MICROBIOLOGY

# **Energy Inputs and Yield Relationship in Greenhouse Okra Production by Bio-priming**

Sumita Pal<sup>1,2\*</sup> and H.B. Singh<sup>1</sup>

<sup>1</sup>Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, 221005, India <sup>2</sup>Department of Bioscience, Integral University, Lucknow, India

\*Corresponding author: sumtapal99@gmaul.com (ORCID ID: 0000-0002-0275-651X)

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#### ABSTRACT

Production of chemical fertilizers is associated with consumption of huge energy. In the present study, an attempt was made to assess the energy consumption in bio-priming mediated okra production under greenhouse conditions. Results showed that among all the treatments, seed priming with *Trichoderma harzianum* (NBRI 1055) along with 90% RDF was not only helpful in reducing fertilizer dose by 10%, but at the same time produced almost similar yield as compared to with 100% RDF. Bio-priming in different combination with chemical fertilizers had a positive impact on the chemical composition of okra. Bio-primed treatments saved 970 to 1670 KJ energy in producing unit produce as compared with 100% RDF. This proved that bio-priming is cost-effective, user, and eco-friendly technique solving numerous problems in agriculture. This technique requires the prerequisite of incorporating organic matter in soil and seen as complementary to inorganic fertilizer source, not as their replacement.

#### Highlights

- Seed priming with *Trichoderma harzianum*(NBRI 1055) have the potential to meet crop nutrient requirements and improve seedling emergence and crop stand establishment, yield, and grain micronutrient enrichment.
- An extra intervention with the existing technology of INM practice could make a huge difference in the intensive input/energy consuming system altogether leading to sustainable high yields saving a lot of energy for future use.

Keywords: Seed bio priming, Okra, input energy requirement, Trichoderma harzianum, growth promotion

Fertilizer use has become the lifeline of agriculture, without its use one can never think about the production level which is required to feed the evergrowing global population. Governments of many countries like India, spend a huge amount of money to provide subsidy on fertilizers. Its other face is that non-judicious application of these chemicals come into common practices. Hence, this leads to problems related to land degradation through accelerated salinity, acidity, nutrient imbalance, etc. and also causing other environmental issues by polluting soil, air, and water. Nutrient status of soil drastically changes as some nutrients get locked in slowly available forms and some exhaust totally

with time; consequently, they remain unavailable for plant's uptake and there is a reduction in nutrient use efficiency. Fertilizer manufacturing industries fall under the most energy-intensive sectors. This is evident from the fact that production of N, P, and K fertilizers consume approximately 78.2 MJ kg<sup>-1</sup>, 17.5 MJ kg<sup>-1</sup>, and 13.8 MJ kg<sup>-1</sup> energy, respectively (Rakshit *et al.* 2015). Thus, loss of fertilizer is not only loss of nutrient but also the loss of money provided in form of subsidy and energy required to produce that amount of fertilizer. To deal with such problems caused by indiscriminate use of fertilizers, microbes are used to increase uptake of nutrient efficiently by solubilizing and mobilizing



nutrients which are present in unavailable forms, thereby reducing the high requirement of fertilizer doses. Moreover, the exploitation of beneficial microbes has become of paramount importance in the agriculture sector for their potential role in food safety and sustainable crop production having multifunctional impact on soil-plant system such as improved nutrient use efficiency, nutrient uptake, plant growth, and plant tolerance to abiotic and biotic stress (Adesemoye and Kloepper 2009; Sarkar et al. 2017). Seed bio-priming emerge as new technology for applying microbes which is simple and easy to use. Among the useful fungi, Trichoderma spp. are recognized for having multiple functions in growth promotion and plant protection purposes (Harman 2006; Harman 2011). In the present set-up, Trichoderma harzianum was used for priming okra seeds. Okra [Abelmoschus esculentus (L.) Moench] is one of the most important vegetable crops in the world, being popular in many tropical and subtropical countries. It is mostly cultivated for human consumption and also for industrial use as fiber particularly in the intensively cropped Indo-Gangetic Plains of Varanasi (Uttar Pradesh, India). The proposed study was undertaken to evaluate the potential of bio-priming in improving energy efficiency in the production process of okra, a commonly cultivated vegetable crop with high intensive resource requirement.

# MATERIALS AND METHODS

# **Experimental soil**

To conduct the pot experiment, bulk surface (0-15 cm) soil was collected from the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi. The soils of Varanasi are formed on alluvium deposited by the river Ganges and have a predominance of illite, quartz, and feldspars minerals. The alluvial soil is characterized by higher silt content. Illitic nature of alluvial sediments is clearly demonstrated by higher K<sub>2</sub>O content. The cropping history of the site from where the soil was collected is rice–wheat. The fertility status of the soil is classed as low to medium with a slightly alkaline reaction. After collecting the soil, it was sieved and 10 kg of soil was filled in each polythene lined earthen pot.

