PREVALENCE AND DISTRIBUTION OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH SOME SPICES

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ABSTRACT

Capsicum seedlings are highly susceptible to a number of viral, bacterial, fungal and pest diseases during nursery stage and after transplanting into field. Keeping in view the above information, the aim of the present investigation was to select the efficient AM fungi for their use in nurseries as biocontrol agent against the fusarium wilt of Capsicum annuum. After 50 days, it was observed that all the treated plant showed less disease incidence as compared to control. The quadruple inoculation i.e. G. mosseae + A. laevis + T. harzianum + F. oxysporum showed the maximum shoot height, root length fresh and dry shoot biomass and fresh root biomass as compared to all treated and control plants. Percent mycorrhizal root colonization and spore number was found to be maximum in double inoculated plants of A. laevis + F. oxysporum. But all the leaves of the control plants was showed the yellowing and wilt but in treated plant leaves was yellowish green.

INTRODUCTION

Vesicular Arbuscular Mycorrhiza :

Mycorrhiza is a three way interaction between plant root, soil and fungus. This symbiotic association offers a viable alternative of a high input agricultural technology employed for the protection of environmentally hazardous fertilizers. Thus, it plays a fundamental role in sustainable farming.

1. VAM as nutrient supplier :

The demand for particular nutrient depends upon plants internal requirements while the supply of nutrients primarily depends upon its availability and mobility in the soil (Marschner and Dell, 1994). The most important benefits of VAM association is the increase in phosphorus uptake by the plant. Phosphorus uptake consists of three sub - processes:

i) Absorption by VAM fungal hyphae from soil. ii) Translocation along the hyphae from external to internal mycelia. iii) the transfer of phosphate to cortical root cells (Barea, 1991). VAM infection improves uptake of Phosphorus (P) (Koide, 1991; Ortas *et al.*, 1996), Nitrogen (N) (Ames *et al.*, 1983), Zinc (Zn) (Kothari *et al.*, 1991; Ortas *et al.*, 2001), Copper (Cu), Potassium (K) (Clark and Zeto, 2000; Liu *et al.*, 2000a). Under conditions of low soil nutrient availability VAM plants accumulate large quantities of Zn, Cu and Co (Faber *et al.*, 1990). Phosphate uptake by root is much faster than diffusion of ions to the absorption surfaces of the root (Bhat and Kaveriappa, 2007). Thus, phosphate depletion zone forms around the plant root. The mycorrhizal hyphae invade the phosphate depletion zone into soil for several cms. Thus, the plant is able to exploit nutrient beyond the nutrient depleted area where root cannot thrive (O'Keefe and Sylvia, 1992). Mycorrhizal fungi can also improve absorption of N from mineral fertilizers and transporting to the host (Ames *et al.*, 1983; Johansen *et al.*, 1993). Its transport and absorption can also increase biomass production in soils with low potassium, calcium and magnesium (Liu *et al.*, 2002).

2. Effect of VAM on Drought :

Drought resistance of plants can be improved by VAM colonization (Bethlenfalvay *et al.*, 1988). VAM inoculated seedling can withstand transplant shock and are more resistant to wilting. VAM fungi exert this influence by increasing the transpiration rate and lowering the stomatal conductance (Auge *et al.*, 1987 a,b) under drought conditions. Tinkler (1984) indicated the many possible reasons for the enhanced water and nutrient uptake rates by mycorrhizal plants. It is because of better distribution of absorbing hyphal network, more favourable geometry of hyphae in comparison to roots, greater surface area and increased functional longevity, chemical alteration in soil rhizosphere, alternative rhizosphere microbial population, uptake kinetics, greater hydraulic conductivities, lower transpiration rates per unit of leaf area, extraction of water from soil to lower water potential and more rapid recovery from water stress.

In watermelon (*Citrullus lunatus*) mycorrhizal colonization was found to improve not only the plant yield and water use efficiency, but also the quality of the fruit (Kaya *et al.*, 2003).

