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Effect of different water application rates and nitrogen fertilisation on growth and essential oil of clove basil (*Ocimum gratissimum* L.)

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ABSTRACT

Ocimum gratissimum L. (clove basil) is a popular medicinal plant with vast potential for use in the pharmaceutical, cosmetic and food industries. The effects of different water application rates, 60% (I_{60}), 70% (I_{70}), 80% (I_{80}), 90% (I_{90}), 100% (I_{100}) and 110% (I_{110}) of field capacity (FC), and nitrogen fertilization, 0 (N_0), 40 (N_2), 80 (N_4) and 160 (N_8) kg of nitrogen/ha, on growth and essential oil of this species were studied. The experiment was carried out for 60 days in a greenhouse in a completely randomised design with three replicates. The isolated volatile constituents were analysed by GC–MS. There was a significant ($P < 0.05$) effect on the growth and production of *O. gratissimum* essential oil for water application rate, nitrogen and the interaction between these two factors. The maximum values of width (8.3 cm), length (11.5 cm) and number of leaves (301.3), leaf dry matter (52.5 g/plant), essential oil content (1.58%) and oil yield (82.9 mL/plant) were obtained in the $I_{110}N_8$ treatment. Twenty compounds were identified in each clove basil essential oils with predominance of phenylpropanoids in the $I_{110}N_8$ treatment and of monoterpenes in the other treatments. A water application rate above field capacity together with nitrogen doses higher than normally recommended improves plant growth and increases the production of its essential oil by modifying the metabolic pathway.

1. Introduction

The essential oils are reported in aromatic plants distributed mainly in the Mediterranean and tropical countries (Pandey et al., 2014). Essential oils as well as their isolated compounds are widely used in cosmetic (Sarkic and Stappen, 2018), food and pharmaceutical industries (Elshafie and Camele, 2017).

The essential oils are complex mixtures of volatile compounds of low molecular weight, which the constituents fall mainly into two distinct chemical classes: terpenes and phenylpropanoids (Moghaddam and Mehdizadeh, 2017). They are produced by various differentiated structures and are usually stored in the trichomes (glandular hairs), epidermal cells (conical-papillate cells), ducts (secretory canal) or cavities (secretory pockets) of plants (Rehman et al., 2016).

Plants collectively produce thousands of essential oils, whose emission and storage allow plants to withstand numerous abiotic and biotic stress conditions and to mediate ecological interactions with the biotic environment (Ormeño and Fernandez, 2012). However, environmental constraints can limit various physiological and metabolic responses like solute and antioxidant accumulation and expression of stress-specific genes, which interfere with essential oils production (Ahanger et al., 2017). The amounts of essential oils produced under stress conditions can be modified, depending on the species and magnitude of the stress (Pradhan et al., 2017).

Under natural conditions, plants rarely experience single abiotic factors one at a time, but are much more likely to be exposed to multiple stressors simultaneously (Castro et al., 2017). The influence of multiple stress factors commonly experienced by plants in field environments is often interactive. Knowledge of how multiple stressors affect secondary metabolite accumulation in plants will provide more information to evaluate the biological roles of these metabolites in mitigating stress and provide criteria for describing their optimum yields and quality (Ncube et al., 2012). Hence, this provides a means of ensuring quality in phytomedicine, which has increased in importance in tropical regions, not just due to its strength as an alternative therapy, but also because of the prevalence of neglected diseases in the local communities (Cheuka et al., 2016).

Ocimum gratissimum L., a dicotyledonous shrub plant, which belongs to the Lamiaceae family, stands out for the quality, quantity, and chemical diversity of the essential oils it produces. These oils have been

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used in applications in the pharmaceutical, cosmetic and food industries. Some of these essential oil compounds have antibacterial, insecticidal and antioxidant properties with high demand on the international market of the fine perfumery industry (Santos et al., 2016). It is also popularly used in herbal medicine for treating several diseases, such as upper respiratory tract infection, fever, piles, cough, diarrhoea and pneumonia (Pandey, 2017).

Despite several studies dealing with the effect of water and nutrient stress on *O. gratissimum* (Osuagwu et al., 2010; Ormeño and Fernandez, 2012; Ade-Ademilua et al., 2013), information about the influence of multiple and interactive stress factors commonly experienced by plants in the field is scarce. In these tropical circumstances, with high potential evapotranspiration rate and soils having low contents of organic matter, crop production is often co-limited by both water and nitrogen availably and inadequate soil management. Consequently, it is estimated that production may be increased by 45–70% for most crops following improvements in water and N management and by understanding their interactions in affecting crop yield (Sadras et al., 2016).

Available water is defined as the difference between the amount of water in a soil at field capacity and the amount of water in a soil at a permanent wilting point (Lopez and Barclay, 2017). Field capacity corresponds to the highest limit of available water and represents the moisture of the soil after drainage of the water contained in the macropores by gravity. This moisture condition favors higher absorption of water and nutrients by plants and therefore has been recommended as content to satisfactory plant growth (Clay and Trooien, 2017).

