Effect of Chlorine Treated Water on Germination and Growth of Cowpea Cultivars (*Vignaunguiculata* L. Walp)

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Abstract— *Effects of different water qualities (WQ_1- WQ_5) of varying chlorine contents were tested on the growth and germination of four varieties of cowpea (Vignaunguiculata) IT03k 131-2 (v_1), IT99k -573-1-1(v_2), UAM09 1046-6-1 (v_3) and UAM09 1055-6 (v_4). The experimental design was factored using Completely Randomized Design (CRD) with three (3) replicates for each treatment of the five (5) water qualities for four (4) cowpea varieties. Growth parameters, germination rate, seedling vigor indices and chlorophyll content were measured. The interactions of cowpea variety and water quality had no significant effect on the any growth parameter tested ($p>0.05$). Variety factor was largely insignificant ($p>0.05$) with minor exceptions. Water quality factor had significant effects on all growth parameters of cowpea ($p<0.05$). Germination rate was highest in pond water (no chlorine) but least in disinfected water when10g and 20g of chlorine were applied. Percentage germination recorded the high values of 98.6% and 95.8% in pond and river water respectively. Water-treated plant without additional chlorine had the same germination with river water (95.8%). 10gCl and 20gCl added to disinfected water reduced cowpea germination to 10.1% and 0.5% respectively. Chlorination had significant effects on seedling height from 7 day to 28 day after planting ($p<0.05$). Seedlings treated with disinfected water were the tallest at 28 day after planting (18.1cm) followed by river and pond water (16.2cm and 16.1cm respectively). Heights of seedlings reduced drastically to 0.5cm on addition of 10g and 20g of chlorine. The two best vigour indices were found among seedlings treated with pond water (1186) and disinfected water (1172). Vigour were significantly reduced when seedlings were treated with additional chlorination ($p<0.05$). The same trend was observed in the germination speed indices of seedlings. Shoot and root weight were also reduced by chlorine. Disinfected water +10g of chlorine and disinfected water +20g of chlorine recorded zero weight (0.000g). The highest chlorophyll content was found in the leaf of plant treated with disinfected water (1.799) followed by river water (1.658) and pond water (1.402). No chlorophyll test was conducted on plants treated with additional chlorine as they died off before maturity when treated with DFW+10gcl and DFW+20gcl. As a result, normal disinfection yielded the same result as pond and river water having no significant effect on the growth parameters evaluated. However, additional chlorination (DFW+10gcl and DFW+20gcl) significantly affected the cowpea cultivars ($p<0.05$).Therefore, municipal water treated with chlorine for drinking should be considered safe for irrigating the crop. However, high chlorine concentrations adversely affect the crop and this outcome may also be applicable to other commercially cultivated crops of huge importance to the economy.*

Keywords— Cowpea, Chlorine, Water quality, Growth parameters.

I. INTRODUCTION

Cowpea is of Africa and Asian origin (perrino *et al.,* 1993, Ogunkanmi *et al*., 2005). In Nigeria, it is grown all over the country but with varying sowing dates. The major cultivation centre includes Kano, Katsina, Bauchi, Borno, Sokoto and Niger in the North, Ibadan, Owo, Benin and Asaba in the South (Rachie, 1985).The cowpea (*Vignaunguculata*) is one of several species of the widely cultivated genus Vigna. Four subspecies are recognized, of which three are cultivated (more exist, including textiles pubescens, and sinensis) (Perrino *et al*., 1993). Most cowpeas are grown on the African continents, particularly in Nigeria and Niger which account for 72% of world cowpea production (FAO, 2015). Cowpea are grown mostly for their edible beans, although the leaves, fresh peas and fresh pea pod can also be consumed, meaning the cowpea can be used as food source before the dried peas are harvested (Ehlers and Hall, 1997).Cowpea is an important source of food for humans in poor arid regions the crop can also be used as feed for Livestock this predominantly occurs in India, where the stock is fed cowpea as forage or fodder (Singh *et al.,* 1997). Cowpea provides a rich source of proteins and calories as well as minerals and vitamins. A cowpea seed can consist of 25% protein and is low in anti-nutritional factors (Rangel, 2003). The diet complements the mainly cereal diet in countries that grow cowpeas as a major food crop (Philips, 2003).