3. VAM fungi as bioremediation of heavy metals :

AM fungal strains tolerant to heavy metals has provided evidence for their rapid adaptation to contaminated soils. Cadmium tolerant *Glomus mosseae* was responsible for uptake, transport and immobilization of cadmium (Joner and Leyval, 1997). *Glomus caledonicum* seems to be promising mycorrhizal fungus for bioremediation of heavy metal contaminated soil (Liaq *et al.*, 2003). Under an elevated lead (Pb) condition, mycorrhizae could promote plant growth by increasing P uptake and mitigate Pb toxicity by sequestrating more Pb in roots (Chen *et al.*, 2005). Copper (Cu) was absorbed and accumulated in the extra radical mycelium of three isolates as observed in study with *Glomus* species (Gonzalez-Chavez *et al.*, 2002). The synergistic effects of *Trichoderma koningii* and AM fungi on the effectiveness of plant resistance to heavy metal in soil have been proved (Wang *et al.*, 2007; Ray and Adholeya, 2006; Al-Garni, 2006; Gohre and Paszkowski, 2006; Gaur and Adholeya, 2004; Lakshmipathy *et al.*, 2004; Arrigada *et al.*, 2004; Srinath *et al.*, 2003; Fracchia *et al.*, 2000).

4. VAM as bioprotector:

Mycorrhizal fungi act as a bioprotector to the root system from attack of pathogenic fungi. The mechanism include –

i) Development of a mechanical barrier to infection by pathogens. ii) Production of antibiotic compounds that suppress the pathogen. iii) Competition for nutrients with the pathogen, including production of siderophores. iv) Induction of generalized host defense mechanism. The presence of VAM fungi increase the tolerance of some plants to nematodes and to *Fusarium, Phytophthora, Rhizoctonia* and other plant pathogens. In pine seedling, the fungus mantle has been found to restrict the penetration of *Phytophthora cinnamomi. Glomus fasciculatum* has been used to control damping off caused by *Pythium* (Kavitha *et al.,* 2004). Root pathogen of black pepper was suppressed using mycorrhizae (Anandraj, 2007). Besides these VAM fungi are also known to reduce bacterial diseases. They acts as the biocontrol agents.

5. Effect of VAM on soil fertility and stabilization:

The fungal component involves, the fungal structure within the cell of the root and the extra radical mycelium in the soil. The later may be extensive under some conditions, but does not form any vegetative structures (Smith and Read, 1997). Extrametrical hyphae of mycorrhizal fungi bind soil particles and thereby improve soil aggregation. An immunoreactive glycoprotein termed "glomalin" (Wright *et al.,* 1996) is secreted by the extra radical hyphae and play a significant role in the production and maintenance of macro aggregates formed by plant root, mycorrhiza and soil particles.

VAM play a critical role in the formation of stable soil aggregates i.e., crucial for soil conservation especially in eroding soil (Dodd, 2000; Mukerji *et al.,* 1996; Manoharachary and Reddy, 1995). AMF have been shown to improve productivity in soil of low fertility (Jeffries, 1987) and are particularly important for increasing the uptake of slowly diffusing ions such as PO_4^{3-} (Jacobsen *et al.,* 1992), immobile nutrient such as P, Zn and Cu (Lambert *et al.,* 1979; George *et al.,* 1994; George *et al.,* 1996; Ortas *et al.,* 1996; Liu *et al.,* 2002). Other nutrient such as cadmium (Guo *et al.,* 1996).

REVIEW OF LITERATURE :

In Capsicum annuum, non VAM plant ceased growing after 4 to 5 weeks whereas VAM inoculated plant increased in weight by 41 -188% and fruit development was delayed in all non-VAM plants as compared with VAM ones (Haas *et al.,* 1985). Suppression of damping off by AMF has been reported in cucumber (Rosendahl and Rosendahl, 1990), ginger (Josph, 1997) and pepper (Odebode *et al.,* 1997).

