The effect and interaction of *Trichoderma* isolates on *Armillaria* fungus of tea

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ABSTRACT

Fungal diseases are a biotic constraint in tea production worldwide. One such disease is Armillaria. The disease causes high mortality and yield losses of tea in almost all growing regions. The most common strain of this fungal pathogen is *Armillaria mellea.* The management of *Armillaria* involves different methods mostly cultural including complete elimination of the pathogen source. These methods are at times non-effective, environmentally-hazardous and require huge financial investment and time to be encountered by tea growers. Biological control involving the use of *Trichoderma* has been found to have some antagonistic effect on the growth of *Armillaria* on the soil. This study aimed at assessing the effect of different *Trichoderma* isolates on *Armillaria* growth under *in vitro* conditions to ascertain its utilization. A total of 14 isolates (T-series) were screened during the study. *Armillaria* isolates (9T2 and 5H1) were obtained in two regions; Tigania and Hamisi tea growing regions in Kenya. Culturing of *Armillaria* was done on Extract Agar while *Trichoderma* on PDA agar. Association was done *in vitro* on nutrient agar and measures of interaction recorded based on radial growth. The setup was undertaken at the Tea Research Foundation, IPDM Laboratory and each treatment replicated three times. Data on radial growth was recorded at intervals of two days for four weeks. Data was analysed using MSTAT-C software. The results indicated that most *Trichoderma* isolates showed antagonistic effect by restricting *Armillaria* growth though there was significant difference ($P = 0.05$). T45 and T19 isolates were the most antagonistic among all other isolates. On the other hand, Isolates T13 and *T. asperrellum* caused minimal effect on growth of *Armillaria*. The most antagonistic *Trichoderma* isolate can be utilized as a biocontrol remedy against *Armillaria*.

Key words: Association, Fungal infection, Isolates, Radial, Tea, Trichoderma

INTRODUCTION

Armillaria species is a fungus that causes root rot on a wide range both natural and artificial forest hosts leading to high mortality and yield losses throughout the world (Hood *et al.*, 1991). Focusing on Africa, records of Armillaria root rot on tea (*Camellia sinensis*) dates back to the 1930s when Leach (1939) reported problems associated with this disease in Nyasaland. Armillaria root rot is common in the South, Central, East and Western African countries (Mohammed *et al.*, 1989) and affects both cash crop plants and forest plantation species (Shaw and Kile, 1991). In Kenya, it affects many plant species in the highlands (Mwangi *et al.*, 1989) and has been a limiting production factor in the tea growing areas. Yield reductions of up to 50% have been reported on smallholder farms due to Armillaria root rot, making it a disease of major economic concern (Onsando *et al.*, 1997). In Kenya, Armillaria root rot on tea plantations is common where the crop is established shortly after deforestation, suggesting that the fungus is native to the country (Goodchild, 1960). Major hosts include species like eucalyptus, pinus, acacia and cupress that are utilized in plantations (Wargo and Shaw, 1985; Hood *et al.*, 1991; Kile *et al.*, 1991). Studies have attributed serious causes of root rots of perennial crops in tropical Africa to species of *Armillaria* with reports showing the pathogen to be affecting tea, coffee, cocoa, rubber,

softwood timbers and other trees (Mohammed and Guillaumin, 1994). Different studies suggest that *A. heimii* and *A. mellea* subspecies *africana* (Ota *et al.*, 2000) are the prevailing *Armillaria* taxa in Africa (Mwangi *et al.*, 1989; Agustian *et al.*, 1994; Guillaumin *et al.*, 1994; Mohammed *et al.*, 1994; Mwangi *et al.*, 1994; Mwenje and Ride, 1996; Abomo-Ndongo and Guillaumin, 1997; Otieno *et al.*, 2003). A recent study in Zimbabwe by Mwenje *et al.* (2003) identified three *Armillaria* taxa as responsible for the occurring root rots on tree crops. In their findings, they considered one taxon to be representing *A. heimii*, another one to represent *A. fuscipes* (this group included isolates previously identified as *A. heimii* but that were similar to those identified as *A. fuscipes* from South Africa and La Réunion). From different studies, *Armillaria* has been regarded as a primary pathogen, stress-induced secondary invader and a saprophyte (Wargo and Shaw, 1985; Shaw and Kile, 1991). Pathogen quantification in rating the level of damage and extent of infestation is important in understanding the pathogenicity of *Armillaria*.

