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High Dilution of Mercuric Chloride Increases Water Permeation, Chlorophyll Content and Growth in Germinating Seeds of Cowpea *Vigna unguiculata* (L.) Walp.

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Abstract Aquaporins (AQP) facilitate water transport in cells and conductance of CO_2 through mesophyll to chloroplast. $HgCl_2$ inhibits AQP activity.As per homeopathic principles $HgCl_2$ at ultra high dilution (UHD) would produce reverse effects. To find out whether mercuric chloride at UHD could increase water permeation, chlorophyll content and growth in cowpea seedlings. Germinating seeds were treated with $HgCl_2$ by both high (0.3%) and ultra low doses (*Merc cor* 200 cH). Water, chlorophyll, protein and sugar content and total biomass of treated and untreated embryos were estimated by standard methods. Percentage of germination was calculated in both the groups. Mercuric chloride at UHD (200 cH) increased germination of seeds, water permeation, biomass, protein, sugar and chlorophyll content in embryos significantly (p<0.05) as compared to the untreated control and 0.3% $HgCl_2$-treated seedlings thus showing mercuric chloride at UHD enhances the activity of AQPs and promotes water permeation, photosynthesis and growth in cowpea seedlings.

Keywords Mercuric chloride, High dilution, Aquaporin.

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Introduction

Synthetic nitrogen fertilizers are extensively used in farm lands to increase growth and yield of crops. The other alternative to increase crop biomass and yield is to promote photosynthesis. In fact, there exists a positive relationship between photosynthesis and crop biomass and yield [1].

During photosynthesis atmospheric CO_2 is diffused into the leaf through stomata and transferred

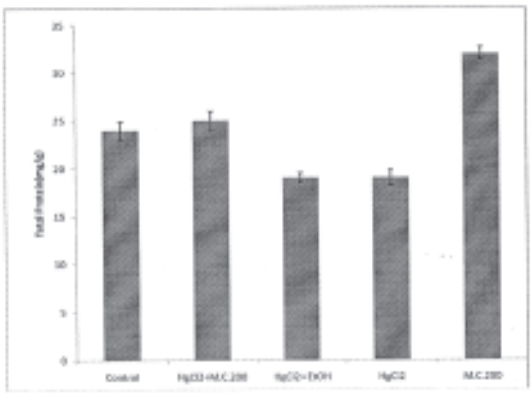

Fig. 1. Total protein contents in embryos of germinating cowpea seeds after 168 h in 5 groups (n=50/group). Significant difference in protein content (p<0.05) one-way ANOVA followed by t-test.

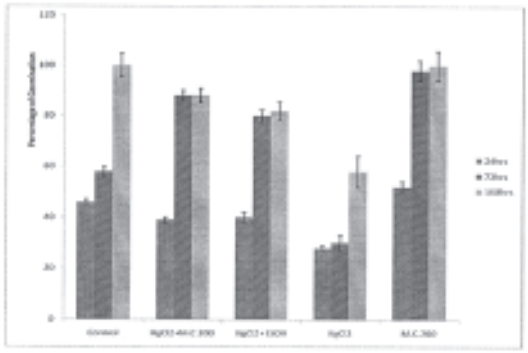

Fig. 2. Percentage of germination of cowpea seeds after 24, 72 and 168 h in 5 groups (n=50/group). Significant difference in germination (p<0.05) one-way ANOVA followed by t-test.

through mesophyll to the chloroplast. Aquaporins or water channel membrane proteins play an important role in the conductance of CO_2 through mesophyll besides water transport [2]. It was reported that mercuric chloride ($HgCl_2$) reduces growth, plant pigment thereby affecting photosynthesis [3]. Mercuric chloride is a non-specific inhibitor of aquaporins. According to the homeopathic principle a substance, which produces some adverse effects on a healthy individual in high doses, would produce exactly the opposite effect at ultra low doses [4]. So, we can anticipate that $HgCl_2$, at ultra high dilution, would enhance aquaporin activity and CO_2 conductance to mesophyll thereby increasing photosynthesis. In our earlier study we demonstrated that some plant growth inhibitors at ultra high dilution (UHD) promoted growth and photosynthesis of crops [5, 7]. The purpose of the present study is to see whether $HgCl_2$ in UHD could increase water permeation through aquaporins, photosynthesis and plant growth. The MT used in this study is a 0.3% solution of $HgCl_2$. *Merc cor* potencies could enhance the activity of the enzyme diastase/α-amylase in terms of breakdown of starch *in vitro* in test tubes in a cell free medium [8, 9].

