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The cumulative use of compost teas and rhizobia against *Megalurothrips jostetti* for yield improvement in field grown cowpea at Ngaoundere (Cameroon)

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Abstract

Cowpea (*Vigna unguiculata*) is a food crop legume, considered as one of the cheapest sources of protein. However, its culture is subject to attack by many pests that drastically reduce yield. An alternative for better pest control and increased yield is the simultaneous use of biopesticides and biofertilizers. The objective of this study was to control the pests, while optimizing yields of this legume in a sustainable manner. To achieve this, compost teas and rhizobia were first produced and characterized. Then a pot experiment was set up in a triplicated randomized block design comprising 11 treatments, in order to determine the ability of compost teas and rhizobia to control insect pests and optimize the yield of this Fabaceae. The characterization of the compost teas revealed that the pH varied around the neutrality with an abundance of alkaloids and terpenoids and the absence of tannins. The three isolates obtained were all Gram negative bacteria and did not absorb the dye Congo red. Compost teas reduced and stabilized thrips (*Megalurothrips jostetti*) population development. Non-aerated compost teas from *Tithonia diversifolia* (PNA) alone, and its association with rhizobia (Rh + TNA) resulted in a reduction of the number of flower buds attacks. Their effects appeared to be limited outside the pods as they did not positively affect the number of seeds attacked. Both PNA and Rh+TNA treatments increased pod number, and seed biomass. Rhizobia and rhizobia + non-aerated compost tea from *Tithonia diversifolia* (Rh+TNA) significantly increased the yield of this plant species.

Keywords: Compost tea; Rhizobia; Gram Negative Bacteria; Thrips; *Vigna unguiculata*

1. Introduction

In Central Africa countries, particularly in Cameroon, the production of food plants is generally unable to meet the nutritional needs of ever-growing populations. The non-achievement of these needs is related to a decrease in agricultural productivity, which thus exposes a significant proportion of the population of these countries to undernourishment and very often to malnutrition [1]; although one of the key objectives of agricultural policy is to ensure exponential growth in yields [2]. Therefore, it is necessary to develop methods that are healthy and accessible to populations, such as application of compost teas to fight against insect pests and bioinoculants (rhizobia) to increase the yield of crops within a food security and sustainable development programs [3, 4].

However, the exploitation of compost teas and rhizobia rarely features in the agricultural programs of many African countries [5, 6]. Although, these biofertilizers play a positive role in controlling insect pests or improving soil fertility in several countries around the world [6, 7]. Generally, the low yield of certain crops is attributed to several factors

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including the inefficiency of fertilizers, the uncontrolled use of pesticides, the activity of insect pests, the poor quality of the soil, as well as weeds [8, 9].

Cowpea (*Vigna unguiculata*) is a very important plant for the populations of Cameroon, whose leaves and/or grains are consumed in all regions of this country. The fresh leaves of this plant are among the most consumed leafy vegetables (in the form of soup) in Adamaoua - Cameroon [10]. Its seeds are very caloric and rich in vegetable proteins, vitamins and mineral elements [11]. These seeds are eaten alone or combined with other vegetables or crushed in the form of koki, or donut [12]. It also plays an important role as a source of nitrogen for cereal crops (such as maize, millet and sorghum), especially in areas characterized by low soil fertility. Roots are equipped with nodules populated by bacteria (*Rhizobium*) which contribute to the fixation of atmospheric nitrogen in a form assimilable by plants [13]. Despite these many assets, this plant is still one of the most neglected and undervalued species by donors. This plant could offer enormous potential in the composition of foods to alleviate malnutrition in areas of chronic malnutrition in Cameroon [14].

In Cameroon, more specifically in Adamaoua, the few works carried out to improve cowpea cultivation are those of [15] on Pesticidal activity of plant extracts and a mycoinsecticide (*Metarhrizium anisopliae*) on cowpea flower thrips and leaves damages in the field, while rhizobia inoculation were used in the field to increase the yield of this culture [6]. To our knowledge, no study has yet been carried out on the joint use of an organic and microbial fertilizer on the cultivation of cowpea in the Adamaoua Region. Therefore, the main objective of this research was to assess the efficiency of four types of compost teas and a rhizobial inoculum against one of the most devastating cowpea pest (*Megalurothrips jostedti*), while improving yield in a plot experiment.

In this work, we will characterized the compost teas from *Tithonia diversifolia* and chicken manures, as well as the Rhizobia in order to determine their influence on the population of the insect pest and the cowpea yield.

2. Materials and Methods

The experiment was carried out in the Guinean Savannah agro-ecological zone at Dang, within the experimental field of the Unit for Apply Apidology (latitude: 7°42.264 N; longitude: 13°53.576 E; altitude: 1124 m a.s.l.) of the Faculty of Science, University of Ngaoundere. The climate is characterized by a rainy season (April to October) and dry season (November to March), with an annual rainfall of approximately 1500 mm. The mean annual temperature is 22°C, while the mean annual relative humidity is 70 % [16]. The plant material was represented by *Vigna unguiculata* seeds of the Bafia variety (Figure 1), with a life cycle of 85 to 95 days provided by the Institute for Agricultural Research and Development (IRAD) at Wakwa-Ngaoundere. Synthetic fertilizer was applied from the 30th day after sowing, at 15 g per plant [6]. Synthetic insecticide solution was obtained by mixing 3 ml of Cyperal (a substance belonging to the family of pyrethroids with the formula $C_{22}H_{19}Cl_2NO_3$ (Figure 2) with 5 liters of borehole water. The solution obtained was stored in plastic bottles for field application. The NPK fertilizer used was of the formula 20:10:20, purchased from a local phytosanitary store. It was applied at 14 days after sowing, at a rate of 10g within the rhizosphere of a plant.

Figure 1 *Vigna unguiculata* seeds of Bafia variety

Figure 2 Cypéral insecticide sachets

2.1. Production and characterization of compost teas

The production process of compost teas takes place in two phases: the extraction phase and the fermentation phase. The presence of oxygen or not during the second phase differentiates between aerated and non-aerated compost teas. The production of compost teas was based on the method of [17]. Chicken manures and *Tithonia diversifolia* based composts used were obtained from the biofertilizers and biopesticides production unit of IRAD in wakwa, headed by Professor Albert Ngakou.

