

## Article

# Plants extracts and a mycoinsecticide (*Metarhizium anisopliae*) in cowpea yield improvement in Guinean Savannah and Sahelian Savannah agro-ecological zones of Cameroun

Raoul BORKEUM BARRY <sup>1, \*</sup>, Katamssadan HAMAN TOFEL <sup>2</sup>, Jean WINI GOUDOUNGOU <sup>3</sup>, NCHIWAN Elias NUKENINE <sup>4</sup>, Manuele TAMÒ <sup>5</sup>, François NDOSINVIAN VANDI <sup>6</sup> and Albert NGAKOU <sup>7</sup>.

<sup>1</sup> Department of Life Sciences, Higher Teacher Training College of Bertoua, University of Ngaoundere, Cameroon; borkeumbarry@gmail.com

<sup>2</sup> Department of Biological Sciences, Faculty of Science, University of Bamenda, Cameroon, tofel-hama@yahoo.fr

<sup>3</sup> Department of Biological Sciences, Faculty of Science, University of Bamenda, Cameroon, winigoudougou@yahoo.fr

<sup>4</sup> Department of Biological Sciences, University of Ngaoundere, Cameroon; elinchiwan@yahoo.fr

<sup>5</sup> International Institute of Tropical Agriculture, Cotonou, Benin, m.tamo@cgiar.org

<sup>6</sup> Department of Biological Sciences, University of Ngaoundere, Cameroon; ndosinvian@yahoo.com

<sup>7</sup> Department of Biological Sciences, University of Ngaoundere, Cameroon; alngakou@yahoo.fr

\* Correspondence: borkeumbarry@gmail.com; Tel.: +237699887666,

**Abstract:** Cowpea yield improvement is done by adding agricultural inputs. The use of natural substances as pesticides is being encouraged to fight against cowpea field pests. The pesticidal potentials of aqueous extracts of *Azadirachta indica* and *Boswellia dalzielii*, *Metarhizium anisopliae*, alone and in combination with plant extracts, as well as the commercial insecticide Decis were tested on two *Vigna unguiculata* varieties in field in two agroecological zones (Guinean Savannah and Sahelian Savannah) of Cameroon. The field trials were arranged in a completely randomized block design with nine treatments including control. Each treatment was replicated four times. *Vigna unguiculata* plants were sprayed at flowering stage thrice with insecticidal products at 5 days interval. Data assessment consisted of counting ramifications per plant, the number of pods per block, and seed yield. All the tested insecticides significantly ( $p < 0.0001$ ) improved the cowpea yield in the two agro-ecological zones. The productions parameters were highly influenced by variety and agroecological zone. The extracts and their combinations were as effective as synthetic pesticide (Decis). Bafia variety recorded the highest ramification rate ( $37.03 \pm 1.59$ ) when treated with the combination of *M. anisopliae* and *A. indica* in Maroua (Sahelian Savannah). The same variety also produced most important pods number ( $90.50 \pm 16.66$ ) in Ngaoundere (Guinean Savannah) with the combination of *A. indica* and *B. dalzielii*. The highest seed yield ( $44.23 \pm 2.31$ ) was recorded in Ngaoundere with B125 variety treated with the combination of the three treatments (*A. indica*, *B. dalzielii*, *M. anisopliae*). *A. indica*, *B. dalzielii*, *M. anisopliae* and their combinations could be considered as potential natural input in the improvement of *V. Unguiculata* yield. This would not only increase *V. unguiculata* yield but also preserves environment from the pollution due to the use of synthetic residual chemicals.

**Keywords:** *Azadirachta indica*, *Boswellia dalzielii*, *Metarhizium anisopliae*, *Vigna unguiculata*, efficacy, yield

## 1. Introduction

Cowpea (*Vigna unguiculata* L. Walp) is a staple food in Cameroon. It is cultivated and consumed across the ten regions of the country and the neighboring countries. The country's cowpea production increased from 40 000 tons in 1997 to 198 201 tons in 2017 [1]. Whereas the production is increasing, cowpea grain yield in farmer's fields is decreasing. Both the cowpea grain and leaves are edible products that are rich and cheap sources of high-quality protein (25-32%) and vitamins [2]. Immature pods and peas are

used as vegetables while several snacks and main dishes are prepared from the grains. The seed is valued as a nutritional supplement to cereals [3]. The freshly harvested leaves are sold in local markets. Cowpea shoots and leaves are rich sources of calcium, phosphorous and vitamin B [4]. The young leaves are especially important in drought-prone regions of Sub-Sahara Africa (SSA) to tide local populations over during the “hungry period” [5]. Cowpea provides farmers with needed cash income as it is one of the first agricultural products to reach the market each year [6]. The leaves and stems are also an important source of high-quality hay for livestock feeding and roots fix atmospheric nitrogen through symbiosis with nodule bacteria [7].

The growth and production of cowpea is seriously hampered by numerous biotic factors including insect pests. Yield losses range from 10 to 100% due to the activities of wide range of insect pests which attack cowpea crop in the field at different growth stages [8]. Cowpea pests colonize the plant from seedling to the developing pods. The pest sucks leaf sap and deposits honeydew on the leaves. This sucking activity results in nutrient drain which causes direct reduction of plant productivity, the number pods and seeds size are reduced [9]. The pest is considered also as important vector of virus on cowpea [10]. Currently, exploration of bio-pesticides is gaining momentum by the agricultural industries in formulating some novel bio-agents for the management of crop pests.

