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Full Length Research Paper

Effect of mycorrhizal inoculum and urea fertilizer on diseases development and yield of groundnut crops (*Arachis hypogaea* L.)

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Diseases (rosette virus disease (RVD), *Cercospora* leaf spot (CLS) and poverty of soils in nutrients are the main causes of groundnut losses (>60%). Among the methods applied in disease management, the biological method is identified as a priority in crop protection research programs. This study was carried out during the 2001 growing season in Yaoundé, Cameroon. The aim was to characterize the development of RVD and CLS on A-26 groundnut variety and also to evaluate yield after mycorrhizal inoculation. A randomized block design with four replicates was used, with two applications of mycorrhizal inoculum. The inoculum contained spores of *Glomus* sp. and *Gigaspora* sp. in concentration of 2.10^3 spores.g^{-1}. A urea treatment and an absolute control were also used. The number of nodules per plant, the root colonization rate (RCR), disease severity and yield were assessed. Results show that RCR was very low in control and urea plots compared to mycorrhizal inoculated plots. Mycorrhizal applications reduced disease infection up to 38.8 and 54.4% respectively, for RVD and CLS. However, plants issued from urea treatment were more stressed and infected. A higher number of nodules (459.1) were recorded on roots of mycorrhizal inoculated plants compared to control and urea treatments which were 218.8 and 237.5 nodules per plant. Mycorrhization of groundnut plants led to a yield increased of up to 628% for dry pods of healthy plants compared to uninoculated plants in control samples. No yield was recorded on infected plants from control and urea treatments, whereas plants infected by RVD and inoculated with mycorrhiza yielded 177.2 Kg.ha^{-1} of dry pods. The mains results of this study show that mycorrhizal symbiosis with groundnut roots increased the resistance of plants to RVD and CLS, and positively influenced the physiology of groundnut plants infected by RVD.

Key words: *Arachis hypogaea*, mycorrhization, disease development, biological control, yield increase.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a leguminous crop which is grown in all tropical and subtropical countries, up to 40˚ N and S. of the equator (Westphal et al., 1985). Seeds are produced underground in pods and they are rich in oil (38 - 60%), protein, and vitamins B and C (Rakipov, 1987). In Cameroon, groundnut production is very low. The yield of groundnut seeds in 1999 was around 0.85 t/ha compared to word's yield (1.05 t/ha) (FAO, 2000). Diseases and poor soils are considered to be the main causes of losses in the groundnut production. Rosette virus disease (RVD) and *Cercospora* leaf spots (CLS) are the major worldwide diseases that infect groundnut plants. In Cameroon, up to 53% losses have been estimated (Fontem et al., 1996). CLS are caused by *Cercospora arachidicola* Hori (early leaf spot) and *Cercosporidium personatum* (Berk and Curt.) Deighton (late leaf spot). Depending of the moment of contamination during the growing season, groundnut plants infected by RVD do not produce pods and consequently, do not give any harvest (Uzunov, 1988; Savary, 1991). Management against phytoviruses is very difficult because viral infection can be transmitted through seeds and also through some insect vectors (*Aphis* sp.).

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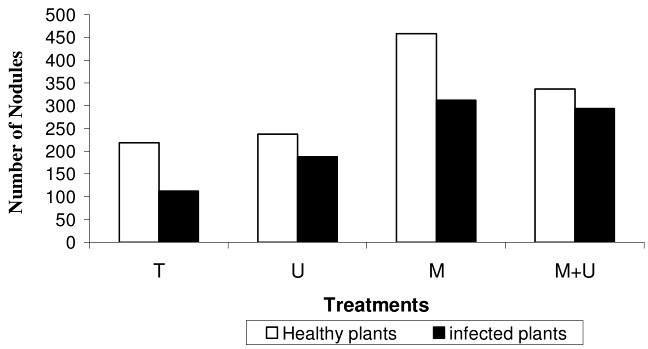

Figure 1. Effect of mycorrhizae and urea fertilizer on nodulation of groundnut crops 7 weeks after inoculation. T = Control; U = Urea fertilizer; M = Mycorrhizal inoculum; M + U = combined treatment.

One of the greatest needs in this regard is how the plants infected by RVD can be grown right up to harvest, that is, to the end of their vegetative cycle. Strullu (1991) showed that the symbiosis between mycorrhizae and roots of many crops has a positive influence in the plant's nutrition and in protection against some diseases. Previous research showed that mycorrhizal symbiosis can ameliorate phosphorus up take and can participate in the formation of pods in some leguminous plants. The main objective of this study was therefore to determine the effect of mycorrhizal inoculation on the development of diseases (RVD and CLS) and on the physiology of groundnut plants infected by RVD.

