Short Communication

Seed biopriming and spraying at fruit set with microbial agents suppress anthracnose disease and improve growth and yield in chilli

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Abstract

Anthracnose caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is a major disease of chilli (*Capsicum annuum* L.) resulting in a yield loss from 8 to 60 percent in India. Most of the commercial chilli varieties are susceptible to the disease. Present study reports potential of seed biopriming and spraying with biocontrol agents at fruit-set stage for the management of anthracnose disease in chilli (var.VellayaniAthulya). Three biocontrol agents *viz*., *Trichoderma asperellum*, *Bacillus subtilis* VLY 62 and *Bacillus amyloliquefaciens* VLY 24 were compared with chemical check (Carbendazim 0.1%) and untreated control for disease management and growth promotion. Seed biopriming and spraying with *B. amyloliquefaciens* at a concentration of 10^8cfu mL^{-1} was the most effective treatment in reducing the disease. Biopriming alone also exhibited significant reduction.

Keywords: *Bacillus* spp., Biopriming, Chilli anthracnose, *Colletotrichum capsici*, *Trichoderma asperellum*.

Chilli (*Capsicum annuum* L.), is a common spice cum vegetable crop used since ancient times for its pungency, flavour and colour. Its fruits are good sources of antioxidants such as vitamin A, flavonoids, β-carotene, α-carotene, lutein, zeaxanthin and cryptoxanthin (Howard et al., 2000). Extracts of chilli fruits are widely used as analgesic for arthritis, burns, headaches and neuralgia. They also boost our immune system, lower cholesterol levels, reduce high blood pressure and increase peripheral circulation (Geetha and Selvarani, 2017). Sahitya et al. (2014) reported that chilli could lessen the risk of cancer by preventing binding of carcinogens to DNA. India is the largest producer, consumer and exporter of chilli in the world and it is cultivated throughout the year (Abarna et al., 2019).

The important diseases that affect chilli include damping off, fruit rot (anthracnose), die back, powdery mildew, bacterial leaf spot, cercospora leaf spot, alternaria rot, fusarium wilt, leaf curl and mosaic. Anthracnose caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is of great importance as it affects fruits, the economic part of the crop. The disease is both seed and air borne; and hinders seed germination and vigour (Asalmol et al., 2001). In India, chilli anthracnose causes yield loss of 8 to 60 percent (Pooja and Simon, 2019).

A promising way to increase agricultural production, while bringing down the use of chemical pesticides which pollute the environment, is biological control (Mishra et al., 2011). Biocontrol agents are environmentally safe and can ensure long-lasting disease management. Many of them are available in the form of carrier based, liquid and polymer entrapped formulations. They are applied to plants in different ways depending upon the formulated product. Seed priming with cell

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suspensions or liquid formulations of biocontrol agents, commonly called as 'seed biopriming' is one of the promising techniques. It integrates biological and physiological aspects of seed treatment. Biological aspect involves inoculation of seed with beneficial microorganisms to protect it and physiological aspect involves controlled seed hydration resulting in enhanced vigour (Ghassemi et al., 2008). Seed biopriming provides protection from seed and soil-borne pathogens, improves the speed and uniformity of germination and final stand of the crop. Biopriming allows the inoculated microbes to colonize the seed prior to planting (Callan et al., 1991; El-Mohamedy, 2004; Ananthi, et al., 2017). The current study was undertaken to assess the efficacy of seed biopriming together with spraying of the biocontrol agents at the time of flowering and fruit set for the management of anthracnose/fruit rot disease in chilli. Such a sequential approach is supposed to provide better protection of the crop during the juvenile stage as well as to its economical produce during the bearing stage. Further, the bacterial isolates used were endospore formers which are supposed to have the ability to survive better than the other bacterial biocontrol agents.

All microbial cultures except the plant pathogen used in the study were obtained from the culture collection of Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. Three biocontrol agents *viz*., *Trichoderma asperellum*, *Bacillus subtilis* VLY 62, and *Bacillus amyloliquefaciens* VLY 24, and chemical check (Carbendazim 0.1%) and untreated control were included in the study. *T. asperellum* was grown and maintained in potato dextrose agar (PDA) medium. Nutrient agar (NA) was used for the growth and maintenance of the *Bacillus* cultures. Incubation of both fungal and bacterial cultures was done at 28^{o}C. The pure culture of the anthracnose pathogen, *Colletotrichum capsici* was procured from the culture collection of Department of Plant Pathology, College of Agriculture, Vellayani and the virulence was tested on leaves and fruits of chilli var. Vellayani Athulya.

T. asperellum spore suspension was prepared by flooding the surface of five-day-old culture in PDA plates with sterile water and scraping the surface with a bent glass rod. The suspension was filtered through sterile muslin cloth. The filtrate was collected aseptically. The spore count was determined using a haemocytometer and was adjusted to 10^8 spores mL^{-1} by adding sterile water (Bankole and Adebanjo, 1996).

Bacillus strains were heavily cross streaked individually on NA plates. The plates were incubated for 48 h, drenched with 10 ml of sterile distilled water and cell suspension was collected in sterile conical flasks. The optical density (OD) value was adjusted to 0.6 at 660 nm using sterile distilled water so that the suspension contained approximately 10^8 cfu mL^{-1} (Anith et al., 2015). Seed treatment was done on the same day.

