|------------|-------|------------|-------|------------|-------|------------|-------|------------|--------|---------|
| No of atom | % | No of atom | % | No of atom | % | No of atom | % | No of atom | % | No of atom | % | |
| 22 | 49.576 | 4 | 9.013 | 1 | 2.153 | 1 | 2.253 | 1 | 2.253 | 14 | 31.548 | 96.896 |

It is observed that the percentage composition of the elements in the dye compound is closely related to the theoretical values.

FTIR Spectroscopic Analysis of the dye

Table 3: Result of FTIR Spectroscopic Analysis of The Dye

| Wave band cm^{-1} | Functional Group | Compound |
|----------------------------|--------------------------------|------------------------|
| 672.25 | C – Br | Bromo |
| 715.73 | C – Cl | Chloro |
| 984.06 | Ring breathing mode | Cyclic |
| 1148.89 | C – X | Aromatic halogen |
| 1278.60 | C – N stretch | Aromatic amine |
| 1364.74 | NO_2 | Aromatic nitro |
| 1460.82 | N = N | Azo |
| 1515.47 | C = C | Aromatic |
| 1700.95 | C = C | Aromatic |
| 1898.94 | C – H out of plane deformation | Aromatic |
| 2203.26 | NH | Amine halohalide |
| 2361.45 | - $\text{N}^+ = \text{N}$ | Diazonium |
| 2463.13 | R – C = N stretch | Nitrile |
| 2567.91 | NH stretch | Amide |
| 2627.94 | $\text{R}_3\text{C}=\text{O}$ | Carbonyl |
| 2696.52 | $\text{R}_2\text{C}=\text{O}$ | Carbonyl |
| 2770.51 | R- COH | Carbonyl |
| 2924.95 | CH stretch | Phenolic |
| 3024.68 | - CH =CH - | Aromatic |
| 3160.17 | C_6H_5 - | Phenyl |
| 3256.32 | NH stretch | Phenol, aromatic amine |
| 3396.27 | NH stretch | Phenol, aromatic amine |
| 3491.99 | R_2NH_3 | Tertiary amine |
| 3616.17 | -OH stretch | Alcohol, phenol |
| 3683.88 | -OH | Alcohol, Phenol |
| 3746.05 | -OH | Alcohol, Phenol |
| 3835.18 | -OH | Alcohol, phenol |
| 3912.81 | -OH | Alcohol, Phenol |

The result of the FTIR in Table 3 supported the suggested structure of the dye, with OH stretch of phenols at 3616.17 to 3912.81 cm^{-1} . The = CH stretch of conjugated aromatics appeared at 3024.68 cm^{-1} . At 2361.45 and 1460.82 cm^{-1} appeared the – N = N stretch of diazonium salt and – N = N – stretch of azo compound. The C – Br stretch of bromine group at 660.47 cm^{-1} confirmed the C – X stretch vibration of aromatic halogen vibration at 1148.89 cm^{-1} .

The UV-Visible Analysis Of The Dye

Table 4 Result of UV-visible of The Dye

| Wave length (λ_{max})(nm) | Bond | Transition |
|--------------------------------------------|--------|-------------------------|
| 243.0 | C = C | $\pi \rightarrow \pi^*$ |
| 312.0 | C = C | $\pi \rightarrow \pi^*$ |
| 479.0 | C – OH | $n \rightarrow \pi^*$ |

UV-visible result of the dye showed the two possible transitions as expected for the investigated Dye 4 (the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$). The transitions depend on the class of the chromophore and the nature of auxochrome in the dye. The presence of OH group in the aromatic ring and two stable azo chromophoric groups (-N=N-)

conjugated with aromatic rings resulted in molecules with intense and highly stable colour throughout the visible region of the light (Bouas-Laurent and Durr, 2001; Coelho *et al*, 2012; Patil *et al*, 2019; Scultz and Julius, 1908). The bands at wavelength 243 and 312 nm were assigned to high energy $\pi \rightarrow \pi^*$ transition. The second band at 479 nm (visible region) was assigned to low energy $n \rightarrow \pi^*$ transition. Increasing conjugation generally involves the absorption at a longer wavelength and finally into the visible region as recorded by Joseph *et al*(1998), Therefore the prepared dye having distinctive peak at 479 nm confirmed it to be conjugated and possess remarkable colour,

