INNSPUB

International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 14, No. 2, p. 1-11, 2022

Open Access RESEARCH PAPER

Effects of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on *Dombeya torrida* growth and biocontrol against *Armillaria* species

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Keywords: Fungi, Inoculation, Colonization

Publication date: February 10, 2022

Abstract

Armillaria root rot disease caused by *Armillaria* species is one the most widespread and important disease of many forest trees. An interaction between arbuscular mycorrhizal fungi (AMF) and *Trichoderma asperellum* have beneficial effects on plant growth as well as to the host plants and may affect plant resistance. The objective of this study was to determine the ability of *T. asperellum* to promote plant growth and reduce *Armillaria* root rot disease in green house condition. *Armillaria* species was isolated from a severely infected *Dombeya torrida* plant and cultured on malt extract agar. Each plant was inoculated by placing four mycelial agar plugs cut-out from a 14-days old *Armilllaria* species after every one month of plant growth. The inoculations of *T. asperellum* were done at 0 day of the experiment then repeated at an interval of 30 days up to the 150 days while AMF was inoculated once at the onset of the experiment. Plants that were co-inoculated with *T. asperellum* and AMF exhibited the highest fresh weights and plant height. The presence of *T. asperellum* increased AMF root colonization compared to plants inoculated with the AMF. The fungal interaction revealed a negative interference between AMF and *T. asperellum* on root colonization of *D. torrida* seedlings. This demonstrated that inoculation of AMF and *T. asperellum* either individually or in combination enhanced plant growth response of *D. torrida* seedlings.

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Introduction

Armillaria species are widely associated with woody plant taxa as pathogens, wood decay agents and even as mutualists with orchids (Baumgartner *et al*., 2011; Mihail, 2013). Members of the fungal genus *Armillaria* species occur in boreal, temperate and tropical forests worldwide, and Armillaria root disease (ARD) is known to occur on all continents except Antarctica (Westwood *et al*., 2012). The *Armillaria* fungi, which are feared by foresters, belong to an important and cosmopolitan pathogens inside and outside the forest. They can attack almost all species of hardwoods and coniferouss of all ages (Gibbs *et al*., 2002). Armillaria root disease is a common disease of both forest and agricultural crops. More than 30 *Armillaria* species exist world-wide (Cruickshank *et al*., 2011). The ARD is mostly present in many forest regions of Canada, affecting over 200 million hectares (Canadian Forest Service, 2010). Kenya has 3.47 million hectares of forest cover FAO (2010): The ARD is an important contributor to tree mortality in the forests and has resulted in loss of forest cover thus a significant economic losses (Bendel and Rigling, 2008). Besides, in forests the Armillaria are important root rot pathogens of tea (*Camellia sinensis* L. O. Kuntze) in the highland areas (altitude >1500 m above the sea level (a.s.l.)) of Kenya (Otieno *et al*., 2003$_b$). *Armillaria* species can survive in the soil, on wood and root debris in the absence of any living host and can cause significant damage to forest trees and several other crops (Pertot *et al*., 2008; Savazzini *et al*., 2009).

The disease assumes importance in one or more of the following ways: which include the roots are killed and hence the trees die in one or more seasons; the base of trunk may be injured to such an h extent that the tree may be blown over under the strain of a heavy crop of fruit; affected trees in bearing usually fail to mature their fruit, particularly in cases of severe infection; or the fruit matures poorly, is stunted and is of an inferior quality; diseased trees often lack the normal amount of foliage; and affected plants make little or no growth (Skovsgaard *et al*., 2010).

The disease remains undetected until symptomatic plants are observed, by which time *Armillaria* species may be widely spread both in soil and within the plant. *Armillaria* species forms rhizomorphs that can go deep into the soil, and mycelium remains protected, beneath the plant bark or inside dead wood, from the action of any control agent (Thomidis and Exadaktylou, 2012). Cultural methods such as removal of plant residues that may harbour the fungus and eradication of diseased plants are recommended for minimizing the risk of the disease, but complete elimination of the inoculum by this method is difficult in established crop stands Its success also depends on early and accurate diagnosis of the disease, which is difficult before the appearance of above ground symptoms. The limited access to the pathogen inside dead plant materials and its extensive rhizomorphs systems also limits the efficacy of chemical fumigants such as carbon disulphide (Otieno *et al*., 2003). In addition, fumigation of infested soil is another method used as a preventive measure of this disease before establishing a new planting season (Adaskaveg *et al*., 1999). However, Guillaumin (1988) noted that to be effective, the fumigants should be injected at least 60 cm deep in the soil but even this does not completely eradicate the fungus. Complete eradication of *Armillaria* sp. from an infested site by use of the above method is therefore difficult and alternative methods of destroying inocula fungus within plant materials in the soil are desirable.

