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ORIGINAL ARTICLE

Differential responses of plumbagin content in *Plumbago zeylanica* L. (Chitrak) under controlled water stress treatments

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A pot experiment was conducted on *Plumbago zeylanica* L. (Chitrak) under controlled water stress environment in greenhouse during the kharif season. The experiment was laid out in completely randomized design with five treatments of different water stress levels i.e. control, 20%, 40%, 60% and 80% and four replications. Out of five stress levels, 80% water stress has influenced root length, dry herbage, plumbagin, potassium and proline content. In control conditions the plant height, number of leaf, total leaf area, stomatal conductance, transpiration rate, photosynthesis, CO_2 utilization, H_2O utilization and chlorophyll were found to be maximum. The impact of water stress on plumbagin content has shown increase trend with respect to different water stress levels that is maximum at 80 % and minimum at control.

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Plumbago zeylanica L. (Chitrak) is a semi climbing perennial herb that grows throughout India, Asia and Africa, also in Hawali, Virgin Island. It is found wild in peninsular India and in low elevations in Taiwan (Anonymous, 1962). It is a small perennial 0.5 to 1.5m tall, erect or climbing under shrub with flexible branches. Stems many, smooth, woody, striate, leaves simple, alternate glaborous, ovate, entire, oblong, 5 to 9.5 cm long and 2 to 6 cm broad. Flowers white, long, glandular, strike like racemes. Inflorescences stalk 20-30 cm long branches on which tubular flowers are borne. Calyx is glandular and toothed while corolla tube 17-22cm long. Fruits cylindrical, small capsule, oblong enclosed in the calyx. Roots finger size thick, blackish purple bark and whitish internally with bitter smell. Flowering season is winter followed by fruiting after a month. The root contains a number of naphthaquinone derivatives (Plumbagin yellow, 3 Chloroplumbagin, 3, 3-biplumbagin, Elliptinone, Chitranone, Droserone, Zeylanone, Iso-zeylanon and Plumbazeylanone. Leaves and stem contain volatile oil. The root possesses obortifacient and vesicant properties (Gupta, 2008). The whole plant and its roots have been used as folk medicine. Plumbagin is present in families of Plumbaginaceae and Droseraceae (Botanical Dermatology Database, 1991). Plumbagin has received an enormous amount of attention in pharmacological research, due to its antimalarial (Likhitwitayawuid *et al*., 1998), anti-microbial (Didry *et al*., 1998), anti-cancer, anti-carcinogenic, anti-mutagenic (Sugie *et al*., 1998) and chemotherapeutic activities (Hakura *et al*., 1994). It is used as diuretic, caustic and excellent of phlegmatic tumors and is useful in rheumatism. It is also used as an irritant of the skin, in the treatment of dyspepsia, piles, anasarca, diarrhea, skin diseases and in minute dosages in stimulant for liver (Gupta, 2008). Cultivation of medicinal plants under water stress conditions is an important factor for controlling levels of phytochemicals (Zheng *et al*., 2007). Environmental stresses trigger a wide variety of plant responses, ranging from altered gene expression and cellular metabolism to changes in growth rates and crop yields. However, some studies on the use of water stress to enhance or increased the production of useful natural products in medicinal plants. Stress is measured in relation to plant survival, crop yield, growth (Bio mass accumulation) or the primary assimilation processes (CO_2 and mineral uptake) which are related to overall growth. The alkaloid content in the plants is presumed to be the resultant of some abiotic stress.

The review literature reveals that the effects of moisture stress on plumbagin content in *Plumbago zeylanica* L. are lacking. Thus the present work was carried out to investigate the effects of water stress treatments on a number of growth parameters, biomass production and biochemical responses of the crop.

MATERIALS AND METHODS

Location and Duration: The pot experiment was conducted at the climate control Green house research area of Medicinal and Aromatic plants under the Department of Crop and Herbal Physiology, JNKVV, Jabalpur, (M.P) during the Rabi season 2007-2008. The soil of experimental pot was sandy loam. Date of transplanting was 15th June 2007 and harvesting was carried out on 25th Feb 2008. Four plants of *Plumbago zeylanica* L. were transplanted in each pot applied with FYM, Biofertilizer and Inorganic fertilizer. Sampling was done at 15 day intervals for growth analysis, phenological and biochemical parameters. The sampling was done at 60, 75, 90,105, 120, 135 and 150 days after sowing.