Composts thus obtained underwent manual sorting and then were sifted through a 0.0001mm mesh sieve to remove impurities. Subsequently 1kg of each compost type was introduced into two 20 liters containers each, then 15 liters of borehole water were added, a spatula for aeration (production of aerated compost tea) was placed in one of the two containers containing each type of compost. The mixtures thus produced were infused for 72 hours. For non-aerated compost teas, the containers were hermetically sealed for 72 hours, while for aerated compost teas, stirring for 15 minutes every 2 hours was carried out using the spatula. The compost teas obtained were stored in the refrigerator at 4°C for further uses.

2.2. Characterization of Compost Teas

2.2.1. pH determination

The pH measurement was carried out according to the protocol of [18]. Hence, 5 ml of each compost tea sample was taken and the electrode of the pH meter was immersed in the liquid and the measurement was made after stabilization of the reading.

2.2.2. Phytochemical tests

Alkaloids

Compost teas (1.0 mL) were dissolved in 2N HCl solutions. The mixture was treated with a few drops of Meyer's reagent (3.0 ml of potassium iodide solution mixed with 2.0 ml of mercuric chloride solution). The creamy precipitate indicates the presence of alkaloids [19].

Polyphenols

In a test tube containing 1.0 ml of compost tea, a few drops of a 2% Ferric Chloride ($FeCl_3$) solution were added. The presence of phenolic compounds materialized by obtaining a purple, blue-green or black solution [20].

Flavonoids

In a test tube containing 1.0 ml of compost tea, a few drops of a 20% NaOH solution were added. The change to yellow which upon addition of acid turns into a colorless solution representing the presence of flavonoids [20].

Terpernoids

1.0 ml of compost teas were mixed in 2.0 ml of chloroform. Then 3.0 mL of concentrated H_2SO_4 was then added to form a layer. A reddish-brown precipitate stain at the formed interface indicates the presence of terpernoids [21].

Saponins

The presence of saponins was determined by the foam test. The compost teas were shaken vigorously with distilled water and allowed to stand for 10 minutes. Formations of a fairly stable emulsion indicated the presence of saponins [22].

Tannins

Compost teas were treated with FeCl3 alcoholic reagent, bluish black, which disappears on adding a few drops of dilute H2SO4 followed by the formation of a yellowish brown precipitate indicating the presence of tannins [19].

2.3. Production and characterization of the rhizobia

2.3.1. Trapping of rhizobia

The soils used were taken from agricultural plots within the university campus from where legumes for previous crops were preciously cultivated. The sampling depth was 20 cm, then the soils were mixed to optimize the diversity of bacteria. The soils thus harvested were introduced into 15 nursery bags at the rate of one kilogram per pot. Subsequently, three cowpea seeds were introduced into each pot and then one week after the plants emerged, the plants were thinned to leave one plant per pot. The pots were watered twice a day using borehole water until the plants flowered.

To harvest the nodules, the method recommended by [23] and [24] was adopted. It was a question of removing and separating 15 plants from their different pots, in order to take the whole plant with all its root part. Thereafter, the soil was cleared manually at the level of the roots, without damaging the nodules, then the roots with their nodules were gently washed with tap water. Finally, the nodules were harvested manually from the roots of each plant, then collected and stored in plastic boxes in order to transport them to the laboratory.

Once in the laboratory, the nodules of each legume were dried for three days in the open air and stored at room temperature in format papers for subsequent isolation of rhizobia [25].

The rehydrated nodules were disinfected on the surface by immersion in 95% ethanol for 5 to 10 second, then were immediately transferred into a bleach solution for 3 minutes, before rinsing (05 times) with sterile distilled water [25]. The aim was to eliminate bacteria sensitive to ethanol and fungi to bleach [26]. The sterilized nodules contained in a pasteurized Petri dish were crushed individually in a drop of sterile distilled water using forceps, disinfected beforehand by immersion in ethanol and under a Bunsen burner [25].

Using a platinum loop, flamed with a Bunsen burner, aliquot from the ground nodules was inoculated onto solid YEMA medium, so as to isolate colonies that were easily identified according to the four-dial method as described by [23]. Petri dishes thus inoculated were then incubated at 28°C in the dark for 3 days to allow growth of colonies.

Colonies belonging to the genus *Rhizobium* were identified by their gummy and translucent morphological appearance, corresponding to the description of [23] and [24]. Pure isolates were inoculated on liquid YEMA medium, in tubes and incubated at 28°C for 3 days, then stored at 4°C [23], for further uses.

2.3.2. Characterization of rhizobia isolates

Characterization of *Rhizobium* isolates was carried out based on the observation of cultural, morphological and biochemical criteria.

Pure *Rhizobium* isolates were subcultured on YEMA medium after 3 days of incubation. Using the standard microbiological technique described by [24], the following morphological criteria were considered for the macroscopic description: shape, color, elevation, size, contour and surface.

Microscopic examination of pure *Rhizobium* isolates achieved by Gram staining and observation under an optical microscope (100X) under immersion oil [27]. Bacteria are classified according to their ability to fix crystal violet. Those with an outer envelope are discolored when washed with ethanol and restain with a fushine dye (Gram-), while those without will retain the dye (Gram+).

The consistency and value of the Gram stain correspond to biochemical differences between the wall of Gram-positive bacteria and Gram-negative bacteria [28].

2.3.3. Congo red absorption test (biochemical characterization)

Isolates were inoculated on YEMA medium containing 0.25% Congo red and incubated in the dark for 72 hours at 28°C, in order to assess their absorption capacity of Congo red [23, 29]. Rhizobia absorb little or no Congo red compared to contaminants that strongly absorb this dye. Such as *Bradyrhizobium*, *Agrobacterium* and other contaminant [29].

2.3.4. The bromothymol blue test as biochemical characterization

Test was used to check the acidification or alkalinization capacity of strains [30]. The isolates were inoculated onto YEMA medium + bromothymol blue for a final concentration of 0.25% and incubated at 28°C. Change in the medium to yellow indicates the acidification of the medium and the change to blue indicates the alkalinization of the medium [31]. It also makes it possible to classify bacteria (*Rhizobiums*) according to their growth rates. Fast-growing bacteria modify the pH of the medium in 24 hours, while slow-growing bacteria acidify the medium late (5 to 6 days) [30].

2.3.5. Production of the rhizobia as inoculum

Rhizobia isolates thus identified were removed using a spatula and then introduced into a glass jar containing liquid medium (YEMA). After 3 days of growth, the jar containing our inoculum was transported to the fields for application.

