Original Research Article

Tithonia diversifolia powder and compost ameliorates plant growth parameters, essential oils, phenols and flavonoids content and anticandida activity of *Ocimum gratissimum* L.

ABSTRACT

Aims: *Ocimum gratissimum* is an aromatic and medicinal plant, well known for its medicinal values such as antifungal properties. This study aimed at evaluating the effect of *T. diversifolia* powder and compost use as bio-fertilizer on the growth parameters, essential oil (EO), total phenolic and flavonoid content and anticandida activity of *O. gratissimum.*

Study design: *O. gratissimum* plants were cultivated for 8 months in an experimental farm designed as a split plot into 4 blocks. Each block was amended (main factor) either with *T. diversifolia* compost (150g/plant), powder (40g/plant) or the synthetic fertilizer NPK (10g/plant) respectively, followed by sprayed (second factor) with same fertilizer at 20g/L, 20g/L and 2g/L or water every two weeks after transplantation. The control block received no amendment and was sprayed with water or the previous fertilizer.

Place and Duration of Study: This work was carried out within August 2019 to October 2020 in Yaoundé-Cameroon.

Methodology: Plant growth parameters (fresh and dry leaves weight, florescent weight and plant height) were evaluated at four and eight months after transplantation. The harvested fresh leaves were hydro-distillated for EO and the hot aqueous extract. Both extracts were used for the evaluation of the anticandida activity while the latter was submitted to total flavonoids and phenolic analyses.

Results: At 4 and 8 months after transplantation, the synthetic fertilizer and *T. diversifolia* compost significantly increased plant growth parameters as compared to other treatments. The plants treated with *T. diversifolia* bio-fertilizer compost showed the highest total phenolic (53.16µg GAE/µL), flavonoid (36.32µg/ GAE/µL) content, and EO yield (0.666%). The EO from *O. gratissimum* treated with *T. diversifolia* compost showed the best inhibitory activity on *C. albicans* NR-29451.

Conclusion: This study showed that *T. diversifolia* compost was a promising organic fertilizer in optimizing the growth, secondary metabolites and anticandida activity of *O. gratissimum.*

Keywords: Tithonia diversifolia, plant growth parameters, secondary metabolites, Ocimum gratissimum, Anticandida bioactivity.

3. Research Papers and Short Notes should follow the structure of Abstract, Introduction, Methodology, Results and Discussion, Conclusion, Acknowledgements, Competing Interests, Authors' Contributions, Consent (where applicable), Ethical approval (where applicable), and References plus figures and/or tables.)

1. INTRODUCTION

Today medicinal plants are important to the global economy, as well as source of income for rural people in developing countries. About 70% - 80% of the people worldwide rely on medicines based on plants for their primary health care needs. These plants constitute a great reservoir of a wide variety of compounds with medicinal and nutritive properties

(Oswagwu and Edeoga, 2012). However the increase, demand for herbal medicines has led to over exploitation for active constituents (Sheelu *et al.,* 2017). On the other hand, the natureof soil fertility characterized by low nitrogen and phosphorus content in the tropical regions remains a major constraint to agricultural production including medicinal plants (Michel *et al.,* 2016). Consequently, there is an increase use of synthetic fertilizer in cultivation of plants (Graveson *et al.,* 2008; Oswagwu, 2008). This practice is commonly accompanied with decrease of soil conditions such as chemical nitrogen stimulates excessive microorganism growth which over time depletes organic matter in the soil and soil acidification. Also high application of synthetic chemical fertilizer leads to accumulation of nitrate or nitrite in plant parts, generation of harmful greenhouse gases (Bhattacharyya *et al.,* 2016).
Organic agricultural practices have been encouraged as an alternative to the increase use of synthetic nitrogen fertilizers (Lotter, 2003). *Tithonia diversifolia* is an Asteraceae family member widely cultivated as ornamentals in many sub-tropical regions. It has been used as a biopesticides and biofertilizer to improve the soil fertility, enhance the availability of minerals/nutrients, and increase the crop yields (Jama *et al.,* 2000; Van Sao *et al.,* 2010; Mwine *et al*., 2011). Though many studies have shown the use of *T. diversifolia* as a bio-fertilizer and bio-pesticide in agriculture, very limited studies have assessed its effects and potential in optimizing medicinal plants yield as well as the properties of the resulting medicinal components. *Ocimum gratissimum* is an herbaceous medicinal plant which belongs to the Labiacae family. The plant is indigenous to tropical areas. It has been used extensively in the traditional system of medicine in many countries. In the North east of Brazil, it is used for medicinal, aromate and culinary purposes. *O. gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, wounds, fungal infections, fever, cold and catarrh (Ijeh *et al.,* 2005). The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, headache, eye diseases, skin diseases, pneumonia and cough (Adebolu *et al.,* 2005). The high demand of this plant therefore poses a serious treat in its availability, calling for the necessity of improving its cultivation. This study therefore aimed at improving the medicinal properties of *O. gratissimum* by evaluating the effect of *T. diversifolia* bio-fertilizer and compost on the growth parameters, total phenolic and flavonoid contents and antifungal activity of *O. gratissimum.*