Pot Experiment: The design was completely randomized design and experiment included five treatments of water regimes at field capacity (FC) with four replicates. The five water stress treatment are 20%, 40%, 60%, 80% and control.

- Control (FC).
- Mild Water Stress (20%).
- Moderate Water Stress (40%).
- Severe Water Stress (60%).
- Very Severe Water Stress (80%).

To calculate the field capacity, at the beginning of the experiments, pots were filled with known weight of mixture of sand: compost, saturated with water and allowed to drain freely for a period of 24 hours, until there was no change in weight. The difference between this weight and soil dry weight (DW) was used to calculate 100% of water holding capacity (WHC) (Liu and Stutzel, 2004). Before the beginning of water stress treatments, all pots watered to FC. At the beginning of the experiment, plants subjected to control water regime were irrigated daily to maintain fully FC (well watered plants), while irrigation of the plants of 20%, 40%, 60% and 80% FC water regime treatments (water stressed plants) was withheld until the field capacity reached 20%, 40%, 60% and 80% FC respectively.

Growth Measurements: TPlant height (cm), number of leaf, total leaf area and LAI by Gardner *et al*., (1985), Biological yield /plant and root yield / plant. Photosynthesis etc were determined by using Infra Red Gas Analyzer of LI-COR Model LI-6400 portable photosynthesis system, USA.

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Biochemical parameters: Chlorophyll by Yoshida *et al*., (1972), Potassium by Black (1965), Proline by Bates *et al*., (1973) and Plumbagin by HPTLC.

Statistical analysis: Analysis of observation taken on different variable was carried out to know the degree of variation among all the treatments. The pooled data was statistically analyzed through completely randomize design (Fisher, 1967).

Table 1 : Growth measurements and biomass production of *Plumbago zeylanica* L. plants under water stress conditions in pot.

Treatments	Plant height	No. of Leaves	Total leaf area	Leaf Area Index	Water Use Effiency
Control	56.25	34.62	21.69	2.89	23.26
20% water stress	55.13	33.31	20.06	2.53	20.46
40% water stress	41.06	20.68	15.44	2.20	16.93
60% water stress	31.50	18.25	11.88	1.64	15.18
80% water stress	22.94	12.56	7.44	1.88	12.93
S.Em±	0.0071	0.0069	0.0084	0.0074	0.0083
C.D 5%	0.0213	0.0209	0.0252	0.0222	0.0251

RESULTS AND DISCUSSION

The moisture stress treatment exhibited the significant influences over growth parameters. The maximum plant height was registered in control (56.2cm) and minimum was registered in 80% moisture stress (22.94 cm). The maximum number of leaves was recorded on control (34.62) and minimum was registered in 80% moisture stress (12.56) due to the turgor pressure of the cell. The maximum total leaf area was found in control (21.68) and minimum in 80% moisture stress (7.44). The maximum LAI was found in control (2.89) and minimum was registered in 80% moisture stress (1.88). There is low increase in plant height under extreme deficit possibly due to reduced cell turgor which affects cell division and expansion (Luvaha *et al*., 2008). The results of the study indicate that water deficit decreased leaf number, leaf area, leaf water content, shoot height, shoot dry weight and chlorophyll concentration. Decreases in leaf number and leaf area are common occurrences in water deficit stressed plants (Luvaha *et al.,* 2008). Reduction in leaf number under extreme water deficit may have been due to reduction in leaf formation. Reduction in number of leaves can be a phenomenon by the plants to reduce transpiration surface hence water loss. Similar results have been observed in mango rootstock seedlings, which show a decline in number of leaves due to drying or senescence of lower mature leaves (Luvaha *et al.,* 2008). Reduced leaf area decreases interception of solar radiation and consequently decreases biomass production for most crops (Masinde *et al*., 2005).