The demand for cowpea production keeps increasing as it is one of the cheapest source of protein and as such the production cannot be narrowed only to seasonal (rainfall) production. The increase in cowpea production also depends on other production practices such as irrigation using other available water qualities (such as pond, river or disinfected domestic water). The roles of water in the seed germination, plant growth and physiological functions cannot be over emphasized (Taylor *et al*., 2007). In the commercial production of cowpea, different types of water from different sources are used in irrigation most of which are disinfected with chlorine. Poor yield may be ignorantly attributed to other factors without prior knowledge of the effect of chlorine content on the general well being of the plant. Water quality varies from source to source. Different sources of water are commonly used by growers to irrigate crops in Nigeria among which are: well water, municipal water and pond water. Chlorination is used to kill certain bacteria and other microbes in tap water as chlorine is highly toxic. In particular, chlorination is used to prevent the spread of water borne diseases such as cholera, dysentery, jaundice, typhoid etc. (EPA, 2014). Disinfection by chlorination can be problematic, in some circumstances. Chlorine can react with naturally occurring organic compounds known as disinfection by products (DBPs). The most common DBPs are trihalomethanes (THMs) and shaloacetic acids (HAAs), which mainly responsible for health hazard (WHO, 2011). This present study was designed to determine the effect of chlorine on germination and growth by comparing chlorinated water with municipal and natural water bodies. The most effective water quality would be recommended for cowpea irrigation.

II. MATERIALS AND METHODS

2.1 Sources of materials

The four varieties of cowpea seeds were obtained from the Molecular Biology Laboratory of University of Agriculture Makurdi. These include: IT03k-131-2 (v_1), IT99k -573-1-1(v_2), UAM09 1046-6-1 (v_3), UAM09 1055-6 (v_4). Four different water qualities used for the experiment were: Disinfected water (chlorinated) WQ_1); River water (WQ_2); Pond water (WQ_3); Disinfected water + 10gcl (WQ_4) and Disinfected water + 20gcl (WQ_5). Disinfected water (chlorinated water) was obtained from the Water Works Department of the University of Agriculture Makurdi. River water was collected from River Benue in Makurdi pond was collected within the university. Chlorine was collected from the quality control department of Consolidated Brewery Plc, Makurdi, Nigeria.

2.2 Elimination of Interfering factors

Soil used for this experiment was steamed sterile at the temperature of $100^{o}C$ for 45minutes. This was done to avoid interference of organic matter with the chlorine. The soil was analysed at the Advanced Soil Science Laboratory, University of Agriculture Makurdi. Soil particle size distribution was determined by Bouyaucos's Hydrometer using sodium hexametaphosphate (algon) as the dispersing agent. (Bouyouco, 1962).Soil and water pH was determined using pH meter (electrometric method).

2.3 Experimental Design

The design was set up as factorial experiment, using Completely Randomized Design (CRD) with three (3) replicates for each treatment of the five (5) water qualities for four (4) cowpea varieties. This was designed such that V_1-V_4 was each tested with WQ_1, WQ_2, WQ3, WQ4, and WQ_5. Six (6) cowpea seeds were planted in 60 pots each, filled with 720g of soil, at a depth of 1.5-2cm (Aguoru *et al*., 2015). Germination count began at 5 days after planting. Germination rate was taken at 5, 7, 9, 10 and 13 days after planting and this was estimated using the formular; $\frac{1}{t_n}(\sum Gn)$ (Dniel, 2007).