2.4. Experimental layout and treatments

Investigations were conducted at Dang, within the experimental field of the Unit for Apply Apidology (latitude: 7°42.264 N; longitude: 13°53.576 E; altitude: 1124 m above sea level) of the Faculty of Science, University of Ngaoundéré, Cameroon. The experiment was set up in a complete randomized block design with 11 treatments and 3 repetitions (Table 1). The experimental plot representing the treatments was a square of (1.10×1.10) m^2 made up 12 holes, for a total area of (20.2 x 5) m^2. Three seeds were sown per hole spaced 30 cm from each other, while lines were 10 cm apart. The distance between the subplots was 30 cm. Using a sterile syringe, each hole of treated plots received 2 ml of inoculum [32]. After application of the inoculum, seeds were covered with soil. Weeding was carried out once every month. Spraying of pesticides (25 ml per plant) and compost tea (25 ml per plant) was performed after every 5 days [33], from August 28, 2020, then was repeated on September 3, 8, 13, 2020. The sprayings during the flowering periods took place on September 18, 23, 29, 2020 and October 05, 2020.

Table 1 Distribution of treatments in the experimental surface

Bloc I	**Bloc II**	**Bloc III**
Rh	C-	Rh + PA
TNA	Rh + TA	TA
PA	Rh + PNA	C+
TA	Rh + PA	PNA
PNA	Rh + TNA	PA
Rh + TNA	C+	Rh + TA
Rh + PNA	TNA	C-
C-	PA	Rh
Rh + PA	PNA	Rh + PNA
C+	Rh	TNA
Rh + TA	TA	Rh + TNA

Rh : *Rhizobium* inoculum ; C- : without inputs or pesticides ; C+: positive control (NPK + Cyperal) ; TNA : non-aerated compost tea based on *Tithonia diversifolia* compost ; PNA : Non-aerated compost tea based on chicken manures compost ; TA : Aerated compost tea based on *Tithonia diversifolia* compost ; PA : non-aerated compost tea based on chicken manures compost ; Rh + TNA : *Rhizobium* inoculum + non-aerated compost tea based on *Tithonia diversifolia* compost ; Rh + PNA : *Rhizobium* inoculum + Non-aerated compost tea based on chicken manures compost ; Rh + TA : *Rhizobium* inoculum + Aerated compost tea based on *Tithonia diversifolia* compost ; Rh + PA : *Rhizobium* inoculum + non-aerated compost tea based on chicken manures compost.

2.5. Assessed Parameters

At harvest, the diversity of cowpea entomofauna, thrips population, number of pods and seeds per plant, seed mass per plant, and yield were assessed.

2.6. Evaluation of influence of treatments on the diversity of cowpea entomofauna

During the growth and development phase of cowpea, the observation of insects took place in the morning on plant organs. The observation and identification of all the insects encountered in the cowpea plots were classified according to their scientific name, genus, species, order and family. Their status, their types depending on whether they were pests or phytophagous were recorded [34].

The identification of the species was based on morphological differences by comparison between the insect samples collected with those appearing in the catalogs and the data of the insect identification software. Insects were subjected to a deep visual examination accentuated on its external morphology (shape, color…) followed by a detailed examination of each part of the body [35].

To assess the thrips *Megalurothrips jostedti*, 10 floral buds, 10 flowers and/or 10 pods were randomly selected from the experimental units at the rate of one floral bud, one flower or one pod per plant previously identified per plot unit, in the squares of infestations, a total of 30 fruiting bodies per treatment. The population of *Megalurothrips jostedti* in each flower was determined by counting thrips present in collected flowers from 07:00-12:00 a.m.

2.7. Evaluation of the impact of treatment on attacked flower buds, deformed pods, damaged seeds

The number of flower buds attacked was assessed at pod formation by counting [7].

The number of deformed pods was counted at harvest by counting in order to study the influence of compost teas on pod pests [36].

The number of seeds attacked per plant was counted after shelling all the pods [37].

2.8. Assessment of yields parameters

The number of pods at harvest was evaluated by counting, since the number of pods component is representative of the growth and development conditions between flowering and the start of seed growth [38].

The number of seeds per plant was assessed by direct counting after decortication and sorting of seeds [39].

The seeds were then weighed with an electronic scale brand ELECTRONIC SOALE at 0.01g sensitivity. Seed yield (kg/ha) was calculated using the following formula:

$$Y = \big((P * 1000)\big)/(SE)$$

where Y = seed dry weight in kg/ha; P = dry weight of seeds per unit of experimental area; SE = experimental area (m^2) and 1 ha = 10.000 m [40].

2.9. Statistical Analysis

Microsoft Excel 2016 software was used to enter the raw data in numerical format and to construct the graphs. Data obtained were processed using the R-commander software which performs the analysis of variance (ANOVA) between the treatments. The correlation coefficient (r) was used to assess the linear relationships between two variables and the t-student test for the comparison of means of two samples.

3. Results and Discussion

3.1. Characteristics of Compost Teas

The pH of compost tea ranged from 7.81 to 7.95 (Table 2) which corresponds to the neutral pH range. These values are closed to those of the pH of compost extracts varying from 7.3 to 7.5 obtained by [41]. According to [42], pH of compost teas is around the neutrality. However, pH is one of the fermentation factors that influence the effectiveness of compost teas, the growth and diversity of microorganisms [43]. In addition, neutral pH is specific to the growth of bacteria, while those of fungi and yeasts are favored by alkaline pH, but also by an acidic pH [44].

Phyto-chemical analysis was performed on compost teas to determine the presence and abundance of certain secondary metabolites including polyphenols, flavonoids, alkaloids, terpernoids, saponins and tanins. Their presence in compost teas is an indicator for the activity of certain microorganisms [45]. The result has revealed the presence of polyphenols, alkaloids, terpernoids and saponins (Figure 3), then the absence of flavonoids and tannins (Table 2). Polyphenols were moderately higher in TA tea than in other compost teas. There was a strong presence of alkaloids in compost teas TA (compost tea based on aerated *Tithonia diversifolia*), PA (compost tea based on aerated chicken manures), PNA (non-aerated compost tea based chicken manures). Likewise, there was increased presence of terpernoids in all compost teas. Saponins were strongly represented in PA and PNA than in TA and TNA.