Biological control (BC) of plant diseases is any means of controlling disease or reducing the amount or effect of pathogens that relies on biological mechanisms or organisms other than man (Hofte and Altier, 2010). The BC of plant diseases has received more consideration in the recent decades because of its efficacy against

fungicide-resistant pathogens in addition to reduced possibility of resistance development (Brimner and Boland, 2003; John *et al.,* 2010). Moreover, many research studies have shown that BC offers an environmentally friendly alternative to protect plants from soil borne pathogens (Yangui *et al.,* 2008). There is significant interest in finding alternatives to fungicides for suppression of soil borne pathogens (Roberts *et al.,* 2007) due to several negative effects, like development of pathogen resistance, hazards to humans, damage to non-target beneficial organisms, and environmental pollution (Brimner and Boland, 2003; Begum *et al.,* 2010). In addition, application of fungicides and fumigants are expensive and can be harmful to plants and their efficacy has been reduced by the appearance of microbial resistance (Sang *et al*., 2008; Quagliotto *et al.,* 2009).

The antagonists are biological agents that reduce the population density or disease-producing activities of the pathogen and therefore use of antagonistic micro-organism for disease control is being studied (Jung *et al.,* 2003). Emerging strategies for plant disease control involve application of antagonistic micro-organisms alone or in combination (De Curtis *et al.,* 2010). Unfortunately, biological control agents (BCAs) applied alone are not likely to perform consistently against all pathogens or under different rhizosphere and / or soil environmental conditions (Roberts *et al*., 2005). A combination of different antagonists is more likely to have a greater diversity of traits responsible for suppression of one or more pathogens resulting in improved control of pathogens (Postma *et al.,* 2009). The BC using microbial inoculants has proved to be a reliable component of integrated management of fungal diseases (Tchameni *et al*., 2011). Among the microbial inoculants, AMF and members of the genus *Trichoderma* have emerged as promising groups of fungi that improve plant nutrition and health (Martínez-Medina *et al*., 2011_a).

Fungi of the genus *Trichoderma* are used extensively in the biocontrol of plant diseases (Wijesinghe *et al*., 2011). They are common components of rhizosphere soil and have been reported to suppress a great number of plant diseases (Martínez-Medina *et al*., 2011_b). *Trichoderma* species have shown antagonistic activities towards a range of agriculturally devastating phytopathogenic microorganisms, including those within the genus *Fusarium*, *Phytophthora*, *Sclerotinia*, *Rhizoctonia* and *Pythium* (Beaulieu *et al*., 2011). The AMF belongs to the phylum *Glomeromycota* and are key components of soil microbiota where they establish mutualistic symbioses with the majority of plants improving the nutritional status of their host and protecting it against several soil-borne plant pathogens (Martínez-Medina *et al*., 2011_b; Tian *et al*., 2011; Veresoglou *et al*., 2012). Alleviation of damage caused by soil-borne pathogens, such as *Phytophthora*, *Fusarium*, *Pythium*, *Rhizoctonia*, *Sclerotium* and *Verticillium* has been reported widely in mycorrhizal plants (Martínez-Medina *et al*., 2011_b).

Several reports have established that the interaction of these two groups of microorganisms may be beneficial for both plant growth and plant disease control. (Martínez-Medina *et al*., 2011_b). A synergistic effect of some saprophytic fungi on AMF spore germination and colonization has been confirmed. For example, it has been reported that some *Trichoderma* strains may influence AMF activity (Martínez-Medina *et al*., 2009a). Although interactions between AMF and rhizobacteria have been extensively studied, interactions of AMF with saprophytic beneficial fungal species have received little attention (Chandanie *et al*., 2009). Dual inoculations of AMF and *Trichoderma* might provide a higher level of protection and wider range of effectiveness by activating several different mechanisms. Limited studies has been done to evaluate the effectiveness of the above fungi to control *Armillaria* species in *D torrida* plants.

Thus, , the aim of this study was to evaluate the efficacy of application of different biocontrol agents: *Trichoderma* species and mycorrhizae, singly or in combination, for the control of *Armillaria* root rot in green house condition so as to contribute to a more suitable and environmentally acceptable alternative for sustainable agriculture and forestry.

