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Growth responses of okra (*Abelmoschus esculentus*) to inoculation with *Trichoderma viride*, mancozeb and *Sclerotium rolfsii* in sterile and non-sterile soils

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Abstract

Okra (*Abelmoschus esculentum*) is an annual vegetable that is cultivated in the tropics and subtropics. Infestation by *Sclerotium rolfsii* can cause serious economic losses to farmers. Therefore, the curative effect of *Trichoderma viride* and mancozeb on *S. rolfsii* on okra plants was monitored in the green house in both non-sterile and sterile soils. Okra cultivated in non-sterile soils were healthier and stronger than those in sterile soils although plants were taller in sterile soil. Generally, the fresh weight of okra leaf, root and stem were higher in non-sterile soil than in sterile soil. The fruit yields of okra in non-sterile soil were higher than those of the sterile soil, irrespective of the treatments. Based on the results of this investigation, it is not recommended to use sterile soil if one is interested in yield of the plant.

Key words – mancozeb – okra plants – *Sclerotium rolfsii* – *Trichoderma viride*

Introduction

Okra (*Abelmoschus esculentum* L.) is an annual herb and vegetable crop grown throughout the tropics and subtropics (Emuh et al. 2006, Sergius & Esther 2014). Okra plays an important role in the human diet by supplying carbohydrate, protein, fats, minerals, vitamins and bioactive compounds (Doreddula et al. 2014, Roy et al. 2014). The fruits also have antidiabetic, anti-inflammatory, and antimicrobial properties (Doreddula et al. 2014).

Sclerotium rolfsii is an important soil-borne phytopathogenic fungus found in tropical and subtropical regions of the world (Buensanteai et al. 2012). It has a wide host range e.g., potato, groundnut, soybean, sunflower, chilli, tomato, cotton, lucerne, wheat, onion (Sarma 2002, Ansari 2005, Maurya et al. 2007, Maddu & Ravuri 2015) and can cause serious yield loss (Rupe 1999).

Sclerotium rolfsii is difficult to control without the use of synthetic fungicides. However, high level application of synthetic fungicides in agriculture has the potential to cause toxic effects on humans and wildlife as well as to cause environmental pollution (Heydari 2007, Faruk 2015). Furthermore, the cost of fungicides, particularly in developing countries, and demand for fungicide-free food, has necessitated a search for substitutes (Vogt et al. 2010).

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Biological control treatment provides long lasting and economical disease protection essentially through production of antibiotics, cell wall degrading enzymes, induction of host resistance and competition with the pathogen for nutrients and space (Daayf et al. 2003, Anand & Reddy 2009). Recent study by Ekundayo et al. (2015) has shown the *in vitro* efficacy of *Trichoderma viride* and mancozeb on *S. rolfsii*. This present investigation was therefore conducted to determine the pathogenic effect of *S. rolfsii* on okra plants, and to seek ways of controlling the infection using *T. viride* and mancozeb, a chemical pesticide in sterile and non-sterile soils in the green house.

Materials & Methods

Sclerotium rolfsii and *Trichoderma viride* were obtained from the Departments of Crop Soil and Pest Management and Microbiology, Federal University of Technology, Akure (FUTA). The isolates were maintained on potato dextrose agar plates at room temperature and were regularly subcultured until use.

Planting of okra seeds in sterile and non-sterile soils in the green house

Soil samples collected from the teaching and research farm of the Federal University of Technology, Akure were sieved to remove stones and other debris. Half of the soil samples were then sterilized for 30 minutes (two times consecutively) using a vertical heating pressure steam autoclave. The other soil sample was left unsterilized. Forty-two pots were used in total and each pot contained 5 kg of soil to which 800 ml of sterile water was added. Seeds of okra were surfaced sterilized by soaking in 1% sodium hypochlorite for 1 minute, then 3% ethanol for 3 minutes and then rinsed in several changes of sterile water. The seeds were thereafter allowed to air dry in the laboratory. Three seeds were planted at the depth of 5 cm in each pot. The experiment was carried out in a temporary greenhouse behind the Department of Microbiology, Federal University of Technology, Akure.

Inoculation of *S. rolfsii, T. viride* and mancozeb

Both the pathogen and antagonist were prepared using cultures incubated for 10 days on malt extract agar medium at 28^{o}C**.** They were then mixed separately with sterile water after which sterile spatula was used to scrape the mycelium. Fifteen days after planting, *S. rolfsii*, *T. viride* as well as fungicide (mancozeb) were applied in different treatments as follows: (i) control, (ii) *T. viride* alone, (iii) *S. rolfsii* alone, (iv) mancozeb alone, (v) *S. rolfsii* + *T. viride* + mancozeb, (vi) *S. rolfsii* + mancozeb, and (vii) *S. rolfsii* + *T. viride*. Both the pathogen and antagonist were added to the soil in the pots with okra plants and each soil received 20 ml of the inoculum (de Figueirêdo et al. 2010). The control received 20 ml of sterile water only. The fungicide was applied below recommended rate (0.2 g/100 ml). Each treatment was replicated in three pots. The plants were watered regularly to prevent drying.

