Biocontrol of wilt disease (Fusarium oxysporum f. sp. lycopersici) in tomato by Glomus fasciculatum

N. RAAMAN*

M. GNANAGURU**

Centre for Advanced studies in Botany,

University of Madras, Guindy campus

Chennai-600 025, India.

Abstract:

The use of arbuscular mycorrhizae (AM) to influence soil borne plant diseases is based on their ability to promote plant health and reduce the damage caused by the pathogens. AM have proved to control different pathogens – nematodes as well as parasitic fungi. We examined the protective effects induced by arbuscular mycorrhizal fungus Glomus fasciculatum against Fusarium oxysporum f. sp. lycopersici causal agent of wilt disease in tomato.

The experimental design was completely randomized factorial combination of G. fasciculatum and F. o. f. sp. lycopersici. Six treatments were used: a. Con - Control - Received heat killed inoculum of AM fungus and pathogen. b. MI - AM fungus (G. fasciculatum) inoculated. c. PI - Pathogen (F. o. f. sp. lycopersici) inoculated. d. DI - Dual inoculation - F. o. f. sp. lycopersici and G. fasciculatum inoculated on the same day. e. PrI - Pre inoculation - F. o. f. sp. lycopersici inoculated 10 d prior to G. fasciculatum inoculation. f. PoI - Post inoculation - F. o. f. sp. lycopersici inoculated 10 d after G. fasciculatum inoculation. Leaf and root samples were collected for physiological and biochemical studies. The samples were randomly collected from 5 seedlings of each experiment, 10, 20, 30, 40, 50 and 60 d after inoculation. PoI and DI plants control the pathogen significantly. Increased level of chlorophyll contents, O-dihydric phenol content, total phenol content, lipid levels, protein content, total and reducing sugar, amino acid content, cytokinin and tomatine content were observed in PoI and DI plants when compared to other treatments. These biochemical changes may play a crucial role to protect tomato plants from pathogens.

Key words: Biocontrol, Fusarium oxysporum f. sp. lycopersici, Glomus fasciculatum, Tomato, Wilt disease, Field trial.

Introduction

In recent years, the management of crop diseases caused by root rot pathogens has become one of the most challenging research areas in plant pathology. Increasing knowledge and concern about environmental consequences of fungicide applications have prompted many scientists to explore the potential for alternative strategies of disease and pest management. Among the suggested strategies, the promising strategies for minimizing damage from plant pathogens are biological control of pathogenic population by microorganisms and induced systemic resistance in plants by inoculation of arbuscular mycorrhizae (AM) fungi (Schwab et al., 1991). AM fungi can induce resistance or increase tolerance to some root pathogens, such as nematodes or fungi (Azcon-Aguilar and Barea, 1997; Trotta et al., 1996). In addition, several studies have demonstrated disease control by AM fungi (Raman, 1996). Schenck and Kellam (1978) reported that the AM fungi reduced the effects of several soil borne pathogens on their hosts. Ross (1972) observed that Phytophthora megasperma was controlled by Glomus etunicatum in soybean. Sikora (1978) reported that G. mosseae reduced the population of Meloidogyne incognita in tomato. Systematic resistance against Phytophthora parasitica in tomato roots by G. mosseae has been reported by Cordier et al. (1998). Lingua et al. (2002) reported that G. mosseae reduced the effect of Yellow disease of tomato caused by phytoplasmas. A significant increase in plant height, leaf number, leaf area, fresh and dry matter of shoot and root of tomato have been recorded on inoculation of Glomus fasciculatum and Acaulospora laevis (Manila and Nelson, 2013).

The response of tomato to AM colonization is generally shows improved growth and well colonized root systems (Trotta et al., 1996; Vigo et al., 2000; Lingua, et al., 2002). Dehne (1977) found that G. mosseae had a marked effect on wilt disease by reducing wilt symptoms, vascular invasion and sporulation of pathogen (F. o. f. sp. lycopersici) in tomato. Zambolim and Schenck (1983) studied the effects of G. mosseae inoculation on reduction of root-infection by soil borne fungal pathogens on soybean. They found that the AM fungus overcome the effects of pathogens namely Macrophomina phaseolina, Rhizoctonia solani and Fusarium solani. The plants colonized by G. mosseae showed more resistance to pathogenic attack than the non-mycorrhizal plants. Fritz et al. (2006) found that mycorrhization of tomato roots could protect plants from early blight in tomato caused by the necrotrophic fungus Alternaria solani.

Goncalves et al. (1991) found that Fusarium solani infection decreased the growth rate and dry weight of bean plants, whereas the growth and weight increased in the plants inoculated with G. macrocarpum. Plant height, dry matter, N and P concentrations of pea and sorghum were high when the seeds were planted in soil, which contained mycorrhizal fungi or both mycorrhizal fungi and Fusarium oxysporum (Fracchia et al., 2000). Hwang et al. (1992) observed reduced shoot dry weight of

Medicago sativa infected by Verticillium albo-atrum and F. oxysporum f. sp. medicaginis, but it increased in mycorrhizal plants. Seedlings inoculated with G. fasciculatum and G. mosseae showed reduced incidence of wilt when compared with non-mycorrhizal plants. Propagule numbers of both the pathogens were fewer in the soil inoculated with AM fungi than in the non-mycorrhizal soil. AM fungi reduced the pathogenicity of Fusarium oxysporum f. sp. lycopercisi in Lycopersicon esculentum and also increased the growth and P uptake of the plants even in the presence of pathogen (Raman and Gnanaguru, 1996). G. versiforme can inhibit Ralstonia solanacearum in tomato plants by enhanced content of soluble phenol (Zhu and Yao, 2004). Fusarium wilt of tomato can be inhibited by AM fungi (Ozgonen et al., 1999) and some rhizobacteria (Duijff et al., 1999; Chin-A-Woeng et al., 2000). Inoculation of both AM fungi and some rhizobacteria is considered as effective symbionts for protecting the plants from root rot pathogen and increased plant growth (Akkopru and Demir, 2005). Bioprotection of AM fungal colonized plants against soil-borne pathogens is the complex interactions between plants, pathogens and AM fungi (Harrier and Watson, 2004). Several genes and corresponding protein products involved in plant defense responses have been extensively studied in the AM symbiosis (Pozo et al., 2002; Guillon et al., 2002). G. intraradices in combination with T. harzianum significantly reduced the root rot disease in tomato in Floride (Datnoff et al., 1995). Arbuscular mycorrhizal fungal inoculation showed the best result against the Fusarium wilt in chickpea varieties (Singh et al., 2010, 2013). The defense related physiological, biochemical and anti-oxidant activities observed in roots of groundnut plant significantly increased by single inoculation of AM fungi or Trichoderma (Doley et al., 2014). The present study aims to investigating the effects of single inoculation of AM fungi Glomus fasciculatum as biocontrol agent on F. o. f. sp. lycopersici casual agent of wilt disease in tomato.

Materials and Methods

Plant, fungal species and site description

AM fungal inoculum was produced with Allium cepa L. (onion) as host plant following the method of Ferguson (1981). Cultures of F. oxysporum f. sp. lycopersici causal agent of wilt disease in tomato were from the Fungal culture collection, Centre for Advance Studies in Botany, University of Madras, Chennai, India. Single spore cultures of F. o. f. sp. lycopersici were also isolated from naturally wilted roots of tomato seedlings. Tomato seedlings were collected from the different localities of Tamil Nadu, India (Paddapai, Chengalpet district; Usilampatti, Madurai district; Srivilliputhur, Virudunagar district; Udamelpet, Coimbatore district).

Fusarium was isolated from infected roots by pour plate technique (Schmitthenner and Hilty, 1962). The fungal mat (10 d old) in petri plate was washed gently with sterile dist water and the conidia were collected along with dist water. Ten mL of conidial suspension (106 conidia/mL) of each strain was inoculated to 40 d old tomato seedlings in the glasshouse condition. The disease development was assessed on every 5 d up to 60 d. On the basis of per cent disease incidence, the highly virulent strain was selected and used for further study. Seeds of tomato (Lycopersicum esculentum Mill.) cultivar Co.1, a susceptible variety to F. o. f. sp. lycopersici was obtained from Department of Horticulture, Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu, India. Cleaned surface sterilized earthen seed pan was filled with sterile soil-sand (2:1) mixture. The soil was taken from the land which contain the pH 6.7; N = 155.68 kg/hactare; P = 17.30 kg/hactare; K = 578.22 kg/hactare. The tomato seeds were surface disinfected with 0.5% mercuric chloride solution for 3 min and rinsed in sterile distilled water. The disinfected seeds were sown on the soil surface of the seed pan (50 seeds/ pan) and covered with thin layer of sand. The pan was watered at regular interval.

Growth of tomato plants

Earthen pots (15 X 30 cm) were cleaned and filled with sterile soil-sand (2:1) mixture. Seedlings (20 d old) were carefully removed from the seed pan and the roots were washed gently with distilled water. The seedlings were transplanted in pots (1 seedling / pot) and kept in glass house (30 ± 1º).