2. MATERIALS AND METHODS

2.1. Study period and site

This work was carried out within August 2019 to October 2020. Field experiments were done in an agricultural experimental farm in Emana, situated in Mfoundi division, Centre region of Cameroon with the geographic localization of 3^{o}55'0" North, 11^{o} 31' 0" East.

2.2. Plant materials

The seeds of *O. gratisimum* were obtained by trashing the seeds from the flowering parts of the plant. *T. diversifolia* leaves were harvested from an experimental farm at IRAD Nkolbison, in Mfoundi division, Centre region of Cameroon. (Add the voucher number of each plant) The fresh plants of *T. diversifolia* were cleaned, dried at room temperature, chopped and finely grinded into powder.

2.3. Chemicals and fungal strains

The synthetic fertilizer (NPK 23-10-5 + $10SO_3^{2-}$) was bought from an agricultural shop in Yaoundé (Mfoundi division, Centre region of Cameroon). Folin-Ciocalteu reagent, Hydrochloric acid, Formaldehyde, Gallic acid, Sodium carbonate (Na_2CO_3), absolute methanol, absolute ethanol (≥ 99.8 % purity) were obtained from incomplete sentence. Dimethyl Sulphoxide (DMSO) and the Sabouraud dextrose Broth (SDB) were purchased from Gaylord chemical company and HiMedia industries, respectively. The candida strains namely *Candida albicans* NR-29451, *Candida albicans* NR-29445 and *Candida albicans* NR-29444.

2.4. Preparation of *T. diversifolia* compost

T. diversifolia compost was prepared using a modified method described by Abad *et al.* (1997) and Akanbi (2002). Briefly, *T. diversifolia* powder was mixed with sawdust in a drum in the ratio of 1:3, and enough water was added to moisten the mixture. The drum was then covered and incubated at 37^{o}C for six months for maturation of compost. It is worth noting that the mixture was macerated every two weeks to increase aeration.

2.5. Preparation of extracts for spray

Spray was performed in order to constantly supply plant leaves with nutrients. For this; 20g of the powder of *T. diversifolia*, 20g of the compost of *T. diversifolia* and 2g of NPK were each dipped into three separate containers containing 1L of water for 24 hours. After 24hours, each container was macerated, filtered with mousse line cloth of 25µ and re-dipped into water for another 24 hours. The mixture was then filtered and the resulting extract was used for spraying the plants on their respective blocks in the farm.