Under unfavorable conditions, secondary metabolites play a main role in protecting plants against pathogens and environmental stresses [46]. Certain terpenes such as gibberellins (diterpenes), sterols (triterpenes), carotenoids (tetraterpenes), and acetic acid (sesquiterpenes) play an important role in plant growth and development [47]. A good example is chrysanthemum pyrethroids that were revealed to act as insecticides [47]. Phenolic compounds have been revealed to be involved in many physiological processes in plants, including reproduction, growth and defense against different biotic and abiotic factors [46]. They have antioxidant activity due to their oxygen reducing properties [48]. Certain flavonoids such as anthocyanins are responsible for the development of petal color to attract pollinators [49]. These metabolites were said to have allopathic activity in that they reduce the growth of neighboring plants or heteropathic activity [47].

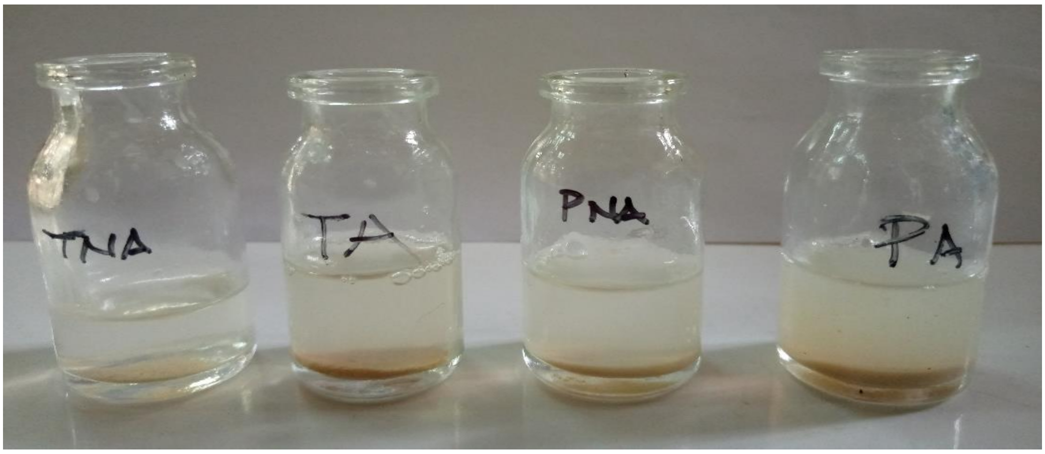


Figure 3 Photochemical test indicating appearance of a creamy precipitate demonstrating the presence of alkaloids

Table 2 pH assessment and Phyto-chemical test of different compost teas

Composts teas	pH	Polyphenols	Alkaloids	Terpernoids	Saponins	Flavonoids	Tanins
TA	7.90	++	+++	+++	+	-	-
TNA	7.91	+	+	+++	+	-	-
PA	7.81	+	+++	+++	+++	-	-
PNA	7.92	+	+	+++	+++	-	-

(+++): strong presence; (++): average presence; (+): low presence; (-): absent; PA: compost tea made from aerated chicken manures; PNA: compost tea made from non-aerated chicken manures; TA: compost tea based on aerated *Tithonia diversifolia*; TNA: compost tea made from non-aerated *Tithonia diversifolia*

3.2. Morphological and cultural characteristics of rhizobia colonies

Growth in YEMA medium is one of the most important phenotypic criteria in the characterization of *Rhizobium* [23]. The isolation of *Rhizobium* from cowpea root nodules revealed 3 *Rhizobium* isolates for this variety. These results are similar to those of [25], who also found 3 isolates for the same cowpea variety.

After incubation on YEMA culture media at 27°C for 3 days, the morphological and phenotypic characters were recorded (Table 3). These isolates form homogeneous colonies along the streaks on YEMA medium. They were are whitish, yellowish and milky in color, circular in shape, regular in outline, with a smooth concave surface and diameters ranging from 0.3 to 0.7 cm. These morphological characteristics of rhizobia are similar to those described by [32]. [50] pointed out characteristics of *Rhizobium* isolated from four legumes (soybean, cowpea, groundnut and Bambara groundnut) grown in Adamawa-Cameroon. Isolate W was whitish in color, circular in shape, regular in outline, with a smooth concave surface and 0.7 cm in diameter, similar to Y and M isolates except for their colors and diameter. The Y isolate was yellowish and has a diameter of 0.3 cm, while the M isolate was milky and has a diameter of 0.4 cm. Differences in color between these isolates might indicate that they do not belong to the same species [51].

Gram staining of all isolates displayed a pink color, indicating that all isolates were Gram negative (−). The results obtained are in agreement with those of [29], who reported that *Rhizobium* a Gram-negative bacteria. These bacteria all have a wall made up of a substance called murein which is a peptidoglycan.

Table 3 Morphological and cultural analyzes of colonies

Plant	Number of isolates	Colors	Elevation	Form	Outline	Surface	Diameters (cm)	Gram staining	Attributes
Vigna unguiculata	3	whitish	convexe	circular	regular	smooth	0,7	-	W
		yellowish	convexe	circular	regular	smooth	0,3	-	Y
		milky	convexe	circular	regular	smooth	0,4	-	M

W: whitish isolates, Y: yellowish isolates, M: milky isolates

Culture of the isolates on YEMA + Congo red medium gave after 72 h, colonies which did not absorb this dye (Table 4), which is a typical characteristic of *Rhizobium*. The growth of isolates on YEMA medium supplemented with Congo red suggests that the strains were pure, since they weakly took the dye [52]. These criteria were observed as characteristics of *Rhizobium* [29, 23].

Regarding growth in YEMA + BBT medium, all the isolates caused a change in the color of the medium towards blue. Bromothymol blue is a colored indicator that can highlight an acid or basic reaction in a pH range extending from 6 to 7.6. An acid reaction results in the change of color to yellow, while an alkaline reaction results in the development of blue color [31]. Fast-growing isolates are considered as acidifying bacteria (color change of BTB to yellow), unlike slow-growing strains which are considered as alkalizing bacteria which give a color change to blue [29, 53, 54]. From this analysis, we noticed that studied *Rhizobium* isolates were slow growers that characterize the genus *Bradyrhizobium* [24].

