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Growth, Essential Oil Content and Antimicrobial Activity of Basil Influenced by Nitrogen Fertiliser and Plant Density

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ABSTRACT

A field experiment was conducted to investigate the effect of different nitrogen (N) rates and plant densities on vegetative growth, yield and essential oil content of basil (*Ocimum basilicum* L.). Three N rates (0, 60 or 120 kg N ha^{-1}) and three plant densities (6,250, 12,500 and 25,000 plants/ha) were assigned to main plots and sub-plots respectively in a split plot design with three replications. Basil essential oil was further evaluated for its *in vitro* effect on *Meloidogyne incognita* and *Fusarium oxysporum*. N fertilization up to 120 kg ha^{-1} significantly ($P > 0.05$) increased yield of above-ground (40.1 t/ha) and leaf fresh biomass (122.6 t/ha), leaf essential oil yield (9.3%), but it did not affect plant height and the number of branches per plant. The increase in essential oil yield induced by N fertilization was related to leaf biomass. Plant density significantly increased the above-ground biomass yield and the plant N content but did not affect plant height and essential oil content. The highest above-ground biomass yield (47.6 t/ha) and plant nitrogen content (2.7%) were recorded with a density of 25,000 plants/ha. Basil essential oil completely prevented egg hatching in *M. incognita* at all concentrations tested while the mycelium growth of *F. oxysporum* was significantly reduced as oil concentration increased. The lowest mycelium growth of 1.5 mm was recorded on plates with 40% essential oil.

Keywords: above-ground fresh biomass, antifungal, essential oil, *Fusarium oxysporum*, nematicidal, *Meloidogyne incognita*, *Ocimum basilicum* L.

INTRODUCTION

Basil (*Ocimum basilicum* L.) is a spice of high economic potential due to its numerous uses. The essential oil (EO) is the most important component of the crop and the oil ratio varies between 0.1 and 0.45% based on climate and soil conditions (Olcay and Emine 2004; Chalchat and Ozan 2008; Kimankara *et al*. 2008; Chang *et al*. 2009; Soran *et al*. 2009; Hamid *et al*. 2011). The EO and oleo-resin may be extracted from leaves or flowers and used for flavouring in liqueurs and for fragrance in perfumes and soaps. However, scientific studies have established that these EOs have potent antioxidant, anti-cancer, anti-viral, and anti-microbial properties (Chiang *et al*. 2005; Bozin *et al*. 2006; Manosroi *et al*. 2006; de Almeida *et al*. 2007; Koba *et al*. 2009; Hamid *et al*. 2011; Hannif *et al*. 2011). In India, it is traditionally used for supplementary treatment of stress, asthma and diabetes (Dube *et al*. 1989). It is also used in the treatment of cold, cough and other respiratory infections. In addition, the plant is used as an insecticide, flea and moth repellent (Lopez *et al*. 2008). This crop of global importance is also a widely consumed spice in Nigeria but its cultivation is neither widespread nor extensive. Production is often restricted to small home gardens. At present, the full potential of the crop for export is yet to be exploited because agronomic practices that influence the quality and quantity of the EO have not yet been studied. Also, many studies have been reported on the effect of basil EO on the growth of human pathogens although there is limited information on their effect on plant pathogenic organisms. Root knot nematode (*Meloidogyne* spp.) is widely distributed causing severe losses on susceptible crops wherever they are cultivated (Trudgill and Blok 2001). *Fusarium oxysporum* is a soil-borne pathogen causing severe losses, especially on Solanaceous crops (Tanovic *et al*. 2007). Control of this pest and pathogen by synthetic chemicals, although effective, is costly, not readily available and frequently environmentally hazardous. This has spurred the search for alternatives to synthetic chemicals. This study therefore aimed to assess the growth and yield performance of basil at different nitrogen (N) rates under different plant densities. It also investigated the toxicity of basil EO on the eggs of *M. incognita* and mycelium growth of *F. oxysporum*.

MATERIALS AND METHODS

The experiment was conducted at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria in May, 2008. Ibadan is located at 7° 30′ N latitude and 3° 54′ E longitude with an altitude of 168 masl. The site has an annual minimum and maximum temperature of 21 and 32°C, respectively. Mean monthly relative humidity ranges between 61 and 83%. The soil is a sandy loam; soil characteristics of the experimental plots are presented in **Table 1**.

