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Microbially Enriched Vermicompost Affecting Seedling and Plant Growth in Aonla and Bael

Neetu Khare[1], D.V. Pathak[2*] and Mukesh Kumar[2]

[1]Department of Microbiology, CCS Haryana Agricultural University, Hisar, 125004, India
[2]CCS Haryana Agricultural University, Regional Research Station, Bawal (Rewari), 123501, India

**Corresponding author*

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A B S T R A C T

The microbially enriched vermicompost was used as potting mixture for raising seedlings of aonla and bael. Seedlings showed significant increase in shoot and root length over the seedlings grown in unamended vermicompost. Aonla and bael seedlings raised in microbial enriched vermicompost were healthier and showed significantly more plant dry weight than seedling raised in vermicompost without addition of microorganisms. *Azotobacter chroococcum*, PSB and *P. maltophilia* population were higher in the rhizosphere of the seedling raised in microbially enriched vermicompost at the time of uprooting the plants. The inoculated strains persisted in the rhizosphere and their count increased at the time of harvest. Microbially enriched compost was proved better potting mixture than unamended vermicompost for raising aonla and bael seedlings. Aonla and bael seedlings raised in microbially enriched vermicompost T5 (*Azotobacter* + PSB + PM4 + AM fungi) recorded 14.8% and 18.7% increase in root length and 22.5% and 39.6% increase in shoot length over control (without enrichment), respectively. Seedlings grown in AM fungi enriched vermicompost had higher infection of AM fungi. The seedlings raised in vermicompost where no AM fungi were added showed very low % infection (1.0-2.1%). Vermicompost enriched with *Azotobacter* + phosphate solubilizing bacteria + AM fungi (T3) showed 40% and 36% VAM infection in aonla and bael, respectively.

Introduction

Vermicompost is being commercialized as potting mixture for different ornamental and horticultural plants; and for raising root stocks and grafting. Vermicompost provides essential nutrients to plants and allows plants to quickly absorb nutrients. The growth characters such as seed germination, seedling vigour index, shoot length, root length, plant fresh weight and plant dry weight were significantly higher in plants amended with enriched vermicompost (Baliah and Muthulakshmi, 2017). This is possible as earthworms grind and uniformly mix nutrients and trace elements in simple form and plant needs only minimal effort to obtain them. Chemical fertilizers are most often detrimental to soil microbes as they adversely affect beneficial organisms and their activity. The vermicompost application is one of the

effective methods to rejuvenate the depleted soil fertility and enrich the available pool of nutrients, conserve more water and maintained soil quality (Makode, 2015). Variable showing statistical differences among treatments with leaf number and plant height during plant vegetative stage when applied with vermicompost (Lopez-Gomez *et al.*, 2012). Vermicompost increases the production of crops and prevent them from harmful pests without polluting the environment. Treatments of humic acids, plant growth promoting bacteria and vermicompost can be used for a sustainable agriculture discouraging the use of chemical fertilizers (Joshi *et al.*, 2014). In addition, chemical fertilizers and pesticides used in both farms and orchards have become a leading cause of water pollution.

Vermicompost enriched with *A. chroococcum* resulted in improvement in plant growth, grain yield, leaf chlorophyll content and nitrate reductase activity of rice followed by enrichment with *A. brasilense* (Mahanta *et al.*, 2012). Horticultural plant cuttings and saplings are raised in sand or soils which are deficient in essential plant nutrients in comparison to potting mixtures. Further, it is difficult to inoculate the adult plant with the bioinoculants.

Inoculation of horticultural plant seedlings may improve their vigour and growth. Seedling pre-inoculation with many beneficial microorganisms may improve their establishment in the field after transplantation. Bael and aonla are two arid zone fruit trees which are widely grown on marginal soils. Inoculation of seedlings with nitrogen fixer, phosphorus solubilizing bacteria and AM fungi may improve the seedling growth and establishment in the field.

Hence, the present investigations were initiated to find the impact of enriched vermicompost on seedlings raised from seeds of bael (*Aegle marmelos*) and aonla (*Phyllanthus emblica*).

