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Correlation of AM Spore Number, Percent Root Colonization and *Azotobacter* Count with Plant Growth, Fruit Yield and Leaf Nutrient Content of Royal Delicious Apple

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Abstract

The investigation was conducted to find out the relationship of soil microflora especially, AM fungi and *Azotobacter* with growth, fruit yield and leaf nutritional status of apple orchards cv Royal Delicious of Shimla and Kullu districts of Himachal Pradesh and to determine the frequency of occurrence and distribution of arbuscular mycorrhizae (AM) and variation in *Azotobacter* count in these orchards. Five apple orchards at six locations namely, Mashobra, Kotkhai, Thanedhar, Bajaura, Naggar, and Seobag in Himachal Pradesh were selected for the studies. A simple linear correlation was worked out between all possible combinations indicated that AM spore population, per cent root colonization and *Azotobacter* count had positive and significant correlation with tree girth and fruit yield, but the relationship was positive but non-significant with N, P, Fe, Cu, Zn and Mn contents of the leaves. However, the relationship with K content of leaf was found to be negative and non-significant.

Key words : Correlation, AM spore number, Root colonization, Fruit yield, Royal Delicious apple.

Arbuscular mycorrhizae (AM) and *Azotobacter* play a significant role in fruit production and have broad-spectrum application. AM fungi are the most common obligate endosymbionts, and their association with many fruit trees such as apple, peach, mango, banana, pineapple, citrus and litchi have been well documented due to increased growth with increased uptake of slowly mobile nutrients especially P, Fe, Cu, Zn and Mn from the bulk of soil. AM fungi are the important components of biodiversity, particularly in tropical and sub-tropical ecosystems. The occurrence and distribution of AM fungi varied with edaphic conditions mainly pH of soil and nutrition (1). Nappi et al. (2) observed higher spore density in top layer of soils, which are related to root quality of grapevines. The inoculation of different AM species increased mycorrhizal colonization in apple roots. Higher percentage of mycorrhizal spores and root infection was observed when apple seedlings were treated with *Glomus macrocarpum* under different phosphorus treatments (3). Covey et al. (4) reported that apple trees had greater colonization in soils with low level of available P than fertile soil. The rate of mycorrhizae formation and extent of infection developed by each fungus could be the reason for differences in the effectiveness of fungi. *Azotobacter* has the ability to utilize wide range of carbon compounds. Their number in Indian soils rarely exceeds 10^4 to 10^5/g soil. The maximum number per gram soil was found to be 1.1×10^4 in Haryana soils (5) and 8×10^5 in forest soils of Karnataka (6). In Egyptian Nile valley clay soils, *Azotobacter* count has been found to be 10^7/g soil (7). Dual application of AM fungi and *Azotobacter* is of greater significance in fruit crops than plants inoculated alone (8). Therefore, the present study was undertaken with the objective of surveying the apple orchards for soil microflora, especially AM fungi and *Azotobacter*, and to assess their relationship with plant growth, fruit yield and nutrient contents of leaves.

Methods

Five full bearing apple orchards of cultivars Royal Delicious of 15 years age group at six locations namely, Mashobra, Kotkhai, Thanedhar areas of Shimla district and Bajaura, Naggar and Seobag areas of Kullu district of Himachal Pradesh were se-

Table 1. Correlation (*r*-values) between AM spore number, per cent root colonization and *Azotobacter* count with tree girth and fruit yield of apple orchards. *Tested at 5% level of significance. **Tested at 1% level of significance.

Location	Parameters	Tree girth	Fruit yield
Mashobra	AM spore number	0.88**	0.39*
	Per cent root colonization	0.62**	0.08
	Azotobacter count	0.65**	0.29
Kotkhai	AM spore number	0.77**	0.90**
	Per cent root colonization	0.84**	0.73**
	Azotobacter count	0.75**	0.44*
Thanedhar	AM spore number	0.84**	0.77**
	Per cent root colonization	0.67**	0.49**
	Azotobacter count	0.68**	0.48**
Bajaura	AM spore number	0.82**	0.62**
	Per cent root colonization	0.73**	0.60**
	Azotobacter count	0.65**	0.77**
Naggar	AM spore number	0.92**	0.40*
	Per cent root colonization	0.47*	0.44*
	Azotobacter count	0.44*	0.54**
Seobag	AM spore number	0.84**	0.64**
	Per cent root colonization	0.62**	0.49**
	Azotobacter count	0.26	0.08

lected for the studies during 2002-2003. In each of these orchards, ten healthy trees were selected for the observations. Soil and root samples were collected at a distance of 30 cm from the tree trunk to 30 cm depth. The soil samples were air dried and passed through 2 mm sieve for the studies.

