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Vesicular-Arbuscular Mycorrhizal Colonization and Growth of Tomato *(Lycopersicon esculentum)* in Autoclaved Soil

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ABSTRAK

Kesan tanah autoklava terhadap pengkolonian kulat vesikal-arbuskular mikoriza (VAM) dan pengeluaran tomato biojisim telah dinilai dalam bekas yang diuji di rumah hijau. Empat rawatan telah diberi iaitu (1) Tumbuhan bebas-VAM dalam tanah autoklava (2) Tumbuhan VAM dalam tanah autoklava (3) Tumbuhan VAM dalam tanah autoklava yang diubah suai dengan tanah tanpa autoklava filtrat bebas-VAM (4) Tumbuhan VAM dalam tanah tanpa autoklava. Tumbuhan VAM yang membesar dalam tanah tanpa autoklava menunjukkan pengkolonian akar paling tinggi iaitu 87.78%, manakala (2) dan (3) masing-masing hanya 55.11% dan 56.94%. Di samping itu, panjang tunas (105.4 cm/tanaman), jumlah ruang daun (740.3 cm²/tanaman) dan biojisim (8.43 g/tanaman) diperolehi dalam tumbuh-tumbuhan VAM yang membesar dalam tanah autoklava. Tumbuhan bebas VAM dalam tanah autoklava kurang membesar. Dalam rawatan (3) dan (4) pembesaran tumbuhan adalah sederhana. Keputusan menunjukkan bahawa pengkolonian VAM dan pengsporaan sesuai dalam keadaan semulajadi tetapi tumbuhan VAM dalam keadaan tanah autoklava menghasilkan pertumbuhan yang maksimum.

ABSTRACT

*The effect of autoclaving soil on vesicular-arbuscular mycorrhizal (VAM) fungal colonization and biomass production of tomato (*Lycopersicon esculentum *Mill.) was assessed in pot experiments under greenhouse conditions. Four treatments were given viz., (1) VAM-free plants in autoclaved soil, (2) VAM plants in autoclaved soil, (3) VAM plants in autoclaved soil amended with VAM-free filtrate of non-autoclaved soil, and (4) VAM plants in non-autoclaved soil. The VAM plants grown in non-autoclaved soil showed the highest root colonization of 87.78% while those under (2) and (3) showed only 55.11 % and 56.94% respectively. On the other hand, significantly higher shoot length (105.4 cm/plant), total leaf area (740.3 cm²/plant) and biomass (8.43 g/plant) were obtained in VAM plants grown in autoclaved soil. VAM-free plants in autoclaved soil had reduced growth. In treatments 3 and 4 plant growth was intermediate. The results indicate that VAM colonization and sporulation were favoured under natural conditions, but VAM plants under autoclaved soil conditions produced maximum growth.*

INTRODUCTION

Partial or complete sterilization of soil often changes its nutrient status and structure (Lopes and Wollum, 1976; Mulder, 1979; Jakobsen and Andersen, 1982). Sterilization also removes some or all of the microorganisms (Bowen and Rovira, 1969). However, enhanced fertility due to autoclaving is available to plants only during their initial stage of growth.

Mosse *et al.* (1969) observed that soil sterilization had a positive effect on the development of vesicular-arbuscular mycorrhizal (VAM) infection and plant growth, but Wilson (1984) reported that autoclaving of soil inhibited VAM spore germination. Little is known about the effect of autoclaving of soil on VAM fungal colonization and biomass production in tomato (*Lycopersicon esculentum* Mill.) which this study attempted to elucidate.