Experimental design

The experimental design was completely randomized factorial combination of G. fasciculatum and F. o. f. sp. lycopersici. Six treatments were used. a. Con - Control - Received heat killed inoculum of AM fungus and pathogen. b. MI - AM fungus (G. fasciculatum) inoculated. c. PI - Pathogen (F. o. f. sp. lycopersici) inoculated. d. DI - Dual inoculation - F. o. f. sp. lycopersici and G. fasciculatum inoculated on the same day. e. PrI - Pre inoculation - F. o. f. sp. lycopersici inoculated 10 d prior to G. fasciculatum inoculation. f. PoI - Post inoculation - F. o. f. sp. lycopersici inoculated

10 d after G. fasciculatum inoculation. G. fasciculatum inoculum, 10 g (soil having 50 spores/g) and pathogen inoculum, 5 mL conidial suspension containing 106 conidia/mL were used for treatment. Control seedlings were inoculated with heat killed conidia. A replicate of 10 seedlings were maintained for each treatment.

Evaluation of disease incidence

Disease incidence of tomato plants inoculated with F. o. f. sp. Lycopersici was evaluated by grading each leaf according to Dimond et al. (1952). Grade 0 – no disease symptom; grade 1 – epinasty and / or slight yellowing of the terminal leaflet; grade 2 – 20-50% of the leaf area yellowing; grade 3- complete yellowing and / or partial wilting; grade 4- leaf fallen or completely non functional. The average grade was computed for the plant as a whole and divided by 0.04 to give a maximum value of 100.

Physiological and biochemical tests

Leaf and root samples were randomly collected from 5 seedlings of each experiment, 10, 20, 30, 40, 50 and 60 d after inoculation. The root samples were washed in tap water to remove the adherent soil particles and rinsed in dist water. The moisture was removed by pressing the roots in between 3 layers of filter paper. For quantification of organic substances, the plant materials were immediately plunged in boiling ethanol (80%) and homogenized. To assay enzyme activity, fresh plant tissues were extracted in buffers in ice cold condition. Chlorophyll content was estimated by following the method of Mahadevan and Sridhar (1986). Reducing sugar content was estimated by following the method of Nelson (1944). Total soluble sugar, free amino acids, O-dihydric phenol and phenol contents were estimated by following the method of Mahadevan and Sridhar (1986). Total soluble protein content was estimated (Lowry et al., 1951). Cytokinin was extracted and estimated by following the methods of Sridhar et al. (1978) and Mahadevan and Sridhar (1986). Isolation of antifusarial substances, tomatine was extracted and purified by Langcake et al. (1972) and tomatine in the residue was confirmed by using TLC and infra red (IR) spectrum analyses. Tomatine was estimated by anthrone method (Stahl, 1969).

Field trial

Based upon glass house experiments, a preliminary field trial was undertaken to find out the effect of G. fasciculatum and F. o. f. sp. lycopersici on tomato plants under field conditions.

Data analysis

All data were analysed using the statistical package for the social sciences (SPSS) (vs. 10.1). Two way ANOVA (F) test was performed to find out

i) the influence of different treatment of G. fasciculatum on F.o.f.sp lycopersici.

ii) Influence of G. fasciculatum on disease severity in different days.

Further, the significant effects among treatments of G. fasciculatum on disease severity were tested by LSD at 5% and 1% level.

Results

Severity of Fusarium wilt disease

The data on disease indeed in tomato are presented in Table 1. G. fasciculatum markedly reduced disease incidence when it was inoculated before the pathogen (PoI) and also at the same time (DI). In PoI plants, only 2% of disease was recorded at 20th d which was completely nullified subsequently. In the PI and PrI plants, 100% disease index occurred at 50th d and the plants died on 54th d, while in DI plants, only 28% disease was recorded at 50th d and 29% on 60th d. symptoms were absent in MI and control plants (Fig. 1 a,b,c).

Chlorophylls

The Chlorophyll levels (Chlorophyll a, chlorophyll b and total chlorophyll) in leaves are presented in Table 2. Chlorophyll a increased progressively in leaves of MI and PoI plants. The PI and PrI plants showed increased level at 10th d and decreased level in the subsequent days. Of the test plants, the maximum chl-a level was recorded in PoI plants followed by MI plants. PrI plants contained least amount of chl-a followed by PI plants.

At early stages, chlorophyll b increased in all the plants. In MI and PoI plants, chl-b increased markedly compared with other experimental plants. Chl-b reduced from 20th d in PI and PrI plants. The maximum level of chl-b was in the PoI plant at 60th d. In DI plants, the chl-b content steadily increased.

Total chlorophyll increased initially in the test plants. The marked increase in total chlorophyll was observed in PI and PrI plants at 10th d and subsequently, reduction was recorded. In MI and PoI plants, high levels of total chlorophyll were recorded which were higher than the control plants. Total chlorophyll gradually increased in DI plants at 60th d, which was only 7% higher than the control. Total chlorophyll in PI and PrI plants were less than the control plants at 50th d.

Reducing sugars

Reducing sugars in leaf markedly increased at 10th d in the leaves of experimental plants, but the amounts varied in different treatments (Fig. 2). Reducing sugars increased along with age of MI, PI and PoI plants. But in PI and PrI plants, it decreased from 20th d onwards. A maximum of reducing sugar recorded in PoI plants followed by MI plants and DI plants. The PI plants had the least amount followed by PrI plants.

In roots, a steady increase was recorded in all treatments except in PI and PrI plants. In these plants, the level of reducing sugars increased at 10th d and then decreased at 50th d. The maximum

amount was recorded in MI plants, followed by PoI plants. Reducing sugars in the DI plants also increased at 60th d.

Total sugar

The quantity of total sugar in leaf steadily increased in MI and PoI plants (Fig. 3). It increased up to 10th d in PI and PrI plants, but subsequently decreased. The maximum level was recorded in PoI plants at 60th d followed by MI plants. Least amounts were observed in PI and PrI plants. The reduction in sugar level was observed in PI and PrI plants on 20th onward. When compared to control plants, in DI plants, sugar level decreased at 20th d as well as on 30th d, then increased and attained higher level than the control.

The total sugar increased in roots of all plants except PI and PrI plants, where it decreased from 20th d onwards. Among the test plants, the PoI plants showed high level at 60th d followed by MI plants. In DI plants, the sugar content increased along with age at 60th d.

Amino acids

In leaves, amino acid in the PoI plants was high and minimum in PI plants (Fig. 4). The PoI plants and MI plants showed amino acid content in increasing order. In the PI and PrI plants, the amino acids increased in 10th d and then decreased at 50th d and lower than the controls. A reduced level of amino acids was recorded at 10th d in DI plants and then it increased.

Amino acids decreased in DI plants at 10th d and then increased in roots. But in other treatments, amino acids increased at 10th d. In MI and PoI plants, it increased further but in PI and PrI, the reduction of amino acids was from 20th d onward. The PoI plants showed maximum level of amino acids at 60th d followed by MI plants. The minimum level was found in the PI plants

O-dihydric phenol

Increased level of O-dihydric phenols was found in the leaves of experimental plants at the initial stage (Table 3). There was fluctuation in both PI and PrI plants. From 10th to 60th d, the level of O-dihydric phenol in DI plants shaply increased. The MI plants showed the highest amount of O-dihydric phenols followed by PoI and DI plants at 60th d. But only few μg of O-dihydric phenols was observed in PI and PrI plants at 50th d.

The DI plant roots showed the maximum amount of O-dihydric phenols and PI plants showed the minimum amount. Increased level was recorded initially in PI as well as in PrI plants and then it decreased. There was a marked reduction at 20th d in the two treatments.

Total phenol

The variations in total phenol in the leaves of plants are presented in Table 4. The increase in the level of total phenol was observed at 10th d in the test plants. The amount decreased in PI and PrI plants from 20th d onwards. The DI plants showed higher amount of total phenol than MI and PoI

plants at all days of analyses except 60th d where it slightly decreased compared with the PoI plants. The phenol contents of PI and PrI plants decreased from 20th d, reached 10% and 15% respectively on 50th d.

Maximum content of total phenol was noticed in PoI plant roots followed by MI and DI plants at 60th d. In PI and PrI plants, phenol decreased at 30th d. A minimum of phenol was in the roots of PI plants followed by PrI plants on 50th d.

Total lipid

Changes in lipid level of leaves of experimental plants are presented in Table 5. The PoI plant leaves had higher amount of lipid than other plants. The MI and PoI plants showed lipid level in increasing order. At 10th d, expect DI plants, all other plants showed increased amount of lipid. In DI plants, the lipid decreased up to 20th d and then increased. In PI and PrI plants, lipid started to reduce from 20th d, the minimum level was found in PrI plants followed by PI plants. The PoI plants had the maximum level followed by MI plants.

At 10th d, expect DI plants, all other plant roots showed increased amount of lipid. In DI plants, the lipids decreased up to 20th d and then increased. In PI and PrI plants, lipid started to reduce from 20th d. The minimum level was found in PrI plants followed by PI plants. The PoI plants had the maximum followed by MI plant.

Total protein

The protein levels in leaves of the experimental plants are presented in Table 6. The PoI plants had higher amount of protein followed by MI plants. In DI plants, the quantity of protein decreased at 10th d and then increased. Fluctuation of protein was in both PI and PrI plants. The minimum protein was found in PI plants followed by PrI plants at 50th d.

The maximum level of protein was recorded in MI plants roots followed by PoI plants and DI plants. The PI and PrI plants showed increased level of protein at 10th d and subsequently decreased significantly. The minimum amount was recorded in PrI plants followed by PI plants.

Cytokinin

The amounts of cytokinin present in the leaves of plants are given in Table 7. Cytokinin level increased along with age in MI, DI and PoI plants, but decreased in PI and PrI plants. A maximum amount was found in PoI plants followed by MI plants. The DI plants had higher amount than the control plants on 50th d. The minimum level was recorded in PrI plants followed by PI.

