DISCUSSION
V. DISCUSSION

Rapid development of a VAM fungal infection could be a major development of response in crops. The role of mycorrhizae as biocontrol agents to feeder root infection by pathogens is an important aspect of our understanding of the overall ecological significance of mycorrhizae (Raman, 1996). The data presented in this thesis showed that the percentage of infection of *Glomus geosporum* was higher in GV plants of both the varieties of cotton. Similar findings were observed by Garcia-Garrido and Ocampo (1989) in the case of VAM and bacterial pathogen in tomato. VAM infection was higher when inoculated simultaneously with *Pseudomonas syringe*. The higher rate of mycorrhizal infection in GV plants compared to GA, G and GAV plants can be explained due to competition between two fungi (*G. geosporum* and *Verticillium dahlia*) to colonize the root for nutrients and root exudates or other mechanisms may operate in the ecological niche on the root surface. The VAM infection was highly reduced in GAV plants which may be due to inefficiency of *Azospirillum brasilense* to control *V. dahliae*. It is more evident from the results that in AV plants population of *A. brasilense* was reduced by presence of *V. dahliae*. According to Hadas and Okon (1987), *A. brasilense* failed to serve as a biocontrol agent. Fallik *et al.* (1988) found that application of pathogen outnumbered *A. brasilense* and inhibited its beneficial effects on maize. In GA plants, the VAM infection was however high because synergistic effect of *G. geosporum* and *A. brasilense*. This type of synergistic effect of VAM and *Azospirillum* has been reported in many plants.*A. lipoferum* and *Gigaspora callospora* in sorghum (Rao *et al.*, 1989). *A. brasilense* and *G. versiforme* in barley (Negi *et al.*, 1990) *A. brasilense* with various VAM fungi such as *Acaulospora* sp., *G. fasciculatum* and *Gig. margarita* in pearl millet (Tilak and Singh, 1988) and *A. chroococcum* and *G. fasciculatum* in tomatoes (El-Shanshoury *et al.*, 1989).
Besides increasing water transport, phosphate uptake etc., VAM fungi improve plant resistance to foliar and root pathogens including, bacteria, fungi and nematodes. In the present investigation, *Verticillium* was completely suppressed in GV plants. The microsclerotia population of pathogen was also not found in the rhizosphere of GV plants. Similar type of VAM fungal effect was observed by Rosendahl (1985) who showed that the VAM fungus *G. fasciculatum* suppressed the root rot peas caused by *Aphanomyces enteiches*. Rosendahl (1985) also found decreased number of oospores of pathogen in VAM inoculated plants. Decreased fusarial incidence in *G. fasciculatum* inoculated tomato plants has been reported by Raman and Gnanaguru (1996). The complete inhibition of *F. solani*, *Rhizoctonia solani* and *Macrophomena phaseolina* in soybean by *G. mosseae* has been demonstrated (Zambolin and Schenck, 1983).