Measurements of the growth parameters after inoculation

Plant heights of the above ground stem portion of the seedlings were measured every 10 days after inoculation till harvesting of the fruits was done. Also, leaf and fruit numbers were counted. Symptoms of disease were also noted. Forty-two days after planting, the fresh weights of stem, root and leaf were determined. The numbers of fruit were counted and their fresh weight determined. Dry weight of both fruits/leaves was recorded after 5 days of drying in an oven. The proximate composition of the fruits under different treatment was also analyzed. The data obtained were subjected to statistical analysis and the means were then separate using Duncan range multiple test.

Results

Comparative effects of *T. viride, S. rolfsii* and manczeb on the growth and yield of okra in sterile and non-sterile soils

At the initial stage, the percentage emergence of okra seedlings in sterile soils was higher than that of non-sterile soil. With time, okra plants cultivated in non-sterile soils were healthier than sterile soils although their height in non-sterile soils was lower than those in sterile soils (esttydayo2010@yahoo.com 1). The tallest plants were in sterile soil treated with *T. viride*. This was followed by sterile soil without inoculation (control). However, the lowest was obtained from non-sterile soil treated with mancozeb after 45 days.

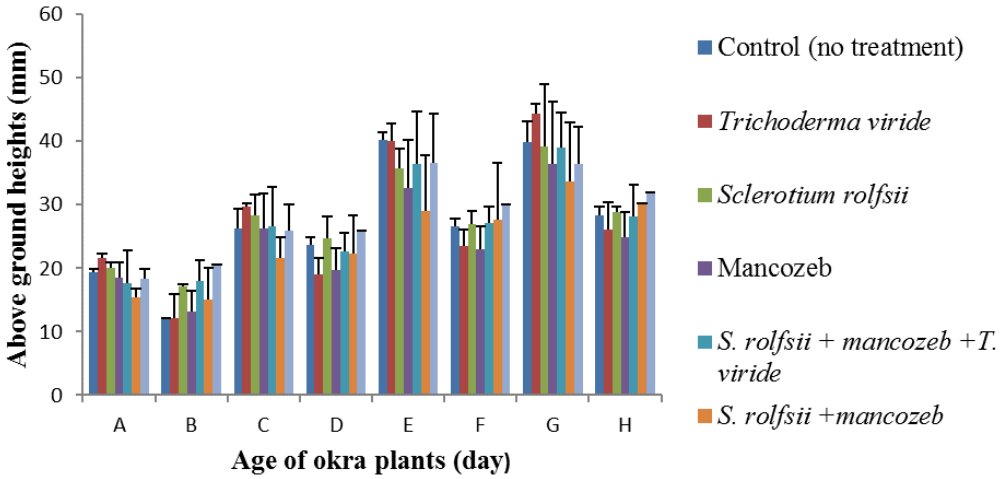

Fig 1 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on above ground heights of okra plants cultivated on sterile and non-sterile soils during experimental trial. Keys, A, Above ground height of okra plants at day 20 after treatments in sterile soils. B, Above ground height of okra plants at day 20 after treatments in non-sterile soils. C, Above ground height of okra plants at day 30 after treatments in sterile soils. D, Above ground height of okra plants at day 30 after treatments in non-sterile soils. E, Above ground height of okra plants at day 40 after treatments in sterile soils. F, Above ground height of okra plants at day 40 after treatments in non-sterile soils. G, Above ground height of okra plants at day 45 after treatments in sterile soils. H, Above ground height of okra plants at day 45 after treatments in non-sterile soils.

Generally, the fresh weights of leaf, root and stem were higher in non-sterile soil than sterile soil except for stem weight of *T. viride, S. rolfsii* as well as *S. rolfsii* + mancozeb + *T. viride* (Fig 2). The highest fresh weight of leaf was obtained from soil without treatment while the least root weight was obtained from the same soil in non-sterile soil.

Similar trend was also observed for the dry weight (Fig 3). The fresh weight of okra fruits in non-sterile soils was higher than that of sterile soil. The highest value was obtained from *S. rolfsii* as well as *S. rolfsii* + *T. viride* treated plants while the least was obtained from *S. rolfsii* + mancozeb treated plants (Fig 4). It was observed in all the treatments that the number of leaves in sterile soils was lower than that of non-sterile (Fig 5).

Figures 6 to 11 show the comparative analysis of the different proximate composition of okra fruits in sterile and non-sterile soils. The highest moisture and fat contents were obtained from sterile soil sample treated with *S. rolfsii* + *T. viride* and *T. viride* (Fig 6 & 7) Also, the highest ash content of okra fruit was obtained from non-sterile soil sample without treatment (Fig 8). The carbohydrate content of okra fruits obtained from mancozeb treated soil sample was higher in non-sterile soil sample than all other treatments (Fig 9). However, the protein contents of all the okra fruits in sterile soil samples were higher than those obtained from non-sterile soil samples except *S. rolfsii* + mancozeb + *T. viride* as well as *S. rolfsii* + *T. viride* treated soils. Similar values were obtained from *T. viride* treated soil both in sterile and non-sterile conditions (Fig 10).

