

EFFECT OF WATER REGIMES ON GROWTH, TOTAL FLAVONOID AND PHENOLIC CONTENT OF PURSLANE (*PORTULACA OLERACEA* L.)

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Abstract

A pot experiment was conducted to investigate the effect of different water regimes on the growth, antioxidant, carbon and nitrogen contents of purslane (*Portulaca oleracea* L.). The experimental design was completely random design using five treatments, namely, continuous field capacity conditions, continuous saturated conditions, continuous flooded conditions, 10 days flooded followed by saturated condition and 10 days saturated followed by field capacity condition with 5 replications each. The results revealed that effects of different water regimes on plant height, leaf length, leaf width, stem diameter, total phenolic content, total flavonoid content, nitrogen content and crude protein content in purslane were significant. However, there were no significant effects of different water regimes on internode length, leaf relative water content, shoot water content and carbon content. Continuous flooded conditions resulted in purslane with the longest internode length (76 mm), highest leaf relative water content (79.85%), highest total phenolic content (3.06 mg GAE/g). Continuous saturated condition resulted in the tallest purslane plants (429.0 mm) and highest shoot water content (90.59%). Continuous field capacity condition produced purslane with longest leaf (26.3 mm), widest leaf (14 mm), highest total flavonoid content (1.35 mg QE/g), highest carbon (40.3%), nitrogen (3.14%) and crude protein content (19.62%). Therefore, continuous saturated condition may be practiced for more yields, but continuous flooded condition is good for nutritional values.

Introduction

Purslane (*Portulaca oleracea* L.) is found naturally in turfgrass areas as well as field crops and lawns (Uddin *et al.* 2009, 2010). In North America, purslane is typically regarded as a weed, but it is consumed as a vegetable in many countries worldwide (Proctor 2013). Purslane has broad acceptability as a potherb in Central Europe, Asia and the Mediterranean regions (Uddin *et al.* 2014). Thus, it is important to evaluate purslane under different water stress conditions since purslane can be considered as a source of food with its benefits.

Studies have shown that purslane has better nutritional quality than other major cultivated vegetables (Liu *et al.* 2000). It is known to be high in antioxidants and is a good source of essential fatty acids and carotenes, which have been shown to be beneficial to human health (Proctor 2013). Purslane also has very high amounts of α -linolenic acid as well as high concentrations of the antioxidants α -tocopherol, β -carotene, and glutathione (Simopoulos *et al.* 1992). Besides being used in the treatment of osteoporosis and psoriasis, it is also used as a purgative, cardiac tonic, emollient, muscle relaxant and anti-inflammatory and diuretic treatments which make it important in herbal medicine (Uddin *et al.* 2014). Plant growth development and production are affected by natural stresses in the form of biotic and abiotic stresses such as

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drought, salinity and others. Drought or water deficiency is one of the most important environmental stresses that influence plant production (Abdalla and El-khoshiban 2007). Purslane is categorized as an annual broad leaf weed (in Bengali: Nunia Sag). In a previous study on weed control in paddy fields it was shown that seeds from broad-leaved weeds largely dominated in all water regime-treated soil samples compared to the other weed groups (Juraimi *et al.* 2012). According to another research done by Juraimi *et al.* (2009), broad-leaved weeds, predominantly *Monochoria vaginalis* and *Limnocharis flava* were the most dominant weeds in most of the water regime treatments in their experiment. Currently, due to climate changes and global warming, more frequent and prolonged drought events are expected in different parts of the world, leading to poorer crop yields and, in more severe cases, food shortages. In this background this study was undertaken to investigate the effects of water regimes on the growth and to compare the antioxidant (total phenolic content and total flavonoid content), C and N content of purslane (*P. oleracea*).

Materials and Methods

The study was carried out under a rain shelter and in the laboratory at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan Campus located at Mile 10, Jalan Sungai Batang, 90509 Sandakan, Malaysia, between July and August 2015. The used soil had organic matter content of 22.02% measured by loss and ignition method (Reddy *et al.* 2008), EC (1.15 dS/m) and pH (5.23) were measured from 1 : 1 and 1 : 2.5 soil-water extract, respectively by glass-electrode method (Jackson 1962, Anderson and Ingram 1966). The topsoil media was pulverized and visible insect pests and plant propagules were removed. The experiment was conducted 25 pots of 20 cm diameter × 30 cm depth. Three uniform seedlings were transplanted into each of the pots containing the soil media. No fertilizers were used in the beginning and all pots were watered to the pot capacity (PC) to facilitate uniform growing up to 30 days.

