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Antifungal activities of *Trichoderma viride* and two fungicides in controlling diseases caused by *Sclerotium rolfsii* on tomato plants

[1*]Ekundayo E. A., [2]Ekundayo F. O. and [2]Osinowo I. A.

[1]Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Nigeria
[2]Department of Microbiology, Federal University of Technology, Akure, Nigeria

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ABSTRACT

Fungal phytopathogens are among the biotic factors that cause serious losses to agricultural crops. The present investigation was aimed at determining the antagonistic effects of three isolates of Trichoderma viride as well as two fungicides against Sclerotium rolfsii causal agent of southern blight of tomato. Also, the efficacy of biocontrol of T. viride on the pathogenicity of Sclerotium rolfsii on tomato plant was compared with the influence of chemical control using fungicide (mancozeb) in this study. Sterile soil sample treated under different conditions were packed inside thirty polyethylene pots and two tomato (Lycopersicon esculentum) seedlings were planted on them. This experimental set up was carried out in a completely randomized design with three replications. Plant height and plant leaves were recorded at interval of ten days for thirty days of growth while fresh weight of plant and root, dry weight of the plant were recorded after thirty days of growth. The antagonistic activities of the three isolates of T. viride were more pronounced at 37^{o}C and pH 4. Trichoderma viride obtained from ginger soil proved very effective in controlling the growth of S. rolfsii but combination of T. viride and mancozeb could be detrimental to tomato plant.

Keywords: *Trichoderma viride, Sclerotium rolfsii*, mancozeb, biocontrol, tomato plant

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INTRODUCTION

Tomato has been on cultivated globally for its fleshy fruits, special nutritive value and protective properties (Hadizadeh *et al*., 2009). It is the world's largest vegetable crop after potato and it tops the list of canned vegetables (Omara, 2010). Tomatoes have been adversely affected by a lot of pests including microorganisms (Howell *et al*., 2003). The conditions suitable for growth and development of the crop are also favourable for the quick development, proliferation and spread of disease. *Sclerotium rolfsii* is a soil borne phytopathogenic fungus that causes diseases of most agricultural crops **(**Fouzia and Saleem, 2005; Kokub *et al*., 2007; Maurya *et al*., 2010). It is the most serious cause of stem rot resulting in significant yield loss of tomato (Rakh *et al*., 2011). Blum and Rodriguez (2004) observed reduction in seed germination and plant growth in soybean. Similarly, Khalequzzaman (2003) recorded a reduction in length of shoot and root, fresh weight of shoot and root with nodules, number of pods, number of nodules and yield in soybean plants inoculated with *S. rolfsii* and *Meloidogyne javanica* as compared to uninoculated plants. Several studies have shown the effectiveness of various fungicides on *S. rolfsii* (Johnson and Subramanyam 2000; Palaiah, 2002). However, fungicides are not normally recommended because they are not economical, cause environmental hazards and deleterious effects on non target organisms. Therefore, biological control of plant diseases is advocated instead of chemical pesticides. *Trichoderma* species have shown

biocontrol potential against many fungal diseases of plants (Dolatabadi *et al*., 2011). This study therefore was undertaken to determine the *in vitro* effectiveness of three isolates of *T. viride* and two fungicides on *S. rolfsii*.

MATERIALS AND METHODS

Collections of samples

Soil sample used for this project work was collected from Crop Soil and Pest Management (CSP) Department planting site of the Federal University of Technology, Akure, Ondo State, Nigeria. Tomato (*Lycopersicon esculentum)* seeds were purchased from the Ondo State Ministry of Agriculture, Akure. Three isolates of *Trichoderma viride* obtained from ginger soil, maize cob and abattoir soil as well as *Sclerotium rolfsii* were collected from Department of Microbiology of the Federal University of Technology Akure, Ondo State. All these isolates were cultured on Potato Dextrose Agar at room temperature.

