

# Bio-Control of *Fusarium* Wilt of Pepper (*Capsicum Annum* (Linn.)) with *Glomus Mosseae* and *Trichoderma Viride*

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## Abstract

Resistant effect of *Glomus mosseae* (mycorrhiza) and *Trichoderma viride* (a saprophytic fungus) as bio-control agents against *Fusarium* wilt disease caused by *Fusarium oxysporum* in pepper was studied in a green house experiment. Growth parameters were used to measure the resistant effect of the antagonists, pepper seedlings were found to be more susceptible to the pathogenic (*Fusarium oxysporum*) infection, especially those inoculated singly with *Fusarium oxysporum* which wilted off 3 weeks after inoculation. Whereas, those used for simultaneous inoculation of *Glomus mosseae* (mycorrhiza) and *Fusarium oxysporum* (pathogen); and concomitant inoculation of *Glomus mosseae* (mycorrhiza) 2 weeks before pathogenic inoculation, were observed to survive the transplanting shock and had better growth with regards to height. Also, all inoculations involving *Trichoderma viride* (either, singly simultaneously or concomitantly) survived, except the concomitant inoculation where pathogen (*Fusarium oxysporum*) was first inoculated before *Trichoderma viride* inoculation. Plants inoculated concomitantly with mycorrhiza first before pathogen had the best values of growth parameter compared to the control, single and simultaneous inoculations. Generally, observation from the study shows that mycorrhiza (*Glomus mosseae*) and *Trichoderma viride* reduced the disease incidence of *Fusarium* wilt of pepper at different conditions of treatments.

**Key words:** Concomitant, Simultaneous, Competition, Antibiosis, Mycoparasitism

## INTRODUCTION

Disease problems and disease control are by no means peculiar to modern agriculture. Also, use of chemical pesticides increases the production cost, thus, making the end products to be more expensive even for the masses to procure, furthermore, chemical control poses risks to human lives and environments (FAO, 2000). Baker and Cook (1974) & (Salami, 2002)

defined biological control as the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state. This is usually accomplished by one or more organisms naturally or through a manipulation of the environment, host or antagonist, or by mass introduction of one or more antagonists. These antagonists often

include actinomycetes, bacteria and some other fungi which have been put to practical use in the field. Tests of different nature ranging from *in-vitro* to *in-vivo* tests have been carried out to show the effectiveness of antagonists in inhibiting the growth and ingress of plant pathogens as well as promoting higher yield. Ladoye (1992) and Odebode *et al.* (2001), for example, inoculated pepper and tomato seedlings with inoculum of mycorrhiza spores and root fragments. This prevented the root - rot infection of these vegetables from been attacked by *Pythium aphanidermatum*. Salami (1999), also showed that *Trichoderma viride* and *Glomus etunicatum* reduced root-rot of tomato seedlings caused by *Phytophthora infestans*. Odebode *et al.* (1997), used *Glomus deserticola* to control the basal stem rot disease of tomato caused by *Sclerotium rolfsii* (sacc.) in a greenhouse experiment. Competition for nutrients between the antagonists and pathogens in the rhizosphere is also a suppressive mechanism against the pathogenic organisms (Salami, 1999). These antagonists confer these potentials on plants and even transferred into the rhizosphere in order to be able to biologically control plant pathogens. Antagonists in biological control of plant pathogens are biological agents with the potentials to

interfere in the life processes of plant pathogens (Cook and Baker, (1983); Salami *et al.* (2005). They have been reported to suppress diseases caused by various pathogens through different mechanisms of action which they exhibit (Whipps and Lumsden, 1991; & Salami *et al.* (2001). These mechanisms include: competition; antibiosis; and mycoparasitism.

