2012 International Conference on Biological and Life Sciences
IPCBEE vol.40 (2012) © (2012) IACSIT Press, Singapore

Response of *Azotobacter , Pseudomonas* and *Trichoderma* on Growth of Apple Seedling

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Abstract. The investigation on the efficacy of biofertilizers (*Azotobacter chroococcum*, *Pseudomonas striata* and *Trichoderma viride*) considering different aspects of seedling growth under apple nursery conditions enabled to reach the positive conclusions. *Azotobacter chroococcum* and *Trichoderma viride* increased the maximum germination per cent of seeds while the maximum survival of seedlings was observed on inoculation with *Azotobacter chroococcum* and *Trichoderma viride*. *Azotobacter chroococcum* and *Pseudomonas striata* was found beneficial in promoting the growth of seedling by increasing the growth rate of length and diameter of seedlings. *Trichoderma viride* also helped against the pests and diseases at the same time provided sufficient moisture in dry spell. The highest growth rate of length and diameter of seedling was observed on inoculation with *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride*. The maximum initiation and increase rate in production of number of leaves was observed on inoculation with *Azotobacter chroococcum* and *Pseudomonas striata*. This treatment also produced large sized leaves. The highest content of N,P,K, Zn and Cu in leaves was analyzed on inoculation with *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride*. The highest reduction of the pests attack was observed on inoculation with *Pseudomonas striata* and *Trichoderma viride*, while the infestation was least in seedling due to antifungal fungistatic organic compounds and toxins on inoculation with *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride*. The good growth of root in seedlings was observed on inoculation with *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride*. The maximum biomass of seedlings (as fresh/dry basis) was observed on inoculation with *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride,* because the maximum shoot and root growth was caused. Thus the 'microbial tripartite' or the combination of three microorganisms gave best results in production of superior quality planting material in healthy condition. The inoculation with *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride* gave better response from seeds germination to full growth of apple seedlings at all stages, whereas the inoculation with 'microbial bipartite' or combination of two i.e. *Azotobacter chroococcum* and *Pseudomonas striata* was the next best treatment in attaining the good growth of apple seedlings.

Keywords: *Azotobacter chroococcum*, *Pseudomonas striata, Trichoderma viride,* Apple nursery, Microbial bipartite, Microbial tripartite

1. Introduction

Apple nursery raising is confined to hills with gentle to moderate slopes under rainfed conditions which poses serious problems of water and nutrient losses. Frequent dry spells during summer and autumn and heavy rains during rainy season result adverse effect in availability of nutrients due to leaching. The apple nursery soils bear poor fertility. The root system is not well developed which affects nutrient uptake and seedlings show the deficiency of nutrients. Under drought conditions many serious pests are infested and in water stress conditions many serious diseases are aggravated. Besides these conditions, the old apple nurseries have come under soil sickness problem. Therefore, apple nursery raising on old sites and in such conditions show poor growth of seedlings with diseases and early mortality. This problem is widespread but the

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dimension varies from nursery to nursery. When the old seedlings are removed from a nursery, some of the root system is left behind in the old site. The soil adhered to such roots consists of number of harmful fungi belonging to oomycetcs and basidiomycetes which rob the vital elements of new seedlings and adversely affect their growth. Number of pests like nematodes, wooly aphid, root borer etc. are also harboured in soil and create harm in synergistic condition which affect the growth of seedlings. The massive application of inorganic fertilizers, insecticides and fungicides have increased the toxicity and polluted the total ecosystem of rhizosphere. Keeping these facts in view the application of some biofertilizers with required doses of organic manure and inorganic fertilizers was tested in the present investigation under apple nursery conditions. The efficacy of biofertilizers on growth of apple seedlings with different aspects were studied at Horticulture Research and Extension Center, Chaubattia, Ranikhet, Almora, India during the years 2005-2006 and 2006-2007.

