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RESEARCH ARTICLE

# Adverse effects of the toxic industrial dye malachite green on the antioxidant and antimicrobial properties of *Allium cepa* L.

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## Abstract

The therapeutic importance of *Allium cepa* L. has been well established through ethnobotanical studies. Malachite Green is a highly toxic dye that finds extensive use in the textile industry as a fabric colorant. *A. cepa* was treated with malachite green solutions (20 mg/l and 40 mg/l) for 45 days. The roots actively take up malachite green and impede the plant's growth in root length, shoot length and fresh weight. *A. cepa* is rich in bioactive compounds, which have superior antioxidant and antimicrobial properties. We observe that the antimicrobial activities decrease by 36% for *Staphylococcus aureus* and *31%* for *Escherichia coli* on the treatment of *A. cepa* bulbs with 40 mg/l malachite green. Antioxidant activity was similarly lowered by 61% under the toxic effects of the dye. Reduction in the efficacy of *A. cepa* in terms of the critical medicinal properties and general retardation in growth is a cause of concern. This article reports a previously unknown aspect of malachite green toxicity and presents the effect of any dye on the medicinal properties of *A. cepa* for the first time.

## Keywords

Antibacterial activity, Antioxidant activity, *Allium cepa*, Malachite Green toxicity

## Introduction

Malachite Green is a triarylmethane dye with a complex molecular structure with aromatic rings, making it a prominent recalcitrant toxicant. It is associated with a plethora of health risks. It is already established that Malachite Green is toxic to aquatic life, land organisms and humans. Long-term exposure to the dye can cause developmental defects, mutations and cancer (1). Several toxicological studies on the dye have shown that malachite green can cause severe irritation to test animals' eyes and mucous membranes (2) and has also been reported as a mutagen (3). The dye has inhibitory effects on the growth of important crops like wheat, rice, pulses etc. (4-6). *Allium cepa* is a common plant used traditionally as a vegetable in households worldwide and has also found extensive use in laboratories for toxicological studies. There are several reports of the red onion *A. cepa* having antibacterial effects on both Gram-positive and Gram-negative bacteria (7, 8) and some fungi (9-11). Essential oils obtained from onion have been found to possess potent antibacterial properties (12). Onion extracts exert hypoglycaemic effects on diabetic rats (13, 14). The anti-oxidant and free radical scavenging activity of red onion have been discussed in several studies (15-17). We tested the effect of Malachite Green on *A. cepa* for a few essential parameters like root length, shoot length, biomass, antioxidant and anti-

bacterial activities. Previously, *A. cepa* mainly had been used to study chromosomal aberrations under the effect of poisonous chemicals (18, 19). The effect of the toxic dye malachite green on the overall growth and two important medicinal properties: antibacterial and antioxidant activity of *A. cepa* has been studied in the present work.

## Materials and Methods

### Chemicals

Malachite Green and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich. Agar-agar was purchased from Merck Millipore. All other chemicals and media components used were obtained from Himedia and were of analytical grade. All experiments were performed using distilled water as and when required.

### Plant specimen

*Allium cepa* L. used for the study were locally obtained.

### Microorganisms

*Escherichia coli (*ATCC 25922*)* and *Staphylococcus aureus* (ATCC 25923) were used.

### Preparation of A. cepa

*A. cepa* bulbs were washed thoroughly in running tap water, and the dry roots and dirt were removed so that only the fresh root primordia remained (20). A stock solution (100 mg/l) of Malachite Green dye was prepared and 20 mg/l and 40 mg/l solutions were obtained by dilution from this stock. The onions were placed in the dye solutions for 45 days so that only the root primordia came in touch with the treatment solutions.

### Estimation of the dye taken up by the A. cepa

Triplicates of *A. cepa* bulbs were treated with the toxicants. The concentration of dye remaining in the solutions was measured by estimating the optical densities using the spectrophotometer (Agilent Technologies, Model: Cary 60 UV-Vis, Model No: G6860A) at 614 nm and then interpolating them on a malachite green (MG) standard curve. The amount of dye thereby calculated from the concentration and volume of dye solutions remaining in the treatment containers after growing *A. cepa* for 45 days. Since the plant took up the dye from the test solutions, the dye absorbed was obtained by subtracting the amount of dye remaining in the treatment container from the initial amounts of dye.

### Evaluation of the effect of dye on phenotypic characteristics of A. cepa

Roots of *A. cepa* emerged first, followed by shoots. The root lengths were observed and measured 5$^{th}$ day onwards and continued till the 45$^{th}$ day with a regular interval of 10 days. The shoot lengths and fresh weights of leaves were recorded on the 45$^{th}$ day of growth. All experiments were performed with the *A. cepa* grown in the dye solutions for 45 days (21).