Table 4 Growth results of isolates on YEMA + RC and YEMA + BBT media

Plant	Isolates	Acidification or Alkalinization (YEMA + BBT)	Dye absorption (YEMA + Congo red)
Vigna unguiculata	W	Blue (Alkalinization)	-
	Y	Blue (Alkalinization)	-
	M	Blue (Alkalinization)	-

(-) no absorption of Congo red

3.3. Influence of rhizobia, compost teas mixture on cowpea entomofauna and yield

The inventory of the cowpea entomofauna in the study area revealed a wide diversity of insects made up of pests, pollinators and predators (Figure 4).

Figure 4 (A) Leaf insect pest (*Pyrgomorpha* spp.), (B) pollinator insect (*Apis mellifera*) and (C) predator insect (*Animalia axiridis*)

The identified insects were grouped in eight (08) orders comprising into 13 families and 17 species. This result is lower than that of [34], who found 08 orders, composed of 27 families and 39 species. The different orders were Coleoptera, Diptera, Heteroptera, Homoptera, Hymenoptera, Lepidoptera, Orthoptera and Thysanoptera (Table 5).

Table 5 Entomofauna of the host plant classified by Order, Family and Species

	Order	Family	Species	Frequencies (%)
	Coleoptera	3	5	29.41
	Homoptera	3	3	17.64
	Hymenoptera	1	1	5.88
	Lepidoptera	2	2	11.76
	Orthoptera	1	2	11.76
	Thysanoptera	1	2	11.76
	Diptera	1	1	5.88
	Heteroptera	1	1	5.88
Total	8	13	17	100

A total of 17 insect species were collected from the different cowpea plots. Coleoptera was the most important order with 05 species (29.41 %), followed by the Homoptera with 03 species (17.64 %) then the Lepidoptera, Thysanoptera and Orthoptera with 02 species (11.76 %) each, and finally the Diptera, Hymenoptera and Heteroptera with 01 species (5.88%) each (Figure 5).

From figure 6 it appears that the NPK + cyperal treatment has a statistically lower number of thrips (p = 0.0007) compared to the other treatments, while *Rhizobium* treatment associated with aerated compost tea made from chicken manures (Rh + TA) has the highest number compared to other treatments. All plots treated with the pesticide cyperal had the best control of *Megalurothrips jostedti* population, whereas the response of thrips to treatments rhizobia and compost teas was weak. The application of compost teas on cowpea could have multiple effects, protective, suppressive and/or repellent, consistent with results found by [55], who revealed 60 % to 80 % control of insect pests of okra (*Abelmoschus esculentus*) with cornstalk compost extract, while those with extracts based on *Manihot esculenta* and *Tithonia diversifolia* weakly affect the pests. Suppression has been attributed to direct pathogen suppression or induction of systemic resistance [56]. Moreover, the protective effect could be due to inhibition of germination of *Megalurothrips jostedti* larvae, mechanisms of antagonism and competition with pathogens, as well as induction of resistance reactions in cowpea [57].

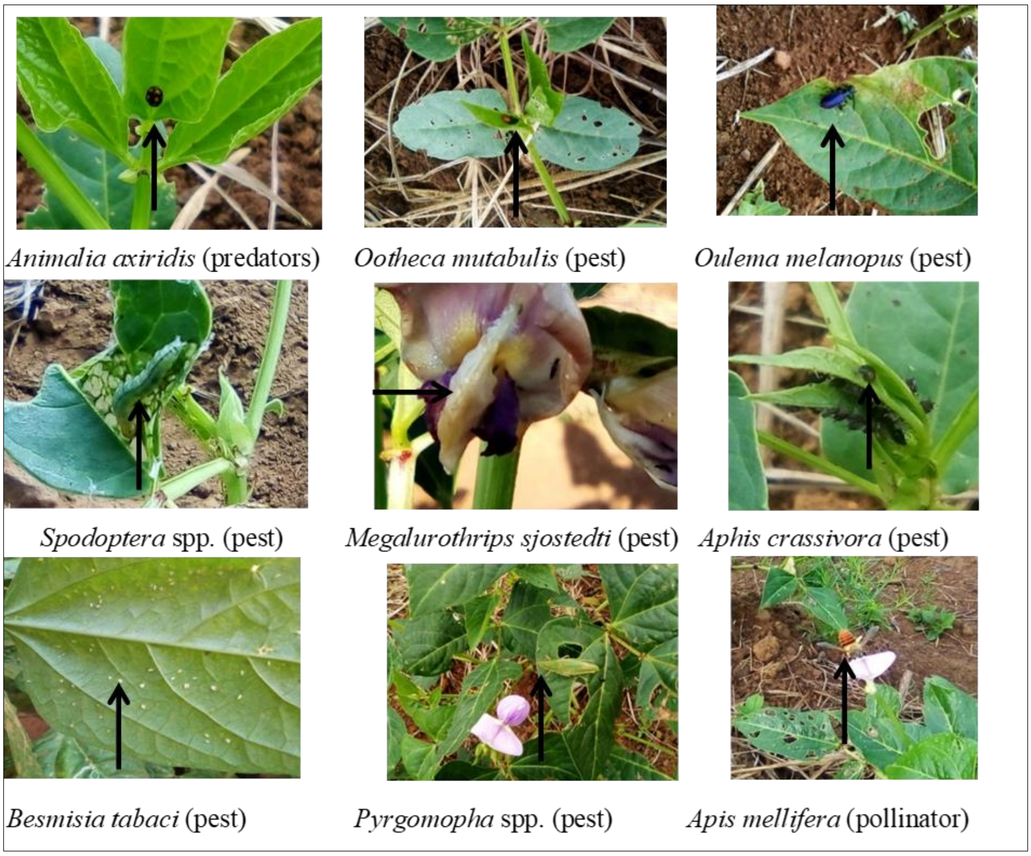


Figure 5 Some insects species found on cowpea leaves and flowers

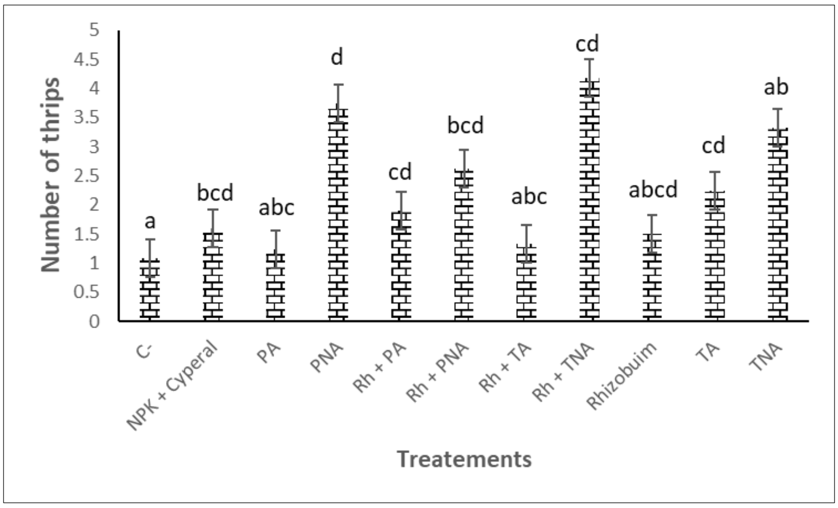


Figure 6 Effect of treatments on the number of adult thrips

Differentiated values of different letters for a given parameter in each column are significantly different at the 5% level.

