Journal of Biological Control, 24 (1): 51–57, 2010

Research Article

Influence of application methods of arbuscular mycorrhiza *Glomus mosseae* in the bio-management of root knot nematode, *Meloidogyne incognita* on black gram (*Vigna mungo* L.) Hepper

C. SANKARANARAYANAN[1*] and RAJESWARI SUNDARABABU[2]

[1]*Division of Crop Protection, Sugarcane Breeding Institute, Coimbatore 641007, Tamil Nadu, India.*
[2]*Department of Nematology, Tamil Nadu Agricultural University, Coimbatore 641003, Tamil Nadu, India.*
**Corresponding author E-mail*: csankar@lycos.com

ABSTRACT: Glasshouse and micro-plot experiments were conducted to find out the influence of different application methods of arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* such as seed pelleting, AMF mixed with soil and as a layer under seed, AMF soil pelleting, and direct application of spores against root-knot nematode *Meloidogyne incognita* on black gram (*Vigna mungo*). In both experiments, application of *G. mosseae* to black gram plants suppressed *M. incognita* soil population by up to 14–49% under pot culture and 35–46% reduction over nematode alone treatments. Variations among different application methods of AMF were observed in terms of plant growth and suppression of the nematode population. Among the methods tested, AMF mixed with soil recorded least gall index (2.8 and 2.2 in pot culture and micro-plot, respectively) and nematode population, (290 and 280 / 200g soil in pot and micro plot condition, respectively).

KEY WORDS: Arbuscular mycorrhizal fungi, biological management, inoculation methods, *Meloidogyne incognita*, *Vigna mungo*

(Article chronicle – Received: 24.07.2009; Sent for revision: 21.10.2009; Accepted: 24.11.2009)

INTRODUCTION

The root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, is one of the important pests of black gram (*Vigna mungo* L.). Earlier investigations suggested that plant-parasitic nematodes including *Meloidogyne* spp. adversely affect nodulation, N_2 fixation and yield in legumes (Hussaini and Seshadri, 1975). Arbuscular mycorrhiza fungus (AMF) occurs profusely in agriculture crops (Smith and Read 1997). The plant-parasitic nematodes and AMF commonly occur together in the roots or rhizosphere of the same plant, each having a characteristic but opposite effect on plant growth. Among the various kinds of organisms engaged in biological control of nematodes, mycorrhizal fungi especially AMF is attracting significant attention as a potential biocontrol agent. The interaction between AMF and nematodes studied by several workers, demonstrated reduction in nematode population (John and Bai, 2004; Kantharaju *et al.,* 2005; Sankaranarayanan and Sundarababu, 1994; Siddiqui and Akhtar, 2007). The AMF inocula is prepared in pots or micro-plots under controlled conditions. Such inocula containing spores and infected root fragments can be inoculated in the soil at the time of planting. Jacson *et al*. (1972); Crush and Pattison (1975); Menge *et al*., (1977) and Hall (1979) inoculated soil with AMF to enhance growth of crop plants. In a pot experiment, Hall (1979) showed that infested soil pellets were a satisfactory method of introducing AMF into steamed soil. Hence, an attempt was made to find out a suitable application method for introduction of AMF, *Glomus mosseae* (Nicol and Gerd.) Gerd and Trappe for effective management of root knot nematode, *M. incognita* on black gram.

MATERIALS AND METHODS

Cultivar selection and pot mixture preparation

The black gram cultivar Co 5 was sown @ two seeds / pot, one was removed after germination. The earthen pots (Ø 20 cm, volume 2 kg) were filled with sterilized (autoclaved at 121°C and 1.5 atm for 2 h) red soil: sand: Farm Yard Manure @ 2: 2: 1 v/v. The soil characteristics were: sandy loam, pH 7.8, electrical conductivity 0.5 dS / m and available N 74 kg acre^{-1}, available P 4 kg acre^{-1} and available K 259 kg acre^{-1}.

