Tropical and Subtropical Agroecosystems, 11 (2009): 423 - 429

Tropical and Subtropical Agroecosystems

EFFECT OF *Trichoderma harzianum* AND ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH IN TOMATO (L*ycopersicum esculentum* MILL) SEEDLINGS, NAPIER *(Pennisetum purpureum L)* AND TEA (C*amellia sinensis* L) CUTTINGS

[EFECTO DE *Trichoderma Harzianum* Y MICORRIZAS ARBUSCULARES SOBRE EL CRECIMIENTO DE PLANTULA DE TOMATE (*Lycopersicum esculentum* MILL) Y ESQUEJES PASTO NAPIER (*Pennisetum purpureum* L) Y TE (*Camellia sinensis* L)]

Margaret W. Mwangi*[1]; Ethel O. Monda[1]; Sheila A. Okoth[2]; Joyce M. Jefwa[3]

[1]Department of Plant and Microbial Sciences, School of Pure and Applied Sciences, Kenyatta University, Box 43844–00100 Nairobi, Kenya, E-mail*; wanjirumargie @ yahoo.com; emonda@iconnect.co.ke; [2]Department of Botany, School of Biological Sciences, University of Nairobi, Box 30197-001000 Nairobi. Kenya E-mail; dorisokoth @ yahoo.com; [3]CIAT-Nairobi TSBF. Off UN Avenue Gigiri P.O.Box 823- 001621 Nairobi. Kenya E-mail; jjefwa @ cigar.org

**Corresponding author*

SUMMARY

A green-house study was conducted to investigate the ability of an isolate of *Trichoderma harzianum* (P52) and arbuscular mycorrhizal fungi (AMF) in enhancing growth in tomato seedlings, tea and napier grass cuttings. The effect of these bio-inoculants on growth was compared with the influence of Diammonium phosphate (DAP) fertilizer and the interactions of these three factors (P52, AMF and DAP). The plants were grown in plastic pots filled with sterilized soils. A completely randomized design was used and growth measurements taken on height shoot and root dry weights. It was observed that isolate P52 and DAP fertilizer individually enhanced growth in tomatoes, tea and napier while AMF only enhanced growth in tomatoes. Combinations of P52 and DAP; P52, AMF and DAP enhanced growth significantly ($P<0.05$). *Trichoderma harzianum* and AMF showed potential for use as biofertilizers to reduce on chemical inputs in the perspective of sustainable agriculture and conservation of natural resources.

Keywords: Arbuscular mycorrhizal Fungi (AMF); *Trichoderma harzianum* (P52); Diammonium Phosphate (DAP); Enhanced growth; Inoculation.

INTRODUCTION

Soil microbial communities are composed of many microorganisms having complex ecological relationships (Ted 1992). Biological relationships may be antagonistic, neutral or beneficial. Beneficial microorganisms of the rhizosphere help the roots to take up nutrients from the soil through fixation, decomposition, mineralization and solubilisation.

Arbuscular mycorrhizal fungi (AMF) stimulate plant growth especially in soil substrates with low fertility mainly due to improved phosphorus absorption (Smith *et al.* 1986). Under experimental conditions commercial crops inoculated with AMF have showed increased growth and yield (Powell and Bagyaraj 1984).

Trichoderma harzianum also a beneficial fungus of the rhizosphere establishes robust root colonization of the root surfaces penetrating into the epidermis that enhance root growth and development, crop productivity and resistance to abiotic stresses through increases of mineral absorption (Harman 2000).

Tomatoes are grown for home consumption and are an important cash crop to both smallholders and medium-scale commercial farmers. The major constraints that causes decline in production are high prices of inputs, poor crop management practices, transportation, marketing, diseases and pests.

Napier grass *(Pennisetum purpureum)* is grown as a fodder crop in Embu region and is also used to stabilize soils and act as wind breaks in the steep terrain of the region. Napier is established vegetatively by stem cuttings or by tillers. However propagation using cuttings though more convenient, is a major challenge in this area because of drying of the cuttings caused by water stress due to inadequate rainfall and slowed growth as a result of low temperatures in most parts of the year.

