

Plants Extracts and a Mycoinsecticide in Cowpea Yield Improvement in Guinean Savannah and Sahelian Savannah Agro-Ecological Zones of Cameroun

Raoul Borkeum Barry^{1*}, Katamssadan Haman Tofel², Jean Wini Goudougou², François Ndosinvian Vandì³, Manuele Tamò⁴, Albert Ngakou³, Elias Nchiwan Nukenine³

¹Department of Life Sciences, Higher Teacher Training College, University of Bertoua, Bertoua, Cameroon

²Department of Biological Sciences, Faculty of Science, University of Bamenda, Bamenda, Cameroon

³Department of Biological Sciences, Faculty of Science, University of Ngaoundere, Ngaoundéré, Cameroon

⁴International Institute of Tropical Agriculture, Cotonou, Benin

Email: *borkeumbarry@gmail.com

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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 *Azadirachta indica* and *Boswellia dalzielii* water extracts, *Metarhizium anisopliae*, alone and in combination with plant extracts, and a commercial synthetic pesticide (Decis®) were tested in field on two varieties of cowpeas, *Vigna unguiculata* in two agroecological zones (Guinean Savannah and Sahelian Savannah) of Cameroon. The field trials were carried out in a full randomized block design including nine treatments and a control. Four replications were made concerning the different treatments and control. At the flowering stage, the cowpea field was sprayed three times with different pesticidal formulations at the interval of five days. The number of ramifications per plant, and that of pods per block and seed yield were determined. The pesticide formulations considerably ($p < 0.0001$) improved cowpea yield in the two agro-ecological zones. The production parameters were highly influenced by variety and agroecological zone. The extracts and their combinations were as effective as synthetic pesticide (Decis®). Bafia variety treated with the combination of *M. anisopliae* and *A. indica* recorded the highest ramification rate (37.03 ± 1.59) in Maroua (Sahelian Savannah). The same variety also produced more important pods number (90.50 ± 16.66) in Ngaoundere (Guinean Savannah) with the binary combination of two plants used in this experiment. The highest seed yield (44.23 ± 2.31) was recorded in

Ngaoundere with B125 variety treated with the combination of the three pesticidal formulations separately (*A. indica*, *B. dalzielii*, *M. anisopliae*). The plant products used in this work, *M. anisopliae* and their mixtures could supersede the synthetic pesticides considering environmental issue in cowpea crop protection. Then, such formulations would not only improve crop productivity but also preserve environment from the pollution due to the use of synthetic residual chemicals.

Keywords

Azadirachta indica, *Boswellia dalzielii*, *Metarhizium anisopliae*, *Vigna unguiculata*, Varieties, Yield

1. Introduction

Cereals and pulses remained the most consumed grains in Sub-Saharan Africa especially in Cameroon. Cowpea (*Vigna unguiculata* L. Walp) is among these consumed grains and one of the highly used pulses in human nutrition in Cameroon. This legume is cultivated and consumed across the ten regions of the country and the neighboring countries. The country's cowpea production raised from 40,000 (1997) to 198,201 tons (2017) [1]. Whereas the production is increasing, cowpea grain yield in farmer's fields is decreasing. All the parts of these plants are used for human or livestock nutrition. Grain and cowpea leaf are highly used in human dietary; these plant parts are rich and cheap sources of high-quality protein (25% - 32%) and vitamins [2]. Immature pods and peas are recognized as legumes; however populations in different localities of Cameroon prefer using cowpea to prepare several snacks and main dishes. This legume permits to compensate the cereals during lacking or insufficiency due to the poor harvest or cereal shortage; then in human nutrition, cowpea becomes a supplement to cereals [3]. Cowpea is endowed with several beneficial nutritional properties; its shoots and leaves are rich sources of calcium, phosphorous and vitamin B [4]. The young leaves are very helpful in some parts of Sub-Sahara Africa (SSA) especially in drought-prone regions to overcome the "hungry period" [5]. Cowpea generates income and cash for some farmers, since it is one of the first agricultural products to reach the market each year [6]. The freshly harvested leaves are sold as vegetables in local markets. The leaves and stems are important source of food for livestock and roots improve the soil fertility by fixing atmospheric nitrogen through symbiosis with nodule bacteria [7].