MATERIALS AND METHODS

Preparation of substrates and raising of seedlings

Moderately fertile sandy loam soil collected from the experimental fields of Bharathiar University, Coimbatore (pH 8.1, EC 0.1 m S cm^{-1}, N 10.5 mg kg^{-1}, P 1.7 mg kg^{-1}, K 38 mg kg^{-1}), mixed with sand at 1:1 proportion was used as the substrate for plant growth. The substrate was sterilized in cloth bundles in an autoclave at 1.5 kg sq cm^{-1} pressure (121 °C) for 1 hour each on three consecutive days and left in the laboratory for seven days to facilitate release of any toxic substances produced during heating. Ten presterilized pots of 18 cm diam. were filled with 6 kg pot^{-1} of autoclaved soil and another 30 pots were filled with non-autoclaved soil-sand mixture.

The field soil contained VAM fungal spores predominantly of *Acaulospora bireticulata* Rothwell & Trappe; A. *sporocarpia* Berch; *Glomus deserticola* Trappe, Bloss & Menge; *G. fasciculatum* (Thaxter & Gerdemann) Gerdemann & Trappe; *G. geosporum* (Nicolson & Gerdemann) Walker; *G. tenue* (Greenall) Hall and *G. sinosum* (Gerdemann & Bakshi Almeida & Schenck (= *Sclerocystis pakistanica* Iqbal & Bushra), having a total spore count of 20.43 (± 0.73) spores g^{-1} dry soil. The VAM fungal species were identified using synoptic keys (Hall, 1984; Morton 1988; Schenck and Perez, 1987) for spores and sporocarps.

All pots were sown with uniform sized seeds of tomato cv. Co. 1 at a density of 5 per pot and watered regularly. One week after germination, they were thinned to maintain one healthy seedling per pot and allowed to grow for 30 days under greenhouse conditions. VAM fungal infection was detected by the method of Philips and Hayman (1970) in the seedlings raised on non-autoclaved soil, while those from autoclaved soil were free from any infection.

Transplanting of seedlings

Two sets of potted soil, i.e. 30 pots of autoclaved and 10 non-autoclaved, were prepared as in the previous experiment. Of these, 10 pots of autoclaved soil were drenched with soil filtrate of non-autoclaved soil at the rate of 100 ml pot^{-1} to facilitate VAM and mycophagous animal free microbial action in the soil. The filtrate was prepared by adding 500 ml of sterilized water to 350 g of non-autoclaved soil and thoroughly mixing. The liquid portion of mixture was decanted and filtered through a 38 mm mesh that retained VAM fungal spores and mycophagous animals but not other microbes (Azcon-Aguilar and Barea, 1985).

The 30-day-old VAM-free tomato seedlings in the autoclaved potted soil were transplanted into 10 pots of autoclaved soil with one seedling per pot as Treatment 1, and out of the 30 VAM seedlings in the non-autoclaved potted soil, 10 pots each with one seedling were transplanted into autoclaved soil (Treatment 2), autoclaved soil amended with filtrate (Treatment 3), and non-autoclaved soil (Treatment 4). There were six replicates for each treatment.

All the transplanted seedlings under different treatments were allowed to grow for another 60 days (90 days in total) without adding any fertilizer, when they were harvested for estimation of VAM colonization, sporulation and growth parameters.

Laboratory analysis

Soil pH, EC and nutrient status were analysed using standard procedures (Misra, 1968; Jackson, 1973). For estimation of VAM colonization, at harvest, 0.20 g (wet weight) was excised from each root system. The rest of the root system was kept for observing dry weight. VAM colonization index (VAM1) was estimated after staining the root samples following the method of Phillips and Hayman(1970) and using the scoring method of Edathil *et al.* (1994). The number of VAM fungal spores of the rhizosphere soil of different treatment plants was estimated by wet-sieving and decanting method (Gerdemann and Nicolson, 1963). Spore counting was done with 100 g dry soil and spore density was expressed as number of spores per gram of soil.

The total leaf area was measured using a leaf area meter and the mean of six replicates of each treatment calculated.