Pepper is needed in households especially in the tropics to enhance or improve intake of balanced diets and it also serves as good source of income to small producers in many developing countries (Atanda *et. al.*, 1990). Demand for pepper fruits has increased considerably, as a result of urbanisation, industrialisation, growth in population and diversification of eating habits (Ladoye, 1992); & Odebode and Shehu (2001). Due to lack of alternative crops that provide the same economic returns to farmers, the repeated cultivation of pepper in fertile land has become unavoidable. The continuous monocropping of pepper plants results in various problems such as deterioration of physico-chemical properties of soil, the accumulation of toxic compounds and the increase of plant pathogens in the soil (Anon; 1982). Pepper has also been subjected to attacks from group of organisms such as fungi, bacteria, viruses, insects and

nematodes (Jones and Watson, 1969; Prabhu and Fillipi, 1995). Wilt disease has been a major constraint to pepper production which leads to reduced income to the farmers. Many organisms have been known to cause wilting in pepper but *fusarium* wilts are more destructive and are much more common in the warm temperate regions, tropics and sub-tropics (Correll, 1991). These problems require replanting of pepper plant and re-constitution of soil to evacuate organisms causing diseases in order to increase the nutrient content of the soil. Disease protection against soil-borne pathogens is one of the benefits of mycorrhiza (Salami *et al.*, 2001). Effects of arbuscular mycorrhiza (AM) infection on plants have been found to be gainful in terms of nutrient uptake and it has inhibitory effect against ingress of pathogens into the plants. It is against this background that this paper attempts to discuss the resistant effect of some antagonists namely: *Glomus mosseae* (mycorrhiza) and *Trichoderma viride* (a saprophyte) on wilt disease caused by *Fusarium oxysporum* (pathogen) on the growth of pepper (*Capsicum annum*).

#### MATERIALS AND METHODS

Healthy looking bell shaped pepper seeds of variety (NHU-2C) were raised in trays (36 x 15 x 10

cm) filled with loamy topsoil sterilized at 121<sup>o</sup>c at 10kg/cm<sup>2</sup> for one hour. They were watered daily and after two weeks, the seedlings were transplanted into black polythene bags with perforations. The pure cultures of *Fusarium oxysporum* and *Trichoderma viride* in slant bottles were sub-cultured into different plates of already prepared Potato Dextrose Agar (PDA) medium respectively. The inocula were prepared as spore suspensions obtained from 3-days old cultures and the concentrations was adjusted to 4.0 x 10<sup>5</sup> spores/ml. with the aid of Hewskley haemocytometer. Mycorrhizal inoculum, *Glomus mosseae*, containing 1,300 spores/100 grammes of dry soil was used for inoculation.

#### Inoculations

Pepper seedlings were inoculated by carefully uprooting with soil so as not to destroy their roots. The roots were then rinsed with distilled water and placed in the beaker containing 50ml of *Fusarium oxysporum* or *Trichoderma viride* suspension as the case may be, for 5 minutes. Culture discs (5mm in diameter) of either *F. oxysporum* or *T. viride* was cut and put into the hole made from where the seedlings were uprooted before being replaced and immediately moistened with distilled water.

Each 30g of mycorrhizal inoculum (used to inoculate each seedling) was divided into three equal parts. One part was dissolved in distilled water in which the seedling roots were soaked for 5 minutes. The seedlings were removed carefully turned into the second part of the mycorrhizal inoculum, this stuck to the wet roots while the third part was put directly into the hole where the seedlings were uprooted and then covered with soil.

#### Experimental design

A completely randomized experiment was designed for this work and 5 treatment factors were used, which are: First treatment- Pepper seedlings inoculated with *Fusarium oxysporum* alone and designated F. Second treatment- Pepper seedlings inoculated with *Trichoderma viride* alone and designated T. Third treatment- Pepper seedlings inoculated with *Glomus mosseae* alone and designated M.

Four threatment-Pepper seedlings inoculated simultaneously with the pathogen and either of the two antagonists used. They were designated T-F; M-F; and/or F-M respectively. Fifth treatment-This was the concomitant inoculation. After 2 weeks, pepper seedlings that were singly inoculated before were re-inoculated (i.e. F alone will be re-inoculated with M; and T

alone will be re-inoculated with F; while M alone will be re-inoculated with F). All of them were tagged FM; TF and MF respectively. Each treatment was replicated thrice and the planting was carried out in the greenhouse with relative humidity that varies between 45% and 75%.

#### Determination of growth parameters

Measurement of growth parameters was done at age 4weeks of the plant which is two weeks after transplanting. These growth parameters measured include: Height; number of leaves; leaf surface area; and girth. They were measured in centimeters (cm) except the leaf surface area that was measured in square centimeters (cm<sup>2</sup>). All the data collected were taken through statistical analyses for adequate results and discussion.

