Original Research Article

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

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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 biofertilizer 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 (150 g/plant), powder (40 g/plant) or the synthetic fertilizer NPK (10 g/plant), respectively, followed by sprayed (second factor) with same fertilizer at 20 g/L, 20 g/L and 2 g/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 hydrodistillated 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* biofertilizer compost showed the highest total phenolic (53.16 µg GAE/µL), flavonoid (36.32 µg// 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, with about 70% - 80% of the people worldwide relying on medicines based on plants for their primary health care needs. Of all these plants, *Ocimum gratissimum*, is a herbaceous medicinal plant which belongs to the Labiacae family and has been used extensively in the traditional system of medicine in many countries (Oswagwu and Edeoga, 2012). 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). It is also used by the Ibos of South eastern Nigeria in the management of the baby's cord, wounds, fungal infections, fever, cold and catarrh (Ijeh *et al.,* 2005). Due to increase demand for herbal medicines, there have been over exploitation of these plants for active constituents (Sheelu *et al.,* 2017) which are naturally present in minute quantities. Agricultural productivity is thus a key element to ensuring the availability, and affordability of these plants accompanied with increased plant medicinal content. The sustainability of the constant increase demand for these plants requires the extensive use of agrochemicals including synthetic fertilizers. Synthetic fertilizers used in cultivation of plants have enhancing effects on their bioactive content including their total phenolics and flavonoids. Yet, the hazardous environmental consequences and high cost of these inorganic fertilizers make them not only undesirable but also uneconomical and out of reach of the poor farmers who still dominate the agricultural sector (Oswagwu, 2008). In recent times, attention has been directed towards organic manure because of the rising cost of inorganic fertilizers coupled with their inability to give the soil the desired sound health. This has led to increased use of organic manure, a readily available alternative, which proves more environmentally friendly (Stephen *et al.,* 2014). *Tithonia diversifolia* is an Asteraceae family member, widely cultivated as an ornamental in many sub-tropical regions has been used as a biopesticide and biofertilizer to improve 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. In many countries such as Uganda and Kenya, this plant species is employed by farmers as a bio-pesticide to replace hazardous and expensive synthetic pesticides (Mwine *et al.,* 2011). Previous studies have shown that the use of this plant species as organic manure on the cultivation of maize in the region Mbujimayi improve the production of maize (Michel *et al.,* 2016). At present, farmers mostly apply organic fertilizers in combination with inorganic nitrogen-based fertilizers such as Urea and NPK often because organic fertilizers alone are believed to dissolve slowly and may not meet up the yield of plants (Stephen *et al,* 2014). There is a need to determine the independent influence of organic fertilizers such as *T. diversifolia* and inorganic nitrogen fertilizers such as NPK on the growth, yield, and quality of plants such as *Ocimum gratissimum* species so as to justify the continuous mixture of both or otherwise. The present study compares the growth, yield (essential oils, total phenolic and flavonoid contents), and anticantidal activity of *O. gratissimum* grown with *T. diversifolia* bio-fertilizer powder/compost and NPK in relation to unfertilized (control) soil.

2. MATERIALS AND METHODS

2.1. Study period and site

This work was carried out from August 2019 to October 2020. Field experiments were performed on an agricultural experimental farm in Emana situated in the Mfoundi division, central region of Cameroon, with a geographic location of $3^{o}55'0''$ north, 11^{o} 31' 0" east.

2.2. Plant materials

The seeds of *O. gratissimum* (Voucher number 5817/SRF/Cam) were obtained by trashing the seeds from the flowering parts of the plant. *T. diversifolia* leaves (Voucher number 57410 HNC) were harvested from an experimental farm at IRAD Nkolbison, in Mfoundi division, central region of Cameroon and identified at the Cameroon National Herbarium. The fresh plants of *T. diversifolia* were cleaned, dried at room temperature, chopped and finely ground 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, and absolute ethanol (≥ 99.8% purity) were obtained from the Department of Biochemistry-University of Yaoundé 1. Dimethyl sulphoxide (DMSO) and Sabouraud dextrose broth (SDB) were purchased from Gaylord Chemical Company and HiMedia Industries, respectively. The Candida strains were *Candida albicans* NR-29451, *Candida albicans* NR-29445 and *Candida albicans* NR-29444 obtained from BEI resources.

