RESEARCH ARTICLE

Rice Cropping in Urban Farming with Special Reference to AM Fungi

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ABSTRACT

Urban farming is no more new to Mumbai and it's suburban. Being a metropolitan city, its expansion is a common trend in every possible direction. A railway is a life line of this city. This local service is divided into Central railways, Western railways, Harbour railway and Trans Harbour railway. From which Trans Harbour railway is comparatively new development connecting Thane to Vashi (New Mumbai). Near the railway tracks some area of land might be available as a free space. On this land various vegetables like Okra (*Abulmoschus esculantus* L.), Methi (*Trigonella longiceps* F.), Bathua or Mayalu (*Chenopodium album* L.) Chawali (*Vigna unguiculata*.), Palak (*Spinacia oleracea* L.). are cultivated during pre and post monsoon period. Whereas in monsoon season, Paddy cultivation (Rice, *Oryza sativa* L.) is a common practice. Such rice cultivation is largely based on stagnant water which gets collected during heavy monsoon.

In present study, one of the urban farms which are near Koparkhairane station on the trans - harbour train route was selected. From the urban farm *Oryza sativa* L. (rice) plants were collected at specific intervals throughout the monsoon period and percent root colonization of arbuscular mycorrhizal fungi from roots was analyzed. Rhizosphere soil was analysed for pH, mineralizable nitrogen along with spore density of arbuscular mycorrhizal fungal chlamydospore. Very high level (as high as 100 %) root colonization by various arbuscular mycorrhizal fungi could be observed. A positive correlation between spore density and percentage root colonization was drawn from the results.

Keywords: Urban farming, mycorrhiza, per cent root colonization, abiotic stresses.

INTRODUCTION

Urban farming is no more new to Mumbai and it's suburban. Being a metropolitan city, its expansion is a common trend in every possible direction. New routes for approach have been developed in recent past. A railway is a life line of this city. Thousands of people use local trains as a convenient mode of transport every day. This local service is divided into Central railways, Western railways, Harbour railway and Trans – Harbour railway. From which Trans – Harbour railway is comparatively new development connecting Thane to Vashi (New Mumbai). New big stations with broad platforms have been constructed for easy use by passengers. Since this line is comparatively new and have been constructed in near past, stations are well maintained, but local tracks pass from area which are under developing stages. Earlier to the development of this local route, this area use to be either an open fields or industrial area. Near the railway tracks some area of land might be available as a free space. Such areas are generally utilized by Indian citizens who are migrated from other parts of India to Mumbai in search of their daily needs and some work. Such people conveniently develop slums for their accommodation and use such areas for garbage disposal and as their backyards. Along with such slum development, many of people often use free space along the tracts for farming. On this land various vegetables like Okra (*Abulmoschus esculantus* L.), Methi (*Trigonella longiceps* F.), Bathua or Mayalu (*Chenopodium album* L.) Chawali (*Vigna unguiculata*.), Palak (*Spinacia oleracea* L.).are cultivated during pre and post monsoon period. Whereas in monsoon season, Paddy cultivation (Rice, *Oryza sativa* L.) is a common practice. Such rice cultivation is largely based on stagnant water which gets collected during heavy monsoon. Along with monsoon as a source, nearby water bodies are used as a source of water fir paddy cultivation. Often these water bodies are polluted with heavy metals, sewage from nearby area, garage effluents etc.

In present study, one of the urban farms which is near Koparkhairane station on the trans - harbour train route was selected. This selection was done on the basis of easy collection of samples from the field and friendly approach of urban farmers from the field. This is because these urban farming practices are totally illegal. Majority of these urban farmers are from very poor families and migrants from North India. Since it is illegal, it becomes very difficult to get samples in enough number and other information as well, as these farmers refuse to give any relevant information. It was very difficult to get photographs as well of the cultivation in month wise manner. Authors could manage to get some few photographs with very great difficulties.

Mycorrhizal biodiversity of petro effluent – irrigated fields were investigated by Kothari *et al* (1997). Petro effluent of Indian Petrochemicals Corporation Limited (IPCL) has been tested for irrigation in the ecoform at IPCL for recycling millions of tonnes of water. Rhizosphere of 6 crops raised in these fields has been evaluated for mycorrhizal biodiversity along with mycorrhization status of these crops. Authors claim this as the first report of mycorrhizal biodiversity of land distributed by petro effluent in India.