Materials and Methods

The study was carried out at Department of Microbiology, CCS Haryana Agricultural University, Hisar under pot house conditions. Vermicompost was enriched with microbial cultures. Earthen pots (15 cm diameter) were filled with soil amended with enriched vermicompost in 1:1 ratio. Seeds of bael and aonla were surface sterilized with 0.2% $HgCl_2$ for 10 min and washed 5-6 times with sterilized distilled water. Ten seeds were sown in each pot. Each treatment had 10 replications. Pots were watered, whenever required. After 60 days of sowing plants were uprooted and plant dry weight, root dry weight, root-shoot length and colonization of *Azotobacter*, PSB, P-36 and *P. maltophilia* PM4 was observed. The infection of mycorrhiza in bael and aonla was checked by the method of Philips and Hayman (1970).

Results and Discussion

Effect of microbially enriched vermicompost on shoot and root growth in aonla and bael

A pot experiment was conducted to evaluate the effect of microbially enriched vermicompost on growth of aonla and bael. Perusal of data indicated significant improvement of root and shoot length of aonla and bael seedlings raised in microbially enriched vermicompost (Table 1). The aonla seedlings raised in T1 control (without enrichment) showed 62 mm and 155 mm shoot length and root length, respectively. The root and shoot lengths of seedlings raised in vermicompost amended with *Azotobacter* + phosphate solubilizing bacteria (T2), *Azotobacter* + PSB + AM fungi (T3), *Azotobacter* + PSB + PM4 (T4) and

Azotobacter + phosphate solubilizing bacteria + PM4 + AM fungi (T5) was significantly higher than control. Maximum shoot and root lengths were observed in T5 (76 mm and 178 mm, respectively).

Aonla seedlings raised in microbially enriched vermicompost T5 (*Azotobacter* + phosphate solubilizing bacteria + PM4 + AM fungi) recorded14.8%. Increase in root length and 22.5% increase in shoot length over control (without enrichment). Similarly trends were observed in Bael seedlings also. Bael seedlings raised in microbially enriched vermicompost T5 (*Azotobacter* + PSB + PM4 + AM fungi) recorded 18.7% increase in root length and 39.6% increase in shoot length over control (without enrichment).

Effect of microbially enriched vermicompost on shoot dry weight and root dry weight of aonla and bael

Shoot weight and root dry weight of aonla and bael plants was estimated after drying at 80^{o}C. In general, seedlings of aonla and bael raised in vermicompost enriched with bioinoculants had showed better growth as reflected by higher shoot and root dry weight (Table 2).

Aonla seedling raised in vermicompost enriched with *Azotobacter* + PSB (T2), *Azotobacter* + PSB + AM fungi (T3) showed significantly higher shoot and root dry weight over the untreated vermicompost. The vermicompost enriched with *Azotobacter* + PSB + PM4 + AM fungi (T5) was proved as the best treatment showing maximum shoot and root dry weight in aonla and bael.

Persistence of inoculated bacteria in rhizosphere of aonla and bael

Viable count of inoculated bacteria such as *Azotobacter,* PSB and *P. maltophilia* was taken at the time of harvest. Results showed that inoculated bacteria were able to persist in the rhizosphere of aonla (Table 3). In all the treatments population of *Azotobacter,* phosphate solubilizing bacteria and *P.maltophilia* was higher than at the time of sowing. Highest population of *Azotobacter* (6.501 CFU/g), PSB (7.175 CFU/g) and *P.maltophilia* (7.761 CFU/g) was observed in vermicompost enriched *Azotobacter* + PSB + PM4 + AM fungi (T5). These populations were significantly more than the control as well as other treatments.

Survival of *Azotobacter,* PSB and *P. maltophilia* in vermicompost was better at the time of harvesting over the samples at sowing time (Table 4). *Azotobacter,* PSB and *P. maltophilia* were able to survive and persist in bael rhizosphere for 60 days. *Azotobacter* population increased from 4.477 to 4.592 CFU/g in control (T1), from 5.479 to 6.121 CFU/g in *Azotobacter* + PSB (T2), 5.490 to 6.256 CFU/g in *Azotobacter* + PSB + AM fungi (T3), 5.414 to 6.231 CFU/g in *Azotobacter* + PSB + PM4 (T4) and 5.845 to 6.487 CFU/g in *Azotobacter* + PSB + PM4 + AM fungi (T5). Likewise, population of PSB and *P. maltophilia* in the bael rhizosphere also increased with enrichment of vermicompost at the time of sowing.