The growth and production of cowpea crop is seriously hampered by numerous biotic factors including insect pests [8] [9]. 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 [10]. Cowpea pests colonize the plant from seedling to mature grain. 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 pods' number and seeds' size are reduced [11]. The pest is considered also an important vector of virus on cowpea [12]. Currently, exploration of biopesticides is gaining momentum by the agricultural industries in formulating some novel bio-agents for the management of the crop pests.

Extracts of many plants could be used as an alternative to synthetic insecticides [13]. *Azadirachta indica* is known as endowed by insecticide properties [14] [15], it is the same case for *Boswellia dalzielii* [16]. However, the fungus *Metarhizium anisopliae* has shown insecticidal properties [17] [18] [19]. 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 with *M. anisopliae* fungus has not yet been tested on cowpea production parameters. The efficacy of pesticides can be influenced by crop variety and agroecological zone, so the determination of their effect could be very 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 in two agro-ecological zones of Cameroon (Central Africa) regarding two local cowpea varieties.

2. Materials and Methods

The experiment was carried out in the Guinean Savannah (Dang-Ngaoundere) and the Sahelian Savannah (Beguele-Maroua) agro-ecological zones of Cameroon. Field work was conducted during two consecutive years (2014 and 2015), and the different dates of work sessions in field are summarized in **Table 1**. Two cowpea varieties: B125 and Bafia were used. The local Bafia was obtained from the sample cultivated locally during subsequent work, and the B125 was provided by the *Institut de la Recherche Agricole pour le Développement* (IRAD) of Maroua-Cameroon. The B125 variety is an early maturity variety with cycle of 75 days, whereas the Bafia variety is an intermediate variety with cycle of 85 to 95 days.

Experimental device and treatments

The plants were cultivated on flat surface of $57.75 \times 25 \text{ m}^2$. The field was constituted by the plots of $4.5 \times 1.5 \text{ m}^2$ for the B125 and $4.5 \times 2.25 \text{ m}^2$ for the Bafia variety separated by the interval of 4 m. Seeds were sown at 50 cm between and 50 cm within the lines, and 75 cm between and 50 cm within the lines respectively for B125 (early maturity) and Bafia (intermediate variety). Insecticidal substances were separately sprayed using a manual gauge sprayer for each of them and the same procedure was employed for their combinations. The spraying of pesticides substances was done early in the morning between 6 and 8 am, three times at five (5) days interval, starting from the first flower appearance.

Table 1. Cropping calendar.

Sites	Sowing	Spraying	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.

The full randomized blocks design was used in this field work; it made up with 9 treatments replicated four times each. The different treatments were: T1 (control representing plots without treatment); T2 (plots treated with *A. indica* leaves water extract); T3 (plots treated with *B. dalzielii* leaves water extract); T4 (plots treated with solution of *M. anisopliae*); 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 ternary combination of the three bioinsecticides *M. anisopliae* + *A. indica* + *B. dalzielii*); T9 (the plots treated with the commercial insecticide Decis® used as reference).

Preparation of insecticidal formulations

The water extracts of *A. indica* and *B. dalzielii* leaves, was obtained using the method recommended by Sahel People Service [20]. In this issue, 5 L of solution was obtained by macerating 1 kg of fresh leaves in 5 L of water during 12 hours. The mixture was filtered and the liquid fraction is collected. The treatment solution is made by diluting the solution obtained after filtration at proportion of 1/10 with water and filtered through a 0.4 mm mesh tissue. The concentration used for treatment was 20 g/L. The *M. anisopliae* solution was obtained using the method 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. Then the solution of *M. anisopliae* was prepared at 10 g/L for this work. The sample of *M. anisopliae* used in experiment was obtained from IITA Cotonou-Benin, while Deltamethrin-based synthetic insecticide (Decis®) was bought from a phytosanitary store and applied at the recommended dose of 0.2 mL/L of water.