Where;

$$t_n = total\ time\ taken$$

$$\sum Gn = cummulative\ germination\ count$$

Seedling length was measured at 7, 14, 21 and 28 days after planting using meter rule in cm. Germination capacity was determined by the number of seedlings emerging from the seeds. Percentage germination was determined by

$$G = \frac{n}{N} X100$$

Where;

n= the total number of seeds during germination test; N=number of seeds initiated

Speed of germination was also determined by the equation;

$$\frac{n_1}{t_1} + \frac{n_2}{t_2} + \frac{n_3}{t_3} + \frac{n_4}{t_4} \dots . \frac{n_5}{t_5}$$

$$GS = \sum(\frac{n_1}{t_1})$$

Where

n=the number of germination seeds on days; t=the number of days during germination period.

The vigor index of seedling was calculated adopting the Baki and Aderson (1973) method. This was expressed as: Seedling Vigor Index (V_1) =germination (%) X seedling length (cm)

i.e.

SV_1= [SL (cm) X G).

Wet shoot and root weight were measured using digital weighing balance (Sekav^{TM}). Chlorophyll test was carried out using spectrophotometer at the wave length of 500nm Chlorophyll extraction followed standard protocol outlined by the Association of Official Analytical Chemists (AOAC, 1984). Chlorophyll test was carried out using spectrophotometer at the wave length of 500nm. Data obtained were subjected to analysis of variance (ANOVA).

III. RESULTS AND DISCUSSION

The effect of chlorinated water on germination and growth of different cowpea cultivars has been successfully investigated using different levels of chlorine. Cowpea varieties only had significant effect ($p \leq 0.05$) on the germination rate at five (5) days after planting and shoot weight per plant. Varieties did not affect other growth parameters measured. Varietal performance shows that germination at 5 days after planting was most significant in the UAM091046-6-1 variety while differences in shoot weight was caused by UAM091055-6 followed by UAM091046-6-1 varieties (table 2). The minor differences observed in the germination rate and shoot weight among cowpea varieties could have genetic basis (Ogunkanmi *et al*., 2005). In the present findings, since the interactions of water quality and variety had no significant effect on the any growth parameter tested ($p>0.05$), varietal differences alone is negligible.

TABLE 1
ANALYSIS OF VARIANCE FOR THE DIFFERENT PARAMETERS MEASURED

Source of variance	Ger @ 5DAP	%Ger	SLH @ 7 DAP (cm)	SLH @ 14 DAP (cm)	SLH @ 21 DAP (cm)	SLH @ 28 DAP (cm)	AVSLH (cm)	SLV INDEX	Ger index	Ger @ 28 days	RW/PLT (g)	SW/PLT (g)
Variety	1.667*	84.579ns	0.015 ns	1.078 ns	6.752 ns	2.493 ns	1.421 ns	11102.740 ns	0.378 ns	1.706 ns	0.0727 ns	0.531*
Water quality	65.900**	3280.296**	75.927**	365.715**	553.350**	363.038**	417.236**	4409284.840**	32.525**	56.983**	2.3013**	9.675**
Water quality X Variety	1.222 ns	46.295 ns	0.277 ns	1.039 ns	3.266 ns	1.215 ns	0.758 ns	5577.060 ns	0.315 ns	0.758 ns	0.1091 ns	0.271 ns

**** = significance at 0.05 (5%) and ** = are highly significance at 0.01 (1%).***
Ger @ 5DAP= Germination rate at five (5) days after planting
%Ger=Percentage germination; SLH @ 7 DAP=Seedling height at 7 days after planting and so on
SLH @ 28 DAP= Germination capacity at 28 days after planting; AVSLH=Average seedling height
SLV INDEX= Seedling vigor index; Ger index=Germination speed index
RW/PLT (g) = Root weight per planting (g); SW/PLT (g) = Shoot weight per plant (g)

