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Performance of *Trichoderma*-enriched bio-organic fertilizer in N supplementation and bottle gourd production in field condition

Abstract

Bio-organic fertilizer plays a significant role to conserve suitable soil environment for plant growth and development. *Trichoderma*-enriched bio-organic fertilizer (BOF) was evaluated for bottle gourd cultivation to assess suitable dose and mode of application at field condition. The experiment was conducted in two successive years (2014/15 and 2015/16) with three replications and eight treatments of standard dose (SD) of NPK fertilizer and different doses of BOF in two modes of application. Application of BOF with 25% N of standard nitrogen dose played superior roles in present study. The highest dose (3kg/pit with 25%N+100%PK) of BOF offered the highest yield which was statistically similar to SD of NPK in both the years. But sole application (SA) of the highest dose at planting time provided 10.25% higher yield of bottle gourd compared to SD of NPK application. Conversely, 13.95 and 7.69% lower yield was monitored in first and second year, respectively by the split (double) application of the same amount of BOF compared to SD of NPK application. *Trichoderma*-enriched bio-organic fertilizer could save at least 75%N fertilizer usages and ensured earlier fruit setting in bottle gourd production. In the present study the highest dose (i. e. 3kg/pit of BOF & 25%N with 100%PK as of standard dose of NPK) and sole application of BOF presented superior performance to split application. *Trichoderma* enriched bio-organic fertilizer could serve prospective and potential roles in promotion of crop cultivation with reducing usages of N-fertilizer in future.

Keywords: bottle gourd, male and female flower, fruit diameter, fruit length, yield, sole, split application

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Sujan Barua,[1] Abul Hossain Molla,[1] Md. Manjurul Haque,[1] M Saiful Alam[2]

[1]Department of Environmental Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh

[2]Department of Soil Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh

Correspondence: Abul Hossain Molla, Department of Environmental Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur -1706, Bangladesh, Email ahmolla60@gmail.com

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Introduction

Excessive inorganic fertilizers and plant protection chemicals usages in crop production induce risks of both human health and environment. Besides, generally 50% of N and 90% of P from applied inorganic fertilizers are fixed up in soils and run off, and ultimately it releases into atmosphere and water sources, which contribute for greenhouse gas generation, eutrophication and soil salinization.[1,2] Application of excessive P induce cadmium in soil that come to food chains.[3] Moreover nitrogen application increase nitrates in ground water which are finally responsible for N-nitroso compounds and ultimately often linked with fatal methemoglobinemia.[4] To minimize these problems the relevant researchers have been trying to develop sustainable safe and environmental friendly technologies that will help to minimize usages of chemical fertilizers for crop production. Accordingly *Trichoderma* spp. enriched bio-organic fertilizers; pellets and suspension are being evaluated to monitor its performance in different crop production.[5–7] In recent years it has achieved recognition as plant growth promoter, enhanced antioxidant compounds,[7,8] increased yield of several crops in field condition.[6,10–12] Besides, some *Trichoderma* rhizosphere-competent strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer usage efficiency, percentage and rate of seed germination, and stimulation of plant defense against biotic and abiotic damages.[13,14] *Trichoderma* metabolites and roots colonization by *Trichoderma* change the proteome and transcriptome of plants,[15,16] which enhance plant growth and development but these mechanisms of *Trichoderma* plant interaction are not quite easy.

Application of *Trichoderma* fungi ensures the favorable environment for growing and proliferation of abundant healthy roots and simultaneously plant derived sucrose which is an important resource provided by the *Trichoderma* spp. Cells.[17] Thus plants become benefited from this relationship through increased root and shoot growth and increased macro- and micronutrient uptake. Therefore, *Trichoderma* spp. are being treated as growth promoting (bio-organic fertilizer) as well as pathogen control agent (mycofungicide) and their application may lower the production cost of crops along with conserving congenial environment. Vegetable cultivation is one of the most important and dynamic wing of agriculture in Bangladesh. Among the cultivated vegetables in Bangladesh, bottle gourd (*Lagenaria* spp.) a cucurbit is a popular crop has now drawing attention to greater extent. Bottle gourd (*Lagenaria* spp.) is a common and delicious winter vegetable in Bangladesh. Generally bottle gourd fruits are used as fruit vegetable, but its leaves tender stem are used as delicious and nutritious leafy vegetable.[18] It is reported as an easily digestible vegetable which keeps the body cool and prevents constipation. Its cultivation and usages are wide in winter but now a day it is cultivating during summer and rainy season also. Bottle gourd is vine crop grow rapidly and their stem become 10-12 meter long which generally need support to climb by the pole along the stem. It prefers well-drained, moist, rich soil and proper fertilizer management practices for successful crop growth and yield.[19] At this situation, slow release nutrient rich fertilizers/compost/bio-organic fertilizer might contribute significant roles for bottle gourd cultivation. Bio-organic fertilizer is defined as the fertilizer contained living cells or latent cells of efficient strains of microorganisms with decompose organic substances that help crop plants in uptake of nutrients by their interactions in the rhizosphere. They help in restoring soil health and thus provide a cost effective way to manage crop yield along with balancing the environment.[20] The integrated nutrient management i.e. minimum usages of chemical fertilizers with organic materials such as value added bio-organic fertilizer, animal manures, crop residues, green manuring and composts are alternatives to avoid excessive usages of nitrogenous and phosphorus contained fertilizers that have enough chance to pollute our soil and environment.[1,2,5,21] Therefore, it is important to evaluate the impact and usages technology of *Trichoderma*-enriched bio-organic fertilizer in different crops. The present study was undertaken to evaluate the performance of *Trichoderma*-enriched bio-organic fertilizer in combinations with chemical fertilizers on growth, yield and yield contributing characters of bottle gourd under field condition and to assess the suitable dose and mode of application of bio-organic fertilizer in bottle gourd cultivation.

