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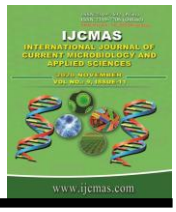

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Effect of Phytohormones and Plant Growth Promoting Microorganisms on Germination and Plant Growth of Aonla (*Emblica officinalis* Gaertn.)

Vibha Kumari*, Vijay Bahadur, Saket Mishra, Samir Ebson Topno and Deena Wilson

Department of Horticulture, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, India

**Corresponding author*

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A B S T R A C T

The present experiment was carried out during July 2019 to March 2020 in horticulture research farm of Department of Horticulture, SHUATS, Prayagraj. The experiment was conducted in Randomized Block Design with 13 + 1 (control) treatment replicated thrice. The phyto-harmones (GA_3 and NAA) and plant growth promoting microorganisms (*Aspergillus niger*, *Pseudomonas fluorescens* and Photosynthetic bacteria (*Rhodopseudomonas palustris*) were used as treatments at different concentrations. From the present investigation it is found that treatment T_{12} [*Pseudomonas fluorescens* + PSB *Rhodopseudomonas palustris* (10ml each in 100ml distilled water)] was found superior in terms of early germination, seedling length, plant spread, shoot length and chlorophyll content of leaves.

Introduction

Aonla (*Emblica officinalis* Gaertn.) is one of the important minor fruit crops of our country. In India, it is known by various names such as Aonla, Amla, Amlika, Dhotri, Emblica and Usuri.

Aonla is a highly nutritious fruit and rich source of vitamin C and also a fair source of minerals particularly iron, phosphorus, calcium and magnesium. It is also the richest source of pectin which is mostly useful in making jam and jellies. It is also used in tannin and dyeing industries.

The area under Aonla is increasing day by day due to its popularization as a medicinal plant (coolant, refrigerant, diuretic and laxative) and also its potential for better adaptation to diversified soil and climatic conditions. There is great demand for genuine true-to-type planting materials in order to optimize production of quality fruits.

Biofertilizers plays an important role in the growth of the seedlings. Use of biofertiizers like *Aspergillus* and *Pseudomonas* enhance the quality and promote growth without deteriorating the soil and produces quality yield. The growth regulators like GA_3 and NAA have been widely used for pre-sowing seed treatments to increase germination and to accelerate vegetative seedling growth.

Application of plant growth regulators in seedling germination of Aonla has become powerful tool to modify several physiological processes in plants which are extensively and profitably used in horticultural crops. They are also used for increasing plant growth and protecting the nursery plants from several insect-pest and diseases.

All plant-related habitats contain a high proportion of plant beneficial micro-organisms, plant pathogens and potential human pathogens. The dynamic changes in these micro-organisms may affect sustainable plant production and plant health (Berg *et al.*, 2005; Mendes *et al.*, 2013). In general, plants drive the composition and structure of rhizosphere bacterial communities through root exudates (Bais *et al.*, 2006; Micallef et al., 2009). In turn, rhizosphere microorganisms can promote the overall health of plant species by promoting crop growth and participating root surface defence protection (Berg *et al.*, 2005; Mendes *et al.*, 2013).

Materials and Methods

The area of Prayagraj district of Uttar Pradesh comes under subtropical belt, which experience extremely hot summer and fairly cold winter. The maximum temperature of the location reaches up to 46℃-48℃ and seldom falls as low as 4℃-5℃. The relative humidity (RH) ranges between 20 to 94 %. The average rainfall in this area is around 1013.4 mm annually However, occasional precipitation is also not uncommon during winter months.

The present investigation was carried out on the "Effect of phyto-harmones and plant growth promoting microorganisms on germination and plant growth of Aonla (*Emblica officinalis* Gaertn.)" under Prayagraj agro-climatic conditions. The experiment was conducted in Randomized Block Design (RBD) with one control and thirteen treatments and three replications at the Research Farm of Department of Horticulture. Total number of treatments was 13+1(control).