Extracts of many plants could be used as an alternative to synthetic insecticides [11]. *Azadirachta indica* has been revealed as an insecticidal plant [12,13], it is the same case for *Boswellia dalzielii* [14]. However, the use of the enthomopathogenic fungus *Metarhizium anisopliae* has shown the insecticidal properties [15,16,17]. These properties confer to these natural products the potentialities to be used as alternative to synthetic pesticides. In order to increase effectiveness of these substances, their different combinations can be explored since the combination of *B. dalzielii* and *A. indica* extracts to the entomopathogenic fungus *M. anisopliae* has not yet been tested on cowpea production parameters. The efficacy of pesticides can be influenced by crop variety and agroecological zone, then the determination of their effect reveals interesting in the framework of integrated pest control. Therefore, the effect of natural substances, *A. indica*, *B. dalzielii* and *M. anisopliae* and their combinations on cowpea production was investigated.

## 2. Materials and Methods

The experiment was carried out in the Guinean Savannah (Dang-Ngaoundéré) and the Sahelian Savanah (Béguélé-Maroua) agro-ecological zones of Cameroon. Trials were conducted during two consecutive years (2014 and 2015), and the field working dates are summarized in Table 1. Plant material consisted of two cowpea seed varieties: the local Bafia cultivated locally during subsequent work, and the B125 provided by the Institute for Agricultural Research and Development (IRAD) of Maroua-Cameroon. The B125 variety was an early maturity variety (75 days), whereas the Bafia variety was an intermediate variety (85 to 95 days).

**Table 1. Cropping calendar**

Sites	Sowing dates	Spraying dates	Dates to maturity
Ngaoundere	26. 07. 2014	B125 : 54-59-64 DAS	20. 10. 2014 (86 DAS)
		Bafia : 64-69-74 DAS	03.11. 2014 (100 DAS)
	01. 08. 2015	B125 : 54-59-64 DAS	30. 10. 2015 (90 DAS)
		Bafia : 59-64-69 DAS	13. 11. 2015 (104 DAS)
Maroua	23. 08. 2014	B125 : 55-60-65 DAS	06. 11. 2014 (75 DAS)
		Bafia : 65-70-75 DAS	22. 11. 2014 (91 DAS)
	24. 08. 2015	B125 : 49-54-59 DAS	04.11. 2015 (75 DAS)
		Bafia : 64-69-74 DAS	18. 11. 2015 (87 DAS)

DAS : Day After Sowing

## 2.1. Experimental layout and Treatments

Plants were grown on flat surface of  $57.75 \times 25$  m<sup>2</sup>. The experimental field was divided into two plots separated by 4 m line. The experimental plots representing the treatments were  $4.5 \times 1.5$  m<sup>2</sup> for the B125 variety and  $4.5 \times 2.25$  m<sup>2</sup> for the Bafia variety. Seeds were sown at 50 cm between the lines, and 50 cm within the lines for the early variety, and 75 cm between the lines and 50 cm within the lines for the intermediate variety. Insecticidal formulations were sprayed using four distinct manual gauge sprayers, each corresponding to a specific insecticidal product. For multi-product treatments, each component was sprayed separately. Treatments were applied early in the morning between 6 and 8 a.m, three times at five (5) days interval, starting from the first flowering.

The experimental design applied to each variety was fully randomized, consisting of 9 treatments, with four replicates each. The different treatments were: T1 (control representing plots that received no insecticidal treatment); T2 (plots treated with aqueous *A. indica* leaves extract); T3 (plots treated with aqueous *B. dalzielii* leaves extract); T4 (plots treated with *M. anisopliae* formulation); T5 (plots treated with the combination of *M. anisopliae* + *A. indica*); T6 (plots treated with the combination *M. anisopliae* + *B. dalzielii*); T7 (plots treated with the combination *A. indica* + *B. dalzielii*); T8 (plots treated with the combination of the three bioinsecticides *M. anisopliae* + *A. indica* + *B. dalzielii*); T9 (the plots treated with the commercial insecticide Decis used as reference).

## 2.2. Formulation of insecticidal products

The aqueous extracts of *A. indica* and *B. dalzielii* leaves, was obtained following the method recommended by Sahel People Service [18]. According to this method, 5 L of solution was obtained by macerating 1 kg of fresh leaves in 5 L of water. The resulting concentrated macerate was then diluted to 10% with water and filtered through a 0.4 mm mesh tissue, for a working concentration of 20 g/L. The *M. anisopliae* based solution was obtained using the formulation described by Ngakou *et al.*, [16] which required the mixture of 50 g of *M. anisopliae* with 700 mL of kerosene and 300 mL of cotton seed oil. For this work, *M. anisopliae* was prepared at a concentration of 10 g/L. The myco-insecticide *M. anisopliae* was obtained from IITA Cotonou-Benin, while Deltamethrin-based synthetic insecticide (Decis) purchased from a phytosanitary store and applied at the dose of 0.2 mL/L of water.

## 2.3. Assessed Parameters

The average number of ramifications per plant, the average number of pod per plot and yield per plot (seeds dry weight) were assessed. The determination of ramification number was performed one day before pod maturity on 10 randomly selected plants on the 2 middle rows [19]. Pods of 10 randomly selected plants from the 2 middle rows were counted, and for the yield, the weight of dry seeds from these pods were assessed [16] using a KERN electronic scale (max : 1000 g; d : 0.1 g).