Five water regime treatments were used in this experiment, namely continuous field capacity condition (T₁), continuous saturated condition (T₂), continuous flooded condition (T₃), 10 days flooded followed by saturated condition (T₄) and 10 days saturated followed by field capacity condition (T₅). Each treatment was replicated 5 times using a complete randomized design.

Thirty-day-old plants' height, single leaf length, single leaf width, internode length, shoot fresh and dry weights, shoot water content were measured (Suzuki *et al.* 2003). Extract of the plant samples of *P. oleracea* was done using methanol solvent. Methanol extracts were prepared using the method described in Crozier *et al.* (1997) with slight modifications. The concentrated extract was then stored at 20 ± 1°C prior to analyses. The total phenolic content of the plant extracts was determined by Folin-Ciocalteu's reagent based on the method described by Singleton and Rossi (1965) with some modifications. Gallic acid was used to produce standard calibration curve. Concentration of 20, 40, 60, 80 and 100 µg/ml of gallic acid was prepared in 95% methanol. Absorbance of total phenolic content of the plant extracts was read using a spectrophotometer. Total phenolic content (C) was calculated as followed and expressed as mg GAE/g dry sample.

$$C = \frac{\text{GAE} \times V \times \text{DF}}{m}$$

where, C = Total phenolic content in mg GAE/g dry sample, GAE = Concentration of gallic acid from the calibration curve in mg/ml, V = Volume of the sample in ml, DF = Dilution factor, and m = Weight of dry sample extract in g.

Total flavonoid content (TFC) was determined according to the procedure of Chang *et al.* (2002), validated by Jaradat *et al.* (2015), with some modifications using quercetin as the

reference standard. Quercetin was prepared and diluted to different concentrations of 6.25, 12.5, 25, 50, 80 and 100 µg/ml using methanol. The concentration of the total flavonoid content in the plant extracts was calculated using the following equation and expressed as mg QE/g dry sample.

$$C = \frac{QE \times V \times DF}{m}$$

where, C = Total flavonoid content in mg QE/g dry sample, QE = Concentration of quercetin from the calibration curve in mg/ml, V = Volume of the sample in ml, DF = Dilution factor, and m = Weight of dry sample extract in g.

For the C and N contents, plant samples were dried in the oven at 70°C for 72 hrs. The oven-dried purslane samples were ground and stored in plastic vials. The C and N content was determined using the Leco CHN628 Analyzer. The crude protein content was calculated from the amount of total nitrogen (%) obtained from the CHN 628 analyzer using the specific conversion factors recommended by Jones (1931) for most food items.

Crude protein (%) = Total nitrogen (%) × 6.25 (Jones 1931)

All data were analyzed using ANOVA with the Statistical Package for Social Science (SPSS) version 21 and treatment means were compared using the LSD test. All statistical analysis was done at 5% level of significance.

Results and Discussion

Plant height showed the significant difference due to the treatments (Table 1). Based on the results, the continuous saturated condition (T₂) had produced the highest plant height. This might be due to the purslane plant favouring saturated soil for its growth. However, there was no significant difference between the continuous field condition treatment (T₁) and flooded 10 days flooded condition followed by saturated condition treatment (T₄). Kamrun *et al.* (2011) observed no difference in the height of tomato plants subjected to different water levels. The authors attributed the lack of response to the fact that when water becomes available after a short period of stress, growth is very rapid such that there will be no net observable reduction in the tomato plants subjected to different water stress. However, Techawongstein *et al.* (1992) had observed suppression of plant height due to water stress in chilli. Similar results were also showed by Baher *et al.* (2002) on *Satureja hortensis* L. herb plant, under severe water stress which significantly reduced the plant height by 31%, compared with the controls.

Table 1. Effect of different water regimes on the plant height, leaf length, leaf width, stem diameter internode length of purslane.

Treatment	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Stem diameter (cm)	Internode length (cm)
T1	275.7d	20.3c	14.0a	4.1d	76.0a
T2	429.0a	25.7ab	13.7a	8.1a	74.3a
T3	326.0c	26.3a	14.0a	6.2b	72.7a
T4	312.3c	21.7bc	11.3b	4.6d	72.3a
T5	390.0b	21.7bc	12.3ab	5.5c	72.7a

In a column, means followed by the same letter are not significantly different at 5% level by least significant differences.