### Determination Antioxidant Activity

10 g of *A. cepa* bulb grown in distilled water and malachite green solutions (40 mg/l and 20 mg/l) were washed thoroughly and then macerated in 80% (v/v) ethanol for 72 hr at 37 °C. The macerated product was then filtered by Whatman No. 1 filter paper and concentrated by Rotary Evaporator at 45 °C. The concentrated extract thus obtained was dried using a freeze dryer at -80°C for 24 hr. A stock solution (1 mg/ml) was prepared using lyophilized *A. cepa* powder. An aliquot of 0.5 ml of this stock was added to 2.5 ml of 0.04% ethanolic DPPH solution. The mixture was incubated in the dark for 30 min at 37 °C, and the absorbance was measured at 517 nm. The percentage inhibition was calculated using the following formula given in Eqn. 1:

$$\% \text{ Inhibition} = [1-(A_{sample}/A_{control})] \times 100 \ldots\ldots\ldots\ldots(1)$$

Where $A_{control}$ is the absorbance of ethanolic control DPPH solution and $A_{sample}$ is the absorbance of sample extracts with DPPH solution.

The percentage of inhibition of the dye-imbibed *A. cepa* was compared to those grown in distilled water (control). Citric acid (1 mg/ml) was selected as standard. The IC50 (concentration of malachite green, which showed 50% inhibition of the antioxidant activity) was also calculated.

### Evaluation of the effect of dye on the antibacterial property of A. cepa

The agar-well diffusion method was employed to estimate the antibacterial efficacy of *A. cepa* bulbs grown in distilled water and test samples against two bacterial strains: *Escherichia coli* ( Gram-negative) and *Staphylococcus aureus* (Gram-positive). The onion bulbs were washed thoroughly in distilled water several times and then crushed by a sterile electric grinder. The product was filtered through Whatman filter paper and powdered through freeze-drying (80 °C). Stock solution (1 mg/l) was prepared from the *A. cepa* powders thus obtained. The microorganisms *E. coli* and *S. aureus* were grown overnight *(*18 hr) in nutrient broth and adjusted to approximately $10^5$ CFU/ml. 1 ml of each of these cultures was used to inoculate 100 ml sterile nutrient agar and plates were poured. Wells of 7 mm diameter were punched using sterile cork borer, and 10 µl of the control and the test samples (1 mg/ml) were loaded in the wells. Streptomycin (150 µg/ml) was taken as a standard to compare the activity of all the extracts. The plates were incubated for 24 hr at 37 °C. The diameter of the inhibition zone was measured.

### Fourier transform infrared spectroscopy (FTIR) of A. cepa

The FTIR of the freeze-dried samples of the treated (20 mg/l and 40 mg/l malachite green) and control *A. cepa* was performed and the spectra were compared. The analysis was performed in the spectral range of 4500 to 500 $cm^{-1}$ to identify the functional groups of the malachite green that could be present in the dye-treated *A. cepa.* Measurements were carried out using an FTIR spectrophotometer (Bruker Alpha model, USA) in the absorbance mode.

## Results and Discussion

### Dye taken up by A. cepa

After 45 days, the dye solutions in which the bulbs were

kept turned almost colorless. The amount of dye that the plants took up through the roots was estimated (Table 1). the dye caused overall weaker growth of the *A. cepa* plants in root length, shoot length and fresh weight.

**Table 1**. Dye absorbed by the *A. cepa* roots after 45 days

| *Initial concentration of dye in the treatment container (mg/l)* | *Initial volume of dye in the treatment container (ml)* | *Initial amount of dye in the treatment container (mg)* | *Final concentration of dye in the treatment container after growing A. cepa for 45 days (mg/l)* | *Final volume of dye in the treatment container after growing A. cepa for 45 days (ml)* | *Final amount of dye in the treatment container after growing A. cepa for 45 days(mg)* | *Amount of dye taken up by the A. cepa plant after 45 days (mg)* |
|---|---|---|---|---|---|---|
| 20 | 250 | 5 | 2.088±0.277 | 9.033±0.503 | 0.019±0.004 | 4.981±0.004 |
| 40 | 250 | 10 | 3.16±0.240 | 11.317±0.775 | 0.0358±.005 | 9.964±0.005 |

Values are expressed as Mean± S.D. where n=3

99.62% and 99.64% of the initial dye (20 mg/l and 40 mg/l respectively) was absorbed by the roots in 45 days. Metal pollutants are taken up by plant roots from the soil (22). Similarly, dyes are also absorbed effectively by root systems. Many researchers have used dyes to track the transport of water inside plants (23). Studies are there on placing *Typha angustifolia* in simulated wastewater containing the toxic reactive dye Reactive Red 141 (RR141) (24). They documented the movement of the dye in the plant after the roots from the simulated wastewater absorbed it. The dye's effects were measured in terms of relative plant growth rate and the emergence of symptoms, including necrosis and chronic or acute wilting (24).