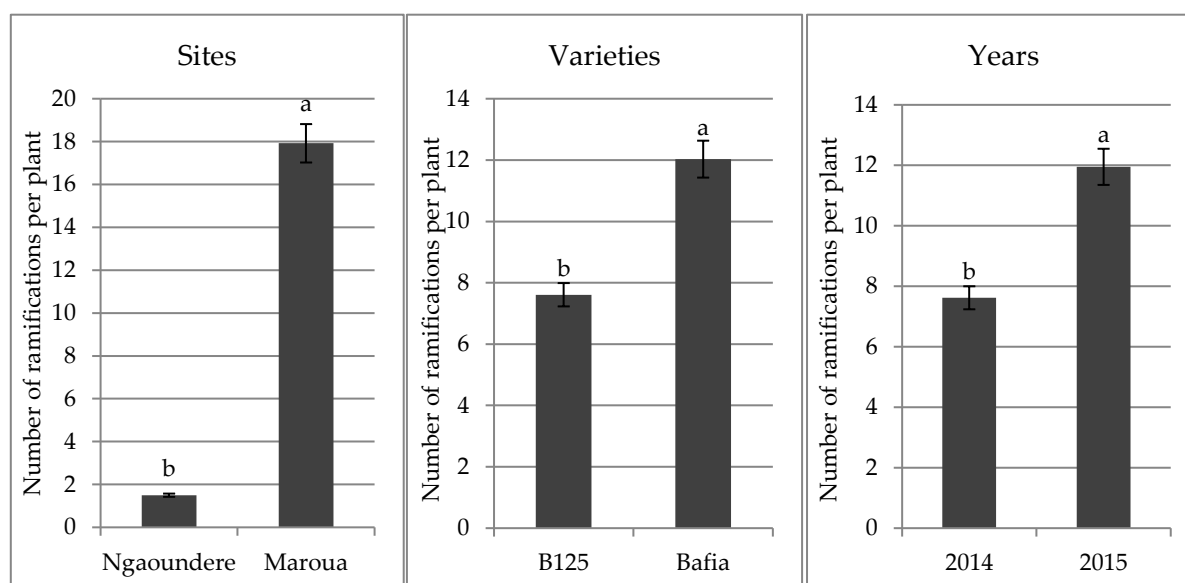
## 2.4. Statistical Analysis

The statistical analysis of data was carried out using the SAS software. The number of ramifications, the number of pods and the yield were subjected to the analysis of variance (ANOVA) to split the averages. The Student- Newman-Keuls test was used to compare the different treatments and T-test, to compare means at twice. The Microsoft Office Excel 2010 was used to draw the diagrams.

## 3. Results

### 3.1. Influence of insecticide formulation on ramifications productions

In general (Figure 1), the highest ramification production was observed in Maroua, ( $t=61.98$ ;  $p<0.0001$ ), and Bafia variety produced more ramifications than B125 variety ( $t=10.93$ ;  $p<0.0001$ ). There were recorded more ramifications in 2015 than 2014 ( $t=10.80$ ;  $p<0.0001$ ). This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.



The bands with different letters are significantly different according Student-Newman-Keuls test ( $P < 0.05$ ).

**Figure 1. Variation of number of ramifications by agro-ecological zones, varieties and years**

In Ngaoundéré (table 2), insecticide treatments significantly ( $p < 0.0002$ ) improved the ramifications production on Bafia variety which were not the same in B125 variety ( $p = 0.1405$ ) in 2014. Compared to negative control, *A. indica* + *B. dalzielii* were the bio-insecticidal treatments which considerably improved ramifications production as far as the synthetic insecticide taken as the positive control. The production of ramifications was lesser in *A. indica*, *M. anisopliae* and *M. anisopliae* + *A. indica* treatments than negative control. In 2015, all different insecticidal treatments significantly improved ( $p = 0.0240$ ) ramification production of B125 variety. Among them, *M. anisopliae* + *B. dalzielii* were the treatments which induced the highest ramification production. Except synthetic insecticide Decis and binary combination *A. indica* + *B. dalzielii*, all the other insecticidal treatments had improved the production of ramifications more than negative control. The different treatments significantly affected Bafia variety ramification in 2014 ( $p = 0.0002$ ) but in 2015 it was not the case ( $p = 0.5288$ ).

In Maroua in 2014, there were no significant difference of ramification production between insecticidal treatments and control ( $p = 0.1165$ ) on B125 variety. It was contrary on Bafia variety ( $p = 0.0021$ ). With 25% of more ramifications produced, *A. indica* treatment was the most effective. This treatment induced much ramification production than Décis. In 2015 (table 2), insecticidal treatments significantly induced more ramification production than control on the two cowpea varieties, B125 ( $p < 0.0001$ ); Bafia ( $p < 0.0001$ ). The 20% of more ramification production induced by *A. indica* and *B. dalzielii* treatment, these two treatments were more effective than Decis. The other bio insecticidal treatments during the two years of experiment in the same way induced higher ramification compared to the reference pesticide Decis. In our Savannah Sahelian zone within the two years experiment (2014 and 2015), the ternary combination *M. anisopliae* + *A. indica* + *B. dalzielii* was very even most effective treatment especially with B125 variety in the second year (year 2015).