Tea is one of the most important beverages in the world and a major cash crop in Kenya. In 2002, Kenya exported 287,000 metric tons of tea that earned the country 34 billion shillings (Kenya Central Bureau

2003). In Kenya, there is continued expansion in tea acreage and replacement of low yielding germplasm with improved clones developed by the Kenya Tea Research Foundation (TRFK). Tea is mainly propagated vegetatively. Single leaf cutting of about 2.5 cm to 4 cm is planted in polythene sleeves and is transplanted when they are about a year old. A major challenge in propagation is drying of the cuttings especially during transplanting due to water stress (Tea Research Foundation 2004).

Demand for alternatives to agro-chemical inputs owing to safety and environmental impact concerns on chemicals have rekindled scientific interest on need for information on the bio-controls. Investigations were conducted to quantify the effects of local isolates of *T. harzianum* and AMF on growth in tomato seedlings, tea and napier cuttings.

MATERIALS AND METHODS

Tomato (*Lycopersicum esculentum* Mill), tea (*Camellia sinensis* L) and napier *(Pennisetum purpureum L)* were chosen as test crops because of their economic importance in Embu District, Kenya.

Source of Inoculum

The AMF inoculum was a mixture containing four different species of the genus *Glomus,* three of *Acaulospora* and one of *Gigaspora* which were obtained from field trap cultures from Embu that had been preserved in a green house at the National Museums of Kenya (NMK). Sterilized sand was added to these field trap cultures in the ratio of 3:1 (sand: field trap culture), to bulk up and improve soil texture. Seeds of *Sorghum vulgare* Pers. were planted in field trap cultures sand mixture held in one litre (13 cm diameter) plastic pots in the green-house. These plant cultures were watered daily and green house sanitation maintained for purity of cultures for a period of four months. After 4 months all the sorghum plants were uprooted and their roots cut into small pieces and then mixed up with soil in the pot. Sterilized sand was again added to the trap cultures in the ratio 3:1 (sand: trap culture), and then seeds of *S. vulgare* were planted in a five litre (20 cm diameter) plastic pot to increase the quantity of inoculum. After one month the seedlings were uprooted and roots cut into small segments. These root segments were thoroughly mixed with soil to form a uniform inoculum consisting of soil, external mycelia and infected root segments which were used to inoculate experimental plants. During inoculation of experimental plants the AMF inoculum was weighed.

The *Trichoderma harzianum* isolate (P52) isolated from the Embu soil was preserved at the School of Biological Sciences University of Nairobi. The isolate P52 was cultured on Potato Dextrose Agar (PDA) at 25^0C for 4 days. Plugs of the isolate were obtained by using a cork borer (0.7 cm) and were mixed with soil during inoculation of experimental plants.

Establishment of green-house experiments

All the experiments were carried out in the green-house. Soil samples used for the growth of the seedlings in the green house were obtained from the tea farms in Embu. Three hundred grams of the soil were taken to the laboratory for chemical characteristics (Table 1). The remaining soil was mixed with sand in the ratio 3:1(soil: sand) to improve the soil texture. The soil was then steam sterilized using an autoclave at 121^0C for 3 hours and procedure repeated after a period of one day. The soils were left for a period of one week to allow for escape of volatile poisonous substances produced during process of sterilization. The soil was used for the growing tea and napier as well as tomatoes in the green house experiment. There were eight treatments applied as follows; *T. harzianum (*P52);- + AMF;- + P52 and AMF;- + P52 and DAP (Diammonium phosphate);- + AMF and DAP;- P52, AMF and DAP; and ;DAP alone and uninoculated control. Each treatment was replicated 24 times and placed in the green house in a completely randomized design. Four seedlings from each treatment were harvested at random each time. Height, shoot and root dry weights were recorded. Plants inoculated with AMF were checked for root colonization by method of Kormaik and McGraw (1982) and root colonization determined by the Grid Line Intersect Method (Giovannetti and Mosse 1980).

Table 1. Chemical analysis of soil samples from tomato, tea and napier farms

Soil sample description	pH H_2O	pH 0.01M $CaCl_2$	%N	%C	K (mg/l)	Na (mg/l)	CEC ($cmol_c$/kg)	P (ppm)
Tea farms	3.90	3.70	0.42	4.75	0.50	Trace	19.60	8.33
Napier farms	4.80	3.80	0.27	3.23	0.25	Trace	16.80	9.58
Tomato farms	4.20	3.70	0.35	3.86	0.50	Trace	19.40	6.67

Tomato Experiment

Certified tomato seeds of cultivar Cal-j were surface sterilized in 1% solution of sodium hypochlorite for 30 sec and rinsed with three changes of distilled water and then dried with sterile blotting paper. The seeds were pre-germinated in 15 x 20 x10 cm trays containing sterilized sand. Twenty eight day old tomato seedlings were transplanted into 500 ml (9 cm diameter) plastic pots, at the rate of one plant per pot in sterilized field soils. The inoculants were at the rates of 30 g of AMF, 20 plugs of P52 while DAP fertilizer at the rates of 0.2 g. Plants were watered regularly and height; shoot and root dry weight taken at 3, 6 and 9 weeks after transplanting.