Fastness Evaluation

Table5: Fastness evaluation data of the dye

| Parameter | Fabrics | | | | | |
|---------------|--------------------------|-------------------|-------------|-----------|---------------|-------|
| | Dr Flannels worsted Wool | Fine worsted wool | 100% cotton | Polyester | Acrylic fibre | Nylon |
| Lightfastness | 5 | 5 | 5 | 5 | 5 | 5 |
| Heatfastness | 5 | 5 | 5 | 5 | 4-5 | 5 |
| Washfastness | 2 | 2 | 2 | 2-3 | 2 | 3 |

Note: Grade 5=excellent, 4-5=very good, 4=good, 3-4=very fair, 3=fair, 2-3=poor, 1-2=very poor

As shown in Table 5, dyed samples exhibited acceptable lightfastness and heatfastness with rating ranging from 1-5 on the international Geometric Grey Scale (Satam *et al*, 2013; Rizk *et al*, 2015). The dye showed excellent lightfastness and heatfastness on all the fibres except on acrylic fibre where it showed very good heatfastness. This means that the materials retained their colour appearances longer, making it more durable and long-lasting. This observation is consistent with the notion that azo dyes containing electron withdrawing substituent on their diazo components exhibit reduced susceptibility to photofading (Gordan and Gregory, 2012).

One of the critical prerequisites for dyed fabrics is their Scorch fastness, which refers to their ability to withstand heat treatment. The synthesized dye demonstrated very good (4 - 5) to excellent (5) fastness to heat-pressing at 60 °C and excellent photofading resistance.

The dye exhibited poor to fair fastness to washing as measured by the international Geometric

DNA treated with the synthetic dye.

It was observed that the dye did not denature the DNA of *E.coli* and *A.niger* after 24 hours. The same dye, at low concentration (12.5-25%) did not denature the human DNA. At higher concentration (50-100%), however, it denatured human DNA after 24 hours. According to Chung *et al*, (1992), the intake of azo dyes can increase the risk of human bladder cancer, splenic sarcomes and nuclear anomalis. Chung (1983) also reported that azo dyes can cause allergic dermatitis, and even DNA damage that results in the formation of malignant tumors.

Effect Of The Dye On the Growth Of *Zea mays*

Table 6: Growth profile of *Zea mays* seeds exposed to different concentrations of the chemically synthesized dye

Table 6: Growth profile of *Zea mays* seeds exposed to different concentrations of the synthesized dye

| Dilution | Dye | | |
|--------------|------------------------|------------------|-------------------|
| | Growth indices | | |
| | No of germinated seeds | Root length (mm) | Shoot length (mm) |
| 100% | 2 | 3.0 | 7.0 |
| 50% | 4 | 8.0 | 10.0 |
| 25% | 5 | 10.0 | 13.0 |
| 12.5% | 5 | 12.0 | 15.0 |
| 6.5% | 6 | 25.0 | 15.0 |
| Control (0%) | 10 | 30.0 | 26.5 |

NB: Root length greater than 5mm and more than 5 germinated seed means no toxicity.

The result from Table 6 showed that the synthesized dye had no toxic effect on the growth of *Zea mays*. This was against the report according to Camen and Daniela (2012) that the pollutants negatively affect the chlorophyll content of plants and they trigger the promotion of chlorophyllase and abscissic acid, both of which can lead to chlorophyll degradation.

Toxicity Assessment On Microbial Strains

The dye was tested for its possible toxicity on some microbes, and the result obtained from this aspect of study is presented in Table 7.