2. Results and Discussion

The stratified seeds of apple were inoculated with *Azotobacter chroococcum, Pseudomonas striata* and *Trichoderma viride* and with their possible combinations; and stratified uninoculated seeds also were sown in well prepared nursery soil mixed with required doses of organic manure and inorganic fertilizers. The highest germination per cent of seeds was observed in combination of *Azotobacter chroococcum + Trichoderma viride* over all the treatments. Similarly the survival of seedling was also encouraged by inoculation of *Azotobacter chroococcm and Trichoderma viride.* The growth of *Azotobacter chroococcum, Pseudomonas striata* and *Trichoderma viride* in the soil has been recorded following the methods described in the Bergey's Manual of determinative bacteriology (1994), Kavimandan et al., (1978) and Kaushik (2000). After sixty days of seed germination further application of biofertilizers with standard doses mixed with well rotten F.Y.M. and placed as basal dressing in the seedlings soil.

Azotobacter chroococcum and *Pseudomonas striata* was found beneficial to buildup the seedling-growth and increased the growth rate of length and diameter progressively. They fight against frequent dry spell conditions by providing sufficient moisture in soil which resulted good rate of growth during summer. *Trichoderma viride* also helped against the attack of pests and diseases during growth of seedling in summer dry spell and in rainy season with more moisture stress conditions. The maximum growth rate of length and diameter of seedling was recorded using *Azotobacter chroococcum + Pseudomonas striata* due to maximum uptake of nitrogen and phosphorus in seedling by extra nitrogen fixation and mobilization of phosphate in soluble form of phosphorus into the soil. However, the highest growth rate of length and diameter of seedling was recorded in treatment with *Azotobacter chroococcum + Pseudomonas striata + Trichoderma viride* over all the treatments. This is in concordance to findings of Mishustin and Shilnikova (1969), Azcon and Barea (1975), Trappe (1977), Lin et al., (1987) and Barbara and Wong (1989) who have also reported a significant increase in population of *Azotobacter chroococcum* , *Pseudomonas striata* and T*richoderma viride* in repeated application during different stages of growth of apple plants and enhanced the survival of seedlings at initial stage. The microorganism treatment combination of *Azotobacter chroococcum + Pseudomonas striata* and *Azotobacter chroococcum, Pseudomonas striata + Trichoderma viride* helped in fast growing of shoot and formed minimum number of branches per seedling which produced the superior quality of seedlings. Formation or initiation of increase in number of leaves which was maximum in treatment combination of *Azotobacter chroococcum + Pseudomonas striata + Trichoderma viride* due to more assimilation and accumulation of food materials. Bagyaraj and Manjunath (1980) , Smith (1980) , Maronek et al., (1981), Mishra and Kapoor (1986) and Verma (1993) have also opined that the association of microorganism like *Azotobacter chroococcum* with *Pseudomonas striata, Azotobacter chroococcum* with vesicular arbuscular mycorrhiza and *Pseudomonas striata* with *Trichoderma viride* were found beneficial in the availability of nutrients.

Azotobacter chroococcum fixed extra nitrogen through roots from atmosphere, *Pseudomonas striata* solubilize the extra phosphorus and both the microorganisms increased potassium content and *Trichoderma viride* made available the micro nutrients like zinc and copper in sufficient amount in soil which resulted the maximum uptake of these nutrients in seedlings. Thus the highest nutrient availability in soil was recorded in treatment combination of *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride*. Inoculation of apple seedlings with the VAM and *Azotobacter chroococcum* + *Pseudomonas striata* encouraged the nitrogen uptake and improved the vegetative growth (Schenck, 1981 and Jayant, 2008).The symptoms of nutrient deficiency was recorded in control treatment due to nursery raising in old sites but minimum range of micronutrients deficiency symptoms was recorded in *Trichoderma viride* and the *Pseudomonas striata.* Starkey (1950), Heeney et al., (1964), Menge et at., (1977) and Campbell, (1985) have also reported the deficiency of zinc, copper and sulphur create serious physiological diseases like little leaf, dieback of shoot, and scorching of leaves and bark in fruit plants.

Azotobacter chroococcum, Pseudomonas striata and Trichoderma viride played an important role in increasing uptake of macro and micro elements in leaves and the maximum content of macro element was analysed with the treatment combination of *Azotobacter chroococcum* + *Pseudomonas striata* and the maximum content of micro element was analysed with *Pseudomonas striata* + *Trichoderma viride.* However, the highest content of N.P.K and Zn and Cu was analysed with treatment combination of *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride*. Sundara Rao (1968) and Dobreiner and Day (1974) have also reported the accumulation of more nutrients in leaves due to the metabolization and assimilation through roots with the help of microorganisms.