**Table 2. Production of ramifications as influenced by pesticides treatments**

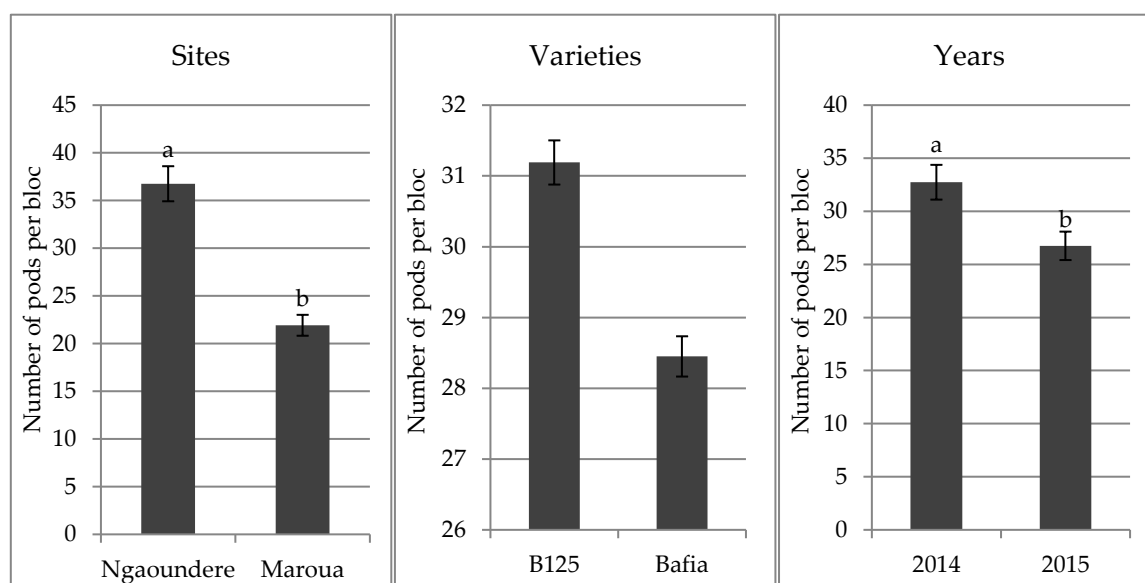
Sites	Treatments	Years					
		2014			2015		
		B125	Bafia	t-value	B125	Bafia	t-value
Ngaoundere	Control	1.80 ± 0.21	2.13 ± 0.30 <sup>ba</sup>	2.2073*	0.85 ± 0.15 <sup>c</sup>	1.00 ± 0.20	1.4696 <sup>ns</sup>
	<i>A. indica</i>	1.83 ± 0.25	1.45 ± 0.24 <sup>b</sup>	2.6858*	1.18 ± 0.18 <sup>c</sup>	0.65 ± 0.19	4.9602*

	<i>B. dalzielii</i>	1.70 ± 0.20	2.13 ± 0.26 <sup>ba</sup>	3.2109*	1.38 ± 0.19 <sup>b</sup>	1.00 ± 0.24	3.0408*
	<i>M. anisopliae</i>	2.15 ± 0.19	1.50 ± 0.26 <sup>b</sup>	4.9442*	1.28 ± 0.19 <sup>b</sup>	0.94 ± 0.19	3.0994*
	<b>M+B</b>	2.08 ± 0.20	2.40 ± 0.36 <sup>ba</sup>	1.9033 <sup>ns</sup>	1.58 ± 0.19 <sup>a</sup>	1.05 ± 0.21	4.5842*
	<b>M+A</b>	1.73 ± 0.20	1.58 ± 0.19 <sup>b</sup>	0.0193 <sup>ns</sup>	1.28 ± 0.17 <sup>b</sup>	1.05 ± 0.15	2.4849*
	<b>A+B</b>	1.37 ± 0.21	2.78 ± 0.25 <sup>a</sup>	10.5783***	0.88 ± 0.17 <sup>dc</sup>	0.63 ± 0.13	2.8614*
	<b>M+A+B</b>	1.83 ± 0.21	2.32 ± 0.33 <sup>ba</sup>	3.0684*	1.20 ± 0.18 <sup>b</sup>	0.72 ± 0.16	4.8820*
	<b>Décis</b>	2.25 ± 0.22	2.85 ± 0.28 <sup>a</sup>	4.1273*	0.80 ± 0.15 <sup>d</sup>	0.82 ± 0.18	0.2090 <sup>ns</sup>
	<b>Means</b>	1.86 ± 0.07	2.12 ± 0.09	5.5856**	1.16 ± 0.06	0.88 ± 0.06	8.0829***
	<b>F</b>	1.54 <sup>ns</sup>	3.92***		2.24*	0.89 <sup>ns</sup>	
	<b>p-value</b>	0.1405	0.0002		0.0240	0.5288	
<b>Maroua</b>	<b>Control</b>	12.75 ± 0.70	12.75 ± 0.82 <sup>ba</sup>	0 <sup>ns</sup>	11.58 ± 0.59 <sup>c</sup>	21.88 ± 1.53 <sup>d</sup>	15.3857***
	<i>A. indica</i>	11.98 ± 0.51	16.65 ± 0.89 <sup>a</sup>	11.1517***	14.68 ± 0.93 <sup>cb</sup>	34.63 ± 1.43 <sup>ba</sup>	28.6475***
	<i>B. dalzielii</i>	11.40 ± 0.69	15.03 ± 0.94 <sup>ba</sup>	7.6253***	15.13 ± 0.88 <sup>cb</sup>	28.90 ± 1.74 <sup>c</sup>	17.2983***
	<i>M. anisopliae</i>	13.30 ± 0.83	11.86 ± 0.83 <sup>b</sup>	3.0050*	16.97 ± 1.00 <sup>b</sup>	26.73 ± 1.15 <sup>dc</sup>	15.6872***
	<b>M+B</b>	12.55 ± 0.63	14.80 ± 1.18 <sup>ba</sup>	4.1201*	15.58 ± 1.06 <sup>b</sup>	30.55 ± 1.61 <sup>bc</sup>	19.0229***
	<b>M+A</b>	10.87 ± 0.98	13.23 ± 0.87 <sup>ba</sup>	4.4112*	15.87 ± 1.02 <sup>b</sup>	37.03 ± 1.59 <sup>a</sup>	27.4377***
	<b>A+B</b>	11.90 ± 0.69	14.58 ± 0.91 <sup>ba</sup>	5.7482**	16.15 ± 1.23 <sup>b</sup>	28.80 ± 1.48 <sup>c</sup>	16.1016***
	<b>M+A+B</b>	10.65 ± 0.47	13.63 ± 0.75 <sup>ba</sup>	8.2470***	21.33 ± 1.10 <sup>a</sup>	29.98 ± 1.54 <sup>bc</sup>	11.1957***
	<b>Décis</b>	12.90 ± 0.83	16.80 ± 1.15 <sup>a</sup>	6.7358***	14.78 ± 0.84 <sup>cb</sup>	22.98 ± 1.31 <sup>d</sup>	12.9071***
	<b>Means</b>	12.02 ± 0.24	14.40 ± 0.32	14.5744***	15.77 ± 0.35	29.05 ± 0.55	49.8975***
	<b>F</b>	1.63 <sup>ns</sup>	3.12**		6.99***	10.72***	
	<b>p-value</b>	0.11	0.002		< 0.0001	< 0.0001	