Table 7: Microbial toxicity of the synthesized dye

| Dilution | Dye | | |
|-----------------|--------------------|-----------------|-------------------|
| | Zone of inhibition | | |
| | E.coli | Bacillus cereus | Aspergillus niger |
| 1000mg/L | 12 | 0 | 12 |
| 5000mg/L | 8 | 8 | 12 |
| 250mg/L | 8 | 8 | 13 |
| 125mg/L | 9 | 12 | 12 |
| 65.5mg/L | 10 | 23 | 9 |
| Control (0mg/L) | 0 | 0 | 0 |

NB: 0-9 = resistant, 10-19 = Intermediate or moderately resistant, 20-above = sensitive or susceptible to the dye.

All the organisms showed moderate resistant to the dye with *E.coli* having greater resistances at the 250 mg/L and 65.5mg/L dilution than other microbial strains.

CONCLUSION

A novel disazo dye, 4-Bromophenylazo-2-naphtholazo-4¹-chlorobenzene, have been synthesized by stepwise diazotization and coupling reactions. The results showed that disazo dyes can be obtained in a high yield from aniline and 4-substituted anilines. The dye obtained showed excellent colourfastness on all the fibres and poor washfastness except nylon where fair washfastness was shown. The synthesized dye is not safe for human consumption and cannot be recommended for food and drug colourings. However, the dye is not toxic to plants and environment.

REFERENCES

- [1] Akpuaka M.U., Orakwue. F.C., Nnadozie U., Oyeka C.A., Okoli I. (2003). Preliminary Phytochemical and Antibacterial Activity of Screening of *Bryophyllum Pinnatum Extracts*. *Journal of Chemical Society of Nigeria*. 23(1) 10-13
- [2] Alessia, C., Luc, M. and Peter, V, (2017). On the stability of medieval inorganic pigments. *Heritage Science* 5
- [3] American Association of Textile Chemists and Colourists, (1994). Technical Manual of American Association of Textile Chemists and Colourists (A.A.T.C.C), Vol.69-12215, Research Triangle Park (North Carolina), USA. 348-349
- [4] Bouas- Laurent, H. and Durr, H, (2001). Organic photochromism, (IUPAC technical report) *Pure and Applied Chemistry*. 73, 639-665
- [5] Carmen, Z. and Daniela, S, (2012). Textile Organic Dye-Characteristics, Pollution Effects and Separation/Elimination Procedure from Industrial Effluents-. A critical Overview. Vol 3. Intech Open; Rijeka, Croatia: 55-86.
- [6] Chung, K. T, (1983). The significance of azo reduction in the mutagenesis and carcinogenesis of azo dyes. *Mutat. Res. Rev. Genet. Toxicol.*; 114:269-281.
- [7] Chung, K. T., Stevens, S. E., and Cerniglia, C. E., (1992). "The reduction of azo dye by intestinal microflora. *Crit. Rev. Microbiology*. 18:175-190
- [8] Coelho, P. J., Castro, M. C. R., Fernandes, S. S., Fonseca, A. M. C. and Raposo, M. M. M, (2012). Enhancement of the photochromic switching speed of bithiophene azo dyes. *Tetrahedron Letter*. 53, 4502-4506
- [9] Fernandez, N.P, (2004). Innovations for home dress making and the popularization of stylish dress, *Journal of American culture*, 17(3):23-33.
- [10] Gopinathan, M. and Thirumurthy, M, (2012). Evaluation of phytotoxicity for compost from organic fraction of municipal solid waste and pulp mill sludge. *Environmental Research, Engineering and Management*, 1 (59): 47-51
- [11] Gordon, P. F. and Gregory, P, (2012). Organic Chemistry in Colour. (Springer Science and Business Media
- [12] Hari, R. M. and Vithal, S. P, (2001). Synthesis and Application of 2-Aminothiodiazole Disperse Dyes for Nylon Fabrics. *Journal of Serbian Chemist*, 66(2):87-93
- [13] Joseph, B. L, Herbert, F. S., David, A. L. and Graham, R. C, (1998). Organic Structural