Sterilization technique

The soil sample used for the planting of tomato (*Lycopersicon esculentum)* was sterilized in an oven (Galenkamp Bs 250) at 180^0C for 3hours while potato dextrose agar, distilled water, and glass wares used in this project were sterilized by autoclaving at 121^0C for 15minutes.

Analysis of the soil sample used

The soil sample used for this project work was analyzed before planting to determine its physiochemical properties using the association of official analytical soil analyst chemist (A.O.A.C., 2000) method.

Determination of sand, clay and silt percentage

Fifty grammes of the soil sample were weighed into a beaker and then mixed with 10ml of sodium hexmeta-phosphate. 900ml of water was added to the mixture in the beaker and left to stand overnight. A 10ml cylinder was filled with the mixture and a hydrometer was used to take the readings and the percentage of sand, clay and silt were calculated (A.O.A.C., 2000).

pH determination

Ten gram of 2 millimeter sieved air dried soil sample was weighed into 100ml beaker, this was done in duplicate. 20ml of distilled was added to one of the beaker and 20ml of 1M potassium chloride was added to the soil sample in the second beaker. These mixtures were several times over a 30 minutes interval. The pH of soil in the beaker containing water was measured by immersing glass electrode into the partly settle suspension beaker (A.O.A.C., 2000).

Determination of total nitrogen

Two gram of the soil sample was weighed into kjeldahl flask. A 10ml of concentrated sulphuric acid was introduced into the flask and one table spoon of catalyst (Copper sulphate) was added. Heat was applied on digestion rack and the sample left to settle for 3 hours until a clear solution was obtained. After digestion, the solution was left to cool and was made to mark 100ml of volumetric flask with distilled water. The solution titrated against 0.1M HCl until end point was reached (A.O.A.C., 2000).

Determination of calcium, magnesium, potassium, and sodium

Atomic absorption spectrophotometer was employed to determine the component of calcium, magnesium, potassium and sodium. Soil sample (1g) was transferred into 100ml conical flask and shake vigorously for 30 minutes; this was followed by the addition of 2ml aqua regia. The conical flask was left to stay for 3 days before being made up to 50ml mark of distilled water (A.O.A.C., 2000).

Determination of phosphorus

Five grams of air dried soil sample was weighed into 250ml conical flask. Bray one solution was added and left ti stand for 1 minute before filtered. Eight milliliter of sample of standard solution or blank was pippeted into a set of well numbered glass vials. 5 drops of PB reagent (ammonium molybdate solution) and % drop of PC reagent ($feSO_4$ solution) were added and carefully mixed. These were allowed to stand for 15 minutes. The samples were read in the colorimeter using a green filter (600 millimicrons weight) against a blank, the standard curve was then calculated. The colorimeter read for standard and phosphorus were determined from the graph (A.O.A.C., 2000).

In vitro* determination of the antagonistic properties of *T. viride* against *Sclerotium rolfsii

The antagonistic property of *T. viride* was determined using the dual culture technique as described by (Gomathi and Ambikapathy, 2011). A plug of 7mm of *S. rolfsii* and *T. viride* was placed in an opposite direction in sterile solidified PDA plates. These plates were then incubated at both 25^{o}C and 37^{o}C for 72 hours using pH of 4, 7, and 9 by adjusting the normal pH of the agar which was 3.6 using 1M sodium hydroxide (NaOH) and pH meter. The percentage inhibition was calculated using the formula;

$$\frac{R_2-R_1}{R_2} \times 100$$

Where R_2 = growth of control

R_1 = zone of inhibition between *Trichoderma viride* and pathogenic organisms

2.4 Compatibility test of *T. viride* and selected fungicides (camazeb and mancozeb) in inhibition of *S. rolfsii*

The food poisoning technique was used to determine the effects of two fungicides on the growth of *T. viride* and *S. rolfsii*. Mancozeb (0.05g) was mixed with sterile PDA after cooling. It was then poured into each petri dish to be used and allowed to gel after which a 7mm plug of the T. viride and *S. rolfsii* were inoculated in opposite direction. The plates were incubated for 72 hours and then observed for result. The above procedure was repeated for camazeb.