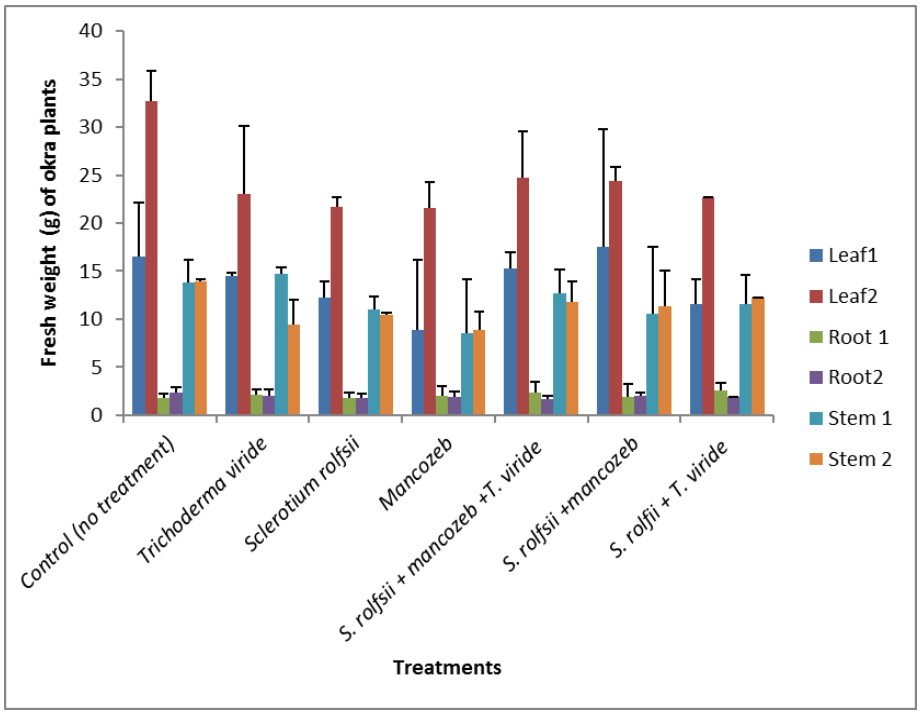

Fig 2 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on fresh weight of okra plants cultivated on sterile and non-sterile soils during experimental trial. Keys, Leaf 1, Leaf weight of sterile soil; Leaf 2 – Leaf weight of non-sterile soil. Root 1, Root weight of sterile soil; Root 2, Root weight of non-sterile soil. Stem 1, Stem weight of sterile soil. Stem 2, Stem weight of non-sterile soil. Leaf 2, Leaf weight of sterile soil. Leaf 2, Leaf weight of sterile soil. Root 2, Root weight of sterile soil. Root 2, Root weight of sterile soil. Stem 2, Stem weight of sterile soil; Stem 2 – Stem weight sterile soil.

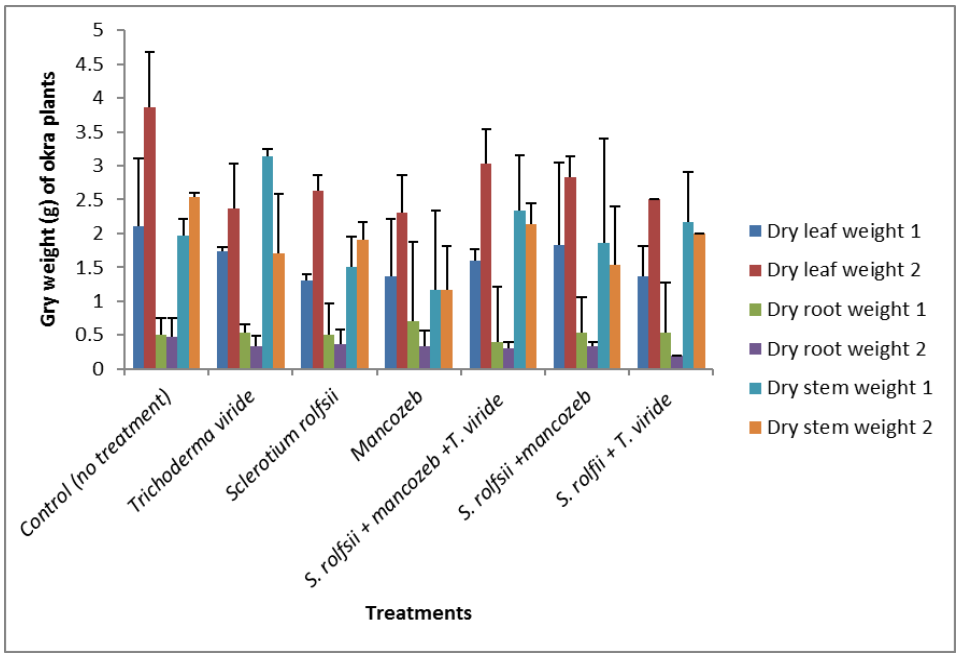

Fig 3 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on dry weight of okra plants grown on sterile and non-sterile soils during experimental trial