During the growth of seedlings the infestation of wooly aphid was prevalent in uninoculated treatment (control). The maximum reduction of this pest attack was recorded in treatment with combination of *Pseudomonas striata* + *Trichoderma viride*. Similarly the infestation of serious diseases like white root rot, collar rot and powdery mildew was prevalent in uninoculated treatment (control). *Azotobacter chroococcum, Pseudomonas striata* and *Trichoderma viride* are known to secrete the antifungal fungistatic compounds, strong organic acids and antibiotics like viridin and phytotoxin respectively which act as synergistic bio-control (antagonistic relation) of the serious diseases and even kill the harmful fungus and pests. Therefore, the treatment with the combination of *Azotobacter chroococcum* + *Pseudomonas striata* + *Trichoderma viride* was found most effective in reduction of serious diseases in seedlings. In the favorable conditions the pathogens infectivity increase and cause diseases like soft rot, root rot, collar rot, powdery mildew etc., have also reported by Nutman et al., 1978.

The seedlings were uprooted in dormant conditions, the length of root and number of rootlets were recorded. *Trichoderma viride* with other microorganisms gave good response in promoting the root growth over control. However, the maximum root length and rootlets per seedling were recorded in treatment combination of *Azotobacter chrooccocum* + *Pseudomonas striata* + *Trichoderma viride* than the other treatments. The maximum biomass of seedlings as fresh and dry weight basis was recorded in treatment with the combination of *Azotobacter chrooccocum* + *Pseudomonas striata* + *Trichoderma viride.* The treatments with microorganisms gave good response in production of total biomass as fresh and dry weight basis than the control treatment.

3. Acknowledgement

Author is thankful to the Indian Council of Agricultural Research (I.C.A.R.), New Delhi for providing financial assistance by way of awarding a Senior Research Fellowship. Author is very grateful to Dr. P. Kaushik Professor of Gurukul Kangri University, Hardwar for technical guidance during Ph. D. thesis works.

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Table 1: Percentage increase in germination of seeds, survival of seedling, growth of shoot and root, Fresh and dry biomass production after inoculation with microorganisms

Microorganisms	Germination of seeds (%)	Survival of seedlings (%)	Length of shoot(cm)	Diameter of shoot (cm)	Branch Number	Leaf Number	Leaf area (Sq. cm)	Root length (cm)	Number of fibrous root	Fresh biomass production Shoot+ Root+ Leaves	Dry biomass production Shoot+ Root+ Leaves

										(gm)	(gm)
Control	40.65	70.00	69.20	2.32	6.00	34.90	24.79	21.62	2.08	63.46	23.88
Azotobacter chroococcum	49.35	79.25	94.30	3.31	4.00	45.59	27.65	22.87	2.48	65.64	27.36
Pseudomonas striata	46.25	76.15	92.05	2.79	5.00	45.50	26.40	30.64	2.74	64.03	26.83
Trichoderma viride	48.40	78.45	88.90	2.72	4.00	44.29	26.27	33.90	4.27	65.01	25.91
Azotobacter chroococcum + Pseudomonas striata	55.05	82.30	103.60	3.95	5.00	51.23	28.78	32.85	3.80	68.86	29.03
Azotobacter chroococcum + Trichoderma viride	61.55	88.40	95.05	3.26	4.00	46.92	27.70	33.79	4.48	67.73	28.41
Pseudomonas striata + Trichoderma viride	56.75	84.55	99.30	3.48	5.00	49.70	28.00	39.89	4.89	68.28	28.81
Azotobacter chroococcum + Pseudomonas striata + Trichoderma viride	60.50	87.00	105.35	4.05	4.00	52.56	28.03	41.82	5.19	74.39	31.14
S. E.	23.08	22.47	4.31	0.15	0.61	10.89	9.05	12.99	5.76	17.00	4.52
C. D. at 5%	1.03	1.45	0.68	0.02	0.20	1.01	1.00	1.65	1.11	1.88	0.97

*This table data are based on the average of two years during 2005-2006 and 2006-2007.