The maximum photosynthesis rate and stomatal conductance were found in control (10.66 mol $m^{-2}s^{-1}$ and 10.04 mol $m^{-2}s^{-1}$) and minimum in 80% water stress (3.87 mol $m^{-2}s^{-1}$ & 2.90 mol $m^{-2}s^{-1}$). Decreased number of stomata under higher moisture deficit condition has been reported by Xu and Zhou (2008) in grasses. Ghannoum *e. al.,* (2003) and Ripley et al., (2007) suggested the reduction in leaf net photosynthetic assimilation by both stomatal and metabolic limitations under moisture stress situation.

Table 2 : Growth measurements and biomass production of *Plumbago zeylanica* L. plants under water stress conditions in pot.

Treatments	Net Photosynthesis	Stomatal conductance	CO_2 Utilization	Transpiration rate	H_2O Utilization
Control	10.66	10.04	9.55	0.46	1.14
20% water stress	9.86	9.50	9.28	0.41	1.07
40% water stress	6.81	6.93	6.89	0.38	0.74
60% water stress	4.39	5.15	5.10	0.31	0.69
80% water stress	3.87	2.90	3.94	0.33	0.57
S.Em±	0.0074	0.0077	0.0074	0.0071	0.0081
C.D 5%	0.0224	0.0234	0.0222	0.0214	0.0245

The maximum CO_2 and H_2O utilization were found in control (9.55 ppm and 0.46 Kap) and minimum in 80% water stress (3.94 ppm and 0.33 Kap). The maximum transpiration rate and water use effiency were found in control (1.14 mol $m^{-2}s^{-1}$ and 23.26) and minimum in 80% water stress (0.57 mol $m^{-2}s^{-1}$ and 12.93).

Maximum root length was found in 80% water stress (16.3cm per plant) and minimum in control (5.4 cm per plant). The increased of root dry weight under drought conditions is in accordance with previous studies in other plant species (Sharp *et al.,* 1990; Boutraa and Sanders, 2001; Liu and Stutzel, 2004). This might be explained by the functional balance theory, proposed by (Brouwer, 1963), that in plant imposed to limited water supply shoots will be checked sooner than that of roots because the latter are closer to the source of water supply limitation, leading to increase in root dry masses due to the increase of assimilates flow to belowground. Harris (1973) observed that retardation of shoot and root growth by moisture stress.

Maximum dry herbage yield was found in control (17.64gm) followed by 20% water stress (17.45 gm) and minimum in 80% water stress (5.90 gm). Decrease in herbage yield under water stress has also been reported in other medicinal and aromatic plants (Singh- Sangwan *et al*., 2001; Fatima *et al*., 2002).

The maximum chlorophyll content was found in control (27.48%) followed by 20% water stress (23.79%) and minimum in 80% (5.54%). Chlorophyll concentration reduced with increase in water deficit. This could be attributed to an increase in oxidative stress. Under more prolonged water deficit, dehydration of plant tissue can result in an increase in oxidative stress, which causes deterioration in chloroplast structure and an associated loss of chlorophyll. This leads to a decrease in the photosynthetic activity (Jafar *et al*., 2004). Reduction in chlorophyll concentration in water stressed plants could indirectly lead to a decrease in photosynthetic activity. Total chlorophyll content reduces by 55% compared to well water plants (Kirnak *et al*., 2001; Cengiz *et al*., 2006).

Table 3: Biochemical profils of *Plumbago zeylanica* L. plants under water stress conditions in pot.

