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Research Article

Antioxidant potential of lettuce treated by Thymol as an allochemical

Abstract

Introduction: Thymol is a phenolic compound with monoterpenes nature (C10H14O) which is one of the main secondary metabolites of the genus of Thymus in Lamiaceae family.

Methods: In this study, antioxidant capability changes, antioxidant compounds level and the total free amino acids of Lettuce were investigated under different levels of Thymol (0.0 and 0.05 mg/ml) treatment.

Results: The results showed that the amount of flavonoids and anthocyanins had drastic decrease with increase of Thymol. The decrease was 15% and 14% in comparison with control group, respectively. On the other hand, the amount of free amino acids increased by 26.47% increase. Antioxidant capacity of lettuce exhibited a drastic reduction under treatment with Thymol, this reduction was 645% than control.

Conclusion: It was concluded that thymol, as an allochemical can be tend to reduce antioxidant potential of lettuce.

Introduction

Thymol is a phenolic compound with monoterpenes nature. Numerous evidences and reports exist in relation with significant biologic activities on this compound. Thymol is one of the main components of Lamiaceae family essence. Previous studies revealed significant allelopathic effects in thymol compounds. This effect may result in allelo-chemical stress in plants [1].

Today, it has been proven that plants' homeostasis would be destroyed under stress and toxic compounds would be agglomerated inside plant's cells [2]. Among these compounds, reactive oxygen species (ROS) can be mentioned. These compounds are potentially harmful when aggregated in the cells. ROSs can severely react with biomolecules such as lipids, proteins and nucleic acids resulting in lipid peroxidation, protein denaturation, and DNA mutation which can lead to disruption of natural metabolism of plant and finally its death [3].

Antioxidant systems of plants' tissues includes ROS scavenging compounds such as flavonoids, anthocyanins, ascorbate, caretoids , antioxidant enzymes such as catalase, superoxide dismutase, peroxidase enzyme detoxifying products of lipid peroxidation including glutathione S- transferase, ascorbate peroxidase [3,4].

In this study, the impact of thymol compounds on antioxidant capacity and also quantity of some antioxidants (flavonoids and anthocyanins) were investigated in lettuce as a model plant for allelopathic studies.

Materials and Methods

Plant cultivation and treatments

After germination of seeds, they were divided into two groups. Control and treatment groups (treated with 0.1 and 0.05 mg/ml of thymol) were separately transferred to peat-containing pots. They were then placed into germinator for 37 days under 6000 Lux illumination and 80% humidity. Control group plants were daily irrigated by Hoagland solution; the treatment group plants, in addition to Hoagland solution, were also irrigated by thymol (0.1 and 0.05 mg/ml), 10 ml/day for each pot. Plants were cultivated up to 7-leaf stage and then they were harvested and the related tests were performed on them.

Measurement of free amino acids

For measuring the concentration of free amino acids, samples were extracted and homogenate in 0.05 molar phosphate buffer solution (pH-6.8). After centrifugation for 20 minutes at 3000 rpm, ninhydrin (350 mg ninhydrin in 100 ml ethanol) identifier was added to supernatant samples in ratio of 1:5. Then it was placed in 70-100°C temperature for 4-7 min.

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after cooling, its absorption was read in the wavelength of 570 nm. Different concentrations of glycine were used for plotting of standard curve [5,6].

Measurement of UV-absorbing compounds: these compounds include flavonoids and anthocyanins:

Anthocyanin evaluation

0.5 g wet leaf sample was ground by acidic methanol (1:99) and the extract was placed in darkness for 24 h (temperature was 25 °C, then it was centrifuged for 10 min at the speed of 4000 rpm and the absorption of supernatant was measured at the wavelength of 550 nm. To calculate concentration, A= εBC was employed in which ε was considered 33000cm/M, B was the width of spectrocell and equals to 1 and C denotes complex concentration in $\mu g.g^{-1}FW$ [6].

