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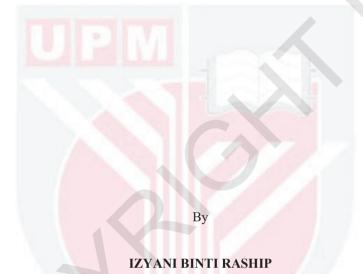
# INFLUENCE OF CYCLICAL WATER STRESS AND DURATION ON GROWTH, PHYSIO-BIOCHEMICAL AND LEAF ANATOMY OF MAS COTEK (*Ficus deltoidea* Jack)

**IZYANI BINTI RASHIP** 

**IPTSM 2019 7** 



## INFLUENCE OF CYCLICAL WATER STRESS AND DURATION ON GROWTH, PHYSIO-BIOCHEMICAL AND LEAF ANATOMY OF MAS COTEK (*Ficus deltoidea* Jack)



Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2018

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## **DEDICATION**

~ \*~

This work is dedicated to my family, who is always there for me

Especially:

Haji Raship Bin Ahmad

HajjahChePuanBintiAwang

HalmiZun

Aishah Othman

MohdNoorshahilHalmi

Nurin Tihani Mohd Noorshahil

Ammar MuhaiminMohdNoorshahil

Hana Humaira MohdNoorshahil

NaurahFaqihahMohdNoorshahil

And

AyraNisrinaMohdNoorshahil Thank you very much May Allah bless us all Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

### INFLUENCE OF CYCLICAL WATER STRESS AND DURATION ON GROWTH, PHYSIO-BIOCHEMICAL AND LEAF ANATOMY OF MAS COTEK (*Ficus deltoidea* Jack)

By

#### **IZYANI BINTI RASHIP**

**June 2018** 

Chairman: Professor Mohd. Razi Ismail, PhDInstitute: Tropical Agriculture and Food Security

Mas Cotek (*Ficus deltoidea*) is a local herb species that has recently gained commercial importance due to its usage in herbal medicine. It has been used as a tonic for women's health, rejuvenation and treatments of pneumonia, migraine, stroke, dysentery, hypertension, diabetes and for improving blood circulation. Currently, commercialization of Mas Cotek as ingredient in bottled drinks, herbal medicines capsules, sachets of tea or coffee and oil, has led to increase in market demand for this species. With increasing consumer demand, efforts must be initiated to increase the supply of raw materials and concomitantly reduces exploitation of the species from its native habitats. Information on the response between the levels of biochemical compounds, growth and leaf anatomical changes with water stress is still lacking for Mas Cotek. All experiments were conducted under a shaded rain-sheltered facility at the Malaysian Agricultural Research Institute (MARDI) Research Station, Serdang. Plant materials of MFD 4 accession, propagated from stem cuttings, were used. In the first experiment, study was undertaken to examine the influence of duration and cyclical water stress on growth, dry matter yield and partitioning; and biochemical compounds of Mas Cotek. The study was carried out using a Randomized Completed Block Design (RCBD) with a 4 x 3 factorial arrangements of four cyclical water stress [cyclical water stress were achieved by withholding irrigation at 0 (well watered), 7, 14, and 21 days intervals] and three durations of the stress imposition (60, 120 and 180 days after treatment) with 6 replications. The effects on growth, dry matter production and partitioning, growth analysis, biochemical compounds and leaf physiological characteristics of Mas Cotek were recorded. Results from the experiments showed that growth of plants were affected significantly by the cyclical water stress treatment. Water stress significantly reduced all growth parameters with higher reductions in longer duration of stress. Data showed that the responses to cyclic water stress was significantly decreased and biochemical compound (vitexin, isovitexin and total phenolic content) were significantly affected by water stress. Seven days CWS can be considered as a best option for manipulation to induce higher production of bioactive compounds of Mas Cotek base on the duration of stress imposition. In the second experiment conducted, the responses on physiological processes and leaf anatomy of Mas Cotek imposed with different water regimes (well water and water stressed) were investigated. Cyclic water stress resulted in significant decrease in photosynthesis rate, stomatal conductance, transpiration rate and water use efficiency as compared to the well watered treatment. Chlorophyll *a*, chlorophyll *b*, total chlorophyll content, SPAD relative chlorophyll content, relative water content, chlorophyll fluorescence and leaf tissues were not significantly affected by water stress except for spongy cells. In conclusion, Mas Cotek plants are not sensitive to short term water stress and able to cope with mild water stress conditions.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

### PENGARUH TEKANAN AIR SIKLIK DAN DURASI KE ATAS TUMBESARAN, PENCIRIAN FISIO-BIOKIMIA DAN ANATOMI DAUN MAS COTEK (*Ficusdeltoidea*)

Oleh

#### **IZYANI BINTI RASHIP**

Jun 2018

Pengerusi Institut Profesor Mohd. Razi Ismail, PhD Pertanian Tropika dan Sekuriti Makanan

Mas Cotek (Ficus deltoidea) adalah spesies herba tempatan yang telah meningkat kepentingannya dari segi komersial kerana penggunaannya dalam perubatan herba. Ia telah digunakan sebagai tonik untuk kesihatan wanita, peremajaan, rawatan radang paruparu, migrain, strok, disentri, hipertensi, diabetes dan untuk melancarkan peredaran darah. Pada masa ini, pengkomersilan Mas Cotek sebagai ramuan minuman dalam botol, kapsul ubatan herba, uncang teh, kopi dan minyak telah membawa kepada peningkatan permintaan pasaran untuk spesis ini. Dengan permintaan pasaran yang semakin meningkat, usaha perlu dimulakan bagi meningkatkan bekalan bahan mentah dan pada masa yang sama, mengurangkan eksploitasi spesies ini dari habitat asal. Maklumat mengenai tindak balas antara tahap sebatian biokimia, pertumbuhan dan perubahan anatomi daun dengan tekanan air masih berkurangan untuk Mas Cotek. Kesemua eksperimen dijalankan di bawah kemudahan struktur lindungan hujan dengan teduhan di Institut Penyelidikan Institut Penyelidikan Pertanian Malaysia (MARDI), Serdang. Bahan tanaman aksesi Mas Cotek MFD 4, yang dibiakkan melalui kaedah keratan ranting, telah digunakan. Dalam eksperimen pertama, kajian telah dijalankan untuk mengkaji pengaruh tempoh masa dan kitaran kekangan air pada pertumbuhan, pembahagian biomas dan sebatian biokimia Mas Cotek. Kajian ini dijalankan dengan menggunakan rekabentuk blok terawak lengkap (RCBD) dengan susunan faktorial 4 x 3 dari empat kitaran kekangan air [kitaran kekangan air dicapai melalui penahanan tempoh pengairan selama 0 (kawalan), 7, 14, dan 21 hari] dan tiga tempoh pemberian kekangan air (60, 120 dan 180 hari selepas rawatan dimulakan) dengan 6 replikasi. Kesan kepada pertumbuhan, penghasilan dan pembahagian biomas, analisia pertumbuhan, sebatian biokimia dan ciri-ciri fisiologi daun Mas Cotek telah direkodkan. Keputusan dari eksperimen menunjukkan bahawa pertumbuhan tumbuhan telah terjejas dengan ketara oleh rawatan kitaran kekangan air. Kekangan air dengan ketara mengurangkan semua parameter pertumbuhan dengan pengurangan yang lebih tinggi dalam tempoh kekangan yang lebih lama. Data menunjukkan bahawa tindak balas terhadap kitaran kekangan air

