

Original Research Article

Studies on the Growth, Production and Component contents of *Chrysanthemum indicum* Using Arduino-controlled moisture content irrigation systems

ABSTRACT

The flowers of *Chrysanthemum indicum* L., when used as herbal medicines, are said to effectively relieve heat, pain, eye fatigue, and inflammation and be detoxifying. Recent studies have been conducted to control the growth environment of plants with the use of computers, Arduino company producing one such open-source control device. In this study, we evaluated the effect of a traditional ck irrigation system and three soil moisture volume content irrigation controlled by Arduino UNO on flowering, growth, production, and indicators of flower quality of *C. indicum* grown in pots in a greenhouse. For the purposes of this study, we aimed to maintain soil moisture volume content to 35%-40%. Irrigation was set to be initiated when soil moisture volume content dropped to 25-30% (Is30% group), 15-20% (Is 20% group) or 5-10% (Is 10% group). Flowering time was earlier in Is 30% and Is 20% plants than the traditional ck plants, while Is 10% plants did not flower throughout the cultivation period. Both the Is 30% and traditional ck plants produced significantly more total number of flowers and total dry weight of the flowers than Is 20% (Is 10% none). Traditional ck flowers has significantly higher content of chlorogenic acid, myricetin and quercetin, indicators of quality. The other indicators, luteolin and apigenin were significantly higher traditional ck and Is 30% plants. This study concluded that the

Arduino UNO's irrigation control system had a better effect on *C. indicum* growth and component content when irrigation was set Is 30%.

KEYWORDS : C. INDICUM, CHRYSANTHEMUM INDICUM; CK, CONTROL; IS, IRRIGATION SYSTEM; DAT, DAY AFTER TRANSPLANTATION

Abbreviations: *C. indicum*, *Chrysanthemum indicum*; ck, control; Is, Irrigation system; DAT, Day after Transplantation

UNDER PEER REVIEW

1. Introduction

Chrysanthemum indicum, a perennial herbaceous species of the Asteraceae family, originates from and is currently found mainly in East Asia[1]. Traditional oriental medicine has used the aerial parts of *C. indicum* to treat vertigo, hypertensive symptoms and several infectious diseases including pneumonia, colitis, stomatitis, carbuncle and fever [2]. Its flowers are also commonly used as a tea to treat some eye diseases in traditional Chinese medicine [3]. *C. indicum* is known to contain several classes of biologically active compounds including essential oils, terpenoids, flavonoids, and phenolic acids [4,5]. Its major active components include flavonoids luteolin and linarin, as well as the phenolic acid chlorogenic acid. Phenolic acids have antibacterial, antiphlogistic, antimutagenic, antioxidant and other biological activities [6].

Drought is one of the main factors restricting plant growth and development. Drought stress reduces the photosynthetic capacity of plants, plant physiology, plant nutrition and reproductive growth [7,8,9]. Furthermore, moderate drip irrigation can improve water efficiency and increase production [10,11]. It is necessary to understand the effect of soil moisture content changes on plants in order to decide optimal irrigation control parameters. Although gravimetry and relative water amount have been used together to assess drought stress, this method of assessment is labor-intensive and time-consuming [10]. It is more practical to construct a system that can be used to monitor soil moisture real time and start irrigation using the most appropriate amount of water when soil moisture drops to a specific level.

Arduino is an open-source hardware and software company that, among many other activities, designs and produces microcontrollers for digital devices. Arduino UNO is one of their open-source microcontrollers that can be used in the design and development of monitoring systems that are much lower in price than commercial monitoring systems to monitor various

environmental parameters [12,13,14]. However, very few agricultural science researchers have used Arduino UNO to design control systems to use in their crop cultivation experiments. Using a fully automatic irrigation system that we developed with Arduino UNO, we were able to create a program to manage three different irrigation methods for the cultivation of *C. indicum*. We then compared the relative effect of these methods on growth, dry weight, and flavonoid content *C.indicum* grown in pots in a green house.

UNDER PEER REVIEW

2. Materials and Methods

2.1. Materials

This study was conducted for five months from Dec. 2019 to Apr. 2020 in a greenhouse at National Pingtung University of Science and Technology (NPUST) in Taiwan (22°38'N, 120°36'E). *Chrysanthemum indicum* seedlings, a gift from Professor Chi-Qiang Wang of the Forestry Department, were certified by the university. They were maintained in the laboratory of the corresponding author in the Department of Plant Industry, NPUST. On 22 Dec. 2019, the cuttings of *C. indicum* were planted in cells filled with a soil mixture of peat moss and sand at a ratio of 3:1. After one month, the cuttings which had grown to around 10 cm high were transplanted into plastic pots (18 cm in diameter and 18 cm high) filled with 2.0 kg of a mixture of soil, sea sand, and peat moss (3 : 2 : 1, v/v). We applied *Hi-Control*, a basic fertilizer containing NH_4NO_3 , P_2O_5 and K_2SO_4 (14 : 11 : 13) 4.5 g/pot once at the beginning of the study period.

2.2. Chemicals and reagents

Chlorogenic acid, myricetin and quercetin were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Apigenin and luteolin were purchased from SunHank Technology Co., Ltd (Tainan, Taiwan). The 95% ethanol was purchased from Taiwan Tobacco and Wine Board (R.O.C.). Methanol was purchased from Riedel-de Haën (Germany). Phosphoric acid was purchased from Kanto Chemical (Japan). Ultra-pure distilled water with a resistivity greater than 18.2 M Ω was obtained from a Millipore mini-Q system (Bedford, MA, USA). Samples for HPLC were filtered separately through a 0.45 μm Millipore membrane filter (Xg-Iwaha-9909-1304-PK, Bio Tech). All other reagents were of analytical grade.

