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Effect of essential oil and powders of *Tithonia diversifolia* on the growth parameters and yield of rice in the field

Dongmo Nanfack Albert[1]*, Nguefack Julienne[1], Fouelefack François Romain[2], Dakolé Daboy Charles[1], Kuate Tueguem William Norbert[4], Nkengfack Augustin Ephraïm[3] and Dongmo Lekagne Joseph Blaise[1]

[1]Department of Biochemistry, University of Yaoundé 1, Cameroon
[2]Department of Biological Sciences, University of Maroua, Cameroon
[3]Department of Organic Chemistry, University of Yaoundé 1, Cameroon
[4]Department of Plant Biology, Laboratory of Biotechnology, Plant pathology and Plant Protection, University of Yaoundé 1, Cameroon

*Corresponding author; e-mail: adongmonanfack@yahoo.com

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ABSTRACT

The high costs of agricultural inputs push to look for alternatives to increase the production of rice. For this, the objective would be to evaluate in the field, the effect of seed treatment using the essential oil and the improvement of plants using *Tithonia diversifolia* powders on rice growth. The experiment was carried out in the Department of Moungo, in the locality of Lelem and Kompina. Before the field experiment, the biochemical parameters of the powders and the soil physicochemical were determined. The experimental design is a completely randomized factorial split-plot consisting of three treatments and two varieties (NERICA 3 and NERICA 8) with four replicates. The present study was carried out during two successive seasons of 2018 and 2019. All data are the meaning of these seasons. It appears that the soil of Kompina is an acid (pH between 5.0 and 5.3) and sandy clay that of Lelem is silty-clay-sandy and slightly basic (pH between 6.8 and 7.9). The yield increase is greater in the treated plots than control. Yield is high in the locality of Lelem than in the locality of Kompina. The 1000 seeds weights are very large in the amended plots (33 g) than the control plots (29 g). It is observed a good emergence rate (96%) in the two localities and some symptoms of the disease transmitted by seeds were observed only in the area of Kompina. From this study, we can retain that the NERICA varieties adapt very well to the rainy conditions of up-land and deserve to be popularized. The positive results of the amendment to the powder and treatment of seeds with essential oil on the growth of rice deserve a renewed interest in the exploitation of this plant resource as biofertilizer and biopesticide to improve rice production.

Introduction

In a rapidly changing socio-economic context in Cameroon, rice production is evolving very slowly. In fact, paddy rice production in Cameroon increased from 181,818 tonnes in 2012 to 359,320 in 2016 (MINADER/DESA, 2017); but, with the social and economic crises in the North-West, South-West, North, and East since 2016, this production is almost reduced to 332,534 tonnes in 2018; on the contrary, the consumption of white rice increased from 500,000 tonnes in 2015 to about 700,000 tonnes in 2018 (Faostat, 2019). Thus, Cameroon depend on imports to meet the needs of this foodstuff. Rice is cultivated mainly under irrigated system, in all agro-ecological zones of Cameroon mainly in; the Sudano-Sahelian zone in the Far North of the country (Yagoua, Kousseri, Lagdo), the humid tropical zone in the Centre- Est-South and the highlands zone in the West (Ndop, Tonga, Santchou) (MINADER/DESA, 2017).

In 1994, scientists of the African Rice Center have successfully obtained a new variety rice type called NERICA (New Rice for Africa) which is promising for countries in Sub Saharan Africa (Sarla and Mallikarjuna, 2005); sub-types of NERICA easily adapted under irrigated and in rainfed upland systems and are being tested in many African counties including Cameroon. Several constraints including the difficulty of accessing to agricultural inputs (fertilizers and pesticides), the insufficiency of healthy rice seeds, and diseases, reduce rice production in Cameroon (Nguefack et al., 2005). These constraints could cause more or less severe damage ranging from 6 to more than 90 % of production losses (Agarwal et al., 1989; Kohls and Percich, 1987), Synthetic chemicals used in the management of rice are sometimes not available, costly for poor farmers and can negatively affect ecosystems (Kagale et al., 2004; Harish et al., 2008).

Plant and plant extracts are amongst other, sources of biological agrochemicals that can be used as bio-fertilizers as well as bio-pesticides to replace their synthetic counterpart in the plant management (Guilter, 1998; Bengyella et al., 2011); regarding to this, *Tithonia diversifolia* has been used as bio-fertilizer and some of its extracts has exhibited antimicrobial activities (Kaho et al., 2011; Linthoingambi et al., 2013); thus, this work, consist to assess the effect of seed treatment with essential oils and soil amendment with *T. diversifolia* powder, under field conditions on the growth parameters (emergence, vigor, tillering, disease index and the production yield) of two NERICA rice varieties; N3 and N8.

Materials and methods

Rice seed samples

Two cultivars of NERICA rice seeds (NERICA 8 and NERICA 3) were obtained from the Institute of Agricultural Research for Development (IRAD). These cultivars were collected and selected according to the protocol described by Fouelefack et al. (2018).