Surface sterilization and pregermination of tomato seeds

The tomato seeds were placed in 70% ethanol for 3 minutes followed by 1% of sodium hypochlorite for one minute and then rinsed in several changes of sterile distilled water. Tomato seedlings (*Lycopersicon esculentum*) were placed on sterile cotton wool soaked with sterile distilled water in a covered sterile petri dish and allowed to grow for five days at room temperature.

Planting of tomato seedlings

The experiment was carried out as factorial experiment in a completely randomized with three replications. The factors considered were as follows: inoculation with *S. rolfsii*, inoculation with *T. viride* and addition of mancozeb. Suspension of both *S. rolfsii* and *T. viride* in 20 millimeter of sterile distilled water were inoculated into some the soil sample after five days of pregermination of tomato seedlings. Four seeds of the tomato seedlings (*L. esculentum)* were planted per polyethylene pot at a depth of six millimeter of 600g sterile soil. The polyethylene pots were watered every morning with 20 millimeter of sterile distilled water to maintain a good soil moisture condition. After growth, all seedlings were thinned to two per pot. Plant height and plant leaves were recorded at interval of ten days for thirty days of growth while fresh weight of plant and root, dry weight of the plant were determined after thirty days of growth.

RESULTS

Physicochemical analysis of soil sample

The percentage composition of sand, silt and clay of the experimental soil was 74.75 $\pm$ 2.93, 16.67 $\pm$ 3.42, and 8.58 $\pm$ 1.41 respectively. The physiochemical analysis of soil sample used was done before and after the experiment. The pH of soil sample used before the experimental trial reduced from 5.99 $\pm$ 0.13 to 5.59 $\pm$ 0.06 after the experiment. The soil sample analysis showed high amount of water holding capacity of 42.48 $\pm$ 0.75 % before experiment reduced to 15.50 $\pm$ 0.56 % after the experiment. The values of potassium, calcium and phosphorus before the experiment were 3.98 $\pm$ 0.43 mg/kg, 2.40 $\pm$ 0.13 cmol/kg and 0.20 $\pm$ 0.04 mg/kg respectively as shown in Table 1.

Antagonistic effects of *T. viride* on *S. rolfsii*

The inhibitory activities of *T. viride* on *S. rolfsii* at 25^{o}C and 37^{o}C are as shown on table 2 and 3. Generally, *S. rolfsii* was best inhibited at 37^{o}C and pH 4. *Trichoderma viride* isolated from maize cob (V_1) inhibited *S. rolfsii* best at pH4 with 77 and 84% inhibition while at pH7, the percentage inhibition 75% and 82% respectively. The least inhibition was observed at pH 9. *Trichoderma viride* isolated from ginger soil (V_2) inhibited the fungi with the percentage inhibition ranging from 64% to 83 respectively at pH4, 61% and 77% respectively at pH 7 and range of 65% and 68% respectively

Effects of fungicide on the growth of *T. viride* and *S. rolfsii*

The combine effect of fungicides and *Trichoderma viride* on *S. rolfsii* as indicated on table 4 shows that both of them could thrive in the presence of the fungicides. The least percentage inhibition of *S. rolfsii* was obtained from *T. viride* from abattoir soil indicating that it was most sensitive to mancozeb. The highest inhibition was obtained from *T. viride* from ginger soil for mancozeb and abattoir soil for camazeb.

Table 1 Physicochemical analysis of soil sample before planting

Parameters determined	Values Obtained
pH	5.99 ± 0.13
OM (%)	1.71 ± 0.38
MC (%)	1.63 ± 0.22
WHC (%)	42.48 ± 0.75
N (%)	0.18 ± 0.02
P (mg/kg)	3.98 ± 0.43
K (mg/kg)	0.20 ± 0.04
Ca (cmol/kg)	2.40 ± 0.13
Mg (cmol/kg)	11.00 ± 0.14
Na (cmol/kg)	0.17 ± 0.05