Higher level of cytokinin in roots was recorded in PoI plants followed by MI plants. The DI plants also showed increased level of cytokinin. The PI plants showed the least amount followed by PrI plants.

Identification of antifusarial substance

The antifusarial assay of methanol extracts of tomato plants by bioassay technique showed only one spot. Further purification of this substance by TLC technique in various solvent systems showed Rf values. Colour reaction matched with tomatine. The R_f values of the sample were 0.74 in ethyl acetate: acetic acid: water, 0.53 in benzene: methanol, 0.37 in chloroform: methanol which coincided with Rf values of authentic sample of α-tomatine (Sigma).

The IR spectral analysis showed 4 absorption bands at 3200, 2900, 1540 and 1410 cm^{-1}. Pure α-tomatine showed 4 absorption bands at 3200, 2900, 1625 and 1410 cm^{-1}. The absorption bands of tomatine isolated from tomato plants were similar to the standard tomatine except the absorption band at 1540 cm^{-1}. Phytoalexin was not detected (Fig. 5).

Changes in tomatine after different treatments are presented in Table 8. The quantity of tomatine increased in MI and PoI plants along with age. In DI plants, tomatine level steadily increased whereas in PI and PrI plants, tomatine level increased at 10^{th} d but subsequently it decreased. The amount of tomatine at 50^{th} d in these plants was 20% less than the control plants. Tomatine level was 926 μg (+51%) and 970 μg (+57%) in the MI and PoI plants, respectively. The DI plants had 790 μg (+29%) of tomatine on 60^{th} d.

Tomatine in roots of MI and PoI plants steadily increased. Maximum amount was found in PoI plants (478 μg; +68%) followed by DI (476 μg; +67%) and MI (448 μg; +58%) plants at 60^{th} d. Least amount was observed in PI plants (169% μg; -36%), followed by 176 μg (-33%) in PrI plants. There was a marked increase in tomatine content in PI and PrI plants at 10^{th} d and subsequently reduction was noticed. An increase of tomatine from 138 μg (+2%) to 230 μg (+35%) in DI plants was observed from 10 to 20^{th} d. Similar effect (140 μg (+3%) to 338 μg (+67%)) was found in PoI plants during this interval.

Antifusarial activity of α-tomatine

The effect of α-tomatine extracted from tomato plants (MI, DI and PoI) was tested for spore germination and hyphal growth inhibition of F. o. f. sp. lycopersici (Fig. 6). At 100 μg/disc, it inhibited hyphal growth. Spore germination was 48% at 50 μg/mL. At 50 μg/disc, the inhibition zone was 25 mm diam. At 75 μg/ mL, spore germination was only 10% and 75 μg/disc caused 38 mm inhibition zone. At 10 μg/mL, it had no effect on spore germination and on hyphal growth. The minimum inhibitory concentration was 25 μg/mL or disc and at this concentration, the spore germination was 73% and inhibition zone was 16 mm diam.

Growth and yield of tomato plants under field condition

The results of field study are given Table 9. The PoI plants were healthy but the PI plants were highly diseased (Fig. 7). The growth rate and yield highly increased in PoI plants (32.5 g; + 136%; 3.95 kg; + 74% respectively). MI plants also showed higher growth rate (28.6 g; +107%). MI plants also showed higher growth rate (28.6 g; +107%) and yield (3.58 g; +58%). The growth (6.4 g; -54%) and yield (0.75 kg; -67%) were highly reduced in PI plants.

Discussion

An early mycorrhizal inoculation, previous to pathogen attack has been shown to be a successful practice to increase disease tolerance/resistance in economically important crop plants (Datnoff et al, 1995, Lovato et al, 1996, Cordier et al, 1998, Akkopru and Denir, 2005). In most investigations, AM fungi decreased the incidence of several root pathogenic fungi and nematodes (Kellam and Schenck, 1980; Azcon-Aguilar and Barea, 1997; Whipps, 2004). In the present study, resistance against F. o. f. sp. lycopersici, induced by G. fasciculatum, depends on the time interval between the mycorrhizal inoculation and inoculation with pathogen. Higher rate of disease incidence along with greater rate of pathogen population was found in PrI plants which mycorrhizal infection was almost absent. The susceptibility of tomato (PrI) plants to Fusarium may be due to reduced metabolic activity which ultimately leads to the death of cells, consequently preventing AM infection, since AM fungi are associated with only living cells.

Chlorophyll content increased in MI and PoI plants, which were higher than the control plants. The increased level of chlorophyll in MI plants was confirmed by the increased rate of photosynthesis, since increase in chlorophyll is usually accompanied by increase in photosynthesis. Mycorrhizal association enhances total chlorophyll in plants (Allen et al., 1981). Inoculation of G. intraradix increased chlorophyll in Citrus aurantium and obviously improved photosynthetic CO_2 fixation and growth (Nemec and Vu, 1990). According to Allen et al. (1984), G. fasciculatum inoculation in Bouteloua gracilis increased photosynthesis up to 80%. The rate of photosynthesis is higher in mycorrhizal plants than in non-mycorrhizal plants (Levy and Krikun, 1980; Kucey and Paul, 1982; Snellgrove et al., 1986). Two of the earlier symptoms in young tomato plants infected by F. o. f. sp. lycopersici are clearing of veinlets and drooping or epinasty of the petioles (Walker, 1971). Levels of total chlorophyll, chl-a and chl-b decline in PI plants. In these plants, initially chlorophyll level increased at 10^{th} d and then decreased. The reduced level of photosynthesis in PI plants may be attributed to reduced P level, lower levels of chlorophyll and water loss.

The MI tomato plants showed an increase in reducing and total sugars both in leaves and roots, but more in leaves. Leaves of AM fungus-infected plants generally contain more sucrose, reducing sugars and starch than non-mycorrhizal plants (Snellgrove et al., 1982; Same et al., 1983; Dixon et

al.,1988; Nemec and Vu, 1990). In leaves and roots of PI plants, initially both total sugars and reducing sugars increased and then decreased (Raman and Gnanaguru, 1996). Fusarium produces a number of enzymes such as exo and endo B-1,4 galactonases and exo B-1,3 and B-1,5 arabinases, that degrade sugar polymers (Mahadevan and Sridhar, 1986). In PoI and DI tomato roots, during the first stage of fungus development, quantity of sugars was not significantly affected. But when G. fasciculatum was well established, sugars increased in infected root. The degree of colonization influenced the carbohydrate concentration of the roots of Trifolium subterraneum (Pearson and Schweiger, 1993). Pearson et al., (1993, 1994) suggested that the outcome of competition may be dependent or at least influenced by the carbohydrate supply of the host rather than an effect of P on the result of competition, since root carbohydrate concentrations were more closely related to the reduction in colonization by AM than shoot P or soil P status. In DI and PoI plants, level of reducing sugars was high. The increased level of reducing sugars in mycorrhizal roots lowers disease incidence (Schenck, 1981). The amount of total sugar in the roots of PoI and DI plants was lower than the leaves. The carbon losses from the root are sufficient to sustain the activities of G. fasciculatum. As hyphal uptake of P occurs, root P content increases, membrane permeability is reduced and more of the carbon is allocated to the mycorrhizal fungus in the root, resulting in less exudation out of the root (Graham et al., 1981). Mycorrhizal-induced reduction in root exudation is correlated with reduction of soil borne disease (Graham and Menge, 1982). Mycorrhizal colonization significantly increased the mineral nutrient concentration, chlorophyll, protein, amino acids, starch, sugars and phenolic content (Manila and Nelson, 2014).

Generally mycorrhizal association increases the amino acid content in plants. An increased level of amino acids was found in MI tomato plants (Raman and Gnanaguru, 1996). Krishna and Bagyaraj (1983) found higher level of amino acids in Arachis hypogea inoculated with G. fasciculatum. Increase in amino acid level was directly correlated with increase in AM fungal infection (Dehne, 1986). Such increase in free amino acids of AM infected plants was observed in G. fasciculatum inoculated tomato plants. Higher level of aspartate and arginine concentrations in G. fasciculatum inoculated Glycine max was found by Pacovsky (1989).

The amino acid content decreased in leaves and roots of PI plants. This indicates that F. o. f. sp. lycopersici by producing protease breaks down the proteins and successfully utilized the amino acids as carbon and nitrogen source. Amino acid content increased in MI, DI and PoI plants. Amino acids like arginine, serine and phenylalanine may influence the mycorrhizal plant resistance to the pathogens (Young and Trappe, 1972). The influence of concentration of amino acids has been proposed as a mechanism regulating mycorrhizal root penetration and colonization (Ratnayake et al., 1978).

In both roots and leaves, protein increased in AM fungus alone inoculated (MI) plants. Vierheilig et al, 1995 have reported that the increase in pathogenesis-related proteins (PRs) induced by AM fungal inoculation to contribute to the plants elevated resistance. Such increased level of protein has been observed in Glycine max with G. fasciculatum (Pacovsky, 1989), Allium cepa and Nicotiana tabacum with G. mosseae (Dumas et al., 1994) and Trifolium pratense with G. mosseae (Arines et al., 1993). The cytochemical studies of Jeanmaire et al. (1988) revealed high protein content in the interface between arbuscular membrane and plant cell. In leaves and roots of PI plants, the protein content initially increased at 10^{th} day and subsequently declined. This is in agreement with Grzelinska (1969) who found that F. oxysporum f. sp. lycopersici reduced the protein content in tomato plant.

