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Research Article

Lactic Acid Bacteria (LAB) and its Curd, as a Low-Cost Input for Plant Growth Promotion Activity in Tomato Crop

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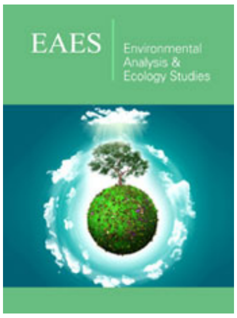
ISSN: 2578-0336



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Submission: September 12, 2023
Published: October 10, 2023

Volume 11 - Issue 4

How to cite this article: SG Borkar*, Pranita N Pardhi and Ajayasree TS. Lactic Acid Bacteria (LAB) and its Curd, as a Low-Cost Input for Plant Growth Promotion Activity in Tomato Crop. Environ Anal Eco stud. 000767. 11(4). 2023. DOI: 10.31031/EAES.2023.11.000767

Abstract

Lactic Acid Bacteria (LAB) are an inhabitant of a diversified environment and plays an important role in the well-being of their habitat. These are the low-cost inputs promoting the health and wellness of humans, animals, aquacultures and crop plants and need special attention and exploration, particularly in financially crunched nations. In developing and underdeveloped countries, the cost of agricultural crop cultivation is increasing day by day, making the farmers debt-ridden to acquire the necessary inputs like crop growth-promoting nutrients and pesticides to be used in agricultural crop production. Different bio-fertilizers and bio-agents are used in the farming system but these are not a part of the human beneficial microbiome. Therefore, a low-cost input for plant growth promotion activity safe for human wellness is a need of the day in the farming system. Lactic Acid Bacteria (LAB), a known human gut microbe consumed in the form of curd, is explored as a low-cost input for plant growth promotion activity for tomato crop in the present investigation.

The LAB curd of the 7^{th}-day incubation period was more effective than the curd of other incubation periods to enhance tomato seed germination (up to 100%). The per cent increase in tomato seedling's radical length was 57.14% while plumule length was 63.63% by the 7^{th} day old curd over the untreated control. The seedling root dip treatment with 7th days curd was more effective in increasing the plant growth parameters in tomato plants, like root and shoot biomass, and number of leaves, thereby overall increasing plant canopy/plant architecture, and yield contributing parameters like number of flowers (where the increase was in the range of 71.42 to 83.33% over untreated control), and number of tomato fruits (where the increase was double or 100% over untreated control).

Lactic acid was the main metabolic constituent of LAB growth in the curd. The concentration of lactic acid in the curd on the 7^{th} day was 70.00µg/10ml curd. The commercial lactic acid concentration of 0.01M was found effective in increasing the tomato seed germination, plant height and number of leaves, thereby indicating that lactic acid was a functional metabolite playing a role in enhancing the plant growth promoting activities. LAB as a gut microbe can be used safely in the agriculture production system as a low-cost input, without threatening the environmental microbial pollution and ecological niches.

Keywords: Lactic acid bacteria; Gut microbe; plant growth promotion; Low-cost input for plant growth; Agriculture; Crop production

Introduction

Lactic Acid Bacteria (LAB) are an inhabitant of a diversified environment and plays an important role in the well-being of their habitat. These are part of the human gut microbiome and used in human wellness [1], in animal farming particularly those of poultry, pig, Ruminant and aquaculture [2], in crop plants production and protection [3], and in dairy product fermentation [4]. In the human gut, the LAB can improve the digestibility of protein in food and enhance the nutritional value of food protein and help the human intestinal tract to absorb the amino acids in dairy products [5], on the plant system it acts as plant growth promoting bacteria or as biocontrol agent [6], and in diary product it acts as lactose coagulator in milk to form curd and other diary fermented products [7]. Thus, there is functional variability of the LAB in different ecosystems and environments. Ferments containing LAB have been used for decades in the agricultural system to improve soils, to control plant diseases and to promote plant growth, however, the functional role of LAB in the phyto-microbiome has yet to be discovered [3]. In the present investigation, we studied the functional role of LAB in promoting plant growth in tomato plants.

