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Effects of thiamine spraying on biochemical and morphological traits of basil plants under greenhouse conditions

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ABSTRACT

Thiamine, as a co-enzyme required to develop many of the metabolic reactions, plays an important role in regulating growth and reducing the effects of environmental stresses on plants. In this project the effect of thiamin (Thi) applied as foliar spray on some morphological and physiological traits was investigated in basil (*Ocimum basilicum* L.). Four levels of Thi including 0 (distilled water), 250, 500 and 750 µM were tested in a randomized complete design with three replications at research greenhouse of University of Guilan, Iran. The results show that Thi application exerted a significant effect on the most studied indices. The amounts of leaf, stem and root dry weights in Thi-treated plants was more than control. Thousand grain weight increased by an increase in Thi concentration, as, its value in 700 µM Thi, was 26% more than control. Thi spraying especially in the concentration of 750 µM improved the leaf nutrients content (N, P and Ca). Chlorophyll b content in leaves affected positively by Thi, and its highest value obtained in 750 µM treatment. The highest and the lowest phenolic compounds were gained in 0 and 750 µM levels of Thi, respectively. A similar trend was observed for total antioxidants, where their content in 750 µM was 13% more than control treatment. Generally, it seems that Thi can be considered as an appropriate growth regulator in basil medicinal plant cultivation.

INTRODUCTION

Thiamine (Thi) or vitamin B_1 is a plant growth regulator required for growth and differentiation of some plant species (Kaya et al., 2015). It has a mitigating effect on environmental stresses and acts as a coenzyme in various metabolic pathways such as sugar and protein metabolism and decarboxylation of α-keto acids, such as pyruvic acid and glutamic acid (Goyer, 2010; Tuna et al., 2013). It also improves the antioxidative defense system and is an important cofactor for the transketolation reactions of the pentose phosphate cycle (Tune et al., 2013). More recently, it has been implicated in tolerance to DNA damage and as an activator of disease resistance in plants (Raschke et al., 2007). In some plant species roots can synthesize Thi, but of other plants cannot synthesize this vitamin (Tune et al., 2013). Thi biosynthesis occurs in the chloroplast of plants. In cells, it exists as three predominant vitamers, namely free Thi, Thi pyrophosphate and Thi monophosphate, although adenylated and triphos-phorylated forms also exist. The cofactor form of the vitamin is Thi pyrophosphate, which is necessary for the activity of enzymes involved in Krebs and Calvin cycles as well as acetyl-CoA and amino acid biosynthesis (Bettendorff et al., 2007; Pourcel et al., 2013).

Thi sprays maintains a larger number of green leaves and, therefore, enhances canopy photosynthesis and hence larger accumulation of photosynthetic compounds during grain filling (Sahu et al., 1993). The results of Abdel-Monaim (2011) in soybean revealed that Thi can improve the vegetative and reproductive growth, and also may play an important role in controlling the charcoal rot disease. So far, the exogenous application of Thi in some plants such as *Lupinus termis* (El-Awadi et al., 2016), *Vicia faba* (Hamada & Khulaef, 2000), *Gladiolus grandifloras* (Sajjad et al., 2015), *Matricaria recutita* (Ranjbar et al., 2014), *Thymus vulgaris* (Reda et al., 2005), and *Calendula officinalis* (Soltani et al., 2015) has shown the progress in growth and development. However, the impact of the foliar application of this vitamin on basil as a main aromatic plant has not been investigated previously in scientific researches.

Basil is an herbaceous medicinal plant from the Lamiaceae family with a wide range of applications in culinary, cosmetic, food, perfumery and medical industries. This plant originated from southern Asia and is cultivated extensively in many countries such as Iran, Egypt, Hungary, France, Indonesia, Greece and Morocco (Bahl et al., 2000; Fallahi et al., 2015). The presence of more than 200 chemicals includes flavonoids, monoterpenes, sesquiterpenes, triterpenes and aromatic compounds in basil oil have been identified. The main components of its oil are eugenol, methyl eugenol, linalool, estragole and anethole, varying by chemotype (Li & Chang, 2016). Regarding the stimulatory role of vitamins in plants growth and development and the lack of comprehensive studies on the effect of these bio-regulators on basil, the aim of this study was to evaluate the effects of Thi foliar application on some vegetative and reproductive growth, physiological and biochemical traits of basil.