Exceptionally wide range of plants in different ecosystems shows association with AM fungi. The latter play a major role in better nutrition, species diversity and survival. Amongst the Angiosperms, about 90 per cent of the families develop AM association. The occurrence of AM fungi differs qualitatively and quantitatively with the change in edaphic factors and type of vegetation (Smith and Smith, 1996). Ecological factors which influence the colonization of AM fungi in the soils are soil pH, temperature, moisture, organic matter content in the soil and soil pollutants. Changes in the soil pH greatly affect the development and functioning of AM fungi. Different species and strains of AM fungi show different responses to soil pH. Higher fungal spore density in acidic soil, while in alkaline soil

the spore density is low has been reported by Dalal and Hippalgaonkar (1995). Higher temperature results into high root colonization in temperate zone whereas it can be just a reverse in tropical region. AM fungi prefer optimum soil moisture for sporulation and growth. Because of it, during winter and summer spore density is low as comparing to rainy season in which it is quite high. Organic matter and soil fertility also play an important role in growth and sporulation of AM fungi. In low fertile soil, AM fungi spore density is higher to that of high fertile soil where it is quite low. Changes in soil fertility due to amendments with mineral fertilizers or organic matter markedly affect the activity and survival of AM fungi. Nitrogen can stimulate or suppress root colonization by AM fungi and sporulation by changing the soil pH. In the presence of sufficient amount of Nitrogen in the soil, addition of Phosphorus suppresses root colonization. More amount of AM colonization is observed in P deficient soil (Sylvia and Neal, 1990).

A systematic study was carried out by Gupta (2001) to determine the effect of AM fungi on growth and phosphorus content in plant tissue of rice. Plant height in inoculated conditions was increased significantly. An improvement in dry biomass production upon inoculation was also observed. Phosphorus content of the plant system was found to be significantly higher in inoculated plants. A positive symbiotic association between rice and AMF inoculated was clearly evident under different soil treatment conditions. Among the AM inoculants, *Sclerocystic dussii* performed best way in increasing growth of rice in pot culture.

In present investigation, roots of *Oryza sativa* L. (Rice) were collected from Koparkhairane from Trans Harbour Route (Thane to Turbhe) railway tracks from urban farms and screened for per cent colonization by mycorrhiza fungi. Similarly, rhizospheric soil samples were also collected and detail investigations were carried out like physical parameters such as soil pH, % nitrogen of soil, Samples were screened for Arbuscular Mycorrhizal Fungi (AMF) spores also and spore density was calculated for each sample.

MATERIAL AND MATERIALS

For the present investigation sample collection was the main fundamental aspect. As discussed earlier, sampling was done throughout the monsoon period at random time (when it was possible as permission from farmers was use to be a chance). So whenever it use to be possible 10 to 15 number of plants from random spots in farms were collected. Plants were collected during the range of 25 to 40 days after sowing, 85 to 95 days after sowing, 115 to 130 days of sowing. The reason behind is same as farmers were some time cooperative where as sometime they use to refuse permission to enter in farms. The plants with root system along with rhizospheric soil were carefully collected in clean, unused plastic bags of suitable size. The samples were carefully labeled showing the records like date of collection, time, etc and were brought in the laboratory. After bringing the samples in laboratory, roots were carefully separated from entire plants, tapped gently to separate soil particles which were adhered to it (which was put in respective soil samples). The root samples were washed under tap water to clean and stored in 70 per cent of alcohol until further use. Soil samples were dried under sunlight and stored in well labeled, sterilized plastic bags.

A. Percentage root colonization was calculated after staining procedure given by Koske (1989), Carol and Stribley (1991). The roots of collected plants were washed thoroughly with running tap water for removal of alcohol which was used as preservative. The properly cleaned roots were subjected to staining procedure. Roots were subjected to 10 per cent of potassium hydroxide and heated in water bath at 90^0 C for one hour to clear the tissue. Such cleared roots were washed with distilled water several times to remove the traces of alkali. In case of dark pigmentation of roots, they were

treated with three per cent hydrogen peroxide for bleaching. It took 5 to 30 minutes depends upon the degree of pigmentation. This step was eliminated in the case of roots which are white or non-pigmented. Once again roots were washed thoroughly for several times with distilled water. Washed roots were kept in 1 per cent hydrochloric acid for about 18 hours to neutralize the effect of alkali used for cleaning the root tissue. Acidification was followed by washing several time the treated roots to remove traces of acid. The roots were then kept in acid glycerol containing 0.05 per cent aniline blue in test tube. Then it was autoclaved for 15 minutes under the pressure of 15 labs at 121^0 C. After that, excessive aniline blue was drained off and excessive stain was removed from stained roots by using acid glycerol. The stained roots were stored in amber coloured bottle in acid glycerol until further use to avoid destaining. The roots were cut into small pieces approximately 1 cm in length and mounted on micro slides using glycerine as mounting medium. After placing the cover slips, the slides were observed under low power of objective of 10 X magnification to confirm the presence of mycorrhizal hyphae and high power objective of 40 X magnification to observe the presence of vesicles, spores etc. The slides were sealed with nail polish. The per cent root colonization was calculated by Nicolson's formula.