Materials and methods

Experimental location and period

The present research project was conducted in research field of the Department of Environmental science, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh in two successive years at the period of October to March in 2014-2015 and 2015-2016 years. Moreover, the experiment was conducted in same field in both the years. The experimental site is situated in the sub-tropical climatic zone. The experimental period was relatively scanty rainfall, low humidity; low temperature (Min. 12-15 and Max. 24-27°C), high light intensity and short day period prevail during this time every year in Bangladesh.

Materials used in the experiment

Bio-organic fertilizer (BOF) i.e. *Trichoderma*-enriched bio-organic fertilizer (*Trichoderma harzianum*T22) was collected from Natore Development Society (NDS) and M/S RASH Agro Enterprise, Natore, Bangladesh for conducting the present experiment. Seeds of field grown variety of bottle gourd recognized as 'Khatlau' were collected from the Lal teer, a prominent private agro seed business center. The fruit of bottle gourd was intermediate to long in size of dark to pale green color with light to pale colored spot that make the fruits attractive appearance.

Nutritional and biological status of soil and Trichoderma-enriched BOF

Nutritional and biological status of the soil of the experimental plot and *Trichoderma*-enriched bio-organic fertilizer (BOF) were analyzed in prior to fix the standard doze of chemical fertilizers for proper growth and development of the studied crop. However, the nutritional status of soil and BOF was analyzed based on procedures of Jackson,[22] Nelson & Sommers,[23] Olsen & Sommers,[24] and microbial status of BOF was assessed based of technique of Martin[7] & Khan et al. [25] The recoded data was presented in Table 1.

Growing seedlings in poly bags

Seeds were soaked in water for overnight and then single seed was sown in each polythene (poly) bag on 15 November 2014 in first year and on 29 October 2015 in second year for rising seedling. Used polythene bags were 9cm diameter and 12cm long. Around 700g of well mixed of clay loam soil and decompose compost (1:1) was taken in each poly bag. After sowing, the poly bags were kept with care until seedling emerged and protect the seedlings from heavy drops of water, severe sunshine and red pumpkin beetle. Light irrigation was done in the late afternoon by using a fine meshed nozzle while it was required.

Table 1 Biochemical status of the soil of experimental field and *Trichoderma*-enriched bio-organic fertilizer

Parameters	Soil	*Trichoderma*-enriched bio-organic fertilizer
pH	6.2	5.78[22]
Organic matter (%)	7.8	-[23]
Organic carbon (%)	0.66	4.54[23]
Total N (%)	0.089	1.36[22]
Available P (ppm)	7.2	7900[24]
Exchangeable K (meq/100g)	0.63	0.42[22]
Fungi (spores/g of BOF)	-	0.53×10^{5} [25]
Bacteria (cfu/g of BOF)	-	2.23×10^{9} [7]

Experimental treatment and design

The experiment was conducted in a randomized complete block design (RCBD) with four replications and eight treatments. The unit plot size was 3.0m×2.0m and plot to plot 0.5m, row to row 0.5m distance were maintained. Single pit was allowed in each plot. The treatments were randomly assigned to different plots of each block.

However, the eight treatments were:

T_1 = Control [No inorganic fertilizer and *Trichoderma*-enriched bio-organic fertilizer (BOF)]

T_2 = Standard dose of NPK (130g of urea, 152g of TSP, 42g of MoP per pit). The standard dose was calculated based on fertilizer guide of Bangladesh Agricultural Research Council.[26]

T_3 = BOF [2kg/pit, total/sole application (TA) i.e. only at planting time] + [25% N + 100% PK (based on T_2)]

T_4 = BOF (2.5kg/pit TA) + [25% N + 100% PK (based on T_2)]

T_5 = BOF (3kg/pit TA) + [25% N + 100% PK (based on T_2)]

T_6 = BOF [2kg/pit, in two equal splits application (SA i.e. two times application, planting time and 35days after transplanting] + [25% N + 100% PK (based on T_2)]

T_7 = BOF (2.5kg/pit in SA) + [25% N + 100% PK (based on T_2)]

T_8 = BOF (3 kg/pit in SA) + [25% N + 100% PK (based on T_2)].