MATERIALS AND METHODS

In this study, the effects of foliar application of four concentrations of thiamin (Thi) (0 or distilled water, 250, 500 and 750 µM) was investigated on growth, pigment and nutrients content and some biochemical indices of purple basil medicinal plant. For this purpose, a pot experiment was carried out at research greenhouse of University of Guilan, Iran, based on a randomized complete design with three replications. Seed sowing was done in 6th January, in the pots with the 33 and 22 cm in diameter and height, respectively. The seed bed was a mixture of soil (Table 1), cow manure and sand with the ratio of 2:1:1. Except for cow manure, no fertilizer was used during the plant's growth. The spraying of thiamin was started from the four-leaf stage and done once every two weeks in the morning, up to the early May. In each spraying date, 20-25 ml of related solution was used per pot, so that, the plants were completely wet. Irrigation was done by intervals of 10 days and plant thinning was performed 20 days after emergence, so that, 6 plants were kept in each pot. The climatic condition of the greenhouse during the plant growth cycle has been shown in (Table 2).

In 4th May, three plants were harvested in each pot for measurement of some vegetative traits including plant height, number of lateral branches, leaf fresh weight, leaf dry weight, root dry weight, main stem diameter (by a ruler with a precision of one millimeter) and stem dry weight. The remained three plants were used to determine the number of florets and thousand seed weight, on May, 28. The pigments (carotenoid, chlorophyll a and chlorophyll b) and nutrients (nitrogen, phosphorous and calcium) content of leaves were measured by the methods of (Minguez-Mosquera & Prez-Galvez , 1998) and (Jones et al., 2001), respectively. The method of (Bakhshi & Arakava, 2006) was used for leaf extraction to determine phenolic compounds and antioxidant capacity. Then, phenols were analyzed by using the method of Folin-Ciocalteau reported by (Tavarini et al., 2008). For this purpose, the absorbance at 760 nm was measured using a spectrophotometer (T80+PG Instrument UV/Vis Spectrometer) and the values were expressed as mg gallic acid/100 g fresh weight. In addition, antioxidant capacity was determined using DPPH free radical scavenging method, which has been described by (Sanchez-Moreno et al., 1999). All chemical indices were determined during vegetative growth stage at the fruit ripening stage. Finally, the experimental data were statistically analyzed using SAS program ver. 9.2 and means were separated via Tukey's HSD (honest significant difference) test at 5% level of probability.

Table 1. Chemical properties of soil used in pots for basil cultivation

Organic carbon (%)	Nitrogen (%)	Calcium (%)	Phosphorous (%)	Potassium (%)	pH	Texture
1.2	1	0.48	0.19	0.55	7.1	Loam Sandy

Table 2. The main climatic condition of greenhouse during basil growth

Day temperature (˚C)	Night temperature (˚C)	Day length (Hrs)	Night length (Hrs)	CO_2 concentration (ppm)	Relative humidity (%)
25	15	16	8	350	40

RESULTS AND DISCUSSION

Vegetative and reproductive growth

Thi foliar application had a significant effect on dry weights of leaf, stem and root and 1000-grain weight of basil (Table 3). Leaf fresh yield increased along with increasing Thi concentration, so that, reached to its maximum of 750 µM, but, there was no significant different between control and Thi spraying at a concentration of 250 µM. The highest leaf, stem and root dry weights were gained at the level of 500 µM, which were significantly 27, 23 and 18% more than control, respectively. However, there was no significant different between two other levels of Thi and control treatment. The maximum of one-thousand-grain weight was gained at the concentration of 750 µM, which was significantly 25% more than control. The differences between control and two other concentrations of Thi were not significant in terms of 1000-grain weight (Table 4). Our findings of the positive effect of Thi on the reproductive and vegetative growth of basil is similar to those obtained on *Vicia faba* by (Hamada & Khulaef, 2000), on *Oryza sativa* by Bahuguna et al. (2012) and on *Lupinus termis* by El-Awadi et al. (2016). Increase in growth and seed production in basil by Thi foliar application, may be related to its role in dividing meristematic stem cells and organ initial cells (Martinis et al., 2016).