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 at a ratio of 1:3, and enough water was added to moisten the mixture. The drum was then covered and incubated at 37°C for six months for maturation of the compost. It is worth noting that the mixture was macerated every two weeks to increase aeration.

2.5. Preparation of extracts for spray

Spraying was performed to constantly supply plant leaves with nutrients. For this, 20 g of the powder of *T. diversifolia*, 20 g of the compost of *T. diversifolia* and 2 g of NPK were each dipped into three separate containers containing 1 L of water for 24 hours. After 24 hours, each container was macerated, filtered with a fine cloth of 25 µ and redipped 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 on the farm.

2.6. Experimental design

The experimental design was a split plot design with three replications consisting of 4 blocks with 6 subblocks per block. Nine (09) *O. gratissimum* seedlings were transplanted per subblock at 0.75 cm intervals. The soil amendments on the 4 blocks included (the main factor) a one-time fertilization with *T. diversifolia* compost (TDC) at 150 g/plant or 6060 kg/hectare, *T. diversifolia* powder (TDP) at 40 g/plant or 1600 kg/hectare, the synthetic fertilizer (NPK 23-10-5 + 10 SO3) at 10 g/plant or 400 kg/hectare and the negative control block without fertilization. The planting was in rows 50 cm apart and 50 cm between hills (40401 plant/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 performed every 2 weeks to maintain the 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, a portion of the harvested plant leaves was air dried while the other was kept fresh and used for extraction of essential oil and hot aqueous extract via the hydro distillation method. The yield of essential oil per fresh plant weight and per hectare was 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 contents

Total phenolic
The total phenolic content was quantified using the method described by McDonald et al. (2001). One hundred microliters (100 µL) of 10% Folin-Ciocalteu reagent was added to 20 µL of 4-fold diluted medicinal plant extract, and after brief homogenization, 80 µL of 20% Na2CO3 was added. After one hour of incubation at room temperature, the absorbance of the blue-coloured complex formed was read at 725 nm. A calibration curve was generated using a gallic acid solution (0.063 to 2.0 mg/mL). The results were expressed in µg equivalents of gallic acid per microliter of extract (µg GAE/µL extract).
Flavonoids
The flavonoid content was precipitated from extracts by adding 80 µL of medicinal plant extract diluted 4 times to 40 µL of 50% HCl in 40 µL formaldehyde (precipitation of flavonoids was performed to obtain the phenolic content without flavonoids to be used in calculating the quantity of flavonoids present in the sample). The mixture was incubated for 24 h at room temperature and then filtered using Falten filter papers. The obtained filtrate represented the phenolic content without flavonoids and was 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 antifungal 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 determined 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) was introduced into the wells. One hundred microliters of either hot aqueous extract (0.39 to 25 mg/mL), essential oils (0.156 to 10 mg/mL) or nystatin (positive control; 0.046 to 3 mg/mL) was added to the respective wells. Then, 100 µL of the fungal suspension (5 $\times 10^3$ CFU/mL) was introduced into the wells. After 48 hours of incubation at 37°C. The 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 are presented as the means ± standard deviations. One-way analysis of variance (ANOVA) coupled with the Newman–Keuls t-Student test was used for comparison of the mean, and differences between means were considered significant at the 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 with fertilizers and sprayed, obtained at either 4 or 8 months of cultivation, displayed better growth parameters compared to other treatments. In the first harvest (4 months after transplantation), plants from fertilization and spray displayed better growth parameters compared to other treatments. Interestingly, the leaf dry weight and inflorescence weight from plants receiving both NPK fertilization and spray application (34.11 ± 9.26 g and 25.44± 2.68 g, respectively) were higher ($P<0.05$) with regard to the other treatments.