Nelson and Achar, (2001) reported the occurrence of VAM fungi on *Brassica oleraceae* for the first time and observed plant biomass, growth and phosphorus uptake were increased by all VAM fungi. Field trials were conducted in 1988 and 1989 to increase growth response, yield and vesicular-arbuscular mycorrhizal (VAM) fungi colonization of cotton (*Gossypium hirsutum*), onion (*Allium cepa*) and pepper (*Capsicum annuum*) in non fumigated soil and it was found that fresh weight of all the crops were highest in VAM inoculated a non fumigated soil (Afek *et al.*, 1991).

In 1999, Aguilera-Gomez and his coworker found that leaf area, leaf number, shoot, root, fruit mass and reproductive growth increased in VAM plants compared to non VAM plants and root colonization was also higher at lower P concentration, while sporulation was unaffected. VAM fungi are more abundant in cultivated land than virgin soil (Mosse and Bowen, 1968; Chaudhury and Panja, 2007). AMF along with other diazotrophs like *Rhizobium leguminosorum* has been reported in common bean in reducing root rot pathogen (*Fusarium solani*) (*Das et al.*, 1997) and in *Vicia faba* in reducing *Botrytis fabae* infection (Rabie, 1998). Even though dual inoculation of AMF and *Azospirillum* could supress the damping off in chilli (Kavitha *et al.*, 2003). Foot rot of black pepper (*Piper nigrum* L.) caused by a soil borne *Phytophthora capsici* (Sarma *et al.*, 1994) and *P. capsici* infection has been biologically controlled by *Trichoderma harzianum* (Sarma *et al.*, 1997). The root rot or wilt of *Coleus forskohlii* caused by *Fusarium* sp. has been controlled by using three biocontrol agents viz., *Glomus mosseae, Pseudomonas fluorescens, Trichoderma viride.* The same treatment also resulted in maximum growth, yield and root forskolin conc. of *Coleus* (Boby and Bagyaraj, 2003). Kapoor *et al.*, (2002) found that the inoculation of two AM fungi *Glomus macrocarpum* and *Glomus fasciculatum* increased the plant growth and conc. of essential oil upto 90% in *Anethum graveolens* and 72% in *Trachyspermum ammi* over control. *Glomus macrocarpum* was more effective than *G. fasciculatum.* In 2004, Kapoor and his coworkers further reported that mycorrhization of these two AM fungi also increased the conc. of essential oils in *Coriandrum sativum* upto 40%. In 2007, Toussaint and his co workers investigated that *Glomus caledonium* increased rosmaric and caffeic acid production in the shoots of *Ocimum basilicum* and *G. mosseae* also increased caffeic acid conc. in *Ocimum basilicum.* Charron *et al.*, (2001) conducted an experiment to study the response of AM fungi and revealed that bulbs of onion inoculated with *Glomus versiforme* were better in yield than those with *G. intraradices.* Sreeramulu (1999) studied the effect of AMF in mineral nutrition of Cardamom seedling and observed increase in seedling growth through increased uptake of plant nutrients. Lewis *et al.*, (1990) has emphasized the role of competition in biological control of *Rhizoctonia solani* by *Trichoderma* species. Azcon-Aguilar *et al.*, (1979) found that inoculation with *G. mosseae* not only affected plant growth and nutrition in *Medicago sativa,* but also enhanced activity of *Rhizobium.* Khaliq and his co-workers in (2001) revealed that the shoot biomass of peppermint (*Mentha piperita)* increased when inoculated with *G. fasciculatum* (145.3%), *G. aggregatum* (131.1%) and *G. mosseae* (87.8%) in comparison to control. *Trichoderma harzianum* and *T. viride* were reported to be effective in controlling the anthracnose fungus *Colletotrichum capsici* (Khamer *et* *al.*, 1990). Furlan and Cardou (1989) found that in onion nitrogen fertilization stimulate root colonization and spore production while plant fertilized with K and phosphorus

reduces root colonization and spore production. The biomass of endomycorrhizal plants is higher than non-mycorrhizal plants. Though the occurrence of symbiotic colonization of AM fungi is well documented by several workers in different parts of India and further from review of literature it is clear that large number of workers have done work on different aspects of AM fungi. But little work has been done on the biodiversity of AM fungi in spices and their use in better yield of *Capsicum annuum.* Keeping in view all these important aspects, the present investigation includes the AM association of some spices from Kurukshetra and its adjoining area with special emphasis on *Capsicum annuum.*

Capsicum annuum

Chillies are grown throughout the world, but native of America. It is grown extensively in India and especially in South India. The word chilli which is of Mexican origin is still under usage in India. In India, chilli was introduced by the Portuguese during the seventeenth century at Goa from which it spread to other tropical countries. It is grown in both rabi and kharif seasons.

