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# Arbuscular-Mycorrhizal Fungi, Rhizobia and *Metarhizium anisopliae* Enhance P, N, Mg, K and Ca Accumulations in Fields Grown Cowpea

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**Abstract:** The concentrations of P, N, K, Mg, Ca in cowpea (*Vigna unguiculata* (L.) Walp.) roots and shoots were assessed at 45 Days After Planting (DAP) in inoculated and uninoculated plants at sowing with rhizobia and Arbuscular-Mycorrhizal Fungi (AMF). Those of harvested seeds from inoculated and uninoculated plants at sowing or sprayed and unsprayed plants at flowering with *Metarhizium anisopliae* were also assessed. Field trials were carried out in a complete Randomized Block Design with four treatments, in the Sudano-Sahelian (zone I), Guinea-Savannah (zone II), monomodal and bimodal humid-forest rainfall (zone IV and V) of Cameroon. The contributions of rhizobia and AMF to cowpea shoots and roots nutrients uptake at 45 DAP significantly accounted for up to 17% for total N, 52% for available P, 19% for Ca, 55% for Mg, 46% for K. Compared to the control, AMF+rhizobia, *M. anisopliae*, AMF+rhizobia and *M. anisopliae* significantly increased ($p = 0.04$) the N, P, Ca, Mg and K seed concentrations in zone-I (2000 and 2001), zone-II (1999), zone-IV (2004) and zone-V (1999 and 2001) at harvest. The two symbionts and *M. anisopliae* almost had the same influence on plant nutrient uptake within agro ecological zones. These results demonstrate the dependency of cowpea on microbial inoculants for nutrient accumulations in cowpea plants. However, more work still need to be carried out to investigate on the mechanisms by which *M. anisopliae* contributes to the increment of nutrient uptake in harvested cowpea seeds.

**Key words:** Agro ecological zones, arbuscular mycorrhizal fungi, cowpea, inoculation, *Metarhizium anisopliae*, nutrient accumulations, rhizobia

## INTRODUCTION

Soil nitrogen, phosphorus, magnesium, calcium, potassium and aluminium contents are widespread constraints to symbiotic nitrogen fixation and endomycorrhizae root colonization (Toro *et al*., 1998; Uliassi and Ruessi, 2002). In most tropical soils which are highly acidic, low

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availability of P, K and Ca may limit plant, rhizobial and mycorrhizal growth, whereas Al and Mn ions may reach toxic levels to plants or symbiotic partners (Fernandes *et al*., 1991). Microbial interactions involving Arbuscular Mycorrhizal Fungi (AMF) and rhizobia are particularly interesting since the two microorganisms colonize the root systems of a common host legume (Xavier and Germida, 2002; Scheublin *et al*., 2004). Cowpea is of major importance to the diet of millions of peoples, many of whom are the world's poorest (Quin, 1997). Cowpea is also important in the nutrient economy low-input cropping systems, since they fix nitrogen through symbiotic association with rhizobia, thereby improving soil fertility and reducing the need of nitrogen fertilizer (Sanginga *et al*., 2000). The process of biological nitrogen fixation by cowpea nodules requires large amount of phosphorus, which can be provided by AMF. Hence, the availability of phosphorus is a primary constraint to nitrogen fixation that contributes to nitrogen economy of many tropical ecosystems (Mortimore *et al*., 1997).

If it is possible to elucidate how soil acidity influences cowpea growth, biological nitrogen fixation and AMF symbiosis, then selection of rhizobia and AMF genotypes may be an appropriate management strategy for sustainable production of cowpea. Attempts have been made to select local rhizobia and AMF (Nwaga, 1997), but these symbionts have not yet been tested on the host cowpea to improve its nitrogen fixation efficiency, phosphorus and other nutrients uptake in different Cameroonian soils. Since low levels of soil available N and P fertilizers are common for both cereals and legumes in tropical soils (Bationo *et al*., 1986), application of P and N fertilizers could therefore be necessary. However, they are toxic to human's life and environment (Mahr *et al*., 2001), or are often expensive due to the lack of locally available resources (Margni *et al*., 2002). To increase cowpea production, it would be desirable for the crop to access a greater proportion of the total soil nutrients pool than is otherwise available to them. Such increment may require the use of low-cost and environmentally friendly microbial inoculants with reference to AMF and rhizobia, which can be strengthened by the mycopesticides *Metarhizium anisopliae* (Metchnikoff) (Deuteromycotina: Hyphomycetes). This entomophagous fungus has shown considerable potential for the control of various insect pests (Lomer *et al*., 1997) and its prospective use against *M. sjostedti* has been reviewed (Tamò *et al*., 2003).