#### RESULTS

High mortality rate was recorded in pepper seedlings inoculated with *Fusarium oxysporum* (pathogen) by wilting off at the end of the third week after inoculation. While those inoculated either singly or simultaneously or even concomitantly with the antagonists survived till the end of the experiment (Table1). The seedlings treated with mycorrhiza (singly, simultaneously or concomitantly), had highest height values, followed by the control and those

**Table 1: Influence of *Glomus mosseae*, *Fusarium oxysporum* and *Trichoderma viride* on the growth parameters of pepper seedlings**

Growth parameters after inoculations from 4 weeks to 11 weeks of age.				
Treatments	Girth (mm)	Number of leaves	Height (cm)	Surface area of leaves(cm <sup>2</sup> )
C	3.1750abc	11.000ab	15.025a	13.875abc
F	0.8500e	1.750d	1.506bc	3.750d
T	2.8250bc	9.375b	2.825bc	9.625c
M	3.8375a	13.250a	3.838b	17.500a
M-F	3.5750ab	11.000ab	3.575b	16.375ab
T-F	2.5500cd	8.375b	2.550bc	11.625bc
F-M	0.6625e	2.250cd	0.738c	4.500d
F-T	0.9000e	1.625d	0.900c	4.125d
TF	1.7250d	4.875c	1.725bc	9.750c
MF	2.4250cd	9.625b	2.425bc	10.875c

**KEYS:**

- C Control  
 F Seedlings inoculated with *Fusarium oxysporum* (pathogen) alone.  
 M Seedlings inoculated with *Glomus mosseae* (mycorrhiza) alone.  
 T Seedlings inoculated with *Trichoderma viride* (antagonist) alone.  
 M-F Simultaneous inoculations with *Glomus mosseae* and *Fusarium oxysporum* at the same time  
 T-F Simultaneous inoculations with *Trichoderma viride* and *Fusarium oxysporum* at the same time  
 F-M Simultaneous inoculations with *Fusarium oxysporum* and *Glomus mosseae* at the same time  
 F-T Simultaneous inoculations with *Fusarium oxysporum* and *Trichoderma viride* at the same time  
 MF Concomitant inoculations with *Glomus mosseae* two weeks before *Fusarium oxysporum* inoculation.  
 FM Concomitant inoculations with *Fusarium oxysporum* two weeks before *Glomus mosseae* inoculation.

treated with *Trichoderma viride*.(Tab. 1 Fig. 1)

Pepper seedlings treated with *T. viride* alone had better height values than those treated concomitantly with *T. viride* and *F. oxysporum* (Fig.1), but lesser than

the control treatment. Concomitant inoculation, where *T. viride* was first inoculated into the pepper seedlings survived the experiment and had better growth in terms of height value (Table 1 & Fig.1) but lesser than those treated with

*Glomus mosseae*. Also, pepper seedling treated with *Glomus mosseae* (mycorrhiza) either singly,