C : négatif control ; A : *A. indica* ; B : *B. dalzielii* ; M : *M. anisopliae* ; M+B : *M. anisopliae* + *B. dalzielii* ; M+A : *M. anisopliae* + *A. indica* ; A+B : *A. indica* + *B. dalzielii* ; M+A+B : *M. anisopliae* + *A. indica* + *B. dalzielii* ; D : Décis. Means with different letters are significantly different according Student-Newman-Keuls test ( $P < 0.05$ ), <sup>ns</sup>:  $P > 0.05$ ; \*,  $P < 0.05$ ; \*\*,  $P < 0.001$ ; \*\*\*,  $P < 0.0001$ .

### 3.2. Influence of insecticide formulation on pods production

In general, the pods productions varied according to sites, varieties and years (Figure 2). This pod production was higher in Ngaoundere than Maroua ( $t=5.58$ ;  $p < 0.0001$ ). Even the pods production of B125 was higher than that of Bafia, this difference was not significant ( $t=0.88$ ;  $p=0.3794$ ). The production was higher in 2014 than 2015 ( $t=2.12$ ;  $p=0.0350$ ).



The bands with different letters are significantly different according Student-Newman-Keuls test ( $P < 0.05$ ).

**Figure 2. Variation of number of pods by Agro-ecological zones, varieties and years**

Different treatments differently affected the pods productions in Ngaoundere and Maroua, and in the same zone this production varied according to the varieties (table 3). Concerning Ngaoundere, results show there is not significant different ( $p=0.8554$ ) amongst the treatments on pod production of B125 variety in 2014. It was not the same case concerning Bafia variety ( $p=0.0492$ ), where used bio-pesticides differently improved pod productions, that production was higher than negative control. On this variety, plots treated with the combination of *A. indica* + *B. dalzielii* had produced pods as far as synthetic insecticide Decis. The combination of *M. anisopliae* + *A. indica* + *B. dalzielii* induced the lesser pod production. There was no significant difference of pod production due to the application of different substances in 2015 concerning the two cowpea varieties ( $p=0.1354$  for B125;  $p=0.4660$  for Bafia). In Maroua, results show there is not significant improving pod production using the different substances in 2014 and 2015, this on B125 variety ( $p=0.7475$  in 2014 ;  $p=0.3069$  in 2015) and on Bafia variety ( $p=0.2837$  in 2014 ;  $p=0.6104$  in 2015). But this pod production in the year for the same bioproduct varied according to the cowpea variety.

**Table 3. Production of pods as influenced by pesticides treatments**

Sites	Treatments	Years					
		2014			2015		
		B125	Bafia	t-value	B125	Bafia	t-value
Ngaoundere	Control	24.25 ± 5.42	25.25 ± 8.13 <sup>d</sup>	0.2506 <sup>ns</sup>	36.75 ± 3.92	15.67 ± 5.61	7.5447***
	<i>A. indica</i>	33.00 ± 6.92	50.75 ± 21.48 <sup>c</sup>	1.9266 <sup>ns</sup>	38.25 ± 3.68	12.25 ± 4.33	11.2074***
	<i>B. dalzielii</i>	26.50 ± 9.31	64.25 ± 25.17 <sup>b</sup>	3.4455*	42.75 ± 3.75	20.00 ± 10.17	5.1388**
	<i>M. anisopliae</i>	29.75 ± 10.13	34.50 ± 8.68 <sup>c</sup>	0.8721 <sup>ns</sup>	26.50 ± 7.44	14.75 ± 5.72	3.0668*
	M+B	30.25 ± 9.50	67.50 ± 5.33 <sup>b</sup>	8.3762***	38.75 ± 8.56	25.75 ± 3.71	3.4132*
	M+A	19.75 ± 5.65	48.50 ± 13.00 <sup>c</sup>	4.9682*	40.25 ± 9.87	12.25 ± 3.73	6.5002***
	A+B	16.25 ± 4.48	90.50 ± 16.66 <sup>a</sup>	10.5423***	40.00 ± 1.08	24.25 ± 3.47	10.6157***
	M+A+B	36.00 ± 13.35	49.33 ± 21.96 <sup>c</sup>	1.2705 <sup>ns</sup>	30.50 ± 10.81	29.00 ± 12.92	0.2181 <sup>ns</sup>
	Décis	27.75 ± 11.60	107.75 ± 24.06 <sup>a</sup>	7.3364***	58.75 ± 5.30	32.00 ± 9.29	6.1262***
	Means	27.06 ± 2.80	60.11 ± 6.62	11.26***	39.17 ± 2.45	20.47 ± 2.43	5.42**

