Inoculum Concentration of *Armillaria mellea* in the Rhizosphere of Intercropped Teak Plantation: the case of the Opro Forest Reserve, Ghana

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ABSTRACT

Teak (*Tectona grandis* Linn. F.) is the most planted timber tree species in Ghana, with over 73,916 hectares of plantation established at the end of 2008. Many of the teak plantations established in the semi-deciduous forest zones were done using the taungya system with various intercrops. Symptoms of *Armillaria* (Vahl: Fr.) root-rot of teak have been reported in the taungya plantations, especially in the semi-deciduous forest zones. The study aimed to determine inoculum concentrations of *Armillaria mellea* in the rhizosphere soil of intercropped teak and compare with sole teak plantation. Mycoflora were isolated from rhizosphere soil of teak intercropped with pepper, okra, maize, yam or cassava at year one to year three sapling stage. Dilution plate technique was used for mycoflora isolation and dilution factor of 10^{-3} was inoculated on potato dextrose agar amended with chloramphenicol (25mg/l) and incubated at 28^0C for 14 days after which *A. mellea* colonies were identified and counted. More *A. mellea* (Vahl: Fr.) colonies were isolated from rhizosphere soils of intercropped teak plantations than non-intercropped. Differences in *A. mellea* colonies were more significant amongst intercrops than amongst age of teak plantation at ($P \leq 0.05$). There were significantly ($P \leq 0.05$) higher numbers of *A. mellea* colonies in rhizosphere soils of teak intercropped with cassava compared with other intercrops. Number of *A. mellea* colonies in rhizosphere soils of intercropped teak did not have linear relation with age of teak plantation. Intercropping could promote *Armillaria* root rot of teak especially with cassava as intercrop.

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Introduction

Teak is a major source of timber in Ghana. Teak wood is profoundly used in the furniture and construction industries in Ghana. It is also used as transmission poles for electric cables, telephone lines and street lights. The teak leaf is known for its medicinal properties (FAO and UNEP, 1981). Teak possesses excellent properties and also has a wide range of uses (Keogh, 1987). Teakwood seasons without splitting, cracking, warping or physically altering shape. It is employed in a wide range of uses such as exterior and interior joinery, window and door frames, flooring, cabinet work, garden furniture, decking, boat building, bridges and railway carriages (Borota, 1991; White, 1991).

Forest plantations in the semi-deciduous forest zones of Ghana are mostly teak. An estimated 73,916 hectares of teak plantations had been established in Ghana by 2008 (FC, 2008). Many of the teak plantations in the semi-deciduous forest zones were established using the taungya system with various intercrops. Taungya system is a means of maximizing land utilization for economic benefits (Djabletey & Adu-Bredu, 2007; Pava, 1993; Lalramnghinglova & Jha, 1996).

In the Opro Forest Reserve of the Offinso forest district of Ghana, teak plantation was established with a planting distance of 3m and intercrop until the teak canopy discouraged intercropping (Owusu, 2011). Five crops namely; cassava, maize, okro, pepper and yam were mainly used as intercrops, majority of the farmers however cultivated maize (Owusu, 2011). The taungya system provided regular maintenance in the plantation due to intercropping activities (Owusu, 2011).

There have been reports of *Armillaria* (Vahl: Fr.) root rot symptoms on teak in plantations in the semi-deciduous forest zones in Ghana. The disease generally ascribed to *Armillaria mellea* has been reported on *Tectona grandis, Pinus elliottii, Acacia albida, Cress sp. Pinus patula* in Zambia, South Africa and Tanzania, Zimbabwe, Kenya, and Ethiopia respectively (Cortzee *et al.,* 2000; Sicoli *et al.,* 2003; Ivory, 1987; Mohammed *et al*., 1989). Adu-Bredu *et al.,* (2008) reported of isolated cases of teak tree die-back in West Africa. Signs of *Armillaria* rhizomorph were observed on the root collar of some four year old and older teak trees in the Opro Forest reserve (Owusu, 2011).