In addition, Thi can increase the accumulation of some osmoregulators in plant tissues which can impose an impact on water potential and consequently can increase the turgor pressure which is needed for cell expansion and thereby plant growth (Sayed & Gadallah, 2002). It has also been reported that the better growth and yield of treated plants by Thi is mainly related to appropriate regulation of photosynthesis and energy-providing reactions (Goyer, 2010; Martinis et al., 2016). Kaya et al. (2015) found that growth improvement in Thi-treated plants is associated with reduced membrane permeability, malondialdehyde and H_2O_2 levels, and altered activities of antioxidant enzymes such as catalase, superoxide dismutase and peroxidase as well as increased photosynthetic pigment concentration and PSII activity (Kaya et al., 2015). In a similar study on maize it was noted that foliar applied of Thi (100 ppm) increased leaf area index, number of green leaves and delayed leaf senescence. In addition, the biological and grain yields increased by Thi, that resulted from increased photosynthetic efficiency and canopy photosynthesis. In that study, it proposed that enhancement in grain yield with Thi spray is due to SH-enhanced photosynthetic efficiency since Thi is an incipient thiol and has cytokinin-like activity (Sahu et al., 1993).

Table 3. Mean squares for the effect of different levels of thiamin on vegetative and reproductive indices of basil

Source of variation	df	Plant height	Leaf fresh weight	Leaf dry weight	Root dry weight	Stem dry weight	Diameter of main stem	Number of lateral branches	Number of florets	1000-grain weight
Treatment	3	155.22^{ns}	2.20*	0.43*	0.07**	0.08*	0.93*	0.66^{ns}	2.00^{ns}	0.15**
Error	8	46.25	0.29	0.07	0.004	0.01	0.19	0.33	1.50	0.01
CV	11	13.03	3.82	13.70	4.57	7.58	13.74	17.32	11.13	7.33

*, ** Significant at 5%, and 1% probability level, respectively.

Table 4. Means comparison for the effect of different levels of thiamin on some vegetative and reproductive growth parameters of basil

Thiamin con. (μM)	Plant height (cm)	Leaf fresh weight ($g.plant^{-1}$)	Leaf dry weight ($g.plant^{-1}$)	Root dry weight ($g.plant^{-1}$)	Stem dry weight ($g.plant^{-1}$)	Diameter of main stem (mm)	Number of lateral branches	Number of floret	1000-grain weight (g)
0	41.66[a]†	13.02[b]	1.7[b]	1.36[b]	1.33[b]	2.62[b]	2.66[a]	10.00[a]	1.38[b]
250	53.33[a]	13.98[ab]	1.52[b]	1.30[b]	1.60[ab]	3.94[a]	3.33[a]	11.00[a]	1.80[ab]
500	56.66[a]	14.77[a]	2.35[a]	1.65[a]	1.73[a]	3.15[ab]	3.66[a]	12.00[a]	1.83[ab]
750	57.00[a]	14.87[a]	2.13[ab]	1.49[ab]	1.58[ab]	2.97[ab]	3.66[a]	11.00[a]	1.86[a]

†Means with the same letter(s) within a column are not significantly different ($P \leq 0.05$) based on Tukey's test.