The dose of BOF was recommended by bio-organic fertilizer company for bottle gourd cultivation.

Land and pit preparation

Well ploughed land was used for the present experiment. A single pit of size 40cm^3 was prepared in each plot. The soil of the pit was prepared properly by mixing assigned treatment materials just a day before of transplanting date of the seedlings.

Application of fertilizer and *Trichoderma*-enriched BOF

The chemical fertilizers urea for N, triple super phosphate (TSP) for P and muriate of potash (MoP) for K were applied at the rate of 130, 152 and 42g per pit, respectively against the treatment (T_2). The dose of P and K were applied at the time of pit preparation to all treatments. In treatment T_2 urea was applied in three equal splits at pit preparation, 28 and 56 days after transplanting (DAT) of seedlings. The TA i.e. total/sole application of *Trichoderma*-enriched BOF was performed at pit preparation. However, in split application (SA) of BOF was carried out as 50% at pit preparation and remaining 50% was at 5weeks after transplanting of seedlings (i.e. at 35 DAT). In treatments (T_3–T_8) the 32.5g urea or 25% of standard dose (i.e. 130g urea) was applied is three equal splits as treatment T_2.

Seedling transplanting and inter-cultural operations

Seedlings were grown in poly bag and two seedlings of 21 days old were transplanted per pit to the main field on 6 December 2014 in first year and 19 November 2015 in second year as per design of the experimental layout. The plants were always kept under careful observation, irrigation, gap filling, weeding, mulching and necessary other cultural operation were done throughout the growing season.

Assessment of microbial population density

Microbial population i.e. fungal and bacterial population density of rhizosphere soils was assayed at the middle stage (i.e. 45 DAT) of the crop bottle gourd based on procedures of Martin[7] & Khan et al.,[25] respectively.

Parameter studied and harvesting fruits

Fruits of all treatments were harvested at edible maturity. Data on the following parameters were recorded to observe the impact of *Trichoderma*-enriched bio-organic fertilizer in combinations with chemical fertilizers on growth and yield of bottle gourd cultivation in field condition. However, the studied parameters were first flower initiation dates and total number of flowers both male and female; fruit length, diameter, weight and number; root and shoot dry weight;[10] and microbial population density in rhizosphere soils.[7,25]

Statistical analysis

The recorded data of various parameters were statistically analyzed using Least Significance Difference (LSD) package program. The significance of difference among the treatments of replicated means was evaluated by LSD test at 5% levels of probability. Tests were conducted using SPSS version 16.

Results and discussion

Sex expression

Male flower - The flower initiation date is presented in DAT, which were almost similar among all treatments except T_1, where the first male flower initiation was delayed 1-2 weeks compared to other treatments in both years (Table 2). In first year it was 56 and the second year 60 DAT. While, in other treatments the male flower initiation was completed within 45-49 DAT in first year and within 47-50 DAT in second year. The flower initiation date was more or less close among the treatments and within the two years. Conversely, the variation of days within the two years in respective of treatments was quite narrow; the maximum was recorded in treatment T_1. The lowest number (i.e. 37 & 30) of male flowers was recorded in control treatment (T_1) in both years. But, the highest number was monitored in treatment T_5 that was 72 in first year and 70 in second years. These were closer to the results of T_2 and T_4 treatments. Moreover, the obtained results clearly implied that the standard dose application i.e. treatment T_2 and total/sole application (i.e. TA) of *Trichoderma*-enriched bio-organic fertilizer treatments (i.e. T_3, T_4 and T_5) provided the maximum number of male flowers than the others. Relatively lower number of flowers was recorded in splits application (SA) of *Trichoderma*-enriched bio-organic fertilizer application treatments (i.e. T_6, T_7 and T_8). Conversely the figures of required days of flower initiation and number of produced flowers in a particular treatment between two successive years were close to each other i.e. deviation was quite narrow except treatment T_1. It might be happened due to similar trend of physical factors (light intensity, temperature, humidity, moisture in soil, rainfall etc) generally prevail in similar pattern in every year at a particular location of Bangladesh. Moreover, same field and same doses of treatments were used in both years. Poor performance in control treatment (T_1, not applied any fertilizers) was noticed due to deficient nutrient there. Total/sole application of *Trichoderma*-enriched BOF (i.e. treatments T_3-T_5) provided similar performance on male flower production as standard dose of NPK treatment (T_2) but number of produced male flowers was quite lower in *Trichoderma*-enriched BOF treatments (T_6-T_8) while it was supplied in split application (Table 2). Perhaps split application of BOF provided insufficient flow of nutrients supply and favorable environment in rhizospheric regions which might induce poor production of flower. Combination of inorganic and bio-organic fertilizers helped to increase uptake of nutrients which promoted faster plant growth of plants leading to enhance reproductive development. Moreover, sufficient bio-organic fertilizer (compost) behaved as slow release of nutrients supplier and conserved favorable environment for proper root growth and development along with reducing load of chemical fertilizers usages.[5,21] The nature of flower initiation among cucurbits is not same. It varies among the members and species. But similar trend of results and findings were reported by Das et al.[27] & Sharma et al.,[28] in pointed gourd. Moreover male flower initiation in cucumber was reported in 35.25 days by Nirmala et al.[29]