Assessed parameters

The average ramifications number per plant, the average pod number 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 [21]. 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 was determined [16] using a KERN electronic scale (max: 1000 g; d: 0.1 g).

Data analysis

The number of ramifications and pods, and the yield were subjected to the analysis of variance (ANOVA) to bring out the effect of different factors (variety, period, zone) on different parameters (ramification, pod production and yield). The Student-Newman-Keuls test was employed to compare the different treatments and T-test to compare means of two modalities of the same factor. The whole statistical analysis was carried by using the SAS system. The Microsoft Office Excel 2010 was used to draw the diagrams.

3. Results

Influence of insecticide formulation on ramifications production

In general (Figure 1), the highest ramification production was observed in Maroua, ($t = 61.98$; $p < 0.0001$). In the same agro-ecological zone, Bafia variety produced more ramifications than B125 variety ($t = 10.93$; $p < 0.0001$). There were recorded more ramifications in 2015 than 2014 ($t = 10.80$; $p < 0.0001$).

In Ngaoundere (Table 2), insecticidal products significantly ($p < 0.0002$) improved the ramifications production on Bafia variety which was not the case for B125 variety ($p = 0.1405$) in 2014. Compared to negative control, the binary combination of the two plants (*A. indica* + *B. dalzielii*) considerably improved ramifications production as far as the chemical, Decis® used 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* was the formulation inducing the highest ramification production. Except synthetic insecticide and binary combination *A. indica* + *B. dalzielii*, all the other insecticidal treatments

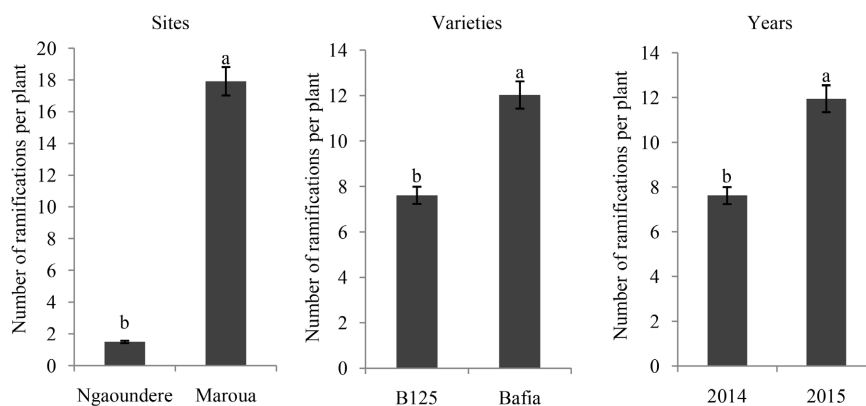


Figure 1. Variation of number of ramifications by agro-ecological zones, varieties and years. The bands with same letter on top for a given factor are not different according to Student-Newman-Keuls test ($p < 0.05$).