AM fungi infection of roots of aonla and bael

Aonla and bael seedling roots were studied for AM fungi infection by staining the root cutting with Trypan blue. Infection was studied in 10 root fragments and percent root infection was calculated (Table 5). The seedling raised in vermicompost where no AM fungi were added showed very low % infection (1.0-2.1%). Vermicompost enriched with *Azotobacter* + PSB + AM fungi (T3) showed 40% and 36% VAM infection in aonla and bael, respectively.

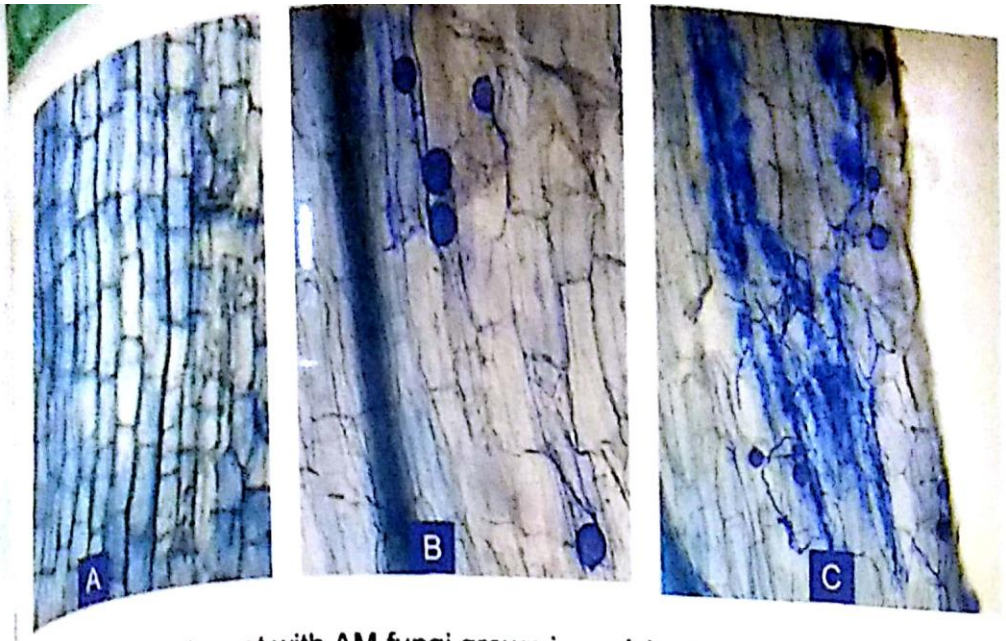

Fig Infection of aonla root with AM fungi grown in enriched vermicompost.
A Vermicompost, B Vermicompost +*Azotobacter*+ PSB+VAM,
C Vermicompost +*Azotobacter*+ PSB+PM4+VAM,

