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EFFECTS OF DROUGHT STRESS ON GROWTH, PHOTOSYNTHESIS AND ALKALOID ACCUMULATION OF *LYCORIS AUREA*

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Abstract

The effects of drought stress on morphological and photosynthetic characteristics, as well as alkaloid contents were analyzed using potted soil water stress test, aiming to provide a scientific basis for the artificial cultivation of *Lycoris aurea* (*L. aurea*). We found that with the increase of drought stress, the plant growth was restrained and that the increase in fresh weight of the bulb, chlorophyll content and maximum net photosynthetic rate of leaves were decreased. However, the contents of two alkaloids, galanthamine and lycorine in the bulb were increased due to drought stress. Mild water stress caused a maximum increase in contents of both galanthamine and lycorine. These results suggest that for artificial cultivation of *L. aurea*, plants should be irrigated with adequate water during their vegetative growth period to promote their vegetative growth while at the later growth stages plants should be irrigated with mild water deficit to increase their alkaloid contents.

Key words: Drought stress, Growth, Alkaloid, *Lycoris aurea*.

Introduction

Lycoris aurea (L' Her.) Herb (*L. aurea*), a perennial herb plant, has been used as a traditional Chinese medicine (Qian *et al*., 1985). Its bulb is rich in galanthamine, lycorine and other alkaloids (Wang *et al*., 2007), and can be used for the treatments of food poisoning and rheumatoid arthritis etc., especially, it has a good effect for the treatment of galanthamine Alzheimer's disease, polio sequelae, myasthenia gravis and other diseases. Furthermore, it has an inhibitory effect on the release of tumor necrosis factor-alpha (TNF-α) and thus, has important medicinal value (Howes & Houghton, 2003; Yuan & Hu, 2009; Liu *et al*., 2010). Additionally, as a good ground cover plants, its bulbs are also rich in starch, snow lotus, lectin and other active ingredients and thus, it has a wide spectrum of applications in the gardening, industry, agriculture and other fields as well (Ji., 2003).

While galanthamine, lycorine and other alkaloids can be artificially synthesized, their synthesis processes are too complicated. The cost of their synthesis was high, yield was low and quality was poor as well as other issues (Kametani *et al*., 1971; Berkov *et al*., 2004). So far, separation and extraction of these alkaloids from the natural *Lycoris* were still the main ways to obtain alkaloids such as galanthamine (Zhou *et al*., 2016). However, the availability of current wild resources can no longer meet the increasing market demand. Thus, artificial cultivation of *L. aurea* is becoming an effective way to solve the conflict between supply and demand, and to protect its wild resources.

It has been shown that the growth and accumulation of natural products strongly depend on the growing conditions, such as the temperature, light, soil moisture and nutrient supply (Dörr *et al*., 2019; Liang *et al*., 2014; Falk *et al*., 2007). In addition, more severe environmental influences such as various stress conditions, also impact on the metabolic pathways responsible for the synthesis and accumulation of secondary products of plants (Selmar *et al*., 2013). Among the environmental and ecological conditions, water is an important ecological factor affecting the yield and quality of secondary products of medicinal plants. Thus, water stress often affects the photosynthetic physiology, active oxygen balance and osmotic regulation of plants, which are finally reflected by the status of their growth and accumulation of natural products (Han *et al*., 2012; Pan *et al*., 2006; Solařová *et al*., 2016).