Control of *Armillaria* involves field cultural practices that aim at elimination of the pathogen from the soils including ring barking during field preparation for newly prepared land, complete removal of trunks known to harbour the fungus and getting rid of diseased plants in tea plantations to avoid spread of the fungus through the rhizomorphs (Home, 1974). In some instances, *Armillaria* has been managed by use of soil fumigants including carbon disulphide, methyl bromide and metham sodium (Ram and Devasahayam, 1974). The costs involved and environmental degradation following these chemicals has led to abandonment of these methods. Biological control of *Armillaria* involving the use of antagonistic fungi could be a potential strategy to reduce their severity and eliminate them among tea plants. One such beneficial fungus that has shown antagonism on *Armillaria* is *Trichoderma* spp. This fungus has been used to reduce *Armillaria* on citrus fruit plantations (Bliss, 1951). This negative interaction between this fungus and *Armillaria* can be optimized to obtain a proper and a sustainable management strategy of this constraint in tea. Antagonism of *Armillaria* by *Trichoderma* shows its potentiality in biological control (Sokolov, 1964). Reaves *et al.* (1990) found that isolates of *Trichoderma* species were antagonist to *Armillaria ostoyae* by reducing colony growth and rhizomorph formation in culture. In addition, Dumas and Boyonoski (1992) investigated the myco-parasite mechanisms of *Trichoderma* species against rhizomorphs of *Armillaria gallica*. They observed events such as direct penetration, coiling of the *Trichoderma* hyphae around the *Armillaria* hyphae and disintegration of rhizomorph content. Onsando and Waudo (1994) found that different isolates of *T. longibrachiatum*, *T. koningi* and *T. harzianum* reduced the mycelium and rhizomorph growth of *Armillaria* infecting tea in Kenya. Raziq (2000) investigated the antagonistic effect of isolates of *T. harzianum*, *T. virens* and *T. hamatum* against *A. mellea* and reported differences between *Trichoderma* in antagonistic effects.

Nonetheless, the efficacy of most biocontrol agents including fungi relies on the type, species and response to the disease (Sokolov, 1964). Biocontrol of *Armillaria* has focused on the use of *Trichoderma* species antagonistic fungi (Hagle and Shaw, 1991). Therefore, this study sought to assess the effect of different *Trichoderma* isolates on *Armillaria* growth under *in vitro* conditions to ascertain the interaction and the potential utilization as a biocontrol agent.

MATERIALS AND METHODS

Soil sampling and *Trichoderma* isolates

Soils with rhizospheres samples in tea growing regions of Kenya in the East and West Rift Valley. Both the top soil and subsoils were sampled and mixed together to obtain a composite sample and from it, a sub-sample taken for *Trichoderma* isolation. The isolation was done using the modified martins Rose Bengal-Streptomycin Agar Medium (Martin, 1950). Streptomycin sample was added at a concentration of 40g/l before pouring out plates. Pure cultures were cultured on Potato Dextrose Agar (PDA) and characterized based on macroscopic features including colour,

mode of concentric zonation of the culture, rate of sporulation and radial growth. Identification of the *Trichoderma* to species was done CAB international mycological institute staff at the UK. From these, a total of 14 isolates were used. The isolates were cultured for five days in PDA before the interaction studies were undertaken. PDA was prepared following the procedures outlined by the IPDM-TRFK agar preparation manual.

