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EFFECTS OF PHARMACEUTICAL EFFLUENT ON GERMINATION, SEEDLING GROWTH AND CHLOROPHYLL CONTENT OF *Sorghum bicolor*

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ABSTRACT
Pharmaceutical industries are a necessity for human and animal health, but some of their operations and discharges may pose environmental pollution problems. The aim of this study was to assess the effects of pharmaceutical effluent on the germination, growth and chlorophyll contents of Sorghum bicolor. This was done by exposing the plant to increasing concentrations of pharmaceutical effluent starting from 0 % to 30 % effluent concentrations. From the results, pharmaceutical effluent caused significant reductions ($p < 0.05$) in all the growth parameters measured. Chlorophyll-a production in the leaves of Sorghum bicolor was significantly ($p < 0.05$) inhibited by pharmaceutical effluent at every concentration except 1 %, while chlorophyll b and total chlorophyll were not inhibited, but promoted with increasing effluent concentrations.While pharmaceutical effluent caused significant ($p < 0.05$) reductions in the plant's germination at higher concentrations (15 %, 25 %, 30 %), it promoted germination at 10 % concentration. Pharmaceutical effluent may be used for irrigation at low concentrations after subjecting it to adequate treatment processes.

Keywords: Industrial effluents, bioactive chemicals, pollutants, environmental degradation

INTRODUCTION

The increasing global human population has brought about increasing number of pharmaceutical industries to cater for various human and animal health needs. However, as desirable as these pharmaceutical industries are, some of their operations and activities may pose environmental pollution problems. Pharmaceutical effluents are wastes generated by pharmaceutical industries during the process of drugs manufacturing (James *et al*., 2013). Pharmaceutical industries do not generate uniform waste due to the variety of medicines produced. Typical wastes generated by pharmaceutical industries are made up of a variety of biologically active chemical components such as antibiotics, lipid regulators, anti-inflammatory, tranquilizers, oil and grease, heavy metals and myriads of other compounds, depending on the drug in production (Lateef, 2004; Sankpal, 2012). Some pharmaceutical effluents are known to contain high concentrations of organic compounds and total solids, mercury, cadmium, isomers of hexa-chlorocylohexane, 1,2-dichloroethane and other solvents. The biochemical oxygen demand (BOD), chemical oxygen demand (COD), suspended solids as well as phenol and pH of pharmaceutical effluent is however not consistent, but varies according to the drugs produced (Osaigbovo, 2006; James *et al*., 2013).

Some of the compounds found in pharmaceutical effluents are potentially capable of causing harm to both aquatic and terrestrial life forms. These compounds may cause reductions in the rate of germination, seedling growth, and pigmentation in plants (Kumar and Bhargava, 1998; Jayakumar, 2010).

Sorghum bicolor is a staple food in many tropical countries including Nigeria. It is an annual grass that grows best under relatively high temperature. Nigeria ranked second in global Sorghum production with a production of 6,897,060 tonnes in 2011 (FAOSAT, 2011; Abubakar and Bubuche, 2013). About 50% of the total area devoted to cereal crop cultivation in Nigeria is occupied by Sorghum (Aba *et al*., 2004). Waste water irrigation is practised in many African countries (Abdulai *et al*., 2011) including Nigeria where peasant farming activities along industrial (including pharmaceutical) wastewater course are common. It is the aim of this study to assess the impacts of pharmaceutical industrial effluents on the germination and growth of *Sorghum bicolor*.

MATERIALS AND METHODS

Sample collection

The seeds of accession NG/SA/DEC/07/204 of *Sorghum bicolor* were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria. The pharmaceutical effluent used for this study was collected from Orange Drugs Limited, Town Planning Way, Ilupeju, Lagos, Nigeria.

Physicochemical analysis of pharmaceutical effluent sample

The effluent sample was analysed for turbidity, conductivity, salinity, pH, temperature, and heavy metals (cobalt, copper, magnesium, lead, cadmium) at the Central Research Laboratory, University of Lagos, Nigeria. Turbidity was determined using H198703 turbidimeter. Temperature was measured by dipping Hanna Instrument digital thermometer into the sample. Salinity was determined with AquaPlus digital salinity meter. Conductivity was read directly from conductivity meter, model 230HT, while pH was read from a calibrated electronic pH meter (Hanna Instrument pHep pocket-sized pH meter). Metal content was determined by Atomic Absorption Spectrometer (Perkin Elmer AnalyteA 200 model).

The result of the physicochemical analysis carried out on the pharmaceutical effluent sample was compared to the standards set by: (1) the Federal Government of Nigeria and referred to as the National Environmental (Chemical, Pharmaceutical, Soap and Detergent Manufacturing Industries) Regulations (2009), (2) Nigerian Federal Environmental Protection Agency (FEPA), and (3) World Health Organisation (WHO) (Dan'azumi and Bichi, 2010).