Flavonoids measurement

Flavonoids level was measured by aluminum chloride colorimetry. 0.2 g of the samples was extracted in 10 ml methanol. Distilled water was added to 0.5 ml of the obtained extract to reach to volume of 5 ml. then 0.3 ml $NaNO_2$ (5%)was added, after 5 min 0.5 ml $AlCl_3$ was added. Finally, 2 ml of 1 molar NaOH and 2 ml distilled water was added and absorption intensity was evaluated at wavelength of 510 nm. Samples' concentration was obtained by quercetin standard curve [7].

Antioxidant activity evaluatioin

0.08% concentration of DPPH was prepared by methanolic solvent at concentrations of 0.125, 0.25, 0.5, 1 and 2 of methanolic extract and 0.1 and 0.5 of control samples of lettuce plant were prepared. 5 cc DPPH were added to each 10 cc of extract and after 30 min, the absorption was read at 517 nm. Then RC_{50} index was calculated using standard curve of DPPH reduction (%) to extract concentration [8]. RC_{50}: is regarded as a concentration of extract that reduce 50% DPPH. DPPH reduction (%) was calculated from following formula:

%R=AD-AS/ AD× 100

AD: DPPH absorption at 517 nm

AS: Absorption extracts at 517 nm

Results

The results indicated that potential of free radical scavenging of lettuce seedling reduced under thymol in a dose dependent manner. Whereas, RC_{50} of the treatment group was 4.30 and 4.78 mg ml^{-1} in two thymol treatment levels, respectively, the control group one was 0.73 $mgml^{-1}$. Thus, the antioxidant potential in treatment groups is 6 times less than untreated plants (Figure 1A).

With the increasing of thymol concentration on lettuce, the level of flavonoids and anthocyanins-decreased to 10 and 17 %, respectively (Figure 1B,C). On the other hand, free amino acids content increased by 26.74% thymol treated lettuce group than control one (Figure 1D).

Discussion

Antioxidant capability of plants to face with oxidative stress due to free radicals, especially ROS, is of crucial importance. This oxidative stress in some cases is caused by a secondary stress such as salt, drought or even allelo-chemical stress [9]. In some plants, antioxidant activities could be due to existence of unknown compounds or synergic interactions between different materials. Regarding their performances, antioxidants are classified into two main groups: primary and secondary antioxidants [10]. Primary antioxidants give their electron or hydrogen to free radicals while secondary ones act as an assistant; this means that they act by donating hydrogen and recovery of primary antioxidant or oxygen scavengers and chelation agents [11].

In addition to flavonoids, saponins, phenols and anthocyanins are among the famous herbal antioxidant. Every plant has vast range of different phenolic compounds and antioxidant properties of each of these materials depend on their chemical structure. For example, flavonoids antioxidant activity would increase with the increasing of hydroxyl groups substituted on B ring [12].

In this study, the level of flavonoids and anthocyanins decreased in lettuce treated with thymol which resulted in drastic decrease of plant's antioxidant activity. In a way that 6-fold reduction can be observed in this parameter in DPPH test. On the other hand, increase of free amino acids can be seen in aero-organs of treated plants which could be a resistive response to thymol treatment. It seems that in thymol-induced stress, plants increased osmolites such as amino acids to enhance the antioxidant capacity. Previous studies on other plants mentioned increase of amino acids such as proline in many biological stresses. This trend is to integrate the membrane and avoid denaturation of proteins, amino acids such as proline interact with enzymes and maintains the protein structure and their activities [13]. It can be expressed that this stress would result in deviation of metabolism path toward production of amino acids, especially those biosynthesized in shicomate path (I.e. tryptophan). This path is close to biosynthesis of flavonoids and anthocyanins.

In this study, plants extraction was performed by methanol and ethanol as their application for *Lactuca sativa L* extraction showed more power for extracting phenolic compounds and the obtained extracts had more inhibition ability [14].