In the present study, we assessed the potential of the two microsymbionts (AMF and rhizobia), as well as that of the mycopesticide (*M. anisopliae*) in improving nutrients accumulation in cowpea (roots/shoots, harvested seeds) grown in different Cameroonian soils.

## MATERIALS AND METHODS

### Plant Material

Plants were raised from inoculated and uninoculated cowpea seeds Bafia local cultivar, Cameroon in experimental fields. The cultivar is semi-erected and matures between 85 and 95 (DAP). The seeds are light brown in colour, medium in size and smooth in texture.

### Agro Ecological Zones of the Study and Soil Characteristics

Experiments were conducted in four out of the five agro ecological zones of Cameroon during the normal cropping seasons: the Sudano-sahelian zone (zone I) in 2000 and 2001; the Guinea-savannah zone (zone II) in 1999 and 2000; the monomodal Humid-forest rainfall zone (zone IV) during the first and second cropping season of 2004 (2004st, 2004nd) and the bimodal Humid-forest rainfall zone (zone V) in 1999 and 2001. These zones were chosen because they represent the most cowpea production area. In each zone, one site was selected for the study. The chemical characteristics of soil in agro ecological zones are indicated in Table 1. These zones were colonized by a wide range of vegetation, site history and climatic conditions. However, none of these sites had a history of inoculation with rhizobia+AMF for at least the three previous years of experimentations.

Table 1: Chemical properties of the soils in agro ecological zones

| | Experimental sites (agro ecological zones) and cropping years | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Nkolbisson-Yaoundé (zone V) | | Dang-Ngaoundéré (zone II) | | Guering-Maroua (zone I) | | Molyko-Buea (zone IV) | |
| Properties of soils | 1999 | 2001 | 1999 | 2000 | 2000 | 2001 | $2004^{st}$ | $2004^{nd}$ |
| pH, $H_2O$ | 5.47 | 5.80 | 5.45 | 4.56 | 5.13 | 5.63 | 6.22 | 6.10 |
| Total C (%) | 1.70 | 0.23 | 2.80 | 2.38 | 0.23 | 1.11 | 2.93 | 2.60 |
| Available P (mg $kg^{-1}$ soil) | 3.00 | 6.40 | 3.00 | 4.10 | 6.70 | 1.00 | 5.80 | 4.91 |
| Total N (%) | 0.14 | 0.20 | 0.18 | 0.15 | 0.03 | 0.05 | 0.32 | 0.21 |
| Ca (cmol $kg^{-1}$) | 1.60 | 1.72 | 3.03 | 2.93 | 2.80 | 1.63 | 2.96 | 2.51 |
| K (cmol $kg^{-1}$) | 0.68 | 0.16 | 0.68 | 0.20 | 0.10 | 0.06 | 0.32 | 0.23 |
| Al (cmol $kg^{-1}$) | 0.02 | 0.07 | 0.04 | 0.05 | 0.06 | 0.08 | 0.01 | 0.03 |
| Mg (cmol $kg^{-1}$) | 0.68 | 0.33 | 1.79 | 1.13 | 0.80 | 0.20 | 1.19 | 0.94 |

mg: milligramme; $2004^{st}$: First cropping season of 2004; $2004^{nd}$: Second cropping season of 2004

The chemical properties of soils were broadly similar, with a slightly acidic soil pH ranging from 4.56 in the Guinea-Savannah to 6.22 in the monomodal Humid-forest rainfall zone. The soil texture in agro ecological zones was described by Ambassa-Kiki (2000): hydromorphic vertisoils in the lowland of diamaré in zone I; ferruginous and hydromorphic yellow or red soils in zone II; ferralitic and yellow or brown soils in zone IV; ferralitic, yellowish or reddish soils in zone V.

## Experimental Set up

Experiments were conducted with two levels of inoculation at sowing (inoculated and uninoculated) and two levels of metarhizial spores spray at flowering (sprayed and unsprayed). Plots were arranged in a Randomised Block Design (RBD) with four replicates per treatment. Each replicate was a 8×8.5 $m^2$ plot with 11 rows spaced at 75×150 cm within and between rows, respectively. The four treatment combinations consisted of: seeds uninoculated at sowing with AMF/rhizobia and plants not sprayed with *M. anisopliae* at flowering as control (C); seeds inoculated at sowing with AMF/rhizobia and plants not sprayed at flowering with *M. anisopliae* (RM); seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae* (Ma); seeds inoculated at sowing with AMF/rhizobia and plants sprayed thrice from flowering with *M. anisopliae* (RMMa).