Napier Experiment

Napier grass cuttings variety Kakamega I was obtained from the Kenya Agricultural Research Institute (KARI) Muguga. The treatments were the same as in tomatoes. The inoculants were at the rates of 50 g of AMF, 20 plugs of P52 while DAP fertilizer was used at the rates of 0.4 g per 1kg (13 cm diameter) plastic pot. Plants were evaluated at 4, 8 and 12 weeks after planting.

Tea experiment

Tea cuttings of the variety 31/8 were obtained from Kangaita Tea Research Foundation of Kenya (T.R.F.K). The cuttings were transported in a cool box and were misted frequently on the way. They were planted in a mist propagator at the National Museums of Kenya. The size of the leaves was reduced by half before planting to reduce the rate of transpiration. Misting was done twice a day for the first one week, then once a day for the next one week, then after every other day for the next one week. The propagator had a humidity of 100% and temperatures were in the range 18^0C to 24.4^0C. The cuttings were transferred to a green house in Chiromo campus, University of Nairobi after 3 weeks and planted in, 500 ml (9 cm diameter) plastic pots which were filled with sterilized field soils. The inoculants were at similar rates to those of napier. Plants were evaluated after 7, 14 and 21 weeks for height, shoot and root dry weight.

Statistical analysis

Data obtained were analyzed by Analysis of variance (ANOVA) using a statistical package (Minitab version 13.1). Treatment mean (dry weights and heights) were separated by Tukeys tests at 5% level of significance. T-tests were also used in the computation of the means in the percentage colonization of the roots by mycorrhizal fungi.

RESULTS

Effect of treatments on plant heights shoot and root weights

Inoculating tomatoes with *Trichoderma* (isolate P52) together with,- AMF and DAP fertilizer enhanced the plant heights as well as root and shoot growth, with DAP fertilizer having the greatest effect, followed by P52 and least being AMF. Combining of P52, AMF and DAP had the highest effect in growth of tomatoes height though this was not significant (Table 2). Diammonium phosphate fertilizer enhanced the shoot and root dry weights over the control at the rate of 377.78% and 700% respectively; P52 at the rates of 116.67% and 200% respectively while AMF improved the weights at the rates of 66.67% and 133.33% respectively. Thus there was a cumulative significant ($P < 0.05$, Table 2) growth effect when two or more of these variables were combined together. Treatment with combinations of P52, AMF and DAP; P52 and DAP; AMF and DAP ($P < 0.05$, Table 2) significantly enhanced the shoot and root dry weights of tomatoes.

Table 2. Effects of treatments on mean heights and mean dry shoot and root weights in tomato

Treatments	Heights (cm)	Dry shoot weights(g)	Dry root weights (g)
Control	21.21a	0.18c	0.03b
DAP	28.47a	0.86b	0.24ab
AMF	26.93a	0.30bc	0.07b
P52	27.43a	0.39bc	0.09b
AMF+DAP	36.49a	1.02ab	0.28a
P52+AMF	29.29a	0.44bc	0.13b
P52+AMF+	40.34a	1.52a	0.33a
DAPP52+DAP	31.33a	1.23b	0.32a

Data are means of 4 replicates. Means followed by the same letter in the same column are not significantly (Tukey, $P \geq 0.05$) different

The effect of each individual treatment (P52, AMF and DAP) on heights of napier was not significantly ($P \geq 0.05$) different from the control (Table 3). However, DAP and P52 applied singly enhanced the heights of napier while AMF performed poorly compared to the control. Application of DAP fertilizer improved shoot and root dry weights in napier at 81.41% and 19.55% respectively while P52 increased at 54.20% and 12.03% respectively. AMF inoculation reduced root

and shoot growth by about a 6.35% and 17.29% below the control respectively. A significant ($P < 0.05$), enhancement was noted in the shoot dry weights in the treatment with combinations of P52 and DAP; P52, AMF and DAP (Table 3). However there was no significant ($P \geq 0.05$) enhancement of the treatments on the dry weights of the roots.