Materials and methods

Source of fungal inoculants, potting medium and host plants

Armillaria species initially isolated from a severely infected *D torrida* plant was kindly provided by Tea Research Foundation of Kenya. The species was isolated using *Armillaria* selective medium and maintained on malt extract agar (MEA) medium at room temperature. The inoculum of AMF which consisted of spores, hyphae, colonized root fragments and *Trichoderma* isolates were provided by Homegrown (K) Limited-Naivasha. In order to obtain fresh active cultures of *Trichoderma* isolates for *in-vitro* test, isolates were sub-cultured on MEA plates and incubated at 25^{0}C for 7 days in the dark.

The dark brown loam soil collected from Sururu forest of Eastern Mau (2716 m a.s.l. 00º38.862 S and 036º01.467 E), was sieved (4 mm), mixed with river sand and compost manure (1:1:1) and sterilized by autoclaving for 1 h at 121º C twice, on two consecutive days (Toshihiro *et al*., 2004). The *D torrida* was used as the host plant and the seeds of these plants were collected from Mau forest complex.

Experimental design and biological treatments

The experiment was conducted. The experiment was conducted in a completely randomized design with three factors (AMF inoculation, *T. harzianum* inoculation and *Armillaria* species) to test the interaction between *T. asperellum* and AMF on fungal colonization, plant growth promotion and *Armillaria* species suppression in a glasshouse at Biological Sciences department, Egerton University. (1) Control (c) (no addition of *Armillaria* species or biocontrol inoculum); (2) *Armillaria* species (A); (3) *T. asperellum* (T); (4) Arbuscular mycorrhizae fungi (M); (5) *T. asperellum* and Arbuscular mycorrhizae fungi (T + M); (6) *T. asperellum* and *Armillaria* species (T+A); (7) Arbuscular mycorrhizae fungi and *Armillaria* species (M+A); (8) *T. asperellum* and AMF and *Armillaria* species (T + M +A). The experiments were conducted in triplicate.

Inoculation of plants with fungal species

Inocula of mycorrhizal comprised 15 g of mycorrhizal inocula (spores, hyphae and infected root systems) or 15 g of autoclaved inoculum plus 10 ml of 15g inoculum filtrate through a 25µm filter to correct the potential differences in microbial communities between mycorrhizal and non-mycorrhizal pots (Wu and Zou, 2010). Inoculum of *T. asperellum* comprising of powder formulation was prepared according to manufactures instruction. Sterile water (100 mL) was added to the 10g of the formulation and mixed with a sterile stirring rod.

The resulting suspension was introduced to potted *D. torrida* seedlings. The inoculations of *T. asperellum* were done at 0, 30, 60, 90, 120 and 150 days from the beginning of the experiment in order to maintain sufficient populations of the antagonist in the soils and thus favour biological control, as suggested by Knudsen *et al*. (1991). *Armilllaria* species inoculum was prepared by growing the culture on malt extract agar (MEA) and incubated at 25 ^{0} C for 14 days old.

Each plant was inoculated after every 30 days by placing four agar plugs cut from 14 days-olds MEA cultures of *Armilllaria* species (Baumgartner *et al*., 2010). The inoculum was introduced to the root zone of potted plants. The three fungal inocula, *Armilllaria* species, and *T. asperellum* were applied singly or in combination according to the treatment.

Plant growth

Selected tree seeds were surface sterilized by immersing them in a 1% solution of sodium hypochlorite for 5 min, rinsed twice in double distilled sterile water (DDSW) and air dried them on sterile filter paper (Coskuntuna and Ozer, 2008). The seeds were placed in half full petri-dishes with water then incubated at 28^{o} C in the dark for two days. Five pre-germinated seeds of *D. torrida* seedlings were each planted in pot filled with potting medium mixed with fungal inocula according to the treatment combination. The seedlings were thinned to one seedling per pot after three weeks. Final harvest was done after 28 weeks and the roots washed in DDSW.