berkurangan dengan ketara dan sebatian biokimia (vitexin, isovitexin dan jumlah kandungan fenolik) telah terjejas dengan ketara oleh kekangan air. Tujuh hari kitaran kekangan air boleh dipertimbangkan sebagai pilihan terbaik untuk dimanipulasi bagi meningkatkan pengeluaran bahan bioaktif Mas Cotek yang lebih tinggi dalam tempoh pengenaan tekanan. Dalam eksperimen kedua yang dijalankan, respons mengenai proses fisiologi dan anatomi daun Mas Cotek yang dikenakan dengan keadaan kekangan air yang berbeza (air yang mencukupi dan kekangan air) telah diselidiki. Kitaran kekangan air mengakibatkan pengurangan ketara terhadap kadar fotosintesis, konduksi stomata, kadar transpirasi dan kecekapan penggunaan air berbanding dengan rawatan air yang mencukupi. Klorofil *a*, klorofil *b*, jumlah kandungan klorofil, kandungan relatif klorofil, kandungan relatif air , fluoresen klorofil dan tisu daun tidak terjejas dengan ketara oleh kekangan air jangka pendek dan dapat mengatasi keadaan kekangan air yang sederhana.

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-IzyaniRaship-

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# LIST OF ABBREAVIATIONS, NOMENCLATURE, SYMBOLS

%	Percent
μg	Microgram
μL	Microliter
μm	Micrometer
μΜ	Micromolar
0C	Degree Celcius
ABA	Abscisic acid
ANOVA	Analysis of Variance
cm	Centimeter
CV	Coefficient of variation
d	Day
dw	Dry weight
EPP	Entry Point Project
g	Gram
gS	Stomatal conductance
GTP	Government Transformation Programme
h	Hour
HPLC	High-performance liquid chromatography
kg	Kilogram
kPa	Kilopascal
L	Liter
LA	Leafarea

	LSD	Least significant different
	LWP	Leaf Water Potential
	m	Meter
	М	Molar
	MARDI	Malaysian Agricultural Research and Development Institute
	min	Minute
	mol	Mole
	NAR	Net Assimilation Rate
	ng	Nanogram
	nm	Nanometer
	PS II	Photosystem II
	RGR	Relative growth rate
	rpm	Revolution per minute
	RSR	Root Shoot Ratio
	RWC	Relative water content
	s	Seconds
	SLA	Specific leaf area
	SLW	Specific leaf weight
	VPD	Vapor Pressure Deficit
C	WUE	Water use efficiency
	WUE	water use enficiency

### **CHAPTER 1**

### **INTRODUCTION**

#### 1.1 Background

The herbal industry is presently recognized as a new source of economic growth for Malaysia following significant increases in demand for herbal products, both at the international and national scenes. In 2008, the market value of Malaysian herbal products stood at RM10 billion with an annual growth rate of 8-15% (Feasibility studies, MARDI). According to *Dasar Agromakanan Negara* (DAN), the herbal market value is projected to increase to RM 32 billion by the year 2020. Due to its high market potential, the herbal industry was identified as the first Entry Point Project (EPP1) for the Agriculture National Key Economic Area (NKEA) under the Malaysian Government Transformation Programme (GTP) in 2011.

Malaysia is able to tap into this lucrative industry as it has a major advantage – a wealth of natural herbal resources from its tropical rainforests which can be fully exploited and utilized, particularly plants with medicinal properties. With the ingrained herbal knowledge of the local and indigenous communities, these treasures can be explored and commercialized in a sustainable manner. Local herbs that have been considered suitable as health supplements include Tongkat Ali (*Eurycoma longifolia*), pegaga (*Centella asiatica*), misai kuching (*Orthosiphon stamineus*), hempedu bumi (*Andrographis paniculata*), Kacip Fatimah (*Labisia pumila*), mengkudu (*Morinda citrifolia*), dukung anak (*Phyllanthus niruri*), halia (*Zingiber officinale*), Mas Cotek (*Ficus deltoidea*) and limau purut (*Citrus hystrix*). One of these highly potential herbs said to be rich in beneficial nutrients and active constituents is Mas Cotek, and this species was selected as the material for the present study.

Mas Cotek, *Ficus deltoidea*, belonging to the plant family Moraceae, also known as the Sleeping Giant, is a well-known herb that has gained commercial importance due to its numerous health benefiting properties. It has been used as a tonic for women's health, rejuvenation and treatments of pneumonia, migraine, stroke, dysentery, hypertension, diabetes and for improving blood circulation (Musa et. al., 2005; Musa, 2005a). Currently, commercialization of Mas Cotek as one of the ingredients in bottled drinks, herbal medicines capsules, sachets of tea or coffee and oil, has led to an increase in market demand for this species.

To fulfill the market demand, there is a need to generate technologies related to the domestication of Mas Cotek both under open and containerized planting systems. Through these technologies, the development of standardized, safe and effective extract of Mas Cotek can be produced which in turn can assist in large scale production of the raw materials required by herbal manufacturers. This ensures that the quantity and

quality of the raw materials meet the minimal requirements specified by herbal manufacturers. Since Mas Cotek is adaptable to bris sandy soil, it can be a lucrative alternative commercial crop to replace crops that do not grow well on bris sandy soil environments, for example tobacco.

With the increasing market demand for this plant, efforts must be initiated to increase the supply of raw materials and at the same time to reduce exploitation of the species from its native habitat. Cultivation under Control Environment (CE) system can result in well-regulated and high quality crop production having high bioactive contents (Robert and John, 1992). Intensive vegetable and fruit production under rain-sheltered environment is presently a common production system in this country where water is supplied according to crop requirements. Mas cotek also can be produced under rainshelter production. The production system practiced in a medicinal herb Feverfew (*Tanacetum parthenium*) under state-of-the art greenhouse conditions has been demonstrated successful elsewhere (David, 2003) where growers managed to produce high quality product with optimal bioactive compounds. Hence, production of Mas cotek under rainshelter may produce higher yield of plant biomass and bioactive compounds.

Production and the level of bioactive compounds within plant parts have been documented in several studies with varying availability of resources such as water, irradiance, temperature and nutrients. Manipulation of a resource or combination of resources with the aim of maximizing production of specific biochemical compounds under control environment production system has been said to be easily implemented (Kirakosyan et al., 2004). This can be applied for Mas cotek production under a rainshelter.