Root length, shoot length, root dry weight, shoot dry weight, root/shoot (R/S) ratio for dry weight and mycorrhizal dependency were recorded. Dry weights were obtained by drying for 24 h at 45°C in a hot air oven. Mycorrhizal dependency (M.D.) was calculated using the formula of Plenchette *et al.* (1983):

$$M.D.= \frac{(\text{DM of VAM plant} - \text{DM of VAM-free plant})}{(\text{DM of VAM-free plant})} \times 100$$

where DM = dry mass.

RESULTS

The highest percentage of VAM colonization and spore density was observed in the VAM plants grown in non-autoclaved soil, while those in autoclaved soil (amended or non-amended with soil filtrate) showed significantly lower root colonization. However, the number of spores was significantly reduced only in the filtrate amended treatment (Table 1). Altogether 17 species of VAM fungi belonging to 3 genera were recorded from the rhizosphere soils of the VAM plants irrespective of treatment conditions (Table 2).

For plant growth, significantly higher ($P < 0.01$) shoot length, total leaf area and biomass were obtained in VAM plants grown in autoclaved soil while lowest growth was recorded in VAM-free plants grown in autoclaved soil. There was no marked difference in root length or R/S ratio among VAM plants and in autoclaved and non-autoclaved soils. However R/S ratios were higher in VAM-free plants and in VAM plants grown in the substrate amended with soil microbes (Table 3; Fig. 1).

The VAM-free plants in autoclaved soil showed good growth initially; they became stunted in due course, but retained a lush green colour unlike plants in non-autoclaved soil which had stunted growth and were pale by the end of the experiment.

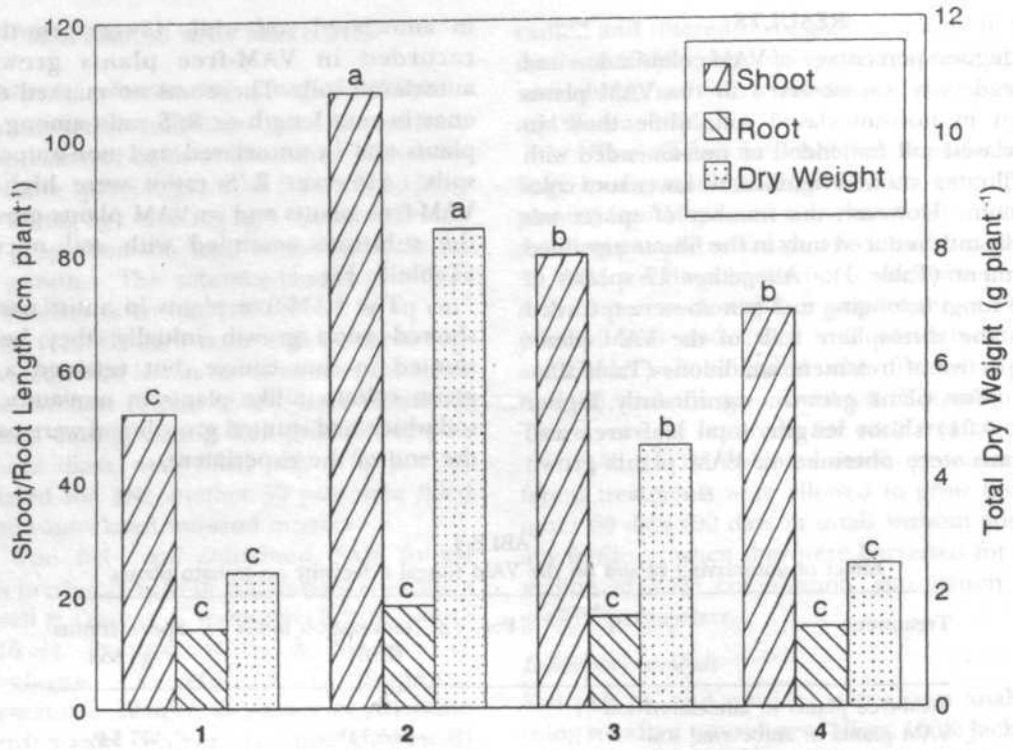
TABLE 1
Effect of autoclaving of soil on the VAM fungal infectivity on tomato plants