$$\text{Per cent root colonization} = \frac{\text{No. of root pieces showing colonization}}{\text{Total no. of root pieces observed}} \text{ X } 100$$

B. Isolation and quantification of AMF spores was carried out by wet sieving and decanting method (Gerdeman and Nicolson, 1963). 25 g of sundried rhizospheric soil of different soil of different plants collected at different localities was taken in separate beakers. Half a litre of water and a pinch of soap powder were added to this. The solution was stirred and was allowed to stand for half an hour. The soil solution was then filtered through sieves of 500, 250, 150, 105 and 55 mm mesh which were kept one above the other. The spores and soil particles which settled on the surface of 150, 105 and 55 mm mesh sieve were washed and collected in separate beakers. The water was again filtered through whatman filter no. 1. This paper was observed under stereoscopic binocular microscope to count the number of spores. Different spores were examined for their taxonomic status by using standard key (Schenk and Perez, 1989).

Rhizospheric soil samples were analyzed for its various physico – chemical properties were estimated.

C. Soil pH was determined by using pH meter model number EQ 614.

D. Mineralizable Nitrogen of soil sample was determined by Jackson's method (1967). 5 g of air dried soil was taken in 500 mL Kjeldahl flask. 5 mL of water was added followed by addition of 15 mL of conc. H2SO4. The setup was kept undisturbed for 30 minutes. To this mixture was added 0.1 g of selenium powder. The digestion was first started over small flame and gradually increased until fumes of sulphuric acid were produced. The flask was removed immediately and added 5 g of potassium sulphate. The flask was againreplaced over the flame and digestion was continued for 1 to 2 hours till the digest has become colourless. The flask was allowed to cool and then 50 mL of water was added. The solution was left for 30 minutes to allow the soil particles to settle down. With the help of pipette the top layer of clear soil extract filtered out and stored in plastic bottles. From the stock 10 mL of soil extract was poured in 500 mL distillation flask. This was diluted by 100 mL of water and 10% NaOH was added to make the mixture in the digestion flask neutral. The mixture was well shook and distillation was commenced. The liberated ammonia was collected in 25 mL of 0.1 N HCl containing 2 to 3 drops of methyl red indicator. The distillation was carried out until the distillate was about $1/3^{rd}$ of the liquid has passed over. When the distillation was over, the condenser tube was rinsed with distilled water to remove any traces of nitrogen if trapped into the

0.1N HCl. A blank without soil extract was carried out in exactly the same manner. The percentage of nitrogen in the soil was calculated on the basis of 5 g soil by using the following formula:

$$\%\text{Nitrogen} = (B-T) \times N \times \frac{0.14}{\text{Weight of Soil}} \times 100$$

Where:

B = Blank titration (ml of alkali used)

T = Actual titration

N = Normality of the standard alkali (0.1)

RESULTS AND DISCUSSION

Oryza sativa L. (Rice) plants were screened for AM fungi association in terms of per cent root colonization and no. of AMF spores per 25 g of rhizospheric soil. These observations are tabulated in table no.1 similarly; rhizospheric soil samples were analyzed for physico – chemical properties such as pH of soil, and mineralizable nitrogen, shown in table no. 2. Table no. 3 shows different genera with species of AMF observed in *Oryza sativa* L. (Rice) plants. Average percent colonization and AMF spore count for *Oryza sativa* L. (Rice) plants (at intervals of collection days) are presented in Figure No. I. During the present investigation AMF spores from 4 genera with 7 species have been encountered which is shown in Figure No. II. Figure No. III shows generic level distribution of AMF of investigation.