Table 3. Effects of treatments on mean heights and mean dry shoot and root weights in napier

Treatments	Heights (cm)	Dry shoot weights(g))	Dry root weights (g)
Control	31.88a	4.41b	1.33a
DAP	45.15a	8.0ab	1.59a
AMF	31.73a	4.13b	1.10a
P52	40.57a	6.80ab	1.49a
AMF+DAP	44.53a	8.28ab	1.75a
P52+AMF	35.27a	5.89ab	1.57a
P52+AMF+	47.03a	8.76a	1.82a
DAPP52+DAP	49.74a	9.24a	2.00a

Data are means of 4 replicates. Means followed by the same letter in the same column are not significantly (Tukey, $P \geq 0.05$) different

With respect to the tea seedlings, application of *Trichoderma* (isolate P52) or - AMF or DAP alone) had no significant effect on height (Table 4) but a combination of *Trichoderma* isolate P52 with DAP and P52 + DAP + AMF significantly ($P < 0.05$) enhanced the height in tea seedlings. *Trichoderma* isolate P52 inoculant- improved shoot and root dry weight by 83.78% and 50% respectively;- while application of DAP fertilizer improved the same by 45.95% and 60% respectively. AMF reduced shoot dry weight by 10% decrease below the control. Significant *Trichoderma* isolate P52 + DAP;- or isolate P52 + AMF + DAP interactions were significant ($P < 0.05$, Table 4) as they improved the tea shoot and root dry weights.

Table 4. Effects of treatments on mean heights and mean dry shoot and root weights in tea

Treatments	Heights (cm)	Dry shoot weights(g)	Dry root weights (g)
Control	5.76b	0.37b	0.10b
DAP	9.49ba	0.542b	0.16ab
AMF	5.66b	0.37b	0.09b
P52	9.37b	0.68ab	0.15ab
AMF+DAP	10.12ba	0.56b	0.12ab
P52+AMF	9.08b	0.57b	0.20ab
P52+AMF+	15.24a	1.21a	0.21ab
DAPP52+DAP	15.88a	1.43 a	0.29a

Data are means of 4 replicates. Means followed by the same letter in the same column are not significantly (Tukey, $P \geq 0.05$) different

Napier roots were colonized in all treatments that received AMF inoculant with vesicles being the main mycorrhizal structures (Fig. 2) but with tomato roots, AMF colonized only roots that were grown in the absence of DAP fertilizer. Paired T-tests showed that combining (isolate P52 and AMF) had higher colonization compared to where AMF inoculant was applied as a single treatment (Table 4).

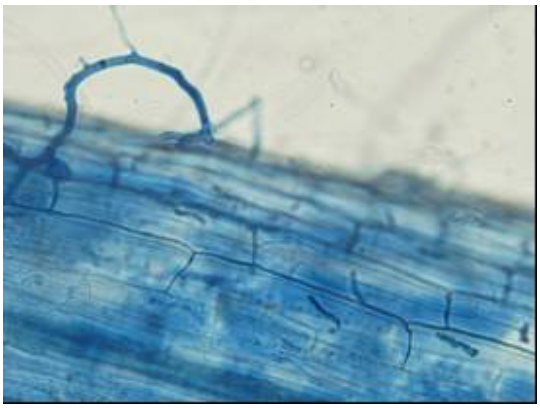

Figure 1. Entry point (appressorium; black arrow) in a tomato root.

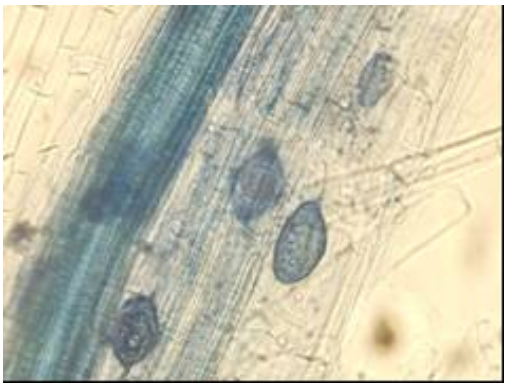

Figura 2 Vesicle (black arrow) in a napier root.