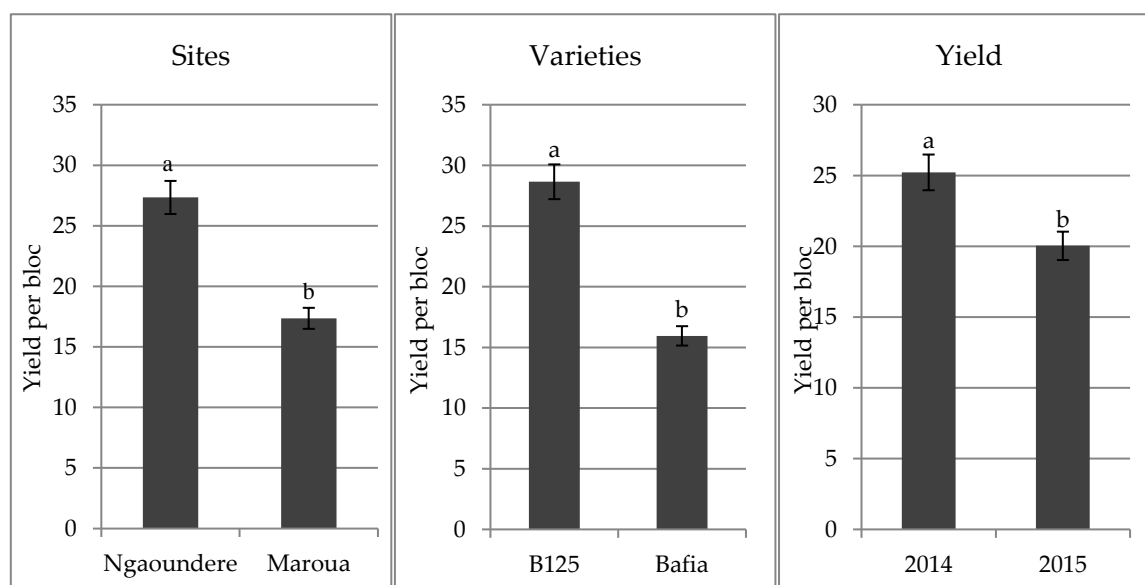


	<b>F</b>	0.49	2.33*		1.74	0.99	
	<b>p-value</b>	0.8554	0.0492		0.1354	0.4660	
<b>Maroua</b>	<b>Control</b>	39.50 ± 7.41	14.75 ± 1.89	7.92 <sup>ns</sup>	21.00 ± 1.68	8.50 ± 6.50	4.56*
	<i>A. indica</i>	30.00 ± 6.54	15.50 ± 4.29	4.54***	28.75 ± 3.94	6.50 ± 0.50	13.72***
	<i>B. dalzielii</i>	29.25 ± 5.50	15.75 ± 1.70	5.74 <sup>ns</sup>	28.50 ± 4.66	5.50 ± 4.50	8.69***
	<i>M. anisopliae</i>	22.00 ± 7.09	11.33 ± 4.37	8.16 <sup>ns</sup>	34.00 ± 6.06	4.50 ± 0.50	11.88***
	<b>M+B</b>	27.50 ± 2.10	19.33 ± 5.55	0.45*	26.00 ± 3.94	-	15.11***
	<b>M+A</b>	21.33 ± 9.28	12.00 ± 4.60	2.89**	24.25 ± 10.86	1.50 ± 0.50	5.12**
	<b>A+B</b>	24.50 ± 8.69	14.00 ± 0.82	2.94**	32.75 ± 4.19	1.50 ± 0.50	18.14***
	<b>M+A+B</b>	24.75 ± 6.71	8.75 ± 1.70	5.66 <sup>ns</sup>	35.00 ± 5.58	2.50 ± 0.50	14.20***
	<b>Décis</b>	32.00 ± 8.50	24.50 ± 7.49	1.62*	41.00 ± 3.58	6.00 ± 1.00	23.06***
	<b>Means</b>	28.24 ± 2.25	15.09 ± 1.41	4.96***	30.14 ± 1.88	4.56 ± 0.96	12.10***
	<b>F</b>	0.63	1.31		1.26	0.80	
	<b>p-value</b>	0.7475	0.2837		0.3069	0.6104	

C : négatif control ; A : *A. indica* ; B : *B. dalzielii* ; M : *M. anisopliae* ; M+B : *M. anisopliae* + *B. dalzielii* ; M+A : *M. anisopliae* + *A. indica* ; A+B : *A. indica* + *B. dalzielii* ; M+A+B : *M. anisopliae* + *A. indica* + *B. dalzielii* ; D : Décis. Means which have different letters are significantly different according Student-Newman-Keuls test ( $P < 0.05$ ), <sup>ns</sup>:  $P > 0.05$ ; \*:  $P < 0.05$ ; \*\*:  $P < 0.001$ ; \*\*\*:  $P < 0.0001$ .

### 3.3. Influence of pesticide formulations on yield

The yield of cowpea crop treated with the different formulations of bioproducts significantly varied according to the sites ( $t = -6.34$ ;  $p < 0.0001$ ), varieties ( $t = 13.45$ ;  $p < 0.0001$ ) and years ( $t = 3.11$ ;  $p = 0.0021$ ) (Figure 3). The yield was higher in Ngaoundere than Maroua and B125 variety recorded higher yield compared to Bafia variety. Concerning the two years of cropping, the first year (2014) recorded higher yield compared to the second year (2015).



The bands with same letter on top for the same factor are not different according Student–Newman–Keuls test ( $P < 0.05$ ).