## Seeds Inoculation and Metarhizial Spore Spray Techniques

Rhizobia inoculum was a mixture of five selected strains formulated by the Applied Microbiology Laboratory and Biofertilizers Unit (UMAB) of the University of Yaoundé I as $VUID_1$, GMXC, $VUXY_1$, $VSXY_1$ and $AHXY_1$. Mycorrhiza inoculum was a mixture of *Glomus* sp. and *Gigaspora* sp., collected from different Cameroonian soils (Mbenoun, 1992; Kamyap, 1997). These inoculants were supplied by Dr. Nwaga, University of Yaoundé I, Cameroon. Seeds inoculation was performed as described by Ngakou *et al.* (2003).

The standard strain of *M. anisopliae* ICIPE 69 (International Center for Insect Physiology and Ecology) was used in all trials. The isolate was obtained under standard Material Transfer Agreement through the Biological control Center for Africa of the International Institute of Tropical Agriculture (IITA) in Cotonou, Benin. Standard material for testing consisted of dry spores powder (50 g) suspended in a mixture of cottonseed oil and kerosene at a ratio of 7/3 (v/v) as an Ultra Low Volume (ULV) formulation (Lomer *et al.*, 1993; Lubilosa, 1997). The suspension involving these components was prepared the day of experiment just before the spraying process. ULV treatments were applied in the field, using a hand-held spinning disc sprayer (Micro-ultra, Micron, UK) at a rate of 125 mL per 68 $m^2$ plot. It was applied thrice at 5 daily intervals from onset of flowering (Lomer *et al.*, 1997). Spraying was done early in the day (7:00-9:00 am) to minimize wind disturbance.

### Samples Preparation and Chemical Analysis

Nutrients analysis was carried out in the soil laboratories of IITA, Yaoundé and Ibadan, Nigeria. Sampling for the assessment of plant biomass was done on 20 randomly selected plants per elementary plot at 45 DAP. Plants carefully removed from the soil with a cutlass were labelled separately per plot, dried in a hot-air oven for 72 h at 60°C and weighed (Athar and Johnson, 1996). Three replicates of cowpea plant samples (100 g of roots and shoots) from inoculated and uninoculated treatments were separately ground into powder in a warring Laboratory blender.

On the other hands, dried pods from 20 randomly selected plants from the middle rows of each plot were sampled at maturity (85-95 DAP) to assess grain yield. The seed samples of four treatments were also prepared in three replicates. In each replicate, each sample consisted of 100 g of seeds per plot, each of which was ground in a warring Laboratory blender.

Analysis of Phosphorus (P) in cowpea plants and seeds was done using the vanadomolybdate yellow method. The total nitrogen content (N) in cowpea plant was determined by an automatic analyzer following wet acid digestion. Magnesium (Mg), calcium (Ca) and potassium (K) contents were analyzed by atomic absorption (IITA, 1989). Response of cowpea to nutrient uptake was calculated by using the method of Plenchette *et al*. (1983) as follows:

$$X \text{ content} = X \text{ content (RM)} - X \text{ content (C)}/X \text{ content (RM)} \times 100$$

Where,

X = The mineral element,
RM = The rhizobia+AMF treatment,
C = The uninoculated treatment.

The nutrient accumulation in plant (root+shoot) expressed in mg plant$^{-1}$ was obtained by multiplying the average biomass per plant by the nutrient content of each element for a particular treatment. The nutrient accumulation in seeds expressed in mg in seed plant$^{-1}$ was obtained by multiplying the nutrient content of each element by the average seed yield per plant for a particular treatment. The effects of different treatments on seed yield at harvest were expressed in percentage.

### 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.

## RESULTS

### Effect of AMF+rhizobia on Cowpea Roots and Shoots Nutrient Uptake at 45 DAP in Four Agro Ecological Zones

Higher nutrient uptake in cowpea roots and shoots was observed in inoculated than in uninoculated cowpea at 45 DAP in all the agroecological zones under study. The effects of inoculation on nutrient concentrations were 50 and 58%, 57 and 59%, 37 and 55%, 32 and 57%, 62 and 58%, respectively for Nitrogen (N), Phosphorus (P), Calcium (Ca), Magnesium (Mg) and potassium (K) for the first (1999) and second (2001) cropping seasons in the bimodal Humid-forest rainfall zone (Table 2). AMF+rhizobia inoculated plants had significantly higher root+shoot N, Ca, P, Ca and K concentrations compared to uninoculated plants in the Guinea-savannah zone during the 1999 and 2000 cropping seasons (Table 3). The effects of inoculation for all the nutrients ranged between 51% and 55% in this zone.