Experimental design and agronomic practises

The experiment consisted of a factorial combination of three N levels (0, 60, 120 kg N/ha) and three plant densities (6,250, 12,500 and 25,000 plants/ha). Treatments of N levels were assigned to the main plots and plant density to sub-plots in a split plot design with three replications. Each plot size was 2 × 2 m, while the spacing between plots and blocks was 1 and 2 m, respectively. Seedlings 10 cm in height, previously raised in trays containing a mixture of topsoil and cured poultry manure (1:1) were transplanted into the field. Each plot consisted of 5 rows of basil seedlings.

Data on plant height, number of branches and leaves was monitored weekly on plants in the 3 middle rows, from which plants were harvested to determine the above-ground fresh biomass yield, 49 days after transplanting, at full bloom and analysed for EO production and percentage plant N. This stage is considered to be the most appropriate for commercial harvest of basil

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Table 1 Soil physico-chemical properties before the experiment.

Characteristics	
pH	6.5
Organic carbon (g.kg^{-1})	0.88
Total nitrogen (g.kg^{-1})	0.94
Available phosphorous (mg.kg^{-1})	5.82
Exchangable bases (Cmol.kg^{-1})	
Ca	0.09
Mg	0.15
Na	0.04
K	0.09
Exchangable bases	
Effective CEC	0.45
Mn (mg.kg^{-1})	28.58
Fe (mg.kg^{-1})	8.87
Cu (mg.kg^{-1})	0.60
Zn (mg.kg^{-1})	2.63
Particle size analysis (g.kg^{-1})	
Sand	810
Silt	120
Clay	70

for EO production (Basker and Putievsky 1978). EO yield and composition were determined from 20 g of dried leaves by hydro-distillation (Guenther 1972). Experimental data was statistically analysed by analysis of variance (ANOVA) using SAS PROC GLM (2002) at $P < 0.05$. Differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$.

Nematicidal and antifungal activity

This trial was conducted in the laboratory and consisted of four concentrations of basil EO (0, 10, 20, and 40%; v/v). Two treatments were used as control, distilled water (0%) and ethanol control which had 4ml of ethanol, the EO extracting solvent and 16 ml distilled water. *M. incognita* eggs were extracted from naturally infected *Celosia argentea* plants. One ml of each concentration was dispensed into a transparent cup containing approximately 50 freshly-laid *M. incognita* eggs. Percentage egg hatch was measured daily for 10 days. One ml of each concentration was mixed with 9 ml of sterile PDA. Thereafter, 6 mm mycelium of *F. oxysporum* grown on PDA for 7 days was used to inoculate each plate. Four plates were inoculated per treatment. Plates were incubated at room temperature for 5 days. Mycelium diameter was measured after 5 days. Data was statistically analysed by analysis of variance (ANOVA) using SAS PROC GLM (2002) at $P < 0.05$. Differences between means were assessed using the least significance difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Performance of vegetative and chemical parameters as influenced by nitrogen fertilization and plant density

The effect of N fertilization and plant density on the vegetative growth of basil is shown in **Table 2**. N fertilization significantly ($P < 0.05$) affected the number of branches and leaves/plant, above-ground fresh biomass yield, leaf fresh biomass, but not plant height (**Table 2**). Generally, these parameters increased as N fertilization level increased. The highest biomass yield was attained at 120 kg/ha. Sifola and Barbieri (2006) also found N fertilisation up to 300 kg/ha increased above-ground biomass, leaf fresh biomass but not plant height. Density had a significant effect on above-ground biomass and fresh leaf biomass but no significant effect on all other parameters measured (**Table 2**). The above-ground biomass also increased significantly with higher plant density. The highest biomass yield (47.6 t/ha) was recorded with a density of 25,000 plants/ha. This indicates that an increase in plant density led to an increase in biomass yield. Spacing significantly affected density and yield-related parameters in different medicinal and aromatic

Table 2 Effect of nitrogen fertilization and plant population of the vegetative character, biomass yield and oil content of basil.