The duration for biopriming of chilli seed for each biocontrol agent was standardized before proceeding to pot culture studies. Biopriming of seed was carried out according to the method described by El-Mougy and Abdel-Kader (2008). Seeds of chilli variety Vellayani Athulya was surface sterilized with 0.1 % mercuric chloride solution for one min and then washed thrice with sterile distilled water. The fungal spore suspension (10^8 spores mL^{-1}) and bacterial cell suspensions (10^8cfu mL^{-1}) were added with carboxy methyl cellulose (CMC) @ 0.1g 10 mL^{-1} which acted as an adhesive. Carbendazim 0.1% was used as the chemical check. For hydropriming, the seeds were soaked in sterile distilled water. Chilli seeds were soaked in the respective treatments for different time durations *viz*., 20 min, 1 h, 2 h, 4 h, 8 h and 16 h. After soaking for each specified time, the seeds were taken out and air dried. Seed without priming served as the control. The germination percentage and biometric parameters of the seedlings were determined using paper towel method.

The germination test was carried out following between paper method (ISTA, 1985). Fifty seeds were placed equidistant between two sheets of the germination paper soaked in water, rolled and tagged and kept for incubation under room temperature. Three replications were maintained for each treatment. Observations on germination percentage and the time taken for germination were taken from 7th day of sowing and continued up to 14th day. The germination percentage was calculated using the formula:

$$\text{Germination (\%)} = \frac{\text{Number of nornal seedling produced}}{\text{Total number of seed used}} \times 100$$

Observations on root length, shoot length, seedling length, seedling dry weight were taken and seedling vigour index was calculated. Primed seeds (10 numbers) were selected from each treatment and were placed equidistant between two sheets of the germination paper soaked in water, rolled, tagged and kept for incubation under room temperature. Three replications were maintained for each treatment. Observations on root length, shoot length and seedling length were taken on the 14th day after sowing. Seedling dry weight was obtained by keeping in hot air oven kept at 60^{o}C for 24 h. Seedling Vigour Index (SVI) was calculated based on the formula given by Abdul-Baki and Anderson (1973).

SVI – I = Germination (%) × Length of seedlings in cm

SVI – II = Germination (%) × Seedling dry weight in g.

After standardizing the duration of priming for each biocontrol agent a pot culture study was undertaken. The most appropriate duration of seed priming was selected based on the results obtained in the previous experiment. The duration of priming was 1h for *T. asperellum* and 4 h for *Bacillus* spp. as well as the chemical check. The concentration of bioagents was fixed as 10^8spores mL^{-1} for *T. asperellum* and 10^8cfu mL^{-1} for *Bacillus* spp. respectively. Seeds were soaked in the respective treatments for the particular duration for priming. After soaking for the specified time, seeds were air dried under shade. Seeds without priming served as the control. The seeds were sown in protrays filled with standard potting mixture containing sand, soil and coir pith compost in 1:1:1 ratio. The protrays were kept in shade house for one month till the seedlings were ready for transplanting.

For the preparation of pathogen inoculum, ten numbers of five mm culture discs of *C. capsici* were inoculated into 100 mL of sterile Potato Dextrose Broth (PDB) prepared in 250 mL conical flasks and incubated at 28^{o}C in a rotary shaker at 150 rpm. Once the medium was completely covered with mycelia, conidial masses were collected and dispersed in 10 mL sterile water aseptically. The concentration of the conidia was determined using a haemocytometer and adjusted to 10^6 conidia mL^{-1} using sterile water.

Bio primed seedlings grown in protrays were transplanted after four weeks to grow bags of size 40cm x 24cm x 24cm filled with standard potting mixture of sand, soil and coir pith compost in 1:1:1 ratio under open condition. The experiment was laid out in completely randomised design (CRD) with four replications. Single plant each was maintained per grow bag. The crop was raised as an irrigated crop. Fertilizer application was done according to the growth stages. Each plant was supplied with 3.2 g urea, 4 g rajphos and 0.8 g muriate of potash (MOP). Half of urea, full rajphos and half of MOP was applied as basal dose in protrays by incorporating with the planting mixture. One fourth of urea and half MOP was applied one week after transplanting. The remaining quantity of urea was applied one month after transplanting.

In addition to seed biopriming, the plants were sprayed twice with the same concentration of cell/spore suspension of the respective biocontrol agents as mentioned above, first on the 60th day of sowing, when almost 50 per cent of the plants possessed

fruit set and the second at 15 days after the first spray. Pathogen inoculation was done 48 h after second spray by spraying the mature fruits with the prepared conidial suspension of *C. capsici*@ 20 mL per plant.

Severity of the disease was determined by calculating the Percent Disease Index (PDI). Disease severity in fruits was scored based on a 0 - 4 scale developed by Vishwakarma and Sitaramaiah (1986) as given below:

0 = healthy
1 = 1-5% of fruit area infected
2 = 6-25% of fruit area infected
3 = 26-50% of fruit area infected
4 = 51-100% of fruit area infected

Disease severity score on leaves was calculated based on the score chart given by Inglis et al. (1988) as given below:

0 = healthy
1 = 1-10% of leaf area infected
2 = 11-25% of leaf area infected
3 = 26-50% of leaf area infected
4 = > 50% of leaf area infected
5 = defoliation

Based on the above scores, the PDI was calculated as: PDI =

$$\frac{\text{Sum of individual scores}}{\text{Total no. of fruits observed x Maximum disease grade}} \times 100$$

Percent Disease Index (PDI) was calculated at 5th, 10th and 15th days after inoculation. Plant height was measured at 40, 80 and 120 days after planting (DAP). Branches per plant were counted at 120 DAS. Three harvests of chilli fruits were taken and observations on number of fruits per plant, fruit yield per plant and 100 seed weight were calculated.