At the present, the researches on *L. aurea* and its related species have been mainly focused on such aspects as biological evolution (Chang *et al*., 2009), chemical composition (Yagi *et al*., 1993; Zhao *et al*., 2016), physiological and biochemical properties (Meng *et al*., 2008; Ru *et al*., 2013) and pharmacological effects *etc*. (Evidente *et al*., 2009; Liu *et al*., 2012; Ma *et al*., 2016; Xu *et al*., 2016). The studies on the effects of environmental factors, such as light, soil and other environmental conditions on the growth of *L. aurea* and the accumulation of medicinal ingredients have been rarely reported. *L. aurea* is a widely distributed ecological species, and it prefers humid environment and has no specific restrictions to the water, light and other environmental conditions, thus, it has a strong adaptability and a wide range of distribution (Quan & Liang, 2017). In the recent years, studies have shown that water has a significant impact on the biosynthesis and accumulation of the active ingredients of medicinal plants, and that drought stress can be beneficial to the accumulation of active ingredients, such as alkaloids, in medicinal plants (Jaleel *et al*., 2007; Liu *et al*., 2017). In this study, we analyzed the effects of drought stress on morphological and photosynthetic characteristics, alkaloid contents by using potted soil water stress test, aiming to provide a scientific basis for the artificial cultivation of *L. aurea*.

Materials and Methods

L. aurea experimental materials used in this study were the same *L. aurea* cultivar domesticated in Huaihua, Hunan, China. In mid-July 2016, we selected several bulbs that were as uniform as possible (≈4.2 cm in diameter), weighed and planted three bulbs in each flowerpot (19 cm in height and 20 cm in top diameter) with 3.5 kg garden soil (loam). The agronomic managements for all the pots were the same. At the beginning of September, 40 pots were selected and transferred to the greenhouse. They were randomly divided into 4 groups with 10 pots in each group. Four precipitation gradients were designed to simulate the precipitation in Huaihua City where the mean monthly precipitations from September to December were 72 mm, 108 mm, 51 mm and 34 mm, respectively, as follows: control group (CK, total water supply during the whole treatment period was 640 ml, 100% of natural precipitation), mild water stress (treatment I, 480 mL, 75%), moderate water stress (treatment II, 320 mL, 50%) and severe water stress (treatment III, 160 mL, 25%). The frequency and the duration of water applications were all the same. Water was applied in every 15 d. Water treatment ended in mid-December. Photosynthetic characteristics and alkaloid content were measured in late December as described previously (Quan *et al*., 2012).

During the drought stress treatment period, the soil samples were collected at the end of each month. The soil moisture content of each treatment group was determined by the drying method. Finally, the average value was taken.

The changes in morphological characteristics of plants, such as leaf color, shape, and withering, *etc*., before and after water treatment were observed and monitored. The changes in fresh weight before and after treatment were measured and calculated. The water contents of bulbs after treatment were measured by the drying method (60℃) and calculated. Finally, the average value was taken.

Chlorophyll content (SPAD value) in leaves of *L. aurea* was determined by chlorophyll meter (SPAD-502 Plus). The photosynthesis-light intensity curve (Pn-PAR) was measured by LI-6400 portable photosynthesis system (Li-Cor, USA). The measurement time was set at 9: 00-11: 30 AM, and the natural conditions of temperature and CO_2 concentration were adopted. The artificial light intensities were set as 0, 50, 100, 150, 300, 500, 700, 900, 1100, 1300, 1700, and 2000 μmol • m^{-2} • s^{-1} for a total of 12 gradients. All the measurements were repeated three times and the average values were taken. Then Pn-PAR light response curves were plotted with the light intensity as abscissa and the net photosynthetic rate as ordinate. The light saturation point, light compensation point and maximum net photosynthesis rate were calculated with reference to Bassman and Zwier (1991). The apparent quantum efficiency was calculated from the initial slope of the Pn-PAR curve.

The cloudiness climate was selected and the transient photosynthetic characteristics under different soil moisture treatments were measured at 10:00 AM in the morning in late December. The CO_2 concentration and temperature in the leaf chamber were maintained at 375 μmol • mol^{-1} and 9.5℃, respectively. The transient photosynthetic rate, transpiration rate, intercellular CO_2 concentration and stomatal conductance etc. were measured and recorded. Three pots were selected for each treatment, three leaves were selected from each pot and the average value was taken.