Treatment details

The experiment design was RBD and there were fourteen treatments (13+1) which are replicated thrice. The treatment details are T_0 (control), T_1 (GA_3 100 ppm), T_2 (GA_3 200 ppm), T_3 (GA_3 300 ppm), T_4 (NAA 100 ppm), T_5(NAA 200 ppm), T_6(NAA 300 ppm), T_7 [*Aspergillus niger* (10ml /100 ml of distilled water)], T_8[*Pseudomonas fluorescens* (20ml/ 100 ml of distilled water)], T_9[Photosynthetic Bacteria *Rhodopseudomonas palustris* (10ml/ 100ml of distilled water)], T_{10} [*Aspergillus niger* + *Pseudomonas fluorescens* (10ml each in 100 ml distilled water)], T_{11}[*Aspergillus niger* + PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)], T_{12}[*Pseudomonas fluorescens* + PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)], T_{13}[*Aspergillus niger* + PSB*Rhodopseudomonas palustris* + *Pseudomonas fluorescens* ((10ml each in 100 ml distilled water)]. The aonla seeds were soaked in each treatment for 12 hours and followed by 5 hours of shade drying.

Seeds were grown in polybags filled with soil, sand and FYM in the ratio 2:1:1, respectively. Data were recorded on various growth parameters.

Results and Discussion

The data recorded on germination and various growth parameters during 2019-2020, the course of investigation have been presented in Table 1.

The effect of phyto-hormones and plant growth promoting micro-organisms on day of germination of Aonla is very obvious and consistent. There was significant difference among the different treatments at 45, 90, 135,180 and 225 days. The results of the experiment are summarized below.

Day of germination

The minimum number of days taken for the germination was recorded in T_{12} [*Pseudomonas fluorescens* + PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] with 6.66 days followed by T_{13} [*Aspergillus niger*+ PSB *Rhodopseudomonas palustris* + *Pseudomonas fluorescens* (10 ml each in 100 ml distilled water)] with 8.33 day. The maximum number of days was observed in control, which took 24 days for initiation of germination.

Even under the most favourable condition the seed germination in Aonla did not attain its maximum, due to the internal condition namely physiological or biochemical factors. Biofertilizers play a prime role in order to overcome causes controlled by chemical or physiological factors. This finding correlates the findings of Nehal M. Elekhtyar (2015) in rice and I. Ketut Widnyana and Cokorda Javandira (2016) in tomato.

Germination percentage

The effect of phyto-hormones and plant growth promoting micro-organism on germination percentage of Aonla is very clear. The maximum germination percentage was recorded in T_{12} [*Pseudomonas fluorescens* + PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] with 72.667 % followed by T_{13} [*Aspergillus niger*+ PSB *Rhodopseudomonas palustris* + *Pseudomonas fluorescens* (10ml each in 100 ml distilled water)] with 70.33 which were significantly superior over control with 33.33 germination percentage.

Seed Germination% increasedby the favourable effect of PGPR (Plant Growth Promoting Rhizobacteria) might be due to Rhizobacteria traits and could prove effective in improving the seed germination rate. (Malleswari and Bagyanarayana 2013) and (Siva Kumar et al., 2012).

Survival percentage

The maximum survival percentage was recorded in T_{12} [*Pseudomonas fluorescens*+ PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] with 69.00 % followed by T_{13} [*Aspergillus niger*+ PSB *Rhodopseudomonas palustris* +*Pseudomonas fluorescens* (10ml each in 100 ml distilled water)] with 66.67 % which were significantly superior over control with 30.33% survival percentage.

Applications of these associations have been investigated in maize, wheat, oat, barley, peas, canola, soy, potatoes, tomatoes, lentils, radicchio and cucumber (Gray andSmith, 2005).

Shoot length

Among the treatments applied, T_{12} [*Pseudomonas fluorescens* + PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] with 25.66 cm increase significantly better shoot length followed by T_{13} [*Aspergillus niger*+ PSB

Rhodopseudomonas palustris + *Pseudomonas fluorescens* (10ml each in 100 ml distilled water)] with 25.03 cm which was significantly superior over control with shoot length 19.13 cm.