Data obtained from the experiments were subjected to analysis of variance (ANOVA). The critical difference (CD) was calculated at 5 per cent level of significance and treatment means were compared. Statistical analysis was done using R based analysis platform of Kerala Agricultural University, GRAPES (General Rshiny Based Analysis Platform Empowered by Statistics; https://www.kaugrapes.com/home) by one way Analysis of Variance (ANOVA) and the treatment means were compared using Duncan's Multiple Range (DMRT) at a probability of 0.05 per cent.

The germination of seeds started from 7th day of incubation and continued upto 14th day. The highest germination percentage, shoot length, root length, seedling length, seedling dry weight, SVI I and SVI II were observed in the case of seeds soaked for 1 h with *T. asperellum* and 4 h for the other treatments *viz.*, *B. subtilis*, *B. amyloliquefaciens,* carbendazim and hydropriming. All the treatments significantly increased the biometric characters compared to unprimed seeds (Tables 1 to 7). Several workers have reported the enhanced effects of seed biopriming in chilli. Biopriming chilli seed with *T. viride*, *Pseudomonas fluorescens* and *B. subtilis* (Bharathi et al., 2004; Dhanalakshmi, 2013; Machenahalli et al., 2014; Ilyas et al., 2015; Chauhan and Patel, 2017; Rai and Behera, 2019), *B. amyloliquefaciens* (Sathya et al., 2016; Gowtham et al., 2018) and *B. pumilus* (Amaresan et al., 2012) enhanced the germination percentage, root length, shoot length, drymatter production and vigour index over non-primed seeds.

It was observed that there was an enhancement in the biometric characters when the soaking time was increased from 20 min to 1h. However, a slight decrease was recorded when primed for 2 h. Thereafter it gradually increased and reached the maximum value at 4 h of priming. After 4 h also a decrease was observed till 16 h except in case of priming with *T. asperellum*, where a decrease at 8 h was followed by an increase at 16 h. Soaking for 8 h and 16 h also improved the growth parameters over control, but the effects were lesser than those of 4 h soaking. This could be due to the seeds being subjected to imbibition injuries. Thus, soaking for 4 h was found to be better as there was an improvement in growth with lesser duration of

priming (Tables 1 to 7). Naik (2015) also standardized the biopriming duration in garden pea. The seeds were soaked in *T. viride* (40 %) and *P. fluorescens* (40 %) and tap water (hydropriming) for 2, 4, 6, 8, 10 and 12 h separately. The maximum effect was noticed when all the treatments were primed for 4 h. Besides, soaking for more than 6 h reduced the quality of seedlings even less than that of the control. The lesser duration of priming (one hour) favoured for *T. asperellum* may be due to the fact that *T. asperellum* being an aerobic fungus, could not survive in the biopriming suspension for longer duration and hence the effect decreased, while other biocontrol agents tested *viz.*, *Bacillus* spp. could survive anaerobic conditions. *Bacillus* spp. produces endospores which can survive for longer periods in adverse conditions (Liu and Sinclair, 1993).

Disease incidence occurred in all treatments including the uninoculated control. This was due to natural incidence of the disease besides that caused by the artificial inoculation. The pots were kept in an open condition in a coconut garden in between the plants. This also favoured the disease occurrence. Disease severity at 15th day of inoculation was the lower for plants bioprimed and sprayed with *B. amyloliquefaciens* which was on par with the chemical check, carbendazim (0.1%). This was followed by bioprimng and spraying with

Table 1. Effect of seed biopriming on germination percentage of chilli seed (var. Vellayani Athulya)

Treatments (A)*	Seed germination (%)						Mean A
	Duration of soaking (B)						
	20 min	1 h	2 h	4 h	8 h	16 h	
T. asperellum	72.22	82.22	73.71	65.89	61.11	71.11	71.04
B. subtilis	75.93	81.11	77.40	91.11	82.22	85.78	82.26
B. amyloliquefaciens	72.96	78.14	75.56	92.59	85.55	83.33	81.35
Carbendazim (0.1 %)	80.73	86.30	77.03	94.52	91.26	81.63	85.24
Hydropriming	72.97	77.41	79.25	87.41	82.97	80.51	80.09
Control (Without priming)	72.96	72.96	72.96	72.96	72.96	72.96	72.96
Mean B	74.63	79.69	75.99	84.08	79.35	79.22	
Treatment effects	SEm (±)	CD(0.05)					
A	0.37	1.03					
B	0.30	0.84					
AxB	0.90	2.53					

**T. asperellum* was used @ 10^8 spores mL^{-1}. Bacterial cultures were used @ 10^8 cfu mL^{-1}. Values are mean of three replications

Table 2. Effect of seed biopriming on shoot length of chilli seedlings (var. Vellayani Athulya) in the roll towel assay