2.6. Experimental design

The experimental design was a split plot design with three replications consisting of 4 blocks with 6 sub-blocks per block. Nine (09) *O. gratissimum* seedlings were transplanted per sub-block at 0.75cm apart. The soil amendments on the 04 blocks included(the main factor) a one-time fertilization with *T. diversifolia* compost (TDC) at 150g/plant or 6060kg/hectare, *T. diversifolia* powder (TDP) at 40g/plant or 1600kg/hectare, the synthetic fertilizer (NPK 23-10-5 + 10 SO3) at10g/plant or 400kg/hectare and the negative control block without fertilization. The planting was in rows of 50 cm apart and 50 cm between hills (40401plant/hectare) for *O. gratissimum*. Plants were also treated by spraying plants (secondary factor) on each block with the same fertilizer used for soil amendment every two weeks after transplantation. Weeding was done every 2 weeks to maintain optimum agricultural conditions of the plants. Plant samples were harvested 4 and 8 months after transplantation and the plant height, fresh weight of herbage, dry weight of herbage and florescent weight were measured **(**Bouvry, 2017). Thereafter, portion of harvested plant leaves was kept fresh while the other was air dried and used extraction of essential oil and hot aqueous extract which were obtained using the hydro distillation method. The yield of essential oil per fresh plant weight and per hectare as determined by Essential oil percentage = (Essential oil weight/plant fresh weight) x 100.
The essential oil yield per plant (ml) was estimated as follows: Oil percentage x density x plant fresh weight.
The essential oil yield per hectare (L) was calculated using the formula: Oil yield per plant x number of plants/ha.

2.7. Determination of the total phenolic and flavonoid content

Total phenolic
The total phenolic content was quantified using the method described by McDonald et al. (2001). One hundred microliters (100µL) of 10% Folin-ciocalteu reagent were added to 20µLof 4 times diluted medicinal plant extract, and after brief homogenization the mixture 80µL of 20% Na_2CO_3were added. After one hour of incubation at room temperature, the absorbance of the blue coloured complex formed was read at 725 nm alongside. Calibration curve was done using gallic acid solution (0.063 to 2.0 mg/mL). The results were expressed in µg equivalence of gallic acid per microliters of extract (µg GAE/µL extract).
Flavonoids
The flavonoid content was precipitated from extracts by adding 80µL of 4 times diluted medicinal plant extract to 40µL of 50% HCl in 40µL formaldehyde (precipitation of flavonoids was in order to get the phenolic content without flavonoids to be used in calculating the quantity of flavonoids present in the sample). The mixture was incubated for 24h at room temperature then filtered using Falten filter papers. The filtrate obtained represented the phenolic content without flavonoids and were quantified using the method described by McDonald et al. (2001). The flavonoid content was then determined using the formula: Total Flavonoïde= T_p (Total Phenols) – T (Phenols without flavonoids).

2.8. Evaluation of the anti-fungal activity of *O. gratissimum* hot aqueous extract and essential oil

The minimum inhibitory concentration (MICs) and the minimum fungicidal concentration (MFC) of the essential oil and extracts of *O. gratissimum* were carried out by the broth microdilution method in 96-well microtiter plates according to the CLSI M27-A3 methodology (CLSI, 2008). Briefly, 100 µL of Sabouraud Dextrose Broth (SDB) were introduced into the wells, and. 100 µL of either hot aqueous extract (0.39 to 25 mg/mL), essential oils (0.156 to 10mg/mL) or Nystatin (positive control; 0.046 to 3 mg/mL) were added into the respective wells. Then, 100 µL of the fungal suspension (5 x10^3 CFU/mL) were introduced into the wells. After 48 hours of incubation at 37°C.MIC was defined as the lowest concentration inhibiting the visible growth of yeasts. All tests were performed in triplicate.

2.9. Statistical analysis

The results obtained were presented as means ± standard deviations. One way Analysis of Variance (ANOVA) test coupled with the Newman Keuls t-Student test were used for comparison of the mean and differences between means were considered significant at 95% confidence level. Analyses were performed using Statistical Package for Social Sciences (SPSS) software version 22.0.

3. RESULTS AND DISCUSSION

3.1. Vegetative growth parameters

In general, plants treated fertilizers and sprayed; obtained at either 4 or 8 months cultivation displayed better growth parameters as compared to other treatments. In the first harvest (4months after transplantation), plants from fertilization and spray displayed better growth parameters as compared to other treatments. Interestingly, the leaf dry weight and inflorescence weight from plants receiving both NPK fertilization and spray with (34.11 ± 9.26g and 25.44± 2.68g, respectively) were higher (P<0.05) with regard to the other treatments.

In the second harvest (8months after transplantation), plants both fertilized and sprayed with TDC organic fertilizer (FS_{TDC}) significantly (P<0.05) increased the fresh leaves weight (310.00 ± 31.51g), dry leaves weight (207.47 ± 14.51g), inflorescence weight (131.24 ± 16.12g) and plant height (156.78 ± 10.46cm)as compared to other treatments.