Rh: *Rhizobium* inoculum; C-: Negative control (without inputs or pesticides); C+: positive control (NPK + Cyperal); TNA: Non-aerated compost tea made from *Tithonia diversifolia* compost; PNA: Non-aerated compost tea made from chicken manures compost; TA: Aerated compost tea made from *Tithonia diversifolia* compost; PA: Non-aerated compost tea made from chicken manures compost; Rh + TNA: *Rhizobium* inoculum + Non-aerated compost tea made from *Tithonia diversifolia* compost; Rh + PNA: *Rhizobium* inoculum + Non-aerated compost tea made from chicken manures compost; Rh + TA: *Rhizobium* inoculum + Aerated compost tea based on *Tithonia diversifolia* compost; Rh + PA: *Rhizobium* inoculum + Non-aerated compost tea made from chicken manures compost.

Table 6 sums up of the effect of the treatments on flower buds, deformed pods and seeds attacked. The aerated compost tea treatments based on chicken manures (PA) and rhizobia combined with non-aerated compost tea based on *Tithonia diversifolia* (Rh + TNA) significantly reduced the of attacked flower buds compared to other treatments. Therefore, non-aerated compost teas could have a suppressive effect on flower bud pests.

Afterwards, *Rhizobium* (Rh) treatment has a number of deformed pods statistically higher than that of all the other treatments. In addition, the number of deformed pods of the NPK + Cyperal and *Rhizobium* treatments associated with non-aerated compost tea based on chicken manures (Rh + PNA) is nil.

Finally, the NPK + Cyperal treatment has a statistically lower number of seeds attacked than the treatments *Rhizobium* associated with non-aerated compost tea based on *Tithonia diversifolia* (Rh + TNA), *Rhizobium* associated with non-aerated compost tea based on chicken manures (Rh + PNA) and other treatments. As a result, compost teas increased the number of attacked seeds. This increase would be linked to the fact that the action of the phytosanitary products being of short duration, there was therefore certainly a reinfestation of the plots by new populations of *megalurothrips jostedti* since the spraying of the compost tea inoculum took place at the start of flowering [7].

Table 6 Effect of treatments flower buds, pods and seeds attacked

Treatments	**Attacked flower buds**	**Deformed pods**	**Seeds attacked**
C-	3.33±2.84 abc	0.58±0.35 ab	2.75±1.15 abc
NPK + Cyperal	2.0±0.57 abc	0	0.66±0.28 a
PA	6.33±2.90 c	0.67±0.22 ab	4.25±1.19 abc
PNA	1.0±0.577 ab	0.25±0.13 a	3.41±0.94 abc
Rh + PA	1.33±0.88 abc	0.25±0.17 a	2.91±1.03 abc
Rh +PNA	2.33±0.33 abc	0	5.75±1.06 c
Rh + TA	1.67±0.33 abc	0.16±0.16 a	1.33±0.75 ab
Rh + TNA	0.67±0.33 a	0.47±0.28 ab	4.91±1.22 bc
Rhizobuim	3.67±1.76 abc	1.47±1.00 b	2.16±0.70 abc
TA	6.0±0.57 bc	0.25±0.13 a	4.0±1.61 abc
TNA	4.33±1.76 abc	0.25±0.17 a	3.66±1.53 abc
P	0.15	0.25	0.05

Differentiated values of different letters for a given parameter in each column are significantly different at the 5% level. ; Rh: *Rhizobium* inoculum; C-: Negative control (without inputs or pesticides); C+: positive control (NPK + Cyperal); TNA: Non-aerated compost tea made from *Tithonia diversifolia* compost; PNA: Non-aerated compost tea made from chicken manures compost; TA: Aerated compost tea made from *Tithonia diversifolia* compost; PA: Non-aerated compost tea made from chicken manures compost; Rh + TNA: *Rhizobium* inoculum + Non-aerated compost tea made from *Tithonia diversifolia* compost; Rh + PNA: *Rhizobium* inoculum + Non-aerated compost tea made from chicken manures compost; Rh + TA: *Rhizobium* inoculum + Aerated compost tea based on *Tithonia diversifolia* compost; Rh + PA: *Rhizobium* inoculum + Non-aerated compost tea made from chicken manures compost.

3.4. Influence of treatments on pod and seed yields

The study of the influence of treatments on the number of pods (Figure 7A) demonstrated that *Rhizobium* treatment associated with non-aerated compost tea with *Tithonia diversifolia* (Rh + TNA) presents a statistically higher number of pods (p = 0.005) compared to chemical fertilizer treatments combined with cyperal pesticides (NPK + Cyperal), non-aerated compost tea based on chicken manures (PA) and *Rhizobium* combined with aerated compost tea based on *Tithonia diversifolia* (Rh + TA). Similarly, the non-aerated compost tea treatment based on chicken manures (PNA) shows a statistically high number (p = 0.0403) compared to the *Rhizobium* treatment aerated compost tea based on chicken manures (Rh + TA). The other treatments did not have a statistically higher number of pods than the negative control (C-). Rhizobia treatments associated with aerated compost tea based on *Tithonia diversifolia* (Rh + TA) and non-aerated compost tea based on hen droppings (PNA) had a better pod yield, while rhizobia treatment associated with aerated compost tea made from chicken manures (Rh + TA) had the lowest. Furthermore, there was a weak positive correlation (r = 0.12669; p < 0.0001) between the number of pods and the number of cowpea thrips. These results are consistent with those of [6], who showed that the inoculation of legumes with rhizobia presented a positive and significant response on the number of pods. Compost teas are able to improve photosynthetic efficiency which is a key factor in amino acid production hence initial root development, nutrient uptake and plant growth [58].