Plant material and preparation of powders

Plant material was constituted of leaves of *Tithonia diversifolia* harvested in March 2017 for the first season of rice culture (March-June) and in June 2019, in Yaoundé Cameroon for the second season of rice culture (September-November). About 300 kg of the harvested leaves were placed on a tarpaulin at room temperature (25-27°C) then, stirred regularly for about three weeks until complete shade dry. The powders were obtained after grinding the dry material, then weighed and stored at 25 °C for later use.

Phytochemical screening: qualitative determination

Standard methods of qualitative analysis described by Harbone (1998) and Edeoga et al. (2005) were used for the determination of secondary metabolites in plant powder.

Preparation of essential oil

The essential oil was obtained from fresh leaves of *T. diversifolia* by the hydrodistillation method for about 4 hours using the Clevenger apparatus: *T. diversifolia* leaves in water are heated at about 100 °C; the essential oil carried by water vapours is condensed in the settling column of the Clevenger apparatus, collected, dried through anhydrous sodium sulphate, weighed and stored at 4 °C for later use

Chemical analyses of powders from leaves of *T. diversifolia* and soil

Samples: The requested analyses were carried out on five (05) samples of soils (1000 g soil samples) taken at different locations of the experimental sites according to the protocol described by

Pauwels et al. (1992). And two (02) samples sources of organic matter (OM), in particular, *T. diversifolia* powder (500 g of plant powder). The samples were taken by the applicant at an experimental site before cultivation, to determine the level of nutrients in the soil and the sources of OM. The samples were packaged and brought to URASCE on 04/15/2019, under the conditions specified by the unit (Pauwels et al., 1992).

Analyses: The analyses carried out in the soil surface samples (0-20 cm) mainly included the contents of sand, clay and silt, the pH in aqueous (pH - H_2O) and saline (pH KCl), the content in organic carbon (CO), the total nitrogen content (Ntot), the content of exchangeable bases (Ca, Mg, K and Na), the exchangeable acidity (AE), the cation exchange capacity (CEC), the rate base saturation (V), the effective cation exchange capacity (CECE) and the assimilable phosphorus content (P Bray II). However, the analyses carried out in the OM source samples consisted of pH, CO, OM, Ntot, N - NO_3^-, N - NH_4^+, total phosphorus (Ptot), Ca, Mg, K, Na, Fe, and S.

Methodology: The physicochemical and chemical parameters contained in the soils were determined according to the methods in force at URASCE, recommended by Pauwels et al. (1992) and complying with ISO, AFNOR NF, and EN standards. It is :

Total nitrogen: mineralization by the acid attack of 2 g of sample, distillation by steam entrainment and determination with sulfuric acid (Kjeldahl method, standard NF ISO 11261);

Assimilable phosphorus; blue ammonium molybdenum colorimetry after

The methodology: The physicochemical and chemical parameters contained in the soils were determined according to the methods in force at URASCE, recommended by Pauwels et al. (1992) and complying with ISO, AFNOR NF, and EN standards. It is :

Assimilable phosphorus; colorimetry with ammonium molybdenum blue after extraction with an acid solution (HCl + NH_4F) of Bray II of 2.5 g of the sample and reading at the wavelength 665 nm (standard NF X 31-130);

Potassium and sodium: flame emission spectrometry by direct reading in the ammonium acetate extract at pH 7 of the sample ((AFNOR NF T 90 - 019 standard);

CEC: by extraction of exchangeable cations, washing with alcohol, replacement of NH4 + ions with K + ions of KCl 1N, distillation by steam entrainment and dosing with 0.01 N sulfuric acids (standard NF EN ISO 23470);

Calcium and magnesium; complexometric and titrimetric method of an ammonium acetate extract at pH 7 of the sample. (AFNOR NF U 44 - 146 standard);

The grain size (sand, clay, and silt); Robinson - Kôhn pipette method after the destruction of organic matter by hydrogen peroxide and iron oxides as well as carbonates with hydrochloric acid (standard NFX 31 - 107);

Organic carbon; oxidation of 0.3 g of sample with potassium dichromate ($K_2Cr_2O_7$) and assay in return of the remaining dichromate with ferrous sulfate heptahydrate ($FeSO_4$, 7 H_2O), (standard AFNOR NFU 44 - 051);

pH; potentiometry using a “Hanna Instruments” brand pH meter in the aqueous extract of ratio 1; 2.5 (ISO 10390 standard).

Rice seed treatments

The rice seeds were treated with an emulsion of 3 % essential oil diluted in 1% agar: They were coated with essential oil emulsion at the rate of 100 µl/g of seed in a tightly closed box for 15 min; then spread on sterile blotting paper and dried at 25 ºC for 24 hours before sowing.