High performance liquid chromatography (HPLC) (Waters 1525, USA) was used to measure the contents of alkaloids in the bulbs of *L. aurea* as described previously (Quan *et al*., 2012) and the optimal chromatographic conditions were established based on the methodological survey in the literature.

The standard solutions of lycorine and galanthamine were precisely prepared at the concentrations of 20.0, 40.0, 60.0, 80.0 and 100.0 μg • mL^{-1}, respectively. Taking concentration (x) as the abscissa and peak area (y) as the ordinate, the linear regression equations of lycorine and galanthamine were formulated as follows: $y = 18069.9529\ x - 9309.1429$, $R^2 = 0.9996$; $y = 9717.7\ x + 12376$, R^2=0.9992, respectively.

Sample preparation was referred to the method of Quan *et al*., (2012). The contents of alkaloids in the bulbs of *L. aurea* under different drought treatments were determined. Three repetitions were set for each treatment group and the average value was taken.

Measured results were averaged with standard error. SPSS and Excel statistical software were applied for processing and analyzing the data. Difference between groups with p<0.05 was regarded statistically significant.

Results

Comparison in soil moisture under different water treatments: Soil water contents were consistently decreased with the severity of water stress (Fig. 1).

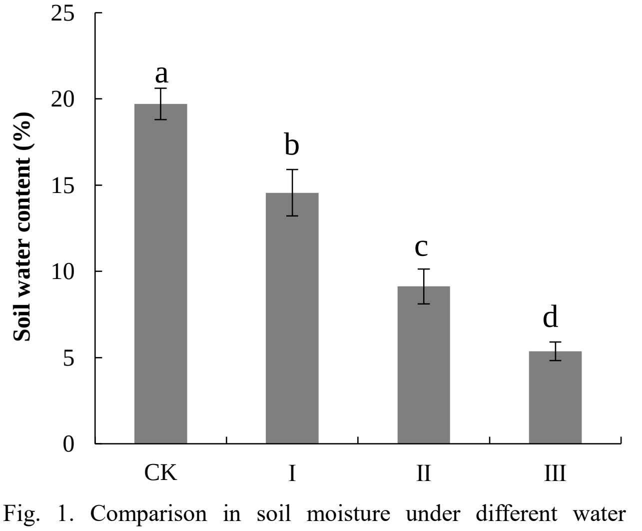

Fig. 1. Comparison in soil moisture under different water treatments.

Comparison of plant morphological characteristics: The morphological characteristics of *L. aurea* under different water treatments were presented in Table 1. There was no significant difference in morphology between mild stress and control treatment. The leaf color was bright and green. The leaf shape was normal and the leaves were rarely withered. Under moderate water stress, some leaves become yellow, curled and withered, indicating that moderate water stress has a significant impact on the growth of *L. aurea* plants. With the increase in the severity of water stress, the plant damage was further aggravated. The leaf color was changed from green to yellow and from yellow to white, most leaves were curled and withered and the bulb moisture content was reduced by 25.53% as compared with that of the control group.

Comparison in the bulb weight between before and after different water treatments revealed that water condition had an effect on the bulb weight of *L. aurea*. Like that of the control group, the bulb weight increased under mild water stress. But with the further increase in the severity of water stress, the bulb weight was decreased in varying degrees.

Comparison in leaf relative chlorophyll content: It can be seen from Table 2, under water stress at different degrees of severity, the relative chlorophyll content (SPAD value) of *L. aurea* leaves was significantly different. With the increase in water stress severity, the SPAD value of leaves of *L. aurea* tended to be decreased obviously, and the SPAD value of *L. aurea* was decreased by as much as 59.96% as compared with that of CK (Treatment III), suggesting that the water stress can reduce the chlorophyll content.

Comparison in leaf light response curve: As shown in Fig. 2, under different water conditions, photosynthetic rate of *L. aurea* was firstly increased with the increase of light intensity and then decreased after reaching the maximum value. Under different light intensities, the photosynthetic rate of CK group was the highest one, while that of severe water stress (Treatment III) group was the lowest one. The photosynthetic rate was decreased gradually with the increase of water stress.

