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Raman spectroscopy for the evaluation of the effects of different concentrations of Copper on the chemical composition and biological activity of basil essential oil

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ABSTRACT

The present study is performed to evaluate the effect of different concentrations of Cu as fertilizer on the chemical composition of basil essential oil and its biological activity including antioxidant and antifungal activities by employing Raman spectroscopy. Moreover, the effect of Cu is also determined on the vegetative growth and essential oil yield. Both, antifungal and antioxidant activities were found to be maximum with essential oils obtained at 0.04 mg/l concentration of Cu fertilizer. The results of the GC–MS and Raman spectroscopy have revealed that the linalool and estragole are found to be as a major chemical compound in basil essential oil. The Raman spectral changes associated with these biological components lead to the conclusion that estragole seems to have dominating effect in the biological activities of the basil essential oil as compared to linalool although the latter is observed in greater concentration.

1. Introduction

Plant growth and development is strongly influenced by availability of nutrients. Fertilizer with specific concentration of micro and macronutrients is extremely important for better quality and high yield of plant biomass [1]. Moreover, these micro and macronutrients significantly affect chemical composition of secondary metabolites of plants including essential oils [2–3]. Previous, studies have shown that application of the chemical fertilizers changed the major chemical constituents of the essential oil extracted from *Satureja hortensis* and *Thymus vulgaris* essential oils [4–5]. Therefore, fertilizer with exact concentrations of micro and macronutrients are extremely important for high yield of plant biomass and better quality of essential oil [1]. Copper is an important micro-nutrient required for normal plant growth. It is involved in several physiological processes and acts as a cofactor for various enzymes and helps in photosynthetic electron transport [6]. However, higher concentration of Copper is phytotoxic to plants, inhibits various physiological functions and reduces plant growth [7–8]. Therefore, it is important to find exact concentration of Cu for optimum plant growth. Essential oil is secondary metabolism of plant, composed of terpenes and oxygenated derivatives of terpenoids, acyclic and cyclic compounds, aromatic, phenolics, acetonides, sulfur and nitrogen-containing compounds [9]. 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 [10].

Food-borne diseases are one of the most growing public health problems around the world. 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 [11]. Essential oils and its 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 [12–13].

Ocimum basilicum L., also known as sweet basil, belongs to family *Lamiaceae*. It is industrially important essential oil producing crop and grown in many regions around the world [14]. Essential oil isolated from these species is extensively used in perfumes and as flavoring agent in food industry [15]. Recently, the potential uses of *Ocimum basilicum* essential oil particularly as antimicrobial and antioxidant agents have also been investigated [16–18]. Monoterpenes and phenylpropanoids are the primary chemical components of its essential oil. However, its chemical composition vary with growing season, colours of flower, maturity stage, nutrient availability and origin of the plants [14,18].

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Vibrational spectroscopy, Raman and FTIR spectroscopy have different applications in the various fields including examination of the spectral changes in any bio-macromolecules like DNA, proteins, lipids simultaneously [19–21]. Recently, vibrational spectroscopy is used for the characterization of the different essential oils [22–29]. In the current study the technique is used for the evaluation of the effect of Cu fertilizer on the chemical composition of *Ocimum basilicum* essential oil extracted from its leaves. 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 Cu fertilizer in comparison with essential oil obtained without any use of Cu fertilizer (control). It is observed that there is significant change in major components of *Ocimum basilicum* essential oil, mainly linalool and estragole. These results are also compared with those of the biological activity of this oil including antioxidant and antifungal activity. Keen 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 Cu on the chemical composition of the essential oil and comparison with the antioxidant and antifungal activities. This work will pave the path for the development of the Raman spectroscopy based analytical 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 moderately controlled temperature 30 ± 2 °C and humidity 55 ± 2% in 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 in. 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, $ZnSO_4$. $5H_2O$, 0.0001, $MnCl_2$. $4H_2O$ 0.00905 and $(NH_4)_6MoO_{24}.2H_2O$ 0.00062 g/l) and iron chelates ($FeSO_4.7H_2O$ 0.00557, NaEDTA.$2H_2O$ 0.00745, Or FeNaEDTA 0.03736 g/l) used to irrigate *Ocimum basilicum* plants. Notably, the concentrations of Cu were varied as 0.000025, 0.00003, 0.000035, 0.00004, 0.000045 g/l whereas all other nutrients were kept constant. After the preparation of the stock solutions, solutions were diluted up to required concentration as describe [30]. 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 isolated by hydro distillation method using Clevenger type apparatus from *Ocimum basilicum* biomass obtained from each treatment. For this purpose, 300 g of the plant biomass was employed. The process of isolation of the essential oil was repeated three times to ensure the reproducibility. All the samples were dried over anhydrous sodium sulphate, filtered, stored in screw capped glass vials and kept at 4 °C for the further analysis.