Treatment	Plant height (cm)	No. of branches	No. of leaves	Above-ground biomass yield (t/ha)	Fresh leaves biomass yield (t/ha)
Nitrogen (t/ha)					
0	60.3	19.3	314.89	25.73	87.78
60	60.8	21.2	322.67	32.86	110.73
120	57.6	20.5	346.33	40.09	122.63
LSD (5%)	NS	1.31	111.67	4.70	13.8
Population (plants/ha)					
6,250	58.81	20.11	368.33	19.49	139.29
12,500	59.26	19.56	363.44	31.58	104.18
25,000	60.63	21.2	252.11	47.61	177.7
LSD (5%)	NS	1.31	111.67	4.70	13.8
P × N	NS	NS	NS	***	***

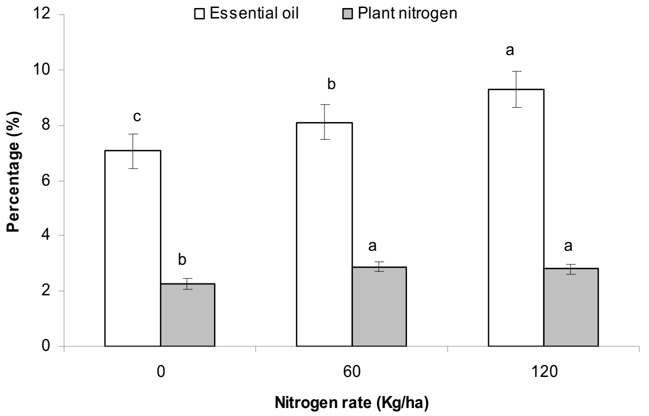

Fig. 1 Effects of nitrogen fertilization on the essential oil and plant nitrogen contents of basil. Means of the same parameter followed by the same letters are not significantly different ($P < 0.05$) using DMRT. n = 9

plants: Japanese mint (Solomon and Beemnet 2011), peppermint (Beemnet *et al*. 2011), artemisia (Zewdinesh 2009), mott elephant grass (Yasin *et al*. 2003) and thyme (Al-Ramamneh 2009). The results of these studies show the significance of identifying optimum requirements of N fertilization and spacing (*syn*. density) for aromatic plants in different parts of the world before initiating commercial economic production. The percentage EO content and plant N increased as N fertilization increased (**Fig. 1**). The increase in leaf N content with increased fertilization also suggests an improvement in the mineral content of fertilized plants compared to unfertilized ones. The increase in EO yield due to N fertilization depended not only on an increase in leaf biomass, but also on an increase in leaf EO concentration, presumably indicating enhanced oil biosynthesis (Sangwan *et al*. 2001). N application generally increases EO yield in aromatic plants by enhancing the amount of biomass yield per unit land area, leaf area development and photosynthetic rate (Ram *et al*. 1995; Meneghini *et al*. 1998; Rao 2001; Sangwan *et al*. 2001). Higher photosynthetic rates occur in crops when the relative investment of leaf N to chlorophyll (Chl/N) had been increased by N fertilization (Mae 1997; Ranjith and Meinzer 1997). Density on the other hand had no significant effect on the EO content of basil but higher densities of 12,500 and 25,000 plants/ha had significantly higher plant N (**Fig. 2**). This result is in agreement with previous reports of Beemnet *et al*. (2011) for peppermint and Sanini *et al*. (2002) for Japanese mint. Density × N interactions were significant for biomass yield, fresh leaf yield, percentage oil content and plant N. These results suggest the enhancement of yield and EO content with N fertilization. These results also indicate that N fertilization and plant density play a large role in the cultivation of basil. Therefore, some of the major economical traits of basil, such as leaf and EO yield, can be im-

Table 3 Effect of basil essential oil on the egg hatch of *Meloidogyne incognita*.

Days (% egg hatch)										
Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Water control	2.5	11.5	21	34.5	42	44	49.5	61	76.5	81
Ethanol control	0	1	7.5	13	18	20.5	22.5	26.5	33.3	40
10%	0	0	0	0	0	0	0	0	0	0
20%	0	0	0	0	0	0	0	0	0	0
40%	0	0	0	0	0	0	0	0	0	0
SE	0	0.86	1.28	0.72	0.64	0.82	0.63	1.08	0.86	0.93

