EFFECT OF METHYL ORANGE ON GERMINATION OF SORGHUM VULGARE AND PHASEOLUS MUNGO

FATIMAH ALSHEHREI

Department of Biology, Jamoum College, Umm Al-Qura University, Makkah 24382, Saudi Arabia.
E-mail: Fmshehrei@uqu.edu.sa

Abstract - Azo dyes are toxic compounds and because of highly persistent to degrade, it can cause many environmental pollutions. Irrigation with waste water include theses dyes can affect soil fertility and growth of plant. In this study, treated and untreated methyl orange were tested to study their effect on the growth of two plants Sorghum vulgare and Phaseolusmungo.A higher concentration of untreateddyeinhibited the elongation of shoot androot of seedlings. Treated dye with bacteria showed a good germination for both plants.

Keywords - Methyl orange, Sorghum vulgare , Phaseolus mungo, Phytotoxicity

INTRODUCTION

Azo dyes are aromatic compounds consist of (N=N) group which responsible for toxicity. The release of azo dyes molecules into the environment causes toxicity to the plants and other organisms (Khaliq et al., 2013). The presence of these chemicals in soil affects the germination process of the plant. Some studies suggest that synthetic water which includes these toxic materials inhibit seed and seedling growth (Wins and Murugan, 2010 and Ventura-Camargo et al. 2013).

(Brown and DeVito,1993) have summarized the mechanisms of azo dye toxicity in few points：

- Some Azo dyes transform to toxic compounds after degradation and breaking down the linkage of azo dye , aromatic amines may be formed and found in solution.
- When free aromatic amine groups form , it will metabolically oxidized without azo reduction and may cause toxicity.
- After oxidation happened, highly reactive electrophilic diazonium salts will produce.

It is important to evaluate the toxicity of azo dyes and its metabolites. (Dave et al.,2014) mentioned several mechanisms for evaluation of toxicity such as using microbes, phytotoxicity, mutagenicity, cytotoxicity, Genotoxicity….etc.

Phytotoxicity tests are used for calculation the percentage of seed germination as a simple and rapid technique and to evaluate the root and shoot elongation (Wang and Keturi,1990 ; Mala and Babu,2005).Some reports used the same techniques to test the tolerance of plants against these compounds (Zhoua and Xianga, 2013).

However, measurement of toxicity by using microorganisms is a suitable method for determination toxicity of dyes and their metabolites, and it is also required for the risk assessment of polluted sediments (Pokharia and Ahluwalia, 2015).
Many reports studied the effect of toxicity of days on plant by using microorganisms. For instant, (Alaguprathana, and Poonkothai, 2017, Bari1 and Bhardwaj 2013 , Ebency et al., 2011 studied the microbial toxicity by using B. subtilis,E. coli, and Azotobacter , they found decrease in toxicity of tris azo Acid Black 210 after decolorization by Providencia sp. SRS82.

Dayama (1987) mentioned that high concentration of industrial effluent reduced the seed germination.
Muhammad and Khan (1985) studied the effective of synthetic water in reducing the germination percentage of two plants; Phaseolusaureus and Abelmoschusesculentus..

(Wins and Murugan, 2010) found that high concentration of textile effluent effect on germination ratio and growth of seed and seedling of black gram Vigna mungo.

From the other hand, azo dyes are also can effect on the chromosomes of plant.Ventura (2009) investigated the mutagenicity of the black dye by using plant A. cepa as a model . The genotoxic effects observed in the meristematic cells of A. cepa before and after the treatment with microbes, however, results show higher frequencies of genotoxic damages after the microbial treatment. (Duttaand Ahmad, , 2016). studied the genotoxicity of azo dyes on the chromosomes of Alliumcepa plant.
Ferraz et al. (2011) studied mutagenetic effect of dye Disperse Orange 1 on Salmonella typhimurium strains viz. TA98, YG1041, TA100 and YG1042. Also, (Gottlieb et al., 2003). studied toxicity of Reactive Black 5 , Acid Orange 7, Food Yellow by using marine bacterium Vibrio fischeri .
Methyl orange is an example of organic and aromatic dye that may present in synthetic effluent as a dissolved toxic material and it impacts germination

process or considered as pollutant that may contaminate the plant seeds and fruits.

Higher Plants consider as an excellent model to estimate mutagenicity of toxic chemicals such as A. cepa which has advantages of low cost and easy to handle.

Since researches interested in this topic are few, this report aims to test the impact of treated and untreated methyl orange on growth of some plants.