The increase in height of plant with application of T_{12} (*Pseudomonas* + PSB) treatment might be due to its stimulating effect for rapid growth or early seedling growth resulted in more cell division and elongation due to a secretion of IAA by bacteria leading to longest shoot because of the capability of bacteria for fixing nitrogen from air and enhanced metabolism process resulted in more energy and growth improvement.

This finding correlates the findings of Khalimi *et al.,* (2012), Vijay Kumar *et al.* (1991) in guava and Pawshe *et al.,* (2007) in Aonla.

Plant spread

The maximum plant spread was recorded in T_{12} [*Pseudomonas fluorescens*+ PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] with 25.90 cm followed by T_{13} [*Aspergillus niger* + PSB *Rhodopseudomonas palustris* + *Pseudomonas fluorescens* (10ml each in 100 ml distilled water)] with 24.63 cm and the minimum was recorded in control with 17.20 cm.

Vigorous shoot growth due to PGPR might have resulted into increase in production of photosynthates; their translocation through phloem to the root zone might be responsible for increasing the plant spread because of cell multiplication and cell elongation at faster rate. Phyto-harmones promote the plant growth, thought to include the ability to produce auxins (Shaharoona *et al*., 2006; Egamberdiyeva, 2007; Gholami *et al*., 2009; Son *et al*., 2014).

Girth of the seedling

The observations which were recorded during the experiment period on the effect of phyto-hormones and plant growth promoting microorganisms on girth of Aonla seedling is that the maximum girth of seedling was recorded in T_{12} [*Pseudomonas fluorescens*+ PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] with 6.00 mm followed by T_{13} [*Aspergillus niger* + PSB *Rhodopseudomonas palustris* + *Pseudomonas fluorescens* (10ml each in 100 ml distilled water)] with 5.36 mm and the minimum was recorded in control with 2.13 mm.

Phyto-harmones increased girth of stem which might be due to stimulation of cambium and its immediate cell progeny by the process of enhancing the rate of cell multiplication. The rate of increase in the dimension of the cell both in pith and cortex region is faster as rather than number of cells per unit area. This type of result was also observed by Das and Pattanaik (2013) in okra. PGPR promote the plant growth, thought to include the ability to produce auxins (Shaharoona *et al*., 2006; Egamberdiyeva, 2007; Gholami *et al*., 2009; Son *et al*., 2014).

Seedling vigour index

The maximum seedling vigor index was recorded in T_{12} [*Pseudomonas fluorescens*+ PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] with 2060.53 followed by T_{13} [*Aspergillus niger* + PSB *Rhodopseudomonas palustris* + *Pseudomonas fluorescens* (10ml each in 100 ml distilled water)] with 1912.67 which were significantly superior over control with 713.33 seedling vigor index.

Seedling vigor index = germination percentage x seedling length (root length+ shoot length)

Table.1 Effect of different treatments on growth parameters of Aonla

Notation	Treatment combination	Day of germination	Germination %	Shoot length (cm)	Plant spread (cm)	Girth of seedling (mm)	Seedling vigor index	Seedling length (cm)	Chlorophyll content of leaves (mg/g)	Survival %
T_0	Control	24.00	33.33	19.13	17.20	2.13	713.33	21.26	0.71	30.33
T_1	GA_3 100 ppm	23.00	40.33	19.46	17.76	3.26	876.33	21.60	0.92	37.00
T_2	GA_3 200 ppm	19.00	43.33	19.90	18.06	3.43	962.40	22.23	0.88	40.33
T_3	GA_3 300 ppm	16.66	48.33	21.03	19.60	4.43	1,135.43	23.50	0.98	45.00
T_4	NAA 100 ppm	20.33	59.23	21.26	19.13	4.13	1,099.93	23.36	0.93	52.00
T_5	NAA 200 ppm	18.66	52.66	22.03	18.86	3.63	1,259.93	23.93	1.03	48.33
T_6	NAA 300 ppm	20.33	62.33	22.56	21.16	3.76	1,491.10	24.83	1.10	61.33
T_7	Aspergillus (10ml/100ml distilled water)	22.66	57.33	21.60	21.56	3.90	1,482.73	23.66	0.95	57.00
T_8	Pseudomonas (10ml/100ml distilled water)	17.33	65.00	23.23	22.53	4.33	1,652.33	25.56	1.10	59.66
T_9	PSB (10ml/100ml distilled water)	17.33	47.33	22.46	22.93	4.76	1,647.47	24.53	1.03	43.66
T_{10}	Aspergillus + Pseudomonas (10ml each in 100ml distilled water)	19.66	60.66	24.46	23.23	4.16	1,428.39	26.83	1.27	57.00
T_{11}	Aspergillus + PSB (10ml each in 100ml distilled water)	14.66	54.56	23.56	20.66	4.43	1,512.93	25.70	1.11	55.66
T_{12}	Pseudomonas + PSB (10ml each in 100ml distilled water)	06.66	72.66	25.66	25.90	6.00	2,060.53	28.40	1.54	69.00
T_{13}	Aspergillus + PSB + Pseudomonas (10ml each in 100ml distilled water)	08.33	70.33	25.03	24.63	5.36	1,912.67	27.16	1.393	66.66
	CD	8.681	12.213	2.393	1.792	2.133	401.826	2.358	0.353	11.586
	SEd	4.2	5.909	1.158	0.867	3.267	194.41	1.141	1.171	5.605
	F-Test	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