There was significant difference in terms of effects of different water regimes on the leaf length and leaf width (Table 1). The results showed that continuous flooded condition produced (T₃) the largest and widest leaf followed by that under continuous saturated condition (T₂). There

are various major abiotic and biotic factors that may have influenced the growth pattern of the leaves, for example, sunlight. Silva *et al.* (2010) had observed that the leaf area decreased under all water stress treatments in *Erythrina velutina* seedlings. Hayatu *et al.* (2014), and Samson and Helmut (2007) also reported that there was significant reduction in leaf area at vegetative and flowering stages of water stressed cowpea.

There were significant differences on stem diameter in different treatments (Table 1). The results showed that continuous saturated condition produced purslane with thickest stem diameter followed by that under continuous saturated condition (T₂). Purslane under continuous field capacity condition (T₁) produced the thinnest stem diameter. Stem diameter changes appear to be a sensitive physiological indicator of plant water stress, since differences in stem shrinkage between two treatments (irrigated 50 and 100%) were detected by Garnier and Berger (1986) in peach trees. According to Gallardo *et al.* (2004), withholding irrigation caused clear responses in the stem diameter in mature tomato and melon plants. The daily maximum stem diameter (MXSD) and daily minimum stem diameter values (MNSD) decreased in the un-watered plants and the maximum daily contraction (MDC) increased relative to the well-watered plants.

The response of internode length to the treatments was not significant (Table 1). In continuous field capacity condition (T₁) treatment, purslane had with longest internode length followed by those under continuous saturated condition (T₂). Those grown under flooded condition followed by saturated condition however produced the shortest internode length in the purslane. According to Nyabundi and Hsiao (1989), well-watered plants had an increase in internode length compared to the moderately and severely stressed plants, for it is well known that as soil water availability becomes limited, plant growth is usually decreased. Soil water deficit causes an overall reduction in the length of internodes and can cause abrupt reductions in internode length if any water stress occurs, as stated by Cull (2010).

There were also no significant differences among the treatments on leaf relative water content (Table 2). This may be due to some unavoidable biotic and abiotic factors that influence the leaf relative water content among the treatments. Field capacity treated purslane showed highest leaf relative water content followed by the continuous saturated condition treatment.

Table 2. Effects of different water regimes on the leaf relative water content, shoot fresh weight, shoot dry weight, and shoot water content content of purslane.

Treatment	Leaf relative water content (%)	Shoot fresh weight (g/plant)	Shoot dry weight (g/plant)	Shoot water content (% per plant)
T1	79.85a	91.26a	10.97a	87.97a
T2	76.17a	68.97b	6.46bc	90.59a
T3	70.44a	42.67c	4.29c	89.63a
T4	72.37a	47.63c	4.82c	89.89a
T5	72.80a	82.93ab	8.06ab	90.29a

In a column, means followed by the same letter are not significantly different at 5% level by least significant differences.

Although no significant differences were observed due to different treatments, the lowest leaf relative water content was found in continuous flooded treatment. According to El Jaafari (2000), water deficit exerts a negative effect on relative water content, thus the ability of the plant to survive severe water deficits depends on its ability to restrict water loss through the leaf epidermis after the stomata have attained minimum aperture. Hayatu *et al.* (2014) reported that leaf relative water content (LRWC) of the water stressed genotypes of *V. unguiculata* were lower than the

unstressed genotypes water stress at vegetative stage that resulted only 22.22% increase in their leaf relative water content while recorded 77.78% reduction.

The results showed that continuous saturated treatment produced the highest shoot water content. Continuous field capacity treatment had lowest shoot water content compared to the other treatments although the differences were not significant. Similar results were reported by Suzuki *et al.* (2003), who found that there were no significant differences between the water stress treatments on rice. However, Sangakkara *et al.* (2010) reported that there was better growth performance of shoots and roots in full irrigated maize plants, thus the shoot water content in full irrigated maize was higher compared to those under 50 and 25% irrigation.

Table 3. Effects of different water regimes on total phenolic content, total flavonoid content, carbon, nitrogen, and crude protein of purslane.