Initiation of female flowers followed similar profile as male flowers (Table 3). Like male flower, the plants of treatment T_5 produced the highest number of female flowers (i.e. 34 & 32) both in first and second year, which were closer to the results obtained in treatment of T_4. Relatively wider variation on number of female flower among treatments was observed in second year than the first year. The lowest number of female flower (23 and 20 in first & second year, respectively) was recorded in control i.e. T_1 treatment. The recorded results implied that the highest dose of BOF (i.e. 3kg/pit, treatment T_5) as total/sole application (TA) produced the highest number of flowers, but same amount of BOF in split application (treatment T_8) produced lower number of both flowers as control treatment (Table 2) (Figure 3). Spit application (SA) of *Trichoderma*-enriched bio-organic fertilizer treatments presented lower number of flowers compared to sole/total application. Insufficient and inconsistent nutrients supply might be happened in split application than the sole application that compelled to produce lower number of flowers. The narrow differences of flower initiation days among the treatments and between the two successive cultivated years were monitored in all treatments except T_1 (Table 3). Also similar profile (i, e. narrow deviation) was noticed for total number of flowers. The T_1 required the maximum days for first flower initiation, 62 DAT in first year and 69 DAT in second year and total number of flowers were the lowest in both years than the others. Flower initiation was happened in close distances (50-52 DAT in first year and 51-53 DAT in second year in remaining treatments. Insufficient/low nutrient supply delayed flower initiation due to insufficient/improper vegetative growth of the plant. Combination of inorganic and bio-organic/organic fertilizers enhanced to uptake different nutrients which promote faster plant growth leading to increase production of higher number of flowers. Similar profiles of findings were noticed in ridge gourd[30] and in muskmelon cucurbits.[31]

Table 2 Performance of male flower initiation per plot of bottle gourd after *Trichoderma*-enriched bio-organic fertilizer (BOF) application

Treat-ment	Initiation of first male flower					Total no. of male flower		
	1st year (2014/2015)		2nd year (2015/2016)		Difference (day)	1st year (2014/15)	2nd year (2015/16)	Difference (#)
	Initiation Date	DAT	Initiation Date	DAT				
T_1	1/2/2014	56	18/1/2015	60	4	37	30	7
T_2	22/1/2114	47	8/1/2015	50	3	67	65	2
T_3	24/1/2114	49	6/1/2015	48	1	59	62	3
T_4	23/1/2114	48	7/1/2015	49	1	70	68	2
T_5	20/1/2014	45	6/1/2015	48	3	72	70	2
T_6	22/1/2014	47	5/1/2015	47	0	45	40	5
T_7	21/1/2014	46	8/1/2015	50	4	40	41	1
T_8	21/1/2114	46	7/1/2015	49	3	45	42	3

Note:

T_1 = Control [No NPK and *Trichoderma*-enriched bio-organic fertilizer (BOF)]

T_2 = Standard dose of NPK (130 g Urea, 152 g of Triple super phosphate and 42 g of Muriate of potash per pit)

T_3 = BOF - 2 kg/pit, sole/total application (TA) + (25% N of T_2 + 100% PK of T_2)

T_4 = BOF - 2.5 kg/pit i.e. 25% higher of T_3, TA + (25% N of T_2 + 100% PK of T_2)

T_5 = BOF - 3 kg/pit i.e. 50% higher of T_3, TA + (25% N of T_2 + 100% PK of T_2)

T_6 = BOF - 2 kg/pit, in two equal splits application (SA) + (25% N of T_2 + 100% PK of T_2)

T_7 = BOF - 2.5 kg/pit i.e. 25% higher of T_6, SA + (25% N of T_2 + 100% PK of T_2)

T_8 = BOF - 3 kg/pit i.e. 50% higher of T_6, SA + (25% N of T_2 + 100% PK of T_2)

[DAT = Days after transplanting, TA (Total application) of BOF was done at pit preparation, SA (Splits application) of BOF was done as: at pit preparation and 35 DAT, and N was applied in three equal splits at pit preparation, 28 and 56 DAT in all treatments].