Key: *OM= organic matter; MC= moisture content; WHC= water holding capacity; N= nitrogen; P= phosphorus; K= potassium; Ca= calcium; Mg=magnesium; Na= sodium*

Table 3: Percentage inhibition of *Sclerotium rolfsii* by *Trichoderma viride* at 25°C

T. viride	**pH4**	**pH7**	**pH9**
V_1	70.56 ±2.41	75.43±1.01	67.75±4.9
V_2	64.07 ±6.80	61.16±2.68	64.56±5.23
V_3	77.92±1.98	70.13±3.04	73.22±7.98

Key: *V_1: Trichoderma viride obtained from maize cob*
V_2: Trichoderma viride obtained from ginger soil
V_3: Trichoderma viride obtained from abattoir soil

Table 4: Percentage inhibition of *S. rolfsii* by *Trichoderma viride* at 37°C

T. viride	**pH4**	**pH7**	**pH9**
V_1	84.24±6.11	82.17±10.67	50.00±8.11
V_2	82.88±6.11	76.66±13.53	68.33±5.81
V_3	88.29±8.11	71.86±2.67	72.50±7.06

Key: *V_1 = Trichoderma viride obtained from maize cob*
V_2 = Trichoderma viride obtained from ginger soil
V_3 = Trichoderma viride obtained from abattoir soil

Fungicides	**V_1**	**V_2**	**V_3**
Mancozeb at (0.05%)	70.64±0.59	88.10±2.05	46.03±4.18
Camazeb (0.05%)	77.77±0.67	80.16±8.36	83.73±3.42

Key: *V_1: Trichoderma viride obtained from maize cob*
V_2: Trichoderma viride obtained from ginger soil
V_3: Trichoderma viride obtained from abattoir soil

Growth characteristics of tomato plant

Plant height, plant leaves, fresh weight of plant and root, dry weight of the plant were recorded at interval of ten days for thirty days of growth. The highest plant height and leaves were observed on soil tomato plant treated with *S. rolfsii* and *T. viride* (ginger soil) while the plant with the least height, plant leaves were observed to be soil tomato plant treated with *S. rolfsii* except for soil tomato plant treated with *S. rolfsii* and mancozeb, soil tomato plant treated with *S. rolfsii*,*T. viride* (maize soil) and mancozeb, soil tomato plant treated with *S. rolfsii, T. viride* (ginger soil) and mancozeb where no growth occurred as shown in Table 4,5 and 6. Fresh weight of plant and root, dry weight of the plant occurs highest on soil tomato plant treated with *S. rolfsii* and *T. viride* (ginger soil) while the least of fresh weight of plant and root, dry weight of the plant was observed on soil tomato plant treated with *S. rolfsii*.

Table 5 Growth characteristics of tomato plant grown under different conditions on the 10th day

Treatment	Plant height(cm)	Leaf number
S_t	7.20 ± 0.12	3.30 ± 0.14
S_tS_r	7.00 ± 0.17	2.00 ± 0.07
$S_tS_rT_m$	8.00 ± 0.21	4.00 ± 0.13
$S_tS_rT_g$	8.20 ± 0.28	4.00 ± 0.16
S_tT_m	7.70 ± 0.14	3.00 ± 0.07
S_tT_g	8.00 ± 0.21	4.00 ± 0.17

Key: S_t= (control); S_tS_r= soil treated with S. rolfsii; $S_tS_rT_m$= soil treated with S. rolfsii and T. viride (maize cob); $S_tS_rT_g$= soil treated with S. rolfsii and T. viride (ginger soil); S_tT_m= soil treated with T. viride (maize cob); S_tT_g= soil treated with T. viride (ginger soil).