In the Sudano-Sahelian zone, the effect of AMF+rhizobia inoculation ranged between 32 and 44% for all the nutrients during the 2000 and the 2001 cropping seasons (Table 4). Here again, nutrient concentration was consistently improved in AMF+rhizobia inoculated cowpea roots and shoots. At

Table 2: Effect of AMF+rhizobia inoculation on nutrients uptake in cowpea roots and shoots at 45 DAP at Nkolbisson-Yaoundé (bimodal Humid-forest rainfall or zone V)

| | | Nutrients uptake (mg plant$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| Cropping seasons | Treatments | N | P | Ca | Mg | K |
| 1999 | C | 1104a | 84a | 587a | 173a | 741a |
| | RM | 2210b | 207b | 929b | 255b | 1955b |
| | LSD (0.05) | 1106 | 123 | 342 | 82 | 1214 |
| | Effect (%) | 50 | 57 | 37 | 32 | 62 |
| 2001 | C | 348a | 24a | 380a | 38a | 289a |
| | RM | 832b | 58b | 849b | 89b | 684b |
| | LSD (0.05) | 484 | 34 | 342 | 82 | 395 |
| | Effect (%) | 58 | 59 | 55 | 57 | 58 |

Values with different letter(s) cases within a column of a cropping season are significantly different at $p<0.05$; C: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*

Table 3: Effect of AMF+rhizobia inoculation on nutrients uptake in cowpea roots and shoots at 45 DAP at Dang-Ngaoundéré (Guinea-Savannah zone or zone II)

| | | Nutrients uptake (mg plant$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| Cropping seasons | Treatments | N | P | Ca | Mg | K |
| 1999 | C | 314a | 43a | 184a | 45a | 247a |
| | 1RM | 651b | 95b | 405b | 96b | 550b |
| | LSD (0.05) | 337 | 52 | 222 | 51 | 303 |
| | Effect (%) | 52 | 55 | 55 | 53 | 55 |
| 2000 | C | 99a | 6a | 115a | 9a | 78a |
| | RM | 201b | 13b | 208b | 19b | 158b |
| | LSD (0.05) | 102 | 7 | 93 | 10 | 80 |
| | Effect (%) | 51 | 52 | 45 | 53 | 51 |

Values with different letter(s) cases within a column of a cropping season are significantly different at $p<0.05$; C: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*

Table 4: Effect of AMF+rhizobia inoculation on nutrients uptake in cowpea roots and shoots at 45 DAP at Guering-Maroua (Sudano-Sahelian zone or zone I)

| | | Nutrients uptake (mg plant$^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| Cropping years | Treatments | N | P | Ca | Mg | K |
| 2000 | C | 282a | 16a | 308a | 27a | 215a |
| | RM | 494b | 28b | 533b | 46b | 383b |
| | LSD (0.05) | 212 | 12 | 225 | 19 | 168 |
| | Effect (%) | 43 | 43 | 41 | 42 | 44 |
| 2001 | C | 139a | 9a | 122a | 12a | 127a |
| | RM | 217b | 13b | 199b | 18b | 187b |
| | LSD (0.05) | 79 | 4 | 77 | 6 | 60 |
| | Effect (%) | 36 | 34 | 38 | 34 | 32 |

Values with different letter(s) cases within a column of a cropping season are significantly different at $p<0.05$; C: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*

At Molyko-Buea in the monomodal Humid-forest rainfall the contributions of AMF+rhizobia inoculation to cowpea nutrient uptake in shoot and root were 63 and 56%, 80 and 81%, 72 and 63%, 60 and 54% and 69 and 61%, respectively during the first and the second cropping seasons in 2004 for N, P, Ca, Mg and K (Table 5). The experimental sites that most responded to nutrients uptake by cowpea plants was Molyko-Buea in 2004, while the one that responded least was Guering-Maroua in 2000. Nutrient concentrations varied from one agro ecological zone to another and within agro ecological zones from one cropping experiment to another. The nutrients that were mostly

Table 5: Effect of AMF+rhizobia inoculation on nutrients uptake in cowpea roots and shoots at 45 DAP at Molyko-Buea (monomodal Humid-forest rainfall or zone IV)

| | Nutrients uptake (mg plant$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Treatments | N | P | Ca | Mg | K |
| **First cropping season ($2004^{st}$)** | | | | | |
| C | 856a | 42a | 724a | 88a | 604a |
| RM | 2234b | 219b | 2646b | 223b | 1956b |
| LSD (0.05) | 1477 | 176 | 1921 | 135 | 1353 |
| Effect (%) | 63 | 80 | 72 | 60 | 69 |
| **Second cropping season ($2004^{nd}$)** | | | | | |
| C | 818a | 56a | 690a | 94a | 672a |
| RM | 1897b | 310b | 1846b | 207b | 1753b |
| LSD (0.05) | 1078 | 254 | 1156 | 113 | 1080 |
| Effect (%) | 56 | 81 | 63 | 54 | 61 |