All soil samples were acidic in pH ranging in between 4.9 to 5.8 which is fairly acidic range. This might be because of very poor quality of water used along with rain water to maintain water level. Abiotic stress can be lower down by mycorrhizal colonization [Zuccarini and Savé, (2015), Dahmosh, (2002)]. Very high (as high as 100%) root colonization has been found out during present investigation. Zero to 43 % root colonization in *Oryza sativa* L. (Rice) plant has been reported by Hajiboland *et al* (2009) where as Rajeshkannan *et al.* (2009) reported it as 37.18%. He also reported that during 35 to 42 days after sowing shows increment in root colonization whereas root colonization percentage gets decreased during 42 to 70 days after sowing in *Oryza sativa* L. (Rice) plants. According to Maiti *et al* (2006) root colonization

Table1: Per cent colonization and spore density in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

Days after sowing	Percent colonization	Spore density
30 days (25 to 40 days)	89.2	224
90 days (85 to 95 days)	93.8	289
120 days(115to130days)	100	304

Table2: Range of physic-chemical parameters (P. C. P.) of rhizosphere soil of *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

Days after sowing / P. C. P	pH	Mineralizable nitrogen
30 days (25 to 40 days)	5.8	2.82
90 days (85 to 95 days)	5.0	2.58
120 days (115 to 130 days)	4.9	1.40

Table3: AM fungi associated with *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

Plants / Spot	**Airoli**
30 days (25 to 40 days)	*Glomus mosseae , Glomus fasciculatum*
90 days (85 to 95 days)	*Glomus mosseae, Acaulospora laevis, Glomus macrocarpum*
120 days (115 to 130 days)	*Glomus mosseae, Glomus macrocarpum, Gigaspora margarita, Acaulospora foveata, Sclerocystis clavispora*

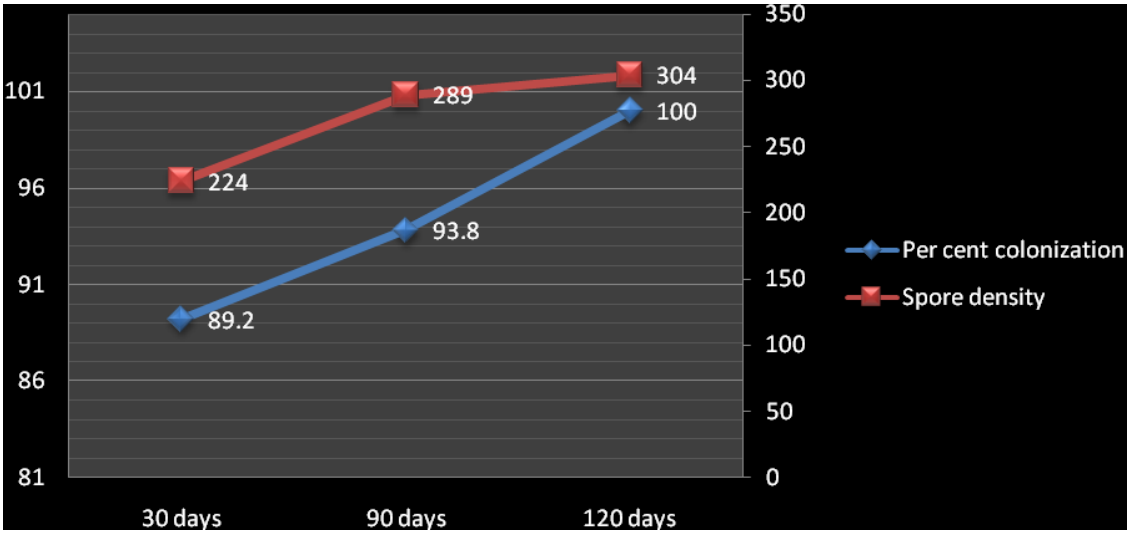

Fig.1: Percent colonization and spore density in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