Armillaria is commonly found in most forest soils. Its distribution spans the globe with 42 described species (Fox, 2000). The fungi attack about 700 plant species, mostly woody plants. Woody plants that have previously been weakened by drought, flooding, poor drainage, frost, repeated defoliation by insects or diseases, other poor soil conditions, excessive shade, polluted air or other chemical injury, or mechanical injury are most susceptible to attack (Hood *et al*., 1991). *Armillaria* is also a known killer of pine, spruce, and fir, especially in plantations where inoculum centres exist prior to planting (Hood *et al*., 1991). *Armillaria* is also known to cause root rot in cassava, okra, pepper, cocoyam, citrus, cocoa, coffee, mango, oil palm and coconut in Ghana (Oduro, 2000).

The loss of fine feeder roots from *Armillaria* root-rot disease deprives affected plants of sufficient nutrients and water, and often results in branch dieback and stag head (Hood *et al*., 1991). The fungus can be of considerable importance in the final death of weakened trees and shrubs (Hood *et al*., 1991). Serious radial and terminal growth reduction of affected plants may occur (Hood *et al*., 1991). *Armillaria* root rot of teak could pose a threat to timber production in Ghana.

The study aimed at identifying intercrops that could predispose teak to *Armillaria* root rot infection in the taungya plantations.

Materials and methods

The study was conducted at the Opro Forest Reserve of the Offinso Forest District in Ashanti Region of Ghana, and at the Plant Pathology laboratory of the Department of Crop and Soil Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The Opro Forest Reserve is located in the dry semi-deciduous forest zone of Ghana. The area has mean annual rainfall of between 1000 mm and 1500 mm. The major rainy season starts from middle of March to July, and the minor season from August to November. The Reserve is approximately 128.94 square kilometers and located between (1° 53'7.078"W, 7° 5'58.111"N and 1°44'37.903"W, 7°15'8.202"N.)

Experimental design

Randomized complete block design was used. There were six treatments and three blocks. The treatments were teak taungya plantations with different intercrops (cassava, yam, okro, maize, and pepper) and non-intercropped teak plantation as a control. The blocks were taungya plantation of different ages (One year old, two year old and three year old). Each block was replicated four times. An Experimental plot of 225 m^2 size which contained 25 teak saplings was randomly selected in the plantation for each treatment. Rhizosphere soils samples were collected from 10 randomly sampled teak saplings within each plot.

Sampling of teak sapling for rhizosphere soil

Rhizosphere soils of the teaks saplings were collected within 20 cm radii from the base and at a depth of 15cm. The soil samples were bulked mixed thoroughly and then sub sampled for each treatment. The Sub samples were then transferred to the laboratory in paper envelopes. The soil samples were air-dried at 25°C for seven days and then examined for mycoflora.

Preparation of culture medium

Potato Dextrose Agar was the medium used to isolate and culture mycoflora of the rhizosphere soil of teak trees. The medium was prepared from Irish potato tubers obtained from a local grocery in Kumasi. The tubers were peeled and cut into pieces and washed clean with tap water. A 200 g weight of the washed peeled potato tuber pieces was put into 2500 ml. Pyrex beaker and 500 ml of distilled water was added and then boiled on a hot plate stove until the potato pieces became very soft. The supernatant was strained through a cheese cloth into a 2500 ml flat bottom flask and then 20 g dextrose and 20 g agar were added and whirled to mix. The potato extract in the 2500 ml flat bottom flask was topped with distilled water to 1000 ml and amended with chloramphenicol (25 mg/l) to check bacterial contaminations and then stoppered with cotton wool and autoclaved at 121°C, 15 psi for 15 minutes to sterilize.

