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REGULATORY EFFECTS OF EXOGENOUS CARBOHYDRATE SOURCES ON GROWTH AND BIOCHEMICAL TRAITS OF SPEARMINT (*MENTHA SPICATA* L.)

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Abstract
The purpose of this study is to investigate the interplay of stress, emotional intelligence, and work-life balance among ambulance personnel. This study is a descriptive-correlational involving 120 ambulance paramedics personnel with at least 2 years of experience and were recruited from two regional ambulance services. Online self-assessment questionnaires were administered from April through June 2023. Descriptive statistics (i.e. frequency distribution, mean, SD) and inferential statistics to explore relationships between variables. Ambulance personnel work long, demanding hours (often exceeding 10 hours daily) with frequent night calls and weekend duties, leading to fatigue and moderate stress (average score 87.07). Though they display high emotional intelligence, their work-life balance suffers (score 41.26), particularly due to work intruding on their personal life. Higher emotional intelligence is linked to slightly increased stress but also slightly better work-life balance. Despite having a high emotional intelligence, ambulance personnel struggle with considerable stress and fatigue due to their busy schedules and poor work-life balance. Although there are links between these parameters, more study is still required. It is still essential for their performance and general well-being to prioritize sleep, breaks, and healthier work habits.

Introduction

Mentha spicata belongs to the family *Lamiaceae* and is commonly known as Menthol mint or Garden mint. *Lamiaceae* is a family of flowering plants and is closely related to the family *Verbenaceae*. Its nomenclature system has huge difficulties in making the correct name due to higher biodiversity. It is an annual plant and is cultivated in the tropical and sub-tropical regions (Mahendran et al., 2021). Its cultivation has much significance, such as for food flavouring, medicinal applications and essential oils applications. Menthol is a component of Mentha spicata, and it has several industrial applications, mainly in food, cosmetics, pharmaceuticals and by- products. There are many types of menthol found in mint, depending on the type of species and cultivation conditions, such as type of soil, irrigation, weather and other agronomic activities. Its growth rate is strongly affected by changes in variables like pH, temperature and nutrition in the soil (Bghbani-Arani and Poureisa, 2024). Mentha species have anti-microbial, anti-inflammatory and anti-oxidant properties, especially when human cells are affected by the reactive oxygen species, produced during metabolism under physiological conditions; these anti-oxidants help to neutralize the free radicals, which destroy proteins, lipids and nucleic acids (Prakash et al., 2016).

Members of the *Lamiaceae* family, such as mint, rosemary, oregano, basil, thyme, and sage, are well known for their medicinal use. Aqueous infusions obtained from mint leaves have been used for the treatment of anorexia, hypertension, and many gastrointestinal diseases (Mimica- Dukic and Bozin 2008). In addition, mint has been used in clinical trials to treat headaches through its analgesic properties (McKay and Blumberg 2006). In-vitro studies have demonstrated that mint extract has potential anti-depressant effects (López-Rubalcava and Estrada-Camarena, 2016). These medicinal effects of mint are closely related to the high content of phenolic compounds. The phenolic s from mint extracts have been shown to exhibit anti-microbial and anti-viral activities (Mimica-Dukic et al. 2008). Carbohydrates are one of the major constituents of plants. They are formed by the photosynthetic activity of plants and represent the largest proportion of organic compounds. Over the past few decades, carbohydrate research has advanced greatly and substantial progress is being recorded year by year. In plants, carbohydrates perform a variety of physiological functions such as cellulose forming part of the structural component, starch as reserve food material providing an energy source, gums and mucilage performing a defensive action to prevent tissue desiccation, *etc.* (Martínez-Vilalta et al., 2016).

Nutrients such as proteins, carbohydrates, vitamins and minerals have a key role in maintaining good health. Numerous studies have shown that plants are the source of these nutrients (Kumar et al., 2017; De and De, 2019). The use of medicinal plants as nutrients can solve the problem of malnutrition in developing countries like Pakistan. The current study was designed to study the effects of different carbohydrate sources on growth and carbohydrate accumulation in mint (*Mentha spicata*).