In the second harvest (8 months after transplantation), plants both fertilized and sprayed with TDC organic fertilizer (FS_{TDC}) significantly ($P<0.05$) increased the fresh leaf weight (310.00 ± 31.51 g), dry leaf weight (207.47 ± 14.51 g), inflorescence weight (131.24 ± 16.12 g) and plant height (156.78 ± 10.46 cm) 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 (nonamended soil and no spray), S_{TDP}: nonamended soil and spray with extract of *T. diversifolia* powder, S_{TDC}= nonamended soil and spray with extract of *T. diversifolia* compost, S_{NPK}: nonamended 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 were prepared at 2% (20 g/L) for *T. diversifolia* powder and compost and 0.2% (2 g/L) for NPK.

Within the first 4MTP, plant treated with synthetic fertilizer (NPK 23-10-5 + 10 SO_3) stimulated higher growth parameters of the plants compared to those treated with *T. diversifolia* bio fertilizer. Nevertheless, 8MTP, there were significant differences in the growth parameters of plants treated with *T. diversifolia* bio fertilizer compared to those treated with the synthetic fertilizer NPK 23-10-5 + 10 SO_3. The significant increase in the vegetative growth parameters per plant in response to *T. diversifolia* bio fertilizer application observed in the present study agreed with the work carried out by Jeptoo *et al*., 2013; Kaho *et al*., 2011; Babajide *et al*., (2012) which obtained an improvement in growth parameters of carrots treated with *T. diversifolia* manure, improvement in the maize yield treated with powder form *T. diversifolia*, and improvement of growth parameters of sesame treated with *T. diversifolia*. In another study Agba (2019) obtained significant increases in dry weight of okra due to another organic fertilizer (poultry manure) application. It could be observed that nutrition also played a key role in the growth and development of all the plants. Lafon et al. (1985) reported that the mineral nitrogen absorbed by the plant is used for the synthesis of amino acids, and plays a very important role in growth. Phosphorus is also an essential element for plant life and of the three major elements, phosphorus is recognized as having an effect on root growth (Dongmo *et al*., 2020). Also, fertilization is one of the most important factors that affect crop yield and plant health (Nawal *et al*., 2014).A study carried out by Nguefack et al. (2020) explains the increase in growth parameters 8MTP as it was shown that fertilization of plants with *T. diversifolia* bio fertilizer compost yields better results due to the fact that compost is more stable since biodegradation has commenced already compared to the powder with a slower nutrient releasing mechanism, thus releasing nutrients (such as N, P, K and other micro nutrients) into the soil for plant uptake as well as providing a constant supply of these nutrients to plants over longer periods of time effectively improving growth parameters compared to NPK 23-10-5 + 10 SO3 whose nutrients are limited and are thus exhausted within shorter periods of time. The above mentioned studies thus explain the higher yields in plants treated with *T. diversifolia*bio fertilizer compared to NPK 23-10-5 + 10 SO_3. Also *O. gratissimum* plants that received treatment of soils by amendment and spraying of the plants with the same fertilizer compared to those that were only treated by soil amendment with no spraying were seen to have higher values in the growth parameters obtained from blocks sprayed with *T. diversifolia* bio fertilizer compared to those blocks sprayed with NPK 23-10-5 + 10 SO_3. This could be explained by the fact that; spraying every two weeks provided foliar nutrients to plants. 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, causes at the same time enhances nutrient uptake from the nutritional environment, thereby increasing the pool of macro and micronutrients in the plant (Renata, 2013) effectively increasing plant yield and health.

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 were prepared at 2% (20 g/L) for *T. diversifolia* powder and compost and 0.2% (2 g/L) for NPK.

