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Contribution of Arbuscular Mycorrhizal Fungi (AMF), Rhizobia and *Metarhizium anisopliae* to Cowpea Production in Cameroon

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Abstract: The objective of this study was to investigate on how the interactions between the microbial symbionts (AMF+rhizobia) and the mycopesticide *M. anisopliae* can affect the cowpea production in varied agro ecological zones of Cameroon. Cowpea of the Bafia local cultivar was grown from 1999 to 2004 in the Sudano-sahelian (zone-I), Guinea-savannah (zone-II), monomodal (zone-IV) and bimodal humid-forest rainfall (zone-V) of Cameroon. Two cropping seasons were experimented in each zone, but in different years except in zone-IV. Experiments were conducted in a Randomised Block Design (RBD) with two levels of inoculation at sowing (uninoculanted seeds and dually inoculated seeds with Arbuscular Mycorrhizal Fungi (AMF) and rhizobia and two levels of spray applications at onset of flowering with the mycopesticide (*Metarhizium anisopliae*), or the insecticide Deltamethrin ®. Results indicate that inoculation significantly increased cowpea biomass in the first and second cropping years, respectively by 38 and 40% in zone-I, 54 and 43% in zone-II, 55 and 46% in zone-IV, 41 and 51% in zone-V at 45 Days After Planting (DAP). Inoculated plants showed a low but significant ($p = 0.01$) response to AMF colonization in all the trials compared to uninoculated plants. Nodules were formed by native and introduced rhizobia while the number and dry weight of nodules were significantly higher ($p<0.01$) in roots of inoculated than those of uninoculated plants. Inoculated and sprayed treatments significantly produced more pods per plant ($p<0.01$) and enhanced the dry weight of pods per plant at harvest ($p = 0.03$) in all trials compared to the control. These results suggest that AMF, rhizobia and *M. anisopliae* are variously efficient microsymbionts and mycopesticides in different Cameroonian soils and may be used as economical and safe bio-inoculants to improve cowpea production in the country.

Key words: AMF, cowpea, growth, inoculation, *Metarhizium anisopliae*, rhizobia, yield

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp. is an economically important grain legume and cash crop with high quality protein (24%) to supplement the staple food crops in most African communities (Bressani, 1985). In a worldwide production of 3 millions tons seed yield harvested from 12.5 millions hectares, Africa accounts for about 68% and Cameroon only 0.4% of this production (Singh *et al*.,

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1997). Cowpea is a far from negligible item of internal trade, but it is an important component of some dietary staples in Cameroon. There is a real need to increase its production in order to improve on human nutrition. Cowpea yields are generally high (1.5 to 3 t ha^{-1}), but the actual yields on small-scale farm in developing countries average just 0.2 to 0.4 t ha^{-1} (Murdock and Kitch, 1993). Insect pests attack, diseases and deficiency of soils in nutrients, particularly phosphorus and nitrogen are the major causes of these yield deficits. Though chemical fertilisers are often used, they are toxic to soil micro-organisms or hazardous to human (Margni *et al*., 2002). One way to circumvent this may be the use of microbial inoculants such as rhizobia and AMF, which have a direct beneficial effect on the plant (Denison and Kiers, 2004).

Grain legumes like cowpea depend on rhizobia for nitrogen supply (Mulongoy, 1985) and AMF which benefits plant by essentially improving its mineral nutrition, water uptake and hormone production (Marschnen and Dell, 1994). The individual effects of rhizobia inoculation (Athar and Johnson, 1996) and mycorrhizal (Naqvi and Mukerji, 1998; Bagayoko *et al*., 2000), on leguminous crops have been examined. In many developing countries, farmers intercrop cowpea with cereal or include it in crop rational cycle to enrich exhausted soils, mainly through nodule dinitrogen fixation without the application of other nutrients such as phosphorus (Israel, 1987). Supplementaring this agronomic practice with other crop management practices such as microbial inoculation could contribute to improve yields. Indigenous strains of rhizobia and AMF coexist naturally, but are not always efficient and therefore, the performances of selected efficient species warrant examination under different field conditions. Research on dual inoculation of plants for re-establishment of soil fertility has shown that inoculation of legumes with both rhizobia and AMF increases plant growth more than any of the inoculum used solely (Azcòn *et al*., 1991; Valdenegro *et al*., 2001). An increase in phosphorus nutrition leading to enhanced nitrogen fixation was attributed to the synergistic effects of the two symbionts (Xavier and Germida, 2003).