# **Experimental details**

The pot experiment was conducted during *kharif* season of 2016 in the greenhouse of Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi in completely randomized block design with six treatments, viz., T<sub>1</sub>: absolute control (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 0:0:0 kg ha<sup>-1</sup>); T<sub>2</sub>: recommended dose of fertilizer (RDF), i.e., N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 120:120:75 kg ha<sup>-1</sup>; T<sub>3</sub>: 70% RDF + priming with Trichoderma harzianum; T<sub>4</sub>: 75% RDF + priming with T. harzianum; T<sub>5</sub>: 80% RDF + priming with *T. harzianum*;  $T_6$ : 90% RDF + priming with *T*. harzianum. The widely cultivated cultivar Shitla Jyoti (DVR-2) was used as a test crop. Three seeds were sown in plastic pots containing 10 kg soil (sand: 67%; silt: 16%; clay: 17%; organic carbon: 4.31 g kg<sup>-1</sup>; pH 7.4). Ten days after emergence, 1 healthy seedling of each cultivar was maintained in each pot. Weeding was done as soon as weeds emerged from the pots. Irrigation was given as per the requirement of the crop which kept the soil moist throughout growth period of okra. The crop was maintained for 90 days, and relevant data on growth and yield were noted at 60 and 90 days after sowing (DAS).

#### Seed treatment with microbial formulation

Seeds of okra were first soaked in sodium hypochlorite solution for one minute, and then rinsed 4-5 times with sterilized water and kept or air dried in laminar air flow. *Trichoderma harzianum* (NBRI 1055) spores were collected in vials by adding 4-5 ml of 0.85% saline water in petri plate. In all vials, 10-15 ml of 1% carboxy methyl cellulose was added and mixed properly. Per treatment 50 dry seeds were taken in petri plates containing autoclaved filter papers. The suspensions were spread uniformly over the seeds followed by drying in laminar air flow and incubating in moist chamber.

# Plant analysis

Okra fruits were frozen in liquid nitrogen and crushed. From the powder obtained, 1 g was taken and treated with 5 mL ethanol 96% w/w for 60 min and then centrifuged ( $11,500 \times g$ , 30 min). On the extracts, total phenols was quantified employing the Folin–Ciocalteu reagent (Swain and Hillis 1959). Later sodium carbonate was added, and after 1 h,

absorbance was noted at 725 nm. Protein content of the fruits was determined by multiplying 6.25 (factor) with nitrogen (N) content (determined by Kjeldahl's method) in fruits. Crude fibre content was determined following the standard procedure of AOAC (2000). Ascorbic acid was estimated by the dye 2,6 dichlorophenol-indophenol (DCIP)-titration method (AOAC 2000). Diacid (HNO<sub>3</sub>:HClO<sub>4</sub> : 9:4) digestion was carried out to estimate total phosphorus (P), potassium (K), iron (Fe), zinc (Zn), copper (Cu), manganese (Mn).

# **Energy calculations**

Efficient energy use in crop production is the ratio of output energy ( $E_{output}$ ) to input energy ( $E_{input}$ ) (Singh *et al.* 2002; Shahan *et al.* 2008).

 $E_{input} requirement = E_f + E_p + E_d + E_m + ... + E_{hp} (KJ unit<sup>-1</sup>)$ 

Where,  $E_f$  is the energy of fertilizers (KJ),  $E_p$  is the energy of plant protection chemicals (KJ),  $E_d$  is the energy of diesel fuel (KJ),  $E_m$  is the energy of machinery (KJ),  $E_{hp}$  is the energy of human power (KJ). In the calculation, we have excluded the solar energy.

# **RESULTS AND DISCUSSION**

# Growth and yield parameters

The perusal of results (Table 1) indicated that okra plants fertilized with recommended dose  $(T_2)$  gave maximum response in growth parameters. Similar observations were recorded in field experiments of Mal et al. (2013) and Nair et al. (2017). The beneficial effect of seed bio-priming along with inorganic fertilizer (90% RDF) i.e., T<sub>6</sub> reflected in enhanced vegetative growth of plant and was at par with T<sub>2</sub>. This may be attributed to the synergistic effect of omnipresent Trichoderma species which plays significant role in nutrients re-cycling and nutrient availability to okra crop by improving the soil physical and chemical conditions and solubilizing the nutrients. Moreover, Trichoderma are also significant sources of growth regulators and major and micronutrients dynamics. Seed treatment with Trichoderma also has been shown to increase the root length probably as a consequence of an increased efficiency in the absorption of phosphorus and nitrogen from the soil (Meena *et al.* 2016). Comparatively less vegetative growth was recorded with  $T_1$ .