Table 1: Growth parameters of *O. gratissimum* at 4 and 8 months after transplantation

Treatment/ parameters	Leaves fresh weight (g)	Leaves dry weight (g)	Fluorescence weight (g)	Plant height (cm)
4 months after transplantation				
Control	18.46±7.08^{a}	6.94±3.13^{a}	7.69±1.74^{a}	39.00±7.94^{ab}
Sprayed only(S)				
S_{TDP}	30.74±5.33^{ab}	7.50±1.71^{a}	10.76±1.29^{ab}	30.33±5.13^{a}
S_{TDC}	56.13±15.53^{b}	14.51±4.15^{a}	12.99±1.64^{b}	50.33±12.66^{abc}
S_{NPK}	47.42±10.26^{ab}	16.81±2.45^{a}	16.62±2.53^{c}	34.33±4.04^{a}
Fertilization only(F)				
F_{TDP}	16.71±4.13^{a}	4.72±1.02^{a}	9.82±2.32^{ab}	40.67±12.50^{ab}
F_{TDC}	47.27±19.72^{ab}	8.62±4.01^{a}	18.71±2.39^{cd}	69.33±10.12^{cd}
F_{NPK}	52.60±27.99^{b}	15.58±7.08^{a}	17.81±0.83^{c}	57.69±8.33^{bcd}
Fertilization + Spray (FS)				
FS_{TDP}	30.31±4.44^{ab}	5.75±0.65^{a}	11.06±0.60^{ab}	44.33±6.66^{ab}
FS_{TDC}	81.73±3.82^{c}	13.71±0.91^{a}	21.92±2.09^{d}	74.00±6.25^{d}
FS_{NPK}	97.05±7.03^{c}	34.11±9.26^{b}	25.44±2.68^{e}	59.67±11.59^{bcd}
8 months after transplantation				
Control	68.12±6.54^{a}	50.89±5.46^{a}	20.44±2.84^{a}	61.38±4.46^{a}
Sprayed only (S)				
S_{TDP}	91.35±1.85^{abc}	67.01±2.31^{ab}	43.90±3.56^{bc}	74.32±7.90^{ab}
S_{TDC}	95.39±4.11^{abc}	73.82±3.33^{ab}	50.43±7.19^{bc}	76.50±9.26^{ab}
S_{NPK}	74.32±4.57^{ab}	53.51±7.41^{a}	46.49±7.43^{bc}	68.13±4.28^{ab}
Fertilization (F)				
F_{TDP}	118.41±7.61^{cd}	101.03±11.04^{ab}	34.38±6.20^{b}	71.17±7.20^{ab}
F_{TDC}	202.03±13.15^{e}	145.59±13.86^{c}	103.75±9.25^{d}	95.49±7.25^{c}
F_{NPK}	96.35±6.26^{abc}	87.42±52.72^{ab}	58.89±5.48^{c}	84.60±4.45^{bc}
Fertilization + pulverisation (FS)				
FS_{TDP}	139.02±22.64^{d}	105.47±14.91^{b}	58.27±0.50^{bc}	69.96±7.04^{ab}
FP_{TDC}	310.00±31.51^{f}	207.47±14.51^{d}	131.24±16.12^{e}	156.78±10.46^{d}
FP_{NPK}	103.57±6.48^{bc}	84.16±7.02^{ab}	61.36±10.84^{c}	92.80±4.05^{c}

Data are means ± standard deviation for three repetitions. Averages with the same letter in the column are not significantly different at p<0.05.

Control = Negative control (non-amended soil and no spray) ,S_{TDP}: non-amended soil and spraywith extract of *T. diversifolia* powder, S_{TDC}= non-amended soil and spray with extract of *T. diversifolia* compost, S_{NPK}: non-amended soil and spray with extract of NPK, F_{TDP}: amended soil with *T. diversifolia* powder and no spray, FS_{TDP}: amended soil with *T. diversifolia* powder and spray with extract of *T. diversifolia* powder, F_{TDC}: amended soil with *T. diversifolia* compost and no spray, FS_{TDC}: amended soil with *T. diversifolia* compost and spray with extract of *T. diversifolia* compost, F_{NPK}: amended soil with NPK and no spray, FP_{NPK}: amended soil with NPK and spray with extract of NPK. The extracts are prepared at 2% (20g/L) for *T. diversifolia* powder and compost and 0.2% (2g/L) for NPK.