Metarhizium anisopliae is an entomophagous fungus (mycopesticide), which contributes to lower the bean flower thrips population *Megalurothrips sjostedti*, thereby contributing to increased plant yield (Ekesi *et al*., 1999 and 2000). However, the combined effect of microbial inoculants and mycopesticide on cowpea growth and yield has not been studied. Such information would be of great interest to developing countries like Cameroon, where microbial inoculants are not yet available to growers, despite current research on selected isolates of rhizobia and AMF intended to inoculate legumes and other plants (Nwaga *et al*., 1997; Nwaga, 2000).

The present study was initiated to study the effects of co-inoculating cowpea seeds at sowing with the microsymbionts (AMF+rhizobia) and spraying plants at onset of flowering with the mycopesticide (*M. anisopliae*) on cowpea production in varied agro ecological zones of Cameroon.

MATERIALS AND METHODS

Agro Ecological Zones of the Study

The studies were carried out from 1999 to 2004 in eight cowpea fields in four agro ecological zones of Cameroon, thus two per zone. Two agro ecological zones were planted every year with one study site per agro ecological zones. The first site was established at IITA Research Station at Nkolbisson-Yaoundé, in the bimodal Humid-forest rainfall zone or zone-V (03° 51.281° N; 13° 32.309° E) during the first cropping seasons (April-July) of 1999 and 2001. In the second site at Dang-Ngaoundéré in the Guinea-Savannah zone or zone-II (11° 27.779° N; 7° 25. 838° E), the trials were conducted during the first cropping seasons (April-July) of 1999 and 2000. The third site was located at the Research Station of the Institute of Agricultural Research for Development (IRAD) at Guering-Maroua, in the Sudano-Sahelian zone or zone-I (10° 37.335° N; 14° 22.326° E). The trials in this site were conducted during the cropping seasons of August-November of 2000 and 2001. The fourth site was located at Molyko-Buea, within the monomodal Humid-forest rainfall zone or zone-IV (04°09.627°N; 09°14.754°E). In this site, trials were conducted during the first (May-August) and second (September - December) of 2004 cropping seasons. The soil was slightly acidic with the pH ranging from 5.13-6.22.

Experimental Design and Plant Materials

The cowpea variety Bafia local purchased from farmers was used in all experiments in this study. It has a semi-erected growth pattern and a growth cycle from sowing to harvest between 85-95 Days After Planting (DAP). Experiments were carried out in a Randomized Block Design (RBD) with five treatments and four replicates each per cropping year. Each replicate was a (8×8.5) m² plot with 11 rows spaced at 75×150 cm within and between rows, respectively. The five treatments were: seeds uninoculated at sowing with AMF+rhizobia and plants unsprayed with *M. anisopliae* at flowering as control or uninoculated; seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae* (AMF+rhizobia); seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae* (*Metarhizium*); seeds inoculated at sowing with AMF+rhizobia and plants sprayed at flowering with *M. anisopliae* (AMF+rhizobia +*Metarhizium*); seeds uninoculated at sowing with AMF+rhizobia and plants sprayed thrice at flowering with the insecticide Deltamethrin ® (Deltamethrin).

Rhizobia and AMF Seeds Inoculation

Rhizobia inoculum was a mixture of five selected strains designated as $VUID_1$, GMXC, $VUXY_1$, $VSXY_1$ and $AHXY_1$ by the Applied Microbiology laboratory and biofertilizers unit (UMAB) of the University of Yaoundé I. Mycorrhizal inoculum was a mixture of *Glomus* sp. and *Gigaspora* sp., collected from different Cameroonian soils (Nwaga, 1997). The inoculants were supplied by Dr. Nwaga, University of Yaoundé I, Cameroon, after selection and isolation tests (Anonymous, 1998a and b). Seeds were co-inoculated by sowing them at the rate of three per hole previously inoculated with mycorrhizal inoculum (Ngakou *et al*., 2003). Two weeks after emergence, seedlings were thinned to two plants per land. Weeding was done as necessary.