Comparison in leaf photosynthesis-light intensity curve of characteristic parameters: According to the light response curve and the corresponding equation calculation, it was found that the water saturation led to the decrease in different degrees of light saturation point, maximum net photosynthesis rate and apparent quantum yield. But light compensation point and dark respiration rate were increased to varying extends (Table 3). Compared with that of the control (CK), the maximum net photosynthetic rates of mild water stress, moderate water stress and severe water stress were decreased by 29.32%, 57.99% and 70.46%, respectively, indicating that water stress is not conducive to photosynthesis.

Comparison in transient photosynthetic characteristics of leaves: With the reduction of soil water content, transient net photosynthetic rate (Fig. 3-A), transpiration rate (Fig. 3-B) and stomatal conductance (Fig. 3-C) displayed a gradually downward trend with significant differences among treatments as compared with that of the control. The transient net photosynthetic rate, transpiration rate and stomatal conductance were decreased by 76.77%, 86.46% and 73.53%, respectively, under severe water stress. The intercellular CO_2 concentration (Fig. 3-D) showed no significant change among the treatments, and was increased slightly with the severity of drought stress.

Table 1. Comparison in morphological characteristics of *L. aurea* under different water conditions.

Treatment	Leaf color	Leaf shape	Leaf shedding	Bulb water content (%)	Bulb weight increment (g/bulb FW)
CK	Bright green	Normal	None	67.45^a	$9.15±0.30^a$
I	Bright green	Normal	Little	65.81^a	$5.53±0.18^b$
II	Yellow	Partially curled	Partial	58.03^b	$-1.31±0.12^c$
III	Yellowish white	Curled withered	Most	50.23^c	$-9.94±0.27^d$

Different normal letters in the same columns indicate the significant differences at 5% probability level

Table 2. Comparison in relative chlorophyll content (SPAD value) of *L. aurea* under different water conditions.

Water conditions	CK	I	Ⅱ	Ⅲ
SPAD value	$50.2±3.2^a$	$46.7±5.6^{ab}$	$35.8±4.4^b$	$20.1±3.0^c$

Different normal letters in the same columns indicate the significant differences at 5% probability level

Table 3. Comparison in photosynthetic parameters of *L. aurea* under different water conditions.

Treatment	P_{max} (μmol·m^{-2}·s^{-1})	LSP (μmol·m^{-2}·s^{-1})	LCP (μmol·m^{-2}·s^{-1})	R_d (μmol·m^{-2}·s^{-1})	AQY (μmol·m^{-2}·s^{-1})
CK	8.92^a	1152^a	12^{cd}	-0.78^a	0.067^a
I	6.01^b	912^b	16^c	-1.01^b	0.069^a
II	3.27^c	894^b	36^b	-1.02^b	0.029^b
III	1.50^d	506^c	84^a	-1.49^c	0.019^c

P_{max} = maximum net photosynthetic rate; LSP = light saturation point; LCP = light compensation point; R_d = dark respiratory rate; AQY = apparent quantum efficiency. Different normal letters in the same columns indicate the significant differences at 5% probability level

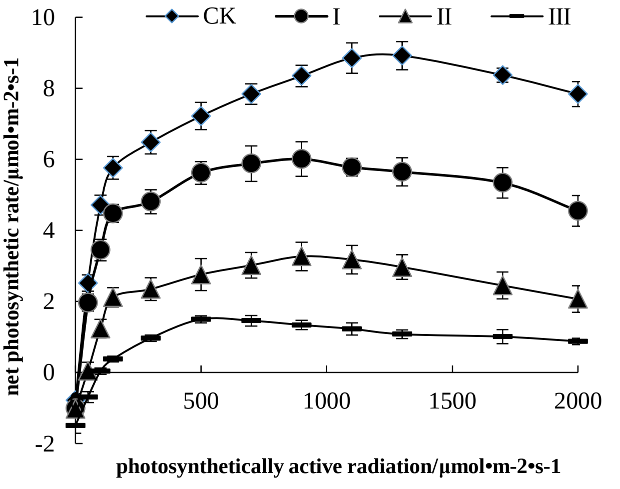

Fig. 2. Light response curves of *L. aurea* under different water conditions.