2.3. Experimental Design

For this study, we created an automatic irrigation system using an open-source microcontroller board, Arduino UNO (Arduino AG) (Fig. 1A). Fig. 1B shows the wiring diagram of our automatic irrigation system using Arduino UNO. Two moisture sensors, the Arduino soil moisture sensor and a ready-made soil moisture sensor (Decagon), were used to detect moisture and collect the data needed to create calibration curves which we used to set Arduino UNO program parameters. A curve coefficient of 0.9934 was considered a high correlation (Fig. 2). The experiment was conducted using four water application levels. One, which we included for comparative purposes, was the traditional ck irrigation system. The other three were Arduino UNO controlled irrigation system (Is). The traditional ck irrigation system used a timer set to add 1.46L per pot twice a day (7:00 and 17:00). The goal of irrigation system was to maintain soil moisture volume content between 35%-40%. The Arduino system was set to start irrigation (each pot 1.46L an hour) when the soil moisture volume content decreased to 25-30% (Is30%), 15-20% (Is 20%) and 5-10% (Is 10%). These four water irrigation treatments were used throughout the entire four-month cultivation period, starting from the time the seedlings were transplanted into the pots. The soil moisture sensor (EC-5, Decagon) was buried 10 cm below the soil surface during the treatment period, with soil moisture volume content was recorded once every 10 min using a data logger (Em 5b, Decagon). The total amount of irrigation water used for each pot was calculated based on the total number of irrigations. WUEd (water use efficiency of dry mass) was calculated using the following formula:

$$\text{WUEd} = \text{Total dry weight} / \text{Total irrigation applied}$$

Five pots of flowers per application group (total = 20 pots) were sampled every three days starting from 42 days after transplantation (DAT) to calculate average total number of flowers and total dry weight of flowers. To do this, opened flower blossoms were collected and dried at 50°C for 48 hr. After drying, the dried blossoms from each group were weighed and dry weight was recorded. After each plant had completed flowering (about 2 to 3 months after transplantation), the entire plant was harvested and plant height, number of leaves, stem diameter and SPAD values were recorded. Afterwards, each plant was physically separated into flowers, leaves, stems, and roots. The diameters of all flowers from each plant was measured and averaged. The length of longest root on each plant was measured and recorded. Image analysis software (Image J, National Institutes of Health) was used to measure areas of all the leaves and an average leaf area size was recorded. Finally, all the parts from each plant were collected and dried in an oven set at 50°C for 48 h. The dry weight was recorded.

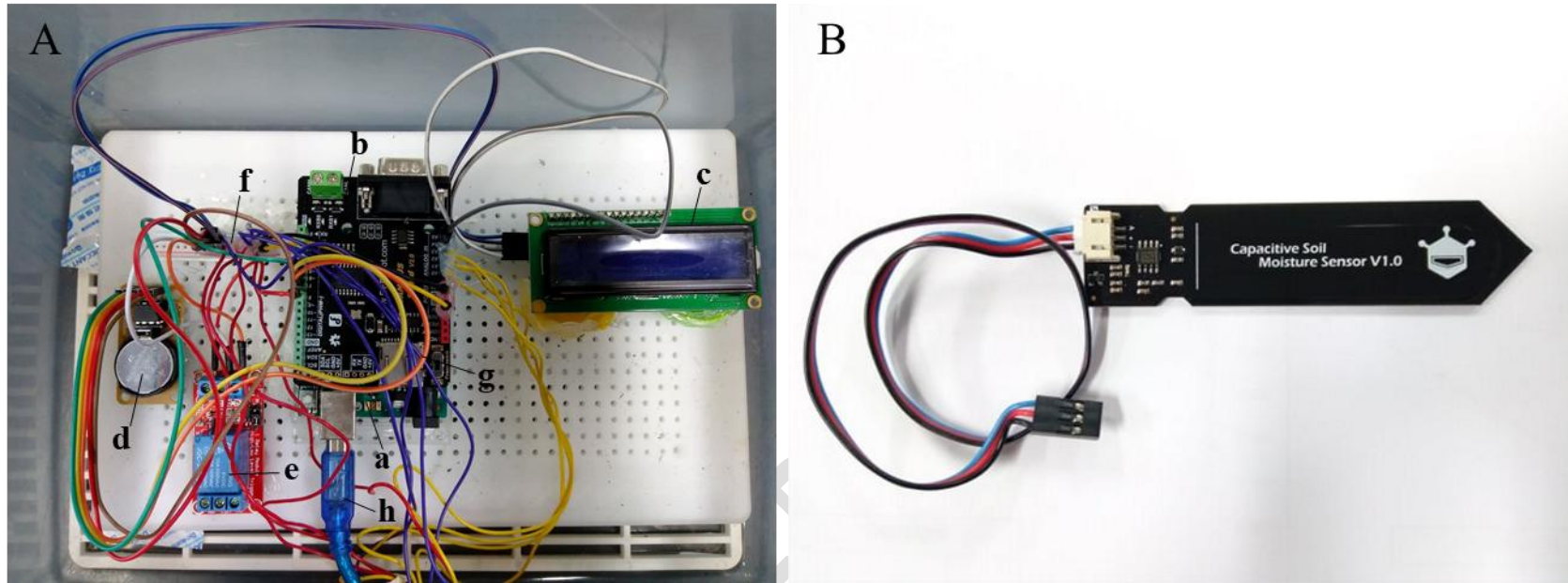


Fig. 1. Automatic irrigation system using Arduino module. The automatic irrigation equipment (A) and soil moisture sensor (B).
Arduino Uno SMD Rev3 (a), CAN-BUS Shield v2.0 communication expansion board (b), LCD dot matrix liquid crystal module (c),
Arduino Real-Time Clock module (d), Arduino 1-way relay module (e), breadboard (f), SD card (g), USB port (h).