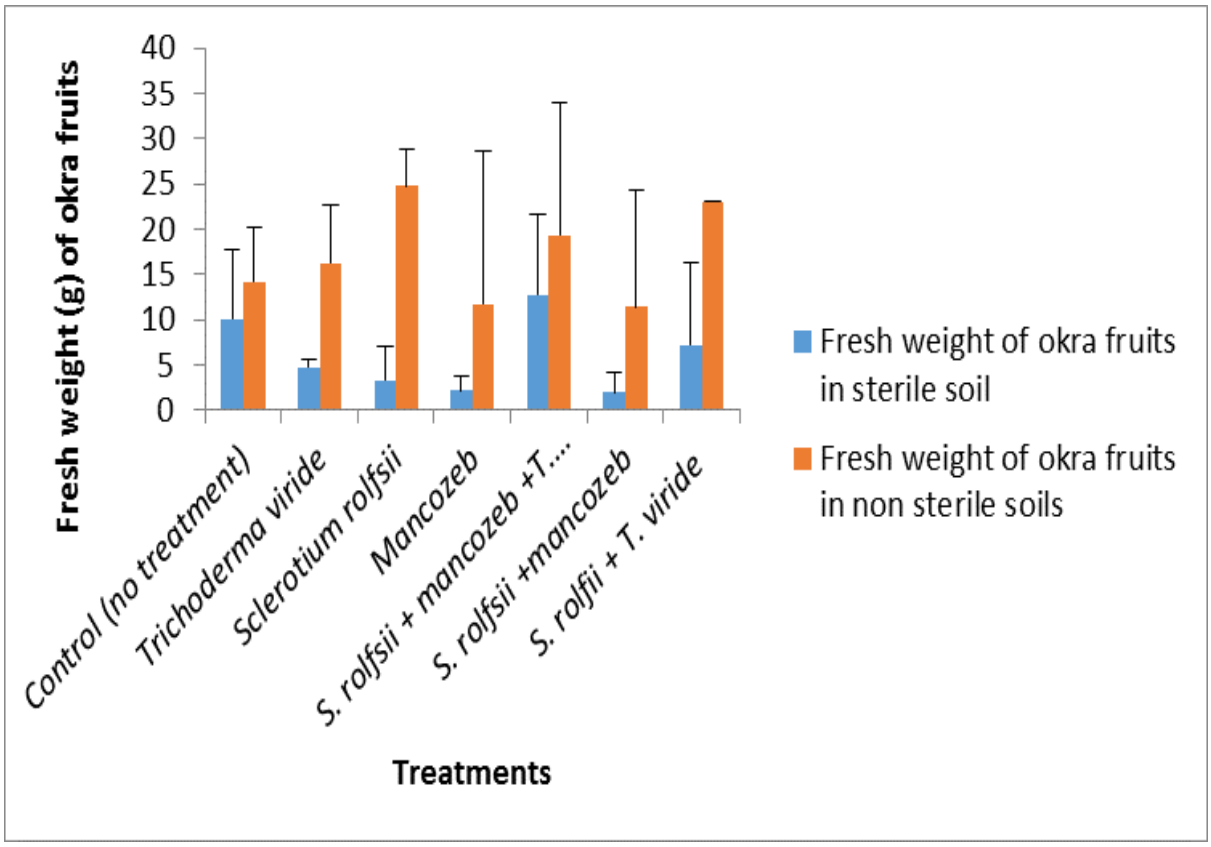

Fig 4 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on fresh weight of okra fruits grown on sterile and non-sterile soils during experimental trial.