Materials and Methods

Seeds of *Vigna unguiculata*, var, were surface sterilized with 0.1% $HgCl_2$, washed properly with sterile water and immersed in sterile distilled water overnight.

Drugs/control

Mercuric chloride was purchased from SRL, Mumbai and the homeopathic drug *Merc cor* 200 cH, a product of Dr Reckeweg, Germany, was purchased from the local market. The UHD drug *Merc cor* 200cH was in 90% ethanol as mentioned in its label. The control consisted of 90% ethanol (Merck, Germany). Both

Table 1. Total biomass, shoot and root length in cowpea seedlings after 168 h of germination in 5 groups (n=50/group). Significant difference (p<0.05) by one-way ANOVA followed by t-test.

Groups	Biomass accumulation (g)	Seedling length in mm (7 Days) Root	Shoot
Group I (Control)	0.964±0.008	28±0.002	82±0.01
Group 2 ($HgCl_2$ + MC 200)	0.952±0.01	27±0.003	48±0.01
Group 3 ($HgCl_2$ + EtOH)	0.932±0.009	12±0.018	52±0.03
Group 4 ($HgCl_2$)	0.904±0.004	9±0.01	38±0.04
Group 5 (MC 200)	0.985±0.003	35±0.01	95±0.02

Table 2. Total amount of chlorophyll types and carotenoids (mg/g dry mass) in the leaves of cowpea seedlings after 168 h of germination in 5 groups (n=50/group). Significant difference (p<0.05) by one-way ANOVA followed by t-test.

Groups	Photosynthetic pigment mg/g dry mass				Total chlorophyll/Carotenoids ratio
	Chlorophyll-*a*	Chlorophyll-*b*	Carotenoids	Total chlorophyll	
Group 1 (Control)	0.828±0.005	0.102±0.001	0.532±0.005	0.925±0.004	1.74±0.01
Group 2 ($HgCl_2$ + MC200)	0.0929±0.03	0.0288±0.007	0.1994±0.04	0.1217±0.04	0.6103±0.007
Group 3 ($HgCl_2$+Alc)	0.0868±0.01	0.00583±0.09	0.6857±0.001	0.1451±0.08	0.2166±0.004
Group 4 ($HgCl_2$)	0.0944±0.09	0.0028±0.08	0.189±0.05	0.0972±0.05	0.5126±0.003
Group 5 (MC200)	1.127±0.01	0.139±0.0014	0.746±0.003	1.26±0.01	1.69±0.01

the drug and the control were diluted with sterile distilled water 1:100 one hour before their application on test seeds. The purpose of dilution was to minimize the effect of ethanol on the test samples while retaining the efficacy of the drug [10].

Treatment

After imbibition seeds were divided into 5 groups and treated with the following preparations.

Group I- Control solution (EtOH), Group II- 0.3% $HgCl_2$ followed by *Merc cor* 200 cH, Group III-0.3% $HgCl_2$ followed by control solution (EtOH), Group IV-0.3% $HgCl_2$, Group V-*Merc cor* 200 cH.

Seeds were dipped in the control / *Merc cor* 200cH. (1:1000) in a petridish for 5 mins, washed thoroughly in distilled water. For groups II, III and IV seeds were kept in 0.3% $HgCl_2$ solution for 15 mins, and then washed. Seeds (n=50/group) of all the gropus were then transferred to petridishes (18.5 cm in diameter), each containing filter paper soaked with 10 ml distilled water. Seeds were allowed to germinate in the dishes at room temperature (28±2°C) for 7 days. The following parameters were observed : (i) Growth of embryos, (ii) Chlorophyll content in the leaves, (iii) Total protein content in embryos, (iv) Water content in embryos, (v) Soluble and insoluble sugars in embryos, The percentage of germination at 24 h, 72 h and 168 h was calculated at room temperature with 12 h photoperiod.

Biomass accumulation was estimated by takin dry weight of seedling after 168 h of growth. Seeds were packed in a piece of paper, kept in a hot air oven at 70°C for 72 ho and then weighed.

Photosynthetic pigment was extracted from the leaves and estimated quantitatively following the process of Litchenthaler and Welburn [11].

Total soluble protein from seeds of each group was extracted following the process of Kee and Nobel [12] with necessary modification. Soluble and insoluble sugars were estimated by the Anthrone

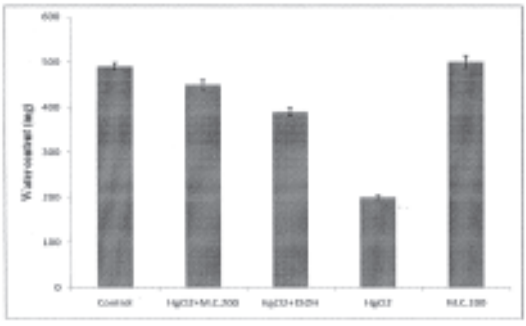

Fig. 3. Total water content in embryos of cowpea seedlings after 168 h of germination in 5 groups (n=50/group). Significant difference (p<0.05) by one-way ANOVA followed by t-test.