Indian Chillies reach over 90 countries in the world. During 2000 -01, 61000 million tones of chillies valued at Rs. 195.25 crores was exported from India, earning a foreign exchange of 13% of spices.

RESULT AND DISCUSSION

In present investigation, soil and root samples of the selected plants collected from different parts of Kurukshetra and its adjoining areas for studying mycorr hizal status and spore biodiversity of AM fungi. Presence of mycorrhizal root colonization was observed in terms of mycelium, arbuscules and vesicles. During root sample studies, mycelium of H and Y-shaped and parallel shaped was observed (Plate 3.5) and vesicles of various shapes like oval, elongated and rounded were observed (Plate 3.6). In studied plants, root colonization ranged from zero to 95.66 percent. Among studied families Liliaceae, Lamiaceae, Fabaceae, Apiaceae and Zingiberaceae (*Curcuma longa*) showed maximum root colonization, Solanaceae and Lauraceae showed average mycorrhizal root colonization, Rutaceae and Zingiberaceae (*Elettaria cardamomum*) were observed to be least mycorrhizal and Brassicaceae were observed to be non-mycorrhizal. Maximum root colonization ranged from 55-100%, average from 40-55% and least from 10-40%.

Only one plant which was lacking mycorrhizal root colonization was *Brassica compestris. Allium sativum* showed 95.66 ± 5.131 percent of mycorrhizal root colonization followed by *Mentha arvensis* (85.66 ± 5.131), *Allium cepa* (71 ± 2.645), *Trigonella- foenum-graceum* (69.86 ± 3.202), *Foeniculum vulgare* (68 ± 7.211), *Curcuma longa* (61.66 ± 10.408), *Coriander sativum* (60.33 ± 4.509) *Capsicum annuum* (55 ± 5), *Cinnamomum tamala* (51.33 ± 10.263), *Murraya koenigii* (35.66 ± 5.131) and *Elettaria cardamomum* (20 ± 10.583) respectively.

For each spice plant sample, 50 gm of rhizospheric soil sample were processed and AM spore number present in soil sample were counted (Table 3.1). The spore number ranged from 73.66 to 459 spores per 50 gm to the rhizospheric soil. The highest spore count was observed in *Allium cepa* (459 ± 9) followed by *Cinnamomum tamala* (339.33 ± 14.502), *Murraya koenigii* (326.33 ± 7.371), *Foeniculum vulgare* (325 ± 13), *Allium sativum* (313 ± 12.529), *Elettaria cardamomum* (285.66 ± 8.504), *Brassica compestris* (284.33 ± 12.096), *Trigonella- foenum-graceum* (266.66 ± 7.505), *Mentha arvensis* (223.66 ± 7.767), *Coriander sativum* (222 ± 13.74), *Capsicum annuum* (112 ± 11.357) and *Curcuma longa* (73.66 ± 7.571) respectively.

The growth of AM fungi and variation in the percentage of AM root colonization may be due to the effect of host chemicals (Rahman *et al*., 2003).

In the present study, plants showing 95.66 percent AM root colonization had fewer no. of AM spore as compared to plants showing 35.66 percent AM root colonization. On the other hand plant like *Brassica compestris* showed zero percent mycorrhizal root colonization but had 284.33 spore number. So, there is no co-relation between the AM spore number and mycorrhizal root colonization.