A sufficiently suitable availability of nitrogen can increase the essential oil contents in *O. gratissimum* due to promotion of terpenoid emissions by rising electron transport rate and leaf photosynthesis, which provide ATP requirements and carbon substrate availability for isoprene synthesis. This is supported by previous studies that reported a direct relationship between photosynthetic carbon products and terpenoid biosynthesis as well as in studies where positive relationships between leaf or soil nitrogen and terpenoid concentration in leaves has been found (Ormeño and Fernandez, 2012). According to Chude et al. (2012) the dose of 90 kg/ha may be recommended for medicinal crops in tropical conditions.

Although water and nitrogen stress have been reported as the main cause of low production of essential oil in crops, largely unpredictable interactions are a fundamental cause of complexity in processes of plant growth and yield of essential oil when affected by environmental stress (Mohidin et al., 2015; Alves et al., 2017; Bortolheiro and Silva, 2017; Fahad et al., 2017; Gong et al., 2018; Khan et al., 2018). This can cause contradictory results of water and nitrogen stress on essential oil production, when treated alone. Implying that the combined effect of various stresses is more diverse, research efforts aimed at understanding this diversity is essential. Therefore, the aim of this work was to evaluate the combined effect of water and nitrogen on yield and quality of the essential oil of *O. gratissimum* in tropical conditions.

2. Materials and methods

2.1. Installation and design of the experiment

The study was carried out in 2016 (September–November) in a greenhouse at Maranhão State University, São Luís, Brazil (2°30′S and 44°18′ W). While conducting the experiment, the mean greenhouse temperature was 28 °C, with maximum temperature of 33.8 °C and minimum temperature of 24.9 °C. The mean relative humidity was 71.4%, with the maximum and minimum relative humidity of 77.3% and 68.2%, respectively.

The soil in the study was classified as Ultisol (Soil Taxonomy: a basic system of soil classification for making and interpreting soil surveys, 1999) and its chemical properties were determined (Carter and Gregorich, 2008), as follows in Table 1.

Table 1
Chemical properties of the soil used in the experiments.

Parameter	Unit	Value
Ca^{2+}	$mmol_c/dm^3$	32.0
Mg^{2+}	$mmol_c/dm^3$	49.0
K^+	$mmol_c/dm^3$	3.30
Al^{3+}	$mmol_c/dm^3$	1.00
H^+	$mmol_c/dm^3$	4.00
Na^+	$mmol_c/dm^3$	5.70
SB	$mmol_c/dm^3$	90.0
CTC	$mmol_c/dm^3$	95.0
MO	mg/dm^{-3}	20.0
P	mg/dm^{-3}	25.0
Ph ($CaCl_2$)		8.30
Na^+/CTC	%	6.00
Al^{3+}/Al^{3+} + SB	%	1.10
V	%	94.7

2.2. Conduction of the greenhouse experiment

The experiment was conducted in plastic pots with a capacity of 10 dm^3 in a completely randomised design with three replicates, a 5 × 3 factorial arrangement, which consisted of five water regimes, taking into a account the percentage of the soil field capacity and three nitrogen doses. The pots were filled uniformly with 10 kg of sieved and homogenised soil. Four seeds of *O. gratissimum* were sown in each vase. To ensure satisfactory development, all plants were fully irrigated until thinning 14 days after sowing, when water application rate and nitrogen fertilization treatments were initiated by keeping two plants per pot. A total of 60% (I_{60}), 70% (I_{70}), 80% (I_{80}), 90% (I_{90}) and 100% (I_{100}) of field capacity (FC) was used. The volumetric soil moisture content at the FC was determined collecting three replicates of soil samples before the planting in volumetric rings with a 100-cm^3 capacity. The samples were saturated, weighed, placed on a tension table and equilibrated at −10 kPa (Thomasson, 1978). This volumetric soil moisture content was considered to 100% FC (I_{100}). The I_{100} treatments were used as controls for adding water at the other water application rate. In order to maintain soil in different water regimes, all pots were weighed and adjusted daily at the same time each day. Nitrogen (as urea source) was applied to roots in doses of 0.0, 2.0 and 4.0 g of nitrogen (N) per pot, which correspond to 0 (N_0), 40 (N_2) and 80 (N_4) kg of N/ha, in four equal fractions at 0, 15, 30 and 45 days after emergence (DAE). We also evaluated an additional treatment with 110% FC (I_{110}) and 8.0 g of N per pot, which corresponds to 160 kg of N/ha (N_8). All plants were harvested between 12:00 a.m. and 2:00 p.m. at 60 DAE, when they showed the highest vegetative growth, that is, in the inflorescence. A voucher specimen (MAR-9037) was deposited in the Herbarium of Maranhão, Biology Department, Federal University of Maranhão, Brazil.

2.3. Evaluation of morphometric parameters

For the morphometric analyses, the following parameters were considered: leaf size (length and width), number of leaves and dry mass of leaves per plant. All determined during the harvest period at 60 DAE. The length and width of the leaves were measured in the middle third of the largest leaves of each plant by means of a ruler graduated in centimetres. The dry mass of the leaves per plant, expressed in grams per plant, was established by weighing after drying all the leaves of the greenhouse plant with air circulation at 40 °C until constant weight.

2.4. Essential oil isolation

All fresh leaves were oven dried at 40 °C until constant weight, crushed and then subjected to hydrodistillation for three hours using a Clevenger-type apparatus, according to the method described in the