Material and Methods

Isolation of LAB from curd sample

The fresh curd was streaked on sterilized Nutrient- Agar medium (Agar, 20g; Sucrose, 20g, Peptone, 5g; beef extract, 3g in 1L of distilled water, pH 7.0) in the sterile petri plates under laminar air flow. These plates were incubated in a BOD incubator at 28±1 ^{0}C for 48hrs. The single LAB colonies formed in the plates were selected and purified further to obtain a pure culture of LAB. The efficacy of the isolated LAB in the formation of curd was tested by inoculating a loopful of pure isolated culture into the milk and incubating at ambient temperature for the formation of curd.

Preparation of LAB solution for tomato seed treatment

A suspension of LAB growth (10^7cfu/mL) was prepared in distilled water and mixed with jaggary solution (10mL LAB growth suspension mixed with 20mL melted jaggary at ambient temperature). The tomato seeds were treated with this solution and dried in the shed for 20 minutes. A germination test was carried out by employing the paper towel method [8]. 100 seeds per replication were kept on a moist paper towel of size 45 x 30cm (10 seeds/row at a distance of 3cm in between seeds x 10 rows) and the paper towel was folded taking due care to not to disturb the seeds during wrapping. The seeds-wrapped towel paper was wrapped in butter paper with the lower end closed with a rubber band while the upper end opened. The wrapped papers were vertically arranged in seed germinator at 30 ^{0}C for germination. Three replications of each experiment were performed.

To determine the effect of Lactic acid on tomato seed germination

Lactic acid solution of 1.0, 0.1, 0.01, 0.001, 0.0001 and 0.00001M was prepared from the commercial lactic acid. 50 tomato seeds were dipped in each concentration of lactic acid solution for 5 minutes and dried in the shed for 20 minutes. These treated seeds were kept on moist blotter paper as described earlier and kept in seed germinator for observation on seed germination, and growth of plumule and radical of the germinating seedlings. The root and shoot length of germinating seedlings were measured up to 13 days.

To determine the effect of different ages of curd on tomato seed germination

The curd of 1,2,3,4,5,7,10,15,20 and 30 days incubated period was used to see its effect on tomato seed germination. 50 tomato seeds were dipped in each curd sample and dried in the shed for 20 minutes. These treated seeds were kept on moist blotter paper as described earlier and kept in a seed germinator for observation on seed germination and growth of plumule and radical of the germinating seedlings. The root and shoot length of germinating seedlings were measured up to 13 days.

Estimation of Lactic acid from curd of various incubation periods

Colorimetric estimation of lactic acid, present in the curd, was done by the method of Mattsson [9] by employing the following steps.

Deproteinization: A 10mL curd sample was taken in a 100mL volumetric flask and 5mL of 0.1N NaOH was added into it. The content was shaken and the volume was made up to 70mL with distilled water. 5.0mL of 10% $ZnSo_4.7H_2O$ was added slowly into it while shaking. The volume was made to 100mL with simultaneous shaking. After 15 minutes the mixture was filtered to obtain clear filtrate (if the filtrate was not clear, the pH was adjusted to 7 with NaOH and re-filter to obtain clear filtrate).

Determination of total lactic acid: 1.0mL of deproteinized sample was pipetted into the test tube with a glass stopper and 10mL distilled water was added to it and mixed thoroughly. A freshly prepared 1% $FeCl_3.6H_2O$ (in water) was added into it, and mixed. The yellow colour developed was read at 375nm in a spectrometer against the blank (without a deproteinized sample). The amount of Lactic acid was read from a standard curve of lactic acid (for preparation of standard curve, standard Lithium lactate solution was prepared corresponding to 10µg lactic acid per mL by diluting 1.0655g lithium lactate in 100mL water).

Determination of the effect of different ages of curd on plant growth parameters of tomato

The curd incubated for 1 day to 30 days was used to see its effect on plant growth parameters. For this 200mL distilled water was added to 100mL curd of various incubation periods and mixed thoroughly. The roots of tomato seedlings of the same age and height were dipped in these curd solutions for individual treatment and were planted in individual earthen pots. The potting soil was saturated with buttermilk of the same curd. Observation on the growth parameters viz. plant height, no. of leaves, no. of flowers, and no. of fruits were recorded after 20, 40, 70 and 90 days of planting.