In case of fruits per plant, fruit weight and fruit yield per plant was found significantly higher in all treatments over control (Table 1). The significantly highest fruit per plant, fruit weight, fruit yield per plant were found in treatment  $T_2$  receiving full application of RDF which was statistically at par with treatment,  $T_6$  i.e., 90% RDF plus bio-priming over other treatments (Plate 1). Contribution of biofertilizers in enhancing growth and yield parameters of okra was reported by Mal *et al.* (2013).

In the present study, seed treatment with *T*. *harzianum* significantly contributed to the improvement in establishment of plant stand and yield of okra. The yield increased by *T. harzanium* application was probably related to increased plant stand establishment and crop growth. Entesari *et al.* (2013) reported that *T. harzanium* application induced profound changes in plant characteristics and encouraged more uniform seed germination and plant growth in soybean. Bio-priming with *T. harzanium* increased the rate of germination of seeds, stimulated root growth, enhanced biomass production, and augmented the plant performances (Meena *et al.* 2017).



**Plate 1:** Effect of bio-priming on maintaining fruit attributes (shape, size, freshness, etc.) of okra

# **Chemical composition**

Chemical composition data presented in Table 2 showed that the protein content in fruits of okra was observed in the range between 13.2 to 14.5%. Protein content showed slight changes, but treatment difference was statistically non-significant by using various sources of nutrients. The protein content in okra fruit was highest (14.5%) in the treatment receiving 90% RDF plus bio-priming ( $T_6$ ).

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Table 1: Effect of seed bio-priming on growth and yield parameters of okra at flowering and harvesting stages

Treatments	Plant height (cm)	Number of leaves plant <sup>-1</sup>	Number of fruits plant <sup>-1</sup>	Root length (cm) at 60 DAS	Number of nodes plant <sup>-1</sup>	Stem girth (cm)	Inter nodal length (cm)	Leaf area (cm² plant -¹) at 60 DAS	Fruit length (cm)	Fruit diameter (cm)	Single fruit weight (g)	Fruit weight (g) plant <sup>1</sup>
$T_1$	81.5	19	14.9	770	11.4	2.14	6.63	3002.9	10.1	1.01	8.9	186
T <sub>2</sub>	120	29	20	950	14.6	3.21	8.26	4043.5	14.5	1.20	16.3	285
T <sub>3</sub>	97.7	22.5	16	825	12.9	2.71	7.4	3420.2	12.8	1.14	15.2	195
$T_4$	104	24.1	17	905	13.0	2.76	7.6	3605.1	13.2	1.16	15.5	249
$T_5$	108	25.3	17.5	915	13.2	3.01	8.0	3800.6	13.9	1.17	15.8	260
$T_6$	116	28.1	19	970	13.8	3.10	8.10	3890.2	14.0	1.18	16.0	275
SEm±	1.79	0.56	0.67	24.08	0.45	0.10	0.20	72.8	0.6	0.10	1.00	7.24
CD (P=0.05)	5.49	1.59	2.04	71.57	1.31	0.27	0.58	216.13	1.73	0.27	3.15	22.81

 $T_1$ : Absolute control (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 0:0:0 kg ha<sup>-1</sup>);  $T_2$ : RDF (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 120:120:75 kg ha<sup>-1</sup>);  $T_3$ : 70% RDF + T. harzianum;  $T_4$ : 75% RDF + T. harzianum;  $T_5$ : 80% RDF + T. harzianum;  $T_5$ : 90% RDF + T. harzianum

Table 2: Effect of seed b	nio-priming on c	phemical compositi	on of okra fruits
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	Protein (%)	Crude fibre (%)	Total phenol (mg g <sup>-1</sup> )	Ascorbic acid (mg ml <sup>-1</sup> )	Macronutrient (%)			Micronutrient (mg kg <sup>-1</sup> )			
Treatment					Ν	Р	К	Fe	Zn	Cu	Mn
T <sub>1</sub>	13.2	7.29	43.1	0.28	0.61	0.091	0.270	3.78	0.114	0.095	0.07
Τ <sub>2</sub>	14.3	7.27	47.79	0.35	0.86	0.115	0.360	5.76	0.189	0.152	0.11
T <sub>3</sub>	13.5	7.01	48.1	0.30	0.78	0.104	0.331	5.68	0.181	0.129	0.09
$T_4$	14.0	7.09	48.5	0.34	0.80	0.108	0.347	5.83	0.230	0.142	0.11
T <sub>5</sub>	14.2	7.11	49.0	0.34	0.85	0.113	0.355	6.08	0.247	0.151	0.12
T <sub>6</sub>	14.5	7.18	49.1	0.35	0.91	0.127	0.375	6.39	0.263	0.197	0.13
SEm±	0.67	0.24	1.39	0.03	0.06	0.003	0.01	0.29	0.005	0.003	0.01
CD (P=0.05)	2.12	0.76	4.37	0.10	0.18	0.01	0.03	0.91	0.02	0.01	0.03