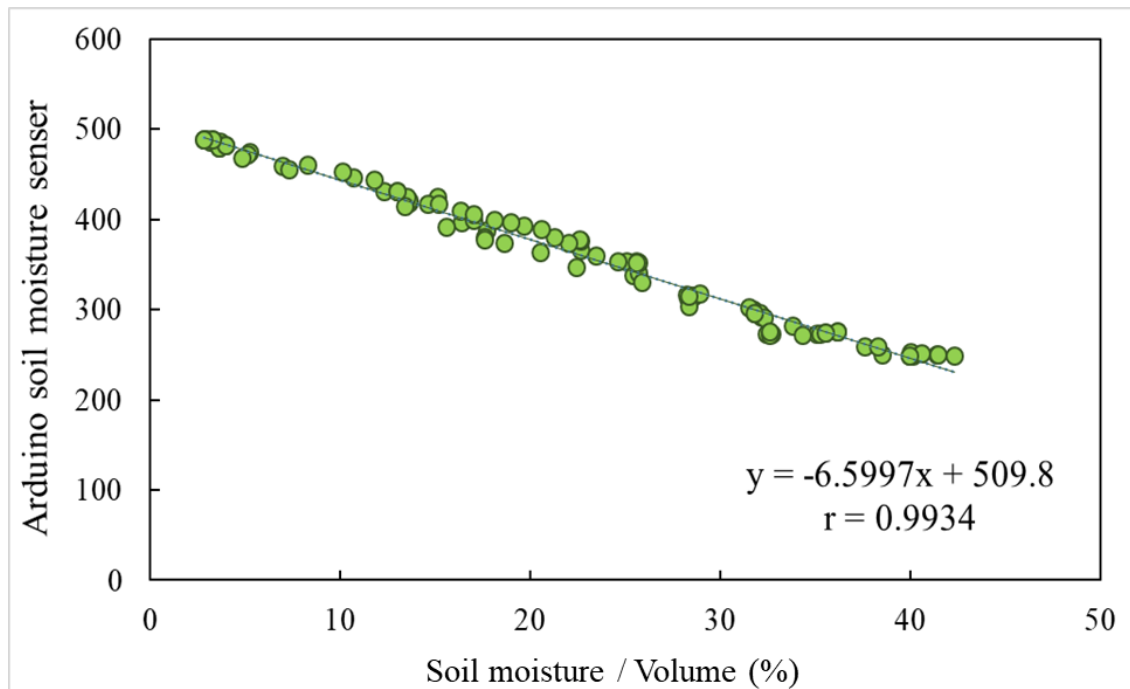


Fig. 2. Calibration curve of Arduino automatic irrigation device sensor and soil moisture sensor.

2.4. HPLC analysis

2.4.1. Preparation of standard solutions

Standard stock solutions were prepared by dissolving the amount of each quality indicator component in a methanol aqueous solution to obtain the desired concentrations: chlorogenic acid (400.0 µg/mL), myricetin (320.0 µg/mL), luteolin (300.0 µg/mL), quercetin (200.0 µg/mL), and apigenin (200.0 µg/mL).

2.4.2. Preparation of sample solutions

The dried flowers were pulverized in a vibrating sample mill (TI-100, CMT) and large particles were removed by sieve (0.2 mm²). 0.5 g of flower powder from each group was placed in a beaker, 100 mL methanol was added, and the sample was shaken for 30 min in a microwave vibrator (DC400, Delta). To concentrate the solutions, they were filtered through NO.2 filter paper and placed in a rotary evaporator (B-491, R-210, Büchi; B403L, Firstek). Then, concentrated solution was added with methanol 10 mL. After centrifuging for 20 second, supernatant was filtered through a 0.45 µm membrane filter creating a sample stock solution.

2.4.3. HPLC Instruments and Conditions

HPLC separation was conducted using a Hitachi system equipped with a pump L-2130 UV/Vis detector L-2420, a photodiode array detector L-2455 and an autosampler L-2200. Peak areas were calculated using D-2000 HSM software.

A reversed phase column Inertsil 5 ODS-2 (Nacalai, 4.6 mm I.D.×250 mm) was placed in the detector's column oven which was set at 40°C. A gradient elution of A (80% Methanol solution and 20% water) and B (10% Methanol solution and 90% water (pH 2.8) was used as follows: 0-5 min, 0-5% A; 5-10 min, 5-20% A; 10-15 min, 20-30% A; 15-20 min, 30-45% A; 20-40 min, 45% A; 40-50 min, 45-55% A; 50-60 min, 55-90% A; 60-65 min, 90-95% A; 65-75 min,

95-100% A; 75-80 min, 100-0% A. Detection wavelength was set at UV 280 nm. The flow rate was 1.0 mL/min. Sample solutions, prepared as described above, were injected (20 µL) into the HPLC column for analysis.

2.4.4. Calibration Method

Each standard component stock solution was diluted using methanol to give sequential concentrations of chlorogenic acid from 25 to 50, 100, 200, and 400 µg/mL for high calibration concentration range and from 1.56 to 3.13, 6.25, 12.5, and 25 µg/mL for low calibration concentration range. Myricetin was diluted using methanol to give sequential concentrations from 18.75 to 37.5, 75, 150, and 300 µg/mL, luteolin from 18.75 to 37.5, 75, 150, and 300 µg/mL, and quercetin from 12.5, 25, 50, 100, to 200 µg/mL for high calibration concentration ranges, and for the low calibration ranges for these three the sequence of concentration ranged from 0.78 to 1.56, 3.13, 6.25, and 12.5 µg/mL. Apigenin was diluted from 12.5 to 25, 50, 100, and 200 µg/mL for high calibration concentration range and from 0.78 to 1.56, 3.13, 6.25, and 12.5 µg/mL for low calibration concentration range.