Total phenols increased in MI plants. This could be attributed to general triggering of pathways of aromatic biosynthesis (Mahadevan, 1991). Benhamou et al., (1994) have suggested that phenols and Chitinase were involved in plant resistance to fungal pathogen induced by AM fungi. Krishna and Bagyaraj (1984) reported an increase in phenols of roots of Arachis hypogea colonized by G. fasciculatum. Fry (1986, 1987) demonstrated that the cell wall bound phenols are important because they are the sites at which the covalent cross links may form by oxidative coupling between wall polymers. Codignola et al. (1989) found that G. versiforme inoculated Allium porrum showed high level of phenols. Both syringic and ferulic acids increased in the AM inoculated A. porrum and Ginkgo biloba. Zhu and Yao (2004) have reported that phenols were induced by G. versiforme in tomato roots in both locally and systemically when challenged with R. solanacearum, and that R. solanacearum growth was inhibited. The phenols increased initially in PI plants but decreased subsequently.

In DI and PoI plants, both phenols and O-dihydricphenols increased. The increased level of phenols was correlated with disease resistance to pathogen (Mahadevan, 1991). In histochemical studies, deposition of phenols was observed in the cortical cells of roots of DI and PoI plants. The observation that colonization by F. o. f. sp. lycopersici was mainly confined to the epidermis and outer cortical area in the roots of DI and PoI plants correlates well with the idea that the phenolic deposits contribute to prevent pathogen ingress towards the vascular stele. Benhamou et al. (1994) suggested that the deposited phenols may act as a barrier to pathogen spread and might display fungitoxic activity against pathogen. Increased accumulation of phenols is important in the resistance mechanism (Sedlarova and Labeda, 2001; Gershenzon, 2002; Zhu and Yao, 2004). Infection by AM fungi significantly increased the lipid content and fatty acid composition in G. mosseae infected roots of Allium cepa, Lolium perenne and Trifolium repens (Cooper and Losel, 1978) and Citrus aurantium (Nordby et al., 1981). Lipids in both leaves and roots of G. fasciculam

infected tomato plants increased. But the amount of lipids was higher in leaves than roots. This corroborates with the observations of Pacovsky and fuller (1988) that G. fasciculatum increased the lipid content more in leaves than roots. The decreased level of total lipids in PI plants may be accompanied by action of lipases of the pathogen. Several fungi degrade lipids by lipases, phospholipidases etc., which hydrolyze the lipids into fatty acids. The fatty acids are presumably utilized by the pathogen directly. The total lipid content in the PI plants decreased in both roots and leaves. Since chloroplasts are rich in lipids, the possibility exists that lipids are degraded along with chlorophyll. But lipids increased in PoI and DI plants, due to AM fungal infection. Beilby and Kidby (1980) showed that unusual lipid derived fatty acids found only in the mycorrhizal roots and suggested that fatty acids may be synthesized by AM fungi (Nagy et al., 1980).

Leaves and roots of PoI tomato plants had high level of cytokinins. Enhanced cytokinin levels in mycorrhizal plants suggest significant impacts on plant growth and development. Allen et al., (1980) reported increase of cytokinin content in plant tissues associated with AM fungi. Mycorrhiza enhances cytokinin synthesis (Raman et al., 1994; Thiagarajan and Ahmed, 1994; Barker and Tagu, 2000). The increased cytokinin levels might have resulted from fungal cytokinin production or inhibition of cytokinin degradation by compounds produced by the fungus. Alternatively, the fungus might have stimulated cytokinin production by the plant as a result of improved nutrition or some signals (Allen et al., 1980).

Decreased level of cytokinins was recorded in the leaves and roots of PI plants. The increased level of disease severity of PI plants may be correlated with the decreased level of cytokinins, because reduced amount of cytokinin would result in decreased chlorophyll content and ion transport, increased chlorophyll degradation and early senescence.

Leaves and roots of PoI plants had more amount of cytokinins followed by MI and DI plants. It may be possible that at least a part of the increased amount of cytokinins in DI and PoI plants resulted due to infection by G. fasciculatum. Increased cytokinins via mycorrhizae may trigger increased root infection (Azcon et al., 1978), since cytokinins are known to reduce resistance to fungal invasion (Haberlach et al., 1978). In addition, cytokinins facilitate P utilization (Menary and Van Staden, 1976) since P levels are known to influence frequency of mycorrhizal infection (Menge et al., 1978). Cytokinins increase chlorophyll content and reduce senescence, prevent chlorophyll degradation, increased ion transport, accumulation of metabolites in the tissues and organs and delay senescence (Mahadevan, 1984). Cytokinins inhibit ethylene formation by preventing proteolysis, deprive the tissue of free methionine, a major ethylene precursor (Tetley and Thimann, 1974). Thus cytokinins preserve the macromolecules such as proteins and polysaccharides from

breakdown. Cytokinin increases can elevate both photosynthetic and transpiration rates by opening stomata (Incoll and Whitelam, 1977).

Tomatine is a steroidal glycoalkaloid and is toxic to microorganisms (Mahadevan, 1984). Tomatine plays a major role in resistance by inhibiting spore germination and production (Smith and MacHardy, 1982). The concentration of tomatine increased in the leaves and roots of MI plants. Mycorrhizal fungal association increased the rate of photosynthesis. Hence increased level of tomatine in MI plants may be the result of enhanced photosynthesis which was influenced by mycorrhizal association.

The increased level of tomatine is an important factor in the resistance of PoI and DI plants to the pathogen. Arneson and Durbin (1968) described that tomatine is a antifusarial agent found in a number of Solanum and Lycopersicon species. Langcake et al. (1972) found the toxicity of tomatine produced by tomato plant roots and leaves against F. oxysporum f. sp. lycopersici. G. fasciculatum induced production of higher amount of tomatine in DI and PoI plants that inhibited the infection of F. oxysporum f. sp. lycopersici. Reduction in disease severity and spore population of pathogen in PoI and DI plants may be due to the efficacy of AM fungal infection which increased the tomatine content.

Even though tomatine increased in the roots and leaves of DI and PoI plants, the rate of AM infection was not reduced. Resistance to wilt disease in tomato plants is influenced by tomatine and resistant cultivars have greater amount of tomatine in the roots and leaves of susceptible cultivars (Hammerschlag and Mace, 1975). Roots and leaves of DI and PoI plants had increased levels of tomatine. AM plays a major role in the enhancement of tomatine accumulation. The increased levels of tomatine, P, phenols, O-dihydric phenols and cytokinin may play a crucial role in the resistance of tomato plants to pathogen and suppress the disease.

In the field trail, G. fasciculatum increased the growth and yield of tomato plants. Various application methods of AM inoculam in field crops were suggested (Powell, 1984; Hayman, 1987; Strulla et al., 1989; Sylvia and Jarstfer, 1992). The successful exploitation of biocontrol agent requires that the biocontrol agent becomes established in the infection court, which is usually the mycorrhizal root (Graham, 1988). When there is a compatible interaction with the biocontrol agent in the rhizosphere, the mycorrhizal fungus in effect becomes and integral part of the biocontrol system. G. fasciculatum is a compatible partner with tomato plants and control the wilt caused by F. o. f. sp. lycopersici effectively. The mechanism of control is not only to improved plant nutrition by mycorrhizal fungi but also to other physiological and biochemical factors associated with AM fungi.

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Figure legends

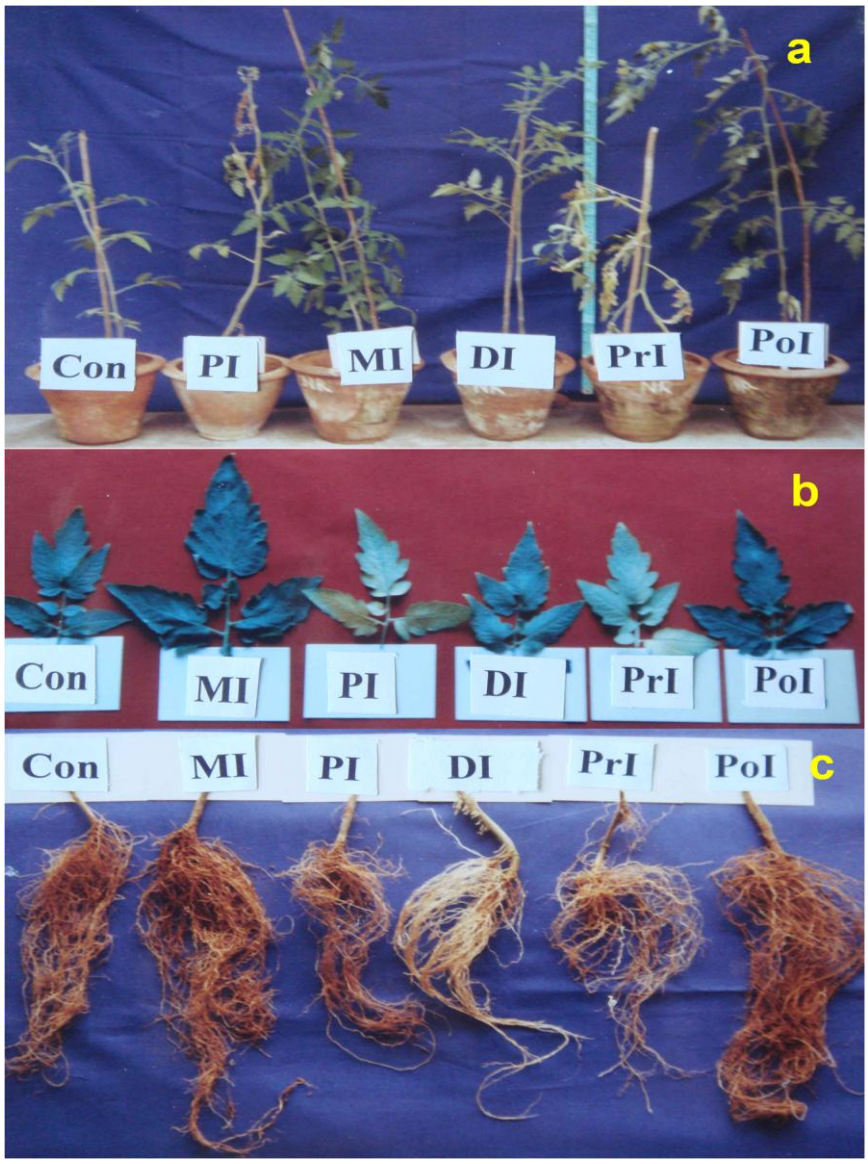


Fig. 1. Various treatments of tomato plants showing growth and disease incidence.