Treatments	Chlorophyll (%)	Herbage/ pod	Root length (cm)	Proline (%)	Potassium (%)	Plumbagin (%)
Control	27.48	17.64	5.40	1.70	1.40	0.0984
20% water stress	23.80	17.45	9.80	1.87	1.55	0.1034
40% water stress	18.47	15.65	13.40	2.35	1.68	0.1291
60% water stress	14.94	10.63	15.20	2.36	1.75	0.1323
80% water stress	5.54	5.90	16.30	2.39	1.89	0.2342
S.Em±	0.0070	0.0085	0.0416	0.0074	0.0085	0.0001
C.D 5%	0.0211	0.0257	0.1255	0.0222	0.0257	0.0003

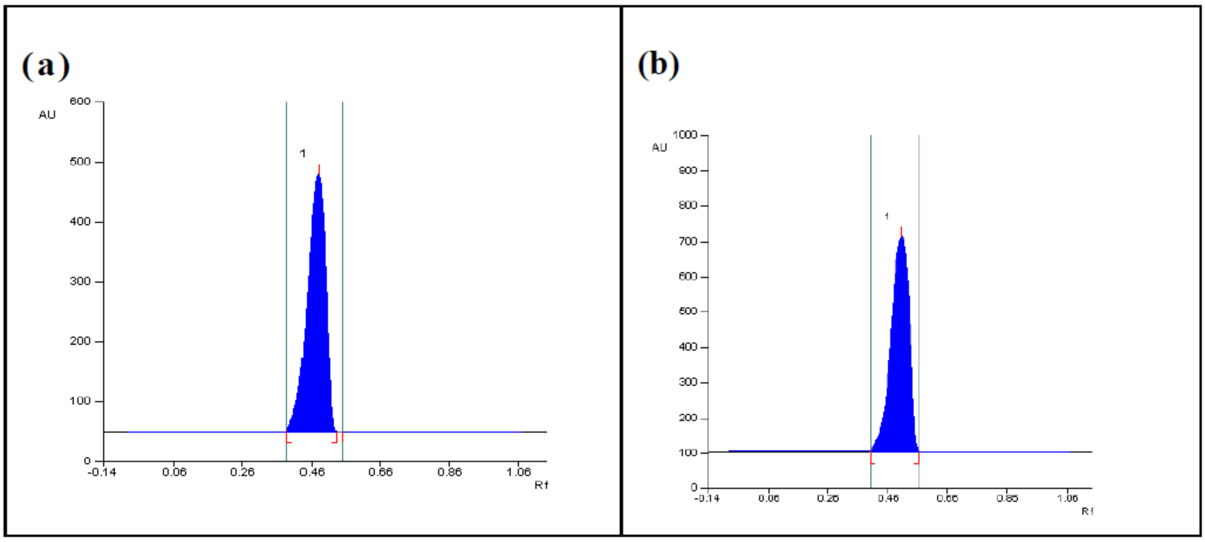

Figure 1: HPTLC chromatogram of plumbagin content in *Plumbago zeylanica*.
(a) Plumbagin Standard (b) Plumbagin in sample.

The maximum proline content was in 80% water stress (2.39%) followed by 60% water stress (2.36%) and minimum in control (1.70%). Water stress induced the accumulation of free proline in plants may be part of a general adoption to water stress (Hare *et al.,* 1998). Amino acid proline is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses. In addition to its role as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions. It may also function as a protein compatible hydrotrope (Srinivas and Balasubramanian, 1995). The concentration of free proline of plumbagin stressed plants was significant increase in response to water stress compared to the control plants. Current study showed that the effect of water stress in pots experiments lead to significant increase in the total free proline. The obtained data were in agreement with (Wu *et al*., 2006) who stated that the content of free proline significantly increased under water stress of *Rosmarinus officinalis* L. Blum and Ebercon (1976) indicated that proline is regarded as a source of energy, carbon and nitrogen for recovering tissues, so it increased under water stress levels. Water deficits induce dramatic increases in the proline concentration of phloem sap in medicinal and aromatic plants, suggesting that increased deposition of proline at the root apex in water stressed plants could in part occur via phloem transport of proline. A proline transporter gene, ProT2, is strongly induced by water and salt stress in *Arabidopsis thaliana* (Rentsch *et al.,* 1996).