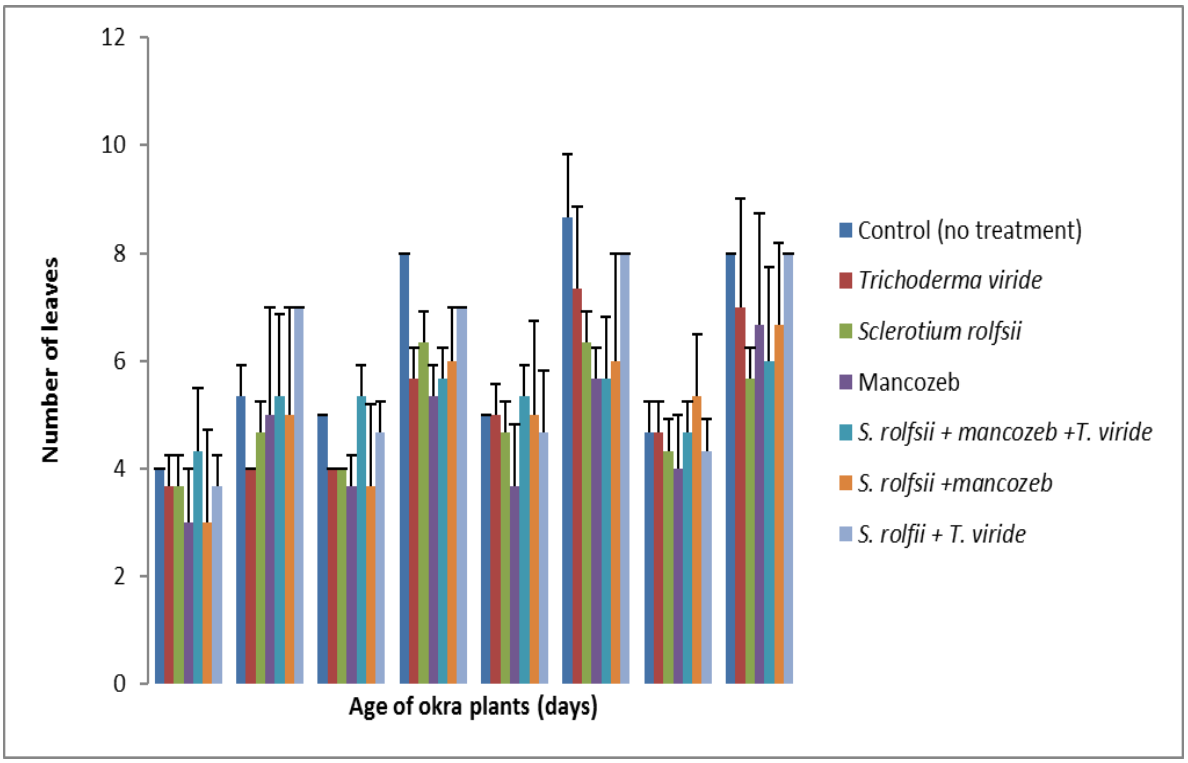

Fig 5 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on leaf numbers of okra plants grown on sterile and non-sterile soils during experimental trial.

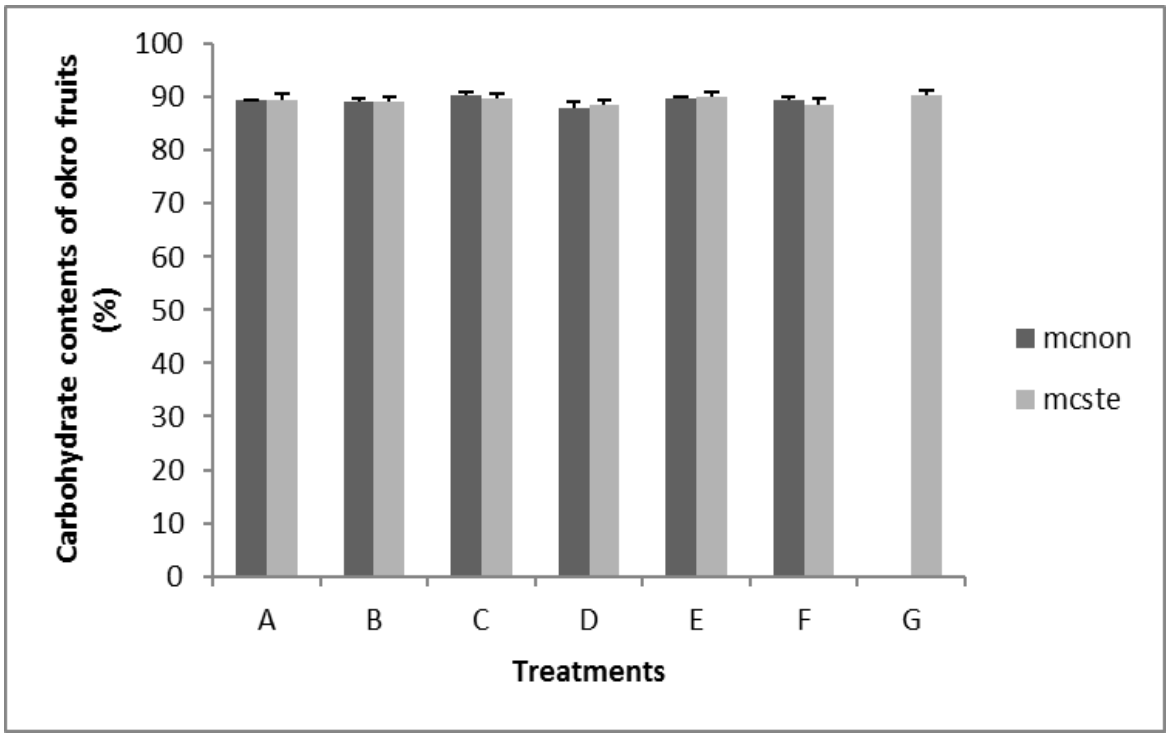

Fig 6 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on the moisture content of okra fruits harvested from sterile and non-sterile soils. Keys, A, Control (no treatment). B, *Trichoderma* viride. C, *Sclerotium* rolfsii. D, Mancozeb. E, *S. rolfsii* + mancozeb + *T. viride*, F, *S. rolfsii* + mancozeb. G, *S. rolfsii* + *T. viride*, Mcnon, Moisture contents of okra fruits in non-sterile soils. Mnste, Moisture contents of okra fruits in sterile soils.