Treatments (A)*	Shoot length of seedlings (cm)						Mean A
	Duration of soaking (B)						
	20 min	1 h	2 h	4 h	8 h	16 h	
T. asperellum	3.46±0.01	4.33±0.08	3.92±0.06	3.86±0.02	3.87±0.03	3.97±0.09	3.90
B. subtilis	3.27±0.01	3.53±0.05	3.24±0.05	3.61±0.04	3.40±0.03	3.20±0.03	3.38
B. amyloliquefaciens	3.23±0.02	3.53±0.09	3.37±0.09	3.59±0.09	3.67±0.07	3.36±0.02	3.46
Carbendazim (0.1%)	3.52±0.02	3.67±0.06	3.22±0.06	3.88±0.05	3.82±0.01	3.79±0.03	3.65
Hydropriming	3.71±0.01	3.80±0.03	3.68±0.03	3.95±0.07	3.19±0.01	3.51±0.04	3.64
Control (Without priming)	3.28±0.04	3.28±0.04	3.28±0.04	3.28±0.04	3.28±0.04	3.28±0.04	3.28
Mean B	3.41	3.69	3.45	3.70	3.54	3.52	
Treatment effects	SEm (±)	CD (0.05)					
A	0.02	0.05					
B	0.02	0.04					
AxB	0.05	0.13					

**T. asperellum* was used @ 10^8 spores mL^{-1}. Bacterial cultures were used @ 10^8 cfu mL^{-1}. Observations taken 14 days after sowing. Values are mean ± standard deviation of three replications.

Table 3. Effect of seed biopriming on root length of chilli seedlings (var. Vellayani Athulya) in the roll towel assay

Treatments (A)*	Root length of seedlings (cm)						Mean A
	Duration of soaking (B)						
	20 min	1 h	2 h	4 h	8 h	16 h	
T. asperellum	8.24±0.05	8.49±0.02	7.74±0.03	7.96±0.02	7.98±0.04	8.23±0.07	8.11
B. subtilis	7.88±0.01	8.12±0.01	7.53±0.04	8.79±0.04	8.26±0.02	8.41±0.02	8.17
B. amyloliquefaciens	6.79±0.06	8.15±0.03	7.45±0.05	8.51±0.06	8.18±0.04	8.36±0.05	7.91
Carbendazim (0.1%)	8.82±0.05	8.92±0.06	9.10±0.10	9.17±0.02	9.02±0.03	8.96±0.05	9.00
Hydropriming	7.74±0.02	8.23±0.04	8.41±0.02	8.79±0.05	8.39±0.03	8.40±0.03	8.32
Control (Without priming)	7.21±0.02	7.21±0.02	7.21±0.02	7.21±0.02	7.21±0.02	7.21±0.02	7.21
Mean B	7.78	8.19	7.91	8.41	8.17	8.26	
Treatment effects	SEm (±)	CD (0.05)					
A	0.02	0.04					
B	0.01	0.04					
AxB	0.04	0.11					

**T. asperellum* was used @ 10^8 spores mL^{-1}. Bacterial cultures were used @ 10^8 cfu mL^{-1}. Observations taken 14 days after sowing. Values are mean ± standard deviation of three replications.

Table 4. Effect of seed biopriming on seedling length of chilli (var. Vellayani Athulya) in the roll towel assay

Treatments (A)*	Length of seedlings (cm)						Mean A
	Duration of soaking (B)						
	20 min	1 h	2 h	4 h	8 h	16 h	
T. asperellum	11.69±0.05	12.83±0.06	11.66±0.04	11.82±0.03	11.86±0.04	12.20±0.10	12.01
B. subtilis	11.15±0.01	11.66±0.07	10.78±0.01	12.41±0.02	11.66±0.04	11.61±0.06	11.55
B. amyloliquefaciens	10.02±0.06	11.69±0.04	10.82±0.06	12.11±0.06	11.85±0.09	11.76±0.07	11.37
Carbendazim (0.1%)	12.34±0.05	12.59±0.04	12.31±0.13	13.05±0.03	12.85±0.03	12.76±0.06	12.65
Hydropriming	11.46±0.02	12.05±0.04	12.09±0.05	12.74±0.11	11.58±0.02	11.91±0.01	11.97
Control (Without priming)	10.49±0.02	10.49±0.02	10.49±0.02	10.49±0.02	10.49±0.02	10.49±0.02	10.49
Mean B	11.19	11.89	11.36	12.10	11.72	11.79	
Treatment effects	SEm (±)	CD (0.05)					
A	0.02	0.07					
B	0.02	0.05					
AxB	0.06	0.16					

**T. asperellum* was used @ 10^8spores mL^{-1}. Bacterial cultures were used @ 10^8 cfu mL^{-1}. Observations taken 14 days after sowing. Values are mean ± standard deviation of three replications.

Table 5. Effect of seed biopriming on dry weight of chilli seedlings (var. Vellayani Athulya) in the roll towel assay

Treatments (A)*	Seedling dry weight (mg)						Mean A
	Duration of soaking (B)						
	20 min	1 h	2 h	4 h	8 h	16 h	
T. asperellum	2.38±0.04	2.46±0.05	2.53±0.03	2.80±0.03	2.52±0.04	2.73±0.05	2.57
B. subtilis	2.11±0.06	2.01±0.05	2.14±0.03	2.07±0.04	2.14±0.01	2.13±0.04	2.10
B. amyloliquefaciens	2.14±0.01	2.10±0.03	2.14±0.05	2.27±0.02	2.12±0.01	2.23±0.03	2.17
Carbendazim (0.1%)	2.02±0.03	2.31±0.02	2.63±0.04	2.88±0.05	2.67±0.02	2.44±0.04	2.49
Hydropriming	2.23±0.02	2.44±0.05	2.23±0.04	2.80±0.11	2.37±0.04	2.40±0.01	2.41
Control (Without priming)	1.91±0.01	1.91±0.01	1.91±0.01	1.91±0.01	1.91±0.01	1.91±0.01	1.91
Mean B	2.13	2.21	2.26	2.46	2.29	2.31	
Treatment effects	SEm (±)	CD (0.05)					
A	0.02	0.04					
B	0.01	0.04					
AxB	0.04	0.11					

**T. asperellum* was used @ 10^8 spores mL^{-1}. Bacterial cultures were used @ 10^8 cfu mL^{-1}. Observations taken 14 days after sowing. Values are mean ± standard deviation of three replications.