Recent studies have shown that water can be manipulated with respect to the amount and frequency of delivery to ensure maximum production of specific bioactive compounds such as antioxidants, flavonoids or those with medicinal values (Nunes et al., 2014; Ramakrishna and Ravishankar, 2011). The present study was undertaken to examine the effects of different watering regimes on growth, physiology, leaf anatomy and production of bioactive compounds in Mas Cotek.

#### Problem Statement and Objectives

1.2

Research on Mas Cotek has focused mainly on the production of bioactive compounds, morphological and phylogenetic analysis. Little or no attention has been given to the species adaptations to watering regimes. It is known Mas Cotek is an epiphyte which enables the species to withstand prolonged water deprivation but knowledge on whether the plant can respond positively to limited water availability in term of its growth, biochemical, physiological responses and leaf anatomy are currently lacking. Thus, the overall objective for the present study was to determine the effects of watering regimes on growth and physio-biochemical characteristic of Mas Cotek. The specific aims of the study were as follows:

- 1. To determine the effects of watering regimes (duration and cyclic water stress) on growth, dry matter yield and partitioning and physio-biochemistry parameters of Mas Cotek;
- 2. To examine the changes in production of selected biochemical compounds (vitexin, isovitexin, and total phenolic content) as affected by watering regimes, and
- 3. To elucidate the effects of different watering regimes on leaf anatomy of Mas cotek.



#### REFERENCES

- Abdulla, M.A., Abdul-Aziz Ahmed, K., Abu-Luhoom, F.M. and Muhanid, M. (2010). Role of *Ficus deltoidea* extract in the enhancement of wound healing in experimental rats. *Biomedical Research*, 21(3): 241–245.
- Abdullah, Z., Hussain, K., Zhari, I. and Rasadah, M.A. (2009). Anti-inflammatory activity of standardised extracts of leaves of three varieties of *Ficus deltoidea*. *International Journal of Pharmaceutical and Clinical Research*, 1: 100–105.
- Adam, Z., Hamid, M., Ismail, A. and Khamis, S. (2007). Effect of *Ficus deltoidea* aqueous extract on blood glucose level in normal and mild diabetic rats. *Malaysian Journal of Health Science*, 5(2): 9-16.
- Adam, Z., Khamis, S. and Hamid, M. (2012). Ficus deltoidea: a potential alternative medicine for diabetes mellitus. Evidence Based Complementary and Alternative Medicine. Vol. 2012, Article ID 632763, 12 pages. https://doi.org/10.1155/2012/632763.
- Adiwirman. (2006). Effects of water Stress on the physiological and biochemical responses of Mangosteen (Garcinia mangostana L.) Plant. PhD Thesis, Universiti Putra Malaysia.
- A'fifah, A.R. (2013). Effects of nutrient deficits on growth, physio-biochemical changes and yield of chilli (Capsicum annuum L.) grown in soilless culture, PhD Thesis, Universiti Putra Malaysia.
- Akhir, N.A.M., Chua, L. S. F., Majid, A.A. and Sarmidi, M. R. (2011). Cytotoxicity of aqueous and ethanolic extracts of *Ficus deltoidea* on human ovarian carcinoma cell line. *British Journal of Medicine and Medical Research*, 1: 397–409.
- Almeselmani, M., Abdullah, F., Hareri, F., Naaesan, M., Ammar, M.A., Kanbar, O.Z. and Saud, Abd. (2011). Effect of drought on different physiological characters and yield component in different Syrian durum wheat varieties. *Journal of Agricultural Science*, 3: 127-133.
- Anjum, S.A, Xie, X.Y., Wang, L.C, Saleem, M.F., Chen Man and Wang Lei. (2011). Morphological, physiological and biochemical responses of plants to drought stress. *African Journal of Agricultural Research*, 6(9): 2026-2032.
- Anonymous. (2009). Third Herbal Asia Bussiness Dialogue. Kuala Lumpur. Forest Research Institute Malaysia (FRIM).
- Araus, J.L., Slafer, G.A., Reynolds, M.P. and Royo, C. (2002). Plant breeding and drought in C<sub>3</sub> cereals: what should we breed for? *Annals of Botany*, 89: 925– 940.

- Aris, S.R.S., Mustafa, S., Ahmat, M., Jaafar, F.M. and Ahmad, R. (2009). Phenolic content and antioxidant activity of fruits of *Ficus deltoidea* var angustifolia sp. *The Malaysian Journal of Analytical Sciences*, 13(2):146-150.
- Bacelar, E.A., Moutinho-Pereira, J.M., Goncalves, B.C., Ferrreira, H.F. and Correia, C.M. (2007). Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. *Environmental and Experimental Botany*, 60: 183-192.
- Baeck, H., Kuenwoo, P., Baeck, H.W. and Park, K. W. (2001). Effect of watering on growth and oil content of sweet basil (*Ocimum americanum* L.). *Horticultural Science and Technology*, 19: 81-86.
- Baher, Z. F., Mirza, M., Ghorbanli, M. and Rezaii, M. B. (2002). The influence of water stress on plant height, herbal and essential oil yield and composition in *Satureja hortensis*. *Flavor and Fragrance Journal*, 17: 275-277.
- Banon, S., Fernandez, J.A., Franco, J.A., Torrecillas, A., Alarcon, A.A. and Sanchez-Blanco, M.J. (2004). Effects of water stress and night temperature preconditioning on water relations and morphological and anatomical changes of *Lotus creticus* plants. *Scientia Horticulturae*, 101: 333-342.
- Benjamin, J.G. and Nielsen, D.C. (2006). Water deficit effects on root distribution of soybean, field pea and chickpea. *Field Crops Res.* 97: 248-253
- Blum, A. (1996). Crop responses to drought and the interpretation of adaptation. *Plant Growth Regulation*, 20(2): 135–148.
- Boutraa, T. (2010). Growth performance and biomass partitioning of the desert shrub *Calotropis procera* under water stress conditions. *Research Journal of Agriculture and Biological Sciences*, 6(1): 20-26.
- Boyer, J.S. (1982). Plant productivity and environment. Science, 218: 543-548.
- Briskin, D.P. and Gawienowski, M.C. (2001). Differential effects of light and nitrogen on production of hypericins and leaf glands in *Hypericum perforatum*. *Plant Physiology Biochemistry*, 39: 1075-1081.
- Buhler, D., and Cristobal, M. (2000). Antioxidant activities of flavonoids. http://lpi.oregonstate.edu/f-w00/flavonoid.html. http://www.quackwatch.org/01QuackeryRelatedTopics/herbs.html.
- Bunawan, H., Amin, N.M., Bunawan, S.N., Baharum, S.N. and Normah, M.N. (2014). *Ficus deltoidea* Jack: A review on its phytochemical and pharmacological importance. *Evidence-Based Complementary and Alternative Medicine*. Vol. 2014, Article ID 902734, 8 pages. http://dx.doi.org/10.1155/2014/902734.