MATERIAL AND METHODS

Climatic conditions

The study was carried out in the experimental station of the department of Plant Biology, University of Yaoundé I (Cameroon), during the 2001 growing season. The experimental site is characterized by an average rainfall of 1900 - 2000 mm per annum, with a bimodal distribution. Annual temperatures range between 21 and 29°C. The soil is classified as the ferruginous tropical soil. According to the description of the physical properties of the soil by IRAD (Institut de Recherche Agricole pour le Développement), it is a well drained sandy loam soil with a clay fraction, pH = 5.52-6.0, total N = 0.038 %, total P = 60-5 µg g^{-1}.

Experimental design and cultural details

Improved, sensitive *A. hypogaea* seeds (A-26) were used. The land was cleared manually and tilled before the experiment was laid out in four treatments with four replicates. Treatments were randomized in each of four replicates. The treatments consisted of: T (no application), U (urea), M (mycorrhizal inoculum), M + U (mycorrhizal inoculum + urea). The groundnut seeds were planted manually at a spacing of 30 × 30 cm thereby given 111,110.00 plants ha^{-1}. Just after planting 1.25 g of urea was applied around each plant. The mycorrhizal inoculum called *Myco 4* is a substratum produced locally by UMAB (Unité de Microbiologie Appliquée et Biofertilisant) of the Biotechnology Center of the University of Yaoundé I (Cameroon). *Myco 4* contains *Glomus* sp. and *Gigaspora* sp. spores in concentration of 2.10^3 spores.g^{-1} of substratum. At the moment of planting, 10 g of *Myco 4* were applied per plant and three weeks after, when plants had developed three or four leaves, 2 kg of *Myco* 4 in 10 L of sterile water were sprinkled around and on the crops.

Measurements and statistical analysis

The parameters measured were disease incidence, severity of RVD and CLS, number of nodules, root colonization by mycorrhizal fungus and groundnut dry pod yields. The disease symptoms appeared in natural conditions. Assessment of disease infection was done by visual examination of groundnut crops using ordinary phytopathological methods according to Tchumakov and Zaharova (1990). RVD and CLS severity were evaluated at four weeks intervals on randomized selected plants in the central lines of each plot. First examination was done 30 days after seed planting (before flowering). Plants infected by RVD before flowering, were particularly observed from the beginning of the infection (first symptoms) to the end of their growth. The average number of these plants was determined at the moment of harvest. The nodule quantity was evaluated seven weeks after seed planting by counting total number of root nodules. To evaluate the nodule number, five infected and five healthy plants were picked from each treatment on the meddle lines. These plants were cleaned with water, after that, their roots were air-dried on filter paper. Nodule counting was done using magnifying lens. After that, roots were cut into pieces (about 1 cm in length), and used to determine mycorrhizal root colonization according to Kormanick and McGraw (1982) method. Plant yield was evaluated at the end of its growth by counting the number of mature pods and measuring their dry weight. After harvest, fresh pods were dried using solar radiations for one week. The collected data were subjected to analysis of variance using the X^2 and the Student-Fisher test for significance.

RESULTS

Nodulation of groundnut crop

Observations during 7 weeks after seeds planting and mycorrhizal inoculation, showed different variations of nodule quantity on groundnut roots depending on treatments (Figure 1). On healthy plants, the highest number of nodules was recorded in the treatment with mycorrhizal inoculation where up to 459.1 nodules per plant was obtained, followed by combined treatment using mycorrhiza and urea simultaneously (337.3 nodules per plant). However, fertilization with urea showed no significant effect on nodulation compared to control where in both treatments 237.5 and 218.8 nodules per plant were recorded, respectively. Meanwhile, crops infected by RVD showed a lesser number of nodules per plant compared to healthy crops. But a higher number of nodules (312.5) was also obtained on roots of RVD infected crops inoculated with mycorrhiza (Figure 1).

