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Growing medium amendments effect on growth, secondary metabolites and anti-streptococcal activity of two species of *Plectranthus*

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ABSTRACT

Plants belonging to the genus *Plectranthus* are versatile aromatic perennial shrubs that are commonly used in cuisine, medicine and landscaping. A study was carried out to determine the effects of different growing medium amendments on growth, secondary metabolites and anti-microbial properties of *Plectranthus amboinicus* (Lour.) Spreng and *Plectranthus* 'Nicoletta'. The greenhouse pot-experiment tested three different amendments to Promix-BX^{TM} growing medium potting mix as follows: 120 g of K-humate; 120 g vermicasts; and 1.16 g $N_{20}P_{20}K_{20}$. Rooted cuttings were grown in 670 g of Promix-BX^{TM} potting mix with or without an amendment, and finally harvested after 100 days. Plants grown in the vermicasts had significantly ($P < 0.01$) high fresh weight as compared with plants grown in the K-humate or the $N_{20}P_{20}K_{20}$ but there was no significant ($P > 0.05$) difference between the two species. Vermicasts application significantly ($P < 0.05$) reduced plant mass density but increased specific leaf area index as compared to the $N_{20}P_{20}K_{20}$, K-humate or the Promix-BX^{TM} alone treatment. Overall, cavacrol production was higher in *P. amboinicus* whereas total carotenoid and phenolic production were higher in *P.* 'Nicoletta'. The $N_{20}P_{20}K_{20}$ treatment gave the most yield of secondary metabolites while the Promix-BX^{TM} alone gave the least. Both species had the same Minimum Bactericidal Concentration (MBC) of *ca.* 500 μg/mL and Minimum Inhibitory Concentration (MIC) of *ca.* 250 μg/mL, which were all higher than that of the pure carvacrol (*ca.* 198 μg/mL). In conclusion, the different growing medium amendments had different degree of effect on the growth and phytochemistry of these two *P. amboinicus* and *P.* 'Nicoletta'. Whereas vermicasts was most effective on plant growth and development, $N_{20}P_{20}K_{20}$ enhanced secondary metabolite content. Both species demonstrated similar effect on growth reduction in *Streptococcus pyogenes*. The results of this pot-experiment are only preliminary. Future research should focus on field plot experiments for general conclusion on cultivation practices.

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1. Introduction

Plants belonging to the genus *Plectranthus* (Family: Lamiaceae), large pubescent and succulent aromatic perennial shrubs, are mostly used as indoor ornamental plants in Canada and other places of introduction (Kaliappan and Viswanathan, 2008). In Asia, Africa and the Caribbean Islands, *Plectranthus* species are commonly used as medicinal and ornamental plants, food and fodder (Lukhoba et al., 2006; Taye et al., 2013). The leaves are added to soup and tea in order to infuse the aromatic and medicinal compounds. These plants are used for the treatment of various diseases and medical conditions such as insect and reptiles bites; digestive, genito-urinary, muscular-skeletal and skin diseases; and respiratory conditions (Lukhoba et al., 2006; Chiu et al., 2012). Amongst the different species of *Plectranthus*, *P. amboinicus* also known as Jamaican thyme, Country borage, Indian mint and Mexican mint amongst others (Brako et al., 1995), is the most researched. *Plectranthus* 'Nicoletta' also known as Nico, is an interspecific *Plectranthus* hybrid used as ornamental plant (Jim, 2006) and common in annual gardens in Canada. Comparatively, Nico has received less research attention, and its alternative uses are not well documented.

Abbreviations: $A^{(1\%/1cm)}$, extinction coefficient for a mixture of solvents arbitrarily set at 2500; Abs, absorbance; DW, dry weight (g); Fc, final count; FW, fresh weight (g); G, sample weight (g); Ic, initial count; LA, leaf area (mm2); PMD, plant mass density (mg g-1); R^2, coefficient of determination; SLA, specific leaf area (mm2 g-1); TCC, total carotenoid content (μg g-1); TPC, total phenolic content (g L-1); V, total volume of extract (ml).

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Organic extraction of Jamaican thyme showed high contents of primary and secondary metabolites contents including carbohydrates, proteins, flavonoids, phenolics and terpenoids (Patel et al., 2010). In recent years, there have been many studies on total carotenoid and total phenolic compounds and their relevance to human health and wellbeing including antimicrobial properties. Many studies confirmed that high total carotenoid content has preventive effect on cancers of the lung, head, neck, prostate and breast (Soares et al., 2015; Vieira et al., 2016). Carvacrol is a phenolic compound found in plants including Jamaican thyme (El-Ahmady, 2014; Pinheiro et al., 2015), and is known to be the major active ingredient in plant essential oils that suppresses inflammation, minimize the effect of microbial pathogens in the human body and serve as treatment for diarrhea (Shubha and Bhatt, 2015). Therefore, there is the need to explore the bioactive compounds and antibiotic potential of *Plectranthus* spp. for culinary and health benefits.