Chromatographic conditions: Chromatographic conditions were set as follows: Column: Waters XSelect HSST3C18 (5 µm, 4.6 × 250 mm); detection wavelength at 286 nm; mobile phase: 0.1% phosphoric acid in water-methanol, gradient elution with methanol 0-10 min 5%-20 %, 10-30 min 20%-40%, 30-50 min 40%-80%, 50-56 min 80%-100%, column temperature: 25°C, flow rate, 1.0 mL/min; and injection volume, 20 µL. The HPLC chromatograms of the lycorine and galanthamine standards were shown in Fig. 4A, and the retention times were 9.437 min and 11.884 min, respectively. The HPLC chromatogram of the alkaloids in the bulbs was shown in Fig. 4B, among which, the chromatogram peak 1 and peak 3 were lycorine and galanthamine, respectively.

Comparison in content of alkaloids in *L. aurea*: Under different water treatments, contents of the main active ingredients i.e. lycorine and galanthamine were shown in Fig. 5. The contents of lycorine and galanthamine were the highest ones under mild water stress, which were increased by 86.56% and 94.00%, respectively, as compared with that of the control (CK), but were decreased with the increase in the severity of water stress. While under severe water stress, contents of both lycorine and galanthamine were increased, indicating that the effect of soil moisture on the contents of active ingredients of *L. aurea* is significant and that mild water stress is conducive to increase in the contents of galanthamine and lycorine. Compared with those of moderate stress (treatment II), the contents of galanthamine and lycorine were increased slightly under severe water stress (treatment III), this effect may be related to the severe wilting of plants under severe water stress, and the smaller bulbs, resulting in effective relative increase in the composition and other factors.

Discussion

It has been shown that plants exposed to water stress do indeed accumulate higher congents natural products, such as alkaloids, phenols, glucosides, isoprenoids, *etc*. However, water stress also reduces growth and biomass production in most plants (Selmar *et al*., 2013). Thus, the higher contents of natural products could be due to either a stress-related decline in biomass production with an unchanged biosynthesis rate of natural products or an authentic enhancement of the total content of secondary metabolites. In this study, we found that compared with the control, water stress did restrain the plant growth of *L. aurea*, but the decrease in bulb weight was far less than the increase of alkaloids, especially under mild water stress, indicating that appropriate water stress is beneficial to the accumulation of alkaloids of *L. aurea*.

Water supply is an important limiting factor for plant growth and accumulation of natural products. Water shortage induces drought stress-related metabolic responses and, due to stomatal closure, the uptake of CO_2 is decreased significantly (Selmar *et al*., 2013). In addition, with the decrease in chlorophyll content and activities of photosynthetic enzyme, the photosynthetic rate of plants was decreased (Zhang *et al*., 2011; Silva *et al*., 2004). Water supply is an important factor affecting plant growth and biomass accumulation (Mirbahar *et al*., 2009), and this is also verified in our study.

However, the reason why water stress can promote the accumulation of secondary metabolites in medicinal plants is complex (Huang & Guo, 2007). Some studies have shown that certain external conditions can induce the expression of related genes in medicinal plant, thereby affecting the synthesis and accumulation of secondary metabolites (Kazuo S. & Y.S. Kazuko, 1997). For example, Lv *et al*., (2019) indicated that mild and moderate water stress promoted the expression levels of genes related to anthocyanin biosynthesis in grape, thus increasing the mass fraction of total anthocyanin in grape berries. Some studies have indicated the plant growth is always restricted under drought stress, and a large number of photosynthetic products accumulate within the body. Plants can use these excess photosynthetic products to synthesize secondary compounds so as to resist adverse environmental conditions (Huang & Guo, 2007). Other studies have indicated that under water deficiency conditions, the consumption of reduction equivalents (NADPH $+H^+$) for CO_2 fixation via the Calvin cycle is declined considerably. Then metabolic processes are shifted towards biosynthetic activities that consume reduction equivalents. As a consequence, the synthesis of isoprenoids, phenols or alkaloids, is enhanced (Selmar *et al*., 2013). Some or all of the above factors may contribute to a maximum increase in contents of galanthamine and lycorine of *L. aurea* under mild water stress. However, more in-depth research is needed to explore this aspect.