- Butler, W. L. (1977). Chlorophyll fluorescence: a probe for electron transfer and energy transfer. In *Encyclopedia of Plant Physiology*, Vol. 5, Springer-Verlag, Berlin, pp. 149–167.
- Chakraborty, U., Dutta, S. and Chakraborty, B.N. (2002). Response of tea plants to water stress. *Biologia Plantarum*. 45(4): 557–562.
- Charles, D.J., Simon, J.E., Shock, C.C., Feibert, E.B.G. and Smith, R.M. (1993). Effect of water stress and post-harvest handling on artemisinin content in the leaves of *Artemisia annua* L. In: J. Janick and J.E. Simon (Eds.) *New Crops* (pp. 628-631). New York: Wiley.
- Chartzoulakis, K., Patakas, A., Kofidis, G., Bosabalidis, A. and Nastou, A. (2002). Water stress affects leaf anatomy, gas exchange, water relations and growth of two avocado cultivars *Scientia Horticulturae* 95 : 39–50
- Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P., Osorio, M.L., Carvalho, I., Faria, T. and Pinheiro, C.C. (2002). How plants cope with water stress in the field. Photosynthesis and growth. *Annals of Botany*, 89: 907–916.
- Chavez, M.M. (1991). Effects of water deficits on carbon assimilation. *Journal of Experimental Botany*, 42: 1-16.
- Choo, C.Y., Sulong, N.Y., Man, F. and Wong, T.W. (2012). Vitexin and isovitexin from the leaves of *Ficus deltoidea* with *in-vivo* aglucosidase inhibition. *Journal of Ethnopharmacology*, 142(3): 776–781.
- Chowdhury, J. A., Karim, M. A., Khaliq Q. A., Ahmed, A. U. and Khan, M. S. A. (2016). Effect of drought stress on gas exchange characteristics of four soybean genotypes. *Bangladesh Journal of Agricultural Research*, 41(2): 195-205.
- Couceiro, M. A., Afreen, F., Zobayed, S. M. A. and Kozai, T. (2006). Effect of harvesting time, temperature, and germplasm. *Plant Science*, 170: 128-134.
- Couceiro, M.A, Afreen, F., Zobayed, S.M.A. and Kozai, T. (2005). Variation in concentrations of major bioactive compounds of St. John's wort: Effects of harvesting time, temperature and germplasm. *Plant Science*, 170: 128-134.
- Dai, Y., Shen, Z., Liu, Y., Wang, L., Hannaway, D. and Lu, H. (2009). Effect of shade treatments on the photosynthetic capacity, chlorophyll fluorescence, and chlorophyll content of *Tetrastigma hemsleyanum* Diels et Gilg. *Environmental* and Experimental Botany, 65: 177-182.
- David, E. (2003). Greenhouse production of Feverfew. A publication of Agriculture and Agri-Food Canada.

- de Abreu, I.N. and Mazzafera, P. (2005). Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. *Plant Physiology & Biochemistry*, 43: 241-248.
- Devkota, A. and Jha. P. K. (2011). Influence of water stress on growth and yield of Centella asiatica. International Agrophysics, 25: 185-212.
- Demirevska, K., Zasheva, D., Dimitrov, R., Simova-Stoilova, L., Stamenova, M. and Feller, U. (2009). Drought stress effects on rubisco in wheat: changes in the rubisco large subunit. *Acta Physiologiae Plantarum*, 31: 1129-1138.
- Devkota, A. and Jha, P.K. (2011). Influence of water stress on growth and yield of *Centella asiatica. International Agrophysics*, 25: 211-214.
- Dube, K.G. (2011). Absolute growth rate, leaf area index, leaf: stem ratio and harvest index influenced by organic manures, biofertilizers and growth regulators in *Stevia rebaudiana* Bertoni. *Asiatic Journal of Biotechnology Resources*, 2(5): 508-521.
- El-Juhany, L.I. and Aref, I.M. (1999). Growth and dry matter partitioning of *Leucaena leucocephala* (lam.) de Wit. trees as affected by water stress. *Alexandria Journal of Agriculture Research*, 44(2): 237-259.
- Ennajeh, M., Vadel, A.M., Cochard, H. and Khemira, H. (2010). Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *Journal of Horticultural Science and Biotechnology*, 85(4): 289-294.
- Evans, J.R., von Caemmerrer, S., Setchell, B.A. and Hudson, G.S. (1994). The relationship between CO2 transfer conductance and leaf anatomy in transgenic tobacco with a reduced content of Rubisco. *Aust. J. Plant Physiol.* 21: 4754–4795.
- Farooq, M., Wahed, A., Kobayashi, N., Fujita, D., and Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*, 29(1): 185–212.
- Fasihuddin, A. and Hasmah, R. (1993). *Kimia Hasilan Semula Jadi dan Tumbuhan Ubatan*. Kuala Lumpur: Dewan Bahasa dan Pustaka.
- Filippou, P., Antoniou, C. and Fotopoulus, V. (2011). Effect of drought and rewatering on the cellular status and antioxidant response of *Medicago truncatula* plants. *Plant Signaling and Behavior*, 6(2): 270-277.
- Fitter, A.H. and Hay, R.K.M. (1987). *Environmental Physiology of Plants*. London: Academic Press.