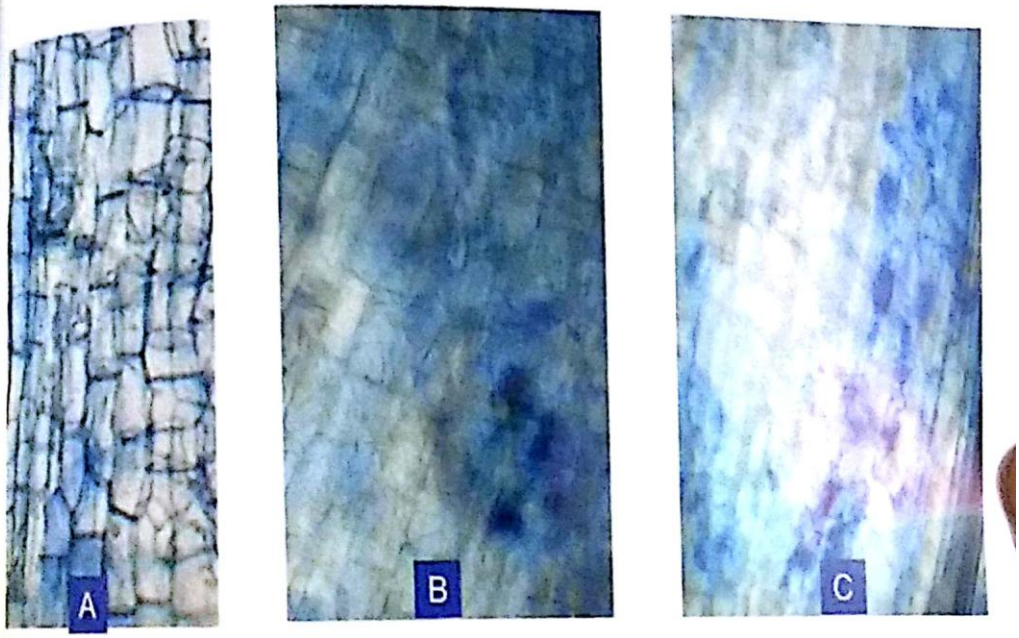

Fig Infection of bael roots with AM fungi grown in enriched vermicompost.
A Vermicompost, B Vermicompost +*Azotobacter*+ PSB+VAM,
C Vermicompost +*Azotobacter*+ PSB+PM4+VAM,

Table.1 Effect of microbially enriched vermicompost on root and shoot growth of Aonla and Bael at 60 DAS

Treatment	Aonla				Bael			
	Shoot length (mm)	% increase over control	Root length (mm)	% increase over control	Shoot length (mm)	% increase over control	Root length (mm)	% increase over control
T1	62	0	155	0	63	0	149	0
T2	71	14.5	164	5.8	70	11.1	165	10.7
T3	74	19.3	172	10.9	76	20.6	171	14.7
T4	74	19.3	172	10.9	73	15.8	168	7.3
T5	76	22.5	178	14.8	88	39.6	177	18.7
CD	4.0	--	4.0	--	5.0	--	5.0	--

T1 (control) = Vermicompost, T2 = Vermicompost + *A. chroococcum* + PSB, T3 =Vermicompost + *A. chroococcum* + PSB+ VAM, T4 = Vermicompost + *A. chroococcum*+ PSB+ *P. maltophilia*, T5 = Vermicompost + *A. chroococcum* + PSB + *P. maltophilia* + VAM.

Table.2 Effect of microbially enriched vermicompost on shoot weight and root weight (on dry weight basis) of Aonla and Bael at 60DAS

Treatment	Aonla		Bael	
	Shoot weight (g)	Root weight (g)	Shoot weight (g)	Root weight (g)
T 1	0.46	0.34	0.50	0.48
T 2	0.52	0.48	0.78	0.64
T 3	0.69	0.52	0.86	0.73
T 4	0.71	0.63	0.97	0.81
T 5	0.78	0.56	1.04	0.83
CD	0.04	0.06	0.04	0.04

T1 (control) = Vermicompost, T2 = Vermicompost + *A. chroococcum* + PSB, T3 =Vermicompost + *A. chroococcum* + PSB+ VAM, T4 = Vermicompost + *A. chroococcum*+ PSB+ *P. maltophilia*, T5 = Vermicompost + *A. chroococcum.* +PSB+*P. maltophilia*+ VAM.