***Metarhizium anisopliae* and Deltamethrin Spray Applications**

Metarhizium anisopliae (ICIPE69 strain) formulated as homogeneous biomass conidial suspension was obtained from the International Center for Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya and mass-produced for our experiments by IITA Benin under standard Material Transfer Agreement. It was applied thrice at 5 day intervals from onset of flowering. The oil-based formulation used was a mixture of 700 mL Kerosene, 300 mL cottonseed oil and 50 g of *M. anisopliae* spores (Lubilosa, 1997). This mixture was applied using a 1 L ultra-low volume (ULV) sprayer, at a rate of 125 mL inoculum per plot. Four replicates each of the control, dual inoculated (AMF+rhizobia) at sowing and sprayed at flowering stage with *M. anisopliae* to give respectively treatments AMF+rhizobia+*Metarhizium* and *Metarhizium*. The insecticide Deltamethrin ® (3 mL/15 L water) was sprayed using the same ULV sprayer, always at a rate of 125 mL inoculum per plot. The spraying process was carried out early in the day (between 7 and 9 am) to minimize wind disturbance.

Assessment of Mycorrhizal Root Infection

The degree of cowpea roots internal colonisation by AMF was rated by observation under a light microscope. Ten plants per plot were collected at 50% flowering (45 DAP), their roots were segmented in approximately 1-2 cm pieces and stored in 50% ethanol. Roots were stained according to Kormanik and McGraw (1982) methods. Each piece of stained root was mounted on a microscope slide and observed at x 40 to 100 magnifications. Data were expressed as percentage of mycorrhizal plant roots colonised per plot (Brundrett *et al*., 1985).

Assessment of Nodulation, Biomass and Inflorescences at 45 DAP

Sampling for assessment of plant biomass, nodulation and the number of inflorescences per plant was done on 20 randomly selected plants per elementary plot at 45 DAP. Plants were carefully removed from the soil with a cutlass and labeled. The number of root nodules and inflorescences per plant were recorded and the whole vegetative part of each plant was dried in a hot-air oven for 72 h at 60°C and weighed (Athar and Johnson, 1996). All root nodules collected from the 20 plants per plot in each replicate, were also dried for 12 hours at 60°C and weighed separately.

Assessment of Pod Yield at Harvest

Dry pods were picked sequentially from experimental plots at maturity (85-95 DAP) to assess the pods yields. Pods from 20 randomly selected plants from the middle rows of each plot were counted and recorded. The pod yield was assessed in ton/ha by multiplying the number of plants per hectare by the pod yield per plant.

Statistical Analysis

Data were subjected to analysis of variance (ANOVA). Means were separated between treatments with the Least Significant Difference (LSD, $p \leq 0.05$), using the Statgraphic plus, version 5.0 (SIGMA PLUS) computer package. Comparisons were made among treatments of the same agro ecological zone conducted the same year. A p-value < 0.05 was generally used to evaluate significance, although higher levels were considered for p value < 0.001. The SPSS statistical program was used to assess the correlation between the growth and pod yield parameters.

RESULTS

Plant Growth Responses to AMF+Rhizobia Inoculation at 45 DAP

Plant biomass, nodulation and the formation of inflorescences were used to assess growth at 45 DAP (Table 1). Plants inoculated with both AMF and rhizobia at sowing produced a significantly

Table 1: Growth responses of cowpea to AMF+rhizobial inoculation at 45 days after planting in four agro ecological zones (I, II, IV and V)