- Galbraith, M.N., Miller, C.J., Rawson, J.W.L., Ritchie, E., Shannon, J.S. and Taylor, W.C. (1965). Moretenol and other triterpenes from *Ficus macrophylla* Desf. *Australian Journal of Chemistry*, 18(2): 226-239.
- Galmes, J., Cifre, J., Medrano, H. and Flexas, J. (2005). Modulation of relative growth rate and its components by water stress in Mediterranean species with different growth forms. *Oecologia*, 145:21–31.
- Gardner, F.P., Pearce, R.B. and Mitchell, R.L. (1986). *Physiology of Crop Plants*. Ames: Iowa State University Press.
- Genty, B., Briantais, J.M. and Da Silva, J.B.V. (1987). Effects of drought on primary photosynthetic processes of cotton leaves. *Plant Physiology*, 83: 360-364.
- Gorai, N., Hachef, A. and Neffati, M. (2010). Differential responses in growth and water relationship of *Medicago sativa* (L.) cv. Gabès and *Astragalus gombiformis* (Pom.) under water-limited conditions. *Emir. J. Food Agric.* 22 (1): 01-12
- Grant, R.H. (1997). Partitioning of biologically active radiation in plant canopies. International Journal of Biometereology, 40: 26-40.
- Hakiman, M. and Maziah, M. (2009). Non- enzymatic and enzymatic antioxidant activities in aqueous extract of different *Ficus deltoidea* accessions. *Journal of Medicinal Plant Research*, 3(3): 120–131.
- Hale, B.K., Herms, D.A., Hansen, R.C., Clausen, T.P. and Arnold, D. (2005). Effects of drought stress and nutrient availability on dry matter allocation, phenolic glycosides, and rapid induced resistance of poplar to two lymantriid defoliators. *Journal of Chemical Ecology*, 31: 2601-2620.
- Harborne, J.B. (1998). Phytochemical Methods: A Guide to A Modern Technique of Plant Analysis. 308 pages
- Hendawy, S.F. and Khalid, K.A. (2005). Response of sage (Salvia officinalis L.) plants to zinc application under different salinity levels. Journal of Applied Science Research, 1(2): 147-155.
- Hendry, G.A.F. and Price, A.H. (1993). Stress indicators: chlorophylls and carotenoids.
  In G.A.F. Hendry and J.P. Grime (Eds.) *Methods in Comparative Plant Ecology* (pp.148-152). London: Chapman & Hall.
- Heschel, M.S.C. and Riginos, C. (2005). Mechanisms of selection for drought stress tolerance and avoidance in *Impatiens capensis* (Balsaminaceae). *American Journal of Botany*, 92: 37-44.
- Heszky, L. (2007). Szárazság és a növény kapcsolata. *Agrofórum*, 18: 37-41. (In Hungarian).

- Hillova, D., Takacsova, M. and Lichtnerova, H. (2014). Stomatal response to water stress in herbaceous perennials. *Plants in Urban Areas and Landscape*. pp 52-56. DOI 10.15414/2014.9788055212623.52–56.
- Hsiao, T.C. (1973). Plant response to water stress. Annual Review of Plant Physiology, 24: 519-520.
- Hsiao, T.C. and Acevedo, F. (1974). Plant response to water deficits, water use efficiency and drought resistance. *Agricultural Meteorology*, 14: 69-84.
- Hu, Y. and Schmidhalter, U. (1998). Spatial distributions of inorganics ions and carbohydrates contributing to osmotic adjustment in the elongating wheat leaf under saline conditions. *Australian Journal of Plant Physiology*, 25: 591-597.
- Hunt, R. (1978). *Plant Growth Analysis*. The Institute of Biology's Studies on Biology, 96. Edward Arnold (Pubs.) Ltd., London.
- Hunt, R. (1990). *Basic Growth Analysis.* (pp. 17-81). London: Academic Division of Unwind Hyman Ltd.
- Hunt, R. (2003). Growth and development: Growth analysis, individual plants. In B. Thomas (Ed.) *Encyclopedia of Applied Plant Sciences* (pp. 579-588). San Diego California: Elsevier Academic Press.
- Hura, T., Hura, K., Grzesiak, S. (2009). Possible contribution of cell-wall bound ferulic acid in drought resistance and recovery in triticale seedlings. *Journal of Plant Physiology*, 166: 1720–1733.
- Xianmin, C., Alderson, P.G. and Wright, C.J. (2008). Solar irradiance level alters the growth of basil (*Ocimum basilicum* L.) and its content of volatile oils. *Environmental and Experimental Botany*, 63(1–3): 216-223.
- Ismail, M.R., Burrage, S.W., Tarmizi, H. and Aziz, M.A. (1994). Growth, plant water relations, photosynthesis rate and accumulation of proline in young carambola plants in relation to water stress. *Scientia Horticulturae*, 60: 101-114.
- Jaafar, H. Z. E., Ibrahim, M.H. and Mohd Fakri, N.F. (2012). Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), maliondialdehyde (MDA) and photosynthetic responses of Malaysian Kacip Fatimah (*Labisia pumila* Benth). *Molecules*, 17: 7305-7322. doi:10.3390/molecules17067305.
- Jagtap, V., Bhargava, S., Streb, P. and Feierabend, J. (1998). Comparative effect of water, heat and light stresses on photosynthetic reactions in *Sorghum bicolor* (L.) *Moench. Journal of Experimental Botany*, 49(327): 1715–1721.

- Jaleel, C. A., Sankar, B., Murali, P.V., Gomathinayagam, M., Lakshmanan, G. M. A. and Panneerselvam, R. (2008). Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus* and impacts on ajmalicine accumulation. *Colloid Surfaces B: Biointerfaces, 62*: 105-111.
- James, L.G., Brian, E.W. and Raymond, C. (2000). Success with container production of twelve herbs species. *Horticulture Information leaflet 509* 7/2000: 10-18.
- Jangpromma, N., Songsri, P., Thammasirir, S. and Jaisil, P. (2010). Rapid assessment of chlorophyll content in sugarcane using SPAD chlorophyll meter across different water stress condition. *Asian Journal of Plant Sciences*, 9(6): 368-374.
- Jenny, L. (2008). Polens: Making Headway In The Herbal product Industry. Bernama.com.
- Jones, H.G. (1992). Plants and Microclimate, Second edition. Cambridge University Press. Pp 428.
- Kalman, D.S., Schwartz, H.I., Feldman, S. and Krieger, D.R. (2013). Efficacy and safety of *Elaeis guineensis* and *Ficus deltoidea* leaf extracts in adults with prediabetes. *Nutrition Journal*, 12(36): https://doi.org/10.1186/1475-2891 12-36
- Katherine, L.A. (2005). Herbs: Organic greenhouse production. A publication of ATTRA National Sustainable Agriculture Information Service.
- Kelly, J. P., Fernandez, R. T. and Miller, W. B. (2000). Drought response of three ornamental herbaceous perennials. *Journal of the American Society for Horticultural Science*, 125(3): 310–317.
- Khalil, S. E., El-Aziz, N. G. A. and Leila, B. H. A. (2010). Effect of water stress, ascorbic acid and spraying time on some morphological and biochemical composition of *Ocimum basilicum* plant. *Journal of American Science*, 6(12): 33-44.
- Khalil, S.K., St.Hilaire, R., Khan, A., Rehman, A. and Mexal, J.G. (2011). Growth and physiology of yarrow species *Achillea millefolium* cv. Cerise Queen and *Achillea filipendulina* cv. Parker Gold at optimum and limited moisture. *Australian Journal of Crop Science*, 5(13): 1698-1706.
- Khalil, A.M. and Grace, J. (1992). Acclimation to drought in *Acer pseudoplatanus* L. (sycamore) seedlings. *Journal of Experimental Botany*. 43: 1591-1602.
- Kharadi, R., Upadhyaya, S.D., Upadhyay, A. and Nayak Preeti Sagar. (2011). Differential responses of plumbagin content in *Plumbago zeylanica* L. (Chitrak) under controlled water stress treatments. *Journal of Stress Physiology & Biochemistry*, 7:113-121.