In summary, in this study, we found that water stress restrained the growth and photosynthesis of *L. aurea*, while mild water stress caused a maximum increase in contents of both galanthamine and lycorine. These results suggest that for artificial cultivation of *L. aurea*, plants should be irrigated with adequate water during their vegetative growth period to promote their vegetative growth while at the later growth stages plants should be irrigated with mild water deficit to increase their alkaloid contents.

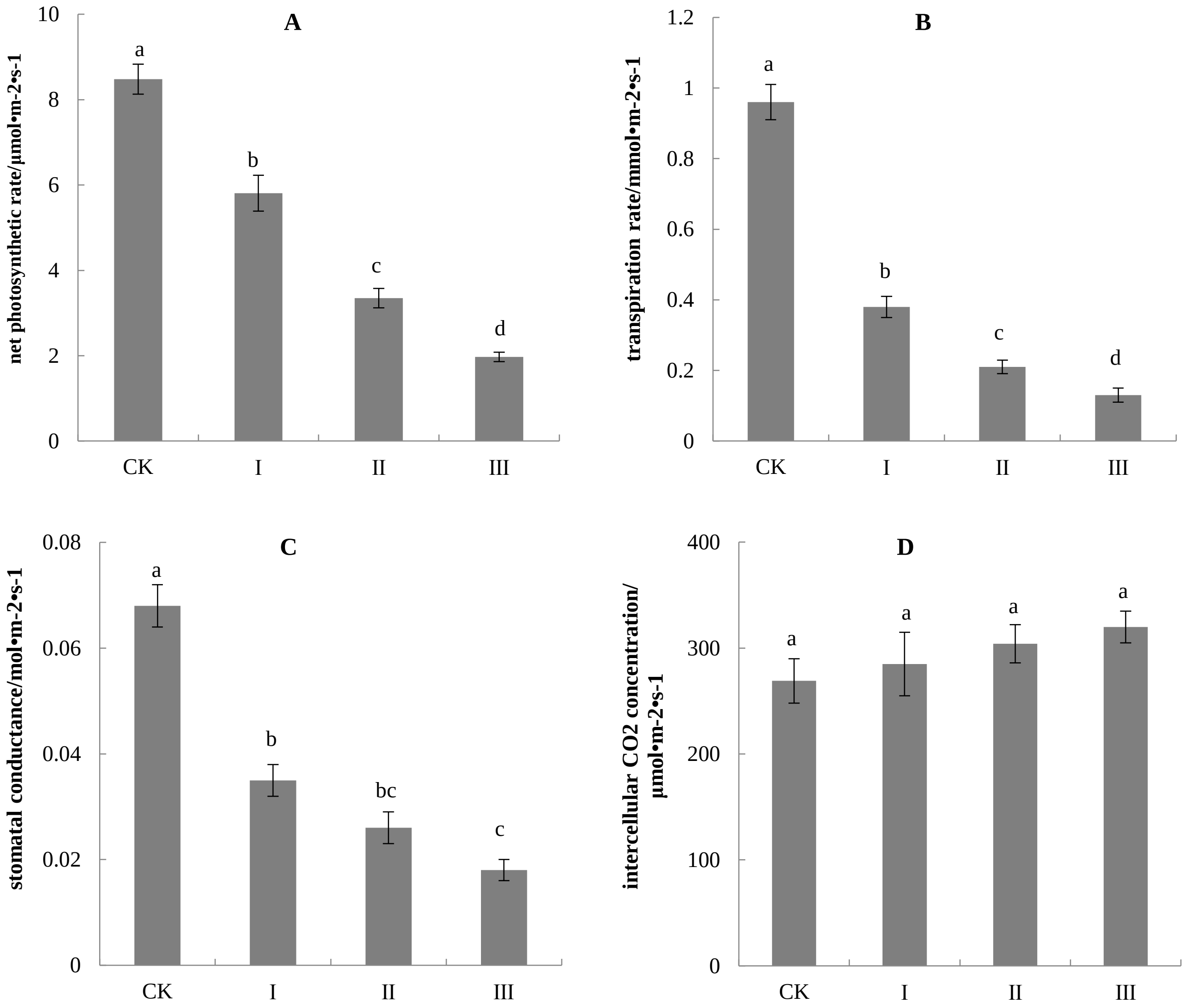