Table 6 Growth characteristics of tomato plant grown under different conditions on the 20th day

Treatment	Plant height(cm)	Leaf number
S_t	12.30 ± 0.22	9.00 ± 0.16
S_tS_r	7.00 ± 0.17	3.00 ± 0.03
$S_tS_rT_m$	15.00 ± 0.25	10.00 ± 0.13
$S_tS_rT_g$	16.00 ± 0.15	13.00 ± 0.23
S_tT_m	13.80 ± 0.07	10.00 ± 0.13
S_tT_g	14.00 ± 0.15	10.00 ± 0.24

Key: S_t= (control); S_tS_r= soil treated with S. rolfsii; $S_tS_rT_m$= soil treated with S. rolfsii and T. viride (maize cob); $S_tS_rT_g$= soil treated with S. rolfsii and T. viride (ginger soil); S_tT_m= soil treated with T. viride (maize cob); S_tT_g= soil treated with T. viride (ginger soil).

Table 7 Growth characteristics of tomato plant grown under different conditions on the 30th day

Treatment	Plant height(cm)	Leaf number
S_t	17.80 ± 0.15	17.00 ± 0.24
S_tS_r	9.30 ± 0.13	7.00 ± 0.12
$S_tS_rT_m$	21.00 ± 0.25	20.00 ± 0.19
$S_tS_rT_g$	27.00 ± 0.39	25.00 ± 0.27
S_tT_m	20.00 ± 0.19	19.00 ± 0.17
S_tT_g	24.50 ± 0.28	22.00 ± 0.23

Key: S_t= (control); S_tS_r= soil treated with S. rolfsii; $S_tS_rT_m$= soil treated with S. rolfsii and T. viride (maize cob); $S_tS_rT_g$= soil treated with S. rolfsii and T. viride (ginger soil); S_tT_m= soil treated with T. viride (maize cob); S_tT_g= soil treated with T. viride (ginger soil).

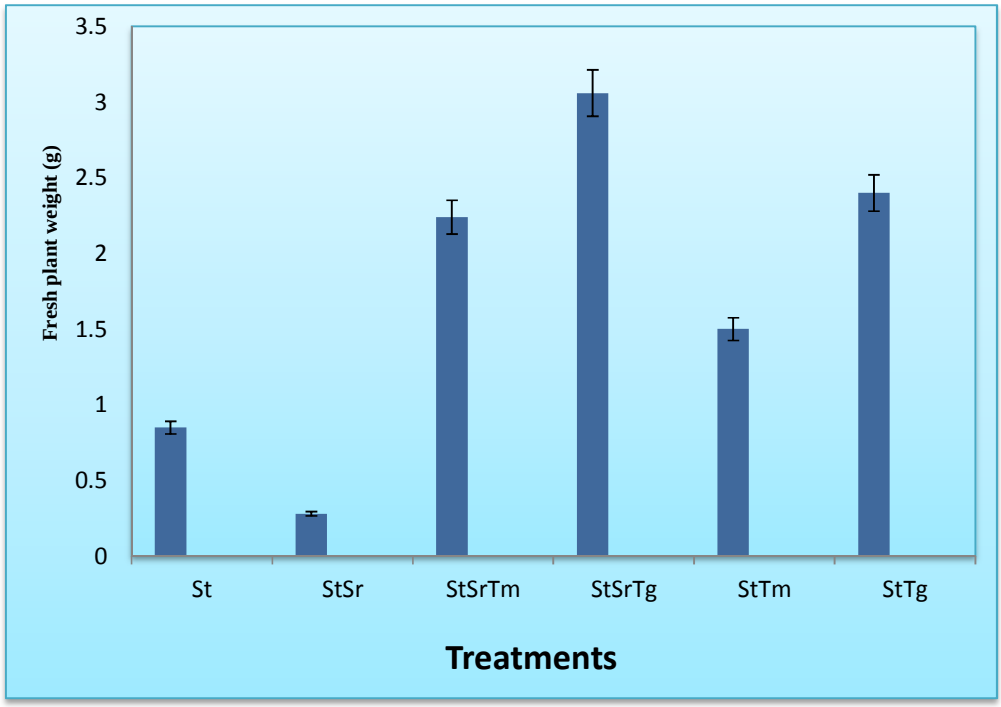

Figure 1 Fresh weight of tomato plant seedlings

Key: S_t= (control); S_tS_r= soil treated with S. rolfsii; $S_tS_rT_m$= soil treated with S. rolfsii and T. viride (maize cob); $S_tS_rT_g$= soil treated with S. rolfsii and T. viride (ginger soil); S_tT_m= soil treated with T. viride (maize cob); S_tT_g= soil treated with T. viride (ginger soil).