Armillaria Isolates

The *Armillaria* species found to be affecting the tea in Kenya belongs to the species *A. mellea*. Fungal inoculums of *Armillaria* were obtained from two tea growing regions and or sites in Kenya – Tigania and Hamisi. These were sourced from diseased parts of plants showing the evident symptoms of the disease. These were collected in the month of March, 2014. During collection, the samples were stored in cooler boxes and transported to the Integrated Pest and Disease Management Laboratory at the Tea Research Foundation of Kenya where they were stored at refrigerated conditions of 4°C awaiting culturing.

The isolates were cultured on Malt Extract Agar, prepared following the IPDM-TRFK standard culture preparation manual. The isolates were left in culture for five days before they were transferred into nutrient cultures for interaction studies with the different *Trichoderma* isolates.

The setup for the interaction studies was laid in a complete randomized design (CRD) with each replicated three times.

Ecological conditions of Tigania and Hamisi

Armillaria isolates were obtained from two tea growing regions in Kenya namely; Tigania and Hamisi. Tigania is located in Meru County, the central highlands of Kenya and it occupies 108.6 km. The area lies within N 0° 6′19.5" E 037°64" 39.6″ and at the altitude of 935 m above the sea level (ASL). The major agro-ecological zones are Lower Midlands 3 and Upper Midland 3 (LM 3 and UM 3). It experiences an annual average temperature ranging from 19.2°C to 22.9°C. The average annual rainfall varies from 1,000 mm to 2,200 mm with long rainy season between March and June and short rainy season between October and December, respectively (Jaetzold *et al.*, 2006). Hamisi is located in Vihiga County in western Kenya. It is situated at latitude 0.0667° and longitude 34.8000°. The area has an average altitude of 1500 m and a mean annual precipitation of 2000 mm. It experiences and average annual temperature of 25°C.

Data collection and analysis

Data on *Armillaria* and *Trichoderma* association was recorded based on the radial growth on the nutrient agar. This was measured at two day intervals for one month. Data was subjected to Analysis of Variance using M-STAT-C computer program and graphically represented.

RESULTS AND DISCUSSIONS

There was a significant difference ($P = 0.05$) for the different associations of the *Trichoderma* isolates with the two *Armillaria* isolates (5H1 and 9T2). For the 5H1 Isolate, there was a negative interaction for *Trichoderma* isolate T45. This showed that T45 was very antagonistic to the 5H1 *Armillaria* Isolate compared to the other isolates. Minimal effect was observed for the *Trichoderma* isolates TA and T26 where it caused a radial growth of 5+ mm. For 9T2 *Armillaria* Isolate, greater antagonistic effect was observed for the *Trichoderma* Isolate T19. T19 also arrested the radial growth for the same *Armillaria* strain. 9T2 *Armillaria* Isolate seemed to interact with minimal antagonistic for TA, T26, T29 and T13 *Trichoderma* isolates. Therefore, it was evident that the different *Armillaria* isolates interact differently with different isolates of *Trichoderma* (Figure 1).