 $T_1$ : Absolute control (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 0:0:0 kg ha<sup>-1</sup>);  $T_2$ : RDF (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O @ 120:120:75 kg ha<sup>-1</sup>);  $T_3$ : 70% RDF + T. harzianum;  $T_4$ : 75% RDF + T. harzianum;  $T_5$ : 80% RDF + T. harzianum;  $T_5$ : 90% RDF + T. harzianum

Crude fibre content is one of the criteria to judge the quality of okra pod. The crude fibre content in fruit of okra showed slightly changes, but treatment difference was statistically non-significant and it was observed in the range between 7.01 to 7.29% (Table 2). Highest total phenol and ascorbic acid content was also obtained in  $T_6$ .

Seed bio-priming with *Trichoderma* also increased macronutrient content when part of the i.e., 10% of the recommended dosage of fertilizer was substituted with microbial mediation (Table 2). *Trichoderma* can enhance nutrient content by virtue of both enhanced decomposition of biomass as well as improving uptake of inorganic fertilizers. In addition of macronutrients, seed bio-priming

with *Trichoderma* can enhance poorly soluble micronutrient content by 1.2 to10.4% for Fe, 22.7 to 30% for Zn, and 9 to 16.6% for Mn. For Cu, 7.28% enhancement occur only when 10% of substitution of recommended dosage of fertilizer have been substituted with seed bio-priming, i.e., in T<sub>6</sub>. Solubilization of macro- and micronutrients (Altomare *et al.* 1999), and thus enrichment of nutritional status (Yedidia *et al.* 2001; Abd-El-Khair *et al.* 2010) of plants by *T. harzanium* is well reported.

#### **Energy requirement**

The objective of nutrient use is to increase the overall performance of okra by providing economically optimum nourishment to the crop while minimizing nutrient losses and supporting agricultural systems with numerous efficiency and productivity by efficient nutrient management technologies and practices. Sustainability through contributions to soil fertility or other soil quality components and energy savings. The data in Table 3 illustrate that even though energy requirement generally decreased as part of total recommended dosage of fertilizer dosage substituted by seed priming, the simultaneous increase in energy efficiency and yield until an optimum fertilizer dosage was attained i.e., 90% of RDF improved over-all system performance. Bio-primed treatment saved 970 to 1670 KJ energy in producing unit produce as compared with 100% RDF. Mihov and Tringovska (2010) also acheived low total energy inputs in greenhouse tomato production with use of biofertilizers. Efficient and effective use of macro and micro nutrients require that both be managed at optimum levels for the specific system. Numerous efficiency and productivity enhancing nutrient management technologies covering both inorganic fertilization and microbiological intervention practices (priming) exist from the present findings, but are still underutilized.

**Table 3:** Energy requirement in producing unit produce of okra as effected by seed bio-priming

Treatment	E <sub>input</sub> requirement (KJ unit <sup>-1</sup> )						
T <sub>1</sub>	0						
T <sub>2</sub>	4320						
T <sub>3</sub>	2650						
$T_4$	2840						
T <sub>5</sub>	3005						
T <sub>6</sub>	3350						
SEm±	77.4						
CD (P=0.05)	216.6						

 $\begin{array}{l} T_1: \ Absolute \ control \ (N:P_2O_5:K_2O @ 0:0:0 \ kg \ ha^{-1}); \ T_2: \ RDF \\ (N:P_2O_5:K_2O @ 120:120:75 \ kg \ ha^{-1}); \ T_3: 70\% \ RDF + T. \ harzianum; \\ T_4: 75\% \ RDF + T. \ harzianum; \ T_5: 80\% \ RDF + T. \ harzianum; \ T_6: 90\% \ RDF + T. \ harzianum \end{array}$ 

# CONCLUSION

The findings revealed that the application of 90% of recommended dose of  $N:P_2O_5:K_2O$  @ 120:120:75 kg ha<sup>-1</sup> plus seed bio-priming was effective in improving the growth and yield attributes of okra crop. *Trichoderma* contributed a considerable increase in the energy output with the yield. The use of seed

bio-priming of beneficial soil microorganisms led to significant decrease not only in energy requirement, but with bio-priming along with inorganic fertilizer (90% RDF), i.e.,  $T_6$  has achieved closed to the total economical yield of fertilized with recommended dose  $(T_2)$ . The bio-priming intervention can reduce the inorganic fertilizer requirement for *A. esculentus* and also combined application of bio-inoculant and inorganic fertilizers may be a sound soil fertility management strategy to get higher yield of the okra crop. Better results are expected with prerequisite incorporation of organic matter, and the technology is seen as complimentary to inorganic fertilizer source not as their replacement. However, more experiments should be conducted in different locations and seasons to draw a valid conclusion regarding the bio-priming for fruit yield improvement of okra.

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