Values with different letter(s) cases within a column of a cropping season are significantly different at $p<0.05$; C: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*

Table 6: Influence of different treatments on Nitrogen (N), Phosphorus (P), Calcium (Ca), Magnesium (Mg) and Potassium (K) accumulation in harvested cowpea seeds in monomodal humid forest rainfall (zone-IV) during the first and second cropping seasons of 2004

| | Nutrient accumulation in seeds (mg in seeds plant$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Treatments | Ca | K | Mg | N | P |
| **First cropping season ($2004^{st}$)** | | | | | |
| C | 26.08a | 222.04a | 39.43a | 657.36a | 12.24a |
| Ma | 54.83b | 416.17b | 83.58b | 1119.67bc | 27.86b |
| RM | 75.74bc | 450.13b | 84.45bc | 1060.47b | 26.11b |
| RMMa | 82.97c | 525.53c | 110.63c | 1139.59c | 24.89b |
| LSD (5%) | 28.13** | 75.40** | 27.05** | 79.12** | 12.74** |
| **Second cropping season ($2004^{nd}$)** | | | | | |
| C | 19.44a | 283.18a | 44.34a | 915.70a | 28.00a |
| Ma | 30.38a | 435.44b | 64.80b | 1225.32b | 92.14b |
| RM | 101.63b | 595.43c | 81.30c | 1523.27c | 141.08c |
| RMMa | 94.3b | 639.40c | 70.15b | 1461.65c | 142.60c |
| LSD (5%) | 63.92** | 152.25** | 11.15** | 236.32** | 50.45** |

Values with different letter(s) within a column for each nutrient of a cropping season are significantly different at $p<0.05$ *: Denotes a statistically significant difference between treatments; **: Denotes a highly statistically significant difference between treatments; C: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; Ma: Seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae*; RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*; RMMa: Seeds inoculated at sowing with AMF+rhizobia and plants sprayed thrice from flowering with *M. anisopliae*

taken up thus mobilized by cowpea plant following inoculation were N and K, while the least were P and Mg in all the four agro ecological zones surveyed. A significant positive correlation was observed between nutrient uptake and mycorrhizal colonization in all the experimental sites ($0.79<r\leq 0.99$; $0.01<p<0.03$).

## Effect of AMF+rhizobia and *Metarhizium Anisopliae* on Nutrient Uptake in Harvested Cowpea Seeds in Four Agro Ecological Zones

AMF/rhizobia (treatment RM) and *M. anisopliae* (treatment Ma) consistently influenced ($p\leq 0.04$) the nutrient concentrations in cowpea seeds (mg in seed plant$^{-1}$) at maturity in experimental fields of agro ecological zones compared to the control (Table 6-9).

The highest nutrient seed concentrations in nitrogen were obtained at Molyko-Buea in zone-IV during the second cropping season in 2004 with up to 1523 mg in seed plant$^{-1}$ for treatment RM, against 1461, 1225 and 916 mg in seed plant$^{-1}$ for treatments RM, Ma and the control (C), respectively (Table 6).

Table 7: Influence of different treatments on Nitrogen (N), Phosphorus (P), Calcium (Ca), Magnesium (Mg) and Potassium (K) accumulation in harvested cowpea seeds in the Guinea-savannah (zone-II) during the 1999 and 2000 cropping seasons

| | Nutrient accumulation in seeds (mg in seeds plant$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Treatments | Ca | K | Mg | N | P |
| **First cropping season (1999)** | | | | | |
| C | 25.05a | 199.75a | 31.02a | 523.11a | 13.63a |
| Ma | 26.50a | 282.94b | 39.71a | 639.87b | 16.21ab |
| RM | 36.12b | 344.55c | 53.38b | 790.52c | 18.75b |
| RMMa | 41.01b | 427.83d | 53.38b | 899.77d | 17.89b |
| LSD (5%) | 9.26** | 61.60** | 13.67** | 109.24** | 4.26* |
| **Second cropping season (2000)** | | | | | |
| C | 1.52a | 7.67a | 1.21a | 23.72a | 0.40a |
| Ma | 2.69b | 14.46b | 2.37b | 45.56c | 1.29a |
| RM | 2.64b | 16.66bc | 2.35b | 44.10ab | 1.60a |
| RMMa | 2.10ab | 18.23c | 1.76ab | 42.59b | 0.91a |
| LSD (5%) | 1.12* | 3.77** | 1.14* | 2.97** | ns |