This study showed that the essential oil yield of fresh leaves of *O. gratissimum* not treated with any fertilizer was 0.252%. Mohr *et al.* (2017) also found similar results (0.18 ± 0.04%) in EO yield in the extract of fresh leaves of clove basil plants. Fokou *et al.* (2014) also found similar EO content (0.19%) in extracts of clove basil plants cultivated in Cameroon. Omobolanle *et al.* (2013) found similar essential oil content of 0.2% in a study carried out in Nigeria as they also showed in comparative study that plants cultivated in shade and subjected to water stress gave a yield of 1% EO; which is also in line with this study in which the combined treatment of plants by soil amendment and spraying with *T. diversifolia* bio fertilizer compost gave a high yield of 0.666 compared to NPK 23-10-5 + 10 SO_3. The high yields of essential oils obtained from plants treated with *T. diversifolia* bio fertilizer compost compared to those treated with NPK 23-10-5 + 10 SO_3 is due to the fact that the biosynthesis of essential oil, likewise other processes taking place in the plant, 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). Nutrition has been observed to play a key role in medicinal plants that synthesize essential oils as nutrients can effectively increase oil yield and quality (Aziz *et al.* 2010, Jabbari *et al.* 2011). Nitrogen uptake and use by plants are dependent, among others, on the type of fertilizer (nitrogen form) and its amount (nitrogen rate). Basil essential oil yield is significantly dependent on the rate of nitrogen, rate of potassium and the interaction between N and K (Rao *et al.* 2007). Compost application in growing basil contributes not only to an increase in essential oil content, but also increases the concentration of linalool and borneol in the oil (Taie *et al.* 2010). It can be seen in this study that the available nitrogen for plant usage is more in the plants treated with *T. diversifolia* compost than that treated with NPK 23-10-5 + 10 SO_3 reason for its higher essential oil yields. Foliar nitrogen application increases essential oil content in thyme and affects its chemical composition (Jabbari *et al.* 2011) so folia application of fertilizers added to that in the soil could contribute to the increased essential oils in plants treated with fertilizers compared to the control.

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

The total phenolic and flavonoid contents obtained from different fertilizing treatments of *O. gratissimum* are 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 than 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 plants (23.83 ± 0.16 µg GAE/µL).

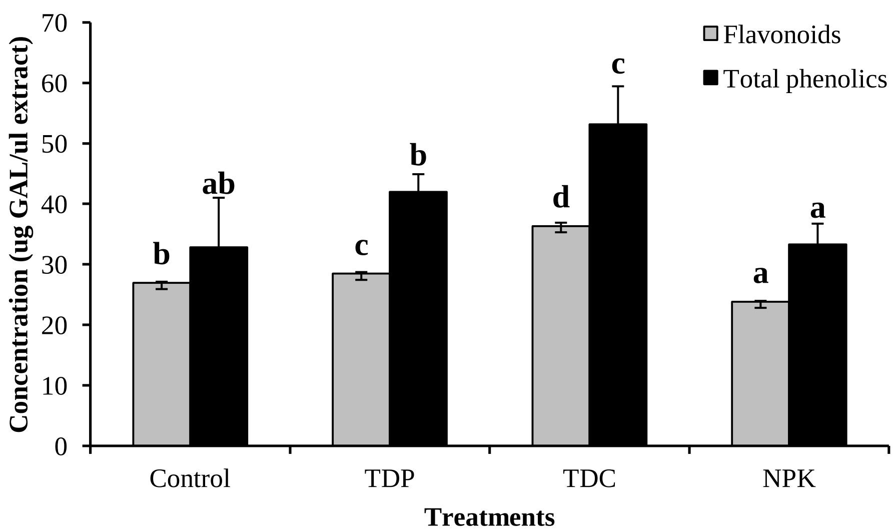

Figure 1: Concentrations of total phenolics and flavonoids according to treatments.

Data are the means ± standard deviation of three repetitions. Bars with different superscript letters 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 were prepared at 2% (20 g/L) for *T. diversifolia* powder and compost and 0.2% (2 g/L) for NPK.