Material & Methods Experimental

design

The experiment was conducted in a Completely Randomized Design (CRD) at the Institute of Biological Sciences (IBS), Gomal University, D.I. Khan, Pakistan. Three small plots for each of the control groups (no sugar and starch treatment), sugar additive and starch additive were made to grow spearmint. These plots were filled with soil, and then fertilizer was added to them. After that, small stems of mint were inserted into that soil. Then the plants were allowed to grow, with daily watering with the help of spray bottles. The initial growth of spearmint in every plot was made homogenous by fine thinning of the plants before the treatments. After treatment, the differences in the growth of samples were observed on a daily to weekly basis.

Additives preparation

Two additives were prepared for the experiment, i.e. sugar additive and starch additive.

Sugar Additive

Sugar additive was prepared by taking 50:50 grams of glucose and sucrose, and get dissolved in 1 litre of water.

Starch Additive

The starch additive was prepared by taking 50:50 grams of corn starch (powdered corn) and wheat starch, and get dissolved in 1 litre of water.

Exogenous application of sugar and starch additives

Each of the spearmint plant samples was treated by the hand-spray method daily for a consecutive 1-2 weeks.

Soil preparation for spearmint growth

Standard soil with the following was used for spearmint growth.

pH = 8.23, soil texture = (Clay=12.5%, Silt=15%, Sand=72.5%), Organic matter = 1.79%.

Parameters studied

Length of plants

The lengths of plants were simply measured by using a ruler/measuring tape. The ruler was placed horizontally on the stem of the plants, and the reading was on it.

Estimation of carbohydrates

The carbohydrates were estimated by the Phenol sulphuric acid method (Giglou et al, 2023), using the following formula;

Amount of carbohydrate present in 100mg of the sample = $\frac{mg\ of\ glucose \times 100}{Volume\ of\ test\ sample}$

Determination of total chlorophyll content

Total chlorophyll contents were determined by the method of Grzeszczuk and Jadczak (2009). A total of 250 mg of fresh leaves was taken and ground with the help of a pestle and mortar with 10 ml of 80% acetone. The homogenate was filtered/using filter paper. The filtrate was stored and utilized for chlorophyll estimation. The colour intensity of the green pigment was observed at 645nm, 663nm and 652nm for chlorophyll 'a, chlorophyll 'b' and total chlorophyll content, respectively, using as pectrophotometer. Chl 'a and Chl 'b' were calculated by the formula;

Chlorophyll a (mg/mL) = 12.7 A663 - 2.69 A645

Chlorophyll b (mg/mL) = 22.9 A645 – 4.68 A633

Where:

A645 = absorbance at a wavelength of 645nm

A663 = absorbance at a wavelength of 663nm

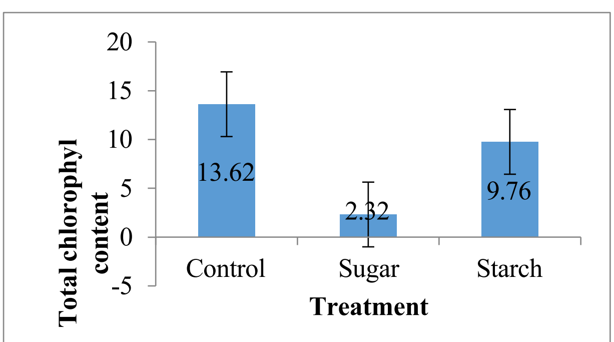
Total Chlorophyll (mg/mL) = Chlorophyll a + Chlorophyll b

Statistical analysis

All data collected was subjected to Statistics v.8.1 for tabular and graphical presentation.

Results & Discussion Length of Plants (cm):

The data regarding the length of plants is presented in Table 1. The statistical analysis of the data showed that the application of carbohydrates significantly affected the length of plants. The plants of the control group were small in length (mean length: 19.65 cm) while the plants of the sugar-applied samples were larger in length (mean length: 23.95 cm). The plants of the starch-applied samples were the largest among all (mean length: 26.3 cm)



(Fig. 1) Our results are in agreement with Masroor et al. (2024), who showed that exogenously applied phenolic compounds can enhance *Mentha arvensis* L. growth, physiological characteristics, essential oil and their active constituents 'production.

Table 1: Plant Length and Total Carbohydrate.

Treatments	PL (cm)	TC (mg/100g)
Starch	26.3	1.68
Sugar	23.95	1.66

PL: Length of plants, TC: Total Carbohydrates

Figure 1: Response of the length of the plant of spearmint in response to carbohydrate treatment.