AM Isolation and Spore Count in Soil

Soil samples were collected from the apple orchards during July to September to estimate spore population. AM spores were isolated from the soil samples by wet sieving and decanting method as suggested by Gerdmann and Nicolson (9). The most probable number (MPN) method was used to enumerate the AM spore count using by 10-fold series of soil dilution ; 100 g soil was diluted in one liter water (1 : 10) and stirred vigorously. Heavier particles were allowed to settle down. The spores were harvested from the suspension by passing the supernatant solution through 450, 300, 200, 150 and 100 meshes. The residue of each sieve was collected. The number of AM 100/g soil was recorded.

Assessment AM Colonization in Root

Whole roots were collected from saplings after plants were excavated to a depth of 10—15 cm using a spade. The samples were placed in a plastic bag and stored in a refrigerator (7 C) on the day of collection, before further use. The washed roots were stored in 50% ethanol. The roots were cleared and stained according to Brundrett et al. (10) with some modifications. The roots were cleared by autoclaving for 20 min at 121 C in a KOH (10% wt/vol) solution and were bleached in a 5% H_2O_2 solution for 3—4 h to remove phenolic compounds before they were stained in 0.05% trypan blue-lactoglycerol solution (3 lactic acid : 3 glycerol : 4 water) for 10 min. The stained roots were preserved in lactophenol to distain the plant material (while the fungal structures retained their blue color) before mycorrhizal colonization was determined, and then examined under biological microscope using LEICA DMLB image analysis software system. The colonization on roots was measured using the gridline intersect method (11, 12). Subsamples of roots (<2mm diameter) were placed in a petri dish with a 0.5 cm grid, and the mycorrhizal colonization was determined as a percentage of root length. Every subsample was counted three times by rearranging the roots in the petri dish, and the average was calculated.

Azotobacter Bacterial Count

Soil samples were collected from rhizosphere

Table 2. Correlation (*r*-values) between AM spore number, per cent root colonization and *Azotobacter* count with leaf nutrients status of apple orchards. * Tested at 5% level of significance. **Tested at 1% level of significance.

Location	Parameters	N	P	K	Cu	Mn	Zn	Fe
	AM spore number	0.02	0.47*	– 0.29	0.44*	0.08	0.18	0.27
Mashobra	Per cent root colonization	0.40*	0.19	– 0.18	0.13	0.03	0.05	0.10
	Azotobacter count	0.42*	0.08	– 0.43	0.11	0.03	0.03	0.05
	AM spore number	0.30	0.91**	– 0.08	0.53**	0.31	0.74**	0.79**
Kotkhai	Per cent root colonization	0.17	0.53**	– 0.09	0.24	0.14	0.28	0.30
	Azotobacter count	0.41*	0.28	– 0.45**	0.20	0.28	0.10	0.04
	AM spore number	0.02	0.46*	– 0.17	0.46*	0.43*	0.13	0.22
Thanedhar	Per cent root colonization	0.17	0.08	– 0.08	0.13	0.03	0.09	0.07
	Azotobacter count	0.43*	0.08	– 0.50**	0.16	0.02	0.17	0.12
	AM spore number	0.04	0.64*	– 0.34	0.20	0.27	0.54**	0.47*
Bajaura	Per cent root colonization	0.11	0.39*	– 0.32	0.28	0.03	0.35	0.20
	Azotobacter count	0.38*	0.32	– 0.46*	0.15	0.24	0.24	0.13
	AM spore number	0.12	0.75**	– 0.12	0.26	0.72**	0.74**	0.10
Naggar	Percent root colonization	0.21	0.02	– 0.13	0.09	0.11	0.11	0.05
	Azotobacter count	0.40*	0.11	– 0.48**	0.13	0.06	0.05	0.15
	AM spore number	0.35	0.44*	– 0.19	0.68**	0.30	0.09	0.71**
Seobag	Per cent root colonization	0.07	0.05	– 0.05	0.09	0.05	0.07	0.23
	Azotobacter count	0.38*	0.17	– 0.43*	0.25	0.09	0.06	0.32

and screened through 2 mm sieve. The samples were pooled and thoroughly mixed to make a represented sample. The serial dilution technique was employed for the isolation of viable *Azotobacter* cells ; 10 g soil from each sample was drawn aseptically and serially diluted to 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. One ml of each dilution was inoculated over Jensen's medium. The medium was sterilized for half an hour at 121 C at 15 psi pressure. Taxonomic identification of *Azotobacter* isolates were according to Bergey's Manual (13).

A sample correlation analysis was worked out for AM spore population, per cent root colonization and *Azotobacter* count with growth, fruit yield and nutritional status of leaf apple orchards according to Snedecor and Cochran (14).