Values with different letter(s) within a column for each nutrient of a cropping season are significantly different at $p<0.05$; *: Denotes a statistically significant difference between treatments; **: Denotes a highly statistically significant difference between treatments; ns: not significant; C: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; Ma: Seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae*; RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*; RMMa: Seeds inoculated at sowing with AMF+rhizobia and plants sprayed thrice from flowering with *M. anisopliae*

Table 8: Influence of different treatments on Nitrogen (N), Phosphorus (P), Calcium (Ca), Magnesium (Mg) and Potassium (K) accumulation in harvested cowpea seeds in the bimodal humid forest rainfall (zone-V) during the 1999 and 2001 cropping seasons

| | Nutrient accumulation in seeds (mg in seeds plant$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Treatments | Ca | K | Mg | N | P |
| **First cropping season (1999)** | | | | | |
| C | 1.97a | 15.89a | 3.29a | 63.78a | 1.16a |
| Ma | 6.20b | 47.16b | 7.04b | 122.50b | 2.31b |
| RM | 9.91c | 57.63c | 8.34bc | 156.28d | 3.50bc |
| RMMa | 5.96b | 44.54b | 9.21c | 140.95c | 2.71c |
| LSD (5%) | 8.71** | 10.47** | 2.17** | 15.33** | 1.15** |
| **Second cropping season (2001)** | | | | | |
| C | 13.78a | 219.06a | 20.40a | 362.16a | 26.50a |
| Ma | 14.90a | 313.39b | 28.31b | 554.28bc | 38.11b |
| RM | 15.84ab | 305.44b | 28.28b | 541.53b | 38.74b |
| RMMa | 17.60b | 338.13c | 30.93c | 594.66c | 43.20c |
| LSD (5%) | 2.7* | 24.73** | 2.62** | 53.13** | 4.46** |

Values with different letter(s) within a column for each nutrient of a cropping season are significantly different at $p<0.05$ *: Denotes a statistically significant difference between treatments; **: Denotes a highly statistically significant difference between treatments; C: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; Ma: Seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae*; RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*; RMMa: Seeds inoculated at sowing with AMF+rhizobia and plants sprayed thrice from flowering with *M. anisopliae*

The highest concentrations of phosphorus were obtained from harvested seeds at Molyko-Buea in zone IV during the second cropping season in 2004 with 142 mg in seed plant$^{-1}$ for treatment RMMa, 141 for treatment RM, 92 for treatment Ma, against 28 mg in seed plant$^{-1}$ for the control.

There was a non-significant difference ($p = 0.27$) between treatments in phosphorus seed concentration at Dang-Ngaoundéré (zone-II) in 2000 (Table 7).

The lowest nutrient seed concentrations were obtained in this zone during the 2000 cropping season with 46, 44, 43 against 24 mg in seed plant$^{-1}$, respectively for treatments Ma, RM, RMMa and the control. Similarly, the lowest seed concentration in phosphorus were 1, 2, 1 and 0.4 mg

Table 9: Influence of different treatments on Nitrogen (N), Phosphorus (P), Calcium (Ca), Magnesium (Mg) and Potassium (K) accumulation in harvested cowpea seeds in the Sudano-sahelian (zone-I) during the 2000 and 2001 cropping seasons

| | Nutrient accumulation in seeds (mg in seeds plant$^{-1}$) | | | | |
|---|---|---|---|---|---|
| Treatments | Ca | K | Mg | N | P |
| **First cropping season (2000)** | | | | | |
| C | 3.48a | 14.35a | 2.46a | 47.74a | 0.98a |
| Ma | 5.88ab | 28.71b | 4.00b | 81.73b | 1.49b |
| RM | 4.41ab | 32.95b | 4.28b | 78.73b | 1.54b |
| RMMa | 7.44b | 39.28c | 4.51b | 86.84b | 1.70b |
| LSD (5%) | 3.96* | 6.33** | 1.54** | 31.09** | 0.51** |
| **Second cropping season (2001)** | | | | | |
| C | 5.04a | 98.16a | 11.63a | 226.00a | 17.79a |
| Ma | 8.36b | 180.96b | 19.32b | 388.65b | 37.35b |
| RM | 10.99c | 194.52b | 21.98c | 465.97c | 40.66b |
| RMMa | 12.76d | 222.48b | 25.99d | 545.37d | 49.23c |
| LSD (5%) | 1.77** | 82.80** | 2.66** | 77.32** | 8.58* |

Values with different letter(s) within a column for each nutrient of a cropping season are significantly different at $p<0.05$ *: Denotes a statistically significant difference between treatments; **: Denotes a highly statistically significant difference between treatments; C: Seeds uninoculated at sowing with AMF+rhizobia and plants not sprayed at all with *M. anisopliae* at flowering; Ma: Seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae*; RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*; RMMa: Seeds inoculated at sowing with AMF+rhizobia and plants sprayed thrice from flowering with *M. anisopliae*