Fertilization is one of the most important factors that affect crop yield and plant health (Nawal *et al*., 2014). The present findings showed the importance of combined fertilization through roots and plant spray on the optimum increase of *O. gratissimum* vegetative growth parameters. Foliar applied nitrogen, most frequently is much more quickly and effectively assimilated and used by plants. This relationship results from the greater permeability of the membranes of the leaf cuticle for urea and other organic molecules than in the case of inorganic ions (Wójcik, 2004). Foliar application of nitrogen, with its quick absorption enhances nutrient uptake from the nutritional environment, thereby increasing the pool of macro and micronutrients in the plant (Renata, 2013).

The highest growth parameters were recorded 8 months after transplantation from plants treated with *T. diversifolia* compost. The least growth parameters were recorded from control plots with no fertilization. The poor development of vegetative growth parameter (leave weight, plant height and inflorescence) observed in control treatment confirmed the report of Agyei and Bayor, (2017). Akanbi (2002) reported that nutrient availability especially nitrogen determine plant vegetative growth such as leaves, branches and plant height. Dongmo *et al*. (2020) reported Phosphorus as an essential element for plant life and one of the three major elements with an effect on root growth. *T. diversifolia* biofertilizer may have increased the availability of soil nutrients for the absorption and utilization by the *O. gratissimum* plants resulting in higher parameters as compared to those that were not treated with *T. diversifolia* biofertilizer. Biofertilizer application improved plant growth parameters due to the constant supply with nutrients as compared to the synthetic fertilizer (Nguefack *et al*., 2020). Similar results also demonstrated improvement in growth parameters of carrots, maize and sesame yield treated with *T. diversifolia* derived manure (Kaho *et al.*, 2011; Babajide *et al.*, 2012; Jeptoo *et al.*, 2013).

3.2. Essential oil yield

Essential oil from leaves harvested from *O. gratissimum* plants treated with TDC had the highest percentage yield (0.666%), yield per plant (1.169 mL) and yield per hectare (47.228 L) with respect to other treatments (Table 2).

Table 2: Essential oil yields of *O. gratissimum* from different treatments

Treatment	**Control**	**TDP**	**TDC**	**NPK**
Essential oil yield (%)	0.252	0.422	0.666	0.315
The essential oil yield per plant (ml)	0.137	0.408	1.169	0.262
The essential oil yield per hectare (L)	5.534	16.483	47.228	10.585

Data are means ± standard deviation for three repetitions.
Control= Negative control; TDP: plants treated with *T. diversifolia* powder; TDC: plants treated with *T. diversifolia* compost; NPK: plants treated with NPK. The extracts are prepared at 2% (20g/L) for *T. diversifolia* powder and compost and 0.2% (2g/L) for NPK.

Treatment of *O. gratissimum* plants with *T. diversifolia* bio fertilizer resulted in an increases yields of essential oils. Nutrition plays a key role in plants biosynthesis including essential oils (Aziz *et al.,* 2010; Jabbari *et al.,* 2011). Moreover, the biosynthesis of essential oils, is dependent on a number of factors such the use of bio-fertilizers among others, the presence of different input substances and enzymes, and the metabolic pathway in which a given group of compounds is formed (Dubey *et al.,* 2000; Ganjewala and Luthra, 2007; Woronuk *et al.*, 2011). *T. diversifolia* compost thanks to its richness in nitrogen and minerals (P, K, Ca, Mg) (Dongmo, 2020), therefore disposes in sustainable manner more nutrients essential for the biosynthesis of essential oils by *O. gratissimum* plants. The noticeable effect of the *T. diversifolia* compost aligns with the study of Taie *et al.* (2010) who showed that compost application in growing basil contributes not only to an increase in essential oil content, but also the concentration of linalool and borneol in the oil.

