Effect of potassium humate and growth stage on phenolic compound and vitamin C accumulation in kale (Brassica oleracea var. sabelllica)

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Abstract. Kale is one of the top economically valuable crops in the world because of its high antioxidant content. Research shows that the antioxidant profile of Brassica crops varies with growth stages due to soil fertility, temperature, light and other agronomic factors. This study aimed to analyze the effect of potassium humate on phenolic compound content, the greatest contributor to the antioxidant properties of the highest-ranking superfood kale (Brassica oleracea var. sabelllica). Our results showed that potassium humate at different growth stages elicited phenolic compounds in the studied samples. Leaves of 22 weeks old plants accumulated phenolic compounds about two times higher than those of 7 weeks old plants. Vitamin C content was increased by potassium humate treatment at 7 weeks. At 22 weeks levels in controls and treated kale leaves did not significantly differ.

1. Introduction

Dark green leafy kale (Brassica oleracea var. sabelllica), one of the oldest Eastern Mediterranean plants belonging to the family Brassicaceae [1], has received great attention from health and nutrition experts because of its nutritional profile. Kale cabbage among the Brassica vegetables is a major natural source of healthy antioxidants such as tocopherols, phenolic compounds, ascorbic acid, carotenoids and glucosinolates [2].

Phenolic compounds contribute largely to kale antioxidant properties. The increased interest of consumers in natural sources of phytochemicals makes the study of the influence of factors that affect the content of these molecules in kale cabbage a very promising area of research. Phenolic compounds positively affect human health because they possess a variety of biological activities that are linked with reducing the risk of cancer, cardiovascular, diabetes and other chronic diseases [2–4]. Phenolic compounds constitute a large group of secondary metabolites widely distributed in the plant kingdom and are considered to be effective antioxidants by their ability to scavenge reactive radicals, chelate iron and inhibit lipid peroxidation [5]. Many studies show that they have anti-allergenic, antiviral, anti-inflammatory, and vasodilating effects [6].

Vitamin C being an antioxidant is used in the prophylaxis of atherosclerosis, glaucoma, stroke and cancer. Humans unlike animals depend on exogenous supplement of this vitamin because of their inability to synthesize it [7,8].

Phenolic compound and vitamin C accumulation in plants are critically influenced by biotic and abiotic factors [9-11]. Agricultural management practices are important factors that also affect crop quality and yield. This includes fertilization, sowing and harvest dates among others [12].
Potassium humate is a commercial product containing mineral elements vital for plant growth and yield. The use of potassium humate has several advantages (such as enzyme activity enhancement, mineral provision, etc.) hence farmers around the world have integrated it into their fertilizer program. Research findings that indicate that application of humic acid leads to a positive effect on the growth and improvement of plants [13] combined with the increased interest of consumers in natural sources of phytochemicals motivated us to study the influence of potassium humate fertilizer on the phenolic compound content vitamin C in kale grown at two growth stages.

The findings can serve as a model for growing nutritionally valuable that may contain the greatest amount of phenolic compounds.

2. Materials and Methods

Kale plants in a field were fertilized with potassium humate (80g/l). The leaves were then harvested at 7 and 22 weeks for phenolic compound analysis.

At a wavelength of 725 nm by the modified Folin-Ciocalteu method phenolic compounds were determined [14]. The calculation was done in terms of gallic acid. By spectrophotometry, the content of vitamin C was determined as the sum of dehydroascorbic and acid ascorbic acid [15]. The experiments were conducted in three independent biological replicates. Statistical data processing was performed using the GraphPad prism software, v. 8.4. The reliability of the difference was determined by the criterion of double-factor analysis of variance *=p≤0.05.

3. Results and Discussion

From our findings, we clearly observed that potassium humate had a positive effect on the studied antioxidant in kale.

![Figure 1](image)

**Figure 1.** The effect of potassium humate on phenolic compound content in kale (*Brassica oleracea* var. *sabellica* (L.)) at week 7 and 22 respectively. Error bars signify 95% confidence intervals. Tukey’s honest significant difference (HSD) post-hoc test was used for multiple pairwise comparisons.