		Agro ecological zones and study years							
		Zone-I		Zone-II		Zone-IV		Zone-V	
Growth parameters	Treatments	2000	2001	1999	2000	2004st	2004nd	1999	2001
Infloresence (per plant)	Control	8.03a	2.95a	6.18a	5.72a	16.22a	17.82a	19.25a	3.95a
	Inoculated	9.95a	3.05a	15.63b	6.79b	24.27b	23.10b	23.40a	10.23b
	LSD 5%	1.91ns	0.1ns	9.45**	1.07*	8.05**	5.27**	4.15ns	6.28**
Nodules (per plant)	Control	2.3a	2.40a	4.70a	1.60a	12.90a	12.50a	0.60a	7.90a
	Inoculated	7.2b	13.61b	16.17b	5.67b	30.55b	26.72b	8.18b	13.61b
	LSD 5%	4.86**	11.20*	11.4**	4.07**	17.7**	14.17**	7.6**	14.7**
Nodules dw (g/plant)	Control	0.16a	0.07a	na	0.04a	0.52a	0.36a	na	0.38a
	Inoculated	0.84b	0.34b	na	0.51b	1.57b	1.60b	na	2.14b
	LSD 5%	0.68*	0.26*	na	0.46**	1.05**	1.22**	na	1.7**
Biomass (g/plant)	Control	7.99a	4.62a	9.53a	2.97a	27.38a	26.43a	38.54a	10.16a
	Inoculated	12.9b	6.42a	19.94b	5.34b	60.82b	49.01b	64.85b	22.90b
	LSD 5%	4.9**	1.8ns	10.4**	2.36**	33.43**	22.58**	26.3**	12.8**
AMF (%)	Control	1.0a	14.25a	25.75a	23.0a	24.1a	19.90a	30.2a	23.15a
	Inoculated	56.25b	51.71b	55.62b	45.9b	61.4b	55.60b	47.0b	49.03b
	LSD 5%	55.25**	37.46**	29.87*	22.9**	37.3**	51.6**	16.7ns	25.9**

Values with different letter within a column for each growth parameter of a particular year are significantly different at p<0.05; *: Denotes a Statistically significant difference between treatments; **: Denotes a Highly statistically significant difference between treatments; Control: no: Not significant; Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; Inoculated: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*; Zone-I: Sudano-sahelian zone; Zone-II: Guinea-savannahh zone; Zone-IV: Monomodal Humid forest rainfall zone; Zone-V: Bimodal Humid; forest rainfall zone; na: not available higher biomass ($p<0.0001$) than uninoculated plants in all agro ecological zones. The highest plant biomass (64.84 g) was obtained from inoculated plants in the Humid-forest rainfall (zone-V) compared to 36.30 g for uninoculated plants. Inoculated plants also produced more inflorescences per plant, though the increase in inflorescence in inoculated plants in the Soudano-sahelian zone was not significant.

Roots colonisation by arbuscular mycorrhizal fungi was generally low in all agro ecological zones (Table 1). Inoculated plants showed a low, but significant ($0.05>p<0.01$) response to AMF infection in all trials compared to uninoculated plants. The degree of roots infection by AMF was 6.1 and 3.3 times, 2.1 and 2.4 times, 1.4 and 2.1 times greater than in the control treatments, respectively for the first and second cropping year in agro ecological zone-I, II, V, 2.5 and 3.6 times during the first and second cropping season in zone IV. The highest infectious experimental site with native AMF was Nkolbisson-Yaoundé in 1999 with 30% AMF colonization, while the lowest roots colonisation due to inoculant strains was 47% recorded in the same zone during the same cropping year.

Root nodules were formed by both naturalized and introduced rhizobia species; but more nodules were formed by introduced than naturalized strains (Table 1). Nodulation was consistently higher ($p<0.0001$) in inoculated than uninoculated plants in all agro ecological zones. There were often 2.3 and 7 fold, 3.2 and 3 fold, 2.3 and 2.2 fold, 4 and 2.8 fold root nodules on inoculated than uninoculated plants, respectively in zone-I, II, IV and zone-V for the first and second experiment in each zone.

The two trials of zone-I and zone-V responded differently to nodulation with an effect of 57 and 86% for zone-I, 75 and 65% in zone-V during the two cropping years. In contrast, the two trials of zone-II had similar responses to inoculation with respect to nodulation with an effect of 67% during the two cropping years. The dry weight of nodules per plant was significantly improved ($0.03 > p\leq 0.001$) by inoculation in all trials tested. Cowpea root nodules were more concentrated on roots of AMF+rhizobia inocolated than uninoculated plants at 45 DAP (Fig. 1). In addition nodules from inoculated plants were distributed on both the main and secondary roots, while those of uninoculated plants were mostly on secondary roots.