3.3. Total phenolic and flavonoid contents of *O. gratissimum*

The total phenolic and flavonoid content obtained from different fertilizing treatments of *O. gratissimum* is presented in Figure 1. The total phenolic and flavonoid contents of the hot aqueous extracts of *O. gratissimum* treated with TDC (53.16 ± 6.30 µg GAE/µL and 36.32 ± 0.59 µg GAE/µL, respectively) were higher as compared to those from plants treated with NPK(33.30 ± 3.45 µg GAE/µL and 23.83 ± 0.16 µg GAE/µL, respectively). However, the flavonoid content from *O. gratissimum* plants treated with NPK (S3T3) (26.93 ± 0.22 µg GAE/µL) was significantly ($P<0.05$) lower than that of the untreated control plant (23.83 ± 0.16 µg GAE/µL).

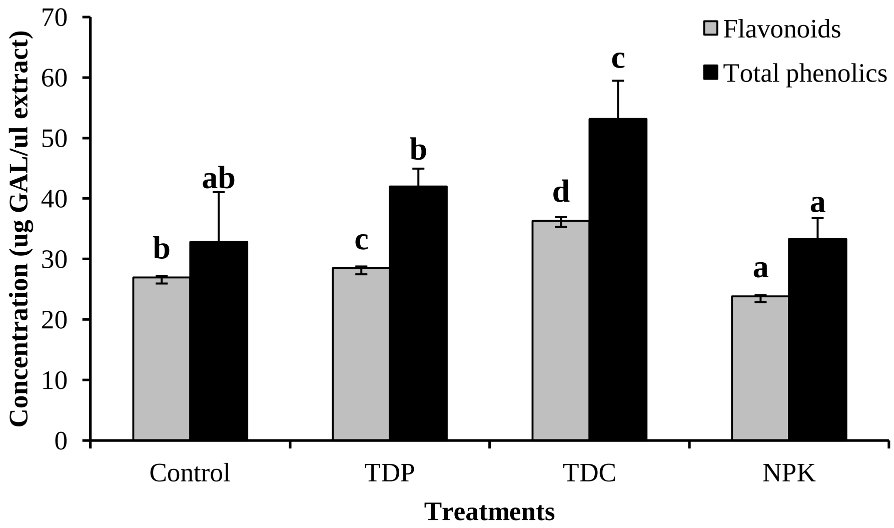

Figure 1: Concentrations of total phenolics and flavonoids according to treatments.

Data are means ± standard deviation of three repetitions. Bars with the different superscript letter for the same parameter are significantly different at $p<0.05$.
Control= Negative control; TDP: plants treated with *T. diversifolia* powder; TDC: plants treated with *T. diversifolia* compost; NPK: plants treated with NPK.
The extracts are prepared at 2% (20g/L) for *T. diversifolia* powder and compost and 0.2% (2g/L) for NPK.

The findings in this study also showed an increased total phenolic and flavonoid secondary metabolite content in plants treated with *T. diversifolia* compost. Koeduka *et al.* (2006) explained that essential minerals are used by plants to build many organic compounds: amino acids, proteins, enzymes, and nucleic acids. These amino acids and enzymes play a key role in the biosynthesis plant secondary metabolites. In studies carried out by Cheng *et al.* (2015), De Jong *et al.*(2015), they showed that in many species, phenylalanine ammonia-lyase (PAL) is an enzyme necessary for secondary metabolite production with some complexity in its signalling pathway. These findings thus explain the increased secondary metabolite content in plants treated with TDC and TDP fertilizers compared to NPK as the bio fertilizers supplies more nitrogen than NPK which is necessary for the synthesis of the amino acid phenylalanine as well as the enzyme phenylalanine ammonia-lyase (PAL) and other enzymes needed in the pathways for biosynthesis of secondary metabolites eventually causing higher production phenolic compounds in those plants.