Table 3 Performance of female flower initiation per plot of bottle gourd after *Trichoderma*-enriched bio-organic fertilizer application

Treat-ment	First female flower initiation					Total no. of female flower		
	1st year (2014/2015)		2nd year (2015/2016)		Difference (day)	1st year (2014/2015)	2nd year (2015/2016)	Difference (#)
	Initiation Date	DAT	Initiation Date	DAT				
T_1	6/2/2014	62	23/1/2015	69	7	23	20	3
T_2	28/1/2014	53	7/1/2015	53	0	27	25	2
T_3	26/1/2014	51	5/1/2015	51	0	26	26	0
T_4	26/1/2014	51	6/1/2015	52	1	31	30	1
T_5	25/1/2014	50	5/1/2015	51	1	34	32	2
T_6	26/2/2014	51	5/1/2015	51	0	25	28	3
T_7	26/2/2014	51	6/1/2015	52	1	24	25	1
T_8	27/2/2014	52	6/1/2015	52	0	25	28	3

Fruit length and diameter

Length and diameter of bottle gourd were influenced by *Trichoderma*-enriched bio-organic fertilizer (BOF) application. In the first year significantly (*P≤0.05*) the highest length of fruit was recorded in T_3 (44.00cm) and T_5 (44.00cm) presented in Table 4. On the other hand the second highest length was recorded in three treatments T_2, T_6 and T_8. The lowest fruit length was monitored in T_1 treatment (36.00cm) followed by T_7 (37.33cm). In case of second year the highest length of fruit was found in T_5 (46.66cm) treatment which was statistically similar to the treatment of T_4. The lowest fruit length was recorded in T_1 treatment (34.66cm) followed by T_6 (36.33cm) treatment. However, the obtained records implied that superior performance was recorded in treatment T_5 in both years. As like as other parameters the fruit length was significantly influenced in treatment T_5 by *Trichoderma*-enriched bio-organic fertilizer application. Generally balanced and sufficient nutrients supply ensures the proper growth and reproductive development, perhaps it was ensured by the treatment of T_5. Fruit diameter was measured in neck, middle and bottom of the bottle gourd. Significantly (*P≤0.05*) the highest diameter in everywhere was recorded only in treatment T_5 in both years. Finally it provided the highest average diameter. However, the treatment T_3 provided statistically similar findings as T_5 in most of the cases, similarly the average diameter attained in second highest there. Conversely, the average diameters of treatments T_2, T_6, T_7 and T_8 were close to each other and the lowest record was attained in T_1 treatment. The increased fruit length, fruit cavity, fruit volume and diameter could be attributed to balanced nutrition, better nutrient uptake and synthesis of more carbohydrates by plants when provided with combinations of inorganic and bio-organic/organic fertilizers, and *Trichoderma*-enriched BOF which influenced on increased fruit length fruit diameter, fruit volume and fruit cavity.[32] Significant increase of leaf chlorophyll content and uptake of different nutrients including micronutrients were reported in tomato by *Trichoderma*-enriched BOF application in field.

Table 4 Performance of fruit length and diameter of bottle gourd in field under *Trichoderma*-enriched bio-organic fertilizer application

Treatment	Average length of fruit (cm)		Diameter (cm)							
			Neck		Middle		Bottom		Average	
	1st year (2014/15)	2nd year (2015/16)	1st year 2014/15)	2nd year (2015/16)	1st year (2014/15)	2nd year (2015/16)	1st year (2014/15)	2nd year (2015/16)	1st year (2014/15)	2nd year (2015/16)
T_1	36.00d	34.66f	12.33e	11.66de	29.00e	29.33de	37.00cd	36.00bc	26.11	25.66
T_2	41.66b	40.33de	13.00e	13.00c	34.00b	35.33ab	40.00b	40.66a	29	29.66
T_3	44.00a	43.00c	17.66ab	15.33ab	35.33b	35.33ab	40.66b	40.33a	31.21	30.33
T_4	39.00bc	45.66ab	13.00e	12.33cd	30.66cd	31.66cd	33.73f	31.33d	25.79	25.1
T_5	44.00a	46.66a	18.33a	16.33a	37.00a	37.00a	42.33a	39.66a	32.55	31
T_6	39.33b	36.33f	15.00c	16.66e	31.33c	30.33de	37.00cd	35.33ab	27.77	27.44
T_7	37.33cd	39.33de	13.33de	15.33ab	29.66de	28.66e	35.00ef	35.66c	25.99	26.55
T_8	42.00b	44.66bc	14.66cd	15.33ab	32.33c	33.66bc	38.33cd	38.33ab	28.44	29.1
CV (%)	2.88	2.46	5.42	4.97	2.54	4.38	3.02	2.5		
LSD at 5%	1.99	1.75	1.4	1.18	1.42	2.45	1.99	1.61		

[DAT = Days after transplanting, TA (Total application) of BOF was done at pit preparation, SA (Splits application) of BOF was done as: at pit preparation and 35 DAT, and N was applied in three equal splits at pit preparation, 28 and 56 DAT in all treatments].