**Figure 3. Variation of yield by Agro-ecological zones, varieties and years**

At Ngaoundere in 2014, all the insecticidal formulations significantly improved yield compared to negative control on the two varieties ( $p < 0.0001$  for B125;  $p = 0.0012$  for Bafia). In B125 variety, the bioformulations induced more than 100% yield increase compared to the negative, it was the same performance reached by synthetic insecticid Decis. On Bafia variety, the single products *A. indica*, *B. dalzielii* and *M. anisopliae* consid-

erably induced yield improvement compared to the negative control; this yield improvement was 25%. However, those yield improvements were lower to those induced by the combined products. The combined bio-formulations improved yield as more as Decis with an increasing of 75%. In 2015, no significantly improving of yield on Bafia variety ( $p=0.4110$ ) was observed. On B125 variety, all pesticides formulations improved the yield and this was varied significantly ( $p<0.0001$ ). Despite 100% of yield increasing, *A. indica* and *M. anisopliae* were the lesser bio-formulations. *M. anisopliae* + *A. indica* + *B. dalzielii* was the most effective bio-formulation with 200% yield increasing compared to the negative control.

The influence of the different treatments in Sahelian Savannah (Maroua) significantly showed more yield improvement compared to the control ( $p<0.0001$ ) on B125 variety in 2014. All biopesticides improved yield by more than 100%, what was higher than the improvement yield induced by the reference pesticide Decis. There was yield increase of Bafia variety treated with all used pesticides, and no significant difference was observed for the different formulations ( $p=0.9293$ ). In 2015, all the treatments induced yield increase compared to the negative control. For a given variety the different pesticidal formulations had statistically the same performance (B125 variety :  $p=0.0706$  and Bafia variety:  $p=0.6328$ ).

Table 4. Yield as influenced by insecticidal treatments

Sites	Treatments	Years					
		2014			2015		
		B125	Bafia	t-value	B125	Bafia	t-value
Ngaoundere	Control	12.12 ± 2.09 <sup>b</sup>	19.65 ± 4.40 <sup>b</sup>	3.7865*	14.65 ± 0.69 <sup>d</sup>	9.00 ± 3.72	3.6595*
	<i>A. indica</i>	30.35 ± 2.68 <sup>a</sup>	24.23 ± 4.02 <sup>ba</sup>	3.1027*	28.20 ± 2.45 <sup>c</sup>	7.65 ± 2.64	13.9759***
	<i>B. dalzielii</i>	35.21 ± 1.89 <sup>a</sup>	26.99 ± 4.01 <sup>ba</sup>	4.5419*	31.10 ± 3.15 <sup>bc</sup>	14.18 ± 8.01	4.8152***
	<i>M. anisopliae</i>	28.06 ± 2.50 <sup>a</sup>	32.81 ± 1.32 <sup>ba</sup>	4.1155*	33.08 ± 3.03 <sup>bc</sup>	9.95 ± 3.44	12.3592***
	M+B	38.93 ± 3.17 <sup>a</sup>	34.01 ± 1.21 <sup>a</sup>	3.5517*	27.10 ± 1.89 <sup>c</sup>	17.88 ± 4.49	4.6359*
	M+A	37.44 ± 4.24 <sup>a</sup>	35.21 ± 1.53 <sup>a</sup>	1.2118 <sup>ns</sup>	31.83 ± 4.08 <sup>bc</sup>	12.40 ± 2.52	9.9246***
	A+B	30.28 ± 1.95 <sup>a</sup>	28.88 ± 2.04 <sup>a</sup>	1.2151 <sup>ns</sup>	30.43 ± 5.78 <sup>bc</sup>	16.73 ± 3.61	4.9243*
	M+A+B	39.07 ± 3.87 <sup>a</sup>	35.67 ± 0.26 <sup>a</sup>	2.1471*	44.23 ± 2.31 <sup>ba</sup>	26.33 ± 11.70	3.6765*
	Décis	41.26 ± 3.10 <sup>a</sup>	36.81 ± 0.59 <sup>a</sup>	3.4541*	48.43 ± 5.06 <sup>a</sup>	23.00 ± 7.13	7.1245***
	Means	32.06 ± 1.86	30.36 ± 1.25	0.76	32.11 ± 1.86	15.19 ± 2.05	6.11***
	F	10.63***	4.88**		7.80***	1.08	
	p-value	< 0.0001	0.0012		< 0.0001	0.4110	
Maroua	Control	13.96 ± 3.75 <sup>b</sup>	5.75 ± 1.73	4.8695*	11.63 ± 1.11	6.50 ± 4.70	2.60*
	<i>A. indica</i>	31.25 ± 2.85 <sup>a</sup>	6.71 ± 2.40	16.10***	22.43 ± 4.24	3.85 ± 0.25	10.71***
	<i>B. dalzielii</i>	30.99 ± 2.93 <sup>a</sup>	7.94 ± 2.68	14.21***	20.08 ± 1.98	4.30 ± 2.70	11.54***
	<i>M. anisopliae</i>	32.05 ± 1.12 <sup>a</sup>	8.88 ± 3.39	15.89***	15.13 ± 3.57	3.05 ± 0.85	8.06***
	M+B	33.19 ± 1.18 <sup>a</sup>	8.12 ± 2.75	20.52***	15.90 ± 0.86	-	45.28***
	M+A	33.91 ± 1.11 <sup>a</sup>	9.43 ± 3.67	15.63***	23.63 ± 5.90	1.20 ± 0.20	9.30***
	A+B	29.89 ± 2.64 <sup>a</sup>	8.39 ± 2.10	15.61***	24.35 ± 6.14	2.20 ± 1.70	8.51***
	M+A+B	36.36 ± 1.22 <sup>a</sup>	9.38 ± 3.08	19.94***	16.83 ± 2.64	1.35 ± 0.15	14.33***
	Décis	38.28 ± 1.99 <sup>a</sup>	11.48 ± 3.42	16.59***	29.30 ± 4.22	4.70 ± 1.10	13.81***
	Means	30.85 ± 1.42	8.41 ± 0.87	13.45***	19.93 ± 1.45	3.39 ± 0.69	10.32***
	F	8.71***	0.36		2.13	0.76	



p-value	< 0.0001	0.92	0.0706	0.6328
C : négatif control ; A : <i>A. indica</i> ; B : <i>B. dalzielii</i> ; M : <i>M. anisopliae</i> ; M+B : <i>M. anisopliae</i> + <i>B. dalzielii</i> ; M+A : <i>M. anisopliae</i> + <i>A. indica</i> ; A+B : <i>A. indica</i> + <i>B. dalzielii</i> ; M+A+B : <i>M. anisopliae</i> + <i>A. indica</i> + <i>B. dalzielii</i> ; D : Décis. Means with different letters are significantly different according Student-Newman-Keuls test (P<0.05), ns: P>0.05; *: P<0.05; **: P<0.001; ***: P<0.0001.				