a. Disease incidence in whole plants

b. Disease incidence in leaves

c. Biomass of roots

Con – Control PrI – Pre inoculation treatment

MI – AM fungal inoculation DI – Dual inoculation

PI – Pathogen inoculation PoI – Post inoculation treatment

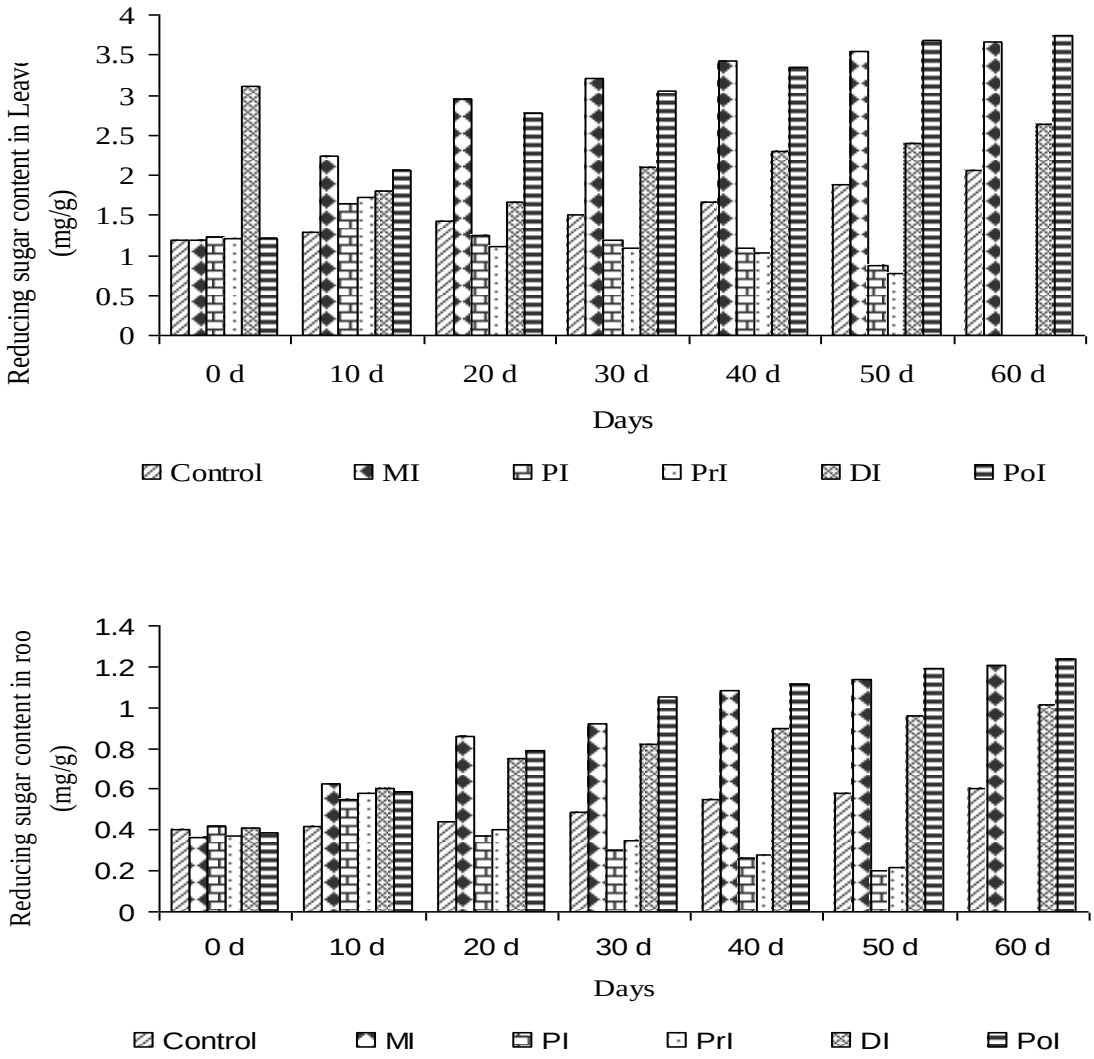
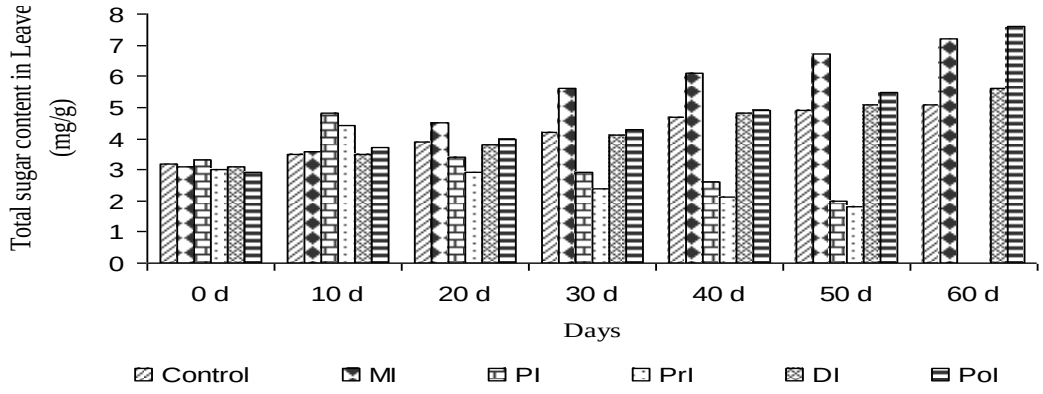


Fig. 2. Changes in reducing sugar content of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.



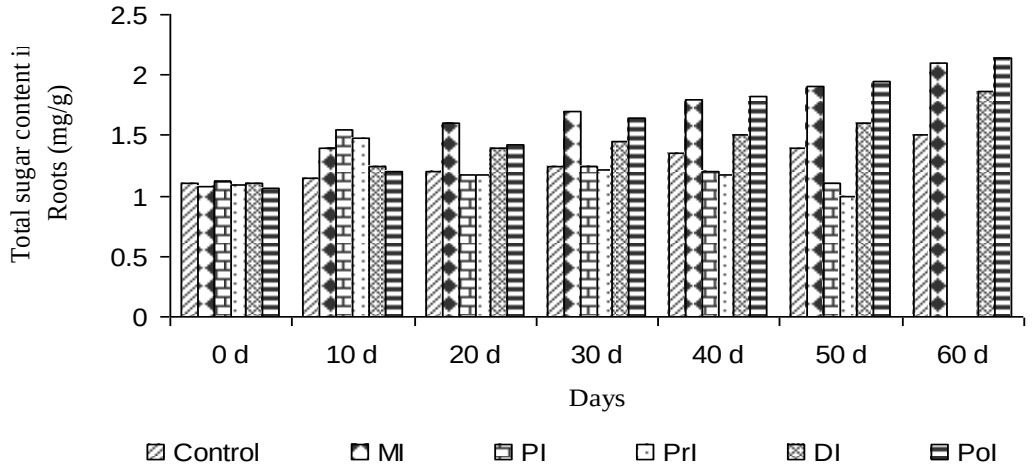


Fig. 3. Changes in total sugar content of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