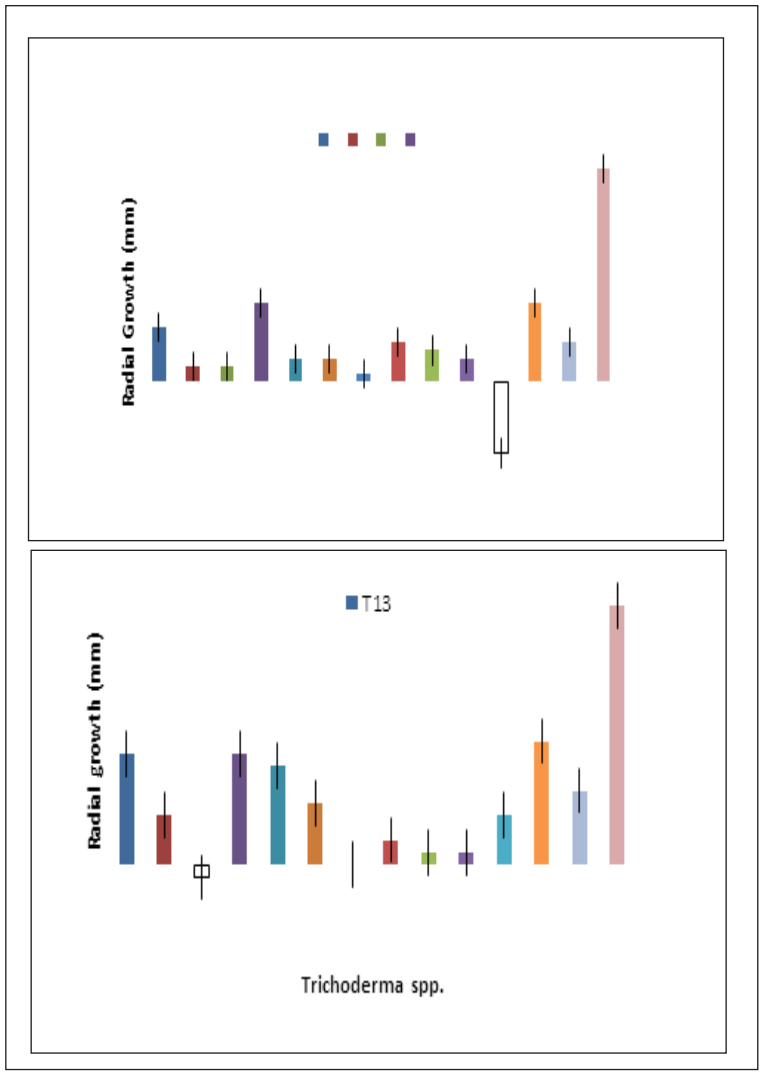

Figure 1: Observed inhibitory effect of metabolites of *Trichoderma* isolates in growth of *Armillaria* isolates within two weeks when two fungi were cultured at the same time. Error bars were used to compare the differences in effect.

After more than a week of interaction, the *Trichoderma* isolates T19 and T45 caused the highest antagonism against *Armillaria* Isolate 9T2. T9 Isolate also arrested the development and radial growth of *Armillaria* 9T2 Isolate. TA, T26 and T29 caused minimal antagonism against *Armillaria* Isolate 9T2. The *Armillaria* strain 5H1 was also arrested for radial growth by Isolate T19. In addition, a high antagonism was observed for the Isolate T45. Minimal antagonism for this *Armillaria* isolate was observed T26.

For both *Armillaria* isolates, *Trichoderma* Isolate T45 showed the highest antagonism while T26 is the poorest in antagonizing *Armillaria*, meaning that it cannot be the best option and strain to be used in controlling *Armillaria* .