DPPH methanolic solution interacted with antioxidants or other proton-donating radicals such as phenol and flavonoids and turned into its non-radical form. Finally, its amount will be reduced and the color changed from dark violet to light yellow. Therefore, the absorption at 517 nm will be reduced. Therefore, the higher amount of remaining DPPH absorption reflects lower antioxidant activity and free radical removal.

Reduction in antioxidant ability of *Lactuca sativa* L could be due to decrease of flavonoids and anthocyanins by thymol treatment.

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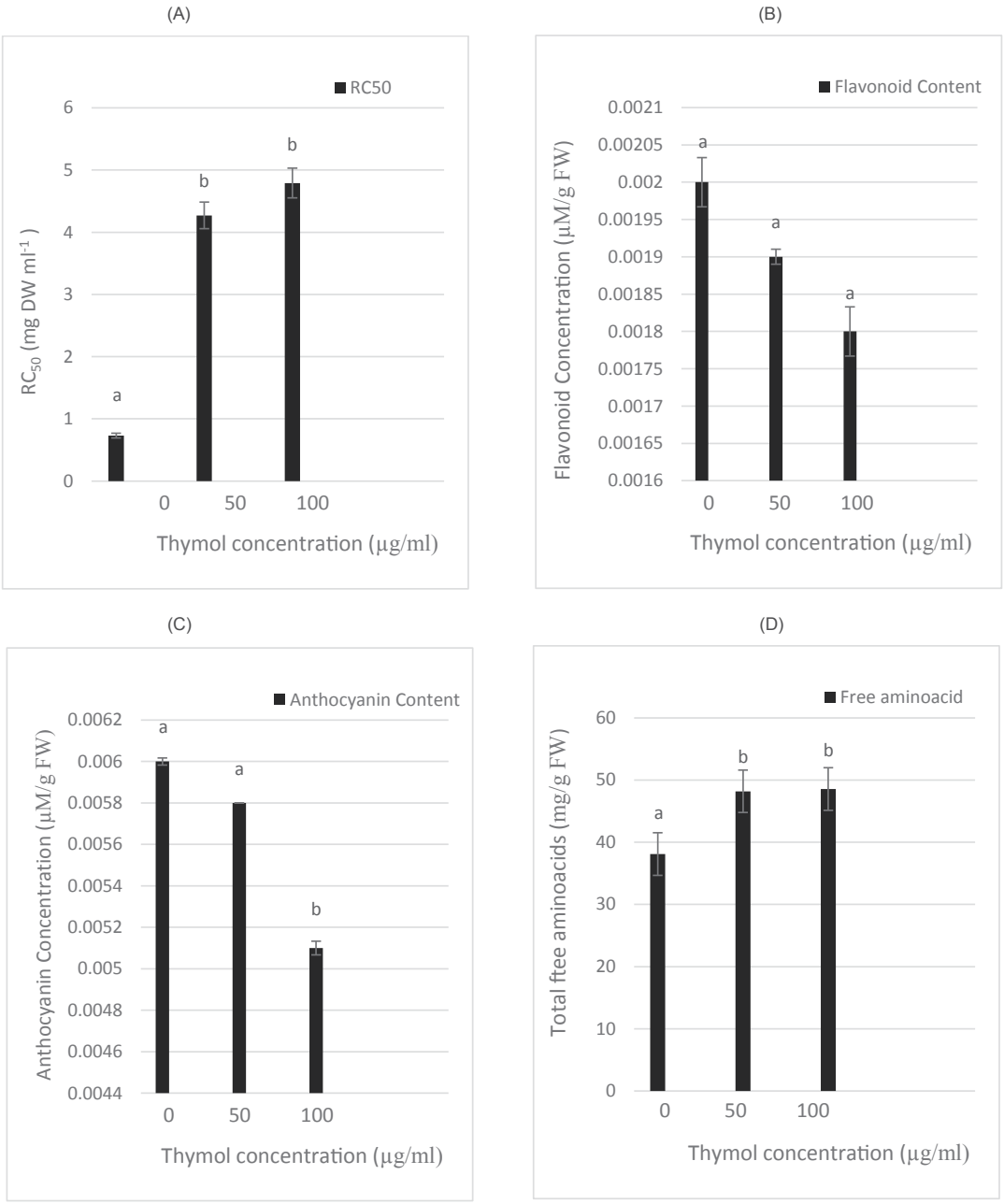