TABLE 2
MEAN PERFORMANCE OF COWPEA VARIETIES

Variety	Ger @ 5DAP	%Ger	SLH @ 7 DAP (cm)	SLH @ 14 DAP (cm)	SLH @ 21 DAP (cm)	SLH @ 28 DAP (cm)	AVSLH (cm)	SLV INDEX	Ger index	Ger @ 28 days	RW/PLT (g)	SW/PLT (g)
UAM091046-6-1	3.533a	63.467a	3.267a	7.133a	8.300a	10.533a	7.210a	686.68a	2.397a	3.266a	0.909a	1.686a
IT99k-573-1-1	2.866b	57.978a	3.220a	6.833a	8.726a	10.667a	6.67a	627.87a	2.163a	3.066a	1.0689a	1.473ba
IT03k-131-2	2.866b	60.167a	3.220a	7.166a	8.106a	10.133a	7.345a	684.08a	2.477a	2.66a	0.949a	1.408ba
UAM091055-6	2.866b	59.056a	3.220a	6.600a	7.140a	9.766a	7.291a	663.78a	2.173a	2.66a	0.9421a	1.226b
Mean	3.033	60.16%	3.221	6.933	8.068	10.275	0.458	665.645	2.303	2.88	0.458	1.4667
LSD	0.539	0.39	0.657	1.454	1.815	1.174	1.066	113.27	0.37	0.803	0.838	0.296

Legend:
Value followed by the same alphabet are not statistically significance at $p \leq 0.05$
LSD= Least Significant Difference

TABLE 3
MEAN SEPARATION OF WATER QUALITY

Water variety	Ger @ 5DAP	%Ger	SLH @ 7 DAP	SLH @ 14 DAP	SLH @ 21 DAP	SLH @ 28 DAP	AVSLH	SLV INDEX	Ger index	Ger C @ 28 days	RW/PLT (g)	SW/PLT (g)
Pd	5.333a	98.611a	5.566a	12.208a	14.300a	16.083b	12.039a	1185.97a	3.892a	4.333a	1.4316a	2.033b
RVW	4.500b	95.833a	4.458^{b}	9.166b	9.800b	16.208b	9.908b	950.03b	3.325b	4.416a	1.2498^{ba}	1.925b
DFW	4.333b	95.833a	5.083^{ba}	11.2500a	14.400a	18.083a	12.204a	1172.92a	3.256b	4.666a	1.1542^{b}	2.375a
DFW+10gcl	0.500c	10.056b	0.500c	1.541c	1.342c	0.500c	1.002c	18.18c	0.539c	0.500b	0.500c	0.000c
DFW+20gcl	0.500c	**0.500c**	0.500c	0.500c	0.500c	0.500c	0.500c	0.500c	0.500c	0.500b	0.500c	0.500c
Mean	3.033	60.16%	3.221	6.933	8.068	10.275	0.458	665.645	2.303	2.88	0.458	1.4667
LSD	0.603	6.390	0.735	1.625	2.03	1.313	0.937	126.64	0.413	0.898	0.2537	0.331

Legend:
Pd = Pond water; Rvw = River water; Dfw = Disinfected water
Dfw + 10gCl = Disinfected +10g of Chlorine
Dfw + 20gCl = Disinfected +20g of Chlorine
Value followed by the same alphabet are not statistically significance at p > 0.05

TABLE 4
CHLOROPHYLL TEST USING 500NM WAVELENGTH

WQ_1(Disinfected water)	WQ_2 (River water)	WQ_3 (Pond water)	WQ_4 (disinfected +10g Cl)	WQ_5 (disinfected +20g Cl)
1.799	1.658	1.402	0	0

As presented in table 3, water quality had significant effects on all growth parameters of cowpea ($p<0.05$). Germination rate was highest in pond water (no chlorine) but least in disinfected water when10g and 20g of chlorine were applied. Percentage germination recorded the high values of 98.6% and 95.8% in pond and river water respectively. Disinfected water- treated plant without additional chlorine had the same germination with river water (95.8%). 10gCl and 20gCl added to disinfected water reduced cowpea germination to 10.1% and 0.5% respectively (figure 1). Chlorination had significant effects on seedling height from 7 day to 28 day after planting ($p<0.05$). For instance, seedlings treated disinfected water were the tallest at 28 day after planting (18.1cm) followed by river and pond water (16.2 cm and 16.1cm respectively). Heights of seedlings reduced drastically to 0.5cm on addition of 10g and 20g of chlorine to disinfected water (figure 2).