The growth promoting substance produced by PGPR have exerted a synergistic action and enhanced the growth promotion of Aonla. Pseudomonas spp. was reported to produce amino acids, salicylic acid and IAA which might have improved the plant growth and seedling vigour. (Sivamani and Gnanamanickam, 1988; O'Sullivan and O'Gara, 1992)

Seedling length (shoots length + root length)

The maximum seedling length was recorded in T_{12} [*Pseudomonas fluorescens* + PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] with 28.40 cm followed by T_{13} (*Aspergillus niger* + PSB *Rhodopseudomonas palustris* + *Pseudomonas fluorescens* (10ml each in 100 ml distilled water)] with 27.16 cm which were significantly superior over control with 21.26 cm length.

Plant growth promoting Rhizobacteria (PGPR) have environmental desirable capacities such as nitrogen-fixation, phosphate and potassium-solubilisation, which makes available for plant root to absorption and utilization of mineral nutrition, which leads to the seedling growth (Lu and Huang, 2010), (Nihorimbere *et al.,* 2011). IAA positively influences root growth and development, thereby enhancing nutrient uptake (Khalid *et al.,* 2004).

Chlorophyll content of leaves

The maximum chlorophyll content was recorded in T_{12} [*Pseudomonas fluorescens*+ PSB *Rhodopseudomonas palustris* (10ml each in 100ml distilled water)] with 1.54 mg/g of fresh seedling, followed by T_{13} [*Aspergillus niger* + PSB *Rhodopseudomonas palustris* + *Pseudomonas fluorescens* (10ml each in 100 ml distilled water)] with 1.393 mg/g of fresh seedling which were significantly superior over T_0 (control) with 0.713 mg/g of fresh seedling.

P. *fluorescens* rapidly utilizes seed and root exudates and produces a wide spectrum of bioactive metabolites. PSB is capable of fixing nitrogen for growth and its genes encode for protein that make up light harvesting complexes and photosynthetic reaction centers.The amount of PSB added promote the plant resistance against disease; increase the content of chlorophyll and seedling growth. Similar findings were observed by Ke *et al.,* (2005) in cucumber, Jun-lin *et al.,* (2012) in eggplant and Yali *et al.,* (2014) in Chinese cabbage.

On the basis of results obtained, it is concluded that the treatment T_{12} [*Pseudomonas fluorescens* + PSB *Rhodopseudomonas palustris* (10ml each in 100 ml distilled water)] found to be best in terms of minimum day to germination (06.66 days), germination percentage (72.66%), survival percentage (69.00%), shoot length (25.66cm), seedling length (28.40 cm), plant spread (25.90 cm), girth of seedling (6.00mm), seedling vigor index (2060.53), and chlorophyll content of leaves (1.54 mg/g).

Application of plant growth regulators and biofertilizers in seed germination of Aonla has great impact on physiological processes in plants which are extensively and profitably used in horticultural crops. They are also used for increasing plant growth and protecting the nursery plants from several insect-pest and diseases.

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