- Kirakosyan, A., Kaufman, P., Warber, S., Zick, S., Aaronson, K., Bolling, S. and Chang, S.C. (2004). Applied environmental stresses to enhance the levels of polyphenolics in leaves of hawthorn plants. *Physiologia Plantarum*, 121: 182– 186.
- Kozlowski, T. T. (1982). Water supply and tree growth. Part. I. Water deficits. *Forestry Abstracts*, 43(2): 57-95.
- Kramer, P.J and Boyer, J.S. (1995). Water Relations of Plants and Soils. London: Academic Press.
- Kro'l, A., Amarowicz, R. and Weidner, S. (2014). Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. Acta Physiologiae Plantarum, 36(6):1491–1499.
- Kulkarni, M., Borse, T. and Chaphalkar S. (2007). Anatomical Variability in Grape (Vitis venifera) Genotypes in Relation to Water Use Efficiency (WUE). American Journal of Plant Physiology. 2: 36-43.
- Kulkarni, M., Borse, T. and Chaphalkar S. (2008). Mining anatomical traits: A novel modelling approach for increase water use efficiency under drought conditions in plants. *Czech Journal Genetic Plant Breeding*. 44 (1):11-21
- Laman, T.G. & Weiblen, G.D. (1998). Figs of Gunung Palung Nasional Park (West Kalimantan, Indonesia). *Tropical Biodiversity*, 5(3): 245-297.
- Lambers, H., Chapin, F.S. and Pons, T.L. (2008). Plant Physiological Ecology. New York: Springer Science.
- Lansky, E.P. and Paavilainen, H.M. (2011). Figs: The Genus Ficus. New York: CRC Press Boca Raton.
- Lawlor, D.W. (1995). Photosynthesis, productivity and environment. Journal of Experimental Botany, 46: 1449-1461.
- Lecouer, J. and Sinclair, T.R. (1996). Field pea water transpiration and leaf growth in response to soil water deficits. *Crop Science* 36: 331-335.
- Lee, J.Y. and Oh, M.M. (2017). Mild water deficit increases the contents of bioactive compounds in dropwort. *Horticulture, Environment, and Biotechnology*, 58(5): 458-466.
- Leport, L., Turner, N.C., French, R.J., Barr, M.D., Duda, R. and Davies, S.L. (2006). Physiological responses of chickpea genotypes to terminal drought in a Mediterranean-type environment. *European Journal Agronomy*. 11: 279–291.

- Li, Q. and Kubota, C. (2009). Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. *Environmental and Experimental Botany*, 67: 59-64.
- 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.
- Marchi, S., Tognetti, R., Minnocci, A., Borghi, M. and Sebastiani, L. (2008). Variation in mesophyll anatomy and photosynthetic capacity during leaf development in a deciduous mesophyte fruit tree (*Prunus persica*)and an evergreen sclerophyllous Mediterranean shrub (*Olea europaea* L.) Trees. 22: 559-571.
- Marjet, E. (2004). Light, nutrient and the growth of herbaceous forest species. Acta Oecologica, 26: 197-202.
- Masacci, A. and Jones, H.G. (1990). Use of simultaneous analysis of gas-exchange and chlorophyll fluorescence quenching for analysing the effects of water stress on photosynthesis in apple leaves. *Trees*, 4:1-8.
- Masinde, P.W., Stützel, H., Agong, S.G. and Frickle, A. (2005). Plant growth, water relations and transpiration of spider plant (*Gynandropsis gynandra* L. Briq) under water limited conditions. *Journal of the American Society for Horticultural Science*, 130(3): 469-477.
- Mat, N., Rosni, N.A., Ab Rashid, N.Z., Haron, N. and Nor, Z.M. (2012). Leaf morphological variations and heterophylly in *Ficus deltoidea* Jack (Moraceae). *Sains Malaysiana*, 41: 527-538.
- Matsui, T. and Singh, B.B. (2003). Root characteristics in cowpea related to drought tolerance at the seedling stage. *Experimental Agriculture*, 39(1): 29–38.
- Milena, H., Go'mez-Aparicio, L., Quero, J.L. and Valladares, F. (2012). Non-linear effects of drought under shade: reconciling physiological and ecological models in plant communities. *Oecologia*, 169: 293–305
- Mirsa A. and Strivastava N.K. (2000). Influence of water stress on Japanese mint. J. Herb, Spices and Medicinal Plants. 7, 1, 51-58.
- Mohamad Zabawi, A.G. (2006). Effects of Plant Available Water on Canopy Development, Biomass Accumulation and Yield of Faba Bean (Vicia faba L). Ph.D Thesis, University of Reading.
- Mohd Lip, J., Nazrul Hisham, D. and Arif Zaidi, J. (2009). Isolation and identification of moretenol from *Ficus deltoidea* leaves. *Journal of Tropical Agriculture and Food Science*, 37(2): 195–201.

- Mohd Norfaizal, G., Khatijah, H. and Muhammad Ruzi, A.R. (2012). Leaf anatomical study of five Macaranga species (Euphorbiaceae). *Journal of Tropical Agriculture and Food Science*, 40(2): 289–296.
- Mosaleeyanon, K., Zobayed, S. M. A., Afreen, F. and Kozai, T. (2005). Relationship between net photosynthetic rate and secondary metabolite contents in St. John's wort. *Plant Science*, 169: 523-531.
- Musa, Y. (2005a). Variability in morphology and agronomy of Mas Cotek accessions found in Kelantan and Terengganu. *Buletin Teknologi Tanaman*. 2: 35-48.
- Musa, Y. (2005b). Mas Cotek (*Ficus deltoidea*). In Y. Musa, M. Muhamad Ghawas, and P. Mansor (Eds.) *Penanaman Tumbuhan Ubatan dan Beraroma* (pp 21-27). Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI).
- Musa, Y. (2006). Evaluation of growth performance and yield potential of selected Mas Cotek (*Ficus deltoidea*) accessions on bris soils. *Journal of Tropical Agriculture* and Food Science, 34(2): 229-235.
- Musa, Y. and Wan Zaki, W.M. (2004). Mas Cotek- Tumbuhan Ubatan Untuk Kesejahteraan Wanita. *Agromedia*, 16: 66-69.
- Musa, Y., Yahaya, H., Wan Zaki, W.M. and Zaharah, A., (2005). Mas Cotek- A new potential medicinal plant. *Buletin Teknologi Tanaman*, 1: 29-36.
- Nacif, D.A.I. and Mazzafera, P. (2005). Effect of water and temperature stress on the content of active constituents of *Hypericum brasiliense* Choisy. *Plant. Physiology and Biochemistry*, 43: 241–248.
- Nahar, K. and Gretzmacher, R. (2011). Response of shoot and root development of seven tomato cultivars in hydrophonic system under water stress. Academic Journal of Plant Sciences, 4(2): 57-63.
- Nahar, K. and Ullah, S.M. (2011). Effect of water stress on moisture content distribution in soil and morphological characters of two tomato (*Lycopersicum esculentum* Mill) cultivars. *Journal of Scientific Research*, 3(3): 677-682.
- Narejo M.N, Puteri Edaroyati, M.W., Siti Aishah, H. and Che Radziah, C.M.Z. (2016). Effects of drought stress on growth and physiological characteristics of roselle (*Hibiscus sabdariffa* L.). *Journal of Tropical Plant Physiology*, 8:44-51.
- Nemali, K.S. and van Iersel, M.W. (2004). Light effects on wax begonia: photosynthesis, growth respiration, maintenance respiration, and carbon use efficiency. *Journal American Society Horticulture Science*, 129(3): 416-424.