Table 2: Percentage decrease in incidence of diseases and pests after inoculation with microorganisms.

Microorganisms	**Incidence of diseases and pests**				
	Powdery mildew (%)	**White root rot (%)**	**Collar Zone rot (%)**	**Leaf spot (%)**	**Wooly Aphids (%)**
Control	6.40	4.80	2.90	2.32	2.60
Azotobacter chroococcum	5.37	0.51	0.41	0.15	1.03
Pseudomonas striata	5.99	3.50	1.55	1.70	1.68
Trichoderma viride	0.85	2.65	0.35	1.03	0.44
Azotobacter chroococcum + Pseudomonas striata	5.91	0.68	0.48	0.25	1.71
Azotobacter chroococcum + Trichoderma viride	0.57	0.67	0.45	1.53	0.44
Pseudomonas striata + Trichoderma viride	0.91	0.75	0.43	0.21	0.56
Azotobacter chroococcum + Pseudomonas striata + Trichoderma viride	0.80	0.79	0.30	0.15	0.46
S. E.	0.19	0.20	0.52	0.48	2.12
C. D. at 5%	0.01	0.01	0.04	0.08	0.62

*This table data are based on the average of two years during 2005-2006 and 2006-2007.

Table 3: Percentage increase in fixation of nitrogen and solubilization of phosphorus in soil by increase of soil moisture and nutrients in soil on inoculation with *Azotobacter chroococcum , Pseudomonas striata* and *Trichoderma viride*

Biofertilizers	**Actions**	**Nitrogen availability increase over control as organic carbon (%)**	**Phosphorus availability increase over control treatment (%)**	**Potash availability increase over control treatment (%)**	**Moisture availability increase over control treatment (%)**	**Increase over control treatment (%)**	
						Zn (ppm)	**Cu (ppm)**
Azotobacter chroococcum	i)Fixing of atmospheric nitrogen ii)Increase moisture iii)Increase uptake of N,K and Zn, Cu	1.41 (0.40)	0.43 (0.002)	2.51 (1.42)	16.01 (2.57)	2.049 (0.159)	0.377 (0.027)
Pseudomonas striata	i)Secreting organic acids and solubilize phosphorus ii) Increase moisture iii)Increase uptake of P and	1.02 (0.01)	0.065 (0.024)	2.49 (1.40)	15.81 (2.38)	2.178 (1.288)	0.435 (0.085)

	Ca						
Trichoderma viride	i)Secreting toxins which reduce harmful soil pathogens and helps in healthy roots system ii) Increase moisture iii)Increase uptake of nutrients	1.02 (0.01)	0.045 (0.003)	2.28 (1.19)	15.57 (2.14)	2.176 (1.286)	0.489 (0.139)
Untreated seeds/soil as control treatment	Minimal action due to low population of all these bio-agents present in soil	1.01	0.041	1.09	13.43	0.890	0.350

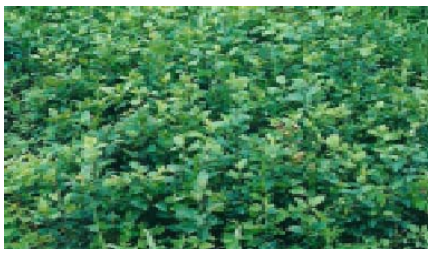
*This table data are based on the average of two years during 2005-2006 and 2006-2007.

