Communication Issue of Journal of Science-FAS-SEUSL (2021) (31-34)

The effect of a commercial biofilm fertilizer on seed germination and seedling vigour of *Solanum lycopersicum* and *Capsicum annuum*.

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Abstract:
Seed vigour is an important determinant on successful plant establishment in the field which determines the crop performance and the final yield. This study investigated the effect of biopriming with a commercial biofilm biofertilizer (Biofilm-R) on seed germination and seedling vigour of two vegetable varieties, Solanum lycopersicum (tomato) and Capsicum annuum (bell pepper). The seed germination parameters were determined using the Ragdoll method and growth performance was tested in soil in a pot trial six weeks after sowing. The means of the control and the bioprimed treatment of each parameter were compared using the T Test ($p \leq 0.05$). Biopriming improved germination parameters of both varieties ranging from 6 - 78% in bell pepper and 133 - 171% in tomato. Seedling parameters too were enhanced by biopriming ranging from 53 - 402% in bell pepper and 35 – 285% in tomato. The results indicate that the Biofilm-R is a potential biopriming agent and it has the potential to continue to support plant growth of both varieties. However, further investigations under field conditions are needed.

Keywords: Biofertilizer, Biofilm, Biopriming

Introduction

The vegetable subsector of Sri Lanka plays an important role in its economy including earning foreign exchange and providing employment for the rural poor in addition to contributing to the health of the nation. The industry faces many challenges from farm to plate and technological innovations at various levels would increase the sector’s contribution to the economy and environment greatly.

Crop production, human nutrition, and food security are key issues in the context of increasing population and climate change. Crop performance and yield depend on successful plant establishment in the field which is determined by seed performance or seed vigour which includes rate and uniformity of seed germination and seedling growth, emergence ability of seeds under unfavourable environmental conditions etc. [1].

Seed priming, a physiological process and a pre-sowing treatment, is becoming popular as an efficient and low-cost technique among farmers and vegetable nursery industry. Seed priming involves different techniques such as hydropriming, halopriming, osmopriming, thermopriming, cold priming, solid matrix priming, and biopriming. The success of the method depends on plant species, priming agent, dosage etc. [2]. Bio-priming, the treatment of seed with beneficial organisms, being an easy and cost effective method, is becoming a potentially prominent technique and offers an attractive option for resource-poor farmers. In addition to enhancing seed quality, it

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also boosts seedling vigour and thus offer an innovative crop protection tool for the sustainable improvement of crop yield [3].

A biofilm is made up of microbial cells and extracellular polymeric substances secreted by them to have structural and biochemical protection. Biofilm biofertilizer (BFBF), an effective and novel product in agriculture, has the ability to reduce chemical fertilizer consumption by 50% while maintaining the existing yields of a variety of agricultural and plantation crops [4]. These BFBFs are generally applied at early or later stages of the crop and not at sowing.

The objective of this study was to investigate if priming with a BFBF affects seed and seedling vigour of two vegetable varieties tomato (*Solanum lycopersicum)* and bell pepper (*Capsicum annuum).*

Methodology

The surface sterilized seeds using 0.25% NaOCl for 30 minutes followed by three washings of both vegetable varieties were soaked overnight separately, half in diluted Biofilm R (1:300, as per the product recommendation) and the other half in water (control). The seed germination potential was tested by the Ragdoll method using pieces of autoclaved (121 ºC for 15 min) Cotton Greige Fabric, having 4 replicates with 30 seeds per replicate. The number of germinating seeds were counted daily from the first day until most of the seeds were germinated and the germination parameters final germination percentage (FGP), daily germination percentage (DGP), mean germination time (MGT), mean germination rate (MGR) and germination index (GI) were determined. Seedling growth performance of both varieties was tested in a pot trial with four replicates per treatment on air dried, crushed and sieved soil (pH-6.7 and salinity 127 ppm) and growth parameters were measured after six weeks of growth. The means of the control and the BFBF treatment of each parameter of each variety were compared using the t-test ($p \leq 0.05$).

Results and Discussion

In the Ragdoll method, all five seed germination parameters of both varieties showed a significant positive effect compared to the control except MGR and MGT for tomato (Table 1). The significant positive effects of the different parameters were ranging from 6% to 78% in bell pepper and 133% to 171% in tomato indicating Biofilm-R as a potential source for biopriming. As the Ragdoll method, has an aseptic condition at least initially and the only factor that determines the priming potential of the microbes in BFBF is their ability to respond to the seed factors which is species specific. This could be a reason for varietal specificity [3] in microbial effects on plants.