Physiologically, plants of *Plectranthus* spp. have good growth performance with respect to seasonal light interception and radiation use efficiency, and can tolerate moderate to high water and nutrient deficit stresses (Taye et al., 2013). As such these plant species are capable of growing under varying environmental conditions. However, not much is known about the growth performance and the bioactivity of the different genotypes following application of chemical fertilizer or organic amendments. More people focus on nutraceuticals and alternative medicine due to the growing interests for organic foods, sustainable environment and concern over harmful effects of chemical residues. Consequently, there has been growing research interests in phytochemical studies and exploitation of potential medicinal and aromatic plants. The present study was carried out to determine the effects of different growing medium amendments on plant growth and secondary metabolites content of two *Plectranthus* spp., namely; *P. amboinicus* and *P.* 'Nicoletta'. Also, to assess the antimicrobial properties of essential oils extracted from these two different species.

2. Materials and methods

2.1. Plant materials

The experiment was carried out in the greenhouse at Dalhousie Faculty of Agriculture campus, Truro between September 2015 and June 2016. The plant materials i.e. *P. amboinicus* (Jamaican thyme) and *P.* 'Nicoletta' (Nico) were obtained from the university greenhouse, and their identities were verified at the Horticulture and Landscape division. A general purpose peat-based potting mix, Promix-BX Mycorise Pro™ with added Mycorrhizal fungi (Premier Horticulture Inc., Pennsylvania USA); dry vermicasts (Gro4 Organics Inc., Toronto ON., Canada); potassium (K) humate (Iron Earth™, Georgetown, Halton Hills ON., Canada); and $N_{20}P_{20}K_{20}$ fertilizer i.e. N_{20} is 20% nitrogen, P_{20} is 20% phosphorus and K_{20} is 20% potassium (Master Plant-Prod Inc., Brampton ON., Canada) were purchased from a local retailer for the study.

2.2. Experimental design

Twelve (12)-cm long apical shoot cuttings (n = 20) of *P. amboinicus* and *P.* 'Nicoletta' obtained from healthy mother plants were dipped in STIM ROOT #2 rooting hormone (Plant Products Co. Ltd., Leamington, ON, Canada) and planted in a flat-tray filled with perlite. The cuttings were exposed to high humidity environment using a mist system for 14 days. The rooted cuttings were transplanted i.e. one plant per 15.2-cm diameter plastic pot filled with approximately, 670 g of Promix-BX™ potting mix. The experimental design was complete randomized design with five replications. The pots were randomly assigned using Minitab v. 17. The Promix-BX™ growing medium potting mix treatments were: control (i.e. no amendment); 120 g of K-humate; 120 g vermicasts; and 1.16 g $N_{20}P_{20}K_{20}$. These application rates were based on manufacturers' recommendations specified on the individual product labels. These treatments were applied in four equal splits at 3-week interval with the first application done at one week after transplanting. In addition, 22.3 g of rock powder was added to each treatment pot to supplement for micronutrients. Each amendment was incorporated into the Promix-BX™ growing medium potting mix. Greenhouse environment conditions were set at 12-h light cycle, 118*10 FC luminous intensity, and 25 °C in the day and 17 °C at night. The plants were watered when the top 3- to 5-cm depth of the growing medium was dry.

2.3. Plant growth

Leaf elongation rate was determined by recording the changes in leaf length every four days until the variation in length was ≤2%. The number of branches recorded at 2 weeks after transplanting of rooted cuttings and at harvest were used to estimate percentage branching. Leaf area was recorded with Li-3100C Leaf Area meter (Li-Cor Inc., Lincoln Nebraska, USA). Leaf greenness (i.e. chlorophyll content) was determined using SPAD 502 Chlorophyll meter (Spectrum Technologies, Inc., Aurora IL, USA). Final harvest was done at 100 days after planting. Plant fresh weight was recorded for each sampled plant per treatment followed by dry weight determination. Leaf tissue samples were oven-dried at 65 °C (model 52100-00; Cole-Parmer Canada Co., Montreal QC, Canada) until equilibrium moisture content was reached, and then weighed. The dried leaf tissues were ground to powder using a coffee grinder, and stored in preservative bags at room temperature for further analysis.

Percentage branching, plant mass density and specific leaf area were calculated using the following formulas from Cornelissen et al. (2003):

$$\%Branching = \frac{Fc - Ic}{Fc} * 100 \quad (1)$$

$$PMD = \frac{DW}{FW} \quad (2)$$

$$SLA = \frac{LA}{DW} \quad (3)$$

2.4. Carvacrol content assay

Dried ground sample (2 g) of each species was placed in individual clean labeled brown borosilicate glass vial with Teflon lined caps and then 10 mL hexane was added. Each sample was incubated at room temperature for three days with occasional shaking. The hexane extract was removed using a Pasteur pipet, and then passed through sodium sulphate containing glass wool pipet filter. Nitrogen was used to evaporate the hexane, then the dried sample was dissolved in 1 mL hexane for further analysis.

The carvacrol content of the hexane extracted samples were analyzed by Gas Chromatography-Flame Ionization Detector (GC-FID) using a Bruker 430 GC-FID (Bruker, Billerica, MA, USA). One microliter of each sample was injected on a BR–1 ms fused silica capillary column with 15 m × 0.25 mm internal diameter, and the film thickness was 0.25 μm (Bruker, Billerica, MA, USA) at a split ratio of 25:1. Helium was used as the carrier gas at a flow rate of 1 mL/min, and as a make-up gas to the detector at 29 mL/min. The flow rates of hydrogen and air to the detector were 30 mL/min and 300 mL/min, respectively. The FID temperature was set at 270 °C. Carvacrol content was analyzed by running an oven temperature program with a total run time of 23.5 min.

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