Table 4: Biofertilizers as Biocontrol agents in Antagonastic relation to Pathogens and Pests

Bio-Control Agents	Actions	Pathogens/Pests	Pathogenic diseases and Pests	Affected seedling parts	Discases and pest incidence (%)	Diseases and pests decrease (%) over control treatment
Untreated seeds / soil		**Fungi :**				
		Podosphaeria leucotricha	Powdery mildew	Tender sten / Leaves	6.40	-
		Dematophora necatrix	White root rot	Roots (Fine roots)	4.80	-
		Phytopthora cactorum	Collar zone rot	Collar zone	2.90	-
		Phyllosticta spp.	Leaf spot	Leaves	2.31	-
		Pests :-				
		Eriosoma lamigerum	woolly aphid	Root	2.60	-
		Physiological diseases:-				
		Chlorosis	Chlorosis	Leaves	5.36	-
		Dieback	Dieback	Tender stem	6.34	-
Azotobacter chroococcum	Produce antifungal and fungistatic compound which reduce soil born fungus.	**Fungi :**				
		Dematophora necatrix	White root rot	Fine roots	0.51	4.39
		Phytopthora cactorum	Collar zone rot	Collar zone	0.40	2.50
		Phyllosticta Spp.	Leaf spot	Spot on leaves	0.14	2.17
Trichoderma viride	Produces Gliotoxin which reduce soil born fungus / pests in saprophitic reaction	**Fungi :**				
		Podosphaeric leucotricha	Powdery mildew	Tender stem / leaves	0.84	5.56
		Phytopthora cactorum	Collar zone rot	Collar zone	0.35	2.55
		Pests :				
		Eriosoma lanigerum	Woolly aphid	Root	0.43	2.17
Pseudomonas striata	Secreting organic acids and increase uptake of micronutrients	Physiological disorder and Zn and Cu deficiency	Chlorosis Dieback	Leaves Tender tip of shoot	0.73 0.64	4.63 5.70

*This table data are based on the average of two years during 2005-2006 and 2006-2007.

Growth in control plot



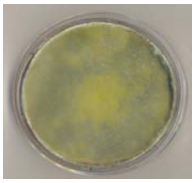
Growth in 'microbial tripartite' treated plot

Growth in 'microbial bipartite'treated plot

Fig. 1: Apple seedlings (*Malus sylvestris*) growth in control plot, treated plot with 'microbial tripartite' of Azotobacter chroococcum, Pseudomonas striata and Trichoderma viride, and 'microbial bipartite' of Azotobacter chroococcum and Pseudomonas striata in nursery at initial stage.

(A)Growth of *Azotobacter chroococcum* on Zenson's medium

(B) Growth of *Pseudomonas striata* on Pikovskaya's medium



(C)Growth of *Trichoderma viride* on Rose Bengal medium

Fig. 2: (A, B and C). Growth of *Azotobacter chroococcum* , *Pseudomonas striata* and *Trichoderma viride* on selective medium.

Fig.3: Incidence of Powdery mildew (*Podosphaeria leucotricha*) showed a grey powder mat of fungal strands and spores on the buds, leaves and twigs of apple seedlings.

Fig. 4: Incidence of white root rot (Dematophora *necatrix*) in apple seedlings.

Fig. 5: Apple seedling infested with wooly aphid.

Fig. 6: Incidence of leaf spot (*Phyllosticta* sp.) in seedling of apple leaves showing lesions on leaf.

a b c d e f g h

Fig. 7: Total growth of seedling on inoculation with microorganisms.(a) Control, (b) Total growth of seedling with *Azotobacter chroococcum*, (c) Total growth of seedling with *Pseudomonas striata,* (d) Total growth of seedling with *Trichoderma viride*,(e)Total growth of seedling with *Azotobacter chroococcum and Pseudomonas striata,(f)* Total growth of seedling with *Azotobacter chroococcum and Trichoderma viride, (g)* Total growth of seedling with

Pseudomonas striata and Trichoderma viride and (h) Total growth of seedling with *Azotobacter chroococcum Pseudomonas striata, and Trichoderma viride.*

a b c d e f g h

Fig. 8: Total root length and number of rootlets after inoculation with microorganisms.(a) Control, (b) Total growth of root with *Azotobacter chroococcum*, (c) Total growth of root n with *Pseudomonas striata,* (d) Total growth of root with *Trichoderma viride*,(e)Total growth of root with *Azotobacter chroococcum and Pseudomonas striata,(f)* Total growth of root with *Azotobacter chroococcum and Trichoderma viride,(g)*Total growth of root with *Pseudomonas striata and Trichoderma viride and (h)* Total growth of root with *Azotobacter chroococcum Pseudomonas striata, and Trichoderma viride.*