Fruit number and yield of bottle gourd

Fruit number and yield of bottle gourd were significantly influenced (*P≤0.05*) by *Trichoderma*-enriched bio-organic fertilizer (BOF) application. Two successive cultivated years provided almost the similar trend of results. Significantly (*P≤0.05*) the highest fruit number per plot was recorded in the treatment T_5 in both years i.e. 10.0 and 11.33 fruits per plot in first and second year, respectively (Table 5). However, significantly the second highest numbers of fruits were attained in treatments T_2, T_6, T_7 and T_8 in both first and second years. Furthermore earlier fruit setting and increased number fruits were noticed in promoted BOF treatment compared to standard dose of NPK application (Figure 1). Within these four treatments statistically similar number of fruits was monitored in respective years. The lowest number of fruits was recorded in treatment T_1, which was statistically similar to the treatments of T_3 and T_4 in both the years. Moreover in both the years statistically similar and highest bottle gourd yield (kg/plot) was attained in three treatments T_2, T_5 and T_8 i.e. standard dose of NPK fertilizer, 3kg/pit (the highest amount) of BOF as sole application (one time) and 3kg/pit of BOF in split (two times) application treatments, respectively. But the second highest yield was attained in treatment T_6 in both the years and the lowest yield of bottle gourd was recorded in control (i.e. T_1) treatment. The fruit yield depends on fruit number per plot, fruit length and fruit diameter. The fruit length and diameter were recorded significantly higher in all cases of two successive years at T_5 treatment (Table 4). The average fruit length and diameter of T_3 was second highest and closer to value of the treatment T_5. But the yield (kg/plot) of bottle gourd in treatment T_3 was significantly poor not similar as T_2, T_5 and T_8 treatments because of average lower number of fruits per plot in both years (Table 5) as well as higher number of deformed fruits. Deformed fruits were observed in almost all treatments but it was maximum in treatment T_3 (not included). Perhaps insufficient nutrients enhanced to produce deformed fruits.

Table 5 Yield of bottle gourd in field under *Trichoderma*-enriched bio-organic fertilizer application

Treat-ment	Average No. of fruit per plot		Wt. of fruit (kg/plot)		Fruit yield (t/ha)		% Yield increased over control		% Yield increased over Standard NPK dose (T2)	
	1st year (2014/15)	2nd year (2015/16)	1st year (2014/15)	2nd year (2015/16)	1st year (2014/15)	2nd year (2015/16)	1st year (2014/15)	2nd year (2015/16)	1st year (2014/15)	2nd year (2015/16)
T_1	3.7d	3.3c	5.4f	4.4e	9	7.4	-	-	-65.3	-65.9
T_2	8.0b	7.7b	15.6a	13.0ab	25.9	21.7	187.9	193.5	-	-
T_3	5.0cd	3.7c	10.3d	9.7c	17.1	16.1	90	118.2	-34	-25.7
T_4	6.0c	4.7c	6.6e	7.0d	10.9	11.6	21.4	57.9	-57.8	-46.2
T_5	10.0a	11.3a	15.4a	14.3a	25.6	23.9	184.2	223.6	-1.3	10.3
T_6	8.0b	7.5b	12.4b	11.7bc	20.7	19.4	129.6	163.2	-20.3	-10.3
T_7	8.3b	8.7b	11.8c	9.5c	19.7	15.8	118.5	114.4	-24.1	-26.9
T_8	8.3b	7.0b	13.4ab	12.0ab	22.3	20	147.8	170.9	-13.9	-7.7
CV (%)	15.8	13.8	3.8	10.1						
LSD at 5%	2.5	1.8	1.25	2.05						

Figure 1 Promotion of bottle gourd production using *Trichoderma*-enriched bio-organic fertilizer application in field [T_2 – standard dose of NPK fertilizer and T_5 - 3kg/pit sole/total application + (25% N of T_2 + 100% PK of T_2)].