Germination experiment

Five seeds of *Sorghum bicolor* each were put in the Petri dishes already lined with cotton wool that had been moistened with different concentrations of effluent (1 %, 5 %, 10 %, 15 %, 20 %, 25 % and 30 %), while the control was moistened with distilled water. Each treatment in the Petri dish was subsequently kept moist with 2 ml of effluent of corresponding concentration everyday to prevent it from drying up, while the control received only distilled water. Each treatment was replicated three times. The germination experiment lasted for 14 days (two weeks). Percentage germination was calculated for each treatment using the formula stated below.

$$\% \text{ Germination} = \frac{\text{No of germinated seeds}}{\text{Total no of seeds sown}} \times 100$$

Measurement of Growth Parameters in *Sorghum bicolor*

Number of leaves and plant height

The number of leaves per plant and the plant height of each seedling were measured and the results were recorded. The number of leaves per plant was counted manually and the plant height was measured using a ruler; measurement was taken from the base of the stem to the apex of the stem.

Weight of seedling

The fresh weight of seedling was determined using electronic weighing balance, while the dry weight was determined by oven-drying the fresh seedling to a constant weight, at 80±5 °C for 48 hours.

Chlorophyll content determination

Chlorophyll a, chlorophyll b and total chlorophyll were determined from the leaf extract using acetone incubation method (Makeem *et al*., 2007). The optical density (OD) of the chlorophyll was determined using spectrophotometer set at 660 nm against a 100 % acetone solvent blank. The concentration of chlorophyll was thereafter calculated using Arnon's equation (Arnon, 1949).

$$\text{Chla (mg/g)} = (12.7\text{Abs } 663) - (2.6\ \text{Abs}645)\text{mlacetone} /(\text{mg})\ \text{leaftissue}$$

$$\text{Chlb (mg/g} = (22.9\ \text{Abs } 645) - (4.68\ \text{Abs } 663)\text{mlacetone} /\text{mgleaftissue}$$

$$\text{Totalchlorophyll (chla} + \text{chlb)} = (20.21\ \text{Abs } 645) - (8.02\ \text{Abs } 663)\text{mlacetone} / \text{mgleaftissue}$$

RESULTS

Physicochemical characteristics of pharmaceutical effluent

The effluent's total dissolved solids (TDS) value of 6010 mg/l was significantly ($p < 0.05$) higher than the acceptable value of 2000 mg/l set by FEPA. The levels of metals were copper: 324 mg/l; magnesium: 285 mg/l; lead: 283 mg/l; cadmium: 229 mg/l; and cobalt: 241 mg/l. These were also significantly ($p < 0.05$) higher than the available recommended values (Table 1).

Table 1: Physico-chemical properties of pharmaceutical effluent compared with Federal Ministry of Environment (FME) standards.

		Maximum permissible levels		
Parameter	**Result**	**National environmental regulations**	**FEPA**	**WHO**
Physical properties				
Colour	Dark brown	Colourless	-	-
Odour	Unpleasant	Odourless	-	-
Turbidity (NTU)	242	-	-	-
Temperature (°C)	23.8	-	-	-
TDS (mg/l)	6010	-	2000	-
Chemical properties				
pH	5.8a	6-9	6-9	-
Conductivity (µs/cm)	12020	-	600	500
Salinity (PSu)	6.99	-	-	-
Copper (mg/l)	324	1.0	1.0	1.0
Magnesium (mg/l)	285	-	-	-
Lead (mg/l)	283	0.1	0.05	0.05
Cadmium (mg/l)	229	0.1	-	-
Cobalt (mg/l)	241	0.5	-	-

a = not significantly ($p < 0.05$) higher than regulatory standard

Germination of *Sorghum bicolor* in pharmaceutical effluent

There was no statistically significant ($p > 0.05$) difference between the mean percentage germination of seeds of the control treatment (86.7 ± 6.7 %), and those of 1 % (86 ± 6.7 %), 5 % (66.7 ± 6.7) and 10 % (93.3 ± 6.7) pharmaceutical effluent treatments (Table 2). However, the mean percentage seed germination of 93.3 ± 6.7 % recorded in seeds of 10 % effluent treatment was the highest. The mean percentage seed germinations of 46.7±13.3, 46.7±17.6, 40.0±11.5, 26.7±6.7 recorded by 15 %, 20 %, 25 %, and 30 % effluent treatmentswere significantly ($p < 0.05$) lower than those of other treatments.