Figure 1: Effects of different thymol concentrations on antioxidant capacity (A), total flavnoids (B), total anthocyanins (C) and amino acid contents (D) lettuce. The same letters in each column represent no significant differences according to Duncan test at 5 percent.

It is probable that lettuce resists against this stress under the influence of thymol by changing the biosynthesis paths and increase of amino acids and antioxidant enzymes.

References

1. Nesrollahi P, Razavi SM (2017) Herbicidal and Anti Pathogenic Potential of Thymol. Sch Acad J Biosci 5: 183-186.
2. Moran EF, Brondizio ES, Mausel P, Wu Y (1994) Integrating Amazonian vegetation, land – use and stellite data. Bioscience 14: 329-339. Link: https://goo.gl/eVrTgl
3. Esfandiari E, Shekan F, Esfandiari, M (2007) the effect of salt stress on antioxidant enzymes activity and lipid peroxidation a wheat seedling. *Journal of Notulae Botanica Horti Agribotanichi cluj-Napoca* 35: 48-56. Link: https://goo.gl/RTQ3Bn
4. Bais HP, Vepechedu S, Gilary RM, Vivanco JM (2003) Allelopathy and exatrac plant invasion: from molecules and genes to species interactions. Science 301: 1377-1380. Link: https://goo.gl/AthoL0
5. Hwang M, Edere GM (1975) Rapid hippurate hydrolysis method for presumptive identification of group streptococci. J Clin Microbiol 1:114-115. Link: https://goo.gl/qJX7CI
6. Wagner GJ (1979) Content and vacuole/extra vacuole distribution of neutral sugars, free amino acids and anthocyanins in protoplasts. Plant Physiol 64: 88-93. Link: https://goo.gl/0X4gSB
7. Toor RK, Savage GP (2005) Antioxidant activity in different fractions tomatoes. Food Res Int 38: 487-494. Link: Link: https://goo.gl/VdXBgj
8. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. Food Sci Technol 28: 25-30. Link: https://goo.gl/yNLJ4p

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9. Jaleel CA, Riadh K, Gopi R, Manivannan P, Inès J, et al. (2009) Antioxidant defense responses: physiological plasticity in higher plants under abiotic constrains. Acta Physiol Planta 31: 427-436. Link: https://goo.gl/pXDsiG

10. Madhavi DL, Singhal RS, Kulkarni PR (1995) Technological aspects of food antioxidants: 158-266.In: Madhavi, D.L, Deshpande, S.S. and Salunkhe, D.K., (Eds.) Food Antioxidants Technological, Toxicological, and Health Perspectives. Marcel Dekker, Inc USA 512. Link: https://goo.gl/cFkWBD

11. Gordon MH (1990) the mechanism of antioxidant action in vitor: 1-18. In: Hudson BJF (Ed.) Food Antioxidant. Elsevier Applied Science USA 329. Link: https://goo.gl/UqSE1d

12. Rajalakshmi D, Narasimhan S (1996) Food antioxidant: Sources and methods of evaluation: 65-83. Link: https://goo.gl/4kdYcs

13. Verbruggen N, Hermons C (2008) Proline accumulation in plants: a review. Amino Acids 35: 753 – 759. Link: https://goo.gl/S8x1Jn

14. Yu JO, Liao ZX, Lei JC, Hu X (2007) Antioxidant and cytotoxic activities of various fractions of ethanol extract of Dianthus superbus. Food Chem 104: 1215-1219. Link: https://goo.gl/6XlhI5

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