Table 6. Effect of seed biopriming on vigour index (SV - I) of chilli seedlings (var. Vellayani Athulya) in the roll towel assay

Treatments (A)*	Seedling vigour index 1 (SVI - I)						Mean A
	Duration of soaking (B)						
	20 min	1 h	2 h	4 h	8 h	16 h	
T. asperellum	844.54±3.25	1054.49±4.88	859.23±3.29	778.574±1.99	724.65±2.77	867.87±7.07	854.89
B. subtilis	846.35±0.98	945.44±5.48	834.15±1.15	1130.95±2.05	958.99±3.49	996.08±4.75	951.99
B. amyloliquefaciens	730.80±4.41	913.19±2.96	817.55±4.39	1120.90±5.17	1014.11±7.74	979.89±5.45	929.41
Carbendazim (0.1%)	996.42±3.88	1086.78±3.05	948.34±10.49	1233.50±2.86	1172.38±2.44	1041.75±5.03	1079.86
Hydropriming	835.99±1.25	932.59±3.16	958.44±2.71	1113.39±9.84	960.79±1.75	959.09±1.11	960.05
Control (Without priming)	765.36±3.41	765.36±3.41	765.36±3.41	765.36±3.41	765.36±3.41	765.36±3.41	765.36
Mean B	836.58	949.64	863.85	1023.78	932.71	935.01	
Treatment effects	SEm (±)	CD (0.05)					
A	4.87	13.66					
B	3.97	11.15					
AxB	11.92	33.45					

**T. Asperellum* was used @ 10^8 spores mL^{-1}. Bacterial cultures were used @ 10^8 cfu mL^{-1}. Observations taken 14 days after sowing. Values are mean ± standard deviation of three replications.

Table 7. Effect of seed biopriming on vigour index (SVI - II) of chilli seedlings (var. Vellayani Athulya) in the roll towel assay

Treatments (A)*	Seedling vigour index 2 (SVI - II)						Mean A
	Duration of soaking (B)						
	20 min	1 h	2 h	4 h	8 h	16 h	
T. asperellum	0.17±0.002	0.20±0.004	0.18±0.002	0.18±0.002	0.15±0.003	0.19±0.003	0.18
B. subtilis	0.16±0.004	0.16±0.004	0.17±0.002	0.19±0.003	0.18±0.004	0.18±0.003	0.17
B. amyloliquefaciens	0.16±0.001	0.16±0.002	0.16±0.004	0.21±0.001	0.18±0.001	0.19±0.002	0.18
Carbendazim (0.1%)	0.16±0.003	0.20±0.001	0.20±0.003	0.27±0.005	0.24±0.002	0.20±0.004	0.21
Hydropriming	0.16±0.001	0.19±0.004	0.18±0.003	0.24±0.009	0.20±0.003	0.19±0.001	0.19
Control (Without priming)	0.14±0.001	0.14±0.001	0.14±0.001	0.14±0.001	0.14±0.001	0.14±0.001	0.14
Mean B	0.16	0.18	0.17	0.21	0.18	0.18	
Treatment effects	SEm (±)	CD (0.05)					
A	0.001	0.005					
B	0.001	0.004					
AxB	0.004	0.011					

**T. asperellum* was used @ 10^8 spores mL^{-1}. Bacterial cultures were used @ 10^8 cfu mL^{-1}. Observations taken 14 days after sowing. Values are mean ± standard deviation of three replications.

B. subtilis and *T. asperellum* respectively. It was also observed that though biopriming alone could bring a reduction in the disease, it was not as effective as biopriming combined with spraying treatments (Table 8).

A similar observation was also noted with respect to the appearance of disease symptoms on the leaves. Biopriming and spraying with *B. amyloliquefaciens* reduced anthracnose infection on leaves (55%) which was on par with biopriming and spraying with *B. subtilis* as observed on 15th day after inoculation. These were on par with that of carbendazim 0.1%. Here also it was observed that, though biopriming alone could bring a reduction of the disease, it was not as effective as biopriming combined with spraying treatments (Table 9).

Several workers have reported the disease suppressing effects of seed biopriming. Meena et al. (2012) studied the effect of seed biopriming with *T. harzianum* (2 x10^9cfu/ml for 24 h) in sorghum against anthracnose caused by *C. graminicola* under field conditions and observed a reduction in disease severity by 28.1 per cent. Chauhan and Patel (2017) observed that PDI of anthracnose or fruit rot caused by *C. capsici* in chilli 30 DAS was reduced by seed

Table 8. Effect of seed biopriming and spraying at fruit set on the severity of anthracnose on fruits of chilli var. Vellayani Athulya

