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Evaluation of the effects of Zinc on the chemical composition and biological activity of basil essential oil by using Raman spectroscopy

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ABSTRACT

The present study is performed to evaluate the effect of different concentrations of Zn as fertilizer on the chemical composition of basil (*Ocimum basilicum*) essential oil and its biological activity including antioxidant and antifungal activities. For this purpose, Raman spectroscopy along with GC–MS and antifungal assays are employed. Moreover, the effect of Zn is also determined on the vegetative growth and essential oil yield. The treatment with 0.09 mg/L of Zn showed maximum vegetative growth (0.24 ± 0.02 kg plant weight and 0.31 ± 0.03 m plant height) while the treatment with 0.15 mg/L of Zn revealed highest essential oil yield (0.48 ± 0.019%) as compared to 0.28 ± 0.005% for control. All essential oils exhibited significant total phenolic contents, total flavonol contents, antioxidant activity measured by hydrogen peroxide scavenging, inhibition of linoleic acid oxidation and DPPH free radical scavenging and Ferric reducing antioxidant power assay Antifungal activity of essential oils was performed by disc diffusion assay against *Aspergillus niger* and *Penicillium notatum* strains. The results of antifungal activity showed that essential oils isolated after treatment of 0.095 mg/L concentration of Zn fertilizer exhibited maximum antifungal activity. Chemical compositions of isolated essential oils were determined by Gas Chromatography-Mass spectrometry (GC–MS) and Raman spectroscopy. The results of the GC–MS and Raman spectroscopy have revealed that the linalool is found to be as a major chemical compound in *Ocimum basilicum* essential oil. Moreover, Raman spectroscopy has identified the effect of the Zn on the concentration of the biological components present in essential oil showing that highest concentration of linalool found in the essential oil obtained from the basil plants which were given 0.095 mg/L of Zn fertilizer treatment that might be responsible for the maximum biological activities of this essential oil.

1. Introduction

Sustainable production of agriculture is an ever increasing concern for farmers. There are many factors that affect vegetative growth, active substances, quality and yield of essential oil in aromatic medicinal plants. Among these several factors, availability of the nutrients is the most significant controllable factor that plays an important role in the biosynthesis, quality and yield of secondary metabolites including essential oil (Chand et al., 2015; Pal et al., 2016). Yield and chemical constituents of essential oil depend on the type of nutrients available to plants (Sakr et al., 2012; Sharafzadeh et al., 2011). Therefore, fertilizer with exact concentrations of micro and macronutrients are extremely important for high yield and better quality of essential oil. Micronutrients such as Mn, Cu, Zn and Fe control various physiological activities of the crop by intruding the level of chlorophyll content in leaves which ultimately influence the photosynthetic activity of the plants and biosynthesis of essential oil (Yadegari, 2014). Previously, it has been reported that application of the chemical fertilizers changed the major chemical constituents of essential oil isolated from *Satureja* and *Thymus vulgaris* (Alizadeh et al., 2010; Baranauskiene et al., 2003). Zinc (Zn) is an essential micronutrient for normal crop growth. It is involved in antioxidant enzymes, carbon assimilation, free radical removal, saccharide accumulation and utilization of carbon in terpenes biosynthesis (Rezaeieh et al., 2016). Its deficiency can severely decrease plant growth and essential oil yield (Ali et al., 2008). Essential oil is secondary metabolite of plant, composed of terpenes and its oxygenated derivatives terpenoids,

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acyclic and cyclic compounds, aromatic, phenolics, acetonides, sulfur and nitrogen-containing compounds (Pandit et al., 2015). It is isolated from different parts of the aromatic plants like flowers, stems, leaves, roots, barks, needles and oleoresins through hydro and steam distillation methods and further used in cosmetics, food, agriculture and pharmaceutical industries (Abad et al., 2012).

Food-borne diseases are one of the most growing public health problems around the world (Hyldgaard et al., 2012). Recently, scientific interest in application of essential oils and its isolated compounds in food preservation have been increased due to adverse health effects of synthetic preservatives (Dobre et al., 2011) Essential oils and their isolated compounds have strong antioxidant potential due to its ability to scavenge free radicals and may play an important role in disease prevention caused by free radicals and reactive oxygen species such as heart disease, brain dysfunction, and cancer (Kamatou and Viljoen, 2010; Roby et al., 2013).

Ocimum basilicum L., also known as sweet basil, belongs to family Lamiaceae. Among 200 species of the genus *Ocimum*, *Ocimum basilicum* is the major essential oil producing crop which is commercially grown in many countries around the world (Singh et al., 2014). *Ocimum basilicum* essential oil has been extensively used in perfumes and as flavoring agent in food industries (Farouk et al., 2016). In folk medicine, different parts of this plant are utilized for the treatment of constipation, diabetes, headaches, diarrhea, warts, coughs, worms and cardiovascular diseases(Bilal et al., 2012). Recently, the potential uses of *Ocimum basilicum* essential oil particularly as antimicrobial and antioxidant agents have also been investigated (Beatovic et al., 2015; Shirazi et al., 2014; Vieira et al., 2014). Monoterpenes and phenylpropanoids are the primary chemical components of the essential oil, but the chemical composition of the essential oil changes with season, colours of flower, leaf and origin of the plants (Hussain et al., 2008; Sajjadi, 2006).