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 ^{ba}	2.2073*	0.85 ± 0.15 ^c	1.00 ± 0.20	1.4696 ^{ns}
	<i>A. indica</i>	1.83 ± 0.25	1.45 ± 0.24 ^b	2.6858*	1.18 ± 0.18 ^c	0.65 ± 0.19	4.9602*
	<i>B. dalzielii</i>	1.70 ± 0.20	2.13 ± 0.26 ^{ba}	3.2109*	1.38 ± 0.19 ^b	1.00 ± 0.24	3.0408*
	<i>M. anisopliae</i>	2.15 ± 0.19	1.50 ± 0.26 ^b	4.9442*	1.28 ± 0.19 ^b	0.94 ± 0.19	3.0994*
	M + B	2.08 ± 0.20	2.40 ± 0.36 ^{ba}	1.9033 ^{ns}	1.58 ± 0.19 ^a	1.05 ± 0.21	4.5842*
	M + A	1.73 ± 0.20	1.58 ± 0.19 ^b	0.0193 ^{ns}	1.28 ± 0.17 ^b	1.05 ± 0.15	2.4849*
	A + B	1.37 ± 0.21	2.78 ± 0.25 ^a	10.5783***	0.88 ± 0.17 ^{dc}	0.63 ± 0.13	2.8614*
	M + A + B	1.83 ± 0.21	2.32 ± 0.33 ^{ba}	3.0684*	1.20 ± 0.18 ^b	0.72 ± 0.16	4.8820*
	Décis	2.25 ± 0.22	2.85 ± 0.28 ^a	4.1273*	0.80 ± 0.15 ^d	0.82 ± 0.18	0.2090 ^{ns}
	Means	1.86 ± 0.07	2.12 ± 0.09	5.5856**	1.16 ± 0.06	0.88 ± 0.06	8.0829***
	F	1.54 ^{ns}	3.92***		2.24*	0.89 ^{ns}	
	p-value	0.1405	0.0002		0.0240	0.5288	
Maroua	Control	12.75 ± 0.70	12.75 ± 0.82 ^{ba}	0 ^{ns}	11.58 ± 0.59 ^c	21.88 ± 1.53 ^d	15.3857***
	<i>A. indica</i>	11.98 ± 0.51	16.65 ± 0.89 ^a	11.1517***	14.68 ± 0.93 ^{cb}	34.63 ± 1.43 ^{ba}	28.6475***
	<i>B. dalzielii</i>	11.40 ± 0.69	15.03 ± 0.94 ^{ba}	7.6253***	15.13 ± 0.88 ^{cb}	28.90 ± 1.74 ^c	17.2983***
	<i>M. anisopliae</i>	13.30 ± 0.83	11.86 ± 0.83 ^b	3.0050*	16.97 ± 1.00 ^b	26.73 ± 1.15 ^{dc}	15.6872***
	M + B	12.55 ± 0.63	14.80 ± 1.18 ^{ba}	4.1201*	15.58 ± 1.06 ^b	30.55 ± 1.61 ^{bc}	19.0229***
	M + A	10.87 ± 0.98	13.23 ± 0.87 ^{ba}	4.4112*	15.87 ± 1.02 ^b	37.03 ± 1.59 ^a	27.4377***
	A + B	11.90 ± 0.69	14.58 ± 0.91 ^{ba}	5.7482**	16.15 ± 1.23 ^b	28.80 ± 1.48 ^c	16.1016***
	M + A + B	10.65 ± 0.47	13.63 ± 0.75 ^{ba}	8.2470***	21.33 ± 1.10 ^a	29.98 ± 1.54 ^{bc}	11.1957***
	Décis	12.90 ± 0.83	16.80 ± 1.15 ^a	6.7358***	14.78 ± 0.84 ^{cb}	22.98 ± 1.31 ^d	12.9071***
	Means	12.02 ± 0.24	14.40 ± 0.32	14.5744***	15.77 ± 0.35	29.05 ± 0.55	49.8975***
	F	1.63 ^{ns}	3.12**		6.99***	10.72***	
	p-value	0.11	0.002		0.0001	0.0001	

C: negative 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: Decis. ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.001$; ***: $p < 0.0001$. For each cowpea variety values of the same column affected by the same upper-case letter are not significantly different between treatments at the indicated level of probability (Student-Newman-Keuls test).

improved the production of ramifications more than negative control. The different treatments significantly affected Bafia variety ramification in 2014 ($p = 0.0002$) but not in 2015 ($p = 0.5288$).

In 2014 in Sahelian Savannah (Maroua), the insecticidal treatments and control recorded the same performance on B125 variety ($p = 0.1165$). It was the contrary on Bafia variety ($p = 0.0021$). With 25% 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 ($p < 0.0001$). 20% 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. In the Savannah Sahelian zone, within the two years experiment (2014 and 2015), the ternary combination (*M. anisopliae* + *A. indica* + *B. dalzielii*) was very effective, and most effective especially with B125 variety in the second year (year 2015).