- Netto, A. T., Campostrini, E., De Oliviera, J.G. and Bressan-Smith, R.E. (2005). Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. *Scientia Horticulturae*, 104:199–209.
- Niu, G., Rodriguez, D. and Mackay, W. (2008). Growth and physiological responses to drought stress in four oleander clones. *Journal American Society Horticulture Science*, 133(2): 188-196.
- Nunes, J.M., Bertodo, L.O.O., Rosa, L.M.G., von Poser, G.L. and Rech, S.B. (2014). Stress induction of valuable secondary metabolites in *Hypericum polyanthemum* acclimatized plants. *South African Journal of Botany*, 94: 182–189.
- Ocampo, E.T.M and Robles, R.T. (2000). Drought tolerance in mungbean II. stomatal movement, photosynthesis and leaf water potential. *Philippine Journal of Crop Science*, 25(1): 7-15.
- Oh, M.J., Abdul Hamid, M., Ngadiran, S., Seo, Y. K., Sarmidi, M. R. and Park, C. S. (2011). *Ficus deltoidea* (Mas cotek) extract exerted anti-melanogenic activity by preventing tyrosinase activity *in vitro* and by suppressing tyrosinase gene expression in B16F1 melanoma cells. *Archives of Dermatological Research*, 303(3): 161–170.
- Oh, M.M., Carey, E.E. and Rajashekar, C.B. (2010). Regulated water deficits improve phytochemical concentration in lettuce. *Journal of the American Society for Horticultural Science*, 135(3): 223–229.
- Omar, M. H., Mullen, W. and A. Crozier (2011). Identification of proanthocyanidin dimers and trimers, flavone C-glycosides, and antioxidants in *Ficus deltoidea*, a Malaysian herbal tea. *Journal of Agricultural and Food Chemistry* 59(4):1363-1369.
- Ong, S. L., Ling, A. P. K., Poospooragi, R. and Moosa, S. (2011). Production of flavonoid compounds in cell cultures of *Ficus deltoidea* as influenced by medium composition. *International Journal of Medicinal and Aromatic Plants* 1(2): 62–74.
- Ong,S.L., Ling, S.L., Poospooragi, A. P. K R. and Moosa, S. (2011) Production of flavonoid compounds in cell cultures of *Ficus deltoidea* as influenced by medium composition. *International Journal of Medicinal and Aromatic Plants* 1(2): 62-74.
- Paez, A., Gebre, G.M., Gonzalez, M.E., and Tschaplinski, T.J., (2000). Growth, soluble carbohydrates and *aloin* concentration of *Aloe vera* plants exposed to three irradiance levels. Environmental Experiment Botany., 44, 133–139.
- Pagter, M., Bragato, C. and Brix, H. (2005). Tolerance and physiological responses of *Phragmites australis* to water deficit. *Aquatic Botany* 81: 285-299.

- Percival, G. and Sheriffs, C.N. (2002). Identification of drought-tolerant woody perennials using chlorophyll fluorescence. *Journal of Arboriculture*. 28(5):215-223
- Pervin, M., Unno, K., Nakamura, Y. and Imai, S. (2016) Luteolin suppresses ultraviolet A- and B-induced matrix metalloproteinase 1- and 9 expression in human dermal fibroblast cells. J Nutr Food Sci 6: 560. doi: 10.4172/2155-9600.1000560
- Petropoulus, S.A., Daferera, D., Polissiou, M.G. and Passam, H.C. (2008). The effect of water deficit stress on the growth, yield and composition of essential oils of parsley. *Scientia Horticulturae* 115: 393-397.
- Poorter, H. (1989). Interspecific variation in relative growth rate: On ecological causes and physiological consequences. In *Variation in Growth Rate and Productivity* of *Higer Plants* ed. H. Lambers, M. L. Cambridge, H. Konings and T. L. Pons, pp. 45-68. The Hague, The Netherlands: SPB Academic Publishing Bv.
- Poorter, H. and Remkes, C. (1990). Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia*83: 553-559.
- Praba, M.L., Cairns, J.E., Babu, R.C. and Lafitte, H.R. (2009). Identification of physiological traits underlaying cultivar differences in drought tolerance in rice and wheat. J. Agron. Crop. Sci. 195: 30-46
- Puértolas, J., Larsen, E.K., Davies W.J. and Dodd, I.C. (2017). Applying 'drought' to potted plants by maintaining suboptimal soil moisture improves plant water relations. *Journal of Experimental Botan* 68: 2413–2424.
- Ramakrishna, A. and Ravishankar, G.A., (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*. 6:1720-1731.
- Rauf, S. and Sadaqat, H.A. (2007) Effects of varied water regimes on root length, dry matter partitioning and endogenous plant growth regulators in sunflower (*Helianthus annuus L.*). Journal of Plant Interactions 2:1, 41-51, DOI: 10.1080/17429140701422512

Robert, A.A and John W.B. Jr. (1992). Greenhouse engineering. Northeast Region

- Samah, O.A., Zaidi, N. T. A. and Sule, A.B. (2012) Antimicrobial activity of *Ficus deltoidea* Jack (Mas Cotek). *Pakistan Journal of Pharmaceutical Sciences* 25: 675–678.
- Sanchez-Blanco, M.J., Alvarez, s., Navarro, A. and Banon, S. (2009). Changes in leaf water relation, gas exchange, growth and flowering quality in potted geranium plants irrigated with different water regimes. *Journal of Plant Physiology* 166: 467-476.