Table 5. Mean squares for the effect of different levels of thiamin on leaf nutrient and pigments content and some biochemical parameters in basil

Source of variation	df	Chlorophyll a	Chlorophyll b	Carotenoid content	Leaf nitrogen content	Leaf phosphorous content	Leaf calcium content	Total antioxidants	Total phenol
Treatment	3	0.01[ns]	0.01*	0.004[ns]	0.58**	0.001**	0.04**	7.88*	24.86**
Error	8	0.006	0.001	0.001	0.04	0.0002	0.005	1.31	2.62
CV	11	6.93	5.37	9.80	6.91	4.26	6.76	3.41	3.67

*, ** Significant at 5%, and 1% probability level, respectively.

Pigments and nutrients content

Thi exerted a significant effect on the leaf nitrogen, phosphorous and calcium content (Table 5). There was an increasing trend by an increase in Thi concentration in terms of all nutrients content. Leaf nitrogen increased by 12, 22 and 28%, when the concentration of Thi enhanced from 0 to 250, 500 and 750 μM, respectively. However, the difference between 750 μM of Thi and control was only statistically significant in terms of leaf nitrogen content. Similarly, Thi at a concentration of 750 μM promoted significantly the leaf phosphorus percentage. The amount of leaf calcium also showed a positive response to Thi foliar application, so that, it's value in the level of 750 μM was 22% higher than control treatment. Overall, application of Thi in the concentration of 750 μM had a significant difference with control, while two other levels of Thi were in a similar significant group with control, although they were higher than control (Fig. 1). In a similar study, Thi application significantly reduced the concentration of Na^+ but increased those of N, P, Ca^{2+} and K^+ in corn (Kaya et al., 2015). In another report, Thi spraying on *Thuja orientalis* increased the percentage of N, P and K (Abdel Aziz et al., 2010). Better root expansion in Thi-treated plants (Table 4) may be a reason for better absorption of nutrients from the soil. Moreover, Thi may protect cell membranes and their binding transporter which leads to more absorption and translocation of minerals (Sayed & Gadallah, 2002; Mady, 2009). In addition, increase in nutrients solubility in the rhizosphere of vitamin-treated plants through the secretion of organic acids into the soil is another reason for more nutrients uptake by the plant (Abd- El Aziz et al., 2007).

The content of chlorophyll b in leaves of basil was also affected significantly by Thi foliar application (Table 5) and its maximum value obtained when Thi was sprayed at a concentration of 750 μM (Fig 2). Similar results were obtained by (Hamada & Khulaef, 2000) in *Vicia faba*, where spraying of 100 ppm Thi enhanced the chlorophyll content and consequently net photosynthesis. In the same way, application of Thi in Calendula increased the content of carotenoids and chlorophyll a and b (Soltani et al., 2015). (Sayed & Gadallah, 2002) also in accord with our results reported that Thi-treated sunflower plants had much more chlorophyll. Thi acts as a precursor of thiamine diphosphate, which serves as a coenzyme in many metabolic pathways such as plant pigment biosynthesis (Tuna et al., 2013). Vitamins have an antioxidant scavenging effect which prevents chlorophyll degradation by the reactive oxygen species (ROS) (Mady, 2009). Chloroplast is the principal cellular organ for generation of ROS, and this may result in degradation of thylakoid membranes and photosynthetic pigments. Thi application counteracts the inhibitory effect of ROS on chlorophyll contents (Kaya et al., 2015). The promotion of carotenoids synthesis - as a protector of chlorophyll against oxidation- by Thi is another reason for the increase in chlorophyll concentration in plants treated by Thi (Farouk et al., 2012). Moreover, overexpression of 2-deoxy-D-xylulose-5- phosphate enzyme which is strongly dependent on Thi-diphosphate, correlates with the accumulation of chlorophyll (Rapala-Kozik et al., 2012).