Results and Discussion

The degree of relationship between AM spore number, per cent root colonization *Azotobacter* count with tree girth, fruit yield and nutrient content of leaf was estimated with simple linear correlation between all possible combinations.

Correlation with Plant Growth and Yield

(Table 1) recorded on the relationship (*r*-values) between soil microflora especially, AM spores and *Azotobacter* count that In Mashobra, AM spores were positively and significantly related with tree girth (r=0.88) and fruit yield (r=0.39), whereas per cent root colonization and *Azotobacter* count exhibited positive and significant relation with tree girth (r=0.62 ; r=0.65) respectively (Table 1). However, in Kotkhai area, AM spores and per cent root colonization were positively and significantly correlated with tree girth (r=0.77) and fruit yield (r=0.90). Similar observations were recorded in Thanedhar area. A positive and significant relationship of AM spores, per cent root colonization and *Azotobacter* count with tree girth and fruit yield were observed in Bajaura and Naggar areas. In Seobag, tree girth (r=0.84) and fruit yield (r=0.64) were positively and significantly related with AM spores, but *Azotobacter* population exhibited weak and non-significant relationship with growth and fruit yield (Table 1). These results are similar to those of Jasrotia et al. (15), who also reported positive and significant relationship in olive, and also in litchi (16). This could be attributed to more dry matter and plant biomass production and also due to enhanced nutrient uptake from soil by roots, improvement in photosynthetic rate and changes in microbial plant hormones induced in the host plant which favoured the establishment of AM symbiosis, whereas, *Azotobacter* produced growth

regulators like IAA and gibberellins, besides, N_2 fixation (17) and hence, positively influenced growth and yield (18).

Correlation with Nutrient Contents of Leaf

The data on the relationship (*r*=values) between soil microflora, especially AM spores, per cent root colonization and *Azotobacter* count with leaf nutrient content revealed that in Mashobra orchards, AM spores were positively and significantly related with leaf P (*r*=0.47) and Cu (*r*=0.44), whereas with leaf N, Mn, Zn and Fe the correlations were positive and non-significant (Table 2). A positive and significant correlation was found between leaf N (*r*=0.40) and per cent root colonization. *Azotobacter* count had also positive and significant relationship with leaf N (*r*=0.42). In Kotkhai orchards, a positive and highly significant relationship was observed between AM spores and leaf P (*r*=0.91), Cu (*r*=0.53), Zn (*r*=0.74) and Fe (*r*=0.79). Per cent root colonization was found to have positive and significant correlation with leaf P only (*r*=0.53). *Aztobacter* count had positive and significant correlation with leaf N (*r*=0.41). In Thanedhar region, the AM spores were positively and significantly related with leaf P (*r*=0.46), Cu (*r*=0.46) and Mn (*r*=0.43). Correlation was positive and significant between *Azotobacter* count and leaf N (*r*=0.43). However, a negative and significant relationship with leaf K was recorded (*r*=0.50). Correlation studies in apple orchards of Bajaura (Table 2) indicated that the AM spores were positively and significantly correlated with P (*r*=0.64), Zn (*r*=0.54) and Fe (*r*=0.47). *Azotobacter* count exhibited positive and significant relation with leaf N (*r*=0.38). AM spores of apple orchards of Naggar were positively and significantly related with leaf P (*r*=0.75), Mn (*r* = 0.72) and Zn (*r*=0.74). *Azotobacter* count exhibited a positive and significant correlation with leaf N only (*r*=0.40). However, correlation with leaf K was found to be negative and significant (*r*=0.48). In Seobag, AM spores exhibited positive and significant relationship with leaf P (*r* =0.44*), Cu (*r*=0.68) and Fe (*r*=0.71), (Table 2). Leaf N, P, K, Cu Mn, Zn and Fe were positively and non-significantly correlated with per cent root colonization. A positive and significant relationship was observed between *Azotobacter* count and leaf N (*r*=0.38). It might be due to reason that mycorrhizae can absorb several times more phosphate than infected roots from soil. Besides P, AM were positively correlated with Fe, Cu, Zn and Mn due to increased root colonization which increased the surface area for nutrient absorption. It can also due to increased root colonization which increased the surface area of the nutrient absorption and hence, positively influenced the nutrient status. Further, AM fungi convert slowly immobile nutrients in the rhizosphere to available form so that these becomes easily available to the plants (19, 20). *Azotobacter* count was positively and significantly correlated with leaf N, whereas the correlation with other nutrient was non-significant. The increase in leaf N content was due to enhanced availability in rhizosphere resulting in better uptake (18, 21).

It can be concluded that the arbuscular mycorrhizal fungi and *Azotobacter* positively and significantly exerted the direct influence on fruit yield, nutritional status of leaf, growth and development of apple orchards.

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