Insecticide formulation effect 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 ($t = 2.12$; $p = 0.0350$).

Different treatments differently affected the pods productions in Ngaoundere and Maroua, and this production varied according to the varieties (Table 3). Concerning Ngaoundere, the results show that there was not significant different ($p = 0.8554$) amongst the treatments on pod production of B125 variety in 2014. That was not the case concerning Bafia variety ($p = 0.0492$), where the used bio-pesticides differently improved pod production, that production was higher than negative control. On this variety, plots treated with the mixture of *A. indica* + *B. dalzielii* produced pods as far as synthetic insecticide. The combination of *M. anisopliae* + *A. indica* + *B. dalzielii* induced the lesser pod production. There

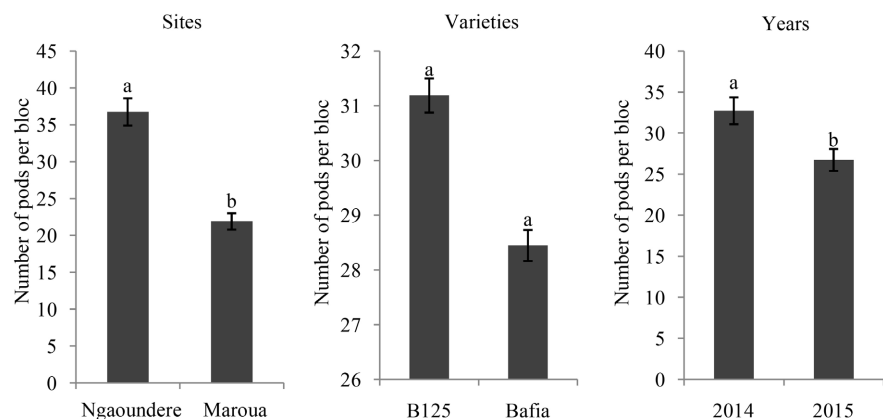


Figure 2. Variation of number of pods by Agro-ecological zones, varieties and years. The bands with same letter on top for a given factor are not different according Student-Newman-Keuls test ($p < 0.05$).

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 ^d	0.2506 ^{ns}	36.75 ± 3.92	15.67 ± 5.61	7.5447 ^{***}
	<i>A. indica</i>	33.00 ± 6.92	50.75 ± 21.48 ^c	1.9266 ^{ns}	38.25 ± 3.68	12.25 ± 4.33	11.2074 ^{***}
	<i>B. dalzielii</i>	26.50 ± 9.31	64.25 ± 25.17 ^b	3.4455 [*]	42.75 ± 3.75	20.00 ± 10.17	5.1388 ^{**}
	<i>M. anisopliae</i>	29.75 ± 10.13	34.50 ± 8.68 ^c	0.8721 ^{ns}	26.50 ± 7.44	14.75 ± 5.72	3.0668 [*]
	M + B	30.25 ± 9.50	67.50 ± 5.33 ^b	8.3762 ^{***}	38.75 ± 8.56	25.75 ± 3.71	3.4132 [*]
	M + A	19.75 ± 5.65	48.50 ± 13.00 ^c	4.9682 [*]	40.25 ± 9.87	12.25 ± 3.73	6.5002 ^{***}
	A + B	16.25 ± 4.48	90.50 ± 16.66 ^a	10.5423 ^{***}	40.00 ± 1.08	24.25 ± 3.47	10.6157 ^{***}
	M + A + B	36.00 ± 13.35	49.33 ± 21.96 ^c	1.2705 ^{ns}	30.50 ± 10.81	29.00 ± 12.92	0.2181 ^{ns}
	Décis	27.75 ± 11.60	107.75 ± 24.06 ^a	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 ^{**}
	F	0.49 ^{ns}	2.33 [*]		1.74 ^{ns}	0.99 ^{ns}	
	p-value	0.8554	0.0492		0.1354	0.4660	
Maroua	Control	39.50 ± 7.41	14.75 ± 1.89	7.92 ^{ns}	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 ^{ns}	28.50 ± 4.66	5.50 ± 4.50	8.69 ^{***}
	<i>M. anisopliae</i>	22.00 ± 7.09	11.33 ± 4.37	8.16 ^{ns}	34.00 ± 6.06	4.50 ± 0.50	11.88 ^{***}
	M + B	27.50 ± 2.10	19.33 ± 5.55	0.45 [*]	26.00 ± 3.94	-	15.11 ^{***}
	M + A	21.33 ± 9.28	12.00 ± 4.60	2.89 ^{**}	24.25 ± 10.86	1.50 ± 0.50	5.12 ^{**}
	A + B	24.50 ± 8.69	14.00 ± 0.82	2.94 ^{**}	32.75 ± 4.19	1.50 ± 0.50	18.14 ^{***}
	M + A + B	24.75 ± 6.71	8.75 ± 1.70	5.66 ^{ns}	35.00 ± 5.58	2.50 ± 0.50	14.20 ^{***}
	Décis	32.00 ± 8.50	24.50 ± 7.49	1.62 [*]	41.00 ± 3.58	6.00 ± 1.00	23.06 ^{***}
	Means	28.24 ± 2.25	15.09 ± 1.41	4.96 ^{***}	30.14 ± 1.88	4.56 ± 0.96	12.10 ^{***}
	F	0.63 ^{ns}	1.31 ^{ns}		1.26 ^{ns}	0.80 ^{ns}	
	p-value	0.7475	0.2837		0.3069	0.6104	