The result of the quantitative analysis of the phytochemical content of *O. gratissimum* plants treated with TDC and TDP biofertilizers were high, with a 61.92% and 27.80% increase respectively in total phenolic content compared to plants treated with NPK. 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 of plant secondary metabolites. In studies carried out by Cheng *et al.* (2015) and 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 supply 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 of phenolic compounds in those plants.

3.4. Anticandida activity

Globally, the antifungal activity of *O. gratissimum* essential oil and hot aqueous extract depended on the 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, the essential oil of *O. gratissimum* treated with TDC exhibited the highest antifungal activity, with MICs between 156.25± 0.00 µg/mL and 1250±0.00 µg/mL against *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 *O. gratissimum* essential oils had an antifungal effect on all tested stains, however, the MIC values varied. The essential oils obtained from TDC have a more potent anti-candidal ability compared to those obtained from 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, the total phenolic and flavonoid contents were increased by 65% in *O. gratissimum* plants treated with TDC .The plants treated with TDC caused a significant increase in the essential oil, phenol and flavonoid content (as such, a consequent increase in the total amount of thymol in essential oils) compared to the plants treated with NPK and as a result increased the anti-candidal activity of essential oils obtained from those plants treated with TDC. 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) which vary according to the genotype of the plant, geographical origin, environmental condition (e.g the use of fertilizer), season of the year, method of extraction of this oil and its preservation. It is also in line with a study carried by Sainsbury and Sofowora, (1971), who reported that the volatile oil of *O. gratissimum* contains mostly phenols, particularly thymol and that these were responsible for its reported antimicrobial properties. Also, a study carried out by Dubey *et al.* (2000) showed that the high antifungal activity of *O. gratissimum* essential oil on pathogenic fungi like dermatophytes, filamentous fungi and yeasts confirmed the excellent fungal growth inhibition properties previously reported as a characteristic of essential oils rich in thymol and/or other phenol derivatives. In contrast to this study Oshim *et al.,* (2019), showed that *C. albicans* strains were resistant to the crude methanol extracts of *O. gratissimum* tested even at very high concentrations of 200000ug/ml and they concluded that the leaves do not contain substances that can exert antimicrobial activity against the test organism because the potency of extract depends on method used to obtain the extract. All these results show that the antifungal potential of *O. gratissimum* against tested fungi is a predictable consequence of its high content in thymol which is as a result of the increased essential oil content in the plants treated with TDC compared to those treated with NPK.

4. CONCLUSION

This study was to investigate the effect of *T. diversifolia* compost/powder and NPK on growth parameters, secondary metabolite yield and anticandical activity of *O gratissimum.* The application of *T. diversifolia* compost/powder optimized the growth parameters (leaves fresh and dry weight, fluorescent weight and plant height) of *O. gratissimum* Also the amount of total phenols, flavonoids and essential oil yield increased when *O. gratissimum* plants were cultivated using *T. diverifolia* compost/powder as a result it improved the secondary metabolite content of the plant unlike in the plants that were cultivated with NPK. Furthermore, the essential oils and extracts of *O. gratissimum* obtained from *T. diversifolia* compost/powder were more susceptible to *C. alblicans* thus showing higher antifungal activities.
Results obtained from the study thus showed that, *T. divesifolia* made bio fertilizer especially the compost is a more promising bio fertilizer in optimizing the growth, secondary metabolites and anti-candidal activity of *O. gratissimum* compared to the synthetic fertilizer NPK and can be used independently as well as not necessarily in combination with with NPK as agrofertilizers.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

DISCLAIMER:

THE AUTHORS HAVE DECLARED THAT NO COMPETING INTERESTS EXIST. THE PRODUCTS USED FOR THIS RESEARCH ARE COMMONLY AND PREDOMINANTLY USED 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. ADDITIONALLY, THE RESEARCH WAS NOT FUNDED BY THE PRODUCING COMPANY; RATHER, IT WAS FUNDED BY THE PERSONAL EFFORTS OF THE AUTHORS.

ETHICAL APPROVAL:

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