Treatments*	Percentage Disease Index (PDI) / Disease severity			Percentage reduction over inoculated control at 15th day
	5th day	10th day	15th day	
T. asperellum (biopriming alone)	22.19 (28.10)^{d}	28.13 (32.03)$_{c}$	40.00 (39.23)^{c}	24.26
T. asperellum (biopriming + spraying)	15.94 (23.52)^{f}	22.19 (28.10)$_{e}$	32.50 (34.75)^{e}	38.46
B. subtilis (biopriming alone)	19.06 (25.88)^{e}	25.00 (30.00)^{d}	37.19 (37.57)^{d}	29.58
B. subtilis (biopriming + spraying)	15.63 (23.28)^{f}	21.25 (27.45)^{e}	31.25 (33.99)^{e}	40.83
B. amyloliquefaciens (biopriming alone)	24.69 (29.79)^{c}	29.69 (33.01)^{c}	40.00 (39.23)^{c}	24.26
B. amyloliquefacien s(biopriming + spraying)	13.13 (21.24)^{g}	19.06 (25.88)^{f}	28.12 (32.03)^{f}	46.75
Carbendazim (0.1%) (priming alone)	29.69 (33.01)^{b}	37.50 (37.76)^{b}	46.56 (43.03)^{b}	11.83
Carbendazim (0.1%) (priming + spraying)	10.00 (18.42)^{h}	17.81 (24.96)^{f}	29.38 (32.82)^{f}	44.37
Inoculated control	34.06 (35.70)^{a}	41.56 (40.14)^{a}	52.81 (46.61)^{a}	
Uninoculated control	0.00 (0.29)^{i}	0.00 (0.29)^{g}	4.63 (12.39)^{g}	
SEm ±	0.41	0.35		
CD (0.05)	1.20	1.01		

**T. asperellum* was used @ 10^{8}s pores mL^{-1}. Bacterial cultures were used @ 10^{8} cfu mL^{-1}. **Assessment made at 15 days after inoculation. All plants in the uninoculated control also showed disease symptoms as a result of natural disease incidence. Values are mean ± standard deviation of four replications. Values followed by same letter in a column are not significantly different at 5% level of significance. Values in parenthesis are arcsine transformed values.

Table 9. Effect of seed biopriming and spraying at fruit set on the severity of anthracnose on leaves in chilli var. Vellayani Athulya

Treatment	Percentage Disease Index (PDI) / Disease severity			Percentage reduction over inoculated control at 15th day
	5th day	10th day	15th day	
T. asperellum (biopriming alone)	3.50 (10.77)^{e}	6.90 (15.23)^{d}	11.30 (19.64)^{cd}	37.22
T. asperellum (biopriming + spraying)	1.90 (7.89)^{e}	4.60 (12.38)^{e}	8.50 (19.95)^{e}	52.78
B. subtilis (biopriming alone)	2.70 (9.44)^{d}	7.00 (15.34)^{d}	10.60 (18.99)^{d}	41.11
B. subtilis (biopriming + spraying)	1.20 (6.24)^{f}	3.70 (11.08)^{f}	8.00 (16.43)^{e}	55.56
B. amyloliquefaciens (biopriming alone)	3.80 (11.24)^{c}	7.90 (16.32)^{c}	11.90 (20.18)^{c}	33.89
B. amyloliquefaciens (biopriming + spraying)	1.00 (5.71)^{f}	3.60 (10.93)^{f}	8.10 (16.53)^{e}	55.00
Carbendazim (0.1%) (priming alone)	5.60 (13.68)^{b}	10.10 (18.53)^{b}	13.70 (21.72)^{b}	23.89
Carbendazim (0.1%) (priming + spraying)	0.60 (4.38)^{g}	2.50 (9.08)^{g}	5.80 (13.93)^{f}	67.78
Inoculated control	7.80 (16.22)^{a}	13.20 (21.30)^{a}	18.00 (25.10)^{a}	
Uninoculated control	0.60 (4.38)^{g}	2.00 (8.11)^{h}	3.20 (10.30)^{g}	
SEm ±	0.34	0.24	0.22	
CD (0.05)	0.97	0.70	0.65	

**T. asperellum* was used @ 10^{8} spores mL^{-1}. Bacterial cultures were used @ 10 ^{8}cfu mL^{-1}. **Assessment made at 15 days after inoculation. All plants in the uninoculated control also showed disease symptoms as a result of natural disease incidence. Values are mean ± standard deviation of four replications. Values followed by same letter in a column are not significantly different at 5% level of significance. Values in parenthesis are arcsine transformed values.

biopriming with *T. viride* and *B. subtilis* compared to hydropriming and control. Seed biopriming with *B. amyloliquefaciens* in chilli resulted in significant protection of 71 percent against anthracnose caused by *C. truncatum* (Gowtham et al., 2018). Biopriming chilli seeds with *Bacillus* sp. strain BSp.3/AM for 6 h reduced the incidence of anthracnose by 20 per cent under greenhouse conditions (Jayapala et al., 2019).