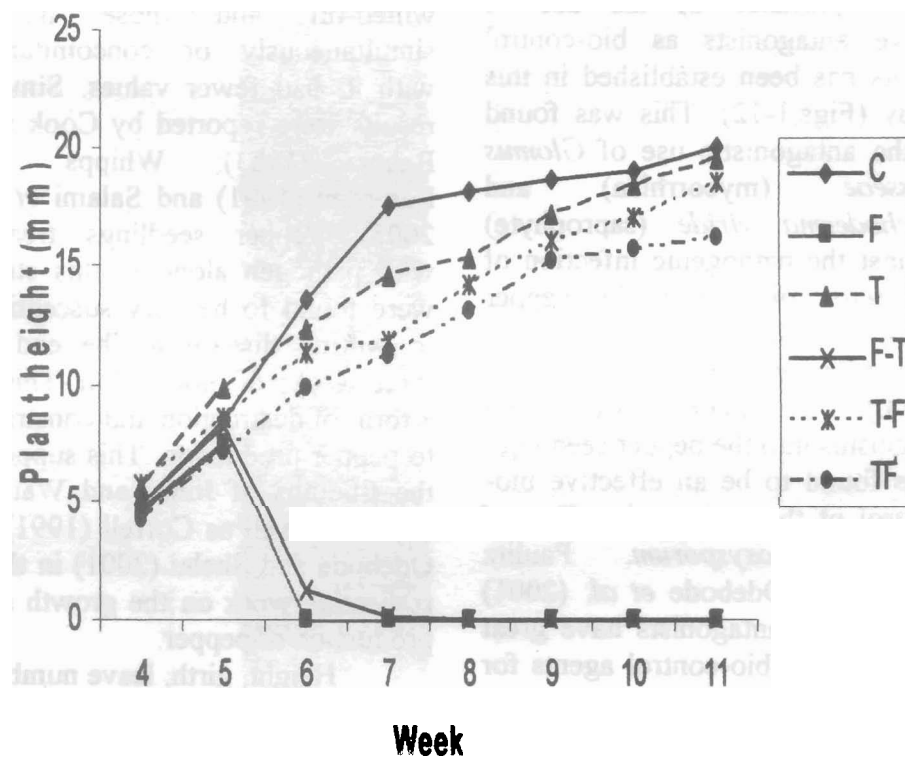


Fig. 1: Plant height measurements showing the effect of *Fusarium oxysporum* and *Trichoderma viride* on the growth of pepper seedlings

simultaneously or concomitantly, had better height values than the control treatment while those treated with pathogen did not survive (i.e. they wilted off) (Fig.1). Pepper plants treated with *Glomus mosseae* (mycorrhiza) had the largest number of leaves, girth and leaf surface area values than other treatments (Fig. 3-8). This

was followed by the control treatment and then those treated with *Trichoderma viride*.

Generally, pepper plants treated with the two antagonists (i.e. *Glomus mosseae* and *Trichoderma viride*) had better growth in all the growth parameters and reduced the disease incidence and severity caused by *Fusarium*

*oxysporum* (pathogen) (Table 1, & Figs. 1-8).

#### DISCUSSION

Importance of the use of native antagonists as bio-control agents has been established in this study (Figs.1-12). This was found in the antagonistic use of *Glomus mosseae* (mycorrhiza) and *Trichoderma viride* (saprophyte) against the pathogenic infection of *Fusarium oxysporum* in pepper seedlings. This supports the findings of Papavizas (1985) and Lynch (1990). Inoculation of these organisms into the pepper seedlings was found to be an effective bio-control of the pathogenic effect of *Fusarium oxysporum*. Paulitz (1992) and Odebode *et al.* (2001) argued that antagonists have great potentials as bio-control agents for the protection of plants against soil-borne pathogens and they also stimulate the growth of plants. *Trichoderma viride* used in this study has been found to be a protective bio-fungicide and not a curative one, thus, it could not penetrate the plant tissues as pathogens do. Mohammadi (1992) and Salami *et al.* (2001) also found this in their work showing the mycostop bio-fungicide present status, in the biological control of plant diseases. Antagonists used in this study have been found to interfere in the life processes of *Fusarium oxysporum* by having

greater values in all the growth parameters and surviving till the end of the experiment. While those treated with the pathogen alone wilted-off and those treated simultaneously or concomitantly with it, had fewer values. Similar results were reported by Cook and Baker (1983); Whipps and Lumsden (1991) and Salami *et al.* (2005). Pepper seedlings treated with pathogen alone in this study were found to be very susceptible to wilting disease at the end of three weeks of inoculation. This is a form of destruction and constraint to pepper production. This supports the findings of Jones and Watson (1969) as well as Correll (1991) & Odebode and Shehu (2001) in their respective work on the growth and production of pepper.

Height, girth, leave numbers and leave surface areas were greatly improved with *Glomus mosseae* (mycorrhiza). This shows that mycorrhiza had positive effect on the growth of pepper plants than the other treatments. This could be attributed to the resistant effect of *mycorrhiza* against *Fusarium oxysporum* (pathogen) and it is the usual benefits of mycorrhiza according to Schonbeck (1979); Thompson (1994) & Salami, (2000). It could be suggested from the results of this study that mycorrhizal plants are nutritionally healthier than non-mycorrhizal plants. This reduces the plants'

disease incidence and severity, it also restricts inward invasion of pathogen into the plants roots.