Total phenol and antioxidants

The content of total phenolic compounds and total antioxidants were affected significantly by the foliar spraying of Thi (Table 5). The highest and the lowest amounts of total phenols were obtained in plants that were treated with distilled water and Thi at a concentration of 750 µM, respectively (Fig. 3). Thi also had an increasing effect on the content of antioxidants and the highest antioxidant capacity was obtained at the highest level of Thi spraying, which was 11% more than control (Fig. 4). However, the differences between all levels of Thi were not significant in terms of total phenols and total antioxidants content (Fig. 3) and (Fig. 4). Kaya et al. (2015) in a study on maize reported that Thi modulates plant antioxidant potential thereby improving salt tolerance. Mansouri et al. (2015) also concluded that exogenous application of Thi increased antioxidant enzyme activities in *Gerbera jamesonii*. These observations may be due to the role of Thi in various metabolic pathways such as sugar and protein metabolism, photosynthesis and cellular respiration (Goyer, 2010; Tuna et al., 2013). Tunc-Ozdemir et al. (2009) found that Thi stimulates the antioxidant defense and consequently protects plant cells against oxidative stress (2009). Moreover, Thi itself can also partly act as an antioxidant to scavenge O_2^- (or OH) and thus affect the cellular response to oxidative stress (Jung & Kim, 2003). Results of Abdel-Monaim et al. (2011) in soybean showed that activity of antioxidant enzymes, including peroxidase, polyphenol oxidase and phenylalanine ammonia lyase were increased in plants treated with the Thi and riboflavin, compared with the control. In a study on Thyme the application of bio-regulators and vitamins such as Thi affected positively the essential oil content and its constituents, phenolic compounds and their components as well as polyphenol oxidase activity (Reda et al., 2005).

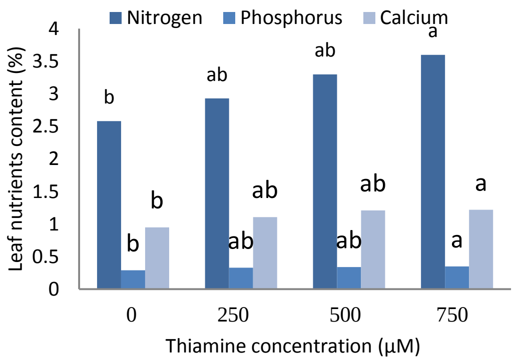

Fig. 1. Effect of different levels of thiamine on leaf nutrients content of basil

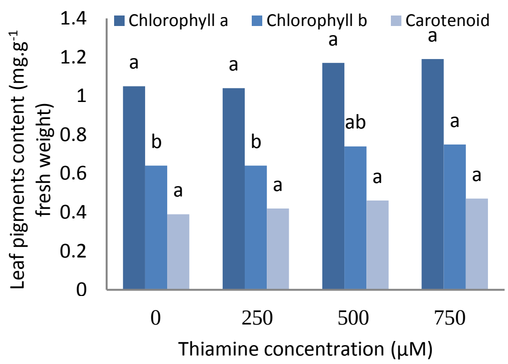

Fig. 2. Effect of different levels of thiamine on leaf pigments content of basil

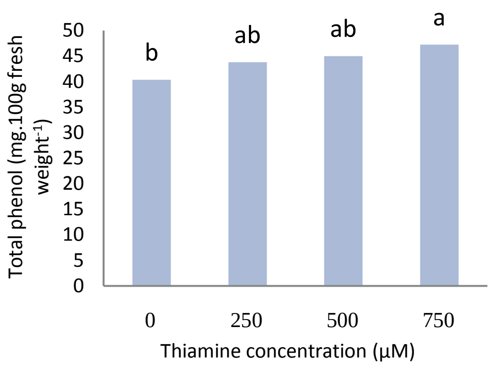

Fig. 3. Effect of different levels of thiamine on total phenolic compounds of basil

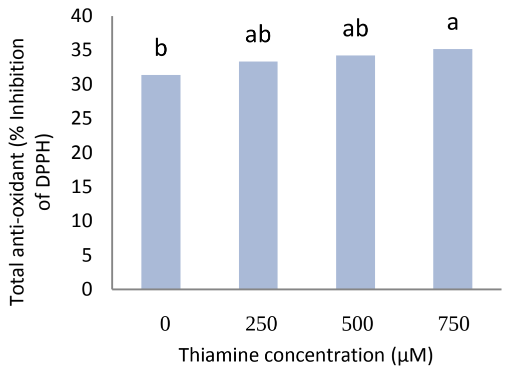

Fig. 4. Effect of different levels of thiamine on total antioxidant content of basil