The symbol * indicates P > 0.05.

Potassium humate caused an increase in the content of phenolic compounds by 98% and 76% at 7 and 22 weeks respectively in comparison with their control variants as shown in figure 1. Researchers have demonstrated that the application of humate increase of biosynthesis of phenolic compounds [16]. This may explain the significant augmentation of phenolic compounds in the studied kale. Humic acid generates reactive oxygen species, which consequently brings about physiological
effects. According to the "oxidative pressure hypothesis", oxidative stress is followed by the biosynthesis of phenolic compounds [17]. Our findings are similar to work done by Ghasemi et al in their experiment on garlic [18]. Thus, it can be concluded that the generation of ROS by humic acid contributed to the rise in the phenolic compound content in kale leaves.

Harvesting time is an important factor in keeping the quality and antioxidant capacity of plants. The phytochemical changes that occur during maturation and the resultant effect on antioxidant activity are important dietary considerations that may affect the consumption of crop plants. Our results showed that at the more advanced vegetative stage phenolic compound content increased. In an experiment by Sellami et al. on sweet marjoram, polyphenol accumulation at the early vegetative stage, late vegetative stage, budding stage and flowering stage were studied. They found out that the phenolic compound content recorded at the late vegetative stage was higher than at the early vegetative stage which is consistent with our studies. This is because the plants accumulate phenolics to prepare for lignifications [19].

Samples treated with potassium humate showed 53\% rise in vitamin C content at 7 weeks as in figure 2. Although at 22 weeks vitamin C content in the treated samples was not significantly different from the controls the levels are higher than in control leaves harvested at 7 weeks. El-Bassiony et al in their work indicated that increased vitamin C in sweet pepper fruits treated with potassium humate is as a result of the potassium component of the fertilizer [20]. Several studies show a positive relationship between potassium fertilizer application and vitamin C content in plants [21]. Potassium plays a critical role in photosynthesis [22]. Jan Bernard stated that there is a direct connection between photosynthesis and vitamin C [23] because ascorbate participates in the scavenging of the excess hydrogen peroxide produced during photosynthesis in high-irradiance conditions by the function of ascorbate peroxidases [11].

4. Conclusion
The results showed that the treatment of kale cabbage with humate has a positive effect on both phenolic compounds and vitamin C content. Kale leaves harvested at a later vegetative stage are a better source of phenolic compounds making them an excellent source of antioxidants while treated...
leaves are richer in vitamin C at 7 weeks than controls. To enhance the nutritional value of kale fertilization with humate as well as harvest time should be considered.