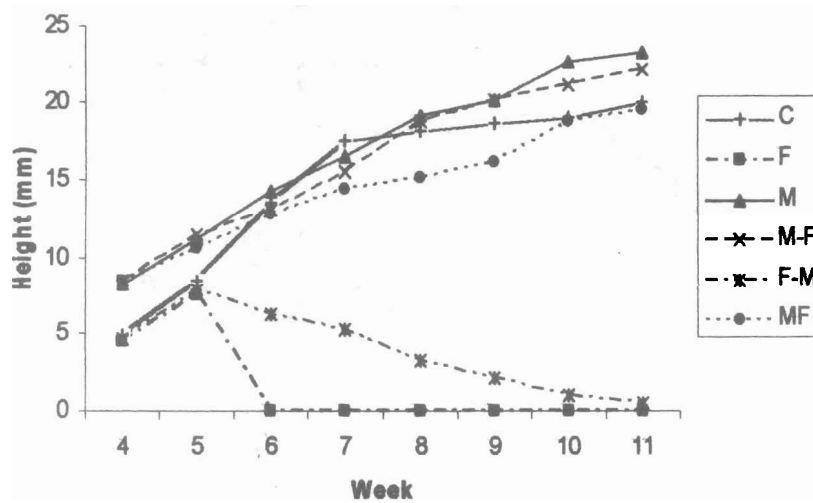


Fig. 2: Plant height measurements showing the effect of *Glomus mosseae* and *Fusarium oxysporum* on the growth of pepper seedlings.

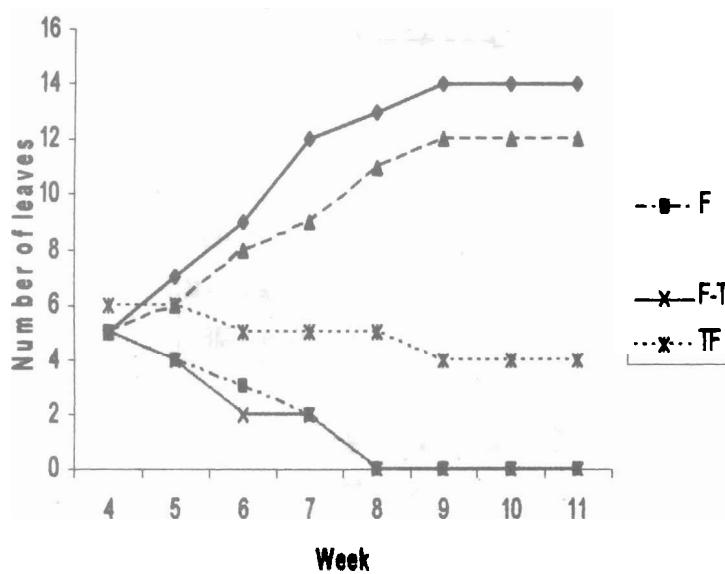


Fig. 3: Number of leaves of pepper seedlings inoculated with *Fusarium oxysporum* and *Trichoderma viride* on the growth of pepper seedlings.

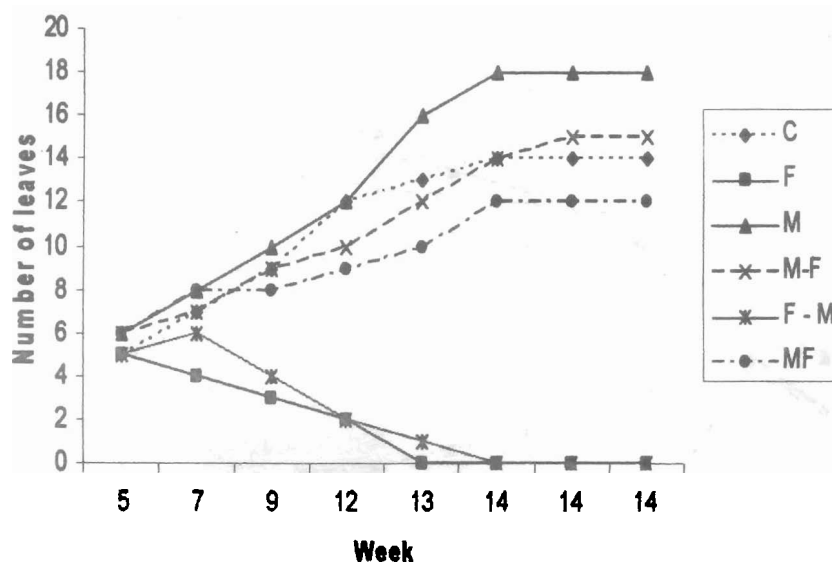


Fig. 4: Number of leaves of pepper seedlings inoculated with *Glomus mosseae* and *Fusarium oxysporum* on the growth of pepper seedlings.