There was a highly significant positive ($p\leq 0.03$; $0.75<r<0.90$) correlation between all the growth parameters during the second (2001) cropping year in zone-I. In zone-II, the number of nodule per plant did not significantly correlate ($p = 0.07$; $r = 0.65$) with the degree of root infection by AMF for the first cropping year. There was a significant positive ($p\leq 0.04$; $0.75<r<0.90$) correlation between other growth parameters during the two cropping years. In zone-V, a positive highly significant ($p<0.01$; $r = 0.85$) correlation between the number of nodule and the plant biomass at 45 DAP was detected during the first cropping year. There was no significant correlation ($p = 0.12$; $r = 0.59$)

Fig. 1: Uninoculated (Ctrl) and AMF+rhizobia inoculated (RM) cowpea roots nodules at 45 days after planting

between the nodule dry weight and root colonization by AMF during the second cropping year. A highly significant ($p<0.01$; $0.85 \le r < 0.99$) and positive correlation between the number and dry weight of nodule per plant, the number of inflorescences per plant, the biomass of plant and the mycorrhizal root colonization was revealed in zone-IV. The dry weight of nodule also significantly ($p<0.01$; $0.77 \le r < 0.99$) and positively correlated with the number of inflorescences per plant, the biomass of plant, the mycorrhizal root colonization. Whereas the number of inflorescences per plant showed a significant ($0.85 \le r < 0.87$) positive correlation with the biomass of plant and the mycorrhizal root colonization, the biomass of plant significantly ($p = 0.01$; $r = 0.80$) positively correlated with the mycorrhizal root colonization at 45 DAP.

Interactions Between Cropping Years and Growth Parameters at 45 DAP

Interactions between cropping years significantly affected the biomass of cowpea plants ($p = 0.00$) at 45 DAP in all the agro ecological zones studied (Table 2). Whereas the plant biomass was higher in the first than the second cropping year in zone-II, IV and V, it was rather lower in the second than the first cropping year in zone-I. Similarly, the number of nodules was higher in the first than the second cropping year in zone-II, while in zone-I and V it was rather lower in the second cropping year than the first. The degree of root infection by AMF was not significantly ($p = 1.14$) affected by the cropping years in any of the agro ecological zone I, IV and V. The number of inflorescences per plant was highly significantly reduced ($p = 0.00$) during the second cropping year in zone-I and V, but was rather increased in zone-II. In contrast it was not affected by the cropping year in zone-IV.

Cowpea Pod Yield Responses at Harvest to AMF+rhizobia Inoculation, *Metarhizium* or Deltamethrin Spray

Treatments *Metarhizium*, AMF+rhizobia and AMF+rhizobia+*Metarhizium* consistently produced more pods per plant ($p<0.006$) in all the trials (Table 3). The effects of *Metarhizium* and AMF+rhizobia+*Metarhizium* were similar in zone-II and V in 1999, while those of AMF+rhizobia and AMF+rhizobia+*Metarhizium* were similar in 1999 and 2000 in zone-II and in 2001 in zone-V. *Metarhizium* or its combination with inoculation had similar responses in 1999 and 2000 in zone-II and in 2001 in zone-I. The insecticide Deltamethrin® contributed to more pods production per plant than any other treatment with an average of 17 pods per plant in zone-I and IV. During the first cropping season in zone-IV, each of treatment *Metarhizium*, AMF+rhizobia and AMF+rhizobia+*Metarhizium*

Table 2: Effect of cropping years on growth parameters in four agro ecological zones (I, II, IV and V)