Growth parameters of *Sorghum bicolor* grown in pharmaceutical effluent

It was observed that the average number of leaves reduced progressively with increase in the concentration percentage of pharmaceutical effluent except in the 10 % effluent treatment which recorded the highest mean number of leaves of 4.3 ± 0.33. There was no significant difference between the mean number of leaves of Sorghum grown in the control and those grown in 1 %, 5 %, 10 %, 15 %, and 25 % pharmaceutical effluent. The mean leaf numbers of 2.0 ± 0.00 and 1.3 ± 0.33 recorded for 25 % and 30 % effluent concentrations respectively were not significantly different from each other.They were, however, significantly ($p < 0.05$) lower than those of other treatments.

The mean seedling height of sorghum was found to reduce with increasing pharmaceutical effluent concentrations. The highest mean seedling height of 17.1 ± 1.42 cm recorded for sorghum in 1 % effluent concentration was the highest. The mean seedling heights of 2.8 ± 0.26 cm and 2.5 ± 1.17 cm recorded for sorghum of 25 % and 30 % effluent concentrations respectively were the lowest.

Results showed that the higher the pharmaceutical effluent concentrations, the lower the mean fresh and dry weights of *Sorghum bicolor*. Sorghum raised in the control treatment recorded the highest and significant ($p < 0.05$) mean fresh and dry weights of 1.7 ± 0.04 g and 0.6 ± 0.05 g respectively. This was followed by Sorghum raised in 1 % effluent concentration with mean fresh and dry weights of 1.5 ± 0.02 g and 0.5 ± 0.03 g respectively. The lowest mean fresh and dry weights of 0.19 ± 0.01 g and 0.07 ± 0.01 g respectively were recorded by Sorghum grown with 30 % effluent concentration (Table 3).

Table 2: Mean percentage germination of *Sorghum bicolor*in pharmaceutical effluent

Concentration	Percentage Germination
Control	86.7 ± 6.7 a
1 %	86.7 ± 6.7 a
5 %	66.7 ± 6.7 a
10 %	93.3 ± 6.7 a
15 %	46.7 ± 13.3 a b
20 %	46.7 ± 17.6 a b
25 %	40.0 ± 11.5 a b
30 %	26.7 ± 6.7 a b

Percentage values having the same letters are not significantly different ($p < 0.05$) (LSD)

Table3: Effect of pharmaceutical effluent on the growth parameters of *Sorghum bicolor*

Concentration	Growth parameters			
	Number of leaves	Height (cm)	Fresh Weight(g)	Dry weight (g)
Control	4.0±0.58a	15.9±1.98a	1.7±0.04a	0.6±0.05a
1%	3.7±0.67a	17.1±1.42a	1.5±0.02a	0.5±0.03b
5%	2.7±0.33a	8.6±1.68b	1.3±0.02a	0.46±0.02b
10%	4.3±0.33a	15.4±1.95a	0.99±0.05a	0.42±0.02bc
15%	3.0±0.58a	6.4±2.44b	0.83±0.12a	0.36±0.04c
20%	2.7±0.33a	5.7±1.81b	0.49±0.02a	0.21±0.01d
25%	2.0±0.00ab	2.8±0.26c	0.42±0.01a	0.13±0.02de
30%	1.3±0.33ab	2.5±1.17c	0.19±0.01b	0.07±0.01ef

Mean leaf numbers with the same letters are not significantly different (LSD)

Chlorophyll content of *Sorghum bicolor* grown in pharmaceutical effluent

Relative to the control, the production of chlorophyll-a in the leaves of *Sorghum bicolor* was significantly ($p < 0.05$) inhibited in all treatments except in the leaves of Sorghum raised in 1% effluent concentration. On the contrary, chlorophyll-b and total chlorophyll production were promoted in leaves of Sorghum grown in 1 %, 5 %, 10 %, 15 % and 20 % effluent concentrations when compared to the control. Chlorophyll-b and total chlorophyll production were highest (0.46 mg/g and 0.39 mg/g respectively) in the leaves of Sorghum grown in 10 % effluent concentration, and was lowest (0.07 mg/g and 0.06 mg/g respectively) in the leaves of Sorghum grown in 30 % effluent concentration.