Many workers have reported the reduction in disease incidence and severity by biopriming together with spraying treatments. Anand and Bhaskaran (2009) observed that spraying of *T. viride* against fruit rot of chilli caused by *C. capsici* reduced the disease incidence by 47.5 per cent and severity by 44.22 per cent over inoculated control. Two sprays with biocontrol agents were found to be more effective than one spray. Seed biopriming @ 2×10^{9}cfu / ml for 24 h followed by two spraying treatments with

T. harzianum in sorghum reduced anthracnose caused by *C. graminicola* by 43.9 per cent under field conditions (Meena et al., 2012). Amaresan et al. (2014) studied the effect of seed treatment and foliar application of talc-based formulations of *B. subtilis* strain BECL11 and *B. amyloliquefaciens* strain BECR2 on the yield and incidence of anthracnose in chilli and compared it with seed treatment of thiram and foliar spray of fytolan (Copper oxy chloride) (2.5g/L) as the chemical check. Considerable reduction (66.67 %) in anthracnose of fruits treated with strain BECL11 compared to control was observed which was lower than the chemical check. The strain BECR2 reduced anthracnose on fruits by 44.46 per cent. The yield was also increased by the treatments BECL11 and BECR2 compared to control. Christopher et al. (2014) studied the effect of seed treatment @ 10g / kg followed by prophylactic spraying @ 0.2 per cent of *B. subtilis* at 25 and 75 days after transplanting on chilli under greenhouse conditions. Incidence of fruit rot was decreased by 28.97 per cent. *B. subtilis* treatment was found better than the chemical check mancozeb. The bacterial biocontrol agents used were endospore forming isolates. Their biocontrol potential has been reported earlier (Nair et al., 2007; Nair and Anith, 2009; Athira and Anith, 2020). The endospore forming ability of these bioagents may help them survive under conditions of limited nutrition, that usually occurs in the phylloplane and fruit surface.

Spray application produced additional benefits over biopriming because it allowed the antagonists to pre-colonise on the surface before the arrival of the pathogen (Ippolito and Nigro, 2000). Biofilm formation is important for the bacteria to act as biocontrol agents (Bais et al., 2004). *Bacillus* spp. were reported to form biofilms on the surfaces of fruits (Hernandez et al., 2019). Biofilms are multicellular matrixes of bacteria surrounded by extracellular polysaccharides called as glycocalyx. This glycocalyx acts as a physical barrier and is strongly anionic, thereby protecting the microcolony from external agents (Arrebola et al., 2010). Biopriming of seed and spray application of *B. amyloliquefaciens* during fruit set was effective for managing the fruit rot caused by *C. capsici* in chilli (Yamamoto et al., 2014). The authors also observed that spray application of *B. amyloliquefaciens* strain S13-3 ($1.5x10^8$ cfu mL^{-1}) reduced the severity of anthracnose of strawberry caused by *C. gloeosporioides* and reported that the lipopeptide antibiotics produced by the strain identified as iturin A, fengycin, mixirin, pumilacidin and surfactin were responsible for the induction of defence related components like pathogenesis-related proteins, chitinase and β-1, 3-glucanase in strawberry leaves. In the present study it was observed that biopriming followed by spraying with *B. amyloliquefaciens* VLY 24 induced effective protection to chilli from fruit rot and anthracnose disease besides improving plant growth and yield. The disease suppression effect of biopriming has been attributed to the induction of systemic resistance in plants by the biopriming agents which colonize the roots of plants (Bharathi et al., 2004; Anand and Bhaskaran, 2009).

Biopriming of chilli seed increased the height and number of branches of chilli plants which in turn increased the yield. It was observed that there was no significant difference between bioprming alone and biopriming together with spraying. The maximum number of primary, secondary and tertiary branches were observed in case of plants treated with *T. asperellum* and this was higher than those treated with carbendazim (Table 10).

Number of fruits per plant was maximum for plants primed with *T. asperellum* followed by *B. subtilis* and *B. amyloliquefaciens*. It was observed that the fruit yield of bioprimed and sprayed plants were slightly higher than that of the corresponding bioprimed plants. This could be due to the fact that more fruits were protected from anthracnose in bioprimed and sprayed plants than plants bioprmed alone. Fruit yield per plant was maximum for plants treated with *T. asperellum* followed by *B. subtilis* and *B. amyloliquefaciens*. The inoculated control

Table 10. Effect of seed biopriming and spraying on height and number of branches of chilli var. Vellayani Athulya

Treatment	Plant height (cm)			Number of branches per plant (120 DAS*)		
	40 DAS	80 DAS	120 DAS	Primary	Secondary	Tertiary
T.asperellum (biopriming alone)	31.38±0.90^{c}	72.38±0.55^{d}	100.13±0.97^{b}	6.25±0.25^{ab}	13.50±0.65^{ab}	28.50±0.96^{a}
T.asperellum (biopriming + spraying)	30.25±0.48^{cd}	71.63±0.75^{d}	100.13±1.03^{b}	6.50±0.29^{a}	14.00±0.41^{a}	28.00±1.08^{a}
B. subtilis (biopriming alone)	30.50±0.65^{cd}	61.75±0.48^{e}	96.88±0.43^{c}	5.25±0.25^{cdef}	12.00±0.41^{bc}	24.50±0.65^{b}
B. subtilis (biopriming + spraying)	31.75±0.48^{c}	61.50±0.54^{e}	96.75±0.32^{c}	5.50±0.29^{bcde}	12.25±0.48^{bc}	25.00±0.41^{b}
B. amyloliquefaciens (biopriming alone)	38.38±0.55^{a}	77.50±0.46^{a}	105.63±0.24^{a}	5.00±0.00^{def}	11.00±0.41^{cd}	22.25±0.48^{c}
B. amyloliquefaciens (biopriming + spraying)	37.88±0.43^{a}	75.88±0.43^{ab}	105.38±0.32^{a}	4.50±0.29^{f}	11.00±0.41^{cd}	21.50±0.65^{cd}
Carbendazim (0.1%) (priming alone)	35.38±0.24^{b}	74.75±0.32^{bc}	105.88±0.32^{a}	6.00±0.41^{abc}	11.75±0.48^{c}	25.25±0.48^{b}
Carbendazim (0.1%) (priming + spraying)	34.63±0.38^{b}	74.13±0.43^{c}	105.50±0.35^{a}	5.75±0.48^{abcd}	11.50±0.65^{c}	25.25±0.48^{b}
Inoculated control	29.13±0.83d^{e}	59.03±0.80^{f}	94.38±0.55^{d}	4.50±0.29^{f}	9.75±0.48^{c}	19.75±0.75^{d}
Uninoculated control	28.50±0.65^{e}	60.13±0.83^{ef}	94.63±0.24^{d}	4.75±0.25^{ef}	9.75±0.75^{d}	19.75±1.11^{d}
SEm±	0.59	0.58	0.55	0.30	0.52	0.75
CD(0.05)	1.71	1.69	1.60	0.88	1.52	2.17