Agro ecological zones	Study years	Growth parameters: Inflorescences (per plant)	Degree AMF (%)	Nodules (per plant)	Biomass (g/plant)
Zone-I	2000	9.20b	29.75a	4.99a	5.06a
	2001	2.10a	33.51a	7.80b	10.47b
	LSD 5%	7.13**	3.76ns	2.81**	5.41**
Zone-II	1999	6.25a	40.68b	10.47b	14.73b
	2000	10.91b	34.43a	3.63a	4.15a
	LSD 5%	4.65***	6.25***	6.84**	10.58**
Zone-IV	2004st	20.20a	42.70a	19.21a	44.10b
	2004nd	20.40a	45.70a	21.71a	37.70a
	LSD 5%	0.21 ns	2.92ns	2.09ns	6.38*
Zone-V	1999	21.32b	36.09a	4.39a	51.70b
	2001	7.09a	38.62a	15.28b	16.53a
	LSD 5%	14.23**	2.53ns	10.89**	35.17**

Values with different letter within a column for each growth parameter are significantly different at $p<0.05$; *: Highly statistically significant difference between cropping years; **: Very highly statistically significant difference between cropping years; ns: on significant; Z-I: Sudano-sahelian zone; Zone-II: Guinea-savannahh zone; Zone-IV: Monomodal Humid forest rainfall zone; Zone-V: Bimodal; Humid forest rainfall zone; 2004 1st: First cropping season; 2004 2nd: Second cropping season

Table 3: Pods production by cowpea plants of the Bafia local cultivar receiving AMF+Rhizobia, *Metarhizium* and Deltamethrin ® treatments in four agro ecological zones (I,II, IV and V)

Agro ecological zones	Study years	Control	Treatments and number of pods per plant at harvest *Metarhizium*	AMF+Rhizobia	AMF+Rhizobia+*Metarhizium*	Deltamethrin	LSD 5%
Zone-I	2000	12.18a	16.22c	14.25bc	14.95bc	17.02c	4.02*
	2001	12.95a	18.37bc	16.52b	20.37c	17.87bc	3.55**
Zone-II	1999	21.37a	30.12b	31.72b	32.21b	na	8.75*
	2000	6.60a	10.02b	8.97ab	9.475b	na	2.85*
Zone-IV	2004st	7.37a	9.88b	11.05b	11.12b	10.75b	2.51**
	2001nd	14.55a	18.51ab	20.93b	20.20b	17.39ab	5.66*
Zone-V	1999	9.93a	13.20a	15.45ab	24.18b	na	10.97**
	2001	13.17a	19.05bc	16.58b	19.38c	20.75c	2.82**

Values with different letter within a row for each study year of a particular agro ecological zone are significantly different at $p<0.05$; *: Statistically significant difference between treatments; **: Highly statistically significant difference between treatments; na: not available; Control: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; AMF+rhizobia: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*; *Metarhizium*: Seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae*; AMF+rhizobia+*Metarhizium*: Seeds inoculated at sowing with AMF+rhizobia and plants sprayed thrice from flowering with *M. anisopliae*; Deltamethrin: Seeds uninoculated at sowing with AMF+rhizobia and plants sprayed thrice from onset of flowering with Deltamethrin ®; Zone-I: Sudano-sahelian zone; Zone-II: Guinea-savannahh zone; Zone-IV: Monomodal Humid forest rainfall zone; Zone-V: Bimodal Humid forest rainfall zone

Table 4: Pods yield (t ha^{-1}) obtained from cowpea plants of the Bafia local cultivar receiving AMF+Rhizobia, *Metarhizium* and Deltamethrin ® treatments in four agro ecological zones (I, II, IV and V)

Agro ecological zones	Study years	Control	Treatments and pod yield at harvest (t ha^{-1}) *Metarhizium*	AMF+Rhizobia	AMF+Rhizobia+*Metarhizium*	Deltamethrin	LSD 5%
Zone-I	2000	1.16a	2.33c	1.93b	2.28c	2.41c	0.27*
	2001	1.34a	1.88b	2.01c	2.13c	1.89b	0.54**
Zone-II	1999	1.63a	2.72b	2.97c	2.66b	na	0.31*
	2000	0.60a	0.99c	0.88bc	0.86b	na	0.13*
Zone-IV	2004st	0.07a	1.05b	1.12b	1.12b	1.00b	0.93*
	2001nd	2.13a	2.61b	3.16c	2.58b	2.56b	0.43*
Zone-V	1999	0.65a	0.87b	1.14c	1.89d	na	0.22**
	2001	1.42a	2.33c	1.93b	2.28c	2.43c	0.35**