The maximum potassium content was in control (1.88%) followed by 20% water stress (1.75%) and minimum in 80% water stress (1.40%). Potassium content was affected by excessive water stress treatment, which resulted in the lowest percentage. These results are confirmed by those of Mirsa and Shrivastava (2000) for Japanese mint plants, Khalid (2001) for *Nigella sativa* L. plants and Hendawy and Khalid (2005) for *Salvia officinalis* L. plants. The maximum plumbagin content was in 80% water stress (0.2342%) and minimum in control (0.0984%). Lim *et al.*, (2006) reported similar findings that ginsenoides content of *Panax quinquefolium* increased by the effect of water stress.

CONCLUSION

The various treatment combinations exhibited a significantly variability in morph-physiological growth parameters, biochemical parameters and economy yield attributing parameters of chitrak. Out of five stress levels, 80% water stress has influenced root length, dry herbage, plumbagin, potassium and proline content. In control conditions the plant height, number of leaf, total leaf area, stomatal conductance, transpiration rate, photosynthesis, CO_2 utilization, H_2O utilization and chlorophyll were found to be maximum. The significant improvement in morph-physiological and biochemical attributes expressed as superior yield attributing parameter which resulted in maximum economic yield of any crop.

REFERENCE

Anonymous, (1962). The wealth of India. Raw Materials, pp: 163-164.

Bates, L.S., Waldren, R.P. and Teave, I.D. (1972). Rapid determination of proline for water stress studies. *Plant and Soil*, **39**:205-207.

Black, C.A. (1965). Methods of Soil Analysis Part-II. American Soc. of Agronomy Inc., Publisher Madison Wisconsin, USA, 1372-1376.

Blum, A. and Ebercon, A. (1976). Genotype responses in sorghum to drought stress. III. Free proline accumulation and drought resistance. *Crop Sci.,* **16**: 379-386.

Botanical Dermatology Database, (1991a). Plumbaginaceae (data of last modification), (1991b). Droseraceae (Sun dew family).

Boutraa, T. and Sanders, F.E. (2001). Effects of interactions of moisture regime and nutrient addition on nodulation and carbon partitioning in two cultivars of bean (*Phaseolus vulgaris* L.). *Journal of Agronomy and Crop Science*, **186**: 229-237.

Brouwer, R. (1963). Some aspects of the equilibrium between overground and underground plant parts. Jaarboek IBS, Wageningen, 31-39.

Cengiz, K., Tuna, L.A. and Alfredo, A. (2006). Gibberellic acid improves water deficit tolerance in maize plants. *Acta Physiologiae Plantarum*. **28**: 331-337.

Didry, N., Dubreuil, L., Trotin, F. and Pinkas, M. (1998). Antimicrobial activity of aerial parts of Drosera peltata Smith on oral bacteria. *J. Ethnopharmacol*., **60**: 91–96.

Fatima, S., Farooqi, A.H.A and Sharma, S. (2002). Physiological and metabolic responses of different genotypes of *Cymbopogan martini* and *C. winterianus* to water stress. *Plant Growth Regl.* **37**: 143-149.

Ghannoum, O., Conroy, J.P., Driscoll, S.P., Paul, M.J., Foyer, C.H. and Lawlor, D.W. (2003). Nonstomatal limitations are responsible for drought induced photosynthesis inhibition in four C4 grasses. *New Phytologist.* **159**: 599-608.

Gupta, D.P. (2008). The herbs, Habitat, Morphology and Pharmacognosy of Medicinal Plants. 357-358.

Hakura, A., Mochida, H., Tsutsui, Y. and Yamatsu, K. (1994). Mutagenicity and cytotoxicity of naphthoquinones for Ames Salmonella tester strains. *Chem. Res. Toxicol*., **7**: 559–67.

Hare, P.D., Cress, W.A. and Staden, J.V. (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ*, **21**: 535-553.

Harris, D.C., (1973). Photosynthesis, diffusion resistance and relative plant water content of cotton as influenced by induced water stress. *Crop Science*, **13**: 570-573.

Hendawy, S.F. and Khalid, Kh.A. (2005). Response of sage (*Salvia officinalis* L.) plants to zinc application under different salinity levels. J. *Appl. Sci. Res*., **1**:147-155.