Fig. 3. Comparison in net photosynthetic rate (A), transpiration rate (B), stomatal conductance (C) and intercellular carbon dioxide concentration (D) of *L. aurea* under different water conditions.

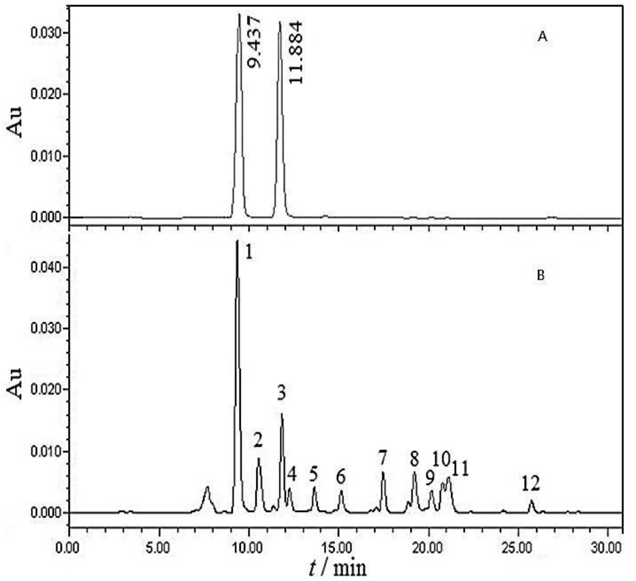

Fig. 4. HPLC chromatogram of lycorine and galanthamine standards (A) and alkaloids in *L. aurea* bulbs (B).

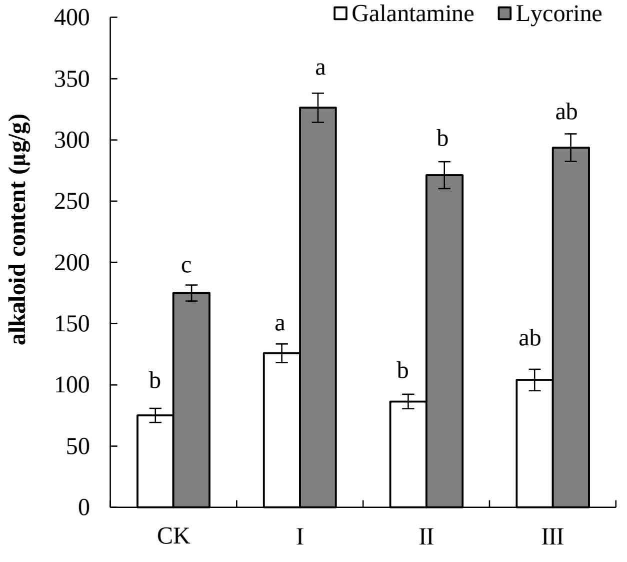

Fig. 5. Comparison in contents of galanthamine and lycorine in *L. aurea* bulbs under different water conditions.

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