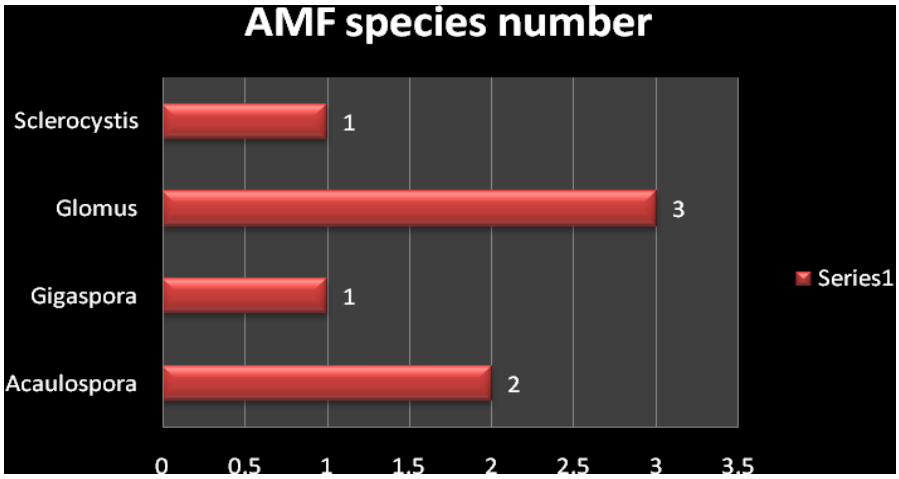

Fig.2: AMF genera with species number in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

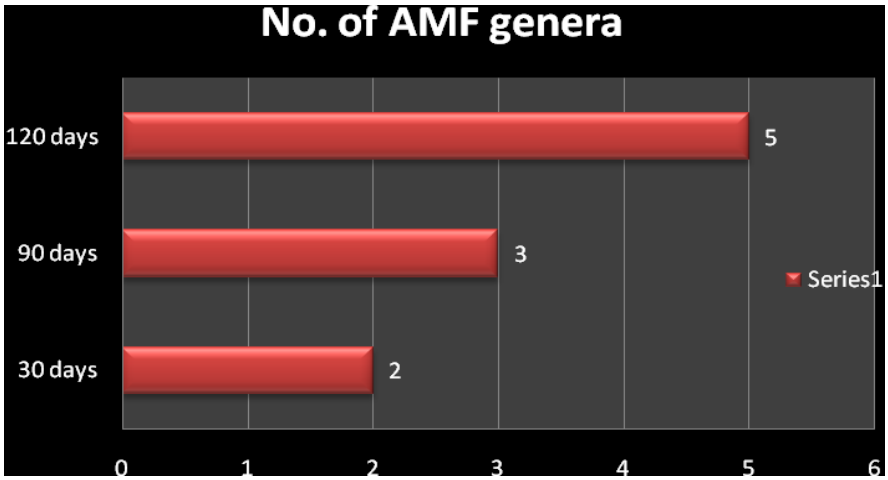

Fig.3: AMF diversity at generic level on days of collection in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts).

percentage in *Oryza sativa* L. (Rice) plant can be 5 to 65 %. Sadhana (2015) reported a gradual increment in percent root colonization from 44.67 to 58.0% from 15 days to 60 days after sowing. Gradual decrease in percent root colonization from 55 to 28% from 1 day to 105 days after sowing has been reported by Solaiman and Hirata (1996). Xu *et al* (2010) reported it as 14%.wher as according to Fernandez *et al* (2011) it ranges from 2 to 44% in rice plant. Muhammad Ali (2008) reported 100 % root colonization in basamati rice but it is in experimental conditions where AM fungal culture was added.

Bhattacharjee and Sharma (2011) reported decrease in percent root colonization up to 90 days after sowing where as increased percent colonization in the period of 90 days to 135 days after sowing. Which ranges from as low as 54 % to as high as 80 %. In this investigation the percentage root colonization in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts) is very high ranging from 89.2 to 100 %. *Glomus mosseae* is the most common in occurrence in soil samples analysed. *Glomus* with 3 species, *Acaulospora* with 1 species,*Gigaspora* with 2 species and *Sclerocystis* with single species observed in *Oryza sativa* L. (Rice) plant from Koparkhairane urban farms (along the railway tracts). Remarkable fact came in light that the farmers apply high amount of urea (a nitrogenous fertilizer) in urban farming for fast growth of the plants. It is well accepted fact that high nitrogen content and low phosphate content of soil increases root colonization of AM fungi. This might be one of the contributory facts to get middle range to high number of AMF spore count and very high per cent colonization (almost 100 %). Generally, a negative correlation is observed in between percent root colonization and spore density. In present study a positive correlation could be observed in them.

CONCLUSIONS

Based on the above observations and results, conclusions can be drawn that mycorrhiza can be used in urban farming for sustainability. This research work is throwing light on scenario of mycorrhiza in such lands where abiotic stress (like polluted land, poor quality of water used for irrigation) is very high. So if local farmers are made available with mycorrhizal cultures for the use in such urban farming, it may give very good results in terms of high yield and rapid growth (as it is well established fact that, mycorrhiza enhance the growth in host).

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