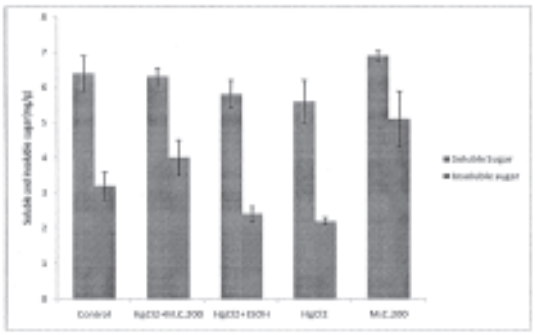

Fig. 4. Total soluble and insoluble sugars in cowpea embryos after 168 h in 5 groups (n=50/group). Significant difference (p<0.05) by one-way ANOVA followed by t-test.

method Hedge and Hofreiter [13]. All the data were analyzed by one way ANOVA followed by t-test.

Results and Discussion

Total soluble protein contents in embryos of different groups are shown in Figure 1. *Merc cor* 200 cH produced maximum protein content followed by $HgCl_2$ + *Merc cor* 200 cH, control, $HgCl_2$ + ethanol and $HgCl_2$. Percentage of germination at 24 h and 72 h was highest with *Merc cor* 200 cH. At 168 h germination percentage was highest with the control followed by *Merc cor* 200cH (Fig. 2). Water content in embryos was highest with *Merc cor* 200 cH followed by control, $HgCl_2$ + *Merc cor* 200 cH, $HgCl_2$ + ethanol and $HgCl_2$ alone (Fig. 3). Sugars, soluble and insoluble, were highest with *Merc cor* 200 cH lowest with $HgCl_2$ alone (Fig. 4). Chlorophyll a, b, total chlorophylland carotenoids, biomass of embryos, shoot and root length were highest in group treated with *Merc cor* 200 cH (Tables 1, 2).

Aquaporins (AQP) are transmembrane water channel proteins which facilitate transport of water molecules through plasma membranes [14, 15]. They are present in both plant and animal cells. Water transport by all types of AQPs except AQP4 are inhibited by mercurial compounds which bind specifically to cysteine residues thereby blocking the pore of AQPs [16]. This phenomenon of reverse effect of a substance at ultra low doses has also been onserved in plants [6, 17–19]. High dilutions of the leaf extract of *Cymbopogon winterianus* (citronella) increased germination and growth of *Sida rhombifolia* seedlings. The dilution 12 cH produced maximum growth [20]. Zinc and silver nitrate are trace elements having biological activity. However, at extremely low does both $ZnSO_4$ and $AgNO_3$ produced growth in *Bacopa monieri* and wheat seedlings, respectively [21]. The UHD of mercuric chloride, i.e. *Merc cor* 200 cH might have enhanced aquaporin activity as a reverse effects of $HgCl_2$ MT and thus increased water permeation in cowpea embryos observed in our study (Fig. 3). *Merc cor* 200 cH-induced increased activity of AQPs has also resulted in increased chlorophyll content in the leaves of germinating cowpea seeds (Table 2). It is known that the rate of photosynthesis is directly proportional to chlorophyll content [22]. So we can conclude that *Merc cor* 200 cH has increased the rate of photosynthesis in the cowpea seedlings. Mild water stress affects stomatal conductance and photosynthesis [23]. Facilitation of AQP activity by *Merc cor* 200 cH might have increased photosynthesis in the test seedlings. Higher photosynthetic activity has led to higher carbohydrate synthesis (Fig. 4). However, understanding the exact mechanism of action of *Merc cor* 200 cH needs further study. *Merc cor* 200 cH appears to be a cheap and safe alternative to chemical fertilizers and holds promise as a facilitator of photosynthesis, growth of plants in arid zones and carbon sequestration from the atmosphere.