CONCLUSION

Basil has many nutritional and medical values because it is rich in phenolic compounds, polyphenols such as flavonoids and anthocyanin and has a wide range of essential oils. Therefore, any practice such as the foliar application of bio-regulators that lead to an increase in the important compounds of this plant can be useful nutritionally and economically. Results of the current study revealed that foliar application of Thi can increase the growth and yield of basil through the increase in pigments content, enhancement of nutrients absorption by the root and improvement of plant antioxidant defense system. Although there were no significant differences between three levels of Thi in terms of many studied traits, its application especially at concentration of 750 µM had a significant different with the control treatment about leaf fresh yield, seed weight, leaf nutrient (N, P and Ca) and chlorophyll b content as well as the amounts of total antioxidant and phenolic compounds. We propose to evaluate the effects of Thi applied to seed soaking or simultaneous seed-soaking and foliar application in coming studies.

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تاثیر محلول‌پاشی تیامین بر خصوصیات بیوشیمیایی و مرفولوژیکی گیاه دارویی ریحان در شرایط گلخانه

حمیدرضا فلاحی ، محمدحسین امینی فرد و عباس جورکش

چکیده

تیامین به عنوان یک کوآنزیم مورد نیاز برای پیش‌برد بسیاری از واکنش های متابولیکی، نقش مهمی در تنظیم رشد و کاهش اثرات تنش‌های محیطی در گیاهان دارد. در این پژوهش تأثیر محلول‌پاشی سطوح مختلف تیامین بر برخی خصوصیات مرفولوژیکی و فیزیولوژیکی ریحان (*Ocimum basilicum* L.) مورد بررسی قرار گرفت. برای این منظور چهار سطح تیامین شامل صفر (آب مقطر)، ۲۵۰، ۵۰۰ و ۷۵۰ میکرومول در قالب طرح کاملاً تصادفی با سه تکرار در گلخانه تحقیقاتی دانشگاه گیلان مورد مقایسه قرار گرفتند. نتایج نشان داد که کاربرد خارجی تیامین اکثر صفات مورد مطالعه را به طور معنی‌داری تحت تأثیر قرار داد. مقادیر وزن خشک برگ، ساقه و ریشه در گیاهان تیمار شده با تیامین بیشتر از گیاهان تیمار شاهد بود. وزن هزار دانه با افزایش غلظت تیامین افزایش پیدا کرد، به طوری که، مقدار آن در سطح ۷۰۰ میکرومولار تیامین در مقایسه با تیمار شاهد ۲۶ درصد بیشتر بود. مصرف تیامین به‌خصوص در غلظت ۷۵۰ میکرومول محتوای نیتروژن، فسفر و کلسیم گیاه را افزایش داد. محتوای کلروفیل b برگ از مصرف تیامین تأثیر مثبت پذیرفت و بیشترین مقدار آن در سطح ۷۵۰ میکرومول به دست آمد. کمترین و بیشترین مقدار ترکیبات فنولیکی به‌ترتیب در تیمارهای شاهد و محلول‌پاشی تیامین با غلظت ۷۵۰ میکرومولار مشاهده شد. روند مشابهی نیز در مورد محتوای کل ترکیبات آنتی‌اکسیدانتی مشاهده شد، به طوری‌که مقدار آن‌ها در سطح ۷۵۰ میکرومولار تیامین حدود ۱۳ درصد بیشتر از تیمار شاهد بود. در مجموع، به نظر می‌رسد مصرف خارجی تیامین به عنوان یک تنظیم کنندهٔ رشد گیاهی می‌تواند در زراعت گیاه دارویی ریحان سودمند باشد.

کلمات کلیدی: آنتی‌اکسیدان کل، ترکیبات فنولیکی، تنظیم کننده رشد، کلروفیل، محتوای نیتروژن برگ