Each dilution contained the above-mentioned standard solution filtered through a 0.45 µm membrane filter. 20 µL volume of each concentration solution was injected into the HPLC system for separation. The calibration curve was plotted using the peak areas of each standard solution (y-axis) against each concentration (x-axis). Linear regression was used to evaluate the equations of $y=ax+b$ and the correlation coefficient (r).

2.4.5. Validation test

2.4.5.1. Precision

Intra- and inter-day variations were chosen to determine the precision of the assay we

developed. Each component standard stock solution was diluted with methanol to three different concentrations, as shown in Table 1. For the intra-day test, each concentration of the five standards was injected into the column three times within 24 h. For the inter-day test, each concentration was injected three times over five days with each injection separated at least 24 h. Both tests were run to check reproducibility. The standard deviation (S.D.) and relative standard deviation (R.S.D., %) were calculated.

2.4.5.2. Accuracy

Each standard stock solution of each component in a series of various concentrations was spiked into a methanol solution of *C. indicum* flowers, and it was shaken for 30 min in a microwave vibrator. The solution was then filtered and subjected to HPLC analysis in triplicate. Using the extract from flowers as an example, the recovery (%) was calculated using the following equation: $[(C3-C2)/C1] \times 100\%$, where C1 is the amount of each standard spiked, C2 the amount of each component in methanol solution of flowers from *C. indicum*, and C3 the total amount of each component in the solution.

2.4.6. Statistical analysis

All statistical operations were performed using SPSS software. Differences between means were tested for by Tukey for the least significant difference (LSD) test. A p-value of 0.05 was considered significant.

Table 1. Results of validation test

Standard	Concentration ($\mu\text{g mL}^{-1}$)	Intra-day RSD (%)	Inter-day RSD (%)	Recovery	
				Mean \pm SD	RSD (%)
Chlorogenic acid	200	0.18	0.30	92.8 \pm 0.6	0.65
	100	0.79	4.10	93.5 \pm 1.2	1.28
	50	0.33	2.10	96.3 \pm 0.4	0.42
Mricetin	160	1.51	3.10	95.2 \pm 1.0	1.05
	80	2.26	1.00	91.3 \pm 0.3	0.33
	40	0.10	3.10	92.4 \pm 0.2	0.22
Quercetin	150	4.00	1.30	98.2 \pm 0.8	0.81
	75	2.90	1.30	96.5 \pm 0.4	0.41
	37.5	3.71	2.30	94.3 \pm 1.4	1.48
Luteolin	100	2.40	4.60	97.6 \pm 0.4	0.41
	50	1.40	2.50	94.4 \pm 1.0	1.06
	25	4.50	3.30	95.2 \pm 1.1	1.16
Apigenin	100	0.45	2.20	96.3 \pm 0.3	0.31
	50	2.30	4.50	94.8 \pm 0.6	0.63
	25	2.57	1.80	97.1 \pm 0.8	0.82

RSD: relative standard deviation.

SD: standard deviation.

3. Results

3.1. The effect of the three different Arduino-controlled irrigation systems and the traditional ck system on total amount of irrigation water used and water use efficiency

Arduino soil moisture volume content parameters were set to irrigate at Is 30%, Is 20% and Is 10%, meaning that water was added when the soil moisture volume content dropped below 30%, 20%, and 10%, respectively, adding enough to maintain a soil volume contents around 35%-40 % (Fig. 3). To do this, the irrigation water was applied at a rate of 1.46 L per hour each time. The total of amount of water used for irrigation for each system during the cultivation period was (in liters) 232.8, 117.1, 24.9 and 16.1 L for the traditional ck, Is 30%, Is 20% and Is 10% systems, respectively. Water use efficiency was 0.18, 0.27, 1.08 and 1.30 g D.W./L, also respectively. Water use was significantly more efficient under Is 20% and Is 10% systems (Table 2).

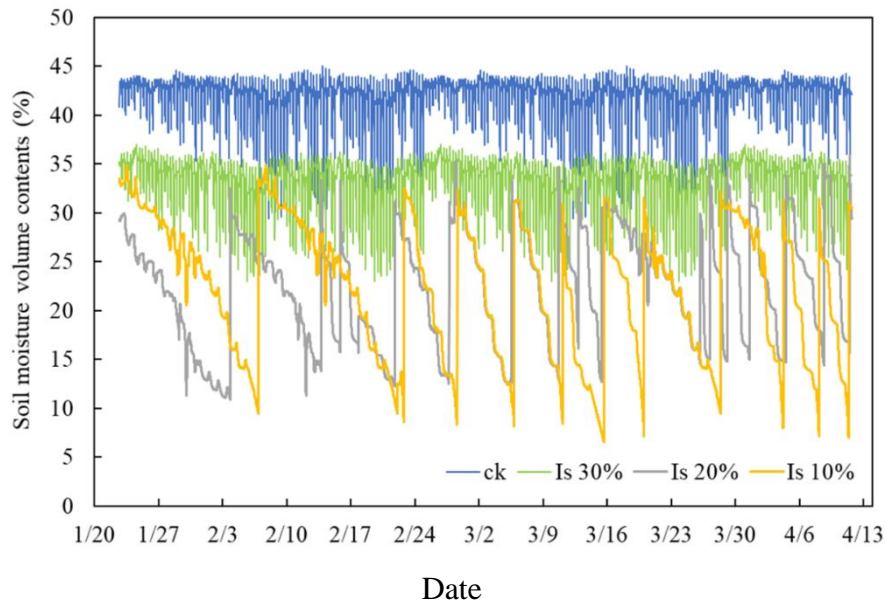


Fig. 3. The effect of Arduino module fully automatic irrigation control on soil moisture volume content.