Isolation of mycoflora of teak rhizosphere soil

Isolation of mycoflora from rhizosphere soils was done using the dilution plate techniques (Watanabe, 2000). Ten grams of the air-dried rhizosphere soil was added to 100 ml of distilled water in a 500 ml conical flask and was shaken by the hand for 10 min. This was taken as dilution 10^{-1}. Using a sterile pipette, 1 ml of the dilution 10^{-1} suspension was transferred into a test tube with 9 ml of distilled water to obtain the dilution 10^{-2}. One ml of the dilution 10^{-2} suspension was transferred into a test tube with 9 ml of distilled water to obtain dilution 10^{-3}. Using a sterile pipette, 0.5 ml of dilution 10^{-3} suspension was pipetted and inoculated on a 9 cm Pyrex Petri dish which contained potato dextrose agar medium amended with chloramphenicol (25 mg/l) and gently swirled to spread the water suspension on the medium. Isolation of mycoflora from teak rhizosphere soil was conducted on one-year-old, two-year-old and three-year-old teak trees for each treatment. The Pyrex Petri dishes were then kept under ambient conditions in the laboratory for 14 days. Identification was done with the aid of a compound microscope (x400) and standard identification manuals (Booth, 1971; Barnett & Hunter, 1972; Watanabe, 2000). Frequency of *A. mellea* colonies was scored using colony counter.

Statistical analysis

Data were transformed using square root transformation where applicable and subjected to two-way Analysis of Variance (ANOVA). GenStat package software version 12 was used for the analysis. The Duncan's multiple range test was used to separate the differences between means at 5% level of significance.

Results and Discussion

Thirteen fungal species which *included Armillaria mellea, Mucor hiemalis, Aspergillus niger, Aspergillus candidus, Aspergillus flavus, Aspergillus ochraceus, Aspergillus tamari, Aspergillus versicolor, Fusarium verticilliodes, Lasiodiplodia theobromae, Penicillium spp, Rhizopus spp and Trichoderma viride* were isolated from the sampled teak rhizosphere soils. The isolated fungi were identified as members of fourphyla namely; Basidiomycota, Deuteromycota, Phycomycota and Zygomycota. This paper discusses the occurrence of *Armillaria mellea* of the Basidiomycota.

Armillaria mellea colonies were isolated from teak rhizosphere soil of the plantations with the intercrops and from those with no intercrops. However, more colonies were isolated from teak with intercrops as shown in figure 1 below. *Armillaria* occurrence is worldwide in natural forests and on planted woody crops

(Hood, *et al.,* 1991; Kile, *et al.,* 1991; Termorshuizen, 2000).

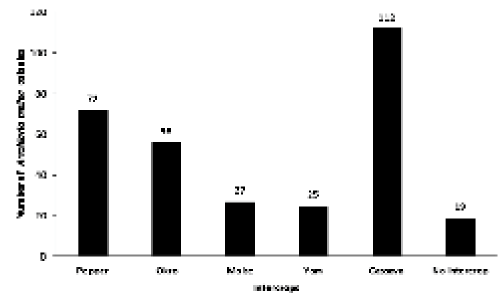

Fig. 1. Number of *Armillaria mellea* colonies isolated from rhizosphere soil of teak of different intercrops.

A total of 125 colonies of *Armillaria mellea* was isolated from rhizosphere soil of one-year-old teak in all the treat-ments. Teak intercropped with cassava recorded the highest mean colony number of 50 followed by pepper as an intercrop with a mean of 29 colonies. Teak intercropped with Yam recorded the least mean colony number of 5.

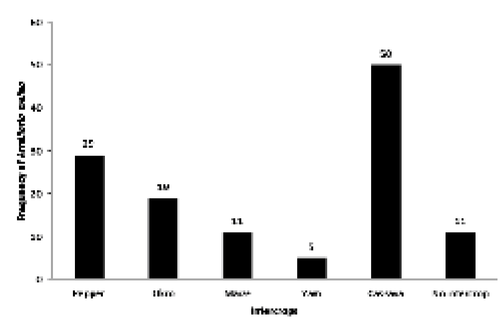

Fig. 2. Number of *Armillaria mellea* colonies isolated from rhizosphere soil of one year teak plantation.

Eighty-five *A. mellea* colonies were isolated from rhizosphere soil of two-year-old teak plantation. Most *A. mellea* colonies were isolated from teak intercropped with cassava, which recorded a mean of 34 colonies followed by pepper with 21 colonies. Teak without intercrops had no *A. mellea* colony in rhizosphere soil.