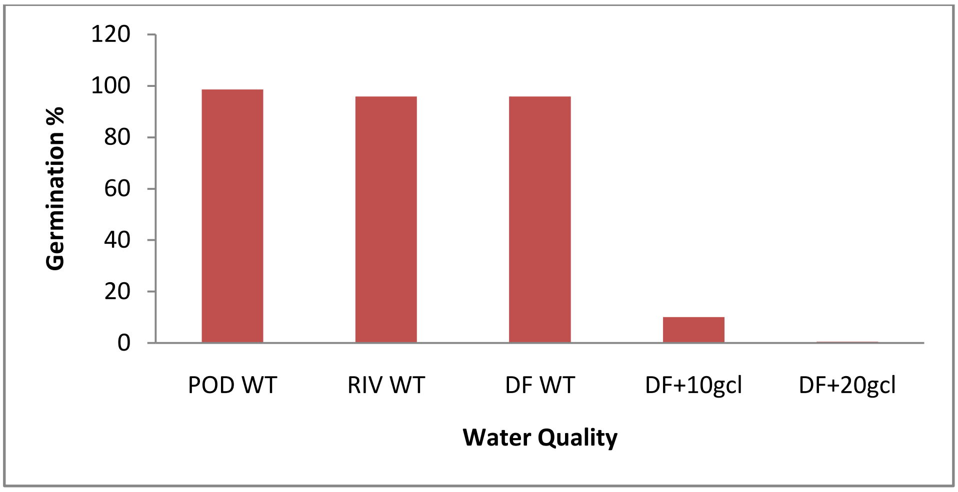

FIGURE 1: Influence of chlorine concentration on germination

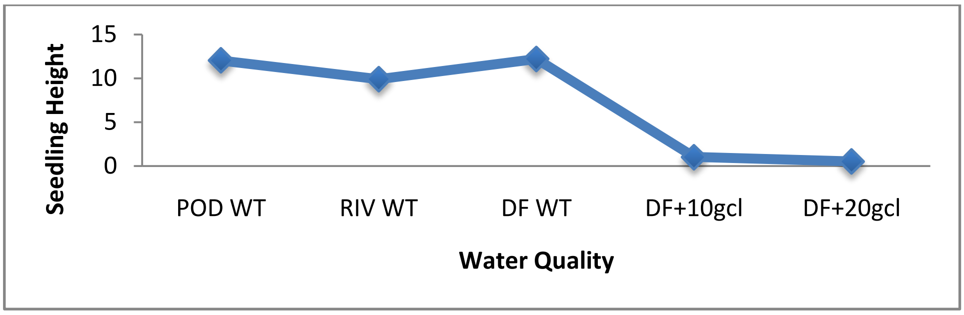

FIGURE 2: Influence of chlorine concentration in seedling height

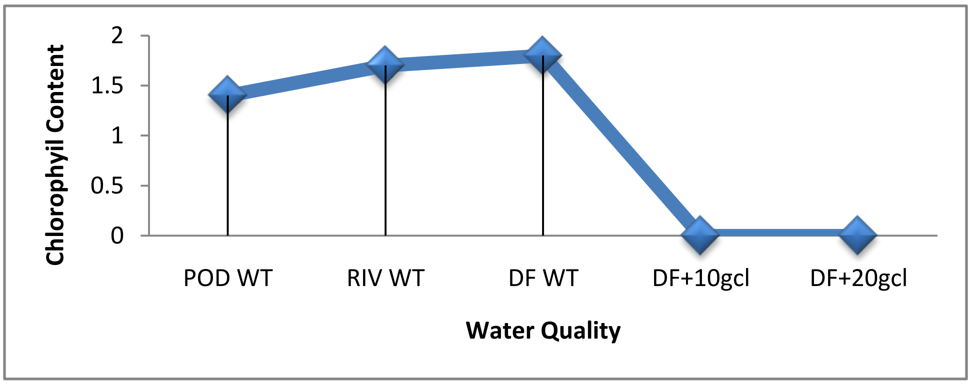

FIGURE 3: Effect of chlorine concentration on chlorophyll

This result could be attributed to the differences in the acidity alkalinity and salt level affecting the pH and overall quality of water treatments. This finding is in line with the report of Bukiv *et al.* (2007) who also found significant differences in seedling growth in white clover and alfalfa genotypes under different pH values of water. However, interaction effect of water quality and variety had no significant effect on any growth parameter tested ($p>0.05$). The present study also aligns with the view of Villagra (1997) that salinity affects seed germination and plant growth. According to Phulari (2013), chlorinated water has inhibitory effect on plants vital process during seed germination. High concentration of chlorine was earlier reported to affect seedling growth and radical length of cowpea (Phulari, 2013).It can be deduced that high concentration of chemical compounds released into the environment may affect the overall plant performances (Aguoru *et al*., 2008, 2015a, 2015b; Egbutah *et al*., 2015). The metabolic activities of all living organisms are catalyzed by enzymes whose functionality depends on water and its quality (Taylor *et al*., 2007). Water is a major limiting factor affecting all life forms, hence its quality assessment is essential (Aguoru and Katsa, 2009; Maduka *et al*., 2014).

The best vigour indices were found among seedlings treated with pond water (1186) and disinfected water (1172). Vigour were significantly reduced when seedlings were treated with additional chlorination to disinfected water ($p<0.05$). The same trend was observed in the germination speed indices of seedlings. Shoot and root weight were also reduced by chlorine. Disinfected water +10g of chlorine and disinfected water +20g of chlorine recorded zero weight (0.000g). According to Olasan *et al*. (2017), seedling vigour is a physiological response attributed to genetic differences. The authors