**T. asperellum* was used @ 10^{8} spores mL^{-1}. Bacterial cultures were used @ 10^{8} cfu mL^{-1}. Values are mean ± standard deviation of four replications. Values followed by same letter in a column are not significantly different at 5% level of significance.

revealed a fruit yield of 412.45 g whereas uninoculated control revealed a yield of 432.32 g. It was observed that biopriming significantly improved the seed weight. The 100 seed weight was highest for treatment with *T. asperellum* followed by *B. subtilis* (Table 11).

Similar observations on growth enhancing and yield improving potential of seed biopriming were reported by many workers. *B. subtlils* isolate AB17 increased the plant height of chilli by 39 per cent over control (Lamsal et al., 2012). Seed biopriming with *B. amyloliquefaciens* in chilli resulted in maximum plant height (18.32 cm) 30 DAS (Gowtham et al., 2018).

Biocontrol agents can enhance plant growth by several mechanisms. Endophytic bacteria such as *Bacillus* species colonize the root surface and increase plant growth (Turner and Backman, 1991, Yashaswini et al., 2021). *Bacillus* spp. can increase the plant growth by the production of plant growth regulators like auxins (Indole Acetic Acid (IAA)) or cytokinins or by the degradation of the ethylene precursor, ACC by ACC deaminase, phosphorus solubilization and siderophore production. IAA improves root growth and development and thereby enhancing nutrient uptake (Amaresan et al., 2012). As stated by Ananthi et al. (2017), an enhancement in growth parameters with biopriming may be

Table 11. Effect of seed biopriming and spraying on number of fruits, fruit yield and 100 seed weight of chilli var. Vellayani Athulya

Treatment	Number of fruits per plant	Fruit yield per plant (g)	100 seed weight (g)
T. asperellum (biopriming alone)	72.75±0.85^{a}	658.37±3.07^{b}	0.74±0.01^{ab}
T. asperellum (biopriming + spraying)	72.50±0.65^{a}	681.01±2.00^{a}	0.75±0.01^{a}
B. subtilis (biopriming alone)	67.00±1.08^{b}	629.25±1.61^{d}	0.73±0.01^{abc}
B. subtilis (biopriming + spraying)	66.25±0.63^{b}	653.16±1.58^{c}	0.72±0.01^{abc}
B. amyloliquefaciens (biopriming alone)	66.75±0.25^{b}	627.77±1.18^{d}	0.71±0.01^{c}
B. amyloliquefaciens (biopriming + spraying)	65.75±0.25^{b}	651.11±0.87^{c}	0.71±0.01^{bc}
Carbendazim (0.1%) (priming alone)	57.50±1.26^{c}	529.14±1.11^{f}	0.70±0.01^{c}
Carbendazim (0.1%) (priming + spraying)	58.00±0.91^{c}	558.15±1.11^{e}	0.70±0.01^{c}
Inoculated control	48.25±1.11^{d}	412.45±2.07^{h}	0.62±0.02^{d}
Uninoculated control	47.75±1.55^{d}	432.32±1.68^{g}	0.62±0.02^{d}
SEm±	1.04	1.74	0.01
CD(0.05)	3.03	5.05	0.03

**T. asperellum* was used @ 10^{8} spores mL^{-1}. Bacterial cultures were used @ 10^{8} cfu mL^{-1}. Values are mean ± standard deviation of four replications. Values followed by same letter in a column are not significantly different at 5% level of significance.

attributed to suppression of deleterious microorganisms and pathogens, production of plant growth regulators like gibberellins, cytokinins and indole acetic acid, increased availability of minerals and ions as well as extensive rooting which helps in water and nutrient uptake.

Priming with carbendazim increased various growth parameters in plants as it stimulates plant growth (Debergh et al., 1993). Carbendazim can act as a growth regulator in tissue culture, exhibited cytokinin like activity in soybean bio-test and stimulated shoot development in micro propagated asparagus. The disease suppressive effect of carbendazim was lower than biopriming. This may be due to the fact that chemicals used for seed treatment mostly acted as contact / systemic fungicides and could not protect the plants from foliar pathogens during later stages of plant growth. The present study revealed that seed biopriming and spraying with a cell suspension of endospore forming bacterial strain *B. amyloliquefaciens* VLY 24 at a concentration of 10^8cfu mL^{-1} at fruit set was found to be most effective for the management of fruit rot / anthracnose of chilli. It also improved the growth and yield of the crop. Thus, it can be concluded that seed biopriming along with spraying of biocontrol agents at fruit set could be used as an eco-friendly measure to produce safe-to-eat crop.

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