Treatment	Per cent colonization index (PDI)	Spore counts g^{-1} soil
VAM-free plants in autoclaved soil	0	0
VAM plants in autoclaved soil	55.11[b]	11.54[a]
VAM plants in autoclaved soil amended with VAM-free filtrate of non-autoclaved soil	56.94[b]	9.51[b]
VAM plants in non-autoclaved soil	87.78[a]	12.39[a]
	P >0.01 CD 16.03	P >0.05 CD 1.83

Values with same alphabet in the same column are not significantly different.

TABLE 2
VAM fungal species recorded from the rhizosphere soil of VAM tomato plants after 90 days of growth

1. *Acaulospora bireticulata* Rothwell & Trappe
2. *A. trappei* Ames & Linderman
3. *Gigaspora albida* Schenck & Smith
4. *Glomus aggregatum* (Schenck & Smith) Koske
5. *G. deserticola* Trappe, Bloss & Menge
6. *G. fasciculatum* (Thaxter & Gerdemann) Gerdemann & Trappe
7. *G. fulvum* (Berk.) Pat.
8. *G. geosporum* (Nicolson & Gendermann) Walker
9. *G. intraradices* Schenck & Smith
10. *G. maculosum* Miller & Walker
11. *G. macrocarpum* (Tul. & Tul) Nicolson & Gerdemann
12. *G. manihotis* Howler, Sieverding & Schenck
13. *G. melanosporum* Gerdemann & Trappe
14. *G. microaggregatum* Koske, Gemma & Olexia
15. *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe
16. *G. tenue* (Greenall) Hall
17. *G. sinosum* (Gerdemann & Bakshi) Almeida & Schenck



1. VAM-free plants in autoclaved soil
2. VAM plants in autoclaved soil
3. VAM plants in autoclaved soil amended with VAM-free extract of non-autoclaved soil
4. VAM plants in non-autoclaved soil

Values are means of four replications
Values with same alphabet are not significantly different at P<0.01

Fig. 1: Effect of autoclaved soil on the growth of tomato seedlings with and without VAM

TABLE 3
Effect of autoclaving of soil on total leaf area, root/shoot ratio and mycorrhizal dependency (M.D.) of tomato plants

Treatment	Leaf area (cm^2)	Root dry weight	Shoot dry weight	R/S ratio	M.D.%
1. VAM-free plants in autoclaved soil	166.20^c	0.60^c	1.79^c	0.34 (±0.001)	0
2. VAM plants in autoclaved soil	740.34^a	2.11^a	6.32^a	0.33 (±0.0002)	252.30
3. VAM plants in autoclaved soil amended with VAM-free filtrate of non-autoclaved soil	409.10^b	1.40^b	3.42^b	0.41 (±0.0005)	90.79
4. VAM plants in non-autoclaved soil	173.91^c	0.62^c	1.86^c	0.33(±0.0007)	3.77
	P > 0.05	P > 0.01	P >0.01		
	CD 138.9	CD 0.34	CD 0.52		

Values with same alphabet in the same column are not significantly different.
Figures in parentheses indicate S.E. of the mean values.

DISCUSSION

Most of the differences observed in plant growth responses were due to root colonization by VAM fungi and soil microbial populations and not to the direct effect of autoclaving. The poor growth of VAM-free tomato plants in autoclaved soil may result from the insufficient uptake of P from the acutely P deficient soils (0.4 mg kg^{-1} soil) in the absence of VAM fungi. On the other hand, Meredith and Anderson (1992) reported that autoclaving soil alone increased plant growth, which was not due to the addition of non-autoclaved soil sieves to autoclaved soil or colonization of plants by VAM fungi. Under their experimental conditions, however, the soil P was high i.e., 41 mg kg^{-1} soil, which increased to 48 mg kg^{-1} upon autoclaving. In the present experiment autoclaving only increased available P levels marginally (from 1.7 mg kg^{-1} to 1.9 mg kg^{-1} soil). The short-lived initial growth increases seen in VAM-free plants grown in autoclaved soil may be indicative of this autoclaving-mediated nutrient release. Under P deficient conditions, VAM colonization is expected to play a vital role in the supply of P to plants, and indeed higher plant growth was observed in VAM plants grown in autoclaved soils (Fig. 1).