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 Cu, 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 [31]. The Calibration curve of Gallic acid is shown in Supplementary information **(S-1)**.

2.3.2. Total flavonol contents

The reaction mixture consisted of 2.0 ml of essential oil isolated after treatment of different concentration of Cu, 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 20 °C [32]. The calibration curve of Quercetin is given in the Supplementary information **(S-2)**.

2.3.3. 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging activity

The DPPH assay was performed as described by [33]. Essential oils isolated after treatments of different concentration of Cu and control were mixed with 1 ml of 0.09 mM DPPH solution and filled up with 95% MeOH, to a final volume of 4 ml. The absorbance of the resulting solutions and the blank were recorded after 1 h at room temperature at 515 nm using a spectrophotometer. Butylated hydroxyl toluene (BHT) was used as a positive control. Inhibition of free radical by DPPH in percent (%) was calculated in the following way:

$I(\%) = 100 \times (A_{blank} - A_{sample}/A_{blank})$ where A_{blank} is the absorbance of the control reaction mixture and A_{sample} is the absorbance of the test compounds.

2.3.4. Total antioxidant contents (FRAP assay)

Total antioxidant contents of essential oil isolated after treatment of different concentration of Cu and control were determined using ferric reducing antioxidant power (*FRAP)* assay [34] with little modifications. Essential oil solution 5 ml (100 mg/ml w/v) in ethanol was mixed with 5 ml of 0.2 m phosphate buffer (pH 6.6). Above reaction mixture was mixed with 5 ml of potassium ferricyanide (1%) and incubated for 20 min at 50 °C. Trichloro acetic acid (10%) 5 ml was added followed by thorough mixing via vortex mixture. Reaction mixture thus obtained was centrifuged for 10 min at 3000 rpm. Supernatant 2.5 ml was mixed with 5 ml of double distilled water and 0.5 ml of $FeCl_3$ (0.1%) solution. Absorbance was measured at 700 nm. Total antioxidant contents were determined from gallic acid curve shown in **S-3**.

2.3.5. Percentage inhibition in linoleic acid system

The antioxidant activity of essential oils isolated after treatment of different concentrations of Cu and blank was determined by the method as described by [35] with modification. The test samples (50 μl) were dissolved to a 1 ml of ethanol, mixed with linoleic acid (2.5%, v/v), 99.5% ethanol (4 ml) and 4 ml of 0.05 M sodium phosphate buffer (pH 7). The solution was incubated at 40 °C for 175 h. The extent of oxidation was measured by peroxide value using the colorimetric method described by [36]. To 0.2 ml sample solution, 10 ml of ethanol (75%), 0.2 ml of an aqueous solution of ammonium thiocyanate (30%) and 0.2 ml of ferrous chloride solution (20 mM in 3.5% HCl) were added sequentially. After 3 min of stirring, the absorbance was measured at 500 nm, using a spectrophotometer. A control was performed with linoleic acid without essential oils. Butylated hydroxytoluene (BHT) was used as positive control. Inhibition of linoleic acid oxidation expressed as percent was calculated as follows:

% inhibition of linoleic acid oxidation =

100 − [(Abs.Increase of sample at 175 h/Abs.Increase of control at 175 h) × 100]