C: negative 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: Decis. ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.001$; ***: $p < 0.0001$. For each cowpea variety values of the same column affected by the same upper-case letter are not significantly different between treatments at the indicated level of probability (Student-Newman-Keuls test).

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 that there is not significant improving pod production using the different substances in 2014 and 2015, that on

B125 variety ($p = 0.7475$ in 2014; $p = 0.3069$ in 2015) and Bafia variety ($p = 0.2837$ in 2014; $p = 0.6104$ in 2015). But the pod production in the present study for a given pesticidal treatment varied according to the cowpea variety alone the year.

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).

At Ngaoundere (Table 4) in 2014, all insecticidal formulations significantly improved the two varieties' yield compared to the negative control ($p < 0.0001$ for B125; $p = 0.0012$ for Bafia). In B125 variety, the formulations induced more than 100% yield increase compared to the negative control, it was the same performance reached by synthetic insecticide Decis. On Bafia variety, the single products; *A. indica*, *B. dalzielii* and *M. anisopliae* considerably 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 bioformulations 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 varied significantly ($p < 0.0001$). Despite 100% of yield increasing, *A. indica* and *M. anisopliae* were the less effective bioformulations. *M. anisopliae* + *A. indica* + *B. dalzielii* was the most effective formulation with 200% yield increasing compared to the negative control (non-treated plot).

The influence of the different treatments in Sahelian Savannah (Maroua) (Table 4) significantly showed more yield improvement ($p < 0.0001$) on B125

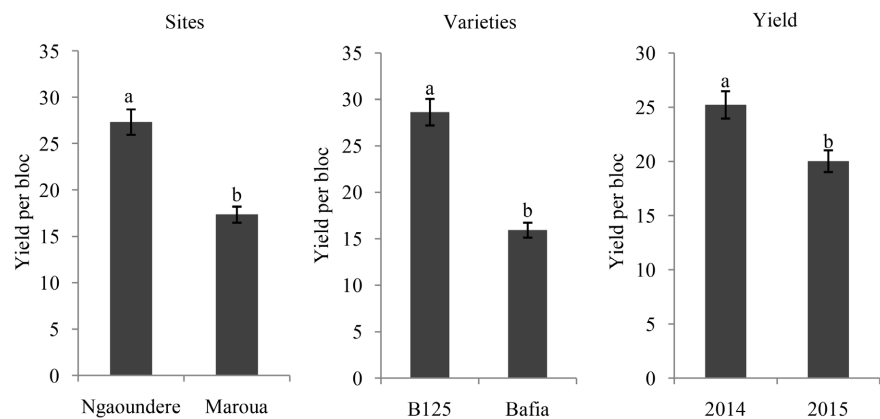


Figure 3. Variation of yield by Agro-ecological zones, varieties and years. The bands with same letter on top for a given factor are not different according Student-Newman-Keuls test ($p < 0.05$).