Study sites and experimental design

The field experiments were conducted in two sites: The first site is located at Lelem Mangwete, in the district of Melong (latitude 5°15’N, longitude 9° 97’ E and altitude 737.00m-2417.98 m), Department of Moungo-North, bordering the Santchou (Fig. 1). The second site is Kompina, in the municipality of Dibombari (altitude 3° 59’- 4° 80’ N, longitude and 9° 45- 9° 83’ E and altitude 02m-92m), Department of South-Moungo bordering Bonabéri (Fig. 2). The climate of both localities is equatorial of Guinean type and the precipitations ranged between 2350-2402 mm/year; the soils are black and predominantly clay-lateritic and ferralitic (Moudingo, 2007).

The field trials were carried out from March to July 2018; the experimental land was cleaned and ploughed; the trial was laid out in a randomized complete split-plot design (Sehgal, 2012; Dagnelie, 2012) with four repetitions, in elementary plot size of 2.5 × 3m (area of 7.5 m^2). The combination of 2 rice varieties and 3 treatments gave a total of 6 (3 x 2) 6 elementary plots per block (Fig. 3). Five (5) seeds per hill were directly sown, with 20 cm spacing between hills in each row and 20cm spacing between rows. Weeds were removed manually at 20^{th} and 45^{th} days after sowing. Fertilizers were applied around each hill 3 times at the 21^{st}; 45^{th} and 70^{th} day; at the rate of 20g/hill for *T. diversifolia* powder and 4g/hill for chemical fertilizer 20.10.10 NPK. Traps and nets were used to fight against rodents and birds.

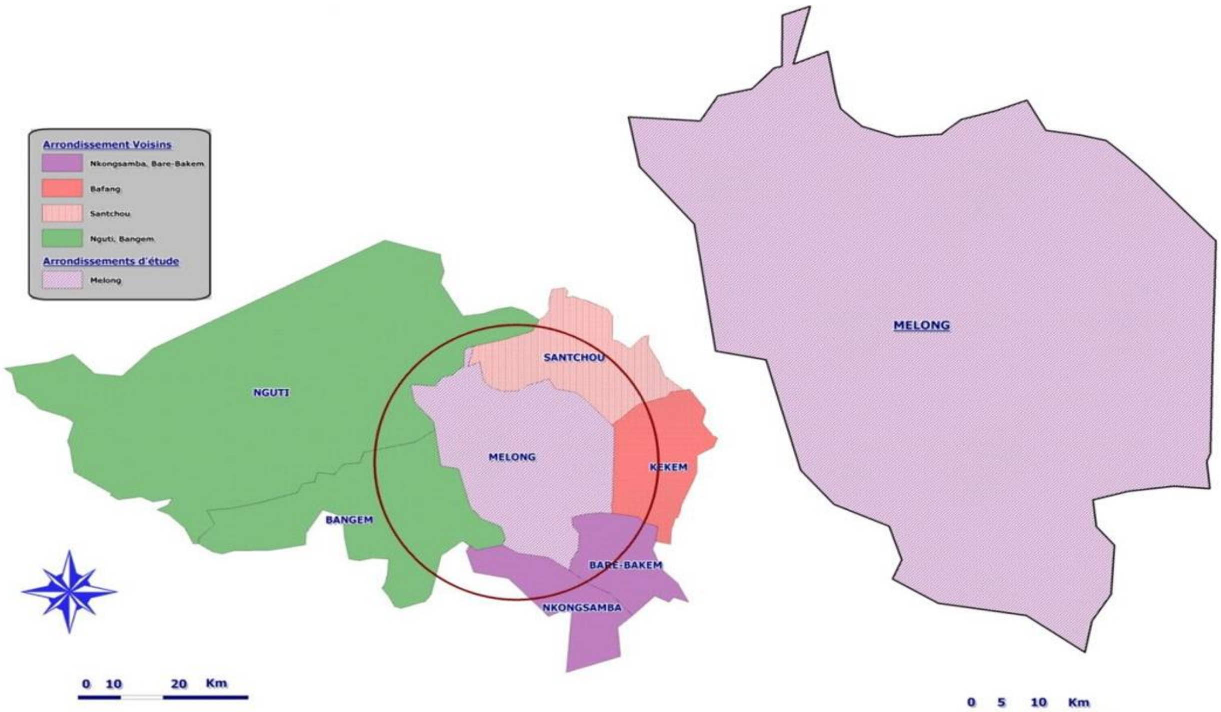

Fig. 1: Location map of the Municipality of Melong (AGRISTAT-MINADER 2010 data).