Mycorrhizal status of groundnut crops

Data obtained in this experiment showed that soil of experimental site had a lower level of mycorrhizal content, since control treatments gave a mycorrhizal root colonization (MRC) rating from 4.5 to 4.0% according to observations done respectively in flowering and ripening

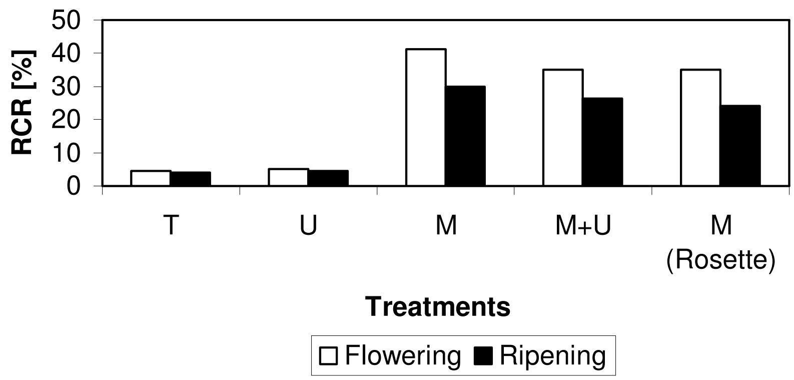

Figure 2. Mycorrhizae root colonization of groundnut crops in flowering and ripening growing phase after mycorrhizal inoculation. RCR- Root colonization rate; T = Control; U = Urea; M = Mycorrhizal inoculum; M+U = Combined treatment; M (Rosette) = Mycorrhizal inoculated groundnut crops infected by rosette virus disease

Table 1. Effect of mycorrhizal inoculum and urea on development of rosette virus disease and *Cercospora* leaf spot on groundnut plants 9 weeks after seeds planting.

Treatment	Rosette virus disease		*Cercospora* leaf spot	
	Severity (%)	Reduction or Increase (%)	Severity (%)	Reduction or Increase (%)
Control	34.3 d	-	33.8 b	-
Urea (U)	42.5 a	23.9**	38.2 a	13.0**
Myco 4 (M)	21.0 c	38.8*	15.4 c	54.4*
M + U	28.2 c	17.8*	11.4 d	66,3*

*Reduction (%) of disease compared to the control, ** Increase (%) of disease compared to the control.
Means followed by different letters along the columns are significantly different at 5 % as per Student-Fisher's test.

phases of groundnut growth respectively (Figure 2). Plants from urea treatment showed lesser increase in MRC compared to the control, but difference was not significant (P = 0.96). The MRC was efficient on healthy plants inoculated with myccorhiza where a colonization rate of 41.25 - 30.0% was recorded, showing an increase up to 9.2 times higher at the flowering phase and 7.5 times higher at the ripening phase than in control. The MRC was much on plants with combined treatment (M + U), but less compared to (M) treatment (Figure 2). The MRC was also efficient on infected plants which were inoculated with mycorrhiza.

Development of rosette virus disease (RVD) and Cercospora leaf spots (CLS)

Plants inoculated with urea fertilizer were more stressed and more sensitive to viral and fungal diseases compared to control treatment (Table 1). Disease severity increased in urea treatment up to 23.9 for RVD and 13.0% CLS as compared to control. Mycorrhizal inoculation had greater effect on development of both diseases studied. The use of mycorrhizal inoculum reduced RVD up to 38.8%, and CLS up to 54.4%. The simultaneous use of mycorrhizal inoculum and urea fertilizer (Table 1) also showed a great effect in disease reduction compared to control samples.

Groundnut yield

In healthy urea fertilized plants, there was an increase in dry pod yield of 33% compared to control (Table 2). Uninfected mycorrhizal inoculated plants gave 3178.12 kg.ha^{-1} of dry pods while a yield increase of 628% was recorded compared to yield obtained in control. There was an increase in groundnut yield of 565% for plants resulting from combined treatment (M + U) compared to control. The most important observation in this experiment is that plants infected by RVD and inoculated with mycorrhiza gave a significant yield of 177.21 kg.ha^{-1} of mature dry pods compared to infected plants in control and urea treatments, where there was no yield (Table 2).

DISCUSSION

The positive reaction of groundnuts to mycorrhizal inoculation shows that natural mycorrhization is greatly reduced in the experimental zone whose soil is probably

Table 2. Evaluation of dry yield of groundnut pods after mycorrhizal and urea applications.