Nematode culture

The inoculum required for raising pure culture of root-knot nematode *Meloidogyne incognita* was obtained

from tomato plants maintained in the glass house of Tamil Nadu Agricultural University, Coimbatore. Roots with conspicuous galls were washed gently and thoroughly with water and examined for the presence of egg masses at 50x under the microscope. Galls showing protruding bodies of mature females covered with gelatinous matrix were dissected and the egg masses were kept individually in embryo cups, which were half filled with water. Egg masses collected were utilized for raising pure cultures. Perennial patterns of the *M. incognita* females were prepared for species confirmation. Tomato *cv*. Co3 seedlings were raised in two kg capacity earthen pots containing autoclaved pot mixture (red soil: sand: FYM @ 2: 2: 1 v/v). Emerging larvae from egg-mass were inoculated in the soil at the base of the tomato seedlings through 0.5cm hole. The pots were maintained at the glass house and regularly irrigated with tap-water. The plants were uprooted gently 45 days after nematode inoculation, carefully washed in water and examined for well developed egg masses. Egg masses were placed in Petri dishes containing adequate water, which were then incubated at room temperature. The larvae hatching out of these egg masses were used for the present study.

AMF inoculum

Arbuscular mycorrhizal fungus *G. mosseae* maintained in the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore was used for present experiments. The stock culture, consisted of living AMF spores, root tissues of previous host and soil, was transferred to earthen pots containing red soil, sand and FYM @ 2: 2: 1. Pearl millet *cv.* WCC 75 seeds were sown and thinned down to eight plants / pots after germination. Number of spores and mycorrhizal colonization was recorded 60 days after planting. Each application consisted of 20g of AMF containing spores, mycelium root along with the soil.

Seed pelleting

Seed pelleting was done as described by Hattingh and Gerdemann (1975). The mycorrhizal inoculum, extracted by wet sieving and decanting methods were collected on 100, 200 and 325 mesh sieves. In addition, roots retained on 20 mesh sieve are fragmented and added to the sieving. Five ml of 1% aqueous solution (w/v) of carboxy methyl cellulose (CMC) was mixed with 35ml of mycorrhizal sieving. This material was poured over blackgram seeds for thorough mixing. The seeds were dried until it could be handled without loss of inoculum.

AMF mixed with soil and as a layer under seed

The AMF inoculum @ 20g / pot was mixed thoroughly with pot mixture before sowing. Top soil was removed to a depth of 5 cm and AMF inoculum was placed between the layers. The soil removed was replaced in the pots thereafter.

AMF soil pelleting and mixing with seed

Soil pellets were prepared (Hall 1979) and mycorrhizal inoculum containing spores, hyphae and soil were mixed with distilled water, until the mixture was malleable and rolled in to pellets. About 20 g of such pellets were placed 2.5 cm below the black gram seeds while sowing. The black gram seeds were hand rolled in slurry of mycorrhizal inoculum and air dried before sowing.

Direct application of spores

The AMF spores extracted by wet sieving and decanting methods were directly inoculated @ 500 spores / pot into the germinated black gram root zone by making small holes in the soil around the plant stem and then covered with sterilized soil (autoclaved at 121°C and 1.5 atm for 2h).

Pot culture experiment

To find out the effects of application methods of *G. mosseae* in *M. incognita* management pot experiments were conducted with the following treatments 1) AMF Seed pelleting, 2) AMF mixed with soil, 3) AMF layer under seed, 4) AMF mixed with seed, 5) AMF soil pelleting, 6) direct application of AMF spores, 7) nematode alone control, and 8) uninoculated control. Three replications were maintained and kept in a randomized design. The earthen pots (Ø 20 cm, volume 2 kg) were sown with black gram *cv*. Co5 @ two seeds / pot. The AMF application was done as per the treatment. After germination the plants were thinned to one seedling / pot. Ten days after germination, second stage juveniles of *M. incognita* were inoculated @ 2000 juveniles / pot at the root zone by making small holes in the soil around the plant stem. The plants were supplemented weekly with Hoagland's nutrient solution (Hoagland and Arnon, 1950) lacking phosphorus. For the confirmation of the results, the whole experiment was repeated with same set of treatments and replications and the data were pooled for statistical analysis.