Determination of colony forming units of T. asperellum on dombeya torrida rhizoplane

The estimation of colony forming units (CFUs) of *T. asperellum* in the rhizoplane were determined using an improved protocol used by Rosa and Herrera (2009). One gram fresh root tissue, previously disinfected in sodium hypochlorite (NaOCl) solution for 6 min, was cut into pieces transferred into 100ml DDW in a test tubes containing then serially diluted, and 0.1 ml of each dilution was finally plated on fresh *Trichoderma* selective medium (TSM) (Elad *et al*., 1981). The plates were incubated for 14 to 18 hours at 25ºC in the dark in triplicate. The population counts of *T. asperellum* colonies on each Petri-dish were recorded, and data expressed as CFUs per gram fresh root.

Armillaria species assay

At the end of the experiments plants were assayed for viability of *Armillaria* species using a modified protocol used by Otieno *et al*. (2003a). One gram fresh root tissue, previously disinfected in NaOCl for 6 min, was cut into pieces and transferred to *Armillaria* semi-selective medium. Inoculated petri dishes were incubated at room temperature in the dark for at least 21 days in triplicate.

Measurement of plant parameters and data analysis

At the end of the experiment plant height, shoot and root fresh weights were measured the roots were separated from the soil by washing and fresh weights were measured. For all data (plant growth parameters, mycorrhizal root colonization), treatments were compared using one-way analysis of variances ($p<0.05$) using Gen stat. the experiment was conducted three times and data from the repeated trials were pooled. The data was subjected to ANOVA and treatment means were compared by Fisher's least significant difference.

Results

Interaction between amf and T. asperellum under greenhouse conditions

Plant shoot and root fresh weight

Growth parameters of *D. torrida* seedlings grown in soils inoculated with AMF and *T. asperellum* were variably affected when examined after 28 weeks. Plants inoculated with *T. asperellum* alone had significantly increased shoot fresh weight compared to the control plants. Single inoculation of AMF did not significantly change shoot fresh weight compared with control plants. Plants co-inoculated with *T. asperellum* and AMF showed the highest fresh weights. Co-inoculation with AMF and *T. asperellum* significantly increased root and shoot fresh weights compared to *T. asperellum* alone or AMF alone (Table 1). Inoculations with *T. asperellum* only resulted in better root weight compared to inoculation with AMF. On the other hand, fresh shoots of plants inoculated with AMF were weightier than control plants. Although no significant changes in the shoot fresh weight due to inoculation with AMF, an increased shoot/root ratio was observed in AMF inoculated plants compared to control plants (Table 1). A significant interaction between the AMF and *T. asperellum* was observed regarding the shoot / root ratio. Combination of *T. asperellum* and AMF resulted in higher increases of root weight than shoot weight as illustrated by

the decrease in the shoot / root ratio. Moreover, smaller fresh shoot / root ratios were recorded in plants inoculated with *T. asperellum.*

Plant height

The other important parameters reflecting the seedling vigour of plants include the direct measurements of plant growth such as height. Although AMF inoculated plants had increased plant height, the difference was not significant from the control. *T. asperellum* inoculants significantly increased the plant height compared to the AMF inoculated plants. Plant height was found to be enhanced by the combined inoculation of AMF and *T. asperellum* compared to individual inoculations. The highest plant height of 82.25 cm was recorded in treatment which received combined inoculation of the two of microorganisms (Table 1).

Table 1. The plant height, shoot and root fresh weight (g), and the shoot/root ratio of *D. torrida* plants inoculated with *T. asperellum* and/or AMF, 28 weeks after planting.

Treatment	Plant height (cm)	Shoot fresh weight (g/plant)	Root fresh weight (g/plant)	Shoot/root ratio
C	61.62ab	13.42a	3.963a	3.538c
A	61.75ab	13.50a	3.975a	3.538c
M	65.38 ab	13.56a	4.013a	3.550c
A+M	52.25a	15.66a	4.575a	3.725c
T	72.00bc	18.26ab	9.050b	2.038ab
A+TA	71.75bc	18.39ab	6.737ab	2.775bc
A+TA+M	74.62bc	21.54bc	14.663c	1.562a
M+TA	82.25c	23.82c	16.150c	1.562a

Data in the same column sharing a letter in common do not differ significantly (P<0.05) by the Fischer's least significant difference test

Treatments: A-*Armillaria* species; C-control; M-mycorrhizae; T-*Trichoderma* species

Root colonization

There were variations in root colonization of *D. torrida* seedlings grown in soils inoculated with AMF and *T. asperellum* when examined after 28 weeks (Table 2). Plants grown in soil inoculated with AMF alone were successfully colonized whereas non-inoculated plants remained non-mycorrhizal. Dual inoculation of *T. asperellum* and AMF significantly reduced the level of root colonisation by AMF (Table 2). Population density (CFU) of *T. asperellum* in the rhizosphere soil of combine inoculated plants (*T. asperellum*-AMF) was almost similar to that of plants inoculated with *T. asperellum* alone. The number of CFUs in the assays performed ranged between 39400 and 44000. There was no significant difference between the CFU of *T. asperellum* associated with roots of single and combine-inoculated plants (*T. asperellum*-AMF) 28 weeks after planting. No mycorrhizal colonization was recorded in control treatment (Table 2).