Table 1. Seed germination parameters of both varieties

Seed germination parameters	Treatment	Bell pepper	Difference (%)	Tomato	Difference (%)
Final Germination Percentage	Control	49.17^{b}		24.99^{b}	
	BFBF	66.67^{a}	35.59	58.33^{a}	133.32

Daily Germination Percentage	Control	4.90[b]		2.56[b]	
	BFBF	7.98[a]	62.85	6.94[a]	171.09
Mean Germination Time (day)	Control	9.75[a]		9.67[a]	
	BFBF	9.16[b]	-6.05	9.17[a]	-3.10
Mean Germination Rate (day-)	Control	0.10[b]		0.10[a]	
	BFBF	0.11[a]	9.00	0.11[a]	9.00
Germination Index (seed/day)	Control	6.57[b]		3.60[b]	
	BFBF	11.71[a]	78.51	9.37[a]	160.28

Note: the values of control and treatment of a particular parameter of a variety, if indicated by different letters are significantly different ($p < 0.05$).

In soil too, all seed germination parameters showed similar effects on both varieties (data not shown). Seedling parameters of both varieties also showed a significantly positive response compared to the controls (Table 2). However, the effects did not differ between the two varieties as in the Ragdoll method. The reason for this could be there is no conducive environment for the microbes of the biofilm in the soil as in the Ragdoll method as the microbes of BFBF have to compete with a large variety of native soil microorganisms. The final effect on the seed or seedling is the outcome of the interactions of the microbes in the BF with the native microbes of soil, the soil abiotic factors and the seed factors and the ability of the BF to influence seed vigour in the natural soil indicates that this could be a potential biopriming agent for the tested varieties.

Table 2. Growth parameters of six week old seedlings of both varieties

Seedling growth parameters	**Treatment**	**Bell pepper**	**% difference**	**Tomato**	**% difference**
Shoot Length(cm)	Control	12.58[b]		20.83[b]	
	BFBF	25.35[a]	101.46	39.69[a]	90.52
Shoot fresh weight (g)	Control	0.44[b]		0.72[b]	
	BFBF	1.61[a]	264.24	2.11[a]	191.42
Shoot dry weight (g)	Control	0.04[b]		0.04[b]	
	BFBF	0.11[a]	196.73	0.13[a]	202.86
Root length (cm)	Control	4.96[b]		7.79[b]	
	BFBF	10.14[a]	104.45	15.13[a]	94.33
Root fresh weight (g)	Control	0.03[b]		0.06[b]	
	BFBF	0.13[a]	267.61	0.23[a]	285.05
Root dry weight (g)	Control	0.01[b]		0.01[b]	
	BFBF	0.04[a]	412.17	0.03[a]	243.85
Total No of Leaves	Control	5.21[b]		7.38[b]	
	BFBF	8.00[a]	53.60	10.00[a]	35.59

Note: the values of control and treatment of a particular parameter of a variety, if indicated by different letters are significantly different ($p < 0.05$).

The possible mechanism of the BF in improving seed and seedling vigour could be the production of plant growth regulators by the BF microbes. The microbes in the BF are probably plant growth promoting microbes (PGPM). PGPMs can produce including gibberellins that induce the activity of certain enzymes that aid the early germination, stimulate the rapid stem and root growth [5], The extracellular polymers of the biofilm aid in the retention of soil moisture in the rhizosphere. Some other mechanisms include fixing atmospheric nitrogen, solubilization of phosphate and other nutrients, repression of soil borne pathogens, improved plant stress tolerance etc. [3]. The same mechanisms may be the reasons for the reported yield benefits with reduced chemical input by using BFBF. Thus, if the biofilm in the biopriming agent can successfully establish in the rhizosphere it may continue to support plant growth throughout, produce higher yields with reduced chemical inputs contributing to the economy and a safer environment.

Conclusion

This study reveals that the use of biofilms as biopriming agents has the advantage of improving both seed and seedling vigour. Improving seedling vigour in natural soil conditions implies that the biofilm could be a potential biofertilizer too for the tested plant varieties and thus contribute to the economy and a greener environment which the other seed priming agents cannot do. However, the effect of this biopriming agent needs to be further investigated under field conditions.

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