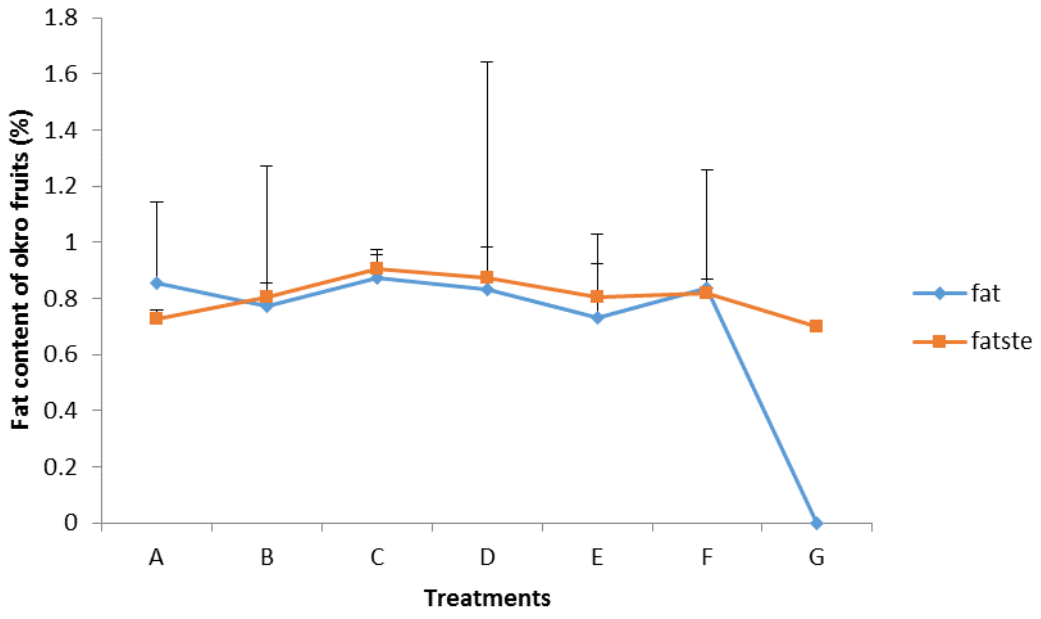

Fig 7 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on the fat content of okra fruits harvested from sterile and non-sterile soils. Keys, A, Control (no treatment). B, *Trichoderma viride*. C, *Sclerotium rolfsii*. D, Mancozeb. E, *S. rolfsii* + mancozeb + *T. viride*. F, *S. rolfsii* + mancozeb. G, *S. rolfsii* + *T. viride*. fat, Fat contents of okra fruits in non-sterile soils. fatste, Fat contents of okra fruits in sterile soils.

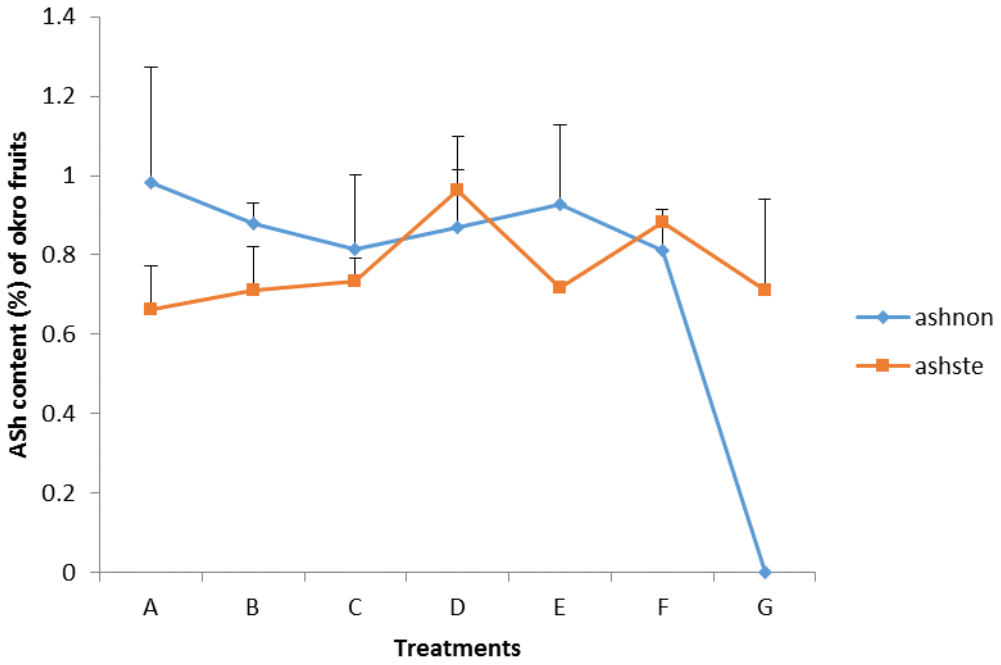

Fig 8 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on the ash content of okra fruits harvested from sterile and non-sterile soils. Keys, A, Control (no treatment). B, *Trichoderma viride*. C, *Sclerotium rolfsii*. D, Mancozeb. E, *S. rolfsii* + mancozeb + *T. viride*. F, *S. rolfsii* + mancozeb. G, *S. rolfsii* + *T. viride*. ashnon, Ash contents of okra fruits in non-sterile soils. ashste, Ash contents of okra fruits in sterile soils.