Total Carbohydrates

The data regarding the total carbohydrates of plants is presented in Table 2. The statistical analysis of the data showed that the application of carbohydrates significantly affected the total carbohydrate content of the plants. The plants of the control group possessed the lowest amount of carbohydrate (0.84 mg/100g) while the sugar samples possessed a high amount of carbohydrate (1.66 mg/100g) which was statistically similar to the carbohydrate content of starch samples (1.68 mg/100g) as shown in Fig. 2. Our results are in agreement with Shittu et al., (2021) who studied effect of the characteristic of mint leaves (*Mentha spicata* L.), and showed that Carbohydrates were more (30.13%) when open sun drying was employed.

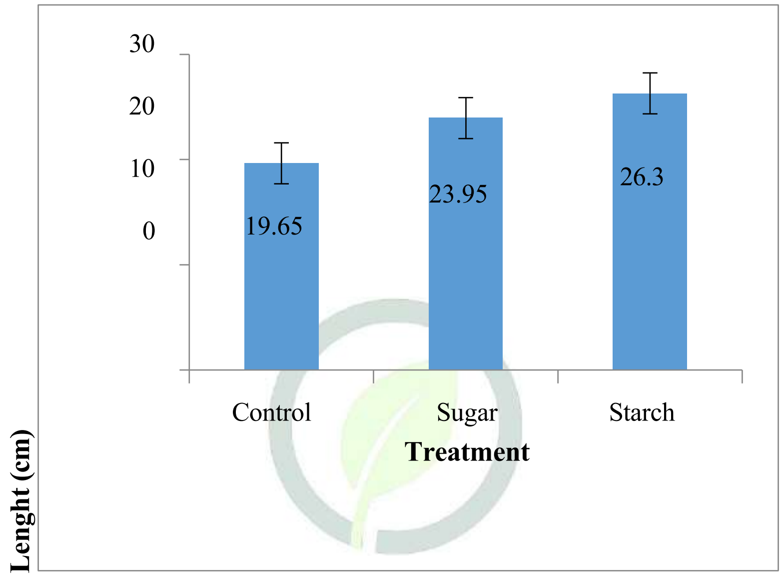


Fig. 2 Variation in total carbohydrate accumulation in spearmint in response to sugar and starch treatments.

Total chlorophyll content

The data regarding the total chlorophyll content of plants is shown in Table 3. The statistical analysis of the data showed that the application of carbohydrates significantly affected the total chlorophyll content of the plants. The plants of the control group showed the highest amount of chlorophyll (13.62) while the plants of the starch sample showed a lower amount of chlorophyll (9.76). The sugar-treated samples showed the lowest amount of chlorophyll (2.32) (Fig. 3). Our findings are supported by the outcomes of Shahani et al., (2021) who studied the influence of zinc and salicylic acid foliar application on total chlorophyll, phenolic components, yield and essential oil composition of peppermint (*Mentha piperita* L.), and showed that exogenous zinc and salicylic acid foliar application decreased total chlorophyll content in *Mentha piperita*.

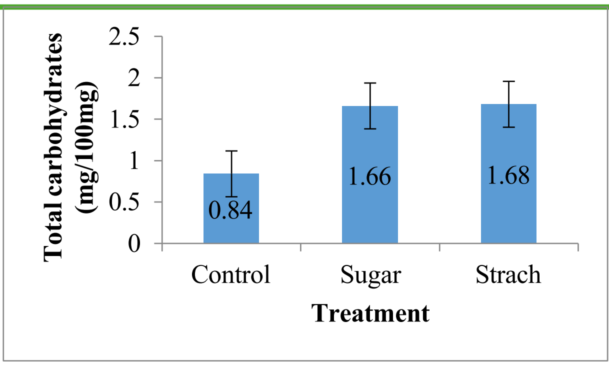


Fig. 3 Variation in total chlorophyll contents of spearmint in response to carbohydrate treatment.

Conclusion

The study concluded that the application of carbohydrates externally to the plants significantly affected the amount of total carbohydrates in them. The plants treated with the carbohydrate additives possesse dhigheramounts of carbohydrates, while the plants of the control group possessed fewer amounts of carbohydrates. Also, it showed positive results on the growth rate and length of the plants. The plants with the application of the carbohydrate additive I (starch) showed the highest growth rate, while the plants with the application of additive II (sugar) showed a growth rate less than starch samples, and the control group plants showed the lowest growth rate. Based on the above results, it is concluded that the external application of carbohydrates on spearmint significantly affects the total carbohydrate content and growth rate of plants.

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