Similar findings were also reported by Kumar *et al*., 2000; Sharma, 2004 and Radhika and Rodrigues, 2005. In 1996, Stutz and Morton reported that the relationship between sporulation and colonization varies with different species as well as host and soil nutrient level. In this investigation, high level of AMF colonization and average number of sporulation and vice-versa was found. This may be due to inadequate fungal biomass and poor root development (Gazey *et al*., 1992).

In the present study five genera of AM fungi were present i.e. *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* (Table 3.2, Plate 3.1, 3.2, 3.3 and 3.4). The sixth genus *Entrophospora* was not reported from the selected spice plants under study. Maximum AM spore biodiversity was reported in *Allium sativum.* Among 12 plants, *Glomus* species were found predominant species present in 10 plants followed by *Acaulospora* and *Gigaspora* both species present in 7 plants, *Sclerocystis* in 2 plants and *Scutellospora* in only *Allium sativum* plant. Of the *Glomus* species, *G. aggregatum* were the most predominant species shown by 3 plants followed by *G. segmentatum* shown by 2 plants. In the present study, 8 species of *Glomus*, 6 species of *Acaulospora*, 2 species of *Gigaspora* and one species of *Sclerocystis* were reported. Other unidentified species of *Glomus, Acaulospora, Gigaspora, Sclerocystis* and *Scutellospora* were also reported.

Thus the genus *Glomus* appears to be the most dominant followed by *Acaulospora*, *Gigaspora*, *Sclerocystis* and *Scutellospora* respectively. Similar with the findings of Mosse and Hayman, 1971, Abbott and Robson, 1991, Gupta *et al*., 2003, Chaturvedi *et al*., 2007. Zang *et al*., 1998b; Gai and Liu 2003, reported that *Glomus* appears to dominate in alkaline and neutral soils while *Acaulospora* sporulates more abundantly in acid soils. Zang *et al*., (1999) reported that *Acaulospora* species being more frequent in latosols, lateritic red earths, or paddy soils and *Gigaspora* and *Scutellospora* species being more common in brown soils or paddy soils.

AM spores isolated from the rhizospheric soil of the spice plants is depicted in table 3.2 and plate 3.1 – 3.5.

REFERENCES

1. Abada, K.A., 1994. Fungi causing damping off and root rot on sugar-beet and their biologicalcontrol with *Trichodema harzianum.* Agri. Ecosyst. Env., **51(3):** 333-337.

2. Abbott, L.K. and Robson, A.D. 1991. Factor influencing the occurrence of vesicular - arbuscularmycorrhizae. Agri. Ecosyst. Env., **35:** 121-150.

3. Adholeya, A. and Gaur, A. 1994. Estimation of VAM fungal spores in soil. Mycorhiza News, **6(1):** 10-11.

4. Afek, U., Menge, J.A. and Johnson, E.L.V. 1991. Interaction among mycorrhizae, soil solarization, metalaxyl and plants in the field. Plant Dis., **75:** 665-671.

5. Aggarwal, A., Parkash, V., Sharma, D. and Shama, S. 2004. Ethnobotanical studies on some wild plants of lower hills of Himachal Pradesh. Applied Botanical Abstracts, **24(2):** 135- 146.

6. Aggarwal, A., Parkash, V., Sharma, D. and Sharma, S. 2005. Vesicular arbuscular mycorrhizal (VAM) aids in growth of *Prosopis juliflora* and *Tecomella undulata* in arid/semi arid climate. J. Mycol Pl. Pathol., **35(1):** 184-187.

7. Aguilera-Gomez, L., Davis, F.T., Olalde-Portugal, V., Duray, S.A. and Phavaphutanon, L. 1999. Influence of Phosphorus and Endomycorrhiza (*Glomus Intraradices*) on gas exchange and plant growth of Chile ancho pepper (*Capsicum annuum* L. Cv. San Luis). Photosynthetica, **36:** 441-449.

8. Al-Garni, S.M.S. 2006. Increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer *Rhizobium* bacterium. African J. Mycol. and Biotech., **5(2):** 139-142.

9. Al-Momany, A. and Al-Radded, A. 1988. Effect of vesicular-arbuscular mycorrhizal on *fusarium* wilt of tomato and pepper. Alexandria J. Agri. Res., **33:** 249-261.