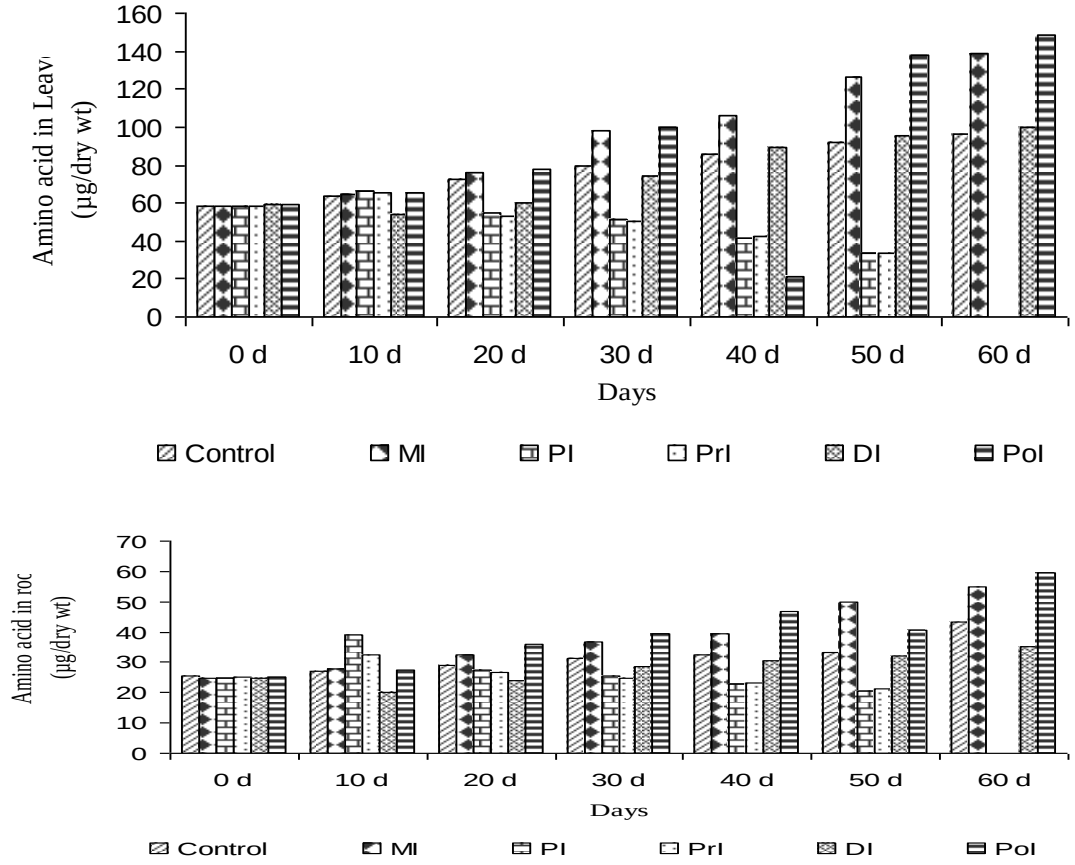


Fig. 4. Changes in amino acid content of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

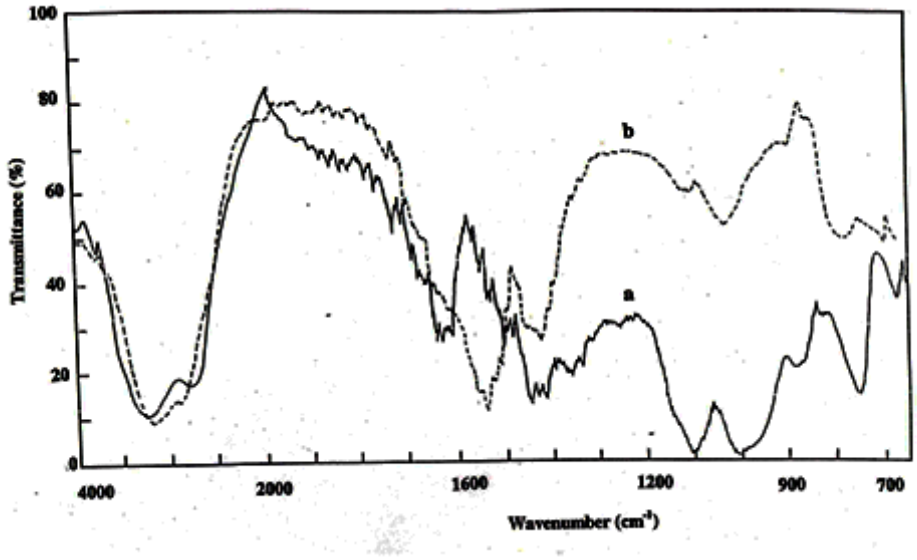


Fig. 5. IR spectrum of Tomatine

a-α-Tomatine

b-extracted Tomatine

Tomatine content

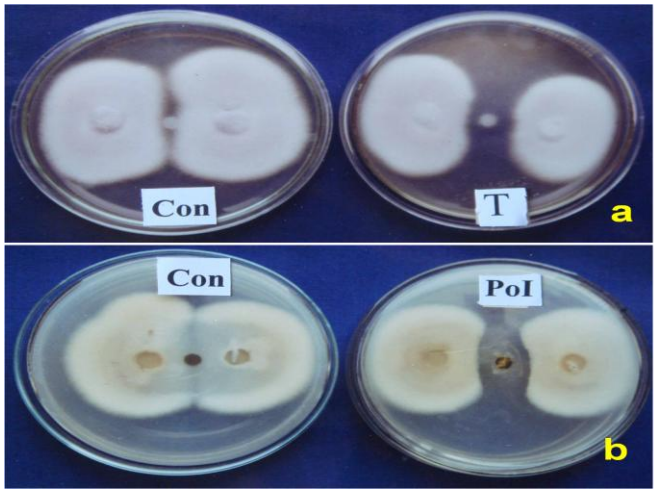


Fig. 6. Antifusarial activity of tomatine.

a. Inhibition of hyphal growth by authentic tomatine sample from sigma (T)

b. Inhibition of hyphal growth by tomatine extracted from PoI plants (PoI)

Con – Control PrI – Pre inoculation treatment

MI – AM fungal inoculation DI – Dual inoculation

PI - Pathogen inoculation PoI – Post inoculation treatment

Fig 7. Field trials of tomato plants

a. PoI tomato plants in bars

b. Infected PI plants showing wilt diseases.

Con – Control PrI – Pre inoculation treatment

MI – AM fungal inoculation DI – Dual inoculation

PI - Pathogen inoculation PoI – Post inoculation treatment

Table 1. Severity of wilt disease in tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

Days	Severity of wilt disease*						
	Con	MI	PI	PrI	DI	PoI	Mean
0 d	0	0	0	0	0	0	0
10 d	0	0	16	18 (+13)	4 (-75)	0	13
20 d	0	0	48	42 (-13)	15 (-69)	2	35
30 d	0	0	74	70 (-5)	22 (-70)	0	55
40 d	0	0	96	88 (-8)	25 (-74)	0	70
50 d	0	0	100	100 (0)	28 (-72)	0	76
60 d	0	0	-	-	29	0	-
Mean	0	0	67	64	21	0	-

LSD	Treatment	Days
P=0.01	26.1	36.8
P=0.05	18.3	25.9

F-values

Treatment (T) = 25.22***

Days (D) = 9.77**

***P = 0.001

**P = 0.005

Con – Control

MI – AM fungal inoculation

PI - Pathogen inoculation

PrI – Pre inoculation treatment

DI – Dual inoculation

PoI – Post inoculation treatment

* Symptoms were scored on a scale of 0 – 100.

Number in parenthesis shows percent increase (+) or decrease (-) over cont

Table 2. Changes in chlorophyll a, chlorophyll b and total chlorophyll content of tomato inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

Days	chl-a, chl-b and total chlorophyll content (mg/g dry weight)						
	Control plant	MI	PI	PrI	DI	PoI	Mean
Chlorophyll a							
0 d	1.80	1.76	1.79	1.75	1.81	1.78	1.78
10 d	2.13	2.21 (+4)	2.29 (+8)	2.19 (+3)	2.92 (+37)	2.16 (+1)	2.32
20 d	2.28	3.17 (+39)	1.95 (-15)	1.98 (-13)	2.13 (-7)	3.05 (+34)	2.43
30 d	2.44	3.36 (+38)	1.85 (-24)	1.78 (-27)	2.48 (+2)	3.12 (+28)	2.51
40 d	2.48	3.43 (+38)	1.68 (-32)	1.52 (-39)	2.51 (+1)	3.41 (+38)	2.51
50 d	2.55	3.38 (+33)	1.42 (-44)	1.39 (-46)	2.58 (+1)	3.57 (+40)	2.48
60 d	2.61	3.79 (+45)	-	-	2.65 (+2)	3.94 (+51)	2.17
Mean	2.33	3.01	1.57	1.52	2.44	3.00	-
Chlorophyll b							
0 d	0.76	0.75	0.79	0.77	0.74	0.76	0.76
10 d	1.04	1.22 (+17)	1.11 (+7)	1.16 (+12)	1.25 (+20)	1.26 (+21)	1.17
20 d	1.16	1.56 (+35)	0.99 (-15)	0.94 (-19)	1.29 (+11)	1.88 (+62)	1.30
30 d	1.28	1.80 (+41)	0.97 (-24)	0.82 (-36)	1.38 (+8)	1.97 (+54)	1.37
40 d	1.34	1.99 (+49)	0.71 (-47)	0.65 (-52)	1.51 (+13)	2.18 (+63)	1.40
50 d	1.39	2.19 (+55)	0.50 (-64)	0.42 (-70)	1.60 (+15)	2.27 (+63)	1.40
60 d	1.46	2.25 (+54)	-	-	1.61 (+10)	2.31 (+58)	1.27
Mean	1.20	1.68	0.72	0.68	1.34	1.80	-
Total chlorophyll							
O d	2.56	2.51	2.58	2.52	2.55	2.54	2.54
10 d	3.17	3.43 (+8)	3.40 (+7)	3.35 (+6)	3.17 (0)	3.32 (+5)	3.31
20 d	3.41	4.73 (+39)	2.94 (-14)	2.92 (-14)	3.42 (0)	4.93 (+45)	3.73
30 d	3.72	5.16 (+39)	2.82 (-24)	2.60 (-30)	3.86 (+4)	5.09 (+37)	3.88
40 d	3.82	5.42 (+42)	2.39 (-37)	2.17 (-43)	4.02 (+5)	5.59 (+46)	3.90
50 d	3.94	5.57 (+41)	1.92 (-51)	1.81 (-54)	4.18 (+6)	5.84 (+48)	3.88
60 d	4.16	6.04 (+45)	-	-	4.46 (+7)	6.25 (+50)	3.49
Mean	3.54	4.69	2.29	2.20	3.67	4.79	-

Chlorophyll a.