Jafar, M.S., Nourmohammadi, G. and Maleki, A. (2004). Effect of water deficit on seedling, plantlets and compatible solutes of forage sorghum cv. speed feed. 4th International Crop Science Congress, Brisbane, Australia, 26th Sep -1st October.

Khalid, Kh.A. (2001). Physiological studies on the growth, development and chemical composition of *Nigella sativa* L. plant. PhD. Thesis, Fac. Agric., Ain -Shams Univ., Cairo, Egypt.

Kirnak, H., Kaya, C., Ismail, T. and Higgs, D. (2001). The influence of water deficit on vegetative growth, physiology, plant yield and quality in eggplant. *Bulgarian Journal of Plant Physiology*. **27**: 34-46.

Likhitwitayawuid, K., Kaeamatawong, R., Ruangrungsi, N. and Krungkrai, J. (1998). Antimalarial naphthoquinones from Nepenthes thorelii. *Planta Med.,* **64**: 237–241.

Lim, W. S., Mudge, K.W. and Lee, J.W. (2006). Effect of water stress on ginsenoside production and growth of American ginseng. *Hort. Technology*, **16**: 517-522.

Liu, F. and Stutzel, H. (2004). Biomass partitioning, specific leaf area and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Scientia Horticulturae,* **102**: 15- 27.

Luvaha, E., Netondo, G.W. and Ouma, G. (2008). Effect of water deficit on the physiological and morphological characteristics of mango (*mangifera indica*) rootstock seedlings. *American Journal of Plant Physiology*. **3**: 1-15.

Masinde, P.W., Stutzel, H., Agong, S.G. and Frickle A. (2005). Plant growth, water relations and transpiration of spider plant (*Gynandropsis gynandra)* under water limited conditions. *J. Amer. Soc. Hort. Sci.* **130**: 469-477.

Mirsa, A. and Shrivastava, N.K. (2000). Influence of water stress on Japanese mint. *J. Herb, Spices and Med. Plants*, **7**: 51-58.

Rentsch, D., Hirner, B., Schmeizer, E. and Frommer, W.B. (1996). Salt stress induced proline transporters and salt stress-repressed broad specificity amino acid permeases identified by suppression of a yeast amino acid permease-targeting mutant. *Plant Cell*, **8**:1437-1446.

Ripley, B.S., Gilbert, M.E., Ibrahim, D.G and Osborne, C.P. (2007). Drought constraints on C4 photosynthesis, stomatal and metabolic limitation in C3 and C4 subspecies of *Alloteropsis semialata*. J.*Exp. Bot*. **58**:1351-1363.

Sharp, R.E., Hsiao, T.C. and Silk, W.K. (1990). Growth of the maize primary root at low water potentials. *Plant Physiology*, **93**: 1337-1346.

Singh- Sangwan, N., Farooqi, A.H.A., Shibin, F and Sangwan, R.S. (2001). Regulation of essential oil production in plants. *Plant Growth Regul.,* **34**: 3-21.

Srinivas, V. and Balasubramanian, D. (1995). Proline is a protein-compatible hydrotrope. *Langmuir*, **11**: 2830-2833.

Sugie, S., Okamoto, K., Rhaman, K., Tanaka, T., Kawai, K., Yamahara, J. and Mori, H. (1998). Inhibitory effects of plumbagin and juglone on azoxymethane-induced intestinal carcinogenesis in rats. *Cancer Lett*. **127**: 177–183.

Wu, X.H., Tang, Z.H. and Zn, Y.G. (2006). Effect of water stress on free amino acid content in *Rosmarinus officinalis* L. *J. North East Forcs. Univ*., **34**: 57-58.

Xu, Z.Z. and Zhou. G.S. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* **59**: 3317–3325.

Yosida, S., Forno, D.A., Cock, J.H. and Gomez, K.A. (1972). *Laboratory Manual of Physiology Studies of Rice*, IRRI, 30.

Zheng, Y., Dixon, M. and Saxena, P.K. (2007). Growing and nutrient availability affect the content of some phenolic compounds in *Echinacea purpurea* and *Echinacea angustifolia*. *Planta Medica*, **10**: 1055.