Micro-plot study

Effect of AMF *G. mosseae* application on *M. incognita* was determined in micro plots (1m circular type) in open field condition. The plots were infested with *M. incognita* population (one nematode g^{-1} soil). The AMF application was done as per the treatments. The following treatments 1) AMF Seed pelleting, 2) AMF mixed with soil 3) AMF soil pelleting, 4) Direct application of AMF spores, 5) Nematode alone, 6) Uninoculated control and four replications were maintained in a complete randomized design. The AMF application was done as per the treatments. All the plots were sown with black gram *cv.* Co5 @ 10 seeds / plot and after germination the plants were thinned to five seedling / plot. For the confirmation of the results, the whole experiment was repeated with same set of treatments and replications and the data were pooled for statistical analysis.

Data on plant growth and yield parameters, nematode reproduction, gall index (Heald *et al*., 1989), AMF spore population, mycorrhizal colonization and total phosphorus content of plants were recorded 70 days after sowing. AMF root infection levels were assessed from randomly selected root material after cutting the entire root into 1cm pieces. Roots were cleared in KOH and stained in tryphan blue (Phillips and Hayman, 1970). Per cent root colonization was determined as observed by Giovannetti and Mosse (1960). The total P content in the plant material was estimated by vanadomolybdate in the nitric acid system (Jackson, 1975). Data on plant growth parameters, nematode reproduction and mycorrhizal colonization were analyzed by analysis of variance (ANOVA) when the conditions for ANOVA (*i.e.*, normal distribution and homogeneity of variances) were met. Experimental results were similar and the data were pooled for analysis. Means were compared using Fisher's protected least significant difference test (LSD) at P = 0.05.

RESULTS AND DISCUSSION

The effect of different inoculation methods of *G. mosseae* on growth parameters of nematode inoculated black gram under pot culture in glasshouse condition are presented in Table 1. In general, application of AMF significantly increased the growth of the black gram plants irrespective of the AMF inoculation methods. Among the treatments AMF mixed with soil recorded highest shoot and root length (36.7 cm and 36.8 cm, respectively) followed by AMF applied as layer under seed (34.9 and 35.3 cm, respectively) and were at par with each others. With regard to shoot weight, maximum shoot weight was recorded when AMF was mixed with soil followed by direct application of spores to black gram roots and were at par with each other. Maximum root weight of the plants was observed when AMF applied as layer under seed followed by AMF mixed with soil. AMF mixed with soil and direct application of AMF spores to the black gram plants gave higher pod number and pod yield (19.5 and 6.6 g, respectively). Root gall index and nematode population were greatest in plants treated with the nematode alone. Reduction of nematode population and gall index reported with application of AMF irrespective of the application methods (Table 2). Lowest gall index and nematode population was recorded when AMF was mixed with soil and the decrease was 44 per cent and 49.1 per cent, respectively over nematode alone treatment. Highest spore and mycorrhizal colonization in the plants was recorded while AMF was mixed with soil. Among the mycorrhizal treatments, AMF mixed with seed recorded high nematode and low mycorrhizal colonization.

Table 1. Effect of application methods of *G. mosseae* on growth of black gram inoculated with *M. incognita* in pot culture

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Pod yield (g)
AMF seed pelleting	33.6	23.2	34.2	10.3	5.2
AMF mixed with soil	36.7	25.8	36.8	12.4	6.6
AMF layer under seed	34.9	23.4	35.3	13.2	5.3
AMF mixed with seed	28.8	16.4	27.1	6.2	4.2
AMF soil pelleting	28.4	16.8	23.4	5.8	3.2
Direct application of AMF spores	32.4	25.3	35.3	11.5	6.5
Nematode alone	15.2	7.4	9.3	3.2	1.3
Uninoculated control	23.4	16.3	23.7	3.1	3.2
LSD (P = 0.05)	2.5	3.8	2.7	0.9	1.2

In case of phosphorus content in black gram plants, all the AMF inoculated plants recorded high shoot and root phosphorus content. Among the treatments, mixing of AMF inoculum with soil recorded highest per cent of shoot and root P content (0.57 and 0.38%, respectively).