LSD	Treatment	Days	F-values
P=0.01	16.3	17.7	Treatment (T) = 86.41808***
P=0.05	12.4	13.4	Days (D) = 23.48852***
			T x D = 11.68243***
			***P = 0.001

Chlorophyll b.

LSD	Treatment	Days	F-values
P=0.01	0.60	0.43	Treatment (T) = 57.83874***
P=0.05	0.65	0.46	Days (D) = 289.5123***
			T x D = 30.25005***
			***P = 0.001

Total Chlorophyll

LSD	Treatment	Days	F-values
P=0.01	1.40	1.10	Treatment (T) = 665.6709***
P=0.05	1.51	1.15	Days (D) = 4026.66***
			T x D = 475.616***
			***P = 0.001

Con – Control PrI – Pre inoculation treatment

MI – AM fungal inoculation DI – Dual inoculation

PI - Pathogen inoculation PoI – Post inoculation treatment

Number in parenthesis shows percent increase (+) or decrease (-) over control.

Table 3. Changes in O-dihydric phenols of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

Days	O-dihydric phenols (μg/g dry weight)						
	Control plant	MI	PI	PrI	DI	PoI	Mean
Leaves							
0 d	54.2	53.9	54.0	54.3	54.1	53.8	54.3
10 d	57.4	64.6 (+13)	65.8 (+15)	66.2 (+15)	68.8 (+20)	66.5 (+16)	64.9
20 d	62.0	79.8 (+29)	61.2 (-1)	63.4 (+2)	90.6 (+46)	86.3 (+39)	73.9
30 d	68.7	91.2 (+33)	67.4 (-2)	66.1 (-4)	98.4 (+43)	100.8 (+47)	82.1
40 d	73.2	110.6 (+51)	65.6 (-10)	63.7 (-13)	105.8 (+45)	110.5 (+51)	88.2
50 d	79.0	126.8 (+61)	62.8 (-21)	60.5 (-23)	112.5 (+42)	116.8 (+48)	93.1
60 d	86.5	134.4 (+55)	-	-	117.1 (+35)	121.2 (+40)	76.5
Mean	68.7	94.5	53.8	53.5	92.5	93.7	
Roots							
0 d	13.1	12.8	12.9	13.2	13.0	12.8	13.0
10 d	14.0	14.6 (+4)	20.4 (+46)	21.5 (+54)	16.8 (+20)	15.6 (+11)	15.5
20 d	14.2	15.0 (+6)	14.2 (0)	14.1 (-1)	19.5 (+37)	19.8 (+39)	16.1
30 d	15.0	16.8 (+12)	14.6 (-3)	14.3 (-5)	21.8 (+45)	24.3 (+62)	17.8
40 d	16.5	19.1 (+16)	12.4 (-25)	13.8 (-16)	24.3 (+47)	26.8 (+62)	18.8
50 d	18.1	23.8 (+32)	10.1 (-44)	10.6 (-41)	26.5 (+46)	27.8 (+54)	19.5
60 d	19.6	27.4 (+40)	-	-	29.8 (+52)	29.1 (+49)	17.7
Mean	15.8	18.5	14.1	12.5	21.7	22.3	-

Leaves

LSD	Treatment	Days
P=0.01	30.0	32.4
P=0.05	22.8	24.7

F-values

Treatment (T) = 30056.98***
Days (D) = 75868.57***
T x D = 12779.82***
***P = 0.001

Roots

LSD	Treatment	Days
P=0.01	7.8	8.4
P=0.05	5.9	6.4

F-values

Treatment (T) = 859.69***
Days (D) = 4184.93***
T x D = 978.17***
***P = 0.001

Con – Control
MI – AM fungal inoculation
PI - Pathogen inoculation
PrI – Pre inoculation treatment
DI – Dual inoculation
PoI – Post inoculation treatment

Number in parenthesis shows percent increase (+) or decrease (-) over control

Table 4. Changes in total phenols of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

Days	Total phenols (μg/g dry weight)						
	Control plant	MI	PI	PrI	DI	PoI	Mean
Leaves							
0 d	117.5	116.8	117.9	118.1	117.5	116.9	117.5
10 d	128.6	140.5 (+9)	146.8 (+14)	145.2 (+13)	156.2 (+22)	139.8 (+9)	142.9
20 d	135.4	162.9 (+20)	141.5 (+5)	145.9 (+8)	186.4 (+38)	149.4 (+10)	153.6
30 d	139.8	183.6 (+31)	135.2 (-3)	131.8 (-6)	204.5 (+46)	171.8 (+23)	161.1
40 d	145.2	195.4 (+26)	142.3 (-2)	136.5 (-6)	215.5 (+48)	189.2 (+30)	170.7
50 d	149.8	210.2 (+40)	134.5 (-10)	126.8 (-15)	219.8 (+47)	205.1 (+37)	174.4
60 d	158.5	216.5 (+37)	-	-	221.6 (+40)	221.7 (+40)	136.4
Mean	139.3	175.1	116.9	114.9	188.8	170.6	-
Roots							
0 d	36.7	36.5	37.1	36.8	37.2	36.9	36.9
10 d	37.4	43.8 (+17)	40.2 (+8)	41.5 (+11)	42.3 (+13)	42.6 (+14)	41.3
20 d	38.5	49.0 (+27)	37.5 (-3)	36.0 (-7)	49.8 (+29)	52.5 (+36)	43.9
30 d	45.0	56.4 (+25)	44.7 (-1)	43.8 (-3)	55.0 (+22)	60.8 (+35)	51.0
40 d	50.0	61.8 (+24)	42.8 (-14)	40.5 (-19)	59.8 (+20)	65.4 (+31)	53.4
50 d	52.6	65.4 (+24)	30.4 (-42)	31.9 (-39)	62.8 (+19)	69.3 (+32)	52.1
60 d	55.4	69.8 (+26)	-	-	66.4 (+20)	72.5 (+31)	44.0
Mean	45.1	54.7	33.3	32.9	53.3	57.1	-

Leaves

LSD	**Treatment**	**Days**
P=0.01	59.1	63.9
P=0.05	45 0	48.6

F-values

Treatment (T) = 30336.44***
Days (D) = 60592.62***
T x D = 22946.52***
***P = 0.001

Root

F-values

LSD	Treatment	Days
P=0.01	16.5	17.9
P=0.05	12.6	13.6

Treatment (T) = 870.82***
Days (D) = 3035.67***
T x D = 542.43***
***P = 0.001

Con – Control
MI – AM fungal inoculation
PI - Pathogen inoculation
PrI – Pre inoculation treatment
DI – Dual inoculation
PoI – Post inoculation treatment

Number in parenthesis shows percent increase (+) or decrease (-) over control.

Table 5. Changes in lipid levels of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

Days	Lipid (μg/g dry weight)						
	Control plant	MI	PI	PrI	DI	PoI	Mean
Leaves							
0 d	14.6	14.5	14.9	14.8	14.6	14.5	14.7
10 d	14.9	13.9 (-7)	15.8 (+6)	15.4 (+3)	12.0 (-20)	13.4 (-10)	14.2
20 d	15.5	15.0 (+2)	13.9 (-10)	13.2 (-15)	10.5 (-32)	15.9 (+3)	14.0
30 d	16.0	16.3 (+4)	13.1 (-18)	12.9 (-19)	12.8 (-20)	16.4 (+3)	14.6
40 d	16.3	18.7 (+15)	12.6 (-23)	12.5 (-23)	14.1 (-14)	18.1 (+11)	15.4
50 d	16.7	20.4 (+22)	10.8 (-35)	10.5 (-37)	16.3 (+1)	21.8 (+31)	16.1
60 d	17.5	22.5 (+29)	-	-	17.9 (+2)	23.8 (+36)	13.6
Mean	15.9	17.3	11.6	11.3	14.0	17.7	-
Roots							
0 d	7.4	7.6	7.6	7.7	7.9	7.2	7.6
10 d	7.6	8.4 (+11)	8.3 (+9)	8.2 (+8)	7.2 (-5)	8.9 (+17)	8.1
20 d	7.9	10.7 (+35)	7.2 (-9)	6.9 (-13)	6.8 (-14)	9.5 (+20)	8.2
30 d	8.5	11.5 (+35)	7.0 (-18)	6.5 (-25)	7.8 (-8)	11.2 (+32)	8.8
40 d	9.0	12.4 (+38)	6.6 (-27)	6.0 (-33)	9.2 (+2)	12.0 (+33)	9.2
50 d	9.4	14.6 (+55)	6.2 (-34)	5.8 (-38)	9.9 (+5)	15.2 (+62)	10.2
60 d	9.9	16.9 (+71)	-	-	11.3 (+14)	17.8 (+80)	9.3
Mean	8.5	11.7	6.1	5.9	8.6	11.7	-

Leaves

LSD	Treatment	Days
P=0.01	5.7	6.1
P=0.05	4.3	4.7

F-values

Treatment (T) = 173.44***
Days (D) = 2232.47***
T x D = 678.16***
***P = 0.001

Roots

LSD	Treatment	Days
P=0.01	3.8	2.9
P=0.05	4.1	3.1

F-values

Treatment (T) = 165.08***
Days (D) = 1620.06***
T x D = 270.44***
***P = 0.001

Con – Control PrI – Pre inoculation treatment
MI – AM fungal inoculation DI – Dual inoculation
PI - Pathogen inoculation PoI – Post inoculation treatment

Number in parenthesis shows percent increase (+) or decrease (-) over control.