Treatment	Healthy crops		Yield of rosette infected plants
	Yield (Kg.ha^{-1})	% of yield increase*	
Control (T)	436.07 a	-	0.00 a
Urea (U)	582.22 b	33.51	0.00 a
Myco 4 (M)	3178.12 c	628.81	177.21 b
M + U	2901.95 cd	565.48	49.04 c

Means followed by different letters along the columns are significantly different at 5 % as per Student-Fisher's test.
*Yield Increase (%) was determined between yield in each treatment and control.

lacks easy accessibility to phosphorus (Habite and Manjunath, 1987). Results obtained in this work concerning nodulation of healthy plants in control treatments are lower than those obtained by Forestier (1976), probable due to soil phosphorus deficiency. In similar works, it has been shown that low level of soil phosphorus reduces nodulation (Nwaga et al., 1998; Caron et al., 1986; Davis et al., 1979). Our results show that introduction of mycorrhizae to the soil enhances nodulation of groundnuts and this is in agreement with results obtained by Betsama (1999). Moreover, a positive correlation exists between nodulation and pod yield; highest yields are observed on plants inoculated with mycorrhiza (Perrin, 1991; Daft and El-Giahmi, 1976).

Results obtained after analysis of diseases show that infection develops less in plots inoculated with mycorrhiza. This leads us to the thought that symbiosis between mycorrhizal fungi and the plants results in the plants acquireing resistance against diseases, mainly CLS especially when infection takes place in the soil (Unestam et al., 1987; Caron et al., 1986; Ross, 1972). The considerable reduction of the severity of CLS and RVD on groundnut plants inoculated with mycorrhiza confirms observations showing the influence of mycorrhiza in plant protection against diseases (Ngonkeu and Nwaga, 1999). Symbiotic association is thus, a biological method of protection against pathogens especially of the soil (Schoenbeck and Dehne, 1981). It is worth noting that mycorrhizal association does not generally ensure total plant protection, but contributes to a net reduction of the gravity of damages (Marais and Kotze, 1976). On the other hand, urea fertilization greatly weakened groundnut plants which presented high disease sensitivity compared to plants from mycorrhizal and control treatments. This should be due to the presence of high amounts of nitrogen which increase plant sensitivity to diseases (Hatcher and Ayres, 1998).

Data obtained on the yield of groundnut dry pods show an increase in yield per hectare of about 628% for mycorrhized plants compared to control, confirming the results of Betsama (1999), who found in similar works that cowpea and peanut yield increases by 20 to 200% compared to control. Simultaneous use of mycorrhizae and urea led to a yield reduction in comparison to the use of mycorrhizae only. This should be due to the high concentration of nitrogen which might have reduced the synthesis of carbohydrates that very much participate in the formation of harvest products (Mooney et al., 1995). Plants inoculated with mycorrhizal substratum produced a dry pod yield 7.3 times greater than that obtained in control, due to the capacity of mycorrhizal fungi to explore the soil and ease accessibility and assimilation of soil nutrients (phosphorus and others) by plant roots (Ross and Gilliam, 1973).

A physiological study on groundnut plants infected by RVD revealed very interesting results. Fauquet and Thouvenil (1980) felt that, if rosette infection appeared in groundnut plants before the 40th day after planting (before flowering), losses in yield would be 100%. In fact, previous works show that early rosette plant infection leads to no pod production, resulting in no yield, even if the plants develop to maturity. In this work, results obtained show that mycorrhized groundnut plants which were infected by RVD before flowering, produced pods which developed to maturity. Our results also show that the increase in the number of mycorrhizal fungi by artificial inoculation in soils on which groundnut is cultivated has a positive effect on the physiology of groundnut plants infected by RVD. This can be explained by the important role played by mycorrhizae on the metabolism of the infected groundnut plant and in supplying it with nutrients (Ross and Harper, 1970; Plenchette, 1991). A detailed study of this phenomenon is necessary in future research. The mycorrhizal inoculum used in this work shows antifungal and fertilizing characteristics of mycorrhizal fungi (*Glomus* sp. and *Gigaspora* sp.) which can serve in biological control of diseases and also as biofertilizer in groundnut production.

REFERENCES

Betsama M (1999). Potentiel des bio-fertilisants rhizobiens et mycorhiziens pour la production de l'arachide (*Arachis hypogaea*) et du niébé (*Vigna unguiculata*) à Yaoundé. Mémoire de DIPES II, Université de Yaoundé I, Yaoundé, Cameroun.
Caron M, Fortin A, Richard C (1986). Effect of phosphorus concentration and *Glomus intraradices* on *Fusarium* crown and root rot of tomatoes. Phytopathol. 76: 942-946.
Daft MJ, El-Giahmi AA (1976). Studies on nodulated and mycorrhizal peanuts. Ann. Appl. Biol. 83: 273-276.

Davis RM, Menge JA, Erwin DC (1979). Influence of *Glomus fasciculatus* and soil phosphorus on *Verticillium wilt* of cotton. Phytopathol. 69: 453-456.