Table 2. The effect of Arduino module fully automatic irrigation control on water use efficiency (WUEd) of *C. indicum*

Treatment	Irrigation applied (L/h)	Total irrigation applied (L)	WUED (g D.W./L)
ck	1.46	232.8	0.18±0.05 ^b
IS 30%	1.46	117.1	0.27±0.03 ^b
IS 20%	1.46	24.9	1.08±0.10 ^a
IS 10%	1.46	16.1	1.30±0.46 ^a

3.2. The effect of the different irrigation systems on plant growth and dry matter

We analyzed growth and dry weights of both the flowers and the rest of the plants. With regard to the flowers, the Is 10% group did not flower at all, the Is 20% and Is 30% groups started flowering on March 3, 2020, producing four flowers and one flower, respectively, and the traditional ck group started flowering on Mar 9 and continued flowering until Apr 26 (Fig. 4A). The average number of flowers produced in the Is 20% group increased slowly and stopped on Apr. 1, lower than the traditional ck group. The average total number of flowers in Is 30% group was found to have continued to increase on Apr 14, exceeding that of traditional ck group. However, by Apr 17, their average total number of flowers was slowly fell below that of traditional ck group. The average flower diameters were 2.48, 3.04 and 2.40 cm for the traditional ck group, the Is 30% and Is 20% groups, respectively (Fig. 5B). The Is 30% group had a significantly larger in average flower diameter. Total dry matter weight of flowers paralleled the total number of flowers. The dry weight was found to have moderately increased in the Is 20% group on April 17 while that of the both the Is 30% and the traditional ck group were found to have sharply increased, that of the Is 30% group exceeding that of the traditional

ck group. By Apr 20, however, the total dry matter of the Is 30% group had not increased significantly and the dry weight of the traditional ck group surpassed that of the Is 30% group. (Fig. 4B). The average dry weight per flower was calculated dividing the average total number of flowers into the total dry weight of flowers. These averages were 0.049, 0.056 and 0.029 g/flower for the traditional ck, Is 30%, and Is 20% groups, respectively, indicating that both the traditional ck and Is 30% systems significantly increased the average dry weight of each flower (Fig. 5A).

Once the flowering survey was completed, we measured the growth and dry weight of the plants in general, the leaves, stems and roots. Average number of leaves and stem diameters increased significantly in both the Is 30% and ck groups. Average leaf area was significantly lower in both Is 20% and Is 10% groups, with also a significant difference between Is 20% and Is 10%. Root length did not differ significantly among the different groups (Table 3). With regard to average dry weights, the leaves of the traditional ck groups weighed an average of 11.54 g/plant, significantly more than the leaves in the other groups. The stems were not found to differ in dry weight among the different groups. The total dry weight of roots were significantly lower in both the Is 20% and Is 10% groups. The average dry weight of root plant was 8.22g in the Is 10% group, significantly lower than the other treatment groups.

Table 3. Effect of Arduino module fully automatic irrigation control on the growth and dry matter of *C. indicum*

Treatment	Plant height (cm plant ⁻¹)	Number of leaves	Stem diameter (mm plant ⁻¹)	SPAD value	Leaf D.W.	Stem D.W	Root D.W	Total D.W	Leaf area (cm ² plant ⁻¹)	Root length (cm plant ⁻¹)
					(g plant ⁻¹)					
Ck	45.9a	279a	3.42a	49.8a	11.54a	7.20a	22.80a	41.54a	4679.2a	27.4a
IS 30%	44.5a	214a	3.36a	50.4a	7.95b	4.97a	16.67ab	31.82ab	2622.2bc	31.8a
IS 20%	36.1b	134b	2.98b	50.1a	7.53b	3.73a	14.38ab	26.87b	2993.2b	30.8a
IS 10%	37.3b	179b	2.78b	51.8a	8.92b	4.10a	8.22b	20.87b	1817.9c	30.8a

Values were means \pm S. D. (n=5).

Means with different letters in the same column are significantly different ($p < 0.05$) by Tukey test (n=5).

Leaf D.W.: dry weight of leaf, Stem D.W.: dry weight of stem, Root D.W.: dry weight of root, Total D.W.: total dry weight.