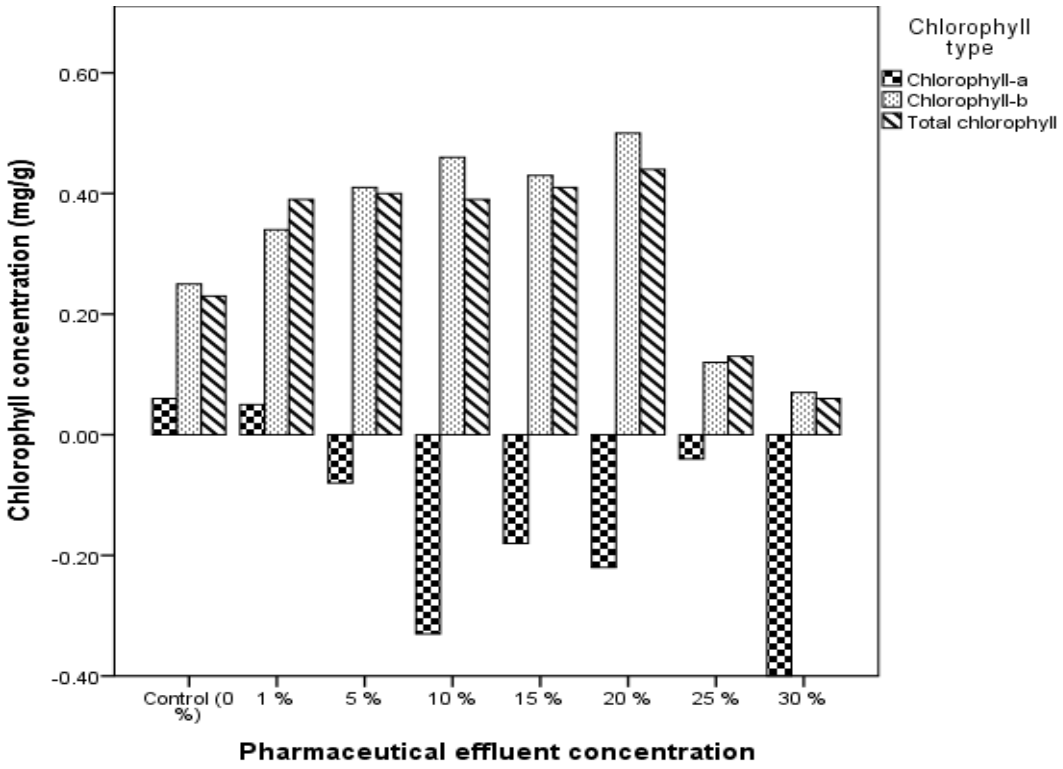

Figure 1: Chlorophyll production in the leaves of *Sorghum bicolor* grown in different concentrations of pharmaceutical effluent

DISCUSSION

The physico-chemical characteristics of the pharmaceutical effluent sample used in this study are obvious signs of pollution. The dark brown colouration, unpleasant odour, low pH, high level of total dissolved solids (TDS), conductivity, salinity, and metal concentrations must have arisen from the biologically active chemicals like antibiotics, lipid regulators, oil and grease, which are used in making drugs (Lateef, 2004; Sankpal, 2012). The pharmaceutical effluent used in this study promoted germination at low concentrations, but inhibited germination at high concentrations. The observed germination-inhibiting effect of pharmaceutical effluent at high concentration is in agreement with the work of Jayakumar (2010) which found that high concentrations of pharmaceutical effluent inhibited the germination of Horse Gram (*Dolichos biflorus*). The germination-promoting effect may be due to the fact that some biological nutrients and salts present in pharmaceutical effluent promote growth and efficient utilization of nutrients at low concentrations (Kumar, 1999). The inhibition of germination at higher concentrations may be due to the overwhelming effect of total solids and heavy metals stress on seed germination process (Indra and Mycin, 2009).

The increase in all the growth parameters (seedling leaf number, height, fresh weight, dry weight) measured in this study at low effluent concentrations, and the decrease of those parameters at high effluent concentrations, are in conformity with previous studies (Sarathchandra *et al*., 2006; Nath*et al*., 2007) which measured the effects of various industrial effluent treatments on crop growth and obtained similar results. The observed reduction in fresh and dry weights of seedlings relative to the control may be partly linked to inability of seedlings to absorb enough water by osmosis due to the high concentration of pharmaceutical effluent salts (Malla and Mohanty, 2010).

The similar increase in chlorophyll-b and total chlorophyll production when pharmaceutical effluent was present at low concentrations is in agreement with the work of Osaigbovo and Orhue (2006) which found similar results with maize plant. The observed increase in chlorophyll-b and total chlorophyll may beattributed to thelow concentrations of trace elements in the pharmaceutical effluent which favour plants' physiology. The reduction in chlorophyll content at higher effluent concentrations may be due to accumulation of heavy metals, especially lead (Pb), which have been reported to inhibit germination, root elongation, seedling development, plant growth and chlorophyll production (Sethy and Ghosh, 2013).

The implication of the results of this study is that pharmaceutical effluent suppresses plant growth when present at high concentrations, but promotes growth at low concentrations. Though no recordof large-scale or commercial use of pharmaceutical effluent in agricultural irrigation in Nigeria was found, peasant farming along wastewater is a common occurrence especially in urban centres. The findings of this study suggests that pharmaceutical effluent may be diverted into farm irrigation use after it has been subjected to proper and adequate treatment and dilution processes. However, further studies on field application need be carried out to confirm these preliminary laboratory findings.

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