Table 2. The AMF root colonization and *T. asperellum* CFUs 28 weeks after planting and *Armillaria* root rot incidence.

Treatment	*T. asperellum* CFUs	AM root colonization	*Armillaria* root rot incidence
A	0.00 a	0.00a	0.00
C	0.00 a	0.00a	0.00
T	44.00b	0.00a	0.00
T+A	42.60b	0.00a	0.00
T+M+A	39.40b	19.40b	0.00
T+M	40.00b	21.80b	0.00
M	0.00 a	41.40c	0.00

Data in the same column sharing a letter in common do not differ significantly (P<0.05) by the Fischer's least significant difference test

Treatments: A-*Armillaria* species; C-control; M-mycorrhizae; T-*Trichoderma* species

Discussion

Interaction between amf and t. asperellum

This study presents the first assessment of AMF and *T. asperellum* to enhance growth and resistance of *D. torrida* against *Armillaria* species,

the causal agent of Armillaria root rot disease under greenhouse conditions. Differences that emerged from measurements of plant growth parameters following the inoculation of plants with *T. asperellum* and AMF provided a number of lines of evidence to show that microbial inoculations could improve growth of *D. torrida* seedlings. In this study, significant increases in plant height, root and shoot weights were observed after inoculation with *T. asperellum* and AMF, although these increases were not similar for all treatments. Microbial inoculants are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake, and support the health of plants (Mader *et al*., 2011). The smallest shoot/root ratios were obtained with the treatment involving the dual inoculation with *T. asperellum* and AMF, showing that the combined inoculation of *T. asperellum* and AMF was not essential for the promotion of plant growth. This may be explained by the different modes of action of *T. asperellum* and AMF and their interaction in dual inoculated *D. torrida* plants. The interaction between AMF and *T. harzianum* and its effect on plant growth may vary depending on the inherent characteristics of the AMF and the *T. harzianum* strain (Martínez-Medina *et al*. (2011_a). Several reports have demonstrated that the interaction of these two groups of microorganisms may be beneficial for both plant growth and plant disease control (Martínez-Medina *et al*., 2009).

Although saprophytic fungi have been reported to influence AMF colonization and host plant response, the effects of the saprophytic fungi on AMF formation differ depending on the inherent characteristic of both agents (Martínez-Medina *et al*., 2011_a). AMF colonization levels of *D. torrida* seedlings were significantly reduced when dually inoculated with *T. asperellum*. This could be due to inhibition of AMF by *T. asperellum* through parasitism. As a biocontrol agent, the mechanism of action of *T. asperellum* is known to be based on mycoparasitism (Mbarga *et al.,* 2012). It could, therefore, be possible for *T. asperellum* to have had a deleterious effect on the AMF as reported by Rousseau *et al*. (1996).

In the current investigations, inoculations with AMF and *T. asperellum* resulted in higher increases of root weight than shoot weight as illustrated by the decrease in the shoot/root ratio. The shoot/root ratio of a plant is a good indicator of plant stress, whereby the lower the shoot/root ratio, the more stressed the plant (Naseby *et al*., 2000). The smallest shoot/root ratios were obtained with the treatment involving *T. asperellum* alone and the dual inoculation with *T. asperellum* and AMF, showing that the combined inoculation of *T. asperellum* and AMF was not essential for the promotion of plant growth. Similarly, root and shoot weights of soya beans were decreased by co-inoculation with *T. pseudokoningii* and *Gigaspora rosea* (Martinez *et al*., 2004). Since the present experiment was conducted under controlled conditions with daily watering, a possible cause of stress could have been nutrient limitations and thus, a decrease in shoot/ root ratio could indicate such stress (Naseby *et al*., 2000). Under these conditions, a larger increase of root weight is related to the expansion of the root system as a result of their search for more nutrients. While other reports indicate positive interactions following the combined inoculation of saprophytic fungal species and AMF (Srivastava *et al*., 2010; Erman *et al*., 2011), the reduced effectiveness of the combined use of the saprophytic fungus *T. asperellum* and the AMF in this study, suggests that the interactions of saprophytic fungi with AMF may differ depending on the AMF, the host plants and the species of the saprophytic fungus.