UNDER PEER REVIEW

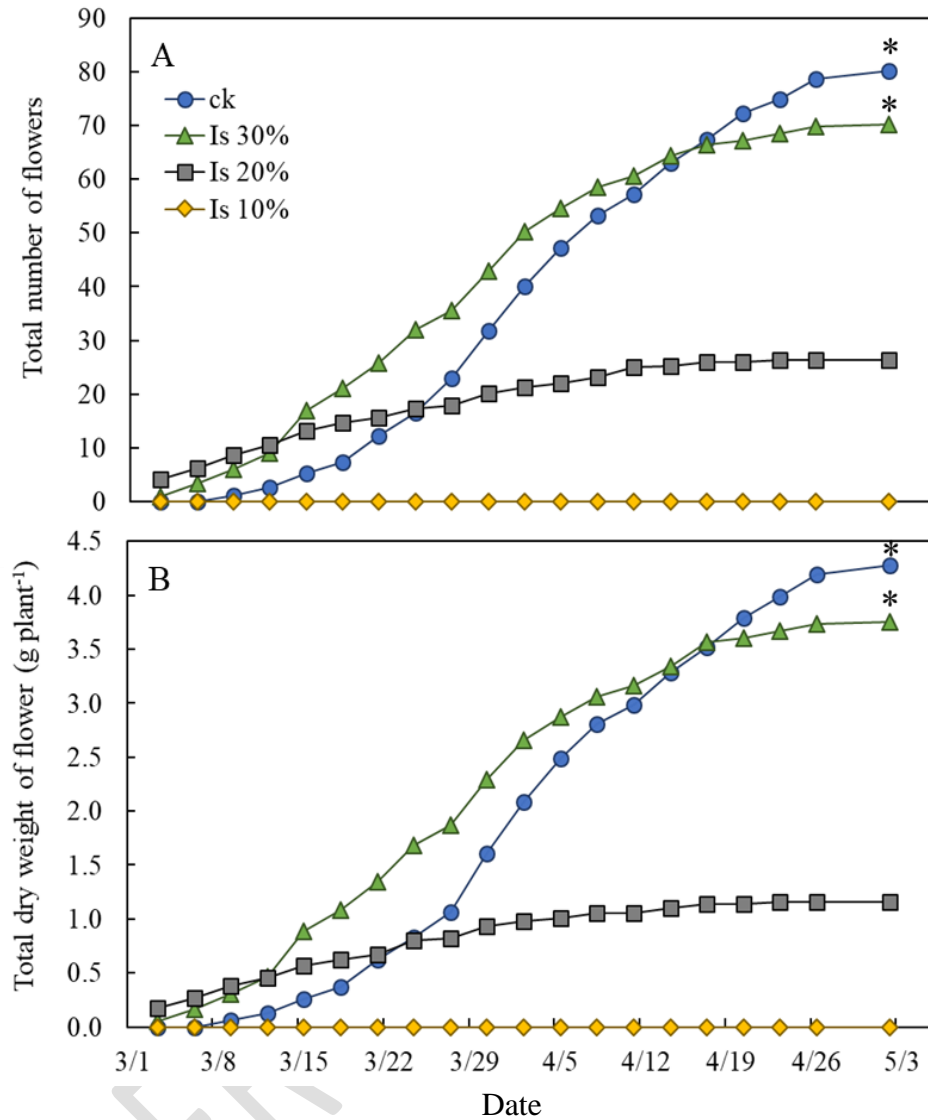


Fig. 4. The effect of Arduino module fully automatic irrigation control on the total number of flowers and total dry weight of flower.

A : Total number of flowers, B : Total dry weight of flower.

* means with different letters in the same column are significantly different ($p < 0.05$) by Tukey test (n=5).

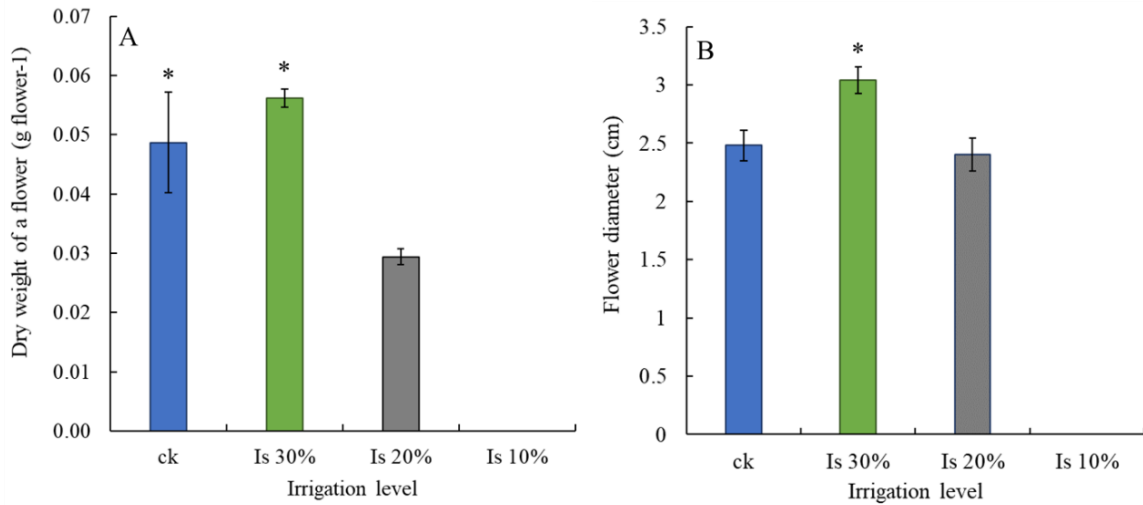


Fig. 5. Arduino module fully automatic irrigation control on the dry weight of a flower and flower diameter of *C. indicum*.

A : Dry weight of a flower 、 B : Flower diameter.

* means with different letters in the same column are significantly different ($p < 0.05$) by Tukey test (n=5).

3.3. HPLC analysis

Figs. 6 and 7 show the HPLC chromatograms of the methanolextracted from the *C. indicum* leaves and flowers, both standard and methanol solutions sampled from the different irrigation systems. Chlorogenic acid, myricetin, quercetin, luteolin, and apigenin compounds were all observed to be well-separated and undisturbed by the other components (flavonoids) under the analysis conditions used by this study. Extract purification was high and separation was efficient. Therefore, it was determined that our HPLC conditions could be used to measure each component.