- Spectroscopy. Prentice-Hall, Inc. Simon & Schuster. A Viacom Company Upper River, New Jersey. P.374
- [14] Mabhai, S. *et al.* (2022) A naphthalene-based azo armed molecular framework for selected sensing of Al³⁺. *New Journal of Chemistry* 46, 6885-6898.
- [15] Mohammed, S.A. and Bello, F, (2010). Colour and Constitution of 4-Chlorothiazole Azo Dyes: Application of ppp method. *Journal of Textile and apparel technology and management*, 6(4): 1-11
- [16] Odilora, C.A., Azih, M.C. and Enaike, J.O, (2001). Extraction and Evaluation of dye from Bixa orellana, *African Journal of Science Technology* (18): 75-78.
- [17] Odilora, C.A. and Ogaga, B.E, (2006). Dye from Bixa orellana: An Investigation into its colouring effects on wool and acrylic fibres. *Nigerian Journal of Biotechnology*, 17; 65-68
- [18] Odilora, C.A. and Omatseye, O.C, (2004). "Extraction and Evaluation of Dye from Lima beans (*Phaseolus linatus* linn). *Applied Sciences*, 3(2): 832-841
- [19] Odilora, C.A., Orakwue, F.C., Obi, J.C., Iloh, E.O. and Okwuego, P.O. (2025). 'Synthesis, Physicochemical and Microbial Evaluation of Phenylazo-1-Naphtholazobenzene (A Novel Disazo Dye). *ChemClass Journal*, 6(2): 479-493
- [20] Odilora, C.A., Orakwue, F.C., Obi, J.C., Iloh, E.O. and Uba, B.O. (2025). 'Synthesis, Physicochemical and Microbial Evaluation of 4-Bromophenylazo-2-Naphtholazo -4¹-bromobenzene (A Novel Disazo Dye). *International Journal of Research and Suatainable Development*, 11(1): 54-63
- [21] OECD, (1984). Guidelines for testing of chemicals 208. Terrestrial plant growth test: Seedling Emergence and Seedling Growth Test., Paris. 1-21
- [22] Omar, A.Z., Mohamed, M.G., Hamed, E.A., and El-atawy, M., (2023) Characterization, DFT calculation and dyeing performance on polyester fabrics of some azo disperse dyes containing pyrazole ring. *Journal of Saudi Chemical Society* 27, 101594
- [23] Patil, V., Padalkar, V. S., Sekar, N., Patil, S. V. and Rajput, (2019). Synthesis of 2-methyl-5-(5-phenyl substituted-1, 3, 4-oxadiazole-2-yl) quinazolin-4-one fluorescent brightening agent: Computational and experimental comparison of photophysical structure. *Journal of Molecular Structure*. 1182, 150157
- [24] Rani, B., Kumar, V., Singh, J., Bish, S., Teotia, P., Sharma, S., and Kela, R, (2014). Bioremediation of dyes by funji isolated from contaminated dye effluent sites for biosability. *Brazilian Journal of Microbiology*, 45(3): 1055-1063
- [25] Rizk, H. F., Ibrahim, S. A. and El-Borai, M. A, (2015). Synthesis fastness properties, colour assessment and antimicrobial activity of some azo reactive dyes having pyrazole moiety. *Dyes and Pigments*. 112, 86-92
- [26] Satam, M. A., Raut, R. K. and Sekar, N, (2013). Fluorescent azo disperse dyes from 3-(1, 3-benzothiazole-2-yl) naphthalene-2-ol and comparison with 2-naphthol analogs. *Dyes Pigment*. 96, 92-103
- [27] Scultz, F. B. and Julius, Z. M, (1908). A Systematic Survey of the Organic Colouring Matters. *2nd edition Macmillan and Co Limited*, St Martins Street, London, p.69.
- [28] Ubah, B. O, (2019). Phylogenetic framework and metabolic genes expression analysis of bacterial isolated from contaminated marine environments of Niger Delta. *Annual Research and Review in Biology*, 30(5):1-16
- [29] Ukponwan, D.O., Odilora, C.A., Offor, M.N. and Freeman, H.S, (1999). Disperse Red 60 Analogs. *Indian Journal of Fibre & Textile Research*, 24: 297-302.