Results

Effect of LAB-culture inoculant on tomato Seed Germination, and on the growth of plumule and radicle of germinating seedlings

The results (Table 1) indicated that the LAB-culture inoculant increased the seed germination percentage. The seed germination in tomato seed starts on the second day and continues up to 13 days, in the seed germinator, depending on the seed's vigour. The LAB-inoculant palleted tomato seeds were used to see the effect of LAB on the growth of plumule and radicle of germinating tomato seedlings. The results (Table 2) indicated that the LAB-treated seed increased the radicle length by 57.14% while plumule length by 63.63% over untreated seed. This clearly indicates that the LAB has plumule and radicle elongation activity and a seedling growth-promoting nature.

Table 1: Effect of LAB on tomato seed germination.

The Incubation Period (in days) of Seeds for Germination	Percent (%) Germination in LAB-Treated Tomato Seeds	Percent (%) Germination in Untreated Tomato Seeds (Control)	Percent (%) Increase in Seed Germination
1	0	0	0
2	9	4	5
3	30	25	5
5	50	45	5
6	62	54	8
9	78	70	8
13	82	73	9

Table 2: Effect of LAB-Culture inoculant on the growth of plumule and radicle of germinating tomato seedling.

Seedling Growth Parameters (Due to LAB Seed Treatment)	In LAB-Treated Seeds	In Untreated (Control) Seeds	Percent (%) Increase Over Untreated Seed
Radicle length (in cm)	5.5	3.5	57.14
Plumule length (in cm)	9	5.5	63.63

Effect of commercial Lactic Acid (LA) on tomato seed germination, and on growth of plumule and radicle of germinating seedlings

Lactic acid being the main metabolite of LAB, the effect of commercial lactic acid on tomato seed germination, and on the growth of plumule and radical of germinating seedlings was studied. The results (Table 3) indicated that commercial lactic acid enhances tomato seed germination. The concentration of 0.01M of lactic acid was more effective than other higher or lower concentrations of lactic acid. At least there was an increase of 30% germination due to 0.01M lactic acid seed treatment in tomato seeds.

Table 3: Effect of Lactic acid on tomato seed germination.

Incubation Period (in days) for Seed Germination	Percent (%) Tomato Seed Germination Due to Lactic Acid Concentration (in M)					Germination of Non-Treated Tomato Seeds
	1	0.1	0.01	0.001	0.0001	
1	0	0	0	0	0	0
2	0	0	20	0	0	10
3	0	20	50	30	20	30
4	10	30	60	30	30	30
5	20	40	60	40	40	40
6	30	40	70	40	40	50
7	40	50	80	50	50	50
8	50	70	80	60	60	60
9	60	70	90	70	70	60
10	60	80	90	70	70	60
11	70	80	100	80	80	70
12	70	80	100	90	80	70
13	80	90	100	90	80	70
S.E. ±	7.87					
CD at 5 %	22.13					

Similarly, there was an increase in the plumule and radical length of the germinating seedlings due to the lactic acid seed treatment. The results (Table 4) indicated that the treatment of lactic acid has an influence on the growth of radicle length and plumule length of germinating seedlings. Lactic acid at 0.01M concentration had increased the radical length by 42.85% and plumule length by 36.36% over the untreated seeds. The lactic acid at 0.00001M concentration did not have any effect on the radicle and plumule length of the germinating seedling and produced the same growth of radicle and plumule as of untreated seed. Other concentrations of LA have a marginal effect on the radicle and plumule length.

Table 4: Effect of lactic acid concentration on the growth of plumule and radicle of germinating tomato seedlings.

Growth of Germinating Seedling (Due to LA Treated seed)	Lactic acid (LA) Concentration (in Mol)						Untreated (control) seeds	Percent(%) Increase in better Treatment Over control
	1	0.1	0.01	0.001	0.0001	0.00001		
Radicle Length (cm)	3.5	4	5	4.5	3.5	3	3.5	42.85
Plumule Length (cm)	5	6.5	7.5	6	5.5	4	5.5	36.36

Estimation of Lactic acid in LAB curd of various incubation ages

Table 5: Production and presence of Lactic acid in curd of various incubation ages.

Age of Curd (in days)	Concentration of Lactic Acid in Curd (µg/mL of Curd)
1	0.63
2	2.06
3	2.33
4	4.33
5	5.33
7	7
10	8.23
15	9
20	8.1
30	7.16

Lactic acid being the main metabolite of LAB, the amount of lactic acid released/produced in LAB curd of various incubation periods (curd ages) was determined by using a standard curve of lactic acid prepared by using a commercial lithium lactate solution. The results (Table 5) indicated that as the incubation period (age) of curd increases, the concentration of lactic acid in the curd also increases. The lactic acid content in 1st-day curd was 6.30µg/1g of curd which increased up to 90µg/10g of curd at 15 days of incubation period /curd age. Thereafter, the concentration of lactic acid decreases. The lactic acid available in 20 and 30 days of curd was 81 and 71.60µg/10g of curd respectively.