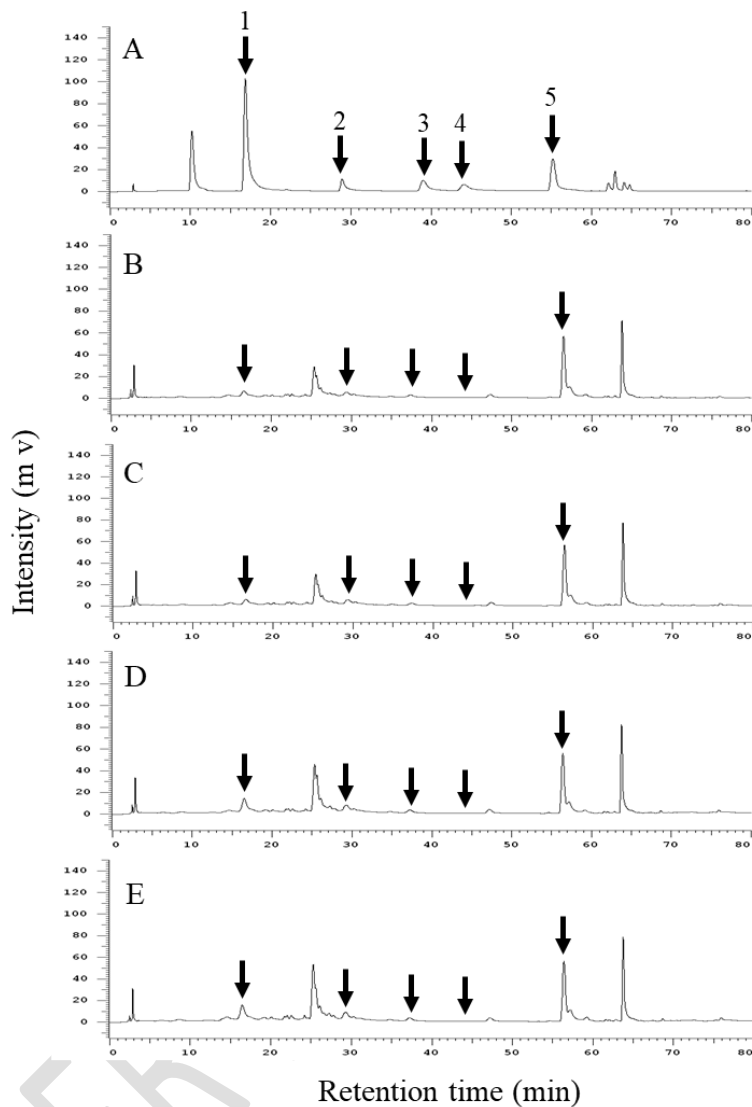


Fig. 6. HPLC chromatograms from leaves of *C. indicum* standard and sample solutions of different irrigation system.

(A)Standard, (B)ck, (C)Is 30%, (D)Is 20%, (E)Is 10%.

1 : Chlorogenic acid; 2 : Mricetin; 3 : Quercetin; 4 : Luteolin; 5 : Apigenin

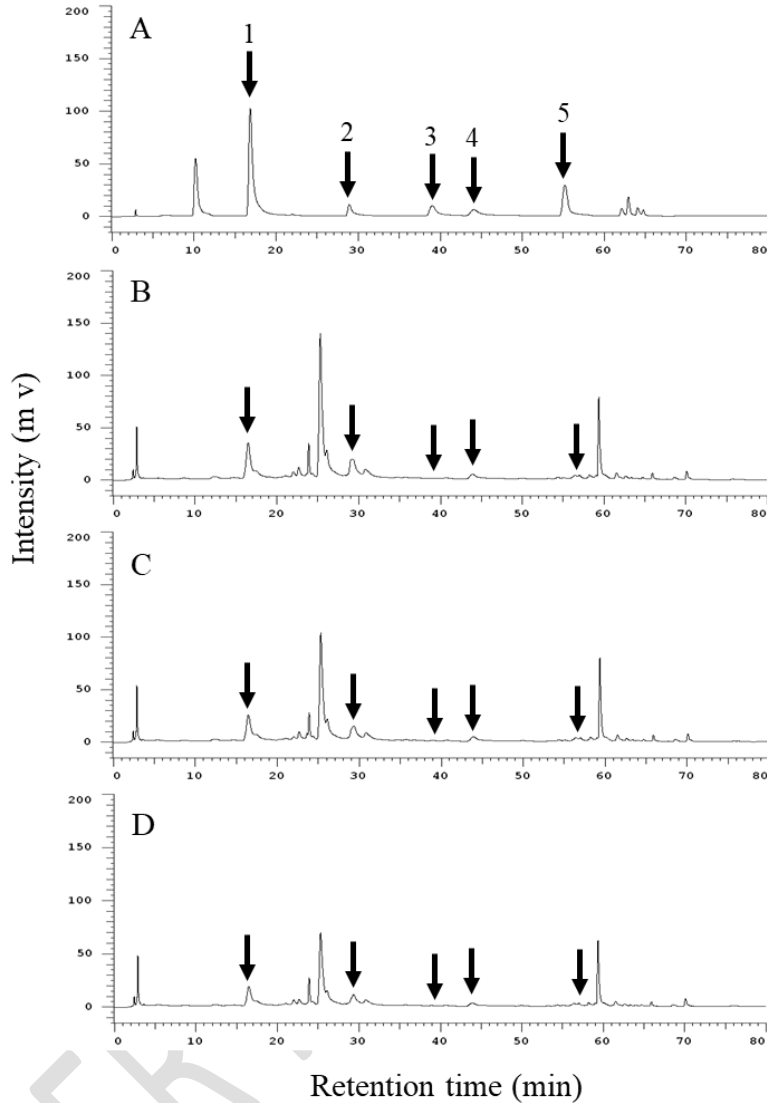


Fig. 7. HPLC chromatograms of from flowers of *C. indicum* standard and sample solutions of different irrigation system.

(A)Standard, (B)ck, (C)Is 30%, (D)Is 20%

1 : Chlorogenic acid; 2 : Mricetin; 3 : Quercetin; 4 : Luteolin; 5 : Apigenin

3.4. Calibration curve

The regression equations and correlation coefficients of the calibration lines for the above-mentioned compounds were as follows:

Chlorogenic acid at concentrations 25.0–400.0 $\mu\text{g/mL}$, $y = 19126x - 296018$, $r = 0.9971$ ($n = 5$).

1.56–25.0 µg/mL, $y = 18499x + 159685$, $r = 0.9997$ ($n = 5$).

Myricetin at concentrations 18.75–300.0 µg/mL, $y = 8350x + 220943$, $r = 0.9982$ ($n = 5$).