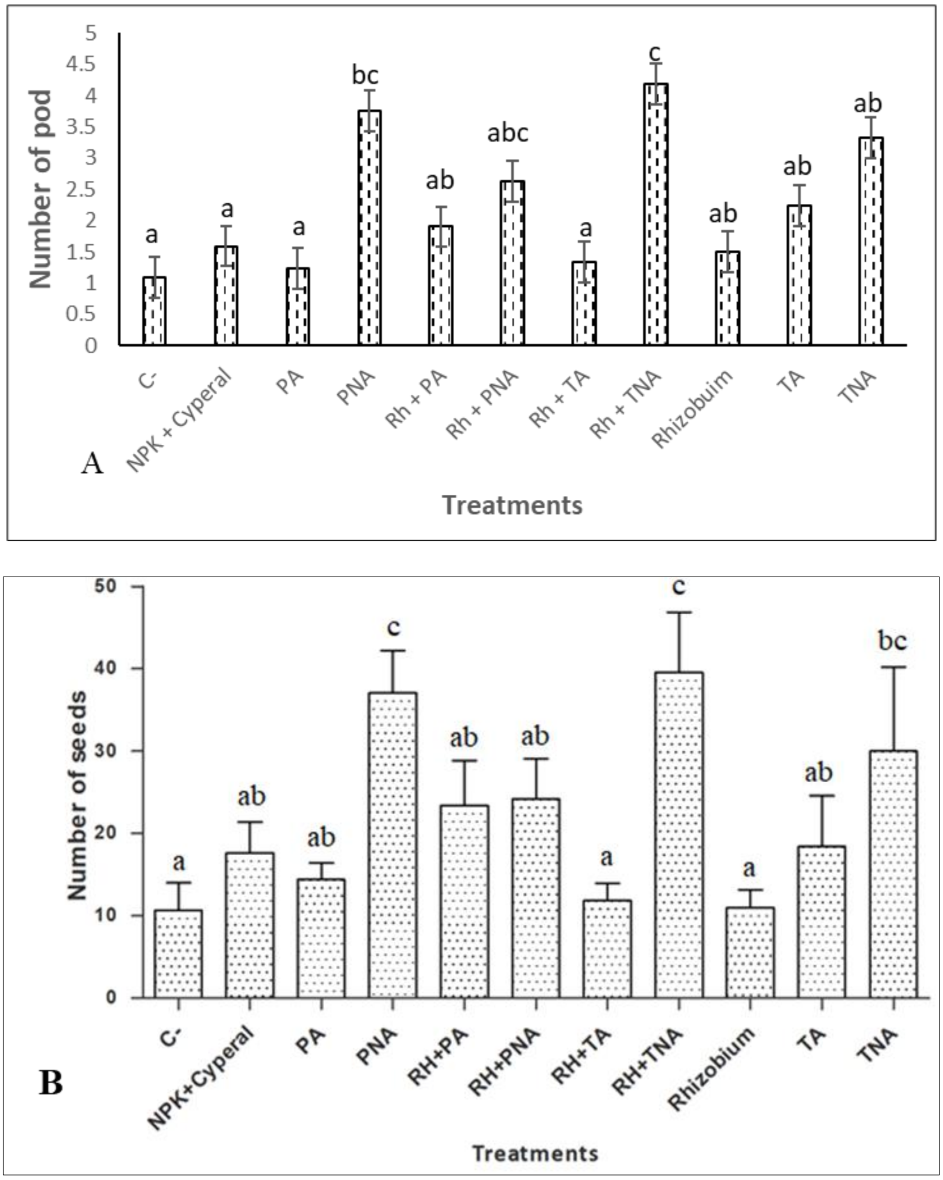


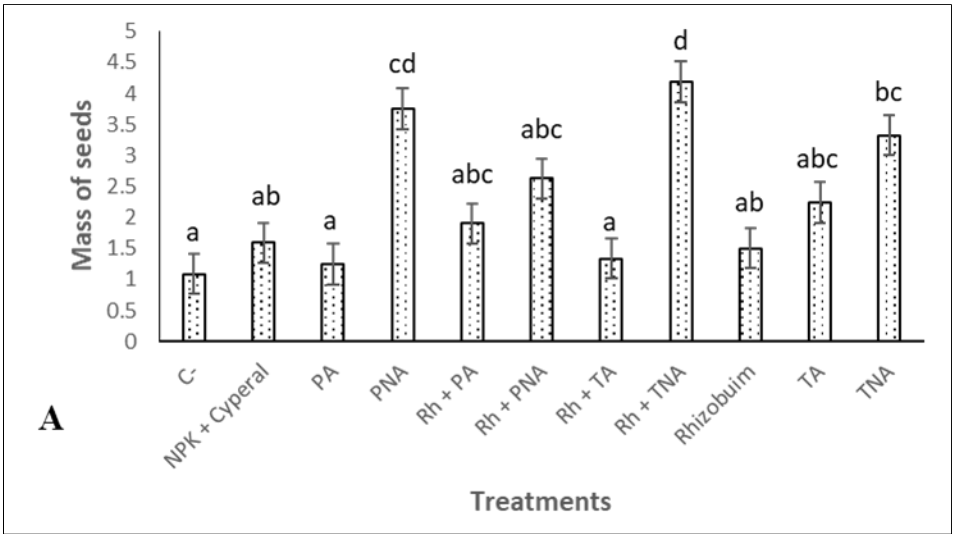
Figure 7 Effect of treatments on the number of pod (A) and seeds (B)

Differentiated values of different letters for a given parameter in each column are significantly different at the 5% level.

Rh: *Rhizobium* inoculum; C-: Negative control (without inputs or pesticides); C+: positive control (NPK + Cyperal); TNA: Non-aerated compost tea made from *Tithonia diversifolia* compost; PNA: Non-aerated compost tea made from chicken manures compost; TA: Aerated compost tea made from *Tithonia diversifolia* compost; PA: Non-aerated compost tea made from chicken manures compost; Rh + TNA: *Rhizobium* inoculum + Non-aerated compost tea made from *Tithonia diversifolia* compost; Rh + PNA: *Rhizobium* inoculum + Non-aerated compost tea made from chicken manures compost; Rh + TA: *Rhizobium* inoculum + Aerated compost tea based on *Tithonia diversifolia* compost; Rh + PA: *Rhizobium* inoculum + Non-aerated compost tea made from chicken manures compost.