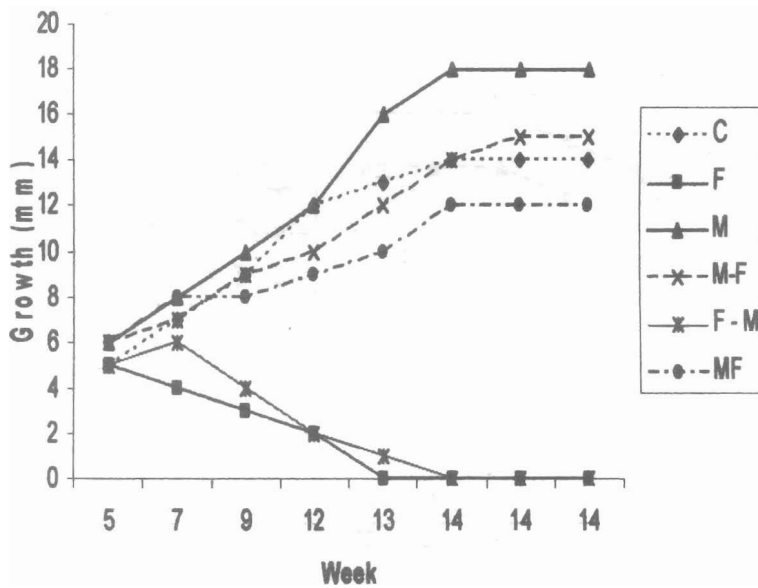


Fig. 5: Plant's girth measurements of pepper seedlings inoculated with *Glomus mosseae* and *Fusarium oxysporum* on the growth of pepper seedlings.

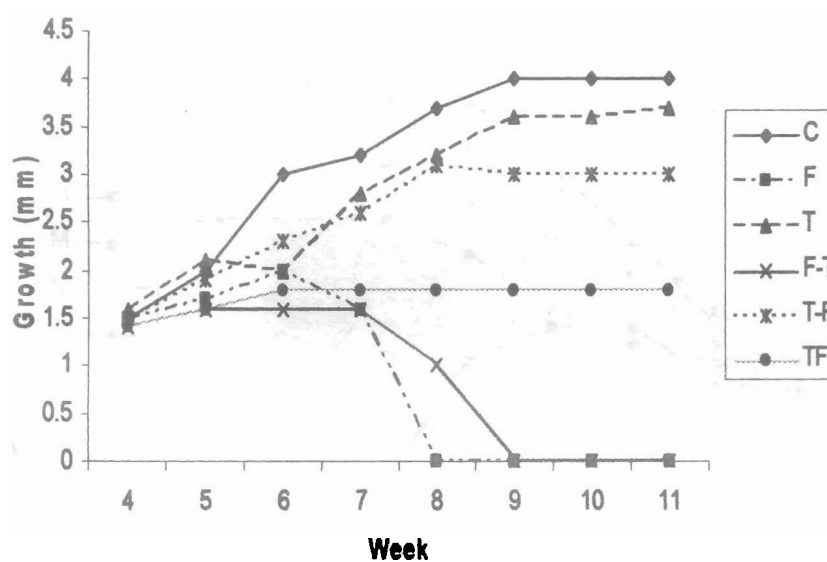


Fig. 6: Plant's girth measurements of pepper seedlings inoculated with *Fusarium oxysporum* and *Trichoderma viride* on the growth of pepper seedlings.

