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

**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,8 increased yield of several crops in field condition.6,9-12 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.) 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 of efficient strains of microorganisms with decompose...
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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. 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. 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 Mujibbar 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 dose 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, Nelson & Sommers, Olsen & Sommers, and microbial status of BOF was assessed based of technique of Martin & Khan et al. 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<sup>25</sup>                         |
| Organic matter (%)      | 7.8  | <sup>23</sup>                              |
| Organic carbon (%)      | 0.66 | 4.54<sup>23</sup>                         |
| Total N (%)             | 0.089| 1.36<sup>25</sup>                         |
| Available P (ppm)       | 7.2  | 7900<sup>24</sup>                         |
| Exchangeable K (meq/100g)| 0.63 | 0.42<sup>25</sup>                         |
| Fungi (spores/g of BOF) | -    | 0.53×10<sup>10</sup><sup>25</sup>        |
| Bacteria (cfu/g of BOF) | -    | 2.23×10<sup>7</sup>                       |

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<sub>1</sub> | Control [No inorganic fertilizer and Trichoderma-enriched bio-organic fertilizer (BOF)] |
|-------------|-----------------------------------------------|
| T<sub>2</sub> | 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. |
| T<sub>3</sub> | BOF [2kg/pit, total/sole application (TA)] i.e. only at planting time + [25% N + 100% PK (based on T<sub>3</sub>)] |
| T<sub>4</sub> | BOF [2.5kg/pit TA] + [25% N + 100% PK (based on T<sub>3</sub>)] |
| T<sub>5</sub> | BOF [3kg/pit TA] + [25% N + 100% PK (based on T<sub>3</sub>)] |
| T<sub>6</sub> | 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<sub>3</sub>)] |
| T<sub>7</sub> | BOF [2.5kg/pit in SA] + [25% N + 100% PK (based on T<sub>3</sub>)] |
| T<sub>8</sub> | BOF (3 kg/pit in SA) + [25% N + 100% PK (based on T<sub>3</sub>)] |

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<sup>3</sup> 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 (T1). The dose of P and K were applied at the time of pit preparation to all treatments. In treatment T1 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 (T3-T5) the 32.5g urea or 25% of standard dose (i.e. 130g urea) was applied in three equal splits as treatment T2.

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 & Khan et al., 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; and microbial population density in rhizosphere soils. 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 T2, 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 T2. The lowest number (i.e. 37 & 30) of male flowers was recorded in control treatment (T1) in both years. But, the highest number was monitered in treatment T2 that was 72 in first year and 70 in second years. These were closer to the results of T1 and T5 treatments. Moreover, the obtained results clearly implied that the standard dose application i.e. treatment T1 and total/sole application (i.e. TA) of Trichoderma-enriched bio-organic fertilizer treatments (i.e. T3, T4 and T5) provided the maximum number of male flowers than the others. Relatively lower number of flowers was recorded in split application (SA) of Trichoderma-enriched bio-organic fertilizer application treatments (i.e. T3, T4 and T5). 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 T1. 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 (T1, not applied any fertilizers) was noticed due to deficient nutrient there. Total/sole application of Trichoderma-enriched BOF (i.e. treatments T3-T5) provided similar performance on male flower production as standard dose of NPK treatment (T1) but number of produced male flowers was quite lower in Trichoderma-enriched BOF treatments (T3-T5) 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 cucurbit is not same. It varies among the members and species. But similar trend of results and findings were reported by Das et al. & Sharma et al., in pointed gourd. Moreover male flower initiation in cucumber was reported in 35.25 days by Nirmala et al.

Initiation of female flowers followed similar profile as male flowers (Table 3). Like male flower, the plants of treatment T1 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 T2. 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. T1 treatment. The recorded results implied that the highest dose of BOF (i.e. 3kg/pit, treatment T3) as total/sole application (TA) produced the highest number of flowers, but same amount of BOF in split application (treatment T2) produced lower number of both flowers as control treatment (Table 2) (Figure 3). Split 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 T1 (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\textsuperscript{30} and in muskmelon cucurbits.\textsuperscript{31}