Table 10: Contribution of treatments Ma, RM, RMMa to seed yield (%) at harvest in the four agro ecological zones

| | Agro ecological zones | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Zone I | | Zone II | | Zone IV | | Zone V | |
| Treatments | 2000 | 2001 | 1999 | 2000 | 2004$^{st}$ | 2004$^{nd}$ | 1999 | 2001 |
| Ma | 43 | 40 | 91 | 48 | 30 | 23 | 44 | 28 |
| RM | 40 | 47 | 93 | 45 | 30 | 35 | 56 | 28 |
| RMMa | 40 | 57 | 94 | 44 | 41 | 32 | 51 | 34 |

RM: Seeds inoculated at sowing with AMF+rhizobia and plants not sprayed at flowering with *M. anisopliae*; Ma: Seeds uninoculated at sowing and plants sprayed at flowering with *M. anisopliae*; RMMa: Seeds inoculated at sowing with AMF+rhizobia and plants sprayed thrice from flowering with *M. anisopliae*; Zone I: Sudano-sahelian zone (Guering-Maroua); Zone II: Guinea-Savannah zone (Dang-Ngaoundéré); Zone IV: Monomodal Humid-forest rainfall zone (Molyko-Buea); Zone V: Bimodal Humid-forest rainfall zone (Nkolbisson-Yaoundé)

in seed plant$^{-1}$, respectively for treatments RMMa, RM, Ma and the control obtained in zone-II during the 2000 cropping season.

*M. anisopliae* (treatment Ma) had the same effect as the control on Ca and P concentrations at Nkolbisson-Yaoundé (zone V) in 2001 (Table 8). Any other treatment consistently improved the nutrient accumulation in seeds as compared to that of the control.

AMF+rhizobia (treatment RM), AMF+rhizobia+*Metarhizium* (treatment RMMa) and *M. anisopliae* (treatment Ma), significantly enhanced the seed of nutrients accumulation in zone-I (Guering-Maroua) as compared to those of the control (Table 9). However, there was no significant difference between the calcium seed concentration of *M. anisopliae*, AMF+rhizobia treatments and that of the control.

The treatment effects on nutrients in harvested cowpea seeds ranged from 41-59% for N, 15-75% for P, 6- 80% for Ca, 21-64% for Mg and 28-72% for K compared to the control in agro ecological zones under study. Seed nutrient concentrations varied from one agro ecological zone to another and within agro ecological zone fro m one cropping season to another.

Increases in seed yield expressed in tons/ha ranged from 23-91% for treatment Ma, 28-93% for treatment RM and 32-94% for treatment RMMa compared to the control (Table 10). Apart from the results obtained in 2000 in zone I, treatment RMMa increased the seed yield in all the other agro ecological zones more than any other treatment.

## DISCUSSION

The results from this study reveal that cowpea nutrient uptake in several agroecological zones in Cameroon can be enhanced through the use of AMF/rhizobia and *M. anisopliae*. Seasonal significant differences were found in nutrient uptake, indicating an environmental influence. Total nitrogen accumulation in cowpea shoots and roots was enhanced by AMF/rhizobia inoculation, in agro ecological zones. A similar increment of nitrogen uptake in *Medicago sativa* was reported by Azcon *et al.* (1991) after AMF/rhizobia inoculation. Legume has a relatively high P requirement for nodule development and nitrogen fixation (Buerkert *et al*., 2001). Most of the earlier studies suggest that roots colonization with efficient AMF significantly improve phosphorus nutrition and consequently nitrogen fixation, probably by stimulating the nitrogenase activity (Xavier and Gerermida, 2004), or by enhancing photosynthetic nitrogen use efficiency (Jia *et al*., 2004).

Concentrations of available P in rhizobia+AMF inoculated plants, or *M. anisopliae* treated plants were increased almost two fold compared to the control treatments. These results differ from those of Hartwig *et al.* (2002), who did not find increased P concentration in *Lolium perenne* whose growth response was increased by mycorrhizae. Besides an increase in P level in inoculated plants with AMF/rhizobia, there was also a greater concentration of Ca, K and Mg in shoots and roots of inoculated plants than those of uninoculated cowpea plants in all agroecological zones. These results are in conformity with previous results of Lop Phavaphutanon *et al.* (1996), who reported enhanced P, K, N, Ca with other plants species following inoculation. A mycorrizal condition was also reported to favour the uptake of K relative to Ca and Mg, which diffuse more rapidly in soil (Smith and Read, 1997).