Quercetin at concentrations 18.75–300.0 µg/mL, $y = 10110x - 236775$, $r = 0.9999$ ($n = 5$).

Luteolin at concentrations 12.5–200.0 µg/mL, $y = 18966x + 76181$, $r = 0.9986$ ($n = 5$).

0.78–12.5 µg/mL, $y = 9578x + 8640$, $r = 0.9970$ ($n = 5$).

Apigenin at concentrations 12.5–200.0 µg/mL, $y = 29731x + 8560$, $r = 0.9995$ ($n = 5$).

0.78–12.5 µg/mL, $y = 33116x + 6177$, $r = 0.9996$ ($n = 5$).

3.5. Precision and accuracy

Comparing three different concentrations of the standard solutions (Table 1), we found the intra-day and inter-day relative standard deviations to range between 0.1–4.5% and 0.3–4.6%, respectively, indicating our HPLC analysis produced consistent and reproducible results. Recoveries all ranged between 91.3% and 98.2%, indicating good accuracy.

3.6. The effect of the different irrigation system on flower components

The traditional ck flowers had the highest contents of chlorogenic acid, myricetin and quercetin, followed by Is 30% and Is 20% flowers. The ck and the Is 30% flowers had the highest contents of luteolin and apigenin, followed by the Is 20% flowers (Table 4).

Table 4. The effect of Arduino module fully automatic irrigation control on important components of *C. indicum* flowers

Treatment	Chlorogenic acid	Mricetin	Quercetin	Luteolin	Apigenin ($\mu\text{g g}^{-1}$)
	(mg g ⁻¹)				
ck	4.06±0.26 ^a	5.78±0.46 ^a	1.00±0.00 ^a	2.68±0.08 ^a	284±0.51 ^a
IS 30%	3.46±0.05 ^b	4.68±0.08 ^b	0.99±0.00 ^b	2.65±0.06 ^a	283±0.35 ^a
IS 20%	3.02±0.06 ^b	4.04±0.10 ^b	0.99±0.00 ^b	2.35±0.07 ^b	281±0.90 ^b
IS 10%	-	-	-	-	-

Values were means \pm S. D. (n=3).

- : no flowering.

Means with different letters in the same column are significantly different ($p < 0.05$) by Tukey test (n=3).

4. Discussion

This study found that the Arduino UNO controlled Is 30% system was much more water efficient than the traditional ck system, that the plants irrigated by either the traditional ck or the Is 30% system produced significantly more flowers with a greater total dry weight of, compared to the other systems. The average flower diameter and average dry weight per blossom of the plants irrigated by the Is 30% system were significantly higher, compared to the flowers irrigated by the other systems. Finally, the traditional ck system produced flowers with significantly higher amounts chlorogenic acid, myricetin and quercetin, compared to the others, while both the ck irrigation system and the Is 30% system produced significantly more luteolin and apigenin.

The agriculture industry is one the largest consumers of the world's water resources [15]. The use of automated irrigation systems can not only make for more effective use of water and reduce water waste but they can also ensure better plant quality and more stable yields [16]. *C. indicum*, a wild species, prefers well-drained soil, has a high drought tolerance and recovers quickly after watering [17,18], explaining its preference for lower water levels and the regular drying out of soil produced by Is 10%, Is 20% and Is 30% systems. This study found that the total amount of water used and the water use efficiency of Is 10%, Is 20%, and Is 30% systems equaled 7%, 10%, and 50%, respectively, of those values achieved by the traditional ck system, meaning they were more water efficient.

The number of flowers that *C. indicum* produces has been found previously to depend on the level and type of irrigation applied [19]. Similarly, we found the flowering to be influenced by irrigation system. We found plants irrigated by Is 20% and Is 30% systems to begin flowering a week earlier than those irrigated by the traditional ck system. The total dry weight of the leaves and average leaf area of plants irrigated by the traditional ck system were significantly higher

than the other groups, indicating that while there was greater plant growth for a longer period of time than the other system, flowering was delayed. With regard to production, both the ck and the Is 30% systems produced significantly higher total number of flowers with a significant higher total dry weight, compared to the Is 10% and 20% systems.

This study found plants irrigated by the traditional ck system had significantly higher levels of chlorogenic acid, myricetin and quercetin, compared to the those irrigated by the other systems. Those irrigated by either the ck or the Is 30% system had significantly higher levels of luteolin and apigenin. Myricetin is an antioxidant and has anti-inflammatory, analgesic, anti-tumor, hepatoprotective and anti-diabetic effects [20]. Apigenin, less toxic than other flavonoids, has a major effect [21]. Luteolin, biochemically either an antioxidant or pro-oxidant, has anti-inflammatory, anti-allergic and anti-cancer effects [22,23]. Quercetin has anti-viral, anti-bacterial and anti-inflammatory, and strong anti-cancer effects [24,25]. Finally, chlorogenic acid, which is one of the most common phenolic acid compounds found in foods and which can be also be extracted from both coffee and tea, is key determinate of tea quality [26,27].

Although the 10% and Is 20% systems made more efficient use of water, the ck and Is 30% systems produced better growth, higher dry weights, and higher concentrations of the important components we studied, suggesting that these two systems can be applied prior to flowering. However, *C. indicum* is currently being studied for its use in leaf tea and essential oils, so future irrigation studies for this plant may want to turn their focus to the effect of the irrigation systems on the vegetative growth stage.

Conclusion

In conclusion, based on the results of this study, although not the most water use efficient of the Arduino Uno-controlled Is systems, Is 30% system was much more water efficient than the traditional ck system, produced more growth and higher dry weights than the other Arduino Uno-controlled Is systems, and nearly as high or higher contents of *C. indicum* components important to production of beverages and the production of pharmaceuticals.

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