Table.3 Persistence of inoculated bacteria in vermicompost rhizosphere of Aonla

Treatment	*A. chroococcum*		PSB		*P. maltophilia*	
	Zero day	60 d	Zero day	60 d	Zero day	60 d
T 1	4.477	4.678	2.241	3.428	ND	ND*
T 2	5.479	6.101	6.121	6.758	ND	ND
T 3	5.490	6.246	6.134	6.803	ND	ND
T 4	5.414	6.368	6.019	6.978	6.001	7.453
T 5	5.845	6.501	6.023	7.175	6.082	7.761
CD for days (D)		0.002		0.002		0.002
CD for treatment (T)		0.003		0.003		0.002
CD for D x T		0.004		0.004		0.002

T1 (control) = Vermicompost, T2 = Vermicompost + *A. chroococcum* + PSB, T3 =Vermicompost + *A. chroococcum* + PSB+ VAM, T4 = Vermicompost + *A. chroococcum* + PSB + *P. maltophilia*, T5 = Vermicompost + *A. chroococcum*+ PSB+ *P. maltophilia* + VA, *ND = not detected

Table.4 Persistence of inoculated bacteria in vermicompost rhizosphere of Bael

Treatment	*A. chroococcum*		PSB		*P. maltophilia*	
	Zero day	60 d	Zero day	60 d	Zero day	60 d
T 1	4.477	4.592	2.241	3.388	ND	ND
T 2	5.479	6.121	6.121	6.726	ND	ND
T 3	5.490	6.256	6.134	6.798	ND	ND
T 4	5.414	6.231	6.019	6.969	6.001	7.438
T 5	5.845	6.487	6.023	7.168	6.082	7.719
CD for days (D)		0.002		0.002		0.004
CD for treatment (T)		0.003		0.003		0.004
CD for D x T		0.004		0.005		0.006

T1 (control) = Vermicompost, T2 = Vermicompost + *A. chroococcum* + PSB, T3 =Vermicompost + *A. chroococcum* + PSB+ VAM, T4 = Vermicompost + *A. chroococcum* + PSB+ *P. maltophilia*, T5 = Vermicompost + *A. chroococcum* + PSB + *P. maltophilia* + VAM.

Table.5 AM fungi infection of roots of Aonla and Bael grown in microbially enriched vermicompost

	Aonla	Bael
Treatment	Root infection %	Root infection %
T 1	1.0	2.3
T 2	1.6	1.8
T 3	40.0	36.0
T 4	1.7	2.1
T 5	56.0	49.0
CD	3.0	2.4

T1 (control) = Vermicompost, T2 = Vermicompost + *A. chroococcum* + PSB, T3 =Vermicompost + *A. chroococcum* + PSB+ VAM, T4 = Vermicompost + *A. chroococcum*+ PSB+ *P. maltophilia*, T5 = Vermicompost + *A. chroococcum* + PSB+ *P. maltophilia*+ VAM

Vermicompost enriched with *Azotobacter* + PSB + PM4 + AM fungi (T5) proved to be the best as it showed 56 and 49 % AM fungi infection in aonla and bael, respectively. It was significantly higher than control (T1).

The root and shoot lengths of aonla seedlings raised in vermicompost amended with *Azotobacter* + PSB (T2), *Azotobacter* + PSB + AM fungi (T3), *Azotobacter* + PSB + PM4 (T4) and *Azotobacter* + PSB + PM4 + AM fungi (T5) was significantly higher than the control. Maximum shoot and root lengths were observed in T5. Similar trends were observed in bael seedlings also. Aonla seedling raised in vermicompost enriched with *Azotobacter* + PSB (T2), *Azotobacter* + PSB + AM fungi (T3) showed significantly higher shoot and root dry weight over the untreated vermicompost. The vermicompost enriched with *Azotobacter* + phosphate solubilizing bacteria + PM4 + AM fungi (T5) was proved best and showed maximum shoot and root dry weight in aonla and bael. *A. chroococcum* as a biofertilizer compensated N fertilizer in mango. *A. chroococcum* application also favored P uptake, K content and micronutrient contents positively (Ahmad

et al., 2004). Co-inoculation of *Rhizobium* and *Glomus* spp. showed a significant improvement in root nodulation, AM colonization, biomass production, N and P contents as compared to the plants those received AM mycorrhiza or *Rhizobium* alone (Rategeri *et al.*, 2005). Kale *et al., (*1992) studied influence of vermicompost application on the available nutrient in a paddy field. There was no significant difference in N content of the soils treated with farm yard manure and vermicompost. However, higher amount of available P was recorded in vermicompost treated soil. Cavender *et al.,* (2003) observed that root and shoot dry weight were increased by vermicompost in the absence of AM fungi in *Sorghum bicolor*. Inoculation with AM fungi and higher amount of vermicompost was responsible for the reduction of dry weight. Gopinathan and Prakash (2014) reported that vermicompost with biofertilizer inoculation improve plant mineral concentration through nitrogen fixation and thereby alters fruit production.