In order to monitor the quality and biological composition of the essential oils many chromatographic methods like Gas Chromatography (GC), and High Performance Liquid Chromatography (HPLC) are used (Andrikopoulos et al., 2001). Although these chromatographic techniques offer great advantages in the analysis of several organic compounds present in the essential oils but there are certain issues like time consumption and cost of analysis which demand for the development of new analytical methods which can efficiently serve the purpose of quality control and are rapid and cost effective methods. Raman spectroscopy has great potential as an analytical technique to fulfill these requirements and is mainly used for qualitative determination of compounds based on their unique spectrum (Siatis et al., 2005). Raman spectroscopy in combination with Multivariate data analysis technique (PCA) is considered as a promising analytical technique (El-Abassy et al., 2009). It is non-destructive, requires minimum or no sample preparation (Strehle et al., 2005) and can be applied to all disciplines of natural sciences (Vargas Jentzsch and Ciobotă, 2014). Recently, the technique is used for the characterization of the different essential oils (Daferera et al., 2002; Schulz et al., 2005; Strehle et al., 2005). In the current study the technique is used for the evaluation of the chemical composition of the essential oil obtained from the leaves of *Ocimum basilicum* plant and effect of the Zn fertilizer on the composition of the essential oil. The technique is used to monitor the variations which occur in major components of *Ocimum basilicum* essential oil by the use of different concentrations of zinc (Zn) fertilizer in comparison with essential oil obtained without any use of Zn fertilizer. In addition to the evaluation of the Raman spectral changes occurring in the essential oil of the *Ocimum basilicum* leaves due to the effect of the Zn fertilizer, these results are also compared with those of the biological activity of this oil which is measured by the antifungal activity of the major biological components present in the oil by employing the pure oil in such assays. Comprehensive review of published literature on *Ocimum basilicum* essential oil showed that there is no report available on employing Raman spectroscopy for the evaluation of the effects of different concentration of Zn on the chemical composition of the *Ocimum basilicum* essential oil along with the comparison of the Raman spectral data with the antifungal activities of the oil. This work will pave the path for the development of the Raman spectroscopy based methods for monitoring the composition of essential oil and their biological activity.

2. Materials and methods

2.1. *Propagation and treatments of* Ocimum basilicum *plant*

The experiments were carried out according to randomized complete block design (RCBD) with ten replications of each treatment in the controlled temperature 30 ± 2 °C and humidity 55 ± 2% in the green house, employing jet fan and heater system, of the Department of Chemistry, University of Agriculture, Faisalabad (31°25′ N, 73°09′ E), Pakistan. Peat moss was used as growth media for appropriate growth of *Ocimum basilicum* plant. Pots were filled with peat moss, sand and soil with 1:1:1 ratio. Purpose of using sand was to soften the soil so that roots can grow appropriately. Firstly peat moss, sand and soil were mixed homogeneously and then this mixture was filled into the pots. Four weeks old *Ocimum basilicum* seedlings were transferred to 24 inches pots (to increase the moisture contents available to plant in soil which causes better plant growth). Amount of macro (KH_2PO_4 0.1361, KNO_3 0.5055, $Ca\,(NO_3)_2$ 1.181 and $MgSO_4$ 0.49296 g/L), micronutrients (H_3BO_3 0.00203, $CuSO_4{\cdot}5H_2O$, 0.00004, $MnCl_2{\cdot}4H_2O$ 0.00905 and $(NH_4)_6MoO_{24}{\cdot}2H_2O$ 0.00062 g/L) and iron chelates ($FeSO_4{\cdot}7H_2O$ 0.00557, $NaEDTA{\cdot}2H_2O$ 0.00745, Or FeNaEDTA 0.03736 g/L) used to irrigate *Ocimum basilicum* plants. Notably, the concentrations of Zn were varied as 0.2, 0.15, 0.10, 0.095, 0.09 mg/L whereas all other nutrients were kept constant. After the preparation of the stock solutions, solutions were diluted up to required concentration as describe by (Hoagland and Arnon, 1950). At flowering stage, plant height (m), weight (kg) and oil yield (%) was recorded, for this purpose plant was cut by leaving first two lateral branches.

2.2. *Isolation of essential oil*

Essential oil was extracted by hydro distillation method using Clevenger type apparatus from 300 g of *Ocimum basilicum* biomass from each treatment. The process of extraction of oil was repeated three times to ensure the reproducibility.

2.3. *Antioxidant activity*

2.3.1. *Total phenolic contents*

To 1.0 mL of each essential oil isolated after treatment of different concentration of Zn, control or Gallic acid standard solution (10, 40, 70, 100 and 130 mg/mL), 5 mL of Folin-Ciocalteu and 4 mL sodium carbonate (7% w/v) were added and samples were shaken to mix the components completely. After keeping all the samples in dark for 30 min, absorbance was measured at 765 nm using a spectrophotometer (model 721D). Reagent solution was expressed as Gallic acid equivalent (GAE) in milligram per gram of dry weight basis (Khan et al., 2012). The Calibration curve of Gallic acid (Fig. S-1) is shown in Supplementary information.

2.3.2. *Total flavonol contents*

The reaction mixture consisted of 2.0 mL of essential oil isolated after treatment of different concentration of Zn, control and Quercetin standard solutions (20, 40, 60, 80 and 100 mg/mL), 2.0 mL of $AlCl_3$ prepared in ethanol and 3.0 mL of (50 g/L) sodium acetate solution. The absorbance was measured at 440 nm after 2.5 h at