Conclusion

Dual inoculation of young *D. torrida* seedlings with the biocontrol agents *T. asperellum* and the AMF were found to have enhanced plant growth

compared to individual inoculations. However, negative interference between AMF and *T. asperellum* on root colonization has been demonstrated, showing their possible incompatibility to occupy the same rhizosphere.

Acknowledgements

The authors are sincerely grateful to the National Commission for Science, Technology and Innovation (NACOSTI) for the financial support for the research work and Tea Research institute and Homegrown (K) Limited-Naivasha for providing the *Armillaria* species and *Mycorrhizae* spores respectively.

References

Adaskaveg JE, Forster H, Wade L, Thompson DF, Connell JH. 1999. Efficacy of sodium tetrathiocarbonate and propiconazole in managing Armillaria root rot of almond on peach rootstock. Plant Disease **83,** 240–246.

Baumgartner K, Coetzee MPA, Hoffmeister D. 2011. Secrets of the subterranean pathosystem of *Armillaria*. Molecular Plant Pathology **12,** 515–534.

Baumgartner K, Bruhn J, Travadon R, Bergemann SE. 2010. Contrasting patterns of genetic diversity and population structure of *Armillaria mellea* sensu stricto in the eastern and western United States. Phytopathology **100,** 708–718.

Baumgartner K, Coetzee MPA, Hoffmeister D. 2011. Secrets of the subterranean pathosystem of Armillaria. Molecular Plant Pathology **12,** 515–534.

Beaulieu R, López-Mondéjar R, Tittarelli F, Ros M, Pascual JA. 2011. qRT-PCR quantification of the biological control agent *Trichoderma harzianum* in peat and compost-based growing media. Bioresource Technology **102,** 2793–2798.

Begum MM, Sariah M, Puteh AB, Abidin MAZ, Rahman MA, Siddiqui Y. 2010. Field performance of bio-primed seeds to suppress *Colletotrichum truncatum* causing damping-off and seedling stand of soybean. Biological Control **53,** 18–23.

Bendel M, Rigling D. 2008. Signs and symptoms associated with *Heterobasion annosum* and *Armillaria ostoyae* infection in dead and dying mountain pine (*Pinus mugo* ssp.Uncinata). Forest Pathology **38,** 61–72.

Brimner TA, Boland GJ. 2003. A review of the non-target effects of fungi used to biologically control plant diseases. Agriculture, Ecosystems and Environment **100,** 3–16.

Canadian Forest Service. 2010. The State of Canada's Forests, Annual Report 2010. Natural Resources Canada, Canadian Forest Service, Headquarters, Ottawa p.45

Chandanie WA, Kubota M, Hyakumachi M. 2009. Interactions between the arbuscular mycorrhizal fungus *Glomus mosseae* and plant growth-promoting fungi and their significance for enhancing plant growth and suppressing damping-off of cucumber (*Cucumis sativus* L.). Applied Soil Ecology **41,** 336–341.

Coskuntuna A, Ozer N. 2008. Biological control of onion basal rot disease using *Trichoderma harzianum* and induction of antifungal compounds in onion set following seed treatment. Crop Protection **27,** 330–336.

Cruickshank MG, Morrison DJ, Lalumiere A. 2011. Site, plot, and individual tree yield reduction of interior Douglas-fir associated with non-lethal infection by Armillaria root disease in southern British Columbia. Forest Ecology and Management **261,** 297–307.

De Curtis F, Lima G, Vitullo D, De Cicco V. 2010. Biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on tomato by delivering antagonistic bacteria through a drip irrigation system. Crop Protection **29,** 663–670.

Elad Y, Chet I, Baker R. 1981. A selective medium for improving quantitative isolation of *Trichoderma* speciesfrom soil. Phytoparasitica **9,** 59–67.

Erman M, Demir S, Ocak E, Tüfenkci S, Oguz F, Akköprü A. 2011. Effects of Rhizobium, arbuscular mycorrhiza and whey applications on some properties in chickpea (*Cicer arietinum* L.) under irrigated and rainfed conditions 1—Yield, yield components, nodulation and AMF colonization. Field Crops Research **122,** 14–24.