10. Al-Raddad, A. 1995. Mass production of *Glomus mosseae* spores. Mycorrhiza, **5 :** 229-237. Ames, R.N. and Schneider, R.W. 1979. *Entrophospora* a genus in the Endogonaceae. Mycotaxon, **8 :** 347-352.

11. Ames, R.N., Reid, C.P.P., Porter, K.K. and Cambardella, C. 1983. Hyphal uptake and transport of nitrogen from two 15 N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungi. New Phytol., **95:** 381-396.

12. Anandaraj, M. 2007. Mycorrhiza for growth enhancement and pathogen suppression in epper.

In: The Mycorrhizae: Diversity, Ecology and Applications, (Eds.), Tiwari, M. and Sati, S.C., Daya Pub. House, Delhi, pp. 349-356.94

13. Ananthakrishnan, G., Ravikumar, R., Girija, S. and Ganapathi, A. 2004. Selection of efficient arbuscular mycorrhizal fungi in the rhizosphere of cashew and their application in the cashew nursery. Scientia Horticulaturae,**2(1-4):** 369-375.

14. Arrigada, C.A., Herrera, M.A., Garcia-Romera, I. and Ocampo, J.A. 2004. Tolerance to Cd of soybean and *Eucalyptus* inoculated with AM and saprobe fungi. Symbiosis, **36:** 285-299.

15. Auge, R.M., Schekel, K.A. and Wample, R.L. 1987a. Rose leaf elasticity changes in response to mycorrhizal colonization and drought acclimatization. Plant Physiol., **70:** 175-182.

16. Auge, R.M., Schekel, K.A. and Wample, R.L. 1987b. Leaf water and carbohydrate status of VA mycorrhizal rose exposed to drought stress. Plant and Soil, **99:** 291-302.

17. Azcon-Aguilar, C. and Barea, J.M., 1996. Arbuscular mycorrhizas and biocontrol of soil-borne plant pathogens, an overview of the biological mechanism involved. Mycorrhiza, **6:** 457- 464.

18. Azcon-G de Aguilar, C., Azcon, R. and Barea, J.M. 1979. Endomycorrhizal fungi and *Rhizobium* as biological fertilizers for *Medicago sativa* in normal cultivation. Nature **279:** 325-326.

19. Bajwa, R. and Read, D.J. 1985. The biology of mycorrhiza in the *Ericaeae*: The peptides as nitrogen sources of the ericoid endophyte and for mycorrhizal and non-mycorrhizalplants. New Phytol., **101:** 459-467.

20. Bajwa, R., Afzal, B. and Javaid, A. 2000. Allelopathy and VA mycorrhiza VI : Comparative effectiveness of *Glomus mosseae* and *G. fasciculatum* in improving crop growth and yield in wheat (*Triticum aestivum L.*) under allelopathy stress. Pakistan J. Bio. Sci., **3(3) :**485-487.

21. Bali, M. and Mukerji, K.G. 1988. Effect of VAM fungi on *Fusarium* wilt of cotton and jute. In: Mycorrhizae for Green Asia. (Eds.) Mahadeavan, A., Raman N. and Natrajan, K. Madras University, Madras, pp. 233-234.

22. Barea, J.M. 1991. Vesicular arbuscular mycorrhizae as modifiers of soil fertility. Adv. Soil. Sci., **15:** 1-40.

23. Barrow, J.R., Havstad, K.M. and McCaslin, B.D. 1997. Fungal root endophytes in fourwing saltbush, *Atriplex canescens*, on arid rangelands of southwestern USA. Arid Soil Res. Rehabil, **11:** 177-185.

24. Berkeley, M.J. and Broome, C.E. 1875. Enumeration of the fungi of Ceylon. J. Linn. Soc., **14 :**129-140.

25. Bethlenfalvay, G.J. and Linderman, R.G. 1992. Mycorrhizae in sustainable agriculture. Madison,Wiscon. Amer. Soc. Agron. (Spl. Publication), pp. 54.