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

| Treatment | 1\textsuperscript{st} year (2014/2015) | 2\textsuperscript{nd} year (2015/2016) | Difference | 1\textsuperscript{st} year (2014/15) | 2\textsuperscript{nd} year (2015/16) | Difference |
|-----------|--------------------------------------|--------------------------------------|-----------|--------------------------------------|--------------------------------------|-----------|
| $T_1$     | 1/2/2014                             | 62                                  | 60        | 4                                    | 37                                  | 30        | 7         |
| $T_2$     | 22/1/2014                            | 47                                  | 8/1/2015  | 50                                   | 3                                    | 67        | 65        | 2         |
| $T_3$     | 24/1/2014                            | 49                                  | 6/1/2015  | 48                                   | 1                                    | 59        | 62        | 3         |
| $T_4$     | 23/1/2014                            | 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         |
| $T_8$     | 21/1/2014                            | 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 $T_1$, TA + (25% N of $T_2$ + 100% PK of $T_2$)
$T_5$ = BOF - 3 kg/pit i.e. 50% higher of $T_1$, 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

| Treatment | 1\textsuperscript{st} year (2014/2015) | 2\textsuperscript{nd} year (2015/2016) | Difference | 1\textsuperscript{st} year (2014/2015) | 2\textsuperscript{nd} year (2015/16) | Difference |
|-----------|--------------------------------------|--------------------------------------|-----------|--------------------------------------|--------------------------------------|-----------|
| $T_1$     | 6/2/2014                             | 62                                  | 23/1/2015 | 69                                   | 0                                    | 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 \( P \leq 0.05 \) the highest length of fruit was recorded in \( T_4 \) (44.00cm) and \( T_5 \) (44.00cm) presented in Table 4. On the other hand the second highest length was recorded in three treatments \( T_4, T_5 \) and \( T_6 \). 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 \( T_8 \) (36.33cm) treatment. However, the obtained records implied that superior performance was recorded in treatment \( T_4 \) in both years. As like as other parameters the fruit length was significantly influenced in treatment \( T_4 \) 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 \( T_4 \). Fruit diameter was measured in neck, middle and bottom of the bottle gourd. Significantly \( P \leq 0.05 \) the highest diameter in everywhere was recorded only in treatment \( T_1 \) in both years. Finally it provided the highest average diameter. However, the treatment \( T_1 \) provided statistically similar findings as \( T_4 \) in most of the cases, similarly the average diameter attained in second highest there. Conversely, the average diameters of treatments \( T_4, T_5, T_7 \) and \( T_1 \) 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/fertilizers, and Trichoderma-enriched BOF which influenced on increased fruit length fruit diameter, fruit volume and fruit cavity. 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)** |
| \( T_1 \) | 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 |

[Fruit number and yield of bottle gourd]

Fruit number and yield of bottle gourd were significantly influenced \( P \leq 0.05 \) by Trichoderma-enriched bio-organic fertilizer (BOF) application. Two successive cultivated years provided almost the similar trend of results. Significantly \( P \leq 0.05 \) the highest fruit number per plot was recorded in the treatment \( T_4 \) 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 \( T_4, T_5, T_6 \) and \( T_4 \) 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 \( T_4 \) and \( T_5 \) in both the years. Moreover in both the years statistically similar and highest bottle gourd yield (kg/plot) was attained in three treatments \( T_4, T_5 \) and \( T_6 \) i.e. standard dose of NPK fertilizer, 3kg/plot (the highest amount) of BOF as sole application (one time) and 3kg/plot of BOF in split (two times) application treatments, respectively. But the second highest yield was attained in treatment \( T_1 \) 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 \( T_1 \) treatment (Table 4). The average fruit length and diameter of \( T_1 \) was second highest and closer to value of the treatment \( T_4 \). But the yield (kg/plot) of bottle gourd in treatment \( T_4 \) was significantly poor not similar as \( T_4, T_5 \) and \( T_4 \) treatments because of average lower number of fruits per

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Maximum percent yield increase over standard dose of NPK (i.e. T1 treatment) was recorded in treatment T6, i.e. Trichoderma-enriched bio-organic fertilizer application. Only treatment T1 provided 10.25% higher fruit yield over standard dose of NPK application. Conversely, the fruit yield of bottle gourd was 7.69, 10.3, 25.66 and 65.93% lower in treatments T2, T3, T4 and T5, respectively compared to T1, 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 T1 was statistically similar to the yields of T6 and T5 treatments. Amount of inorganic and Trichoderma-enriched bio-organic fertilizer was same T6 and T5 treatments but application mode of BOF was different. However, the treatments T1 and T6 were consisted 75% less N compared to T5 treatment; it means that practicing of T1 and T6 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, in mustard and in tomato yield and quality in field condition were reported. Increased yield of several crops such as in rice, in wheat and in soybean 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. Moreover, Vinale et al. 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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**Table 5 Yield of bottle gourd in field under Trichoderma-enriched bio-organic fertilizer application**