Values with different letter within a row for each study year of a particular agro ecological zone are significantly different at $p<0.05$; Control: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; na: not available; AMF+rhizobia: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*; *Metarhizium*: Seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae*; AMF+rhizobia+*Metarhizium* : Seeds inoculated at sowing with AMF+rhizobia and plants sprayed thrice from flowering with *M. anisopliae*; Deltamethrin: Seeds uninoculated at sowing with AMF+rhizobia and plants sprayed thrice from onset of flowering with Deltamethrin ®. Zone-I: Sudano-sahelian zone; Zone-II : Guinea-savannahh zone; Zone-IV: Monomodal Humid forest rainfall zone; Zone-V: Bimodal; Humid forest rainfall zone

increased pod production by 33% compared to the control. In zone-V in 1999 and 2001, in zone-II in 1999 and in zone-I in 2000 and 2001, AMF+rhizobia+*Metarhizium* increased the number of cowpea pods per plant more than treatments *Metarhizium* and AMF+rhizobia compared to the control (Table 3). The effects of treatments *Metarhizium* and AMF+rhizobia+*Metarhizium* were not different in zone-II in 1999 and in zone-IV in 2004. In contrast, they had similar effects in zone-I in 2000 and in zone-IV during the first cropping season. Treatments *Metarhizium*, AMF+rhizobia, AMF+rhizobia+*Metarhizium* and Deltamethrin significantly increased ($p<0.03$) pods yield at harvest (Table 4). The effects of AMF+rhizobia and Deltamethrin were either similar to that of AMF+rhizobia+*Metarhizium* in zone-V, or to that of *Metarhizium* in zone-I and II. Treatments *Metarhizium*, AMF+rhizobia, AMF+rhizobia+*Metarhizium* and Deltamethrin contributed to 37, 39, 44 and 49% increased pod yield respectively, compared to that of the control. The number of pods per plant showed a significant and positive correlation with the dry weight of pods during the 1999 cropping season in zone-II ($r = 0.87$, $p = 0.00$) and zone-V ($r = 0.98$, $p = 0.00$), during the 2000 cropping season in zone-I ($r = 0.56$, $p = 0.01$) and zone-II ($r = 0.89$, $p = 0.00$) and during the 2001 cropping season in zone-V (r = 0.91, p = 0.00). In zone-IV, the number of pods per plant significantly correlated with the dry weight of pods per plant in the first (r = 0.92; p<0.01) and the second (r = 0.80; p<0.01) cropping seasons.

DISCUSSION

Differences were observed between the number and dry weight of nodules formed by inoculated with AMF+rhizobia and those of native AMF+rhizobia in the soil. These differences can be explained by specific nitrogen and phosphorus requirements provided respectively by the two symbionts (rhizobia and AMF) to the host cowpea, or by host-specific interactions among legumes, root nodules and AMF (Valdenegro *et al*., 2001; Scheublin *et al*., 2004). These results are similar to those of Xavier and Germida (2002 and 2003) who reported a positive and synergistic interaction among the members of the tripartite symbiotic association (*Rhizobium*-AMF-legume), but contradict those of Jia *et al*. (2004) who did not find any significant effect on nodule mass in the presence or absence of AMF.