Table 2. Effect of application methods of *G. mosseae* against *M. incognita* on black gram under pot culture

Treatments	Gall index*	Nematode population /200g soil	Spore population / 50g soil	Mycorrhizal colonization (%)	Total P content (%)	
					Shoot	Root
AMF seed pelleting	3.3	370	120.0	56.0	0.56	0.36
AMF mixed with soil	2.8	290	160.0	66.0	0.57	0.38
AMF layer under seed	2.9	330	140.0	60.0	0.56	0.30
AMF mixed with seed	3.5	490	80.0	43.0	0.41	0.30
AMF soil pelleting	3.6	450	110.0	49.0	0.38	0.30
Direct application of AMF spores	3.2	360	120.0	56.0	0.51	0.31
Nematode alone	5.0	570	0.0	0.0	0.19	0.17
Uninoculated control	0.0	0	0.0	0.0	0.28	0.18
LSD(P = 0.05)	0.69	20.1	8.5	3.1	0.04	0.03

*Gall index 1-5 scale (1-no gall; 2- 1-25% galls; 3- 26-50% galls; 4- 51-75% galls and 5- >75% galls, Heald *et al*., 1989)

Table 3. Effect of application methods of *G. mosseae* on growth of black gram inoculated with *M. incognita* under micro plot

Treatments	Shoot length (cm)	Shoot weight (g)	Root Weight (g)	Pod yield (g)
AMF seed pelleting	32.4	20.6	9.8	4.2
AMF mixed with soil	35.6	22.6	11.3	6.1
AMF soil pelleting	33.4	21.4	12.4	5.2
Direct application of AMF spores	29.8	19.8	9.8	3.2
Nematode alone	14.8	7.1	3.1	1.5
Uninoculated control	22.6	12.3	5.1	3.2
LSD (P = 0.05)	1.9	0.6	1.2	0.45

The result of effect of different inoculation methods of AMF against *M. incognita* under micro plot condition on growth of black gram is presented in Table 3. Application of AMF had generally increased the growth of black gram plants as in pot culture experiments. Among the six treatments, the maximum shoot length and shoot weight was recorded (35.6 cm and 22.6 g, respectively) when AMF was mixed with soil and was significantly superior over other treatments. In case of root character, plants inoculated with AMF as layer under seed recorded higher (12.4 g). The maximum pod yield (6.1 g) was recorded in AMF mixed with soil treatment followed by AMF as layer under seed. These two methods of inoculation were significantly superior over seed pelleting and direct application of spores to the blackgram plants. In general under micro plot condition, inoculation of AMF in different methods reduced the gall index and nematode population (Table 4). Among the methods, mixing of AMF inoculum with soil had least gall index and nematode population (2.2 and 280, respectively). AMF inoculum mixed with soil treatment favoured the development of spores and mycorrhizal colonization (140 spores and 60%, respectively). Seed pelleting of black gram seeds with AMF inoculum recorded low spore count and mycorrhizal colonization (110 and 45%, respectively). Inoculation of AMF to black gram plants recorded high shoot and root phosphors content than nematodes alone treated plants. AMF mixed with soil treatment had a high shoot and root phosphorus content (0.51 and 0.36%, respectively).

Plants inoculated with nematodes alone were stunted in growth, with low shoot and root weight, while plants inoculated with mycorrhiza irrespective of inoculation methods showed the opposite trend. All the dual inoculated plants (AMF and nematode) were superior to nematode and uninoculated control treatment in improving biomass. The increased biomass due to AMFungi has already been documented several workers (Elliiott *et al*., 1984; Sankaranarayanan and Rajeswari Sundarababu, 1994).