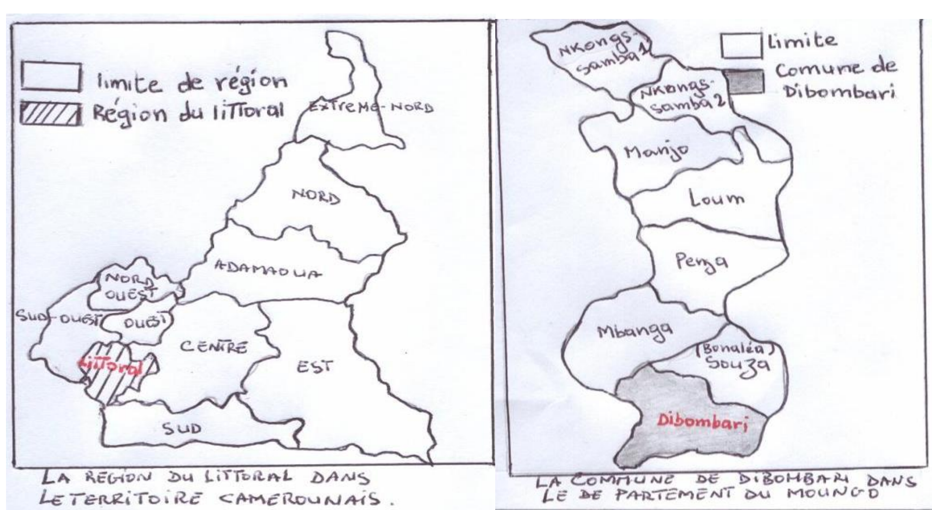

Fig. 2: Location map of the Municipality of Dibombari (AGRISTAT-MINADER 2010 data).

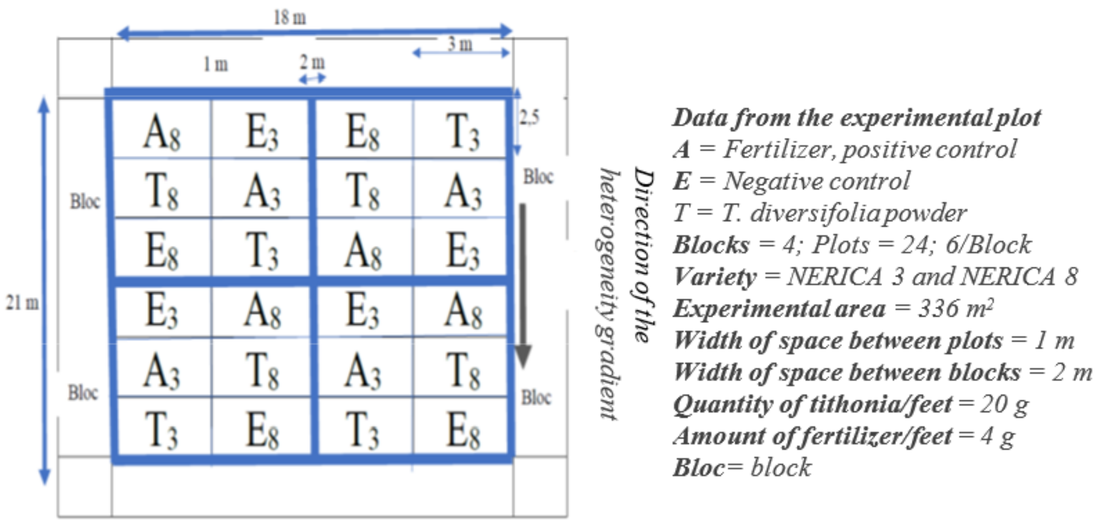

Fig. 3: Experimental design (Dagnelie, 2012).

Field data collection

The data were collected in a meter square of each plot; data were recorded on emergence, this was done by a complete count of the total number of plants emerged after two weeks after sowing; the appearance of the plants after emergence was evaluated after month of sowing on a scale of 1 to 5 (1 = weak vigor, 2-3 = medium vigor, 4-5 = vigorous plants); the vigor index of each concentration tested has been calculated by multiplying the germination rate (%) and seedling length; The chlorophyll rate was took using chlorophyll-meter on the 45th day after sowing; the number of tillers was noted on the 60th day after sowing by an exhaustive count of the number of plants that contain 1 m² in the middle of each plot, previously randomly marked during the assessment of emergence within the interior lines of each (25 plants/m²) parcel; the disease index: the degree of infection of the plants was estimated by scores between 0 and 9 assigned after visual observation and inspired by the standard IRRI rice evaluation system (1980 and 1996). This rating reflected an average visual assessment of the density of lesions on the individual leaves. It was done on the 60th, 80th and 110th day after sowing on a scale of 0 to 9: 0 = no symptoms; 1-3 = few symptoms (presence of brown spots about 1 mm in diameter); 4-6 = average presence of symptoms (less than 26 % of the leaf area affected); 7-9 = high presence of symptoms (26 to 100 % of the leaf area affected); at the end, production by elementary plot was obtained after harvest by weighing paddy rice from 1 m² internal sowing lines_ covering an area of 7.5 m² (IRRI, 1980 and 1996).