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 ^b	19.65 ± 4.40 ^b	3.7865*	14.65 ± 0.69 ^d	9.00 ± 3.72	3.6595*
	<i>A. indica</i>	30.35 ± 2.68 ^a	24.23 ± 4.02 ^{ba}	3.1027*	28.20 ± 2.45 ^c	7.65 ± 2.64	13.9759***
	<i>B. dalzielii</i>	35.21 ± 1.89 ^a	26.99 ± 4.01 ^{ba}	4.5419*	31.10 ± 3.15 ^{bc}	14.18 ± 8.01	4.8152***
	<i>M. anisopliae</i>	28.06 ± 2.50 ^a	32.81 ± 1.32 ^{ba}	4.1155*	33.08 ± 3.03 ^{bc}	9.95 ± 3.44	12.3592***
	M + B	38.93 ± 3.17 ^a	34.01 ± 1.21 ^a	3.5517*	27.10 ± 1.89 ^c	17.88 ± 4.49	4.6359*
	M + A	37.44 ± 4.24 ^a	35.21 ± 1.53 ^a	1.2118 ^{ns}	31.83 ± 4.08 ^{bc}	12.40 ± 2.52	9.9246***
	A + B	30.28 ± 1.95 ^a	28.88 ± 2.04 ^a	1.2151 ^{ns}	30.43 ± 5.78 ^{bc}	16.73 ± 3.61	4.9243*
	M + A + B	39.07 ± 3.87 ^a	35.67 ± 0.26 ^a	2.1471*	44.23 ± 2.31 ^{ba}	26.33 ± 11.70	3.6765*
	Décis	41.26 ± 3.10 ^a	36.81 ± 0.59 ^a	3.4541*	48.43 ± 5.06 ^a	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 ^{ns}	
	p-value	<0.0001	0.0012		<0.0001	0.4110	
Maroua	Control	13.96 ± 3.75 ^b	5.75 ± 1.73	4.8695*	11.63 ± 1.11	6.50 ± 4.70	2.60*
	<i>A. indica</i>	31.25 ± 2.85 ^a	6.71 ± 2.40	16.10***	22.43 ± 4.24	3.85 ± 0.25	10.71***
	<i>B. dalzielii</i>	30.99 ± 2.93 ^a	7.94 ± 2.68	14.21***	20.08 ± 1.98	4.30 ± 2.70	11.54***
	<i>M. anisopliae</i>	32.05 ± 1.12 ^a	8.88 ± 3.39	15.89***	15.13 ± 3.57	3.05 ± 0.85	8.06***
	M + B	33.19 ± 1.18 ^a	8.12 ± 2.75	20.52***	15.90 ± 0.86	-	45.28***
	M + A	33.91 ± 1.11 ^a	9.43 ± 3.67	15.63***	23.63 ± 5.90	1.20 ± 0.20	9.30***
	A + B	29.89 ± 2.64 ^a	8.39 ± 2.10	15.61***	24.35 ± 6.14	2.20 ± 1.70	8.51***
	M + A + B	36.36 ± 1.22 ^a	9.38 ± 3.08	19.94***	16.83 ± 2.64	1.35 ± 0.15	14.33***
	Décis	38.28 ± 1.99 ^a	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 ^{ns}		2.13 ^{ns}	0.76 ^{ns}	
	p-value	<0.0001	0.92		0.0706	0.6328	

C: negative 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: Decis. ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.001$; ***: $p < 0.0001$. For each cowpea variety values of the same column affected by the same upper-case letter are not significantly different between treatments at the indicated level of probability (Student-Newman-Keuls test).

variety in 2014. All biopesticides improved yield by more than 100%, that 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 treated blocks have showed significant yield increase when compared to the non-treated ones. For a given variety the different pesticidal formulations had statistically the same performance (B125 variety: $p = 0.0706$ and Bafia variety: $p = 0.6328$).