In the present investigation, lot of variations among different inoculation methods of AMF was observed in terms of plant growth and nematode suppression. Inoculation of AMF mixed with soil, AMF layer under seed and direct inoculation of AMF spores to the roots gave better results in improving plant growth than the other inoculation methods. The simple reason is that, placing AMF inoculum in the root proliferation zone (5-13 cm below the seed) which covers the entire root system with mycorrhizal inoculum resulted in more infection point, creating a more uniform and complete infection of the root. Ross and Harper (1970) also reported the similar type of findings in soybean. Placing AMF inoculum as layer also gave better results in terms of improving plant growth parameters and yield and placing AMF inoculum as layers has been effectively used for soybean (Ross and Harper, 1970), white clover (Powell, 1976) and on barley (Clarke and Mosse, 1981). Application of AMF by any method tested here had reduced the nematode population compared to nematode alone treatment. In our earlier studies we found that reduction of *M. incognita* population observed in black gram with AM Fungi (Sankaranarayanan and Rajeswari Sundarababu, 1994 and 2000). Such a reduction of nematode population and gall index resulted in increased growth of the plants. Such a depression of nematode population and gall numbers reflect in enhancing the plant height and root length as observed in the current study. The dual inoculation of AM fungus and nematode has reduced the disease incidence than nematode alone treatment. This reduction in the severity of disease caused by *M. incognita* in mycorrhizal plants might be due to the altered biochemical constituents in the host plant (Sikora and Schonbeck, 1975; Siddiqui *et al*., 1999) or improved plant nutrition especially phosphorus (Hussey and Roncadori, 1982) or alteration of compounds of root exudates or alteration of the physiological components of AMF root due to increased lignin levels in the exodermis of mycorrhizal plants (Dehne and Schonbeck, 1975). Presence of increased quantities of sugars, amino acids, like phenylalanine and serine and phosphorus may each or collectively play a role in suppressing the development of *M. incognita* in mycorrhizal plants (Krishnaprasad, 1971). The same kind of mechanism might had operated to reduce the nematode population in our study. Maximum spore, mycorrhizal colonization, shoot and root phosphorus content was recorded in plants where AMF inoculum was mixed with soil followed by placing AMF in layer under seed. This might be due to the complete and uniform infection of roots by AMF inoculum in the root proliferation zone, covering entire root system with inoculum resulted in more infection point and thus creating a more infection of the root (Timmer and Layden, 1978).

Table 4. Effect of application methods of *G. mosseae* against *M. incognita* on black gram under micro plot

Treatments	Gall index*	Nematode population / 200g soil	Spore population / 50g soil	Mycorrhizal colonization (%)	Total P content (%)	
					Shoot	Root
AMF seed pelleting	3.2	340	110.0	45.0	0.48	0.31
AMF mixed with soil	2.2	280	140.0	60.0	0.51	0.36
AMF layer under seed	2.5	310	130.0	50.0	0.50	0.33
Direct application of AMF spores	3.2	320	120.0	45.0	0.42	0.29
Nematode alone	5.0	520	0.0	0.0	0.21	0.18
Uninoculated control	0.0	0	0.0	0.0	0.31	0.21
LSD (P = 0.05)	0.50	16.8	5.6	2.5	0.02	0.01

*Gall index 1-5 scale (1 - no gall; 2 - 1-25% galls; 3 - 26-50% galls; 4 - 51-75% galls and 5- >75% galls, Heald *et al.* 1989)

ACKNOWLEDGEMENT

The senior author is grateful to Council of Scientific and Industrial Research (CSIR), New Delhi, India for the financial assistance in the form of Senior Research Fellowship.

REFERENCES

Clarke, C. and Mosse, B. 1981. Plant growth response to vesicular arbuscular mycorrhiza XII. Field inoculation responses of barley at two soil P levels. *New Phytologist,* **87**: 695-703.

Crush, J. R. and Pattison, A. C. 1975. Preliminary results on the production of vesicular arbuscular mycorrhizal inoculum by freeze drying, pp. 485-493. In: Sanders, F. E., Mosse, B. and Tinker, P. B. (Eds.). *Endomycorrhizas*, Academic Press, London, UK.

Dehne, H. W. and Schonbeck, F. 1975. Untersuchungen Uber den Einfliss der entotropen mycorrhizal auf die Fusarium welke der tomato. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, **82**: 630-632.

Elliiott, A. P., Bird, G. W. and Safir, G. B. 1984. Joint influence of *Pratylenchus penetrans* and *Glomus fasciculatum* on the ontogeny of *Phaseolus vulgaris*. *Nematropica*, **14**: 111-119.

Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist,* **84**: 489-500.

Hall, I. R. 1979. Soil pellets to introduce vesicular arbuscular mycorrhizal fungi into soil. *Soil Biology and Bio-chemistry*, **11**: 85-86.

Hattingh, M. J. and Gerdemann, J. W. 1975. Inoculation of Brazilian sour orange seed with an endomycorrhizal fungus. *Phytopathology*, **65**: 1013-1016.