The VAM plants transplanted onto non-autoclaved substrate showed the highest VAM colonization among the treatments (Table 1). Possible explanations are: (1) VAM inoculum in soil facilitates faster infection in newly formed roots, (2) mycophagous animals, like collembola, feeding on the extramatrical hypae induce faster spread of VAM fungi within root tissues, or (3) beneficial interaction between soil microbes facilitates VAM infection. The beneficial interaction of microbes is discounted as the addition of soil microbes (non-autoclaved soil filtrate) failed to promote VAM colonization in autoclaved soil (Table 1). Groth and Martinson (1983) reported that preexisting infection sites on the root surface restrict further infections in their vicinity. Accordingly, the mycophagous animals may indirectly enhance the internal spread of VAM fungi in the root cortex and so increase root colonization through grazing of extramatrical hypae.

The grazing of mycophagous animals and the activity of parasitic fungi may have major impact on the efficiency of VAM plants in non-autoclaved soil, as they showed poor growth in spite of the highest percent VAM colonization. Clark and Mosse (1981) made similar observations when they found no definite correlation between the amount of fungal infection inside the root and its enhancement of plant growth. In Treatment 3, the VAM plants showed significantly higher growth (compared with those in non-autoclaved soils) in the presence of non-mycophagous soil microbes (Fig. 1). Thus, with moderate root colonization, the growth of VAM plants in autoclaved substrates (Treatments 2 and 3) registered significantly higher growth over those in non-autoclaved soil due to higher VAM efficiency. Again, when soil microbes (soil filtrate) were added to the autoclaved substrate (Treatment 3), plant growth decreased significantly compared with Treatment 2 (Fig. 1), suggesting competition for inorganic nutrients between plants and microbes. This agrees with reports of growth depression in big bluestem grass following additions of sievings of non-sterile soil (Hetrick *et al.*, 1986, 1988; Bentivenga, 1988). Earlier workers have suggested that higher growth of VAM plants in autoclaved soil may be due to the elimination of pathogens, mycophagous animals and/or microbial competitors for inorganic nutrients (Schenck *et al.*, 1975; Atilano *et al.*, 1976; Daniels and Menge, 1980; Warnock *et al.*, 1982; Groth and Martinson, 1983; Hetrick *et al.*, 1986, 1988; Afek *et al.*, 1990; Hetrick and Wilson, 1991). In contrast, Warnock *et al.* (1982) found significant growth stimulation in leek *(Allium porrum)* due to the addition of sievings.

It has been reported that efficient mycorrhizal colonization reduced root/shoot ratio (Fitter, 1977; Douds and Chaney, 1982; Hall *et al.*, 1984) since extramatrical mycorrhizal hyphae act as a supplemental root system and, consequently, root biomass would decrease. However, there was no difference between R/S ratios of mycorrhizal and non-mycorrhizal plants (Table 3), as was also reported by Seo *et al.* (1988). The apparent decrease in R/S ratio in VAM plants as evident in Fig. 1 does not hold good as the root length indicates merely the tap root length but not the total root length.

CONCLUSION

The results of this study established that VAM infection and not autoclaving of soil has the major influence on growth of tomato plants in a

P deficient soil. The suppressed growth of VAM plants with the addition of soil microbes (non-autoclaved soil filtrate) was also evident. VAM efficiency was mainly the result of the presence of extramatrical hyphae and the efficient VAM symbiosis, which reduced R/S ratio, and not the extent of per cent root colonization.

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