The significant increase in the number and dry weight of nodules per plant suggests that AMF+rhizobia inoculants were more efficient and competitive in all the agro ecological zones compared to those naturally available in the soil. According to Kiers *et al*. (2003); Denison and Kiers (2004), microbial inoculants must always compete with indigenous ones, which are often less infective. Nodulation in uninoculated plants indicates the presence of indigenous species in all the experimental soils surveyed. Poor nodulation in uninoculated plants may be attributed to constraints on nitrogen fixation such as the slight acidity of soils that may have reduced the activity of rhizobacteria (Uliassi and Ruessi, 2002). Fewer nodules observed in zone-I and zone-II, may be an indication of inadequate infection or lower multiplication of rhizobia and AMF under drought conditions. In dry soils, such as those of these zones, the absence of normal root-hairs may be inadequate for strains infection. This is in conformity with the results of Athar and Johnson (1996) who reported that drought can markedly reduce nodulation in *Alfalfa* accessions

Extensive mycorrhizal root colonisation occurred in both uninoculated and inoculated plants, but lower colonisation was observed in inoculated cowpea plants in zone-V. This low mycorrhization usually occurs in conditions, where competition between indigenous and introduced strains is often high (Thiagarajan and Ahmad, 1993; Tawaraya *et al*., 2001). Despite the low infection of cowpea roots, significant growth responses were observed in inoculated plants. Similar findings were reported for pigeon pea grown in different soils (Ahiabor and Hirata, 1994). Generally, colonisation of roots by AMF favours nodulation by rhizobia and increases weight of nodules (Sanginga *et al*., 1999).

Higher mycorrhizal colonisation and nodulation in inoculated cowpea resulted in improved growth. These may be ascribed to the synergistic effect of both symbionts and is similar to what was observed in a dual inoculation of legume trees with AMF+rhizobia (Naqvi and Mukerji, 1994). Many previous studies have also demonstrated that inoculation of legumes with both rhizobia improves plant growth (Klironomos, 2003; Van Der Heijden and Scheublin, 2007). Though the control plants had good root colonisation resulting from mycorrhization by indigenous species, the resulted biomass was significantly lower than that of plants inoculated with AMF+rhizobia. Similar results were attained by Valdes *et al*. (1993) in *Leucaena* plants inoculated with rhizobia and AMF. These results suggest that high colonisation may not be the only factor involved when considering the mycorrhizal and rhizobial responses. The effect of mycorrhiza on plant growth depends to some degree on the balance between the development of root and the concentration of available phosphate and perhaps other nutrients (Rao and Tak, 2001), while that of rhizobia depends among others on soil temperature. The responses of inoculation to growth varied from one agro ecological zone to another in conformity with the observations of Klironomos (2003) who indicated that the mycorrhizal performances vary widely depending on the environmental constraints.

The number and dry weight of pods per plant from inoculated plants were significantly higher than those of control plant. The low pod production and pod dry weight in uninoculated plants may be attributed to the low nodulation and low mycorrhizal colonisation. These results show that inoculation of cowpea seeds at sowing, or spraying plants at flowering with metarhizial spores and/ or the combination of both treatments can significantly increase pods yields. The insecticide Deltamethin ® significantly increased the pod yield in zone-I, IV and V, thus had a similar effect as treatments AMF+rhizobia or AMF+rhizobia+*Metarhizium*. This is because Deltamethin ® is a wide range insecticide that acts on several cowpea pests at once, contributing to reduction of crop yield losses (Omongo *et al*., 1998). The present results support the synergistic interactions between rhizobia and AMF that impacted on nodulation, plant biomass, number of inflorescences per plant at 45 DAP and the cowpea pod yield. This agrees with the findings of Valdenegro *et al*. (2001) in other legumes and those of Nwaga *et al*. (2003) in cowpea. There are several possible explanations to this synergistic effect, of which the phosphorus nutrition and the reduction of thrips populations by inoculated and metarhizial spray treatments (Ngakou *et al*., Submitted to Crop Protection) appear to be of greater importance. The physico-chemical differences between agro ecological zones could also explain these findings.

CONCLUSIONS

Repeated experiments in agro ecological zones demonstrated that selected rhizobia and AMF inoculants were more competitive than naturalized strains and had the same impact on the host plant, though at different degree of efficiency. These results suggest that the synergistic or additive interactions among the components of the tripartite symbiotic association (rhizobia-AMF-cowpea) increased plant productivity with a boost of effect accounting for by the entomophagous fungi *M. anisopliae*. Therefore, for a suitable optimization of growth and yield of cowpea or other legumes in low fertile soils such as those of Cameroon, effective and compatible rhizobia and AMF and *M. anisopliae* strains is recommended.

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