FAO. 2010. Forest Resources Assessment Report.

Gibbs JN, Greig BJW, Pratt JE. 2002. Fomes root rot in Thetford Forest, East Anglia: past, present and future. Forestry **75,** 191–202.

Guillaumin JJ. 1988. The *Armillaria mellea* complex. In: Smith, I. M., Dunez, J., Phillips, D. H., Lelliot, R. A., Archer, S. A. (Eds.), European Handbook of Plant Diseases. Blackwell, Oxford pp. 520–523.

Hofte M, Altier N. 2010. Fluorescent pseudomonads as biocontrol agents for sustainable agricultural systems. Research in Microbiology **161,** 464–471.

John RP, Tyagi RD, Prevost D, Brar SK, Pouleur S, Surampalli RY. 2010. Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum* f. sp*. adzuki* and *Pythium arrhenomanes* and as a growth promoter of soybean. Crop Protection **29,** 1452–1459.

Jung WJ, An KN, Jin YL, Park RD, Lim KT, Kim KY, Kim TH. 2003. Biological control of damping-off caused by *Rhizoctonia solani* using chitinase-producing *Paenibacillus illinoisensis* KJA-424. Soil Biology and Biochemistry **35,** 1261–1264.

Knudsen GR, Eschen DJ, Dandurand LM, Bin L. 1991. Potential for biocontrol of *Sclerotinia sclerotiorum* through colonization of sclerotia by *Trichoderma harzianum.* Plant Disease **75,** 466–470.

Mäder P, Kaiser F, Adholeya A, Singh R, Uppal HS, Sharma AK, Srivastava R, Sahai V, Aragno M, Wiemken A, Johri BN, Fried PM. 2011. Inoculation of root microorganisms for sustainable wheat–rice and wheat–black gram rotations in India. Soil Biology and Biochemistry **43,** 609–619.

Martinez A, Obertello M, Pardo A, Ocampo JA, Godeas A. 2004. Interactions between *Trichoderma pseudokoningii* strains and the arbuscular mycorrhizal fungi *Glomus mosseae* and *Gigaspora rosea*. Mycorrhiza **14,** 79–84.

Martínez-Medina A, Pascual JA, Lloret E, Roldán A. 2009. Interactions between arbuscular mycorrhizal fungi and *Trichoderma harzianum* and their effects on *Fusarium wilt* in melon plants grown in seedlings nurseries. Journal of the Science of Food and Agriculture **89,** 1843–1850.

Martínez-Medina A, Roldán A, Pascua JA. 2011_b. Interaction between arbuscular mycorrhizal fungi and *Trichoderma harzianum* under conventional and low input fertilization field condition in melon crops: Growth response and *Fusarium wilt* biocontrol. Applied Soil Ecology **47,** 98–105.

Martínez-Medina A, Roldán A, Albacete A, Pascual JA. 2011_a. The interaction with arbuscular mycorrhizal fungi or *Trichoderma harzianum* alters the shoot hormonal profile in melon plants. Phytochemistry **72,** 223–229.

Mbarga JB, Martijn Ten Hoopen G, Kuaté J, Adiobo A, Ngonkeu MEL, Ambang Z, Akoa A, Tondje PR, Begoude BAD. 2012. *Trichoderma asperellum:* A potential biocontrol agent for *Pythium myriotylum*, causal agent of cocoyam (*Xanthosoma sagittifolium)* root rot disease in Cameroon. Crop Protection **36,** 18–22.

Mihail JD. 2013. Comparative bioluminescence dynamics among multiple *Armillaria gallica*, *A. mellea*, and *A. tabescens* genets. Fungal biology **117,** 202–210.

Naseby DC, Pascual JA, Lynch JM. 2000. Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities. Journal of Applied Microbiology **88,** 161–169.

Otieno W, Jeger M, Termorshuizen A. 2003_b. Effect of infesting soil with *Trichoderma harzianum* and amendment with coffee pulp on survival of *Armillaria*. Biological Control **26,** 293–301.

Otieno W, Termorshuizen A, Jeger M, Othieno CO. 2003_a. Efficacy of soil solarization, *Trichoderma harzianum*, and coffee pulp amendment against. *Armillaria* species. Crop Protection **22,** 325–331.

Pertot I, Gobbin D, De Luca F, Prodorutti D. 2008. Methods of assessing the incidence of Armillaria root rot across viticultural areas and the pathogen's genetic diversity and spatial–temporal pattern in northern Italy. Crop Protection **27,** 1061–1070.