II. MATERIAL AND METHODS

Chemicals:
Methyl orange was purchased from Merck. The molecular weight is 327.33 and the formula is $C_{14}H_{14}N_3NaO_3S$ .Structure of Methyl orange is shown in fig 1

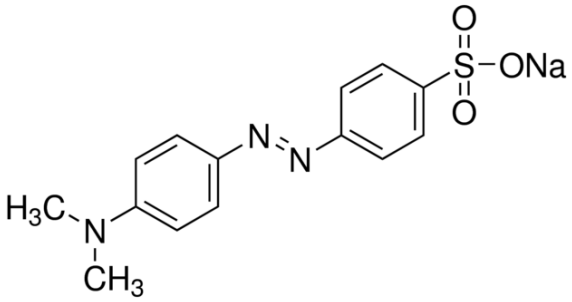


Fig.1 Chemical structure of Methyl orange.

Bacteria:
Gram positive bacteria, Bacillus sp and Gram negetive bacteria, Pseudomonas spwereisolated from synthetic waste water which obtained from textile dyeing factory. Serial dilution was carried out to isolate the bacteria. Isolated bacteria were sub cultured on nutrient agar at 37° C for two days.

Tested Plants:
Two plants,Sorghum vulgare and Phaseolus mungowere used in this study to examine the toxicity of azo dye on these plants.

Identification of Bacteria:
The selected bacteria were examined base on microscopic and physiological characteristics as described by Bergey's manual of determinative bacteriology (9th edition).

Phytotoxicity Studies:
In this experiment, the effect of azo dye was evaluated by studying germination of seed of three plants P. vulgaris and P. sativum.The seeds were germinated in pots containing 1kg of soil. Three replicates of ten seeds of each plant were treated as a follow:

1. Untreated samples: 10 ml of methylene orange was used to treat the seed of three plants.
2. Treatment of azo dyes samples: Isolated bacteria were maintained on basal medium. The basal medium composition (g/L) : methyl orange dye 0.1; $(NH_4)2SO_4$ 0.28; NH_4C_{l0}.23; KH_2PO_4 0.067; $MgSO_4{\cdot}7H_2O$ 0.04; $CaCl_2{\cdot}2H_2O$ 0.022; $FeCl_3{\cdot}6H_2O$ 0.005; yeast extract 0.2; NaCl 0.15;$NaHCO_3$ 1.0; and 1 mL/L of a trace element solution containing (g/L) $ZnSO_4{\cdot}7H2O$ 0.01; $MnCl_2{\cdot}4H2O$ 0.1;$CuSO_4{\cdot}5H_2O$ 0.392; $CoCl_2{\cdot}6H_2O$ 0.248; $NaB_4O_7{\cdot}10H2O$ 0.177; $NiCl_2{\cdot}6H_2O$ 0.02 with glucose 0.1 as a source of carbon and peptone 0.1 as a source of nitrogen. 10 ml of this medium was taken and added for each plant.
3. Plain water: 10 ml of water was used for watering the tested plants.

Germination of seeds was daily recorded. At the end of the germination experiment, the shooting percentage and root length of seedlings was measured.

III. RESULTS AND DISCUSSION

Treated and untreated methyl orange were tested to study their effective on germination of two plants after irrigation for one week. The measurements of shoot and root of S. vulgare and P. mungowere recorded in table (1).

Tested plant	Treatment	Plumule length (cm)	Radicle length (cm)	Percentage of Germination (%)
Sorghum vulgare	Untreated dye	4.40 ± 0.11	2.17 ± 0.10	60
	Treated dye	7.19 ± 0.09	5.43 ± 0.03	80
	Water	±8.55 0.23	6.09 ± 0.34	100
Phaseolus mungo	Untreated dye	2.25 ± 0.03	1.4 ± 0.22	40
	Treated dye	3.66 ± 0.10	2.18 ± 0.03	90
	Water	4.31 ± 0.11	3.67 ± 0.06	100

Table (1) Toxicity of Methyl orange and its biodegraded product on tested plants

In S. vulgare, untreated dye was effected the germination percentage with 60 % and with percentage of 40% in case of P. mungo. Shoot and root elongation was affected by the dye concentration, while the percentage of treated dye showed good germination with 80 % and 90 % for S. vulgare and P. mungorespectively. The results showed that treated dye was not considered to be

toxic to seed germination since good growth was observed when compared withwater, the percentage of germination was the best with 100%.

Many reports indicate that textile dyes have toxic effect on the germination rates and biomasses concentration of several plants species (Umesh et al.2016 , Shahet al.,2013&Shyamalaet al.,2014). Also, (Parshetti et al., 2006 & Shedbalkar and Jadhav , 2011) studied the phytotoxicity of malachite green and its metabolites on germination of Triticum aestivum, Phaseolus mungo and Ervum lens L.seeds and compared that with samples treatment by P.ochrochloron and with water samples. They found that malachite green dye and its degradation metabolite product affected significantly the length plumule and radical indicates less toxicity of the degradation product towards plant seedling and growth. Azo dyes such as methyl red and Disperse Brown had serious hazards on plant seeds, and therefore untreated dyeing effluents could not be use for the agriculture purpose.

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