The results of the effect of the treatments on the number of seeds (Figure 7B) emerges from this that the non-aerated compost tea treatments based on chicken manures (PNA) and the *Rhizobium* treatment associated with non-aerated compost tea based on *Tithonia diversifolia* (Rh + TNA) present a number of seeds statistically higher (p = 0.0002) compared to the negative control (C-) and other treatments. Furthermore, there was a weakly positive correlation (r = 0.049; p = 0.2834) between the number of seeds and the number of thrips. But in the case of the number of seeds and the number of pods this correlation (r = 0.795; $p < 0.0001$) was strong. [59] and [60] demonstrated that compost teas contain bioactive molecules such as humic and fulvic acids that improve plant growth and yield.

3.5. Variation of seed biomass as affected by treatments



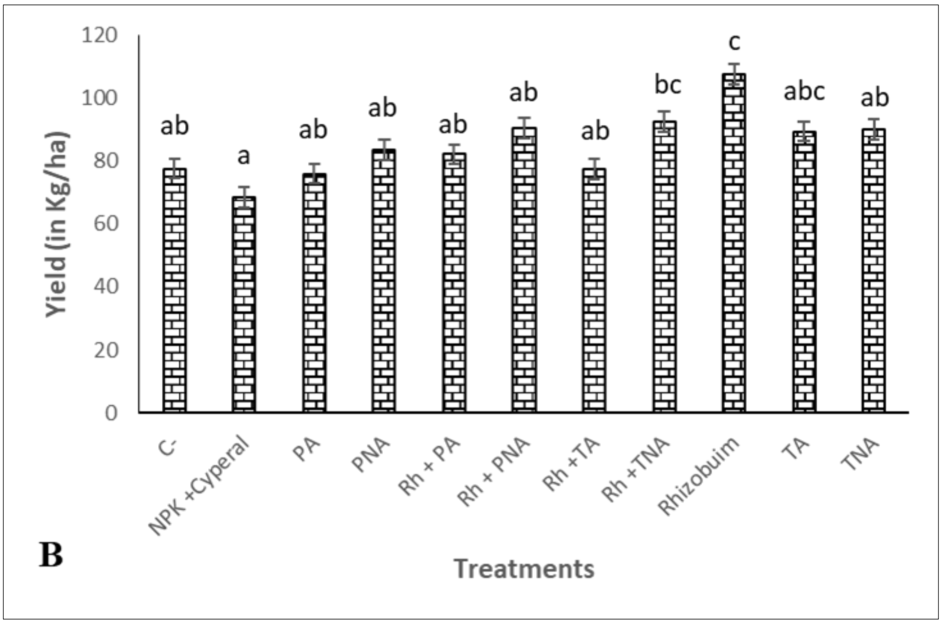


Figure 8 Changes in seed biomass as influenced by treatments (A) and yield (kg/ha) (B)

Differentiated values of different letters for a given parameter in each column are significantly different at the 5% level.

Rh: *Rhizobium* inoculum; C-: Negative control (without inputs or pesticides); C+: positive control (NPK + Cyperal); TNA: Non-aerated compost tea made from *Tithonia diversifolia* compost; PNA: Non-aerated compost tea made from chicken manures; TA: Aerated compost tea made from *Tithonia diversifolia* compost; PA: Non-aerated compost tea made from chicken manures compost; Rh + TNA: *Rhizobium* inoculum + Non-aerated compost tea made from *Tithonia diversifolia* compost; Rh + PNA: *Rhizobium* inoculum + Non-aerated compost tea made from chicken manures compost; Rh + TA: *Rhizobium* inoculum + Aerated compost tea based on *Tithonia diversifolia* compost; Rh + PA: *Rhizobium* inoculum + Non-aerated compost tea made from chicken manures compost.

Figure 8A shows the results of the influence of treatments on cowpea seeds biomass (g). Rhizobia treatments associated with non-aerated compost tea based on *Tithonia diversifolia* (Rh + TNA) and non-aerated compost tea for chicken manures (PNA) had a statistically higher seed mass compared to the negative control treatments (C-), NPK + Cyperal and the other treatments. In addition, there was a strong positive correlation and significant (r = 0.8153785; P < 0.0001) between the number of seeds and the number of pods of the different treatments. These results were consistent with those found by [61], [62] and [63], who indicated that humic acids in compost teas increase the weight of grape, melon, pepper and peach fruit, respectively.

Figure 8B shows the effect of treatments on the yield (kg/ha) of the cowpea crop, and reveals that the yield of the rhizobia treatment had a statistically significant difference compared to the other treatments except the treatments of aerated compost tea from *Tithonia diversifolia* (TA), non-aerated compost tea based on *Tithonia diversifolia* (TNA) and rhizobia associated with non-aerated compost tea based on *Tithonia diversifolia* (Rh + TNA). Similarly, rhizobia treatment associated with non-aerated compost tea from *Tithonia diversifolia* significantly increased the seed yield compared to aerated compost tea based on *Tithonia diversifolia* (TA) and compost tea non-aerated from *Tithonia diversifolia* (TNA). Rhizobia alone and *Rhizobium* associated with non-aerated compost tea from *Tithonia diversifolia* (Rh + TNA) showed a better yield compared to the other treatments. These results are in agreement with those found by [64], [65], who showed an increase in the yield of legumes such as groundnuts and cowpeas inoculated with rhizobia.

4. Conclusion

The need to identify sources of resistance to *M. sjostedti* in cowpea remains a priority in integrated pest management development programs in Cameroon. Thus, the results obtained present various promising approaches to cowpea pests, particularly flower bud thrips control, in addition to compost teas, rhizobia inoculum and their different combinations, which have reduced and stabilize the adult thrips population on plants. These biopesticides and biofertilizers have also contributed to the suppression of attacks by pests responsible for pod deformation, and could therefore be proposed to replace synthetic insecticides commonly used for long-term monitoring of this major pest of cowpea in the field.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Author's contributions

Albert Ngakou and Tchuenguem Fohouo Fernand-Nestor conceived research, contributed in drafting the manuscript and revising it critically for important intellectual content. Mohamadou Moussa, Kengni Beaudelaine Stephanie and Steve Takoukam Toukam conducted experiments, collected data in the field, analyzed and interpreted the results.

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