In woody horticultural plants improvement in their survival rate and quality was higher, when inoculated with AM fungi (Azcon *et al.*, 1997). In microbially enriched vermicompost organic carbon decreased due to degradation of organic matter by microbial activity, total N and P contents were increased due to inoculation of *A. chroococcum*, PSB and AM fungi, respectively (Khare *et al.*, 2014). VAM fungi, *G. mossae* showed improved plant height, dry matter as well as P, N and Zn concentration with no or low level of phosphorus application in papaya plant (Mohandas, 1992). Nitrogen, phosphorus utilization efficiency and N, P, Ca and Mg uptake of the grass treated with phosphorus enriched vermicompost were higher (Sabrina *et al.,* 2013). Seed germination and plant growth parameters in guava (*Psidium guajava*) were positively affected by bioinoculants alone or in coinoculation with FYM as well as vermicompost. The bioinoculants tested were *A. chroococcum*, phosphate solubilizing bacteria, *Pseudomonas maltophilia* and VAM (Pathak *et al.,* 2009). Pre-inoculation of tomato transplants with AM fungi improved yield (Al-Karki, 2006). An application of the basal dose of vermicasting with mulching has an immediate effect on the crop. More germination, better tillering heavy flowering and fruiting, resistant/tolerance to pest's attack and higher yield had been reported by the use of vermicompost in different field crops, plantation crops, orchards etc. (Bhawalkar, 1992).

Leaf area, number of strawberry suckers, number of flowers, shoot weight, and total marketable strawberry yields increased significantly in plots treated with vermicompost compared to those that received inorganic fertilizers only. The improvements in plant growth and increase in fruit yields could be due to increase in soil microbial biomass after vermicompost applications (Arancon *et al.,* 2004). Paula *et al.,* (2007) observed that vermicompost significantly increased germination rates (176%) and improved the marketability of fruits at 40% and 100% substitution rates due to the lower incidence of physiological disorders ('blossom end rot' and fruit cracking).

The seedling raised in vermicompost where no AM fungi were added showed low infection (1.0-2.1%). Vermicompost enriched with *Azotobacter* + PSB + AM fungi (T3) showed higher VAM infection in aonla and bael. Vermicompost enriched with combination of *Azotobacter* + PSB + PM4 + AM fungi (T5) proved to be the best AM fungi infection in aonla and bael, that was significantly higher than control (T1), *Azotobacter* + PSB (T2) and *Azotobacter* +

PSB + PM4 (T4) where *P. maltophilia* was not added. The increase in root biomass because of inoculation with *Glomus leptotichum* and *G. intraradices* was 51.4 and 91.4 %; respectively when compared with uninoculated plants. Improvement of plant growth with inoculation of AM fungi has been reported in a number of forest tree species and medicinal plants (Vasanthakrishna *et al.,* 1995; Ranjan *et al.,* 2000).

Enhanced plant biomass and P uptake because of AM fungal inoculation has been reported by earlier worker in fruit tree species and a few medicinal plants (Vasanthakrishna *et al.,* 1995; Sailo and Bagyaraj, 2005). However, there was no effect of introduced mycorrhizal fungi on non-sterilized soil (Silveira and Lima, 1996). *Glomus* spp when inoculated with *A. chroococcum*, modifies the rhizosphere favorably to improve soil nutrient availability and consequent uptake by plants and thus result in better growth, fruit yield and quality of kinnow (Usha *et al.,* 2004). Kalmegh seedling in presence of AM fungi generally showed an increase in plant growth and andrographolide (active ingredient) concentration over those grown in the absence of inoculation of soil with AM fungi (Chiramel *et al.,* 2006).

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