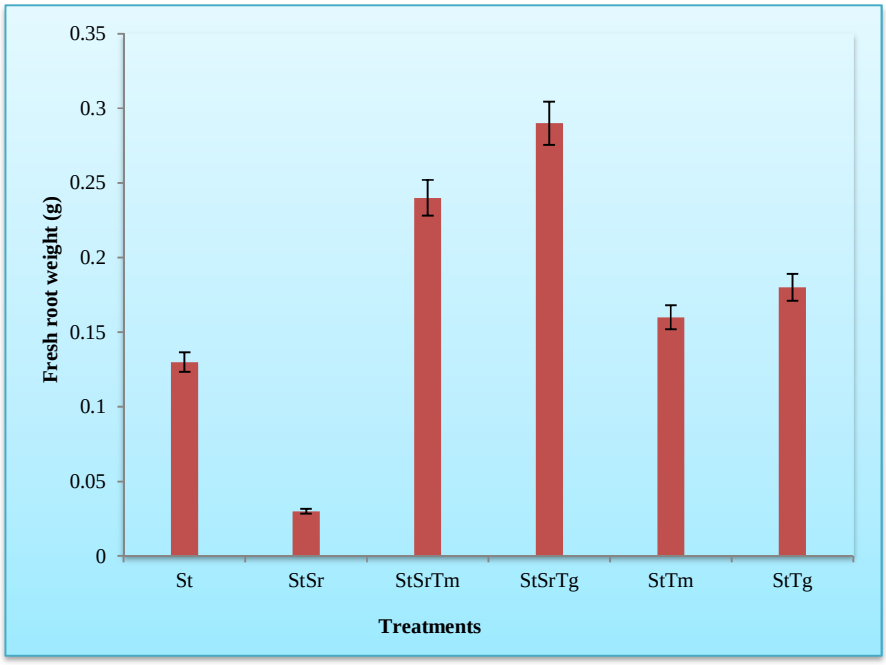

Figure 2 Fresh root weight of tomato plant seedlings

Key: S_t= (control); S_tS_r= soil treated with S. rolfsii; $S_tS_rT_m$= soil treated with S. rolfsii and T. viride (maize cob); $S_tS_rT_g$= soil treated with S. rolfsii and T. viride (ginger soil); S_tT_m= soil treated with T. viride (maize cob); S_tT_g= soil treated with T. viride (ginger soil).