Maximum percent yield increase over standard dose of NPK (i.e. T_2 treatment) was recorded in treatment T_5 i.e. *Trichoderma*-enriched bio-organic fertilizer application. Only treatment T_5 provided 10.25% higher fruit yield over standard dose of NPK application. Conversely the fruit yield of bottle gourd 7.69, 10.30, 25.66 and 65.93% lower in treatments T_8, T_6, T_3 and T_1, respectively compared to T_2 i.e. standard dose of NPK fertilizer application. The findings of the present research project clearly presented that the *Trichoderma*-enriched bio-organic fertilizer 3kg/pit as sole (one time) application provided the 10.25% higher yield compared to the standard dose of NPK application. But the achieved yield at treatment T_5 was statistically similar to the yields of T_2 and T_8 treatments. Amount of inorganic and *Trichoderma*-enriched bio-organic fertilizer was same T_5 and T_8 treatments but application mode of BOF was different. However, the treatments T_5 and T_8 were consisted 75% less N compared to T_2 treatment; it means that practicing of T_5 and T_8 treatments would help to save 75% of N usages in bottle gourd cultivation. Above 50% of N fertilizer usages was supplemented by *Trichoderma*-enriched compost application in maize,[5] in mustard[6] and in tomato yield and quality[10] in field condition were reported. Increased yield of several crops such as in rice,[9] in wheat[11] and in soybean[12] were reported by *Trichoderma* spp application. Application of *Trichoderma* spp and enriched bio-organic fertilizer augmented to solubilize of different nutrient elements and enhanced harboring of microbes in rhizosphere soils.[7] Moreover, Vinale et al.[33] reported that the application of *Trichoderma* spp dramatically increased the number of fruits per plant in pepper and tomato grown in greenhouse than untreated control. It has been reported that the strains of different *Trichoderma* species showed

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variable responses in cucumber, loofah and bitter gourd.[34] Increase yield of wheat was found similar to the findings of Sallam et al.[35] where the formulation of *Trichoderma* spp treatments enhanced green yield of bean plants compared to control. The increase in yield can also be attributed to the application of *Trichoderma harzianum* (Th3) bioformulation along with the farm yard manure helped in increasing the colonies by providing nutrient to *Trichoderma* thereby increasing the plant growth and yield of wheat. Generally 20% decrease in yield is expected in plants cultivated in organic systems than conventionally produced crops.[3] But in the present experiment would not conducted with sole application of bioorganic fertilizer, therefore percent yield losses only in *Trichoderma*-enriched bio-organic fertilizer application was not computed.

Dry matter production

Impact of *Trichoderma*-enriched bio-organic fertilizer application on dry matter production of bottle gourd was presented in Table 6. The highest shoot dry weight (1102.70 and 1201.70gplot^{-1} in first and second year, respectively) of bottle gourd was recorded in T_5 treatment in both years followed by T_2 (1024.70gplot^{-1}) treatment. On the other hand the lowest shoot dry weight was recorded in T_1 in both years. Like shoot weight, the highest root dry weight (15.33gplot^{-1}) was noticed in T_5 treatment in first year and T_6 (19gplot^{-1}) in second year. But statistically similar root dry weight of T_6 was observed in T_5 treatment in second year. The lowest root dry weight was found in T_2 and T_4 in first year, and T_3 in second year. Conversely, both first and second year the highest total dry matter 1118.03 and 1220.36gplot^{-1} of bottle gourd was recorded in T_5 treatment, respectively. The second highest was recorded in T_2 of first year and second year in T_2 and T_8 treatments. The lowest record was achieved in T_1 in both year also. Like other parameters the root and shoot weight was influenced significantly and attained maximum at T_5 treatment. Similar amount of *Trichoderma*-enriched bio-organic fertilizer but different application mode in treatment T_8 provided second height total dry weight as T_2 treatment except first year. Perhaps these treatments provided sufficient available nutrients as well as favorable rhizosphere environment in spite of biotic and abiotic stresses by increasing microbial populations.[13,14] *Trichoderma* promoted growth of primary root length and root branching in maize and beans by inducing lateral root growth. In plants auxins have been demonstrated to initiate lateral root growth[36] and the observed effects of *Trichoderma* in promoting lateral root development is similar to in vitro experiments performed by Hexon et al.,[37] that showed that *Trichoderma* spp. produced indole-3-acetic acid (IAA) that promoted lateral root formation in *Arabidopsis thaliana.* Increased root size resulted into increased shoot size which translocate into increased shoot biomass production indicating a beneficial effect of *Trichoderma* inoculation on plant growth and development. The positive influence of *Trichoderma* on root system architecture would therefore relate to increased yield of plants. *Trichoderma* enhanced root biomass production and increased root hair development has also been reported by Björkman et al.[38] Similar trend of results was noticed in corn shoot dry weight using *T. harzianum.*[39]

Table 6 Dry matter production of bottle gourd as influenced by *Trichoderma*-enriched bio-organic fertilizer (BOF) application