### *Effect of dye on the early shoot and root growth and fresh weight of leaves of A. cepa*

Root lengths are considered standard indices of toxicity estimation. It is observed that the roots growing from the control (distilled water) showed rapid growth and grew more than 9 cm till the 25$^{th}$ day. Roots emerging from onion bulbs fed with dye solutions showed strikingly weaker growth with an 80% reduction in root length when treated with 40 mg/l malachite green solution. The growth inhibition intensity depended on the dye concentration: the 40 mg/l dye solution inhibited root growth more than the 20 mg/l dye solution (Fig. 1).

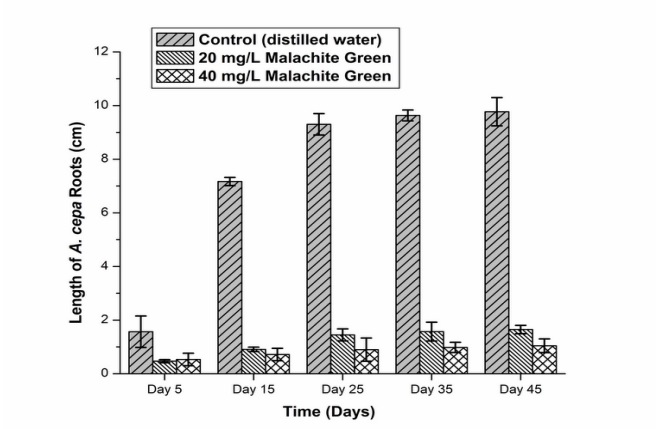


**Fig. 1.** Root lengths of *A. cepa* grown in control (distilled water) and malachite green solutions (20 mg/l and 40 mg/l) at different time intervals.

The shoot lengths and fresh weights were also affected by the presence of dye (Table 2). Up to 63.8% inhibition of the shoot growth was observed in plants treated with 40 mg/l malachite green. The fresh weights were much higher in the control shoots than those of the dye-treated ones: 85% lower fresh weight was recorded for plants grown in 40 mg/l malachite green. Hence, it is evident that

**Table 2**. Shoot lengths and fresh weight of *A. cepa* leaves on the 45th day

| *Allium cepa* | Shoot length (cm) | Fresh weight (g) |
|---|---|---|
| Control (grown in water) | 36.34± 5.119 | 14.843± 1.331 |
| Grown in 20 mg/l of MG | 17.94± 5.46 | 4.553± 1.449 |
| Grown in 40 mg/l of MG | 13.28± 4.612 | 1.27± 0.287 |

Values are expressed as Mean± S.D. where n=3

Dyes are phytotoxic compounds, meaning they exert inhibitory effects on plant growth. Lucerne (*Medicago sativa)* and Chinese cabbage *(Brassica chinensis)* grown in the presence of malachite green showed poor germination percentage and significantly smaller root and shoot lengths when compared to the control (water) (25). Seed germination and root elongation were tested for malachite green toxicity in *Nicotiana tabacum* and *Lactuca sativa* (26). It was observed that germination of both *N. tabacum* and *L. sativa* seeds was suppressed by the dye by 36 % and 75 % respectively (26). Similar results were observed in the case of azo dyes. A phytotoxicity assay was performed to evaluate the toxic effects of Direct Red 81on *Lemna minor* (27). In the presence of dye, 90% growth inhibition was recorded after 10 days incubation, and the germination of *Vigna radiata*, *Raphanus sativus* and *Abelmoschus esculentus* seeds was reduced in the presence of dye DR81, to only 30, 30 and 36 % of seeds germinating respectively (27).

### *Effect of dye on antioxidant and antibacterial activity of A. cepa*

Extracts of *A. cepa* grown in distilled water showed almost double the antioxidant activity (85.729±0.766%) compared to the ones treated with the 40 mg/l dye solution (48.316±2.517%). The IC50 value is 20.97 mg/l malachite green (Fig. 2).