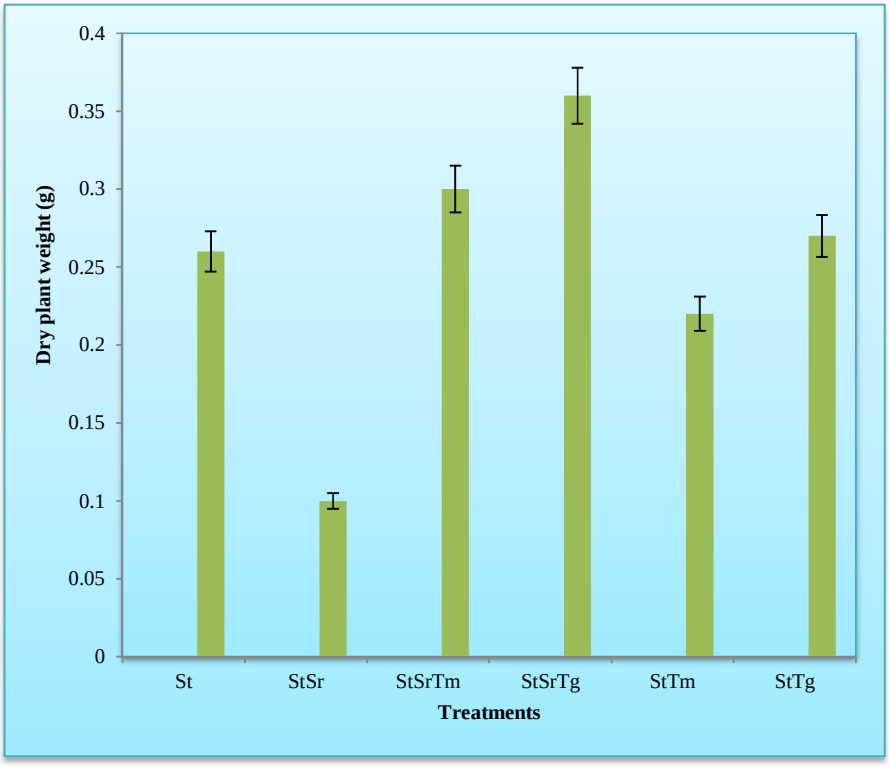

Figure 3: Dry weight of tomato plant seedlings

Legend S_t= soil planted with tomato (control); S_tS_r= soil tomato plant treated with Sclerotium rolfsii; $S_tS_rT_m$= soil tomato plant treated with S. rolfsii and Trichoderma viride (maize soil); $S_tS_rT_g$= soil tomato plant treated with S. rolfsii and T. viride (ginger soil); S_tT_m= soil tomato plant treated with T. viride (maize soil); S_tT_g= soil tomato plant treated with T. viride (ginger soil).

DISCUSSION

The soil sample used was sandy loam and it was slightly acidic. This indicated that combination of soil quality, soil nutrients and water are the major determinants of plant growth and distribution (Jean, 2010).

Biological control is a good alternative for sustainable agriculture to overcome the problems of public concern associated with pesticides and pathogens resistant to chemical pesticides (Akhtar and Siddiqui, 2008). Several researches have been made on the antagonistic properties of fungi especially the fungus *Trichoderma*. These *Trichoderma* strains have important potential as antagonists. *Trichoderma* species show several antagonistic mechanisms towards pathogens (Chaube *et al*., 2003; Brozóvá, 2004). For instance, some *Trichoderma* species have been successfully tested on controlling *Fusarium oxysporum* or *Sclerotium cepivorum, Botrytis cinerea* under field conditions (Ávila-Miranda *et al*., 2006). Although the *T. viride* used against the pathogenic organisms were isolated from three different sources (V_1- maize plant soil, V_2- ginger plant soil and V_3- abattoir soil), the one isolated from the abattoir soil was observed to be more effective at antagonizing these pathogenic organisms followed by the one isolated from the maize plant soil while the one isolated from the ginger plant soil seems to have the weakest ability to antagonize the pathogenic organisms. The difference in the ability of these strains of *T. viride* may be as a result of genetic properties and environmental conditions attributed to each strain that is, the environment in which they are isolated and slight changes in the genetic makeup of the organisms may be related to the antagonistic ability of the organisms.

The highest plant height and plant leaf numbers was recorded in soil treated with *S. rolfsii* and *T. viride* from rhizosphere of ginger soil in comparison with *T. viride* from maize cob. This indicated that *T. viride* can be used as biocontrol agent reducing the effect of pathogens on the plant, increased the resistance in plant and also stimulate plant growth by enhancing uptake of water in plant (Chet *et al*., 2007). The antagonistic activities of *Trichoderma* could be attributed to the production of antibiotics and fungal cell wall degrading enzymes (Chutrakul *et al*., 2008; Sharma *et al.*, 2009).The least plant height, and plant leaves were obtained from soil treated with *S. rolfsii*. This might due to the pathogenicity of this fungus causing obstruction in water uptake, nutrients absorption on the root system of the plant leading to stem weakness, reduced plant growth (Campbell, 2003). The highest fresh weight plant and root, dry weight of the plant were obtained from soil treated with *S. rolfsii* and *T. viride* (ginger soil) which also indicated that *T. viride* supported the growth of the plant while the least fresh weight plant and root, dry weight of the plant was recorded in soil treated with *S. rolfsii* indicating that *S. rolfsii* is pathogenic to the plant growth (Howell *et al*., 2003). *Trichoderma viride* supported the development of all the tomato planted except the ones inoculated with mancozeb. They are common inhabitant of rhizosphere and contribute to the control of many soil borne plant diseases caused by fungi (Chet *et al*., 2007).