Effect of different ages of curd on tomato seed germination

The results (Table 6) indicated that the curd aged from 4^{th} to 7^{th} days having 4.33 to 7.0µg lactic acid was more effective than any other incubated age curd. There was 100 per cent seed germination with these curd-treated seeds. The increase was 30% over the untreated seeds. These results also coincide with the result of 0.01M commercial lactic acid seed treatment where a 30% increase in seed germination was observed over untreated control. These results clearly indicate that lactic acid produced in curd has a direct effect on accelerating seed germination in tomato seeds.

Table 6: Effect of curd age on tomato seed germination.

Incubation Period (in days) for Seed Germination	Percent Seed Germination in Different Ages (in days) of Curd Having Different Lactic Acid Concentrations (in µg/mL of curd)										Non-Treated Seeds
	1^{st}	2^{nd}	3^{rd}	4^{th}	5^{th}	7^{th}	10^{th}	15^{th}	20^{th}	30^{th}	
	0.63	2.06	2.33	4.33	5.33	7	8.23	9	8.1	7.16	
1	0	0	0	0	0	0	0	0	0	0	0
2	10	10	10	10	20	20	10	0	0	0	10
3	20	20	20	20	30	30	20	0	0	0	20
4	20	20	30	30	30	30	30	0	0	0	30
5	40	40	40	50	50	50	30	10	10	0	40
6	40	40	50	60	60	60	40	20	10	0	50
7	50	50	60	70	70	70	50	20	10	0	50
8	50	60	70	70	80	80	60	30	20	0	60
9	60	60	80	80	80	80	70	30	20	0	60
10	60	70	80	90	90	90	80	40	20	0	60
11	70	80	90	90	100	90	80	40	20	10	70
12	80	80	90	90	100	100	80	50	20	10	70
13	80	90	90	100	100	100	90	50	30	20	70
S.E	7.36										
C.D at 5%	20.44										

Effect of lactic acid in LAB-curd on plant growth parameters

On plant height and number of leaves: The curd having lactic acid concentration (per 10g curd) of 6.30µg (curd age 1 day), 53.30µg (curd age 5 days), 70.00µg (curd age 7 days), 90.00µg (curd age 15 days) were tested for their effect on the plant growth parameters. The results (Table 7) indicated that the application of 7th-day-old curd with 70.00µg lactic acid was more effective in enhancing the tomato plant growth parameters. The plant height and number of leaves were more on the 7th day of incubated curd treatment than the untreated control. The increase in plant height was 20% while in number of leaves was 25%. The low concentration of 6.30µg lactic acid has a negative effect on the plant growth parameters. The reason for this negative effect needs further investigation.

Table 7: Effect of Lactic acid (in curd) on tomato plant growth.

Curd Age (Lactic Acid Conc. in ug)	Effect on Plant Growth Parameters	
	Height (in cm)	No. of Leaves
1 day (6.30)	20	6
5th day (53.30)	32	9
7th day (70.00)	36	10
15th day (90.00)	28	7
No curd (control)	30	8

On Root and Shoot Biomass: The results (Table 8) indicated that a maximum increase in root and shoot biomass was observed in the plant treated with 7th-day-old curd. The increase in root biomass was 28.57% while in shoot biomass was 44.73% over the untreated control. The root and shoot biomass results also confirm plant growth parameter results of 7th-day curd treatment.

Table 8: Effect of curd age on root and shoot biomass of tomato plant.

Growth parameters	Age of Curd in Days						Untreated Seedling (Control)	Percent Increase in Better Treatment Over Control
	1st	5th	7th	15th	20th	30th		
Root Biomass (gm/plant)	1.9	4.2	4.5	2.3	1.7	1.2	3.5	28.57
Shoot Biomass (gm/plant)	36.5	47	55	41.6	35.2	32.1	38	44.73

Effect of curd root dip treatment on tomato plant growth parameters

On the growth of plumule and radical: The results (Table 9) indicated that the 7th-day-old curd was more effective in enhancing the radical and plumule length of germinating seedlings. The per cent increase in radical length due to this curd was 57.14% while the plumule length was 63.63%. The 15, 20 and 30th days old curd had a negative effect on the root and plumule growth. The 3rd day's old curd had a 42.85% increase in radicle length and 36.36% in plumule length which was equivalent to growth obtained by 0.01M lactic acid concentration as mentioned in the earlier table.