found out that wet biomass of groundnut (*Arachis hypogaea*) in relation to moisture content of the seedling are a major determinant of its overall vigour. In addition, seedling vigour could be affected by external influences (environment) as they interact with genes. This view corroborates the present investigation. As a result, normal disinfection yielded the same result as pond and river water having no significant effect on the growth parameters evaluated. However, additional chlorination (DFW+10gcl and DFW+20gcl) significantly affected the cowpea cultivars ($p<0.05$).

As given in table 4, the highest chlorophyll content was found in the leaf of plant treated with disinfected water (1.799) followed by river water (1.658) and pond water (1.402). No chlorophyll test was conducted on plants treated with additional chlorine as they died off before maturity when treated with DFW+10gcl and DFW+20gcl (figure 3). In the work of Cayanan *et al.* (2008), normal level of chlorine treatment did not affect leaf chlorophyll content after 11weeks of growth. Frink and Bugbee (1987) reported phytotoxic effects of chlorine on several greenhouse crops and their free chlorine thresholds which range from $2.mg.L^{-1}$ to $77mg.l^{-1}$. In the present study, the effect of excess chlorine on chlorophyll synthesis can be seen as inhibitory. This might have affected the photosynthetic apparatus with resultant effect on the level of substrate (sugar) needed in ATP production to power metabolic activities (Taylor *et al*., 2007). Hence, the affected plants failed to survive to maturity.

IV. CONCLUSION

In conclusion, this present study has revealed that the low level of chlorine in municipal water supply does not affect cowpea germination and growth with 95% confidence limit. Therefore, municipal water treated with chlorine for drinking should be considered safe for irrigating the crop. This may also be applicable to other crops. However, high chlorine concentrations adversely affect the crop. In addition more highly demanded crops should be tested under greenhouse and field conditions and at lower concentration of chlorine other than the level used in this experiment.

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