#### 4. Discussion

The formulated plant-based products in this study have influenced the different investigated production parameters. There were more ramifications in Maroua than Ngaoundere in the two years (2014 and 2015). The most rainfall in Ngaoundere leaches the soil, that weakens plants growth, and the Bafia variety has tendency to crawl in Maroua to resist at the drought [20]. The absence of significant difference between treatments in Ngaoundéré on B125 variety in 2014, on Bafia variety in 2015, and on B125 variety in Maroua in 2015, could be due to the late application (after vegetable growth) of different formulated pesticides. This is similar with Sharah and Ali [21] observations. Difference observed between different treatments in Ngaoundere on Bafia variety in 2014, and on B125 variety in 2015, were the result of the growing plant which is not always uniform.

Concerning production of pod, the higher production observed in Ngaoundere than Maroua was due to the variation of growing plant in different agro-ecological zone [22]. In Ngaoundere, the well full-blowing of Bafia variety in 2014 has induced a better production of pods than B125 variety. But in 2015, the soil leaching weakened Bafia variety growth and reduced the pod production compared to B125 variety. In Maroua, the Bafia variety with his intermediate cycle has been interrupted by the shoddiness of the rainfall season, then it did not produce more pods. The combination properties effects of in *A. indica* + *B. dalzielii* treatment in 2014 on Bafia variety in Ngaoundere, allowed to this treatment to produce much pods than Decis. The presence of kerosine in *M. anisopliae* treatment weaken plants' development, so treatments which were combined with *M. anisopliae* produced less pods, but much than *A. indica* treatments which, according to Bambara and Tiemtore [23], is more effective in stocking than yield. The most resource in gum of *B. dalzielii* [24] makes it viscous, favourable to his adhesion that would allow *B. dalzielii* treatment to be more effective than *A. indica* treatment. According to Ibrahim [25], the adhesion is an important factor of the effectiveness of a treatment.

As regard cowpea yield, it was higher in Ngaoundere than Maroua. That shows the influence of the agro-ecological zone on crop production [22]. Then the cowpea yield in this study was affected by soil, climate and environmental conditions which characterised the different zones. The rainfall season in Maroua is shorter than the one of Ngaoundere, the intermediate cycle of Bafia variety has no more full bloom there. In Ngaoundere in 2014, with good meteorology conditions, B125 variety produced as well as Bafia variety. On B125 variety, the proprieties of different biopesticides have permitted them to improve yield as well as synthetic pesticide Decis. The lower efficiency of yield improving with *A. indica* and *B. dalzielii* extracts on Bafia variety compared to the other insecticidal treatments, were due to the fact that biopesticides have lower efficiency than synthetic pesticides as show by Dereval *et al.* [26]. The authors observed that the low remanence of biopesticides and the fact that their effectiveness depend to environment conditions make them less effective than synthetic chemical pesticides. These results are similar as Bambara and Tiemtore [23] who show that *A. indica*, *Euphorbia balsamifera* and *Hyptis spicigera* were less effective than Decis. The presence of kerozene which have propensity to burn with high temperature was the cause of the helpless of *M. anisopliae* treatment. Treatments in combination, by the synergy of their different constituents, induced improving yield as well as Decis. This is similar to Barry *et al.* [17] who showed that the combination of *M. anisopliae* with other extracts improved the pesticidal efficacy than alone. In 2015, the low production of Bafia variety was due to the high rainfall which leached the soil. The synthetic pesticide Decis induced more yield improving than

biopesticides in B125 variety [23, 26]. Moreover, this pesticide has large spectrum and it is systemic. Among biopesticides, the higher yield observed in combination of *M. anisopliae* + *A. indica* + *B. dalzielii* could be caused by the synergic activities of their different constituents [16, 17]. The lower yield observed in *A. indica* treatments was due to the fact that, the efficacy of *A. indica* products was reduced in field [23]. This could be to the fact that the field is not a close space as stores which increase the evaluability of active components content in pesticide. Also the field environment condition, are very fluctuating which greatly affected the efficacy of pesticide compared to storage. In Maroua, plants have benefited from properties of treatment as *A. indica* and *B. dalzielii* extracts, that permitted them to produce too much than the other pesticides treatments on B125 variety in 2014. The low rainfall of Maroua reduces the leaching of these biopesticides. But when the rainfall is very weak, the need of plant of water is not well satisfied which reduced the crop production as observed in the present study on the two cowpea varieties in year 2015.

### 5. Conclusion

The aqueous extracts of *A. indica* and *B. dalzielii*, the mycoinsecticide *M. anisopliae*, and their various combinations have resulted in improving the cowpea full-blow. These bio-insecticides also contributed to the improving of his yield, and could therefore be proposed to substitute the commonly used synthetic pesticides in field in Guinean savannah and in Sahelian savannah. However, intermediate cycle plants as Bafia variety would be recommended to Guinean savannah than Sahelian savannah, and arrangement ought to be taken into consideration to reduce soil leaching in Guinean savannah characterised by heavy rainfall.

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