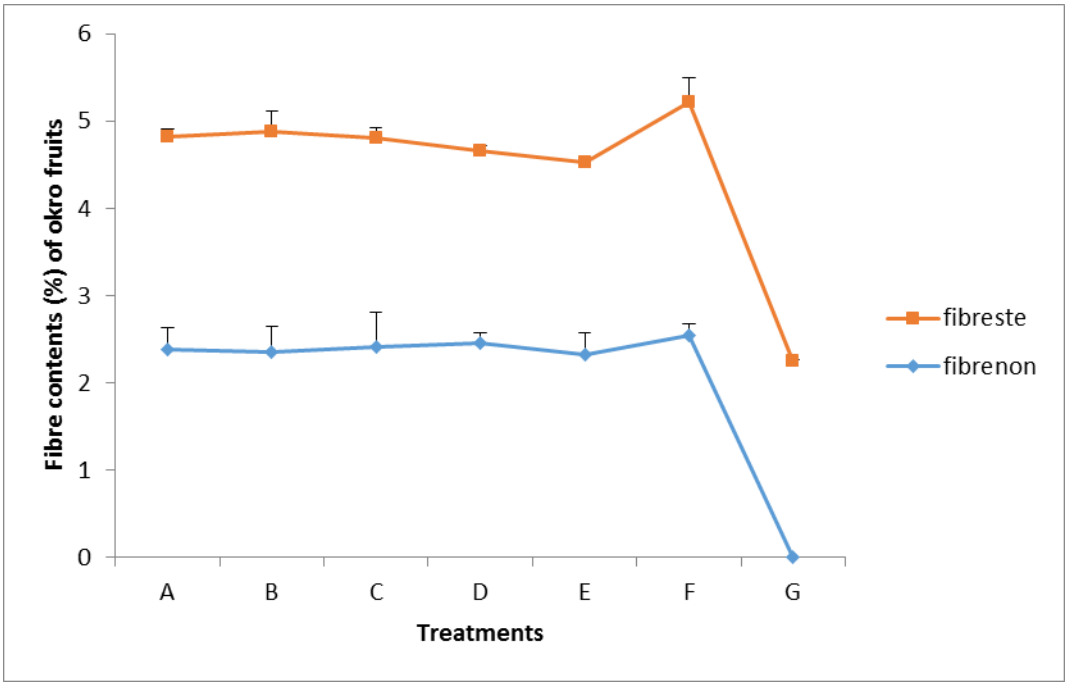

Fig 9 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on the fibre content of okra fruits harvested from sterile and non-sterile soils. Keys, A, Control (no treatment). B, *Trichoderma viride*. C, *Sclerotium rolfsii*. D, Mancozeb. E, *S. rolfsii* + mancozeb + *T. viride*. F, *S. rolfsii* + mancozeb. G, *S. rolfsii* + *T. viride*. fibrenon, fibre contents of okra fruits in non-sterile soils. fibreste, fibre contents of okra fruits in sterile soils.

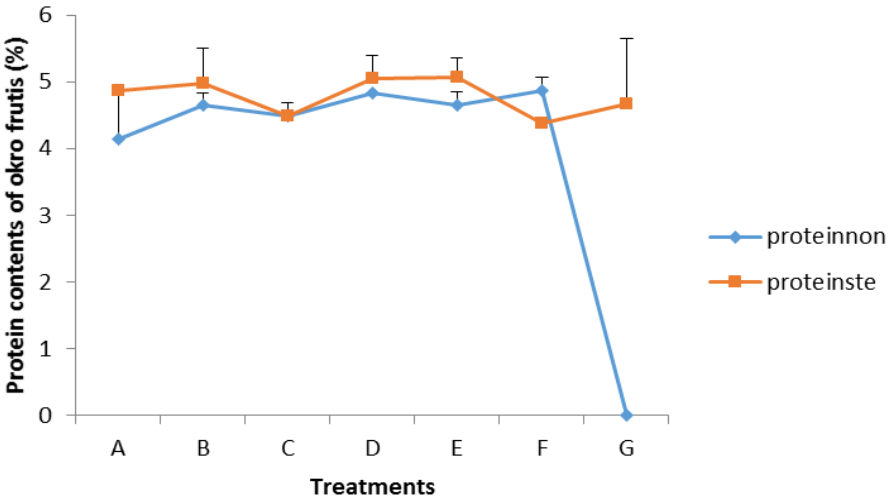

Fig 10 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on the protein content of okra fruits harvested from sterile and non-sterile soils. Keys, A, Control (no treatment). B, *Trichoderma viride*. C, *Sclerotium rolfsii*. D, Mancozeb. E, *S. rolfsii* + mancozeb + *T. viride*. F, *S. rolfsii* + mancozeb. G. *S. rolfsii* + *T. viride*. proteinnon, protein contents of okra fruits in non-sterile soils. proteinste, protein contents of okra fruits in sterile soils.