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References
[1] Gorka S, Samnotra RK, Kumar S, Chopra S and Gupta M 2018 Analysis of genetic diversity in kale (Brassica oleracea L var acephala) genotypes of jammu and kashmir region based on morphological descriptors (Int J Curr Microbiol Appl Sci vol 7) pp 2176–2181
[2] Nawaz H, Shad M A and Muzaffar S 2018 Phytochemical Composition and Antioxidant Potential of Brassica (Brassica Germplasm-Charact, Breed and Util vol 24) pp 7–26
[3] Michalak M, Gustaw K, Waśko A and Polak-Berecka M 2018 Composition of lactic acid bacteria during spontaneous curly kale (Brassica oleracea var sabellica) fermentation (Microbiol Res vol 206) pp 121–130
[4] Hagen S F, Borge G I A, Solhaug K A and Bengtsson G B 2009 Effect of cold storage and harvest date on bioactive compounds in curly kale (Brassica oleracea L var acephala) (Postharvest Biol Technol vol 5) pp 36–42
[5] Garcia L M, Ceccanti C, Negro C, De Bellis L, Incrocci L and Pardossi A 2021 Effect of drying methods on phenolic compounds and antioxidant activity of Urtica dioica L leaves (Horticulutae vol 7) p 10
[6] Aberoumand A and Deokule S S 2008 Comparison of phenolic compounds of some edible plants of Iran and India (Pakistan J of Nutr vol 7) pp 582–585
[7] Chambial S, Dwivedi S, Shukla K K, John P J and Sharma P 2013 Vitamin C in disease prevention and cure: An overview (Indian J of Clinical Biochem vol 28) pp 314–328
[8] Khan H, Hussain F H S abd Samad A 2019 Cure and prevention of diseases with vitamin C into perspective: An overview (J Crit Rev vol 7) pp 289–293
[9] Gouvea D R, Gobbo-Neto L and Lopes N P 2012 The Influence of Biotic and Abiotic Factors on the Production of Secondary Metabolites in Medicinal Plants (Plant bioactives and drug discovery: principles, practice, and perspectives vol 17) p 419
[10] Ramakrishna A and Ravishankar G A 2011 Influence of abiotic stress signals on secondary metabolites in plants (Plant signaling & behavior vol 6) pp 1720–1731
[11] Fenech M, Amaya I, Valpuesta V and Botella M A 2006 Vitamin C content in fruits: Biosynthesis and regulation (Frontiers in plant sci vol 9)
[12] Deryng D, Sacks W J, Barford C C and Ramankutty N 2011 Simulating the effects of climate and agricultural management practices on global crop yield (Global Biogeochem Cycles vol 25)
[13] Patil R B, Kadam A S and Wadje S S 2011 Role of potassium humate on growth and yield of soybean And black gram (Int J Pharma Bio Sci vol 1) pp 243–246
[14] Aydın S 2020 Total phenolic content, antioxidant, antibacterial and antifungal activities, ft-ir analyses of Brassica oleracea L var acephala AND Ornithogalum umbellatum L (Genetika vol 52) pp 229–244
[15] Kapusta-Duch J, Kusznierewicz B, Leszczyńska T and Borczak B 2016 Effect of Culinary Treatment on Changes in the Contents of Selected Nutrients and Non-Nutrients in Curly Kale (Brassica oleracea Var acephala) (Jof Food Processing and Preservation) pp 401280–401288
[16] Schiavon M, Pizzeghello D, Muscolo A, Vaccaro S, Francioso O and Nardi S 2010 High molecular size humic substances enhance phenylpropanoid metabolism in maize (Ze a mays L) (J Chem Ecol vol 36) pp 662–669
[17] Treutter D 2005 Significance of flavonoids in plant resistance and enhancement of their biosynthesis (Plant biol vol 7) pp 581–591
[18] Ghasemi K, Bolandnazar S, Tabatabaee S J, Pirdashti H, Arzanlou M and Ebrahimzadeh M A
2015 Antioxidant properties of garlic as affected by selenium and humic acid treatments (New Zeal J Crop Hortic Sci vol 43) pp 173–181

[19] Sellami I H, Maamouri E, Chahed T, Wannes W A, Kchouk M E and Marzouk B 2009 Effect of growth stage on the content and composition of the essential oil and phenolic fraction of sweet marjoram (Origanum majorana L) (Ind Crops and Prod vol 30) pp 395–402

[20] Bassiony E, Fawzy Z, Abd E H, Samad E and Riad G S 2010 Growth, Yield and Fruit Quality of Sweet Pepper Plants (Capsicum annuum L) as Affected by Potassium Fertilization (J of Am Sci vol 6) pp 722–729

[21] Shehata S A, El-Mogy M M and Mohamed H F Y 2019 Postharvest quality and nutrient contents of long sweet pepper enhanced by supplementary potassium foliar application (Int J Veg Sci vol 25) pp 196–209

[22] Zahoor R, Dong H, Abid M, Zhao W, Wang Y and Zhou Z 2017 Potassium fertilizer improves drought stress alleviation potential in cotton by enhancing photosynthesis and carbohydrate metabolism (Environ Exp Bot vol 137) pp 73–83

[23] Ijdo J B H 1936 The influence of fertilizers on the carotene and vitamin C content of plants (Biochem J vol 30) p 2307