- Sanchez-Rodríguez, E., Ruiz, J. M., Ferreres, F., and Moreno, D. A. (2012). Phenolic profiles of cherry tomatoes as influenced by hydric stress and rootstock technique. *Food Chem.* 134: 775–782.
- Schaper, H. and Chacko, E.K. (1991). Relation between extractable chlorophyll and portable chlorophyll meter readings in leaves of eight tropical and subtropical fruit-tree species. J. Plant Physiol. 138: 674–677.
- Selmar, D. and Kleinwächter, M. (2013). Influencing the product quality by applying drought stress during the cultivation of medicinal plants. *Industrial Crops and Products* 42: 558–566.
- Seyed, Y., Lisar, S., Rouhollah Motafakkerazad, Mosharraf M. Hossain and Ismail M. Rahman M. (2012). Water stress in plants: Causes, effects and responses, In *Water Stress* ed. Ismail Md. Mofizur Rahman. Rijeka, Croatia: Intech Europe.
- Shamsul, K., Tajuddin, A.P. and Mazlina, M.Y. (2003). *Tumbuhan Ubatan Tradisional Malaysia* Edisi 1. Institut Biosains, Universiti Putra Malaysia.
- Shao, H.B, Chu, L.Y., Jaleel, C.A., Manivannan, P., Panneerselvam, R. and Shao, M.A. (2009). Understanding water deficit stress-inducedchanges in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. Crit. Rev. Biotechnol., 29: 131-151
- Sinclair, T.R. and Ludlow, M.M. (1985). Who taught plants thermodynamics? The unfulfilled potential of plant water potential. *Aust. J. Plant Physiol.* 33:213-217.
- Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144-158.
- Sofo, A., Dichio, B., Xiloyannis, C. and Masia, A. (2005). Antioxidant defences in olive trees during drought stress: changes in activity of some antioxidant enzymes. *Functional Plant Biology*. 32(1) 45-53
- Starman, T. and Lombardini, L. (2006). Growth, gas exchange, and chlorophyll fluorescence of four ornamental herbaceous perennials during water deficit conditions. J. Amer. Soc Hort. Sci. 131 (4): 469-475.
- Starr, F., Starr, K. & Loope, L. (2003). Ficus deltoidea (Mistletoe fig). U.S. Geological. Survey, Biological Resources Division. Retrieved 9 December 2012 http://www.hear.org/starr/hiplants/reports/html/ficus\_deltoidea.html
- Suryati, S., Nurdin, H., Dachriyanus, D. and Hj Lajis, M. (2011). Structure elucidation of antibacterial compound from Ficus deltoidea Jack leaves. *Indonesian Journal* of Chemistry, vol. 11, no. 1, pp. 67–70.

- Taylor I.B. (1991) Genetics of ABA synthesis, in: Davies W.J., H.G., Jones (Eds.), Abscisic acid. Physiology and Biochemistry, 23–38.
- Turner, N.C. (1981). Techniques and experimental approaches for the measurement of plant water status. *Plant and Soil* 58:339-366.
- Uyub, A.M., Nwachukwu, I.N., Azlan, A.A. and S. S. Fariza (2010). *In vitro* antibacterial activity and cytotoxicity of selected medicinal plant extracts from Penang island Malaysia on metronidazoleresistanthelicobacter pylori and some pathogenic bacteria. *Ethnobotany Research and Applications* 8: 95–106.
- Vattem, D. A., Ghaedian, R., and Shetty, K. (2005). Enhancing health benefits of berries through phenolic antioxidant enrichment: focus on cranberry. *Asia Pac J Clin Nut.* 14(2): 120-130.
- Villagra, P.E. and Cavagnaro, J.B. (2006). Water stress effects on the seedling growth of *Prosopis argentina* and *Prosopis alpataco*. *Journal of Arid Environments* 64: 390-400.
- Visintini, Jaime M.F., Redko, F., Muschiett, L.V., Campos, R.H., Martino, V.S. and Cavallaro L.V. *In vitro* antiviral activity of plant extracts from Asteraceae medicinal plants. *Virology Journal* 10:245.
- Vurayai, R., Emongor, V., and Moseki, B., (2011). Effect of water stress imposed at different growth and development stages on morphological traits and yield of bambara groundnuts (*Vigna subterranea*). Am. J. Plant Physiol. 6: 17-27.
- Wang, M.L., Jiang, Y.S., Wei, J.Q., Wei, X., Qi, X.X., Jiang, S.Y. and Wang, Z.M. (2007). Effects of irradiance on growth, photosynthetic characteristics and artemisinin content of *Artemisia annua* L. *Photosynthetica* 46 (1): 17-20.
- Winkel, S. B. (2002). Biosynthesis of flavonoids and effects of stress. Plant Biology., 5, 218-223. Plant physiology and biochemistry., 43, 977-984.
- Witham, F.H., Blaydes, D.F. and Devlin, R.M. (1986). Chlorophyll absorption spectrum and quantitative determinations. In. Exercise in plant Physiology. Second edition. Boston, pp. 128-131.
- Wullschleger, S.D., Yin, T.M., DiFazio, S.P., Tschaplinski, T.J., Gunter, L.E., Davis, M.F. and Tuskan, G.A. (2005). Phenotypic variation in growth and biomass distribution for two advanced-generation pedigree of hybrid poplar. Canadian J. For. Res. 35: 1779-1789.
- Yin, C.Y., Wang, X., Duan, B.L., Luo, J. and Li, C. (2005). Early growth, dry matter allocation and water use efficiency of two sympatric *Populus* species as affected by water stress. *Environmental Experimental Botany*.53: 315-322.

- Zaharah, S.S. (2006). Effects of root restriction and water stress on growth performance, and physiological and biochemical responses of mango (*Mangifera indica* cv. Chokanan), MSc Thesis, Universiti Putra Malaysia.
- Zaharah, S.S. (2006). Effects of root restriction and water stress on growth performance, and physiological and biochemical responses of mango (*Mangifera indica* cv. Chokanan), MSc Thesis, Universiti Putra Malaysia.
- Zain, N.A.M., Ismail, M.R., Puteh, A., Mahmood, M. and Islam, M.R. (2014). Impact of cyclic water stress on growth, physiological responses and yield of rice (*Oryzasativa* L.) grown in tropical environment. *Ciencia Rural* [online] 44: 2136-2141.
- Zewdie, S., Olsson, M. and Masresha, F. (2007). Growth, gas exchange, chlorophyll a fluorescence, biomass accumulation and partitioning in droughted and irrigated plants of two enset [Ensete ventricosum (Welw.) Cheesman] clones. *Journal of Agronomy*. 6(4):499–508.
- Zlatev, Z. (2009). Drought-induced changes in chlorophyll fluorescence of young wheat plant. *Biotechnol* 23: 437-441.
- Zobayed, S. M. A., Afreen, F., and Kozai, T.(2005). Temperature stress can alter the photosynthetic efficiency and secondary metabolites concentrations in St. John's wort.
- Zobayed, S.M.A., Afreen, F. and Kozai, T. (2007). Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition. *Environmental and Experimental Botany* 59: 109-116.
- Zulkarami, B. (2018). Enhancing grainfilling in rice using growth enhancer under water stress condition, PhD Thesis, Universiti Putra Malaysia.
- Zunoliza, A., Khalid, H., Zhari, I. and Rasadah, M.A. (2009). Anti-inflammatory activity of standardized extracts of leaves of three varieties of Ficus deltoidea. *International Journal of Pharmaceutical and Clinical Research* 1(3): 100 – 105

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