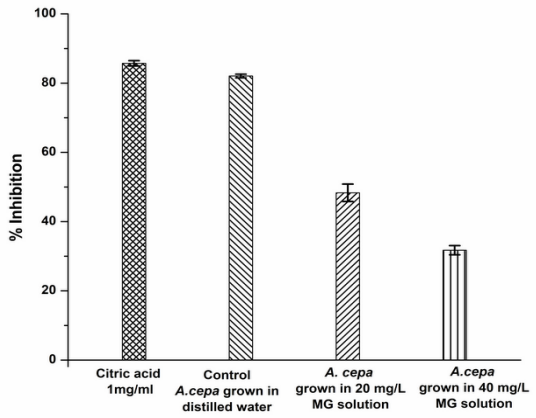


**Fig. 2.** Antioxidant activity of control and dye-treated *A. cepa* extracts.

*A. cepa* grown in distilled water showed prominent inhibitory activity against both Gram-positive and Gram-negative bacteria. On the other hand, imbibition of the dye resulted in diminished antibacterial efficacy. Much smaller inhibition zones were found in the dye-treated onions indicating partial loss of antibacterial effect. When the dye-treated (40 mg/l) *A. cepa* extracts were tested, the diameters of inhibition zones decreased by 35% and 31% for *S. aureus* and *E. coli* respectively (Table 3 and Fig. 3).

**Table 3**. Antibacterial Assay of *A. cepa* extracts grown in distilled water and dye solution.

| Test solutions | Diameter of Inhibition Zone (mm) | |
|---|---|---|
| | ***S. aureus*** | ***E. coli*** |
| Standard (Streptomycin 150 µg/ml) | 18.667±0.764 | 22.833±0.289 |
| *A.cepa* grown in distilled water | 12.833±0.289 | 18.667±0.577 |
| *A.cepa* grown in malachite green (20 mg/l) | 10.11±0.23 | 13.02±0.564 |
| *A.cepa* grown in malachite green (40 mg/l) | 8.2±0.11 | 12.833±0.289 |
| Sterile distilled water | 0 | 0 |

Values are expressed as Mean± S.D. where n=3

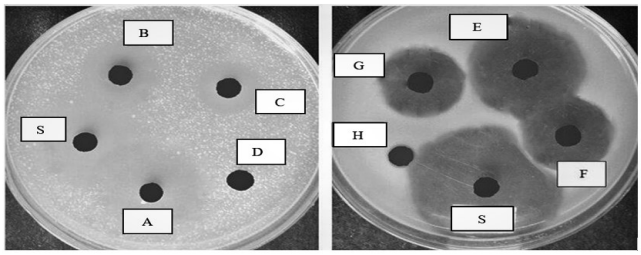


**Fig. 3.** Plate 1: *S. aureus* lawn showing zones of inhibition around Streptomycin 150 µg/ml (S), extracts of *A. cepa* grown in distilled water *(*A), 20 mg/l MG (B) and 40 mg/l MG (C), sterile distilled water (D); Plate 2: *E. coli* lawn showing zones of inhibition around Streptomycin 150 µg/ml (S), extracts of *A. cepa* grown in distilled water (E), 20 mg/l MG (F) and 40 mg/l MG (G), sterile distilled water (H).

The production of secondary metabolites and the physiological parameters of plants are affected by the presence of dyes or heavy metals in the growth medium. A surge in the production of Reactive Oxygen Species (ROS) was observed in the *Lemna minor* plants grown in the presence of Direct Red 81 (27). Reports are on studied the effect of cadmium on the antioxidant activity of *Erica andevalensis.* The presence of cadmium in the growth medium increased the total antioxidant capacity of the plant (28). Toxicants may inhibit the production and accumulation of important bioactive plant compounds. For example, seedlings of *Hypericum perforatum* growing in a media supplemented with 50 mM Nickel, can make or accumulate on 15-20 times lesser hyperforin, hypericin and pseudohypericin (29). Hypericin is a potent antiviral and antibacterial agent, while hyperforin is a natural antidepressant (30). Phytochemical components are known to interact with external agents, which have either positive or negative impacts on their activity, bioavailability, solubility, metabolism, cellular uptake and efflux (31). The biological effects may be reduced if phytochemicals and synthetic compounds are combined in improper ratios (32, 33). A decrease in efficacy may occur if the participant compounds form hydrogen bonds at active hydroxyl groups that reduce their capability to scavenge free radicals (32) or have incorrect orientation or distribution in lipid/water phases to facilitate the interaction (34). Reports are on heavy metals in the growth medium of *Andrographis paniculata* altered the plant's chlorophyll, carotenoid content and antioxidant properties (35).