Yield estimation

The harvest was carried out on the 110th ± 10 days for the varieties used. For each elementary plot of 8 lines, only middle lines were collected in the area of 1 m². The product of each plot was packed in a polyethylene bag. For each treatment the seeds were dry, weighed and yield was estimated using the formula of Kamau et al. (2011).

$$\text{Yield (t ha}^{-1}) = \text{weight (kg/m}^2) \times 10\,000 \text{ m}^2/\text{ha} \times 1 \text{ t}/1000 \text{ kg.}$$

Statistical analysis of data

The data were subjected to analysis of variance (ANOVA) and the separation of means with the smallest significant difference (LSD), $p < 0.05$, performed using the program SPSS 16.0 software.

Results

Soil analysis from the two experimental sites

The soils of Lelem site are a bit basic and suitable for the production of many crops because the optimal pH is located at an average between 7.00 and 8.00. The assimilable phosphorus contents are high as well as the base saturation rates. As the soil is not very basic, a drop in control is, therefore, necessary on these soils. Nitrogen and organic matter contents are also significant. An organic amendment in the form of chicken droppings is necessary, with an addition of nitrogen because the organic matter, although significant is less important because of its relatively significant C/N ratio for all soils (Table 1).

The soil of Kompina site is very acidic (pH is located at an average between 5.3 and 5.2) and marginal for the production of any crop because the optimal pH of many crops is located at an average of 7.0. Consequently, the assimilable phosphorus contents are low as well as the base saturation rates. An increase in pH is, therefore, necessary on these soils with the addition of phosphorus. Nitrogen and organic matter contents are also low. An organic amendment in the form of chicken droppings is necessary, with an addition of nitrogen because the organic matter, although low is of poor quality because of its very high C/N ratio for all soils (Table 2).

Table 1. Lelem soil analytical sheet.

N°	1	2	3	4	5
Codes	Lelem 1	Lelem 2	Lelem 3	Lelem 4	Lelem 5
Texture (%)					
Sand	14	15	10	15	13
Silt	50	54	54	52	52
Clay	36	31	36	33	35
Textural class	LAS	LAS	LAS	LAS	LAS
Coarse items (%)	0	0	0	0	0
Soil reaction					
pH water	7.5	6.8	7.1	7.5	7.9
pH KCl	8.0	7.5	8.0	8.0	8.8
ΔpH	0.5	0.7	0.9	0.5	0.9
Organic Matter					
CO (%)	31.00	35.00	36.00	34.00	33.00
MO (%)	61.00	70.00	72.00	68.00	66.00
N (g/kg)	2.15	2.60	3.70	2.80	2.90
C/N	16.41	15.46	16.00	15.14	16.37
Exchangeable cations and acidity (meq/100 g)					
Ca	3.32	2.52	3.25	2.50	2.40
Mg	80.00	79.00	77.00	76.00	76.00
K	37.20	38.15	38.25	39.10	37.15
Na	1.03	1.04	1.08	1.04	1.03
S	2.91	3.16	4.20	3.08	2.92
AE	2.12	2.08	2.10	2.19	2.12
Cation exchange capacity (meq/100 g)					
CEC	2190	21.82	21.48	21.50	21.56
CECE	5.03	4.24	4.30	4.17	3.04
V	12.2	12.20	13.00	12.30	12.20
Assimilable phosphorus (mg/kg)					
P (Bray II)	16.00	18.50	19.80	19.15	17.39

Chemical analyses of *Tithonia diversifolia* leaf powders

The powder of *T. diversifolia* is basic (pH 9.1) and knowing that the optimal pH of the majority of culture is located at an average of 7.0, its application like any other chemical fertilizer must require a study of the soil. The powder of *T. diversifolia* contains phosphorus, nitrogen, and potassium in relatively small proportions. Note that *T. diversifolia* is very rich in potassium (7.3%) compared to phosphorus (0.6%) and nitrogen (4.4%) (Table 3).

Obtaining *Tithonia diversifolia* powder

The powder of the leaves of *T. diversifolia* was obtained after drying and grinding of the dry plant material obtained. From 300 kg of fresh *T. diversifolia* material, we obtained 50 kg of powders which corresponds to a yield of 16.66%. Low yield of less than 50%.

Table 2. Analytical sheet of Kompina soils.

N°	**1**	**2**	**3**	**4**	**5**
Code	Kompina 1	Kompina 2	Kompina 3	Kompina 4	Kompina 5
Texture (%)					
Sand	74	75	73	75	73
Silt	06	07	06	07	06
Clay	20	17	19	18	21
Textural Class	AS	AS	AS	AS	AS
Coarse items (%)	0	0	0	0	0
Soil reaction					
pH water	5.0	5.3	5.2	5.2	5.0
pH KCl	4.3	4.3	4.4	4.5	4.2
Δ pH	-0.7	-1.0	-0.8	-0.7	-0.8
Organic Matter					
CO (%)	1.54	1.07	1.92	1.65	1.10
OM (%)	2.65	1.84	3.31	2.84	1.90
N (g/kg)	0.30	0,26	0.41	0.36	0.28
C/N	51	41	47	46	40
Exchangeable cations and acidity (meq/100 g)					
Ca	1.32	1.52	2.25	1.50	1.40
Mg	0.36	0.45	0.62	0.44	0.34
K	0.20	0.15	0.25	0.10	0.15
Na	0.03	0.04	0.08	0.04	0.03
S	1.91	2.16	3.20	2.08	1.92
AE	0.12	0.08	0.10	0.09	0.12
Cation exchange capacity (meq/100 g)					
CEC	17.90	19.82	20.48	20.50	17.56
CECE	2.03	2.24	3.30	2.17	2.04
V	11	11	16	10	11
Assimilable phosphorus (mg/kg)					
P (Bray II)	8.00	13.50	7.82	10.15	8.39