References

1. Long SP, Zhu XG, Naidu SL, Ort DR, Ort DR (2006) Can improvement in photosynthesis increase crop yields. Pl Cell Environ 29 : 315—330.
2. Flexas J, Ribas–Carbo M, Hanson DT, Bota J, Otto B, Cifre J, Mc Dowell N, Medrano H, Kaldenhoft R (2006) Tobacco aquaporin NtAQPI is involved in mesophyll conductance to CO_2 *in vivo*. Pl J 48 : 427—439.
3. Priya M, Balakrishnan V (2014) Response to biochemical characteristics by mercuric chloride on *Clitoria ternatia* L. Int J Curr Microbiol App Sci 3 : 179—183.
4. Sukul NC, Sukul A (2004) High dilution effects : Physical and biochemical basis. Kluwer Academic Publ Pvt Ltd, New Delhi.
5. Sukul NC, Singh RK, Sukul (Chunari) S, Sen P, Bhattacharyya A, Sukul A, Chakravorty R (2009) Homeopathic potencies promote growth of ladys finger plants, *Ablemoschees esculentus*. J Nat Hist 5 : 90—97.

6. Sukul NC, Sukul S, Mondal S (2011) Plant growth inhibitors at ultra high dilution enhance chlorophyll content and alter leaf protein profile. J Altern Med Res 3 : 189—194.
7. Sukul C Soma, Mondal S, Sukul NC (2015) A potentized drug enhances growth and yield in rice : A field study. J Altern Med Res 7 : 337—344.
8. Sukul NC, De A, Sukul A, Sinhababu SP (2002) Potentized Mercuric chloride and Mercuric iodide enhance α-amylase activity *in vitro*. Homeopathy 91 : 217—220.
9. Konar A, Sarkar T, Sukul NC, Sukul A, Chakraborty I (2015) Transfer of the effect of potentized Mercuric chloride on α-amylase fromone test tube to another through capillary water. Int J High Dilution Res 14 : 4—11.
10. Sukul S, Mondal S, Sukul NC (2012) *Sepia* 200cH in 1:1000 dilution counters the effect of salt stress in cowpea seedlings but vehicle 90% ethanol proves ineffective in the same dilution. Int J High Dilution Res 11 : 237—240.
11. Litchenthaler HK, Welburn AR (1983) Determinations of total carotenoids and chlorophyll *a* and *b* of leaf extracts in different solvents. Biochem Soc Trans 603 : 591—592.
12. Kee SC, Nobel PS (1986) Concomitant changes in high temperature tolerance and heat shock proteins in desert succulents. Pl Physiol 80 : 596—598.
13. Hedge JE, Hofreiter BT (1962) Estimation of carbohydrate. In : Whistler RL, BeMiller JN, eds. Carbohydrate Chemistry , New York, Academic Press.
14. Maurel C (2007) Plant aquaporins : Novel functions and regulation properties. FEBS Letters 581 : 2227—2236.
15. Katsuhara M, Hanba Y, Shiratake K, Maeshima M (2008) Expanding roles of plant aquaporins in plasma membranes and cell organelles. Functional Pl Biol 35 : 1—14.
16. Hirano Y, Okimoto N, Yasui M et al. (2010) Molecular mechanisms of how Mercury inhibits water permeation through aquaporin-1:Understanding by Molecular Dynamics Simulation. Biophys J 98 : 1512—1519.
17. Nyitrai P, Boka K, Gaspar L, Sarvari E, Keresztes A (2004) Rejuvenation of aging bean leaves under the effects of low dose stressors. Pl Biol (Stutty) 6 : 708—714.
18. Nyitrai P, Boka K, Gaspar L, Sarvari E, Lena K, Keresztes A (2003) Characterization of the stimulating effect of low dose stressors in maize and bean seedlings.J Pl Physiol 160 : 1175—1183.
19. Sukul NC, Chattopadhyay S, Das C, Sukul C, Sukul A, Ghose S, Sinhababu SP (2007) Enhancement of photosynthesis and plant growth by potentized drugs. Environ Ecol (suppl 3) 25 : 520—524.
20. Marques RM, Bonato CM, Marques, Silva G (2008) Effects of high dilutions of Cymbopogon winterianus jowitt (Citronella) on the germination and growth of Sida rhombifolia. Int J High Dilution Res 7 : 31—35.
21. Gupta VK, Ray JR, Singh VK, Pathak SD, Nayak C, Darokar MP (2016) Dose-dependant effect of homeopathic drug $ZnSO_4$ on plant growth *Bacopa monnieri.* Ind J Res Hom 8 : 19—23.
22. Baker NR, Oxborough K (2004) Chlorophyll fluorescence as a probe of Photosynthetic productivity. Chapter 3, Springer, The Netherlands, pp 66—79.
23. Taiz L, Zeiger E (2006) Plant physiology sinauer associates Inc Publ, Sunderland, Massachusetts.