Postma J, Stevens LH, Wiegers GL, Davelaar E, Nijhuis HE. 2009. Biological control of *Pythium aphanidermatum* in cucumber with a combined application of Lysobacter enzymogenes strain 3.1T8 and chitosan. Biological Control **48,** 301–309.

Quagliotto L, Azziz G, Vaz P, Perez C, Ducamp F, Cadenazzi M, Altier N, Arias A. 2009. Three native *Pseudomonas fluorescens* strains tested under growth chamber and field conditions as biocontrol agents against damping-off in alfalfa. Biological Control **51,** 42–50.

Roberts DP, Lohrke SM, Meyer SLF, Buyer JS, Bowers JH, Baker CJ, Li W, Souza JT, Lewis JA, Chung S. 2005. Biocontrol agents applied individually and in combination for suppression of soil borne diseases of cucumber. Crop Protection **24,** 141–155.

Roberts DP, McKenna LF, Lakshman DK, Meyerc SLF, Kong H, Souza JT, Lydon J, Baker CJ, Buyer JS, Chung S. 2007. Suppression of damping-off of cucumber caused by *Pythium ultimum* with live cells and extracts of *Serratia marcescens* N4-5. Soil Biology and Biochemistry **39,** 2275–2288.

Rosa DR, Herrera CJL. 2009. Evaluation of *Trichoderma* speciesas biocontrol agents against avocado white root rot. Biological Control **51,** 66–71.

Rousseau A, Benhamou N, Chet I, Piché Y. 1996. Mycoparasitism of the extra-matrical phase of *Glomus intraradices* by *Trichoderma harzianum*. Phytopathology **86,** 434–443.

Sang MK, Chun S, Kim KD. 2008. Biological control of Phytophthora blight of pepper by antagonistic rhizobacteria selected from a sequential screening procedure. Biological Control **46,** 424–433.

Savazzini F, Longa CMO, Pertot I. 2009. Impact of the biocontrol agent *Trichoderma atroviride*SC1 on soil microbial communities of a vineyard in northern Italy. Soil Biology and Biochemistry **41,** 1457–1465.

Srivastava R, Khalid A, Singh US, Sharma AK. 2010. Evaluation of arbuscular mycorrhizal fungus, fluorescent Pseudomonas and *Trichoderma harzianum* formulation against *Fusarium oxysporum f. sp.lycopersici* for the management of tomato wilt. Biological Control **53,** 24–31.

Tchameni SN, Ngonkeu MEL, Begoude BAD, Nana LW, Fokom R, Owona AD, Mbarga JB, Tchana T, Tondje PR, Etoa FX, Kuate J. 2011. Effect of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on cacao growth and resistance against black pod disease. Crop Protection **30,** 1321–1327.

Thomidis T, Exadaktylou E. 2012. Effectiveness of cyproconazole to control Armillaria root rot of apple, walnut and kiwifruit. Crop Protection **36,** 49–51.

Tian H, Drijber RA, Niu XS, Zhang JL, Li XL. 2011. Spatio-temporal dynamics of an indigenous arbuscular mycorrhizal fungal community in an intensively managed maize agroecosystem in North China. Applied Soil Ecology **47,** 141–152.

Toshihiro A, Maldonado-Mendoza IE, Dewbre GR, Harrison MJ, Saito M. 2004. Expression of alkaline phosphatase genes in arbuscular mycorrrhizas. New Phytology **162,** 525–534.

Veresoglou SD, Chen B, Rillig MC. 2012. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biology and Biochemistry **46,** 53–62.

Westwood AR, Conciatori F, Tardif JC, Knowles K. 2012. Effects of Armillaria root disease on the growth of *Picea mariana* trees in the boreal plains of central Canada. Forest Ecology and Management **266,** 1–10.

Wijesinghe CJ, Wijeratnam RSW, Samarasekara JKRR, Wijesundera RLC. 2011. Development of a formulation of *Trichoderma asperellum* to control black rot disease on pineapple caused by (*Thielaviopsis paradoxa)*. Crop Protection **30,** 300–306.

Wu QS, Zou YN. 2010. Beneficial roles of arbuscular mycorrhizas in citrus seedlings at temperature stress. Scientia Horticulturae **125,** 289–293.

Yangui T, Rhouma A, Triki MA, Gargouri K, Bouzid J. 2008. Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. Crop Protection **27,** 189–197.