Table 3. Chemical composition of powdered leaves of *T. diversifolia*.

N°	**Elements analyzed**	**Experimental values**
1	pHwater	9.1
2	pHKCl	8.5
4	pHwater/pHKCl	1.1
3	Nitrogen (%)	4.4
4	Phosphorus (%)	0.6
5	Potassium (%)	7.3

Phytochemical screening

The results of phytochemical screening have shown that the leaves of *T. diversifolia* are rich in phenols, flavonoids, tannins, lipids, saponins, anthocyanins, and alkaloids.

Extraction of essential oils of *T. diversifolia*

The yield finally obtained from hydrodistillation is 6%. The organoleptic characteristics obtained by the panel show that the colors of the essential oils go from light yellow to *T. diversifolia*, whereas the flavors are sweet and pungent.

Growth parameters and yield

For field work, the results of antimicrobial activities have shown that only essential oils were more effective than aqueous extracts of the leaves of *T. diversifolia* against pathogens in rice seeds. Then the extract of essential oil of *T. diversifolia* was used for the treatment of rice seeds by coating the seeds for 30 min, then for field work leave 24 hours at room temperature before sowing. the leaf powders of *T. diversifolia* were used as fertilizer by soil amendment. The tables below (Tables 4 and 5) show the results of the production characteristics in the field.

Table 4. Emergence, Appearance of plants, tillering, disease index and production of two cultivars of rice seeds treated with essential oil and amendment by *T. diversifolia* powders in Lelem locality.

Treatments	Emergence (%)	Seedling appearance	Tillering	Sizes (cm)	Vigor	Illness index	Weight per plots (kg)	Yield (tha-1)
Powder								
N3	98.0±2.0	4.7±0.6	37.3±1,5	158.3*±1.5	232750*±5.0	0.7±0.6	4.0±0.1	1.3*±0.1
N8	96.0±4.0	4.7±0.6	37.3±2.5	158.6*±1.2	227686.6*±9.0	0.0±0.0	11±0.1	3.7*±0.2
Fertilizer								
N3	94.7±4.0	4.7±0.6	38.3*±1.5	160.3*±0.6	227673.3*±1.9	0.3 ± 0.6	6.0±0.1	2.0*±0.6
N8	97.0±2.6	4.7±0.6	39.3*±0.6	160.0*±1.0	232800*±2.2	0.3±0.6	10.5±0.1	3.5*±0.2
Control								
N3	91.3±1.2	4.0±0.0	29.0±1.0	150.6±0.6	206413.3±0.5	3.4± 0.6	3.0±0.1	1.0±0.1
N8	95.3±5.0	4.3±0.6	29.6±0.6	150.0±1.0	214500±4.5	1.2±0.6	5.0±0.1	1.7±0.1

The data in the Table are the means ± SD of four repetitions over an area of 7.5 m^2. *: Denotes the statistically significant difference ($P<0.05$) between treatments and untreated control with a superior treatment.

Table 5. Emergence, Appearance of plants, tillering, disease index and the production of two cultivars of rice seeds treated with essential oil and amendment by powders of *T. diversifolia* in Kompina locality.

Treatments	Emergence (%)	Seedling appearance	Tillering	Sizes (cm)	Vigor	Index diseases	Weight per plots (kg)	Yield (tha-1)
Powder								
N3	94.0±0.0	4.7±0.6	30.3±1.5	100.3*±1.5	9428.2±5.0	1.6±0.6	4.0±0.1	1.3*±0.2
N8	94.0±0.0	4.7±0.6	30.3±2.5	100.6*±1.2	9456.4±9	0.0±0.0	8.0±0.1	2.7*±0.6
Fertilizer								
N3	92.0±0.0	4.7±0.6	32.3*±1.5	100.3*±0.6	9227.6±1.9	1.3±0.6	4.0±0.1	1.3*±0.1
N8	92.0±0.0	4.7±0.6	32.3*±0.6	100.0*±1	9200.0±2.2	1.3±0.6	8.5±0.1	2.8*±0.5
Control								
N3	90.0±0.0	4.0±0.0	29.0±1.0	100.6±0.6	9054.0±0.5	1.4±0.6	3.0±0.1	1.0±0.2
N8	92.0±0.0	4.3±0.6	29.7±0.6	100.0±1	9200.0±4.5	1.2±0.6	4.0±0.1	1.3±0.3

The data in the table are the means ± SD of four repetitions over an area of 7.5 m^2. *: Denotes the statistically significant difference ($P<0.05$) between treatments and untreated control with a superior treatment.