Table 6. Changes in total protein content of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

Days	Protein (mg/g dry weight)						
	Control plant	MI	PI	PrI	DI	PoI	Mean
Leaves							
0 d	3.29	3.28	3.29	3.20	3.20	3.26	3.25
10 d	3.40	3.43 (+1)	3.02 (-11)	3.07 (-10)	3.00 (-12)	3.43 (+2)	3.23
20 d	3.65	3.89 (+7)	3.17 (-13)	3.23 (-12)	3.48 (-5)	3.78 (+4)	3.53
30 d	3.85	4.22 (+10)	3.38 (-12)	3.45 (-10)	3.65 (-5)	4.13 (+7)	3.78
40 d	3.95	4.85 (+23)	3.15 (-20)	3.22 (-19)	3.95 (+1)	4.98 (+26)	4.02
50 d	4.10	5.20 (+27)	2.98 (-27)	3.03 (-26)	4.17 (+2)	5.24 (+28)	4.12
60 d	4.32	6.22 (+44)	-	-	4.43 (+3)	6.47 (+50)	3.57
Mean	3.79	4.44	2.71	2.74	3.70	4.47	-
Roots							
0 d	1.96	1.90	1.94	1.97	1.93	1.98	1.95
10 d	2.10	2.09 (-1)	2.15 (+2)	2.12 (+1)	2.06 (-2)	2.02 (-4)	2.09
20 d	2.20	2.49 (+13)	1.32 (-40)	1.35 (-37)	1.87 (-15)	2.20 (0)	1.91
30 d	2.32	2.98 (+28.5)	1.43 (-38.4)	1.50 (-35.3)	2.22 (-4.3)	2.88 (+24.1)	2.22
40 d	2.38	3.35 (+41)	1.17 (-51)	1.19 (-50)	2.43 (+2)	3.20 (+35)	2.29
50 d	2.47	3.78 (+53)	1.10 (-56)	1.07 (-57)	2.58 (+5)	3.50 (+42)	2.42
60 d	2.70	3.92 (+45)	-	-	2.78 (+3)	4.25 (+57)	2.28
Mean	2.30	2.93	1.30	1.31	2.27	2.86	-

Leaves

LSD	Treatment	Days
P=0.01	1.2	1.3
P=0.05	0.9	1.0

F-values

Treatment (T) = 1874.12***

Days (D) = 8458.67***

T x D = 2253.19***

***P = 0.001

Roots

LSD	Treatment	Days
P=0.01	0.90	0.98
P=0.05	0.70	0.75

F-values

Treatment (T) = 481.48***
Days (D) = 7664.91***
T x D = 944.64***
***P = 0.001

Con – Control
MI – AM fungal inoculation
PI - Pathogen inoculation
PrI – Pre inoculation treatment
DI – Dual inoculation
PoI – Post inoculation treatment

Number in parenthesis shows percent increase (+) or decrease (-) over control.

Table 7. Changes in cytokinin level of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

Days	Cytokinin (μg/g dry weight)						
	Control plant	MI	PI	PrI	DI	PoI	Mean
Leaves							
0 d	32.8	32.5	33.1	32.9	32.6	32.7	32.8
10 d	35.1	38.3 (+9)	29.5 (-16)	30.6 (-13)	36.4 (+4)	38.6 (+10)	34.8
20 d	38.5	49.1 (+28)	21.8 (-43)	20.4 (-47)	41.8 (+9)	51.2 (+33)	37.1
30 d	41.7	54.8 (+31)	22.6 (-46)	18.5 (-56)	48.5 (+16)	60.4 (+46)	41.1
40 d	43.9	63.6 (+45)	18.4 (-58)	16.9 (-62)	56.2 (+28)	68.9 (+57)	44.7
50 d	46.4	71.4 (+54)	16.2 (-65)	15.7 (-66)	60.6 (+31)	76.5 (+65)	47.8
60 d	48.2	75.8 (+57)	-	-	63.7 (+32)	81.5 (+69)	44.9
Mean	40.9	55.1	20.2	19.3	48.5	58.5	-
Roots							
0 d	18.6	18.4	18.5	18.9	18.6	18.5	18.6
10 d	20.5	22.5 (+10)	19.8 (-3)	19.4 (-5)	20.2 (-2)	21.9 (+7)	20.7
20 d	23.8	30.2 (+27)	16.2 (-32)	16.5 (-31)	25.7 (+8)	32.4 (+36)	24.1
30 d	26.2	39.6 (+51)	12.8 (-51)	13.2 (-50)	31.3 (+20)	44.8 (+71)	28.0
40 d	29.4	44.7 (+52)	10.4 (-65)	10.9 (-63)	35.8 (+22)	49.2 (+67)	30.1
50 d	31.7	48.4 (+53)	8.9 (-72)	9.3 (-71)	39.3 (+24)	53.6 (+69)	31.9
60 d	33.6	51.6 (+54)	-	-	43.5 (+30)	57.8 (+72)	31.1
Mean	26.3	36.5	12.4	12.6	30.6	39.7	-

Leaves

LSD	Treatment	Days
P=0.01	18.3	19.8
P=0.05	13 9	15.1

F-values

Treatment (T) = 41.87***

Days (D) = 362.46***

T x D = 33.13**

***P = 0.001

**P = 0.005

Roots

LSD	Treatment	Days
P=0.01	13.4	14.5
P=0.05	10.2	11.0

F-values

Treatment (T) = 82.17***
Days (D) = 448.42***
T x D = 43.48**

***P = 0.001
**P = 0.005

Con – Control
MI – AM fungal inoculation
PI - Pathogen inoculation
PrI – Pre inoculation treatment
DI – Dual inoculation
PoI – Post inoculation treatment

Number in parenthesis shows percent increase (+) or decrease (-) over control.

Table 8. Changes in tomatine content of tomato plants inoculated with G. fasciculatum and F. o. f. sp. lycopersici.

Days	Tomatine (μg/g dry weight)						
	Control plant	MI	PI	PrI	DI	PoI	Mean
Leaves							
0 d	492	487	489	490	486	488	489
10 d	530	524 (-1)	592 (+12)	576 (+9)	538 (+2)	522 (-2)	547
20 d	556	570 (+3)	562 (+1)	554 (0)	578 (+4)	580 (+4)	567
30 d	578	622 (+8)	520 (-10)	548 (-5)	608 (+5)	672 (+16)	591
40 d	590	738 (+25)	468 (-20)	406 (-31)	668 (+13)	784 (+33)	609
50 d	603	804 (+33)	395 (-35)	386 (-36)	750 (+24)	916 (+52)	642
60 d	650	926 (+51)	-	-	790 (+29)	970 (+58)	550
Mean	566	667	432	423	631	705	-
Roots							
0 d	114	112	115	116	113	110	113
10 d	136	144 (+6)	142 (+5)	148 (+9)	138 (+2)	140 (+3)	141
20 d	170	198 (+17)	166 (-2)	162 (-5)	230 (+35)	216 (+27)	190
30 d	202	272 (+35)	191 (-6)	196 (-3)	342 (+69)	338 (+67)	257
40 d	246	350 (+42)	186 (-24)	190 (-23)	404 (+64)	390 (+59)	294
50 d	263	402 (+53)	169 (-36)	176 (-33)	452 (+72)	436 (+66)	316
60 d	284	448 (+58)	-	-	476 (+68)	478 (+68)	281
Mean	202	275	138	141	308	301	-

Leaves

LSD	Treatment	Days
P=0.01	231.5	250.2
P=0.05	176 2	190.2

F-values

Treatment (T) = 67.55***

Days (D) = 215.40***

T x D = 83.57**

***P = 0.001

**P = 0.005

Roots

LSD	Treatment	Days
P=0.01	1160.5	125.9
P=0.05	88.6	95.7

F-values

Treatment (T) = 5019.15***
Days (D) = 5593.74***
T x D = 943.05***
***P = 0.001

Con – Control
MI – AM fungal inoculation
PI - Pathogen inoculation
PrI – Pre inoculation treatment
DI – Dual inoculation
PoI – Post inoculation treatment

Number in parenthesis shows percent increase (+) or decrease (-) over control.

Table 9. Effect of inoculation of G. fasciculatum and F. o. f. sp. lycopersici on growth and yield of tomato plants under field conditions.

Characters	Treatment			
	Con	MI	PI	PoI
AM population	208 b	684 c (+229)	169 a (-19)	694 c (+234)
Disease severity	-	54	-	-
Shoot dry weight^{-1} g	13.8 b	28.6 c (+107)	6.4 a (-54)	32.5 d (+136)
Yield / bar $^{-1}$ (15 plant) (g)	2.27 b	3.58 c (+58)	0.75 (-67)	3.95 d (+74)

Con – Control
MI – AM fungal inoculation
PI - Pathogen inoculation
PoI – Post inoculation treatment

Number in parenthesis shows percent increase (+) or decrease (-) over control.
Row mean followed by the same letter do not significantly different at the 0.05 level by DMRT.