DISCUSSION

The ability of *T. harzianum* to enhance root growth has been reported in maize, where a strain of *T. harzianum* induced about twice as many deep roots as compared to uninoculated control (Harman 2000). *T. harzianum* strains were also reported to have increased the height, shoot and root dry weight by 26%, 14%,

and 29% respectively in tomato seedlings transplanted into pots in the green-house (Ozbay and Newman 2004**).** *T. harzianum* ability to increase the rate of plant growth and development including causing more robust growth of roots has been known (Harman 2000). Altomare *et al*. (1999) and Yedida *et al*. (1999) noted that *T. harzianum* increases the solubility of phosphate and micronutrients such as zinc, copper, iron and manganese ions, all plant nutrients with low solubility. This increase in nutrients in the soil solution may enhance growth of the roots and that of the above-ground biomass. Increased growth could also have been due to production of growth hormones by the fungus as was suggested by Windham *et al. (*1986).

Diammonium phosphate (DAP) fertilizer was consistently good and improved all the plant growth parameters in the three plants. Applied singly it enhanced the heights, dry weights of shoots and roots in tomatoes, tea and napier. A good supply of phosphorous (P) and ammonium ions is associated with increased root growth (Samuel *et al*. 1993). Phosphorous is indispensable for growth and plays an important role in the formation of new wood and roots, transformation of energy, and is also involved in the utilization of nitrogen (Eden 1965).

Colonized napier roots in all treatments were found to have vesicles as major mycorrhizal structures (Plate 2). This indicates a symbiosis that is dormant and hence less effective as was suggested by Dubsk'y *et al*. (2002).

Graham and Syvertsen (1985) suggested that most plants having a course root system and few root hairs have a likelihood of forming mycorrhizal associations.- The lack of infection in tea with few root hairs and a course root system could possibly be explained by slow or delayed colonization.

AMF established faster, enhanced growth in tomato roots. Croll *et al.* (2008) noted that although AMF have a wide host range, there exists some degree of host specificity. Tomatoes may have a rhizosphere that stimulates colonization and subsequent functioning of AMF species by production of exudates. Observed enhanced growth due to mycorrhizal infection in tomatoes confirms other reports (Smith *et al.* 1986; George *et al.* 1992) that AMF symbiosis with host plant has an improved growth effect mainly attributed to improved phosphorous and micronutrient uptake and growth in their host (Eden 1965). Enhanced growth in tomatoes could also have been as a result of AMF promoting lignifications and production of other polysaccharides as reported by Denhe and Shonbeck (1979).

Comparing the three P52, AMF and DAP fertilizer, it is to be noted that DAP fertilizer ranked highest in enhancing growth, followed by isolate P52 and then AMF, which enhanced growth only in tomatoes. DAP fertilizer it is a highly soluble fertilizer and release both N and P which are assimilated by plant roots. Combinations of isolate P52 and DAP ÷ or P52 + DAP + AMF showed significant ($P < 0.05$) cumulative enhancement on growth of all three crops. *Trichoderma* spp. were stimulated by manuring with minerals and farmyard manure (Domsch and Gams 1972) and this may have been the case in this study. There was synergetic enhancement of root dry weights by treatments with combinations of AMF and P52. Such stimulatory effect by *T. harzianum* on plant roots colonization by AMF has been reported in roots of *Zea mays* (Vazquez *et al*. 2000).

There was less colonization of tomato seedlings roots in treatments with combinations of AMF and DAP fertilizer due to increases in available P which nullified the need for the symbiotic association (Koide and Li 1990). Nevertheless this treatment enhanced growth as the tomato roots absorbed phosphorous and other mineral elements directly. It seems that tomatoes relied on mycorrhizae for absorption of water and mineral salts but in the presence of fertilizer this dependence was reduced and the seedlings absorbed the fertilizer directly.

Table 5. Mean percentage colonization of napier and tomato roots by arbuscular mycorrhizal fungi by T-tests

Plant	Treatments	Percentage colonization
Napier	AMF	38.7a
	P52+AMF	41.8b
	AMF+DAP-F	37.9a
	P52+AMF+DAP-F	41.5b
Tomato	AMF	49.19a
	P52+AMF	58.44b
	AMF+DAP-F	Entry points
	P52+AMF+DAP-F	Entry points
Tea	AMF	Entry points
	P52+AMF	Entry points
	AMF+DAP-F	Entry points
	P52+AMF+DAP-F	Entry points

Data are means of 4 replicates. Means followed by the same letter in the same column are not significantly (T-test, $P \geq 0.05$) different

CONCLUSION

T. harzianum enhanced growth in the three crops while AMF was only effective on tomatoes only. These results show that *T. harzianum* has potential to improve on crop growth and requires further investigation under different agroecosystems and crops.