Knowledge of mechanisms involved in the biological control system is the key for enhancement of biocontrol agents. More than one mechanism may operate to suppress a pathogen in a control system. The complete elimination of disease in GV plants may be due to the increased levels of P, phenols, amino acids etc. Host susceptibility to infection or tolerance to disease is influenced by the nutritional status of the host and fertility level of the soil (Cook and Baker, 1982). Srihuttagum and Sivachidamparam (1991) reported that the disease severity of *F. oxysporum* in peas was reduced by the application of P. The increased level of P enhanced the wall thickening in the cortical cells of the roots which prevents the penetration of the pathogen (Schenck, 1981). Increased level of P was observed in GV plants. Growth benefits of the plants by VAM fungi are often attributed to improve P nutrition (Bolan, 1991). P not only influence plant growth as nutrient but also reduce pathogenic infection (Reuveni et al., 1993). The GV plants had higher concentration of P in both roots and leaves. Therefore, mycorrhizal fungus helped to supplement the P nutrition of plants through their active external hyphae beyond the depletion zone surrounding the absorbing root and root hairs (Cooper, 1984). Further, the
external hyphae of the VAM fungi may have more absorbing sites of P than the surface of the roots (Cress et al., 1979). Mosse et al. (1976) and Waidyanath et al. (1979) reported that mycorrhizal infection increased P level in the shoots. Microscopical observation revealed the presence of arbuscules in GV, GA, G and GAV plants. The percentage of arbuscules in relation to total colonization of VAM was higher in GV plants. According to Liu (1995) the percentage of arbuscules of G. versiforme in the roots of cotton and the arbuscules and total colonization ratio were positively correlated with increase in verticillium wilt resistance. The arbuscules transfer P from the fungus to the host (Harley and Smith, 1983). The rapid formation of arbuscules may be one of the reasons for the rapid uptake and accumulation of P in GV plants. In contrast to GV plants, GAV plants and A plants had decreased amount of P than the control plants. The decreased level of P nutrient may be due to destruction of root cells by pathogen because Verticillium is a necrophile which destroys the cells and from which obtains its nourishment. The proliferation of Verticillium blocked the xylem vessels so that the conductivity was affected. Declined conductivity in the xylem was observed Verticillium infected tomato roots. In the present investigation low rate of moisture content was observed in GAV, AV and V plants whereas in GV plants maximum moisture content was encountered.

In GV plants, increased level of phenols and o-dihydric phenol was also correlated with disease suppression because phenols induce the plant resistance to pathogen (Mahadevan, 1982; Krishna and Bagyaraj, 1986). Increased level of phenols in GV plants is important in the resistant mechanism. The amino acid level in GV plants may also be accounted for disease resistance. These plants had higher level of free amino acids in both roots and leaves. Higher amount of amino acids, especially arginine found in root exudates of mycorrhizal plants, has been shown to reduce the chlamydospores production in Thielaviopsis basicola (Baltruschat and Schenbeck, 1972). The amino acids like arginine,
serine and phenylalanine were shown to be responsible for the mycorrhizal plants resistance against pathogens (Young et al. 1972).

In both roots and leaves the protein content was higher in GV plants. This is comparable to the findings of Wyss et al. (1990) who showed that G. mosseae produced specific proteins in soybean during cellular interaction. Similar observations of increase in protein content due to G. fasciculatum infecting in soybean was made by Pacovsky (1989). In leaves and roots of V, AV and GAV plants the protein content initially increased and subsequently reduced. Amino acid content was also decreased in the roots and leaves of these plants. This indicates that pathogen successfully utilized the amino acids.

_A. brasilense_ inoculated plants (A and GA) showed good plant growth. In addition to increasing many root parameters, plant inoculation with _Azospirillum_ affected many foliage parameters (Kapulnik et al., 1981 a). These changes were directly attributed to positive bacterial effects on mineral uptake by the plants. Enhancement in the uptake N,P,K and Fe by _Azospirillum_ was proposed to cause an increase in foliar dry matter and accumulation of minerals in stem and leaves (Sarig et al., 1988). In the present investigation, high mineral uptake and accumulation especially of N and dry matter yield in _A. brasilense_ inoculated plants were noticed. _Azospirillum_ has a potential as successful competitor and a good survivor in the rhizosphere (Bashan and Levanony, 1990). However, as pointed by Fallik et al. (1988) _A. brasilense_ fail to control verticillium wilt in cotton, since disease incidence was observed in AV and also in GAV plants. Eventhough _A. brasilense_ is synergistic with _G. geosporum_ in enhancing the mineral uptake and growth in GA plants, it did not assist _G. geosporum_ to combat verticillium disease in GAV plants. In barley plants growing soil, _Azospirillum_ populations are relatively small (Negi et al., 1987). This may be the probable reason that _V. dahliae_ reduced _Azospirillum_
populations in AV and GAV plants. Further work needed to elucidate the mechanism involved in the disease occurrence in GAV plants.