FAO (2000). Rapport technique sur les rendements des cultures en Afrique au Sud du Sahara. Rome, Italie.

Fauquet C, Thouvenil P (1980). Les maladies virales des plantes cultivées en Côte-d'Ivoire. Acad. Sc., Paris, France.

Fontem DA, Iroume RN, Aloleko F (1996). Effet de la résistance variétale et des traitements fongicides sur la cercosporiose de l'arachide. Cahier Agric. 5: 33-38.

Forestier J (1976). Optimisation des conditions de production de l'arachide. Cahiers ORSTOM, Paris, France.

Habite M, Manjunath A (1987). Soil solution, phosphorus status and mycorrhizal dependency in *Leucaena leucocephala*. Appl. Environ. Microbiol. 53: 797-801.

Hatcher PE, Ayres PG (1998). The effect of fungal infection and nitrogen fertilization on the carbohydrate composition of *Rumex obtusifolius* leaves. Eur. J. Plant Pathol. 104: 553-559.

Kormanick PP, MC-Graw AC (1982). Quantification of vesicular-arbuscular mycorrhizae in plants root. In: Schenck NC (eds), Methods and principles of mycorrhizae research. The Amer. Phytopathol. Soc., St Paul Minnesota, USA, pp. 59-93.

Marais LJ, Kotze PM (1976). Ectomycorrhizae of *Pinus patula* as biological deterrents to *Phytophthora cinnamomi*. S. Afr. For. J. 99: 35-39.

Mooney HA, Schulze ED, Fichtner K (1995). Growth, photosynthesis and storage of carbohydrates and nitrogen in *Phaseolus lunatus* in relation to resource availability. Oecologia. 104: 17-23.

Ngonkeu ME, Nwaga D (1999). Production végétale durable après inoculation par les bio-fertilisants mycorhiziens. In : Mbofung C.M.F. (ed). Comité Camerounais de Biosciences. Biosc. Proc, Yaoundé, pp. 193-202.

Nwaga D, Ambassa Kiki R, Nsangou F (1999). Réponse du niébé à l'inoculation par les rhizobia et les mycorhizes en champ sur sol ferrallitique. In : Mbofung CMF (ed). Comité Camerounais de Biosciences. Biosc. Proc., Yaoundé, pp. 186-198.

Perrin R (1991). Mycorhizes et protection phytosanitaire. In : Strullu DG (ed), Les mycorhizes des arbres et plantes cultivées. Tech. et Documentation, Lavoisier, Paris, pp. 93-126.

Plenchette C (1991). Utilisation des mycorhizes en agriculture et horticulture. In : Strullu DG (Ed), Les mycorhizes des arbres et plantes cultivées. Tech. et Documentation, Lavoisier, Paris, pp. 131-180.

Rakipov N (1987). Biochimie des cultures tropicales. Editions MIR, Moscou, URSS.

Ross JP (1972). Influence of *Endogone* mycorrhizae on *Phytophthora* root rot of soybean. Phytopathol. 62: 896-897.

Ross JP, Gilliam JW (1973). Effect of *Endogone* mycorrhizae on phosphorus uptake by soybeans from inorganic phosphates. Soil Sci. Soc. Am. Proc. 37: 237-239.

Ross JP, Harper JA (1970). Effect of *Endogone* mycorrhiza on soybean yields. Phytopathology, 60: 1552-1556.

Savary S (1991). Approche de la pathologie des cultures tropicales: l'exemple de l'arachide en Afrique de l'Ouest. KARTHALA-ORSTOM, Paris, France.

Schoenbeck F, Dehne HW (1981). Mycorrhiza and plant health. Gesunde Pflanz. 33: 186-190. Strullu DG (1991). Vers un modèle unifié de fonctionnement des symbioses mycorhiziennes. Physiol. Vég. 35: 655-672.

Tchumakov AE, Zaharova TI (1990). Elucidation and statistical analysis of disease development. In : Plant disease damages. Agroprom-Izdat, Moscow, pp. 5-60.

Unestam T, Chakravarty P, Damm E (1987). Mycorrhizal protection of pine roots against pathogens. Proceedings of Workshop, 7th NACOM, pp. 267-268.

Uzunov IS (1988). Tropical phytopathology. RUDN-Izdat, Moscow.

Westphal E, Embreets J, Ferwerda JD, Van Gibmeesus HAE, Mutsaers HJW (1985). Cultures vivrières tropicales avec référence spéciale au Cameroun. Pudoc, Wageningen.