Similarly, malachite green was observed to affect the antioxidant and antimicrobial properties of *A. cepa.* Potential health benefits offered by red onion extract are thus compromised in the presence of malachite green in the growth medium. The exact mechanism and chemistry of this dye-phytochemical interaction remain to be studied. One theory is that genes involved in producing specific secondary metabolites are activated in response to environmental stresses that activate signaling pathways (36).

Several bioactive compounds of *A. cepa* exhibit antioxidant or antibacterial properties (37-40), but which dye-phytochemical interaction is responsible for the reduced therapeutic efficacy is yet to be determined. Polyphenols of *A. cepa* are active against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Salmonella typhimurium* (41). Some have an indirect stimulatory effect; for example, histidine is present in *A. cepa* (42). L-Histidine does not have antibacterial properties on its own but can turn Rifampicin-resistant bacteria to sensitive when mixed with Rifampicin (43). *A. cepa* also contains xylose (44). Xylose enhances the activity of chloramphenicol and tetracycline antibiotics (45). Some bioactive compounds present in *A. cepa* serve as precursors of antibacterial or antioxidant agents. For example, glycine does not have antioxidant activity but serves as an initial amino acid for glutathione, a strong antioxidant (46). Compounds like beta carotene, isorhamnetin etc. have antibacterial and antioxidant properties (47, 48).

While the effect of heavy metals on the medicinal properties of plants has been studied previously by many authors (29, 30, 36, 49), the present article is the first study that reports the inhibitory effects of a toxic dye on the medicinal properties of a plant.

### *FTIR of the dye-treated and control A. cepa*

The FTIR spectra of all the sets of samples were compared (Fig. 4). A characteristic di/tri sulfide peak is present in the control *A. cepa* (50) around 1100 $cm^{-1}$. A characteristic peak corresponding to the -OH stretching is present in the MG and MG treated *A. cepa* around 3700 $cm^{-1}$ (51) which is ab-

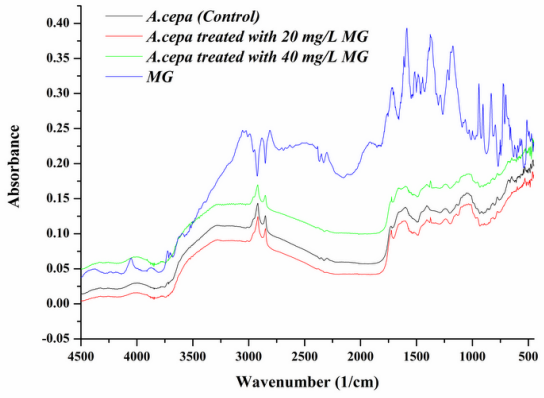


**Fig. 4.** Comparison of FTIR spectra of *A. cepa (*control) and treated with malachite green (20 mg/l and 40 mg/l).

sent in the control *A. cepa.* In *A. cepa* treated with MG, a peak around 3580 $cm^{-1}$ has been observed due to –OH groups of oxalic acid of Malachite Green oxalate (52). At 1724 $cm^{-1}$, a sharp peak corresponding to the C=O stretching of the alpha and beta unsaturated esters of malachite green are observed in the dye treated *A. cepa,* but is absent in the control (51). The FTIR spectra of the dye and the *A. cepa* treated with the dye show peak at 1371 $cm^{-1}$ which represents the C-N of the aromatic tertiary amines of malachite green, is not found in the control *A. cepa* (53). Therefore, the FTIR spectra confirmed the presence of malachite green signature peaks in the dye treated *A. cepa* and absence of those peaks in the control *A. cepa*.

## Conclusion

The heavily used toxic industrial dye Malachite Green has adverse effects on the prime growth parameters like root and shoot lengths and fresh weight of leaves of *A. cepa* L. While several toxicological studies with malachite green have already established it as a highly poisonous dye having several deleterious effects on a wide range of organisms ranging from aquatic life to humans, this study confirms that the dye has a profound negative impact on two of the most important pharmacological properties of *A. cepa* L. the antioxidant activity and antibacterial effect. Our study indicates that both of these activities are reduced by the toxic industrial dye. It can thus be concluded that the adverse effects of malachite green are not only confined to the visible phenotypic characters but also extend to the pharmacological properties of bioactive compounds present in *A. cepa*.

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## Authors contributions

SB and PB conceived and designed the study and prepared the manuscript. NB and SB performed the experiments and collected the data.

## Compliance with ethical standards

**Conflict of interest**: Authors do not have any conflict of interests to declare.

**Ethical issues**: None.

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