ACKNOWLEDGEMENTS

This work was supported by the Below- Ground Biodiversity project with co–financing from Global Environmental Facility (GEF).

REFERENCES

Altomare, C., Norvell, W. A., Bjorkman, T., Harman, G. E. 1999. Solubilization of phosphates and micronutrients by plant growth promoting and biocontrol fungus *Trichoderma harzianum* strain 1295-22. Applied Environmental Microbiology. 65:2926-2933.

Croll, D.; Willie, L., Gamper, H. A., Mathimaran, N., Lammers, P. J.,Corradi, N., Sanders, I. R. 2008. Genetic diversity and host preferences revealed by simple sequence repeat and mitochondrial markers in a population of arbuscular mycorrhizal fungus *Glomus intraradices*. New Phytologist. 178: 672-687.

Domsch, K. H., Gams, W. 1972. *Fungi in agricultural soils*. Longman Group Limited, London.

Dubsk'y, M.; Sr'amek, M., Vos'atka, M. 2002. Inoculation of Cyclamen (*Cyclamen persicum)* and *Euphorbia pulcherrina* with arbuscular mycorrhizal fungi *Glomus spp.* and *Trichoderma harzianum*. Rostlinna Vyroba. 48: 63-68.

Eden, T. 1965. Tea. Longmans, London.

George, E., Marschner, H.; Jakobsen, I. 1992. Role of Arbuscular mycorrhizal fungi in uptake of phosphorous and nitrogen. Critical Reviews in Biotechnology. 15: 257-270.

Giovannetti, M., Mosse, B. 1980. An evaluation of techniques to measure VA infection in roots. New Phytopathology. 84: 489-500.

Graham, J. H. , Syvertsen, J. P. 1985. Host determinants of mycorrhizal dependency of citrus rootstock seedlings. New Phytologist. 101:667-676.

Harman, G. E. 2000. Myths and dogmas of biocontrol. Changes in perceptions derived from research on *Trichoderma harzianum T*-22. Plant Disease. 84:377-393.

Kenya Central Bureau of Statistics 2003. Statistical Abstract, Ministry of Planning and Development.

Koide, R. T., Li, M. 1990. On host regulation of the arbuscular mycorrhizal symbiosis. New Phytologist. 114: 59-65

Kormaik, P. P., McGraw, A. C. 1982. Quantification of vesicular arbuscular mycorrhizae in plant roots. In: Schenick, N. C. (eds). *Methods and principles of mycorrhizal research.* The American Phytopathological Society, St. Paul p.37-45.

Ozbay, N., Newman, E. S. 2004. Effect of *T. harzianum* strains to colonize tomato roots and improve transplant growth. Pakistan Journal of Biological Sciences. 7 (2): 253-257.

Powell, C.; Bagyaraj, D.J. 1984. *Vesicular Arbuscular Mycorrhiza.* CRC Press, Florida.

Samuel, L.T., Werner, L. N., James, D. B., John, l. H. 1993. Soil fertility and fertilizers. 5th Edition. Prentice-Hall of India, New Delhi.

Smith, S. E., Walker, N. A. 1986. Effect of mycorrhizal infection on plant growth, nitrogen and phosphorous nutrition in glass house grown *Allium cepa* L. New Phytopathology. 103: 359-373.

Tea Research Foundation of Kenya, 2004. Quarterly Bulletin volume 9, No. p.4

Ted, St. J. 1992. The importance of mycorrhizal fungi and other beneficial microorganisms in biodiversity projects. Paper presented at the Western Forest Nursery Associations meeting at Fallen Leaf Lake, September 14-18, 1992.

Vazquez, N. M., Cesar, S., Azcon, R., Barea, J. M. 2000. Interactions between Arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillium, Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activity in the

rhizosphere of the maize plant. Applied Soil Ecology. 15: 261-272.

Windham, M. T.; Elad, Y., Baker, B. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology. 76: 518-521.

Yedida, I.,Benhamou, N., Chet, I. (1999). Induction of defense response in cucumber plants (*Cucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum*. Applied Environmental Microbiology. 65:1061-1070

.

Submitted October 07, 2009 – Accepted January 14, 2010
Revised received February 15, 2010