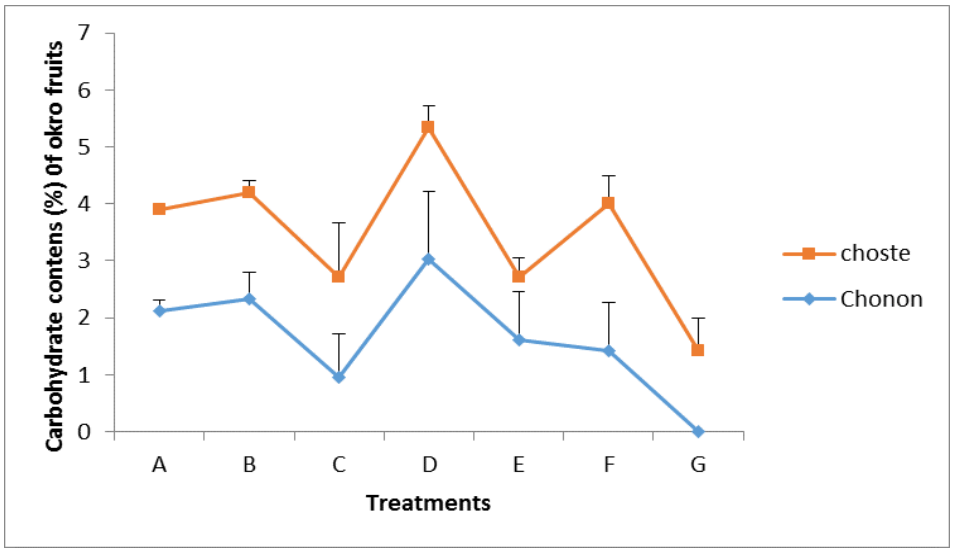

Fig 11 – Comparative effects of *T. viride*, mancozeb and *S. rolfsii* on the carbohydrate content of okra fruits harvested from sterile and non-sterile soils. Keys, A, Control (no treatment). B, *Trichoderma viride*. C, *Sclerotium rolfsii*. D, Mancozeb. E, *S. rolfsii* + mancozeb + *T. viride*. F, *S. rolfsii* + mancozeb. G, *S. rolfsii* + *T. viride*. chonon, carbohydrate contents of okra fruits in non-sterile soils. choste, carbohydrate contents of okra fruits in sterile soils.

Discussion

About 30% of plants are destroyed by plant pathogens of which fungi are the most common. These diseases are often controlled by the use of fungicides which are known to be persistent in the environment (Talla et al. 2015). Control of soil-borne pathogens by the addition of antagonistic microorganisms to the soil is also a potential non-chemical means for plant disease control. (Shahanaz et al. 2008). Among fungi, *Trichoderma* species are the most successful biofungicides and about 60% have been commercialized (Verma et al. 2007). The antagonistic activities of

Trichoderma spp. against many soil-borne plant pathogens have been established (Harman et al. 2004, Murkerjee et al. 2012, Faruk 2015).

Control of soil-borne pathogens by the introduction of antagonistic microorganisms to the soil is a potential means of controlling plant diseases (Shahanaz et al. 2008). There was 100% seed germination in okra seeds cultivated on sterile soils irrespective of the treatment. Similar observation has been made by Williams-Linera & Ewel (1984). It was also noted that there was increase in shoot length of okra of *T. viride* treated soil, in agreement with the report of Shahnaz et al. (2008). Qin et al. (2014) observed a significant increase in seedling height of *Cerasus sachalinensis* with inoculation of biological agent. However, there were varied results in the assessment of other growth parameters. This may be due to the fact that there was nutrient depletion as a result of soil sterilization by autoclaving (He & Cui 2009) resulting in manganese toxicity (Williams-Linera & Ewel 1984). Soil sterilization is known to alter the physicochemical properties of soil, plant growth and community structure of newly developed microbes (Mahmood et al. 2014).

Plant growth and development have been shown to be affected by soil environment which is rich in soil microorganisms (Qin et al. 2014). Generally, there was seedling mortality in non-sterile soils but with increase in planting days, the plants were healthy irrespective of the treatment. This may not be unconnected with the synergistic roles of the indigenous microorganisms of the soil in question. Carvalhais et al. (2013) reported that plants grown in the presence of whole soil microbial communities exhibited enhanced shoot growth when compared to plants cultivated on sterile soil. This is also consistent with related studies by Persello-Cartieaux et al. (2001) and Ryu et al. (2005). These soil microbes promote plant growth and/or improve health by a variety of mechanisms including phosphate solubilization (Richardson et al. 2009), IAA production (Spaepen & Vanderleyden 2011), siderophore biosynthesis (Dey et al. 2004), antibiotics production (Chen et al. 2009, Scholz et al. 2011), ACC deaminase activity (Glick et al. 2007, Siddikee et al. 2010), increase in photosynthetic efficiency (Zhang et al. 2008) and induction of systemic resistance (Wang et al. 2009, Phi et al. 2010, Zamioudis & Pieterse 2012) in plants.

It was also observed that the yield of okra was more in non-sterile soils compared to sterile soils. This may be due to the fact that some of the nutrients required for plant growth have been lost during sterilization. It has been stated that manipulation of soil biota, such as soil sterilization, may have complex effects as they alter soil properties as well as microorganism communities (He & Cui 2009). Troelstra et al. (2001) observed that sterilization results in nutrient flushes due to its killing ability on microbes. Also, sterilization may decrease plant growth, which in turn decreases nutrient uptake by plants. The effect of sterilization may depend on the nature of the soil (He & Cui 2009). The presence of other beneficial microorganisms in non-sterile soil samples might have led to the high yield of okra. Microbes and plants have been shown to establish a multitude of interactions with one another which may be beneficial or detrimental (Carvalhais et al. 2013). These microorganisms might have helped in the solubilization of phosphates for plant uptake (Richardson et al. 2009).

In view of the results of the present investigation, it is necessary to introduce blends of antagonistic microorganisms for use in controlling different isolates of *S. rolfsii.* The biological control efficiency of *T. viride* could be enhanced by either physical of chemical mutation provided the mutants will not have adverse effect on non-target organisms. Although sterilization of the soil samples retarded the yield of okra in this research, this procedure can be used safely for preparing soil for uses that do not require evaluation of growth.

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