Heald, C. M., Bruton, B. D. and Davis, R. M. 1989. Influence of *Glomus intraradices* and soil phosphorus on *Meloidogyne incognita* infecting *Cucumis melo*. *Journal of Nematology,* **21**: 69-73.

Hoagland, D, Arnon D. I., 1950. The water culture method for growing plants without soil. *California Agricultural Experimental Station Circular,* 347.

Hussaini, S. S. and Seshadri, A. R. 1975. Interactionships between *Meloidogyne incognita* and *Rhizobium* spp. on mungbean. *Indian Journal Nematology*, **5**: 189-199.

Hussey, R. S. and Roncadori, R. W. 1982. Influence of *Aphelenchus avenae* on vesicular arbuscular mycorrhizal growth response of cotton. *Journal of Nematology*, **13**: 48-52.

Jackson, K. L. 1973. *Soil chemical analysis*. Prentice Hall of India Pvt. Ltd., New Delhi.

Jackson, N. E., Frankalin, R. E. and Miller, R. H. 1972. Effects of vesicular arbuscular mycorrhizae on growth and phosphorus content of three agronomic crops. *Soil Science Society of America Journal*, **36**: 64-67.

John, A. and Bai, H. 2004. Evaluation of VAM for management of root knot nematodes in brinjal. *Indian Journal of Nematology*, **34**: 22-25.

Kantharaju,V., Krishnappa, K., Ravichandra, N. G. and Karuna, K. 2005. Management of root-knot nematode, *Meloi-dogyne incognita*, on tomato by using indigenous isolates of AM fungus, *Glomus fasciculatum*. *Indian Journal of Nematology*, **35**: 32-36.

Krishnaprasad, K. S. 1971. Effect of amino acid and plant growth substances on tomato and its root-knot nematode *Meloidogyne incognita*. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Bangalore, 143pp.

Menge, J. A., Lambright, H. and Johnson, E. L. V. 1977. Utilization of mycorrhizal fungi in citrus nurseries. *Proceedings of International Society for Citriculture*, **1**: 129-132.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedure for clearing root and obtaining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society,* **65**: 158-161.

Powell, C. L. 1976. Mycorrhizal fungi stimulate clover growth in New Zealand hill country soils. *Nature*, **264**: 436-438.

Ross, J. P. and Harper, J. A. 1970. Effect of endogone mycorrhiza on soybean yields. *Phytopathology,* **60**: 1552-1556.

Sankaranarayanan, C. and Rajeswari Sundarababu. 1994. Interaction of *Glomus fasciculatum* with *Meloidogyne incognita* inoculated at different timings on blackgram (*Vigna mungo*). *Nematologia Mediterranea,* **22**: 35-36.

Sankaranarayanan, C. and Rajeswari Sundarababu, 2000. Influence of moisture and pH on the biocontrol efficiency of VA-mycorrhiza *Glomus mosseae* against *Meloidogyne incognita* on black gram. *Journal of Biological Control*, **15**: 69-72.

Siddiqui, Z. A. and Akhtar, M. S. 2007. Effects of AM fungi and organic fertilizers on the reproduction of the nematode *Meloidogyne incognita* and on the growth and water loss of tomato. *Biology and Fertility of Soils*, **43**: 603–609.

Siddiqui, Z. A., Mahmood, I. and Khan, M. W. 1999. VAM fungi as prospective biocontrol agents for plant parasitic nematodes, pp. 47-58. In: *Modern approaches and innovations in soil management*. Rastogi, Meerut, India.

Sikora, R. A. and Schonbeck, F. 1975. Effect of vesicular arbuscular mycorrhiza (*Endogone mosseae*) on the population dynamics of the root-knot nematodes (*Meloidogyne incognita*) and *M. hapla*, pp. 158-166. In: *International Plant Protection Congress* (8th) Moscow. Reprints and information section V. Moscow, USSR.

Smith, S. E. and Read, D. J. 1997. *Mycorrhizal Symbiosis,* 2nd edn. Academic, San Diego, USA.

Timmer, L. W. and Leydon, R. F. 1978. Stunting of citrus seedlings in fumigated soil in Texas and its correction by phosphorus fertilization and inoculation of mycorrhizal fungi. *Journal of American Society for Horticulture Sciences*, **103**: 533-537.