Treatment	Total phenolic content (mg QAE/g)	Total flavonoid content (mg QE/g)	Carbon (%)	Nitrogen (%)	Crude protein (%)
T1	3.06a	0.96b	27.99a	2.35b	14.66b
T2	1.85b	1.29a	39.13a	2.29b	14.30b
T3	1.63c	1.35a	40.30a	3.14a	19.62a
T4	1.51d	1.21a	40.18a	2.49b	15.54b
T5	1.33e	1.04b	39.59a	1.57c	9.82c

In a column, means followed by the same letter are not significantly different at 5% level by least significant differences.

The treatments had a significant effect on total phenolic content (Table 3). The results showed that continuous field capacity condition (T₁) produced the greatest total phenolic content compared to the other treatments. Flooded condition followed by field capacity condition produced purslane with lowest phenol content. According to Alam *et al.* (2014), the total phenolic content in purslane ranged from 0.96 ± 0.04 to 9.12 ± 0.29 mg GAE/g dry weights. Uddin *et al.* (2012) reported that total phenol content (TPC) in purslane varied from 174.5 ± 8.5 to 348.5 ± 7.9 mg GAE/100 g dry weight. Oliveira *et al.* (2009) showed that total phenolic content of purslane stems and leaves was very different ranging from 78.3 to 633.9 mg/kg dry weight. Lim and Quah (2007) reported that total phenolic compounds of stems and leaves of *P. oleracea* ranged from 127 ± 13 to 478 ± 45 mg GAE/100 g fresh weight of plant.

There were significant differences of the treatments on total flavonoid content (Table 3). The results showed that continuous flooded condition treatment produced the greatest total flavonoid content followed by the continuous saturated treatment and 10 days flooded condition followed by saturated condition (T₄). The lowest flavonoid content was found in the purslane under continuous field capacity condition (T₁). According to Alam *et al.* (2014) found that the total flavonoid content in purslane plants ranged from 0.13 ± 0.04 to 1.44 ± 0.08 mg RE/g dry weights. Uddin *et al.* (2012) showed that the flavonoid contents were also markedly higher in the methanolic extract with a value of 49.18 mg rutin equivalent/g DW compared to the ethanol extract at 41.3 mg rutin equivalent and water extract with a value of 28.7 mg rutin equivalent/g DW. Huang *et al.* (2007) identified three flavonoids in purslane e.g. isorhamnetin, quercetin and kaempferol.

The effect of the treatments was not significant on the carbon content (Table 3). The results showed that continuous flooded condition (T₃) produced the highest carbon content in purslane, whereas continuous field capacity condition (T₁) produced purslane with the lowest carbon content. Schlesinger (1991) noted that carbon content of biomass is found to be between 45 and 50% by

oven-dry mass. McCree and Troughton (1966) estimated carbon content of 39.6% in *Trifolium repens* L. plants, Terry and Mortimer (1972) estimated a carbon content of 42.1% for sugar beet leaves, while Turgeon and Webb (1975) found a value of 45% for cucumber leaves.

The response of treatments to nitrogen content in purslane was significant (Table 3). The results showed that continuous flooded treatment produced purslane with the highest nitrogen content. The lowest nitrogen content was found in the plants under 10 days saturation followed by field capacity condition. In the study on the nitrogen contents of edible wild plants, Tosun *et al.* (2003) reported a nitrogen content of 1.07% in *P. oleracea*, 2.26% in *Malva neglecta*, and 9.45% in *Urtica dioica*. Kaya *et al.* (2004) also reported the nitrogen content to be 4.20% in *M. neglecta* and to be 3.64% in *Papaver dubium*.

There were significant differences among the treatments for crude protein content in purslane (Table 3). The results showed that continuous flooded treatment produced purslane with the highest crude protein content. The lowest crude protein content was found in purslane under 10 days saturation followed by field capacity condition. In the study by Ezekwe *et al.* (2011), purslane was shown to contain 22.9% crude protein content, which was higher than those of other forage or vegetable crops like alfalfa and legumes, traditionally used as animal feed.

The continuous saturated condition resulted in better growth compared to the other treatments in terms of plant height, leaf length, leaf width, stem diameter and internode length. Continuous flooded condition showed higher chemical composition compared to the other treatments in terms of total flavonoid content, nitrogen content and crude protein. Thus, continuous saturated condition is recommended to farmers if the farmers are more concerned about yield, while continuous flooded condition is recommended if the nutritional values are of more concern.

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