4. Discussion

The formulated plant-based products in this study have influenced the different investigated 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 plant growth, and the Bafia variety has tendency to crawl in Maroua to resist the drought [22]. 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 of different formulated pesticides. This is similar with Sarah and Ali [23] observations. Difference observed between the 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 the production of pod, the higher one was observed in Ngaoundere, that was due to the variation of growing plant in different agro-ecological zone [24]. 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, and 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 more pods than Decis. The presence of kerosine in *M. anisopliae* treatment weaken plants' development, so treatments that were combined with *M. anisopliae* produced less pods, but more than *A. indica* treatments which, according to Bambara and Tientore [25], is more effective in stocking than yield. The most resource in gum of *B. dalzielii* [26] makes it viscous, favourising its adhesion that could make *B. dalzielii* treatment to be more effective than *A. indica* treatment. According to Ibrahim *et al.* [27], the adhesion is an important factor for the effectiveness of a treatment.

Concerning cowpea yield, it was higher in Ngaoundere than Maroua. That shows the influence of the agro-ecological zone on crop production [24]. 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 that of Ngaoundere, the intermediate cycle of Bafia variety has no more full bloom there. 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.* [28]. 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 to Bambara and Tientoré [25] who showed that *A. indica*, *Euphorbia balsamifera* and *Hyptis spicigera* were less effective than Decis®. The presence of kerozene which has propensity to burn with high temperature was the cause of the helplessness of *M. anisopliae* treatment. Treatments in combination, by the synergy of their different constituents, induced improving yield as well as Decis®. These findings are similar to Barry *et al.* [19], these authors pointed out 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 on B125 variety [25] [28]. Moreover, this pesticide is systemic one with a large spectrum. Among biopesticides, the higher yield observed in combination of *M. anisopliae* + *A. indica* + *B. dalzielii* could be attributed to the synergic activities of their different constituents [18] [19]. The lower yield observed in *A. indica* treatments was due to the fact that, the efficacy of *A. indica* products was reduced in field [25]. This could be to the fact that the field is not a close space as stores which increase the availability of active components content in pesticide. Also, the field environment conditions are very fluctuating which greatly affected the efficacy of pesticide compared to storage. In Maroua, cowpea crops have benefited from properties of treatment as *A. indica* and *B. dalzielii* extracts, that permitted them to produce more than the other pesticides treatments on B125 variety during the year 2014. The low rainfall of Maroua reduced the leaching of these biopesticides. But when the rainfall is very weak, the need of plant in water is not well satisfied which reduced the crop production as observed during the year 2015 on the two cowpea varieties.

After all, biopesticides are biodegradable [29] and easily accessible (as regard *A. indica* and *B. dalzielii*), and also can be produce by the farmers [30]. So, it is a really economic gain due to expensive cost of synthetic pesticides which even have harmful effects on the environment.

5. Conclusion

The aqueous extracts of *A. indica* and *B. dalzielii*, the mycoinsecticide *M. anisopliae*, and their different mixtures considerably induced improvement of cowpea crop productivity. The different products used in this study positively affect pulses yield. Considering all positive effects of the different formulations on the two cowpea varieties in the two agroecological zones, they could be proposed to substitute the commonly used synthetic pesticides in field in Guinean and Sahelian savannah. However, intermediate cycle plants such as Bafia variety would be recommended in Guinean savannah than Sahilian savannah, and arrangement ought to be taken into consideration to reduce soil leaching in Guinean savannah characterised by heavy rainfall.

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Conflicts of Interest

Authors have declared no relevant conflict of interest that may have influenced the study.

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