Table 9: Effect of curd age on the growth of plumule and radical of germinating tomato seedling.

Growth of tomato Seedling (in curd Treated Tomato Seeds)	Age of Curd (in days)										Untreated Seed (Control)	Percent (%) Increase in Better Treatment Over Control
	1st	2nd	3rd	4th	5th	7th	10th	15th	20th	30th		
Radical Length (cm)	3.5	4	5	5	5	5.5	3	2	2	1	3.5	57.14
Plumule Length (cm)	5	6.5	7.5	8	9	9.5	7	-	-	-	5.5	63.63

On other plant growth parameters: The results (Table 10) indicated that there was an increase in all the plant growth parameters by curd treatment. The curd of 7 days was more efficient in increasing the plant growth-promoting parameters. The increase in respect of the number of flowers was in the range of 71.42 to 83.33% over untreated (control) whereas the per cent increase in the number of fruits to the tune of 100% over untreated control was noticed. From these results, it is seen that the curd of 7 days' age is beneficial and should be used to enhance the plant growth-promoting parameters in tomatoes.

Table 10: Effect of curd treatment as root dip on plant growth parameters.

Plants at Days After Curd Treatment	Plant Growth parameters	Age of Curd (in days) Used for Root Dip Treatment										Non-Treated (Control) Plant	Percent (%) Increase in Better Treatment Over Control
		1st	2nd	3rd	4th	5th	7th	10th	15th	20th	30th		
0	Height (cm)	13	13	13	13	13	13	13	13	13	13	13	0
	No. of leaves	5	5	5	5	5	5	5	5	5	5	5	0
20	Height (cm)	30	35	37	45	48	46	44	37	30	30	30	60
	No. of leaves	18	17	18	20	20	22	23	20	15	14	15	53.33
40	Height (cm)	62	60	66	83	82	86	70	65	62	62	50	66
	No. of leaves	25	25	25	22	25	28	28	23	20	18	20	40
70	Height (cm)	70	85	86	88	95	90	85	70	65	65	60	58.33
	No. of leaves	30	30	27	29	32	34	31	28	25	24	26	30.76
	No. of flowers	1	3	8	10	12	12	8	7	5	3	7	71.4

90	Height (cm)	72	88	90	92	100	98	87	76	70	72	68	47.05
	No. of leaves	25	25	25	22	28	25	26	24	20	18	20	40
	No. of flowers	3	5	7	9	11	10	6	4	2	2	6	83.33
	No. of fruits	1	3	7	9	12	12	8	6	4	2	6	100

Discussion

Effect of LAB-inoculant, and Lactic acid on tomato seed germination, and on the growth of Plumule and Radical of germinating tomato seedling

The tomato seed priming with LAB-culture inoculant increases the tomato seed germination percentage. Though the LAB-priming seed could not minimize the germination period, it increased the tomato seed germination by 9%. Lactic acid being the primary metabolite of the LAB, the *in vitro* assessment of lactic acid on seed germination indicated that lactic acid increases seed germination. The concentration of lactic acid was an important factor influencing the seed germination. The untreated tomato seeds had 70% seed germination whereas the lactic acid-treated seeds at 0.0001M, 0.001M, 0.01M, 0.1M and 1M concentrations gave 80, 90, 100, 90 and 80% seed germination respectively. Thus, there was an increase of at least 10-30% in seed germination due to lactic acid seed treatment over untreated seed. The maximum seed germination i.e., 100% was obtained with 0.01M lactic acid solution and therefore seems to be the optimum concentration of lactic acid to increase the seed germination in tomato seeds. In our experimentation, the LAB-treated tomato seed had 82% seed germination. When the percentage of seed germination was compared with the lactic acid concentration, it seems that the seed priming with LAB produced the lactic acid to the tune of 0.001 or 1M concentration which had 80% seed germination. Asma Saleh El-Mabrok [10] and Asma Saleh El-Mabrok et al. [11] reported that chilli seeds treated with *Lactobacillus plantarum* had a high germination percentage in chilli varieties. Similarly, Tsudam et al. [12] reported that LAB improves the germination rate in chilli seeds.