Treatment	Shoot (g/plot)		Root (g/plot)		Total dry wt. (g/plot)	
	1st year (2014/2015)	2nd year (2015/2016)	1st year (2014/2015)	2nd year (2015/2016)	1st year (2014/2015)	2nd year (2015/2016)
T_1	126.7h	192.7g	10.3d	11.3c	137.0h	204.0g
T_2	1024.7b	1002.7b	6.3f	10.6c	1031.0b	1013.4b
T_3	902.7d	803.0e	8.3e	7.0e	911.0d	810.0e
T_4	801.7f	902.7d	6.3f	10.0cd	808.0f	912.7d
T_5	1102.7a	1201.7a	15.3a	18.7a	1118.0a	1220.4a
T_6	844.0e	700.0f	14.0b	19.0a	858.0e	719.0f
T_7	726.0g	983.3c	9.0e	8.3de	735.0g	991.6c
T_8	966.7c	1000.0b	12.7c	15.0b	979.4c	1015.0b
CV (%)	15.6	18.1	4.8	8.1	15.8	18.62
LSD at 5%	6.7	8.3	0.9	1.8	6.9	10.3

Microbial population in rhizosphere soil

Microbial population i.e. fungal and bacterial population in rhizosphere soils of bottle gourd was assayed in middle stage of the crop at 45 DAT in one year (second) only. The population density of these two groups of microbes was influenced by quantity and mode of application of *Trichoderma*-enriched bio-organic fertilizer (BOF) in the present experiment. The highest figure of fungal population was recorded in treatment T_8 but which was statistically similar to all *Trichodema*-enriched bio-organic fertilizer added treatments except T_4 (Table 7). Significantly (*$P \leq 0.05$*) the lowest number of fungal population was monitored in T_1 i.e. control treatment. At treatment T_2 (i.e. standard dose of NPK fertilizer application) significantly higher number of fungal population than the T_1, but lower number than T_5, T_6 and T_8 treatments. For bacterial population, the highest number was monitored in treatment T_6, which was statistically alike to T_5, T_7 and T_8 treatments. Statistically the second highest bacteria population in rhizosphere soil was noticed T_3 and T_4 treatments. The lowest number of bacterial population was recorded in treatment T_1, which was statistically similar to T_2 treatment. In the present experiment, the dose of *Trichoderma*-enriched bio-organic fertilizer along with inorganic fertilizers in T_3 and T_6 was same i.e. 2kg/pit of

BOF, similarly in T_4 and T_7 2.5kg/pit of BOF, and in T_5 and T_8 3kg/pit of BOF. Only mode of application of BOF was different. In treatments T_3, T_4 and T_5, the total amount of BOF was applied at planting time. Conversely, in treatments T_6, T_7 and T_8 the total amount of BOF was applied in equal two installments at planting time and at 35 DAT. The present obtained results implied that the higher amount of BOF i.e. treatments T_5 and T_8 maintained statistically highest number of microbial populations (both fungi and bacteria) in rhizosphere soils by irrespective of application mode (Table 7). Moreover the split application of BOF maintained increased microbial population (both fungi and bacteria) in rhizosphere soil compared to sole/total application. Perhaps split application helped to maintained population trend in sustainable way but while sole application of BOF was done, then intensive competition for food and spaces among the population might be happened that resulted declined trend of population density. Besides microbial population was assessed in rhizosphere soil that was collected at 45 DAT, just after second instalment of BOF application was done at 35 DAT. It might influence the microbial population in split application treatments. Microbial population in rhizosphere soils was assayed by several authors as usages of organics improved the productivity of crops as well as microbial population soil.[40] The enhanced microbial populations and their activities in rhizosphere soils increased root growth and uptake of higher amount of nutrients by excreting organic acids and phosphates could release elements from complexes presently in soil and increase nutrient availability to plants.[41,42]

Table 7 Microbial population at 45 DAT of rhizosphere soil of bottle gourd as influenced by *Trichoderma*-enriched bio-organic fertilizer (BOF) application

Treatment	Population number (spores/cfug^{-1} of soil)	
	Fungi	Bacteria
T_1	8.50×10^{5}d	2.47×10^{9}d
T_2	14.61×10^{5}c	3.06×10^{9}cd
T_3	17.33×10^{5}abc	5.13×10^{9}b
T_4	15.66×10^{5}bc	4.55×10^{9}bc
T_5	18.33×10^{5}ab	6.94×10^{9}a
T_6	18.33×10^{5}ab	7.36×10^{9}a
T_7	18.00×10^{5}abc	6.96×10^{9}a
T_8	20.00×10^{5}a	7.17×10^{9}a
CV (%)	12	16.87
LSD at 5%	3.39	7.91

Conclusion

Excessive usage of expensive chemical fertilizer could be avoided to a significant extent without compromising the yields by employing the *Trichoderma*-enriched bio-organic fertilizer. Combined application of *Trichoderma*-enriched bio-organic fertilizer with reduced rate of N fertilizer enhanced vegetative, reproductive growth and yield of bottle gourd by slow and steady release of nutrients to the plants than the sole application of NPK fertilizer. *Trichoderma*-enriched bio-organic fertilizer application enhanced earlier fruit setting and could save at least 75% N i.e., decrease cost of cultivation by minimizing N fertilizer usages that would reduce toxicity in food and environment.

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Conflict of interest

Author declares that there is no conflict of interest.

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