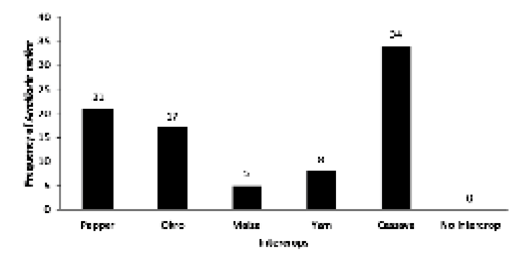

Fig. 3. Number of *Armillaria mellea* colonies isolated from rhizosphere soil of two year old teak plantation

Out of the 101 colonies of *A. mellea* isolated from rhizosphere soil of three-year-old teak plantation, 28 colonies were from teak intercropped with cassava and 22 from teak intercropped with pepper. Teak with no intercrop had the least average *A. mellea* colonies of 8.

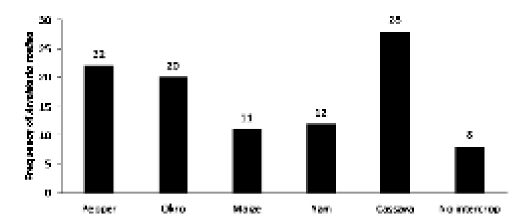

Fig. 4. Number of *Armillaria mellea* colonies isolated from rhizosphere soil of three year old teak plantation.

TABLE 1

Frequency of Armillaria mellea colonies from teak rhizosphere soils in year one to three plantations.

Mean number of Armillaria mellea colonies			
Intercrops	*one*	*two*	*three*
Pepper	29b	21b	22b
Okro	19c	17c	20b
Maize	11d	5d	11c
Yam	5e	8d	12c
Cassava	50a	34a	28a
No intercrop	11d	0e	8c
LSD (P ≥ 0.05)			

Note: Numbers with the same letter in a column are not significantly different at (P ≥ 0.05) according to Duncan's Multiple Range test.

More *A. mellea* colonies were isolated from rhizosphere soils of intercropped teak plantations than non-intercropped. Differences in *Armillaria mellea* colonies were more significant amongst intercrops than amongst age of teak plantation at (P ≤ 0.05). There were significantly (P ≤ 0.05) higher numbers of *A. mellea* colonies in rhizosphere soils of teak intercropped with cassava compared with other intercrops. Higher numbers of *A. mellea* colonies in teak with intercrops suggested that the intercrops promoted *Armillaria* infection of teak in the teak taungya plantations in the Opro Forest Reserve.

Intensive intercropping in the first year of plantation establishment causes severe disturbance to the land and depletes the soil of its nutrients and water resources. The resources depletion could create stressful conditions for teak. Intercropping is reduced in older plantation because of larger teak canopy. *Armillaria* species have been regarded as stress-induced secondary invaders (Wargo & Shaw, 1985; Shaw & Kile, 1991; Morrison *et al*., 1992; Gregory *et al*., 1991). This could be why more *A. mellea* colonies were isolated from rhizosphere soils of one year old teak plantation than the older plantations.

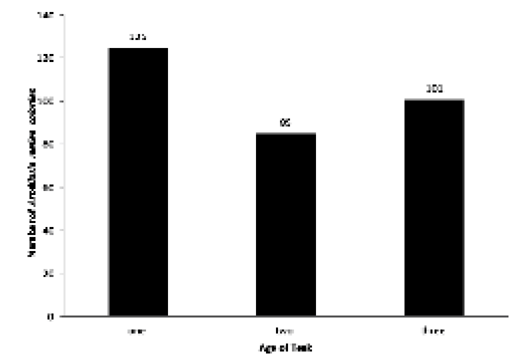

Fig. 5. Number of *Armillaria mellea* colonies isolated from rhizosphere soil of teak of different ages.

Conclusion

The study has shown that intercropping increased *Armillaria mellea* inoculum concentration in the rhizosphere soil of teak in the taungya plantation at the Opro Forest Reserve. Intercropping with cassava promoted proliferation of *A. mellea* the most. Therefore, intercropping could promote *Armillaria* root rot of teak especially with cassava as an intercrop.

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