Further, our results indicated that LAB-inoculant treated seed had increased length of radical and plumule as compared to untreated seeds. The increase in radical length was up to 57.14% and plumule length by 63.63% over untreated seeds. This result clearly indicates that LAB has plumule and radical elongation activity and growth-promoting nature. The lactic acid being the main metabolite produced by LAB during its growth, the commercial lactic acid of different concentrations when tested for their effect on the growth of radical and plumule of germinating tomato seedlings indicated that it had influenced the growth of radical length and plumule length of seedling. Lactic acid at 0.01mol concentration had increased the radical length by 42.85% and plumule length by 36.36% over the untreated seed. However, the lactic acid at 0.0001mol concentration did not have any effect on the radical and plumule growth of germinating seedlings and produced the same growth of radical and plumule as in the case of untreated seeds. These results clearly indicate that seed priming with LAB not only increases seed germination but also increases the length of radical and plumule of germinating tomato seedlings. Hamed et al. [13] reported that LAB applied as seed treatment resulted in the elongation of both shoot and root length and the increase in the number of secondary roots as well as the increment in total fresh weight of tomato plants.

Effect of Curd age on tomato seed germination, and on growth of plumule and radical of germinating tomato seedling

The curd of different ages (incubation period) contained different amounts of lactic acid. The 5-day-old curd was more effective in increasing the seed germination percentage. 4 days, 5 days and 7 days curd had produced 100% seed germination. However, the period required for seed germination varied with the curd age. 5 days old curd required 11 days, 7 days old curd required 12 days and 4 days old curd required 13 days for 100% seed germination. The curd of 15, 20, and 30 days had a negative effect on the tomato seed germination over untreated seed. The 5-day-old curd was better than other curd for priming tomato seed for 100% seed germination with a reduced germination period. There is no literature available on the effect of curd age on seed germination. Therefore, this is the first report on the effect of curd age on tomato seed germination.

The tomato seed priming with curd of 7th days old was more effective in enhancement of radical and plumule length of germinating seedlings. The per cent increase in radical length due to the 7th-day-old curd was 57.14% and the plumule length by 63.63%. The curd of the 1st day, 15th day, 20th day and 30th day had a negative effect on radical and plumule growth. The curd of 3rd day had a 42.85% increase in radical length and 36.36% in plumule length over untreated control. This increase in radical and plumule length was equivalent to the radical and plumule length obtained by 0.01mol lactic acid concentration. At present there is no literature available on the effect of curd age on growth of plumule and radical of germinating seedlings.

Effect of curd as root dip treatment on plant growth parameters in tomato

There was an increase in all plant growth parameters by curd root dip treatment. The curd of the 7th day was more efficient in increasing the plant growth-promoting parameters like plant height, number of leaves, root and shoot biomass, number of flowers and tomato fruits. The root biomass was 28.57% more and the shoot biomass was 44.73% more than the untreated control. The increase in the number of flowers was in the range of 71.42 to 83.33%, whereas the per cent increase in the number of fruits was to the tune of 100% over untreated control. Thus, the root dip treatment of tomato seedlings with LAB was found effective in increasing the growth parameters as well as yield enhancing parameters particularly flower and fruits in tomato plants. The lactic acid at concentrations of 53.30µg (produced by 5-day-old curd) and 70.00µg (produced by 7-day-old curd) was effective in increasing the plant height, number of leaves, flowers and fruits in tomato plants. Naseby et al. [14] suggested that LAB with other beneficial microbes in soil may provide an almost constant nutrient source for the plant, thereby leading to plant health. Stephane et al. [15] reported that treatment of soil with LAB supports plant growth. Hamed et al. [13] reported the efficacy of LAB as PGPB, as bio-protection of tomato seed and the soil drench to support plant growth.

Conclusion

Lactic Acid Bacterial (LAB) inoculant and curd containing LAB helped in seed germination and increased radical and plumule length, number of leaves, flowers and fruits in tomato plants. The functional metabolite involved in enhancing these parameters is lactic acid secreted by LAB in the ferment. The LAB ferment of 7 days had more quantity of lactic acid as compared to other ferments which was appropriate for the enhancement of seed germination, and plant growth and yield contributing parameters.

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