Growth parameters and yield in Lelem site

Impact of treatments of rice seeds with essential oil and amendment with *T. diversifolia* powders on emergence, the appearance of plants, degree of tillering, disease index, and yield in the locality from Lelem are represented in Table 4. It observed that the powder and essential oil increase the yield compare to control (Table 4).

Growth parameters and yield in Kompina site

Impact of treatments of rice seeds with essential oil and amendment with *T. diversifolia* powders on emergence, the appearance of plants, degree of tillering, disease index and yield in the locality of Kompina are represented in Table 5. It observed that the powder and essential oil increase vigor and yield compare to control (Table 5).

It appears from his two tables (Tables 4 and 5) that Emergence and Aspect of plants are very important for both sites. In the Lelem site (Table 4), the plots with plants amended to the powder of *T. diversifolia* had a great vigor compared to the negative control. In the Kompina site, the plots had almost the same vigor. In the Lelem site, tillering and size were very important and the maximum tillering average was 39 and the maximum mean size was between 150 - 65 cm.

The largest tillering and size were obtained in the almond plots with *T. diversifolia* powders compared to the untreated ones. While in the site of Kompina the tillering and the size decreased considerably and without significant difference for each treatment. In the Lelem site, the Disease Index was negligible. No symptoms of the seed-borne diseases were noted such as Helminthosporiosis and fusariosis. The plants were very vigorous and the climate was very favorable for normal development. In contrast, at the Kompina site, the Disease Index was between 1-2 on the scale based on the standard IRRI rice rating system (1980 and 1996).

The plants were not very vigorous and the climate was not very favourable for the normal development of the plants. We noted some attacks of the plants and also of the paddy produced (Fig. 4). In the Lelem site, Production (per elementary plot in kg) and the yield per experimental plot were very high in the plots amended with *T. diversifolia* powders compared to the unamended control (Table 4); whereas in the Kompina site, Production (per elementary plot in kg) and the yield per experimental plot were also high in the plots amended with *T. diversifolia* powders compared to the unamended control (Table 5). However, this production was very high at the Lelem site than at the Kompina site. This low production at the Kompina site could be explained by the presence of different climates at the two experimental sites.

Fig. 4. Helminthosporiosis symptoms observed in Kompina site.

Weight of 1000 grain

The average weight of 1000 seeds of the two different sites is shown in the table 6 below. The plots amended with chemical fertilizer and powder *T. diversifolia* had a higher weight than the untreated plots. The average weight of 1000 seeds was relatively high in Lelem site than in the Kompina site (Table 6).

Variation of chlorophyll rate

Table 7 represents the chlorophyll rate of each treatment in the two different sites. From this table, the chlorophyll rate is very high in the plots treated with fertilizer and powder of *T. diversifolia* than in the untreated plots. The chlorophyll rate is very high in Lelem site than in the Kompina site (Table 7).

Table 6. The weight of 1000 seeds of each treatment.

Treatments	Powder of *T. diversifolia*		Fertilizer		Control	
Varieties	N3	N8	N3	N8	N3	N8
Weight (g) in Lelem site	33±0.2	32±0.1	31±0.1	33±0.2	29±0.1	29±0.1
Weight (g) in Kompina site	30±0.1	31±0.0	31±0.00	30±0.1	29±0.1	29±0.0

The data in Table 6 are the mean ± SD of four replicates.

Table 7. Chlorophyll variation rate for each treatment.

Treatments	Powder of *T. diversifolia*		Fertilizer		Control	
Varieties	N3	N8	N3	N8	N3	N8
Chlorophyll in Lelem site	34±0.7	36±0.5	35±1.2	36±1.0	27±0.6	26±0.4
Chlorophyll in Kompina site	26±0.2	25±0.1	27±0.2	26±0.3	23±0.1	24±0.4

The data in Table 7 are the mean ± SD of four replicates.

Discussion

The treatment of seeds with essential oil and the amendment of the soil with the powder of leaves of *T. diversifolia* improved the various parameters in the field such as the emergence, chlorophyll rate, the appearance of the plants, the degree tillering, disease index and production. Significant differences in the density of the plants and the degree of tillering had repercussions in the same order of magnitude on the production except in the case of control without amendment. The synthetic fertilizer stimulated the emergence and tillering of the plants after the amendment. Powder of *T. diversifolia* has shown the best effect with a slight superiority to synthetic fertilizer. This similarity and superiority of this fertilizing form of *T. diversifolia* compared to synthetic fertilizer could be attributed to their content in active elements and compounds and to their great stability. This result confirms the effect of essential oil on the pathogens of rice seeds. According to the previous work, the chemical analysis of this oil, by Gas Chromatography, shows that it is rich in phenolic compounds and terpenoids. These compounds have strong antimicrobial power (Hulin et al., 1998; Nyegue et al., 2014). Phytochemical screening also shows that *T. diversifolia* powder is rich in polyphenols, terpenoids, alkaloids, saponins, lipids, and tannins with very important biological activities (Yoon et al., 2010), hence the considerable reduction of the symptoms of seed-borne diseases in the field. The results obtained in the field correlated the observations under laboratory conditions.