CONCLUSION

Biological control agents are perceived to have specific advantages over synthetic fungicides because of less non target and environmental effects, efficacy against fungicide-resistant pathogens and reduced probability of resistance development.

REFERENCES

[1] Hadizadeh, I., Peivastegan, B., Hamzehzarghani, H. **2009**. *Am. J. Appl. Sci.*6 (5): 857-861.
[2] Omara, K.J. **2010**. Manual for the identification of tomato species in tropics. ASM press Washington D.C. pp 24-30.
[3] Howell, T.L., Ash G.J., and Muray G.M., **2003**. *Australian J. Exp. Agric.* 43(2): 163-168.
[4] Fouzia, Y, Saleem, S. **2005**. *Pak. J. Bot*., 37(1): 175-180.
[5] Kokub, D., Azam, F., Hassan, A., Ansar, M, Asad, M. J., Khanum, A. **2007.** *Pak. J. Bot*., 39(5): 1849-1866**.**
[6] Maurya, S., Singh, U. P., Singh, R., Singh, A.,Singh, H. B. **2010**. J. *Plant Pro.Res*.50 (2): 206-209.
[7] Rakh, R.R., Raut, L.S., Dalvi, M., Manwar, A.V. **2011**. *Rec. Res. Sci. Technol*.3 (3): 26-34.
[8] Blum, L.E.B, Rodriguez-Kabana.R. **2004**. *Fitopatologia Brasileira*, 29: 66-74.
[9] Khalequzzaman, K.M. **2003**. *Pak. J. Pl. Pathol.*, 2: 56- 64.
[10] Johnson, M., Subramanyam, K., **2000**. *Annals of Plant Protection Sciences*, 8: 255-257.
[11] Palaiah, P. **2002**. Studies on variability in *Sclerotium rolfsii* Sacc. causing stem rot of groundnut. *M.Sc.(Agric.) thesis*, University of Agricultural Sciences, Dharwad, pp.73-78.

[12] Dolatabadi, K.H., Goltapeh, E.M., Varma, A., Rohani, N. **2011**. *J. Agric.Tech.* 7(1): 73-84.
[13] AOAC **2000**. Association of official analytical chemicals officials methods of analysis. 17th edition. Washington D.C. 2200pp.
[14] Gomathi, S., Ambikapathy, V. **2011**. *Adv. Appl. Sci. Res.* 2 (4):291-297.
[15] Jean, M.A. **2010**. Essential biology with environmental physiology. pp. 214-216.
[16] Akhtar, M.S., Siddiqui, Z.A. **2008**. *Mycorrhizae: Sustainable Agriculture and Forestry*, 61–97.
[17] Chaube, H.S., Mishra, D.S., Varshney, S., Singh, U.S. **2003**. *Ann. Rev. Phytopathol*. 2:1-42. Brozóvá, J. **2004**. *Plant Pro. Sci*. 2:63-74.
[18] Ávila-Miranda, M.E., Herrera-Estrella, A., Peña-Cabriales, J.J. **2006**. *Soil Biology and Biochemistry* 38:1823-1830.
[19] Chet, I.A., Brotman, A., Viterbo, Y. **2007**. Proceeding of 5th international conference on biopesticides stakeholders perspectives. New Delhi.
[20] Chutrakul, C., Alcocer, M., Bailey, K., Peberdy, J.F. **2008**. *Chemistry and Biodiversity* 5: 1694- 1706.
[21] Sharma, K., Kumar, M., Misra, R.M. **2009**. *J. Phytopathol.* 157: 51-56.
[22] Campbell, M. **2003.** *Australian Broadcasting Corperation Research* 23: 04-011.