Mycorrhizal plant accumulates some hormones in the tissue which stimulate plant growth (Raman et al., 1994; Thiagarajan and Ahmad, 1994). Leaves and roots of GA plants had a higher amount of cytokinin followed by GV and G plant. Allen et al. (1980) reported increase of cytokinin content in plant tissues associated with infection of VAM fungi. Cytokinins are also known to increase the chlorophyll content, prevent chlorophyll degradation, increase Fe transport and accumulation of metabolites and delayed senescence (Mahadevan, 1984). The increased level of chlorophyll in the cases of GV plants followed by GA and G may be due to the higher concentration of cytokinin in these plants. Mycorrhizal association is known to enhance the total chlorophyll content in plants (Allen et al., 1981). Increase level of chlorophyll in Citrus aurantium infected with G. intraradix has been reported (Nemec and Vu, 1990). Eventhough biologically significant level of cytokinin was found in Azospirillum inoculated pearl millet (Tien et al., 1979; Horemans et al., 1986), in the present investigation, cytokinin content was not significant in A plants.

Photosynthetic rate increased in GV, GA and G plants. Improvement of photosynthesis by VAM colonization may be due to a number of physiological changes. Increased respiratory losses (Snellgrove et al., 1982) from the VAM symbiont could increase sink demand for photosynthates and provide higher photosynthetic rates (Herold, 1980). According to Cockburn et al. (1967) P level enhanced the photosynthesis because orthophosphate in chloroplast is essential for carbon assimilation, the possibility arise that carbon assimilation is limited by P availability at times when light and CO₂ are sufficient. The reduced level of photosynthesis in V, AV and GAV plants may be attributed to the deficiency of P, lower content of chlorophyll, water loss etc.
The total sugar and reducing sugar content in leaves and roots of GA, GV and G plants were in higher amount than in other sets of experimental plants. Soluble carbohydrate in roots of onion was increased due to inoculation of Endogone. The present investigation also supports the observation of Same et al. (1983) that G. fasciculatum inoculated Trifolium subterraneum had higher carbohydrate. McArthur and Knowles (1993) also observed G. fasciculatum increased total sugar, reducing and non-reducing sugars in Solanum tuberosum.

In leaves and roots of V, AV and GAV plants initially both the total sugar and reducing sugars increased and then decreased. According to Mace et al. (1981) wilt pathogen produce a number of enzymes that are capable of degrading the natural sugar polymers.

Increased level of phenol oxidase and phenylalanine ammonia-lyase has been observed in GV plants and increased level of these enzymes may also cited as the reason for suppression of disease. According to Mahadevan and Sridhar (1986) end product of the reactions of these enzymes are closely associated with the defense mechanism of plants. The conversion of phenol quinones by phenol oxidase may be responsible for a general resistance in higher plants towards invading pathogen. The PAL activity reflects the enhanced synthesis of phenols in VAM symbiosis. The PAL activity in V, AV and GAV plants initially increased and then declined. The decreased rate of PAL activity in these plants during the later stages resulted in lower amount of phenols because phenols are produced during this enzyme catalysing reaction. It can be possible that VAM infection develops a defense mechanism and provides resistance to pathogens. According to Volpin et al. (1994), G. intraradix induced a defense response in alfa alfa plants.
Based on the present study, it is concluded

1. *Azospirillum* can be used as a growth promoter but not as a biocontrol agent.

2. VAM fungi can be used as biofertilizers and as well as biocontrol agents.

3. Increased content of total and reducing sugar, high level of P, phenols and O'dihydric phenols, cytokinins and enzymes such as pheno oxidase and PAL are responsible for the resistance developed by cotton plants on inoculation of *G. geosporum* against *V. dahliae*. However, further work will reveal the molecular mechanism of biocontrol of verticillium wilt by VAM fungus.