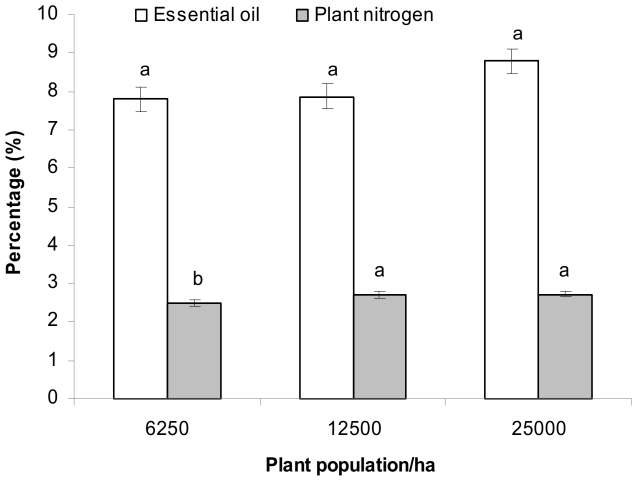

Fig. 2 Effects of plant population on essential oil and plant nitrogen of basil. Means of the same parameter followed by the same letters are not significantly different ($P < 0.05$) using DMRT. n = 9

proved through proper manipulation of N application and plant density.

The EO of basil inhibited the hatching of *M. incognita* eggs (**Table 3**). No eggs hatched at all concentrations tested. However, eggs hatched were recorded in the two controls with ordinary water recording higher % egg hatching than the ethanol suspension control. Sangwan *et al*. (1990) in their review reported that basil EO has nematicidal activity. Plant EOs and their constituents have been suggested as alternative sources for nematode control (Isman 2000; Chitwood 2002). Various compounds, including alcohols, aldehydes, fatty acid derivatives, terpenoids and phenolics exist in these EOs and jointly or independently they contribute to nematicidal activity (Chitwood 2002). The effect of basil EO on the growth of *F. oxysporum* is shown in **Fig 3**: 24 h after plating, plates with EO recorded significantly lower mycelium growth; least growth (0.1 mm) was recorded with plates containing 40% (v/v) EO. Control plates impregnated with only water had the highest mycelium growth (1.4 mm). This observation occurred throughout the study period up until 120 h. Generally, an increase in EO percentage resulted in a significant reduction in mycelium growth. These inhibition effects depend on the qualitative and quantitative composition of EOs. Reuveni *et al.* (1984) studied the fungistatic activity of EOs from *Ocimum basilicum* chemotypes against *F. oxysporum f. sp. vasinfectum* and *Rhizopus nigricans*. The antifungal activity of basil EO was determined mainly by the percentage of the main components: cineol, linalool, methylchavicol and eugenol. Dube *et al.* (1989) also investigated the antifungal activity of basil EO, with the ability of killing aflatoxin-producing strains of *Aspergillus flavus* and *Aspergillus parasiticus*. The nematicidal and antifungal potential of basil EO has been demonstrated.

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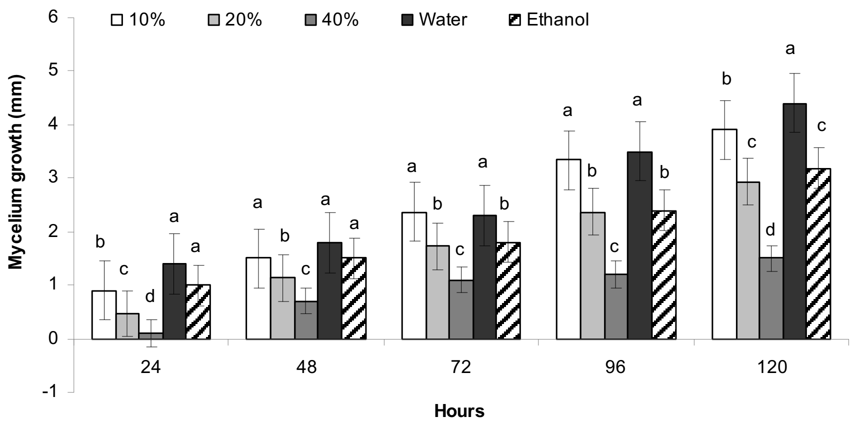

Fig. 3 Effect of basil essential oil on the mycelium growth of *Fusarium oxysporum.* Treatment means followed by the same letters are not significantly different ($P < 0.05$) using DMRT. n = 4

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