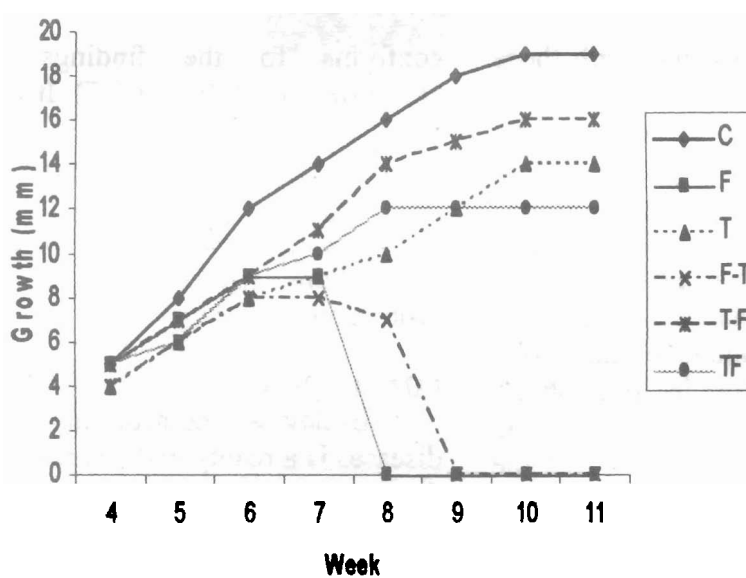


Fig. 7: Leaf surface area of pepper seedlings showing the effect of *Fusarium oxysporum* and *Trichoderma viride* on the growth of pepper seedlings.

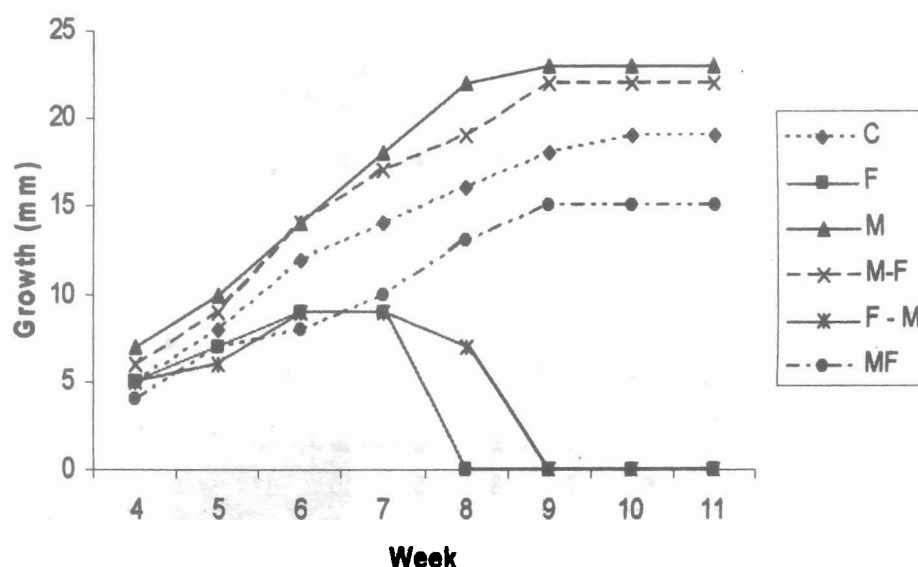


Fig. 8: Leave surface area of pepper seedlings showing the effect of *Glomus mosseae* and *Fusarium oxysporum* on the growth of pepper seedlings.

This result is consistent with those of Safir (1968); Dehine and Schonbeck (1979) & Salami *et al.* (2005). Time of inoculation as well as the mode of inoculation has significant effect on the inoculated pepper plants. They play important role in pepper plant's resistance or susceptibility (Odebode and Shehu 2001). Especially in this study, where both simultaneous and concomitant inoculations (with the antagonists first inoculated into the seedlings) were found to be more resistant than the single inoculation with the pathogen. Also, concomitant inoculations induced more resistance into pepper plants than all other inoculations. This

conforms to the findings of Odebode *et al.* (1995; 1997). It was discovered from this result that prior establishment of antagonists in pepper seedlings before *Fusarium oxysporum* (pathogen) inoculation makes for proper protection.

#### CONCLUSION

Biological control of plant diseases is a reality and has reached diverse stage of application. Progress has been made and there is need to consolidate on this. Biological control should be boldly approached with the mind of making it to work, not merely as a field for academic discoveries. It is

most pertinent therefore, to promote this idea of biological control among peasant farmers and study it carefully to perfect its accomplishment. For instance, mycorrhiza which has proven to be very useful in agricultural programme such as in nutrient uptake, growth parameters, inhibition of pathogens and improvement of yield should be embraced, improved upon and infused into the agricultural programme in the developing countries where there is much demand for self sufficiency in food production, security and safety.

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