3.4. Anticandida activity

Globally, the antifungal activity of *O. gratissimum* essential oil and hot aqueous extract depended on treatment applied to plants and the type of fungal strain with *C. albicans* NR-29445 being the most resistant fungal strain (Table 3).Among all the extracts obtained from the different treatments, essential oil of *O. gratissimum* treated with TDC exhibited the highest anti-fungal activity with MICs between 156.25± 0.00 µg/mL to 1250±0.00 µg/mL on *C. albicans* NR-29451 and *C. albicans* NR-29444/ NR-29445, respectively. The essential oil obtained from plants treated with NPK (MIC of 625± 0.00 to 1250±0.00 µg/mL) had greater inhibitory activity on *C. albicans* than that of *O. gratissimum* treated with TDP biofertilizer.
Similarly, the hot aqueous extract obtained from plants treated with TDC organic fertilizer showed the highest anti-candida activity with an MIC of 6250± 0.00 µg/mL on *C. albicans* NR-29451 when compared to the extracts from plants treated with TDP and NPK (MIC = >25000 µg/mL). Hot aqueous extract obtained from control plants and NPK showed no antifungal activities at the tested concentrations.

Table 3: MIC and MFC of the essential oil and hot aqueous extract of *O. gratissimum*

Treatments	*C. albicans* NR-29451	*C. albicans* NR-29444	*C. albicans* NR-29445
Essential Oil (MIC/MFC)			
Control	1250 ±0.00/ >10000	2500±0.00/ >10000	2500±0.00/ >10000
TDP	156.25±0.00/ >10000	1250±0.00/1250±0.00	1250±0.00/ >10000
TDC	2500 ±0.00/ >10000	1250±0.00/ >10000	2500±0.00/10000±0.00
NPK	1250±0.00/10000±0.00	625±0.00/625±0.00	1250±0.00/10000±0.00
Hot aqueous extract (MIC/MFC)			

Control	>25000/ NA	>25000/ NA	>25000/ NA
TDP	>25000/ NA	25000±0.00/ >25000	>25000/ NA
TDC	6250 ±0.00/ >25000	25000±0.00/ >25000	25000±0.00/ >25000
NPK	>25000/ NA	>25000/ NA	>25000/ NA
Nystatin	3.9±0.00/31±0.00	7.8±0.00/15.6±0.00	2±0.00/31±0.00

C. albicans: *Candida albicans*; MIC: Minimal Inhibitory Concentration; MFC: Minimal; NA: not applicable.
Data are means ± standard deviation for three repetitions.
Control= Negative control; TDP: plants treated with *T. diversifolia* powder; TDC: plants treated with *T. diversifolia* compost; NPK: plants treated with NPK.

The essential oils obtained from *O. gratissimum* plants treated with TDC showed a more potent anti-candida activity as compared to those obtained from plants treated with NPK. This study suggested that plants treated with TDC caused a significant increase not only in the amount of essential oil, but to that of bioactive anti-fungal compounds in general. In fact, total phenolic and flavonoid content were increased by 65 % in *O. gratissimum* plants treated with TDC. The volatile oil of *O. gratissimum* contains mostly phenols particularly thymol and eugenol (Sainsbury and Sofowora, 1971) which are responsible for its reported antimicrobial properties. This study is in line with a study carried out by Suhr and Nelson (2003) who showed that the yield of essential and antimicrobial activity depend on both intrinsic and extrinsic factors such as the chemical composition of oil (eugenol, thymol, methyl eugenol, Γ-terpinene amongst others and are also components of *O. gratissimum* essential oil) and vary according to the genotype of the plant, environmental condition (e.g the use of fertilizer), season of the year, method of extraction of this oil and its preservation.

4. CONCLUSION

The present findings showed that, *T. divesifolia* fertilizers and especially the compost form was a more promising organic fertilizer in optimizing the vegetative growth, total phenolic and flavonoid contents and anti-candida activity of *O. gratissimum.*

COMPETING INTERESTS DISCLAIMER:

AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USE PRODUCTS IN OUR AREA OF RESEARCH AND COUNTRY. THERE IS ABSOLUTELY NO CONFLICT OF INTEREST BETWEEN THE AUTHORS AND PRODUCERS OF THE PRODUCTS BECAUSE WE DO NOT INTEND TO USE THESE PRODUCTS AS AN AVENUE FOR ANY LITIGATION BUT FOR THE ADVANCEMENT OF KNOWLEDGE. ALSO, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY RATHER IT WAS FUNDED BY PERSONAL EFFORTS OF THE AUTHORS.

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