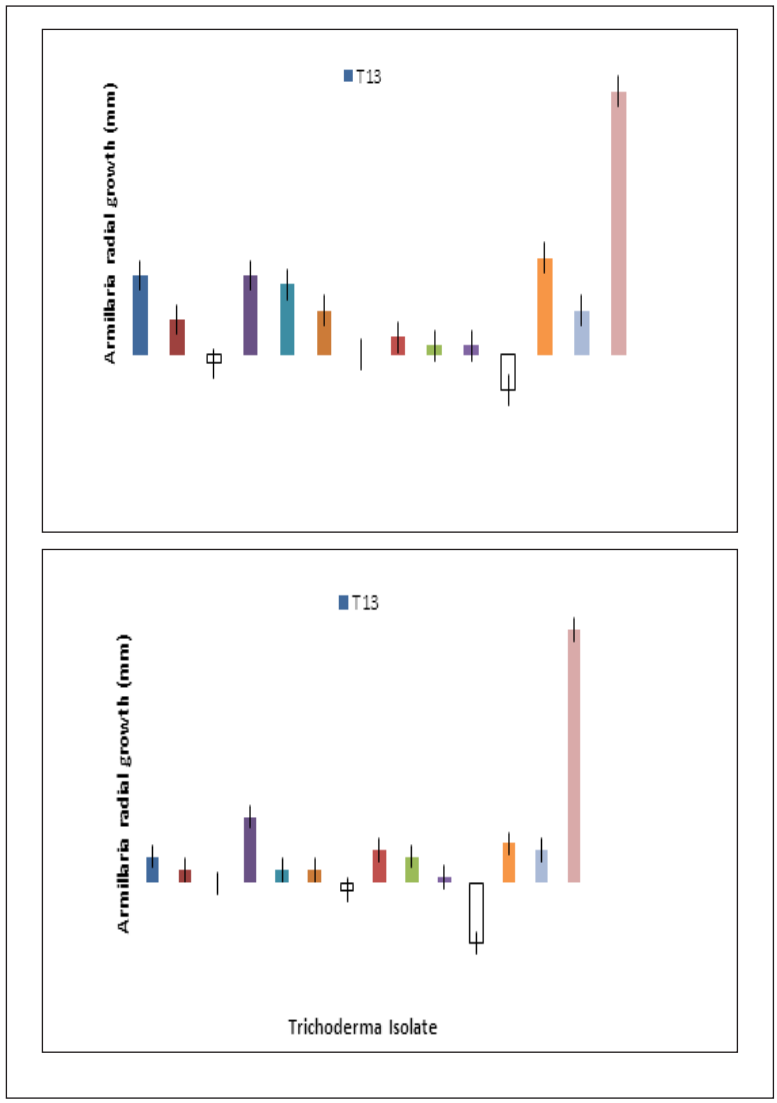

Figure 2: Observed inhibitory effect of metabolites of *Trichoderma* isolates in the growth of *Armillaria* isolates after two weeks when the two fungi were cultured at the same time. Error bars were used to compare the differences in effect.

DISCUSSION

Trichoderma spp. has been globally studied as a biocontrol agent against plant pathogenic fungi. *Trichoderma* antagonism results from different ways including: tolerance to environmental factors (Munnecke *et al.*, 1981); and, ability to degrade soil organic compounds, resistance to inhibitors and metabolic versatility, production of various toxic compounds, antibiotics and enzymes (Bellows, 1996; Howell, 2003). These allow *Trichoderma* to compete, parasitize and antagonize many fungi (Elad and Kapat, 1999).

While *Armillaria* is antagonized by *Trichoderma,* it is a fungus that has the ability to produce antibiotic compounds of resistance and possesses a hyphal component that allows it to colonize and grow very fast. Nonetheless, *Trichoderma* has a faster growth that permits it to overcome and compete more than the *Armillaria* fungus. From the study, all the tested *Trichoderma* isolates were able to cause antagonism on the *Armillaria*. A similar case has been observed against other

fungus like *Sclerotinia sclerotiorum* (Tu, 1980), *Rhizoctonia solani* (Elad *et al.*, 1983), *Rosellinia necatrix* and *Agaricus bisporus* (Cook and Baker, 1983). The current study shows evidence of mycelial impact on the growth of *Armillaria* by the *Trichoderma* fungus. Studies have revealed *Trichoderma* as having the compounds 6-pentyl-α-pyrone (6-PAP), viridin, viridol, gliovirin, heptelidic acid and gliotoxin (Howell *et al.*, 1993) though the production of individual compound varies with the species and type of *Trichoderma*.

CONCLUSION AND RECOMMENDATION

The current study found evidence of negative interaction and antagonism of *Trichoderma* against *Armillaria* fungus. It was observed that some *Trichoderma* isolates perform better than others in reducing *Armillaria*'s spread and growth. Therefore, isolates T45 and T19 should be multiplied and compounded and possibly packaged for use in the control of *Armillaria* fungus.

The researchers recommend that: while *Trichoderma* was antagonistic against *Armillaria*, the variation in the extent of variation might have resulted due to the different compounds responsible for the antagonism. Further studies should focus on characterization of the responsible compounds causing this effect.

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