Concerning the total harvest weights and the weight of 1000 seeds, the amendments which gave the best harvest results are those amended to *T. diversifolia* powder and synthetic fertilizer on the two cultivars. Regarding only NERICA 8, their harvest represents 41.5% and 39.6%, respectively of the total weight of the harvest. Harvest is high compare to control no amended which represents 18.9% of the total weight of the harvest. These results can be justified by the fact that *T. diversifolia* powder is rich in fertilizing elements such as Nitrogen (N), Phosphorus (P) and Potassium (K) from which their origin comes either from the soil or from nutrients provided. Compared to the control plots where the only source of nutrients in the soil. Its elements go into synthesis and production in the plant. The richness in these nutrients in these treated plots may also explain why the plants in these plots were vigorous, with a high chlorophyll content compared to the untreated plots. Besides, fertilization is one of the most important factors that affect crop yield and plant health (Nawal et al., 2014).

Mengel and Kirkby (1982) indicated that nitrogen is an essential element for plant growth. 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. Nitrogen is also a fundamental element of plant tissue, it enters the formula of many constituents of structure (proteins), function (enzyme and coenzymes) and reserves (many grains). Nitrogen plays a role in the development of the vegetative apparatus (foliage), the place of synthesis of carbohydrates by the phenomenon of photosynthesis (Mehari, 1997). For the production of cereals and in the case of rice, 2/3 of the nitrogen absorbed by the plant is supplied by the soil (Russo, 1996). Phosphorus is also an essential element for plant life. Of the three major elements, phosphorus is recognized as having an effect on root growth, this shows the importance of localized phosphate manure, or rich soil on the rapid growth of plants. A good supply of phosphorus for the root system is necessary (Ben Tassil, 1981). Potassium is a major element essential for plant growth, its main functions in the plant are photosynthesis and translocation (assimilates, opening and closing of stomata and enzyme activation) (Mengel and Kirkby, 1982). The major nutrient requirements for the rice plant are known (Brown, 1993). The fertilizer formula used varies from one rice farmer to another, depending on the nature of the soil and the previous crop (Hsieh, 1992).

Regarding production, it was very high at the Lelem site than at the Kompina site despite the amendment. This low production at the Kompina site can hardly be explained by the presence of a very hot climate compared to that of Lelem, which is less hot. The season could also be one of the factors determining production, in the Lelem site, cultivation was carried out in the first rainy season (March-July) characterised by a high quantity of water, on the other hand, in the Kompina zone, cultivation was carried out in the second rainy season (September-November) characterised by a low quantity of water. Also the soil could also have a very great influence on this production. According to analyses, the soil of Kompina is very acidic compared to the soil of Lelem which is a little basic. Also, the C/N ratio is very high (C/N > 40 %) in the Kompina experimental site than in the Lelem site (C/N <15 %). This means that in the Lelem site, organic matter is more bioavailable to the plant than in the Kompina site. According to N'Dayegamiye et al. (2005) when the ratio C/N is low, the quantity of organic matter is bioavailable. In addition, the content of Mg, N, P, K is high in the Lelem site, which results in good photosynthesis and good production.

These results make it possible to recommend the use of the essential oil of *T. diversifolia* as a biopesticide and the powder *T. diversifolia* as a biofertilizer to control rice seeds to improve the quality and quantity of rice produced in Cameroon, where farmers are limited resources. Given the possibility of domesticating *T. diversifolia* on a large scale, their new users added to their use in traditional pharmacopeia and as flavorings in food would increase the income of farmers. However, an economic evaluation of such treatments would be appropriate to better understand their impacts.

Conclusion

At the end of this work, which focused on the evaluation in the field of seed treatments using the essential oil of *T. diversifolia* and the soil amendment using powders from the same plant, it shows that the treatments of the plots amended with the powder and seeds treated with essential oil from the same plant had a positive impact on the improvement of growth parameters and production yield; as well as reducing the incidence of the disease. These results were almost similar to the plots amended by fertilizer and seeds treated with essential oil from the same plant and superior to the results of untreated plots. We recorded a large production in the Lelem site than in the Kompina site. Given these results, it remains to explore this natural resource to produce a biofertilizer or a biopesticide. For this, we have soon to deeply characterize the powders of *T. diversifolia* and to test in several areas.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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