The analysis of nutrient concentration in cowpea seeds at harvest revealed an increase in N, P, Ca, Mg and K in cowpea seeds during some cropping seasons. Accumulation of nutrients in seeds did not vary too much between agroecological zones. However, differences between treatments within agroecological zones were observed, especially for Mg in the bimodal Humid-forest and the Sudano-sahelian zones and P, Ca and Mg in the Guinea-Savannah zone. Nutrient concentration was sometimes lower in RMMa plants than in other treated plants although RMMa plants show greater biomass (data not shown). Therefore, the significant decreases in P, Ca and Mg concentrations observed during some cropping seasons could be attributed to the dilution effect, in which the larger nutrient-deficient plants do not concentrate elements as much as smaller nutrients-deficient plants. These results are similar to those of Quatrini *et al.* (2003), who reported a dilution effect during nutrient uptake by cowpea, maize, sorghum, millet and soybean.

The role of AMF in nutrient uptake has been attributed to increases in total absorption surface of infected plants, leading to improvement of its access to immobile nutrients such as P, Cu, Zn, Ca and N in areas beyond the depletion zones (Guo *et al*., 1996). This function is disproportionally important for such nutrients as N, P, Ca, K and Mg, which have narrow diffusion around roots (Lambert *et al*., 1979). The effect of AMF inoculation on the concentrations of K, Ca, Mg, generally depended on phosphorus availability in the soil. Without phosphorus or with very low phosphorus concentration, AMF inoculation resulted in higher P, K, but lower Ca concentrations in inoculated plants compared to uninoculated plants (Bagayoko *et al*., 2000), whereas at higher P-level, low concentrations of P, K and Zn can occur. The benefits of mycorrhizae vary and decrease with a number of variables, such as low temperature, wet soil and P fertilization (Toaro *et al*., 1998). An intensive root colonization and enhancement of plant P content are usually not expected in soils with high soluble P content, where root infection by AMF is slowed down by soil P availability (Smith and Read, 1997).

The present results indicate that AMF+rhizobial and *M. anisopliae* can increase nutrient uptake efficiency despite environmental constraints. This finding correlates well with growth enhancement allocated to AMF/rhizobia inoculation and *M. anisopliae* at 45 DAP (Nwaga *et al*., 2003). The concentrations of Ca and Mg found in cowpea seeds was by far, higher than the average values described in literature, which ranges from 0.5-0.8% for Ca and 0.2-0.3% for Mg (Azcon *et al*., 1991).

This may be expected because of high concentration of these elements in the experimental soils as shown in Table 1. In the present study, the mineral content was analysed in the whole cowpea plant including roots, stems and shoots. The high concentration of K and Ca also may be the consequence of mycorrhizal improvement of P supply (Azcon *et al*., 1991).

Many crop plants show mycorrhizal dependency, defined by (Thompson, 1991) as the degree to which a host relies on the mycorrhizal condition to produce maximum growth at a given level of soil fertility. The prevailing consensus is that improvement of phosphorus uptake by the host plant resulting from AMF infection enhances nodulation and nitrogen fixation, the later being stimulated by inoculated plants (Asimi *et al*., 1980). The available P concentration in experimental soils in which cowpea was grown in this study was not too high (Table 1) to impede the development of AMF. Cowpea may depend much more on microbial inoculants for its higher rooting systems. The existence of a positive and significant correlation between mycorrhization and nutrient accumulation in cowpea suggests that enhanced P nutrition may be the most probable cause of both improved plant growth and yield in cowpea. The various mechanisms accounting for this increment have been reported to include faster movement of soil phosphorus into AMF hyphae and solubilization of soil phosphorus by the release of organic acid and phosphatase enzymes to help hydrolyse phosphate from inorganic compounds (Bolan, 1991; Joner *et al*., 2000). However, the extra phosphorus in mycorrhizal plants could be due mostly to better soil exploration by the extrametrical mycelium than to the ability of the fungus to utilize or mobilize sources of soil P not available to the plant (Koide and Mosse, 2004). Van Der Heijden *et al*. (2006) have rather suggested the improvement of soil structure and the regulation of the make-up of plant community to explain the mechanisms attributed to these changes.

## CONCLUSIONS

The results obtained from this study suggest that AMF+rhizobia, *Metarhizium* and/or AMF+rhizobia+*Metarhizium* have potential in increasing nutrients accumulation in cowpea shoot+roots and in harvested seeds in various agro ecological zones of Cameroon. Further research are needed to explore the mechanisms by which *Metarhizium anisopliae* contributes to the increment of nutrient uptake in cowpea tissues.

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