| Treatment | 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) |
| T1        | 3.7d                          | 3.3c                   | 5.4f              | 4.4e                           | 9                             | 7.4                        | -                     | -                     |
| T2        | 8.0b                          | 7.7b                   | 15.6a             | 13.0ab                         | 25.9                         | 21.7                      | 187.9                  | 193.5                 |
| T3        | 5.0cd                         | 3.7c                   | 10.3d             | 9.7c                           | 17.1                         | 16.1                      | 90                    | 118.2                 |
| T4        | 6.0c                          | 4.7c                   | 6.6e              | 7.0d                           | 10.9                         | 11.6                      | 21.4                  | 57.9                  |
| T5        | 10.0a                         | 11.3a                  | 15.4a             | 14.3a                          | 25.6                         | 23.9                      | 184.2                  | 223.6                 |
| T6        | 8.0b                          | 7.5b                   | 12.4b             | 11.7bc                         | 20.7                         | 19.4                      | 129.6                  | 163.2                 |
| T7        | 8.3b                          | 8.7b                   | 11.8c             | 9.5c                           | 19.7                         | 15.8                      | 118.5                  | 114.4                 |
| T8        | 8.3b                          | 7.0b                   | 13.4a             | 12.0ab                         | 22.3                         | 20                        | 147.8                  | 170.9                 |
| CV (%)    | 15.8                          | 13.8                   | 3.8              | 10.1                           |                              |                           |                       |                       |
| LSD at 5% | 2.5                           | 1.8                    | 1.25             | 2.05                           |                              |                           |                       |                       |

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Figure 1 Promotion of bottle gourd production using Trichoderma-enriched bio-organic fertilizer application in field [T1 - standard dose of NPK fertilizer and T2 - 3kg/pit sole/total application + (25% N of T1, 100% PK of T1)].
variable responses in cucumber, loofah and bitter gourd. Increase yield of wheat was found similar to the findings of Sallam et al., 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. 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 1201.70g/plot) in first and second year, respectively) of bottle gourd was recorded in T3 treatment in both years followed by T6 (1024.70g/plot) treatment. On the other hand the lowest shoot dry weight was recorded in T1 in both years. Like shoot weight, the highest root dry weight (15.33g/plot) was noticed in T1 treatment in first year and T6 (19g/plot) in second year. But statistically similar root dry weight of T9 was observed in T1 treatment in second year. The lowest root dry weight was found in T1 and T3 in first year, and T9 in second year. Conversely, both first and second year the highest total dry matter 1118.03 and 1220.36g/plot of bottle gourd was recorded in T1 treatment, respectively. The second highest was recorded in T9 of first year and second year in T1 and T9 treatments. The lowest record was achieved in T1 in both year also. Like other parameters the root and shoot weight was influenced significantly and attained maximum at T1 treatment. Similar amount of Trichoderma-enriched bio-organic fertilizer but different application mode in treatment T9 provided second height total dry weight as T1 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. 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 and the observed effects of Trichoderma in promoting lateral root development is similar to in vitro experiments performed by Hexon et al. 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. Similar trend of results was noticed in corn shoot dry weight using T. harzianum.

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      | 2nd year      | 1st year               | 2nd year                | 1st year | 2nd year |
|           | (2014/2015)   | (2015/2016)   | (2014/2015)            | (2015/2016)             | (2014/2015) | (2015/2016) |
| T1        | 126.7h        | 192.7g        | 10.3d                  | 11.3c                  | 137.0h   | 204.0g   |
| T2        | 1024.7b       | 1002.7b       | 6.3f                   | 10.6c                  | 1031.0b  | 1013.4b  |
| T3        | 902.7d        | 803.0e        | 8.3e                   | 7.0e                   | 911.0d   | 810.0e   |
| T4        | 801.7f        | 902.7d        | 6.3f                   | 10.0cd                 | 808.0f   | 912.7d   |
| T5        | 1102.7a       | 1201.7a       | 15.3a                  | 18.7a                  | 1118.0a  | 1220.4a  |
| T6        | 844.0e        | 700.0f        | 14.0b                  | 19.0a                  | 858.0e   | 719.0f   |
| T7        | 726.0g        | 983.3c        | 9.0e                   | 8.3de                  | 735.0g   | 991.6c   |
| T8        | 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 T9 but which was statistically similar to all Trichoderma-enriched bio-organic fertilizer added treatments except T4 (Table 7). Significantly (P<0.05) the lowest number of fungal population was monitored in T1 i.e. control treatment. At treatment T9 (i.e. standard dose of NPK fertilizer application) significantly higher number of fungal population than the T1 but lower number than T6, T5 and T9 treatments. For bacterial population, the highest number was monitored in treatment T4, which was statistically alike to T6, T5 and T9 treatments. Statistically the second highest bacteria population in rhizosphere soil was noticed T1 and T6 treatments. The lowest number of bacterial population was recorded in treatment T9, which was statistically similar to T1 treatment. In the present experiment, the dose of Trichoderma-enriched bio-organic fertilizer along with inorganic fertilizers in T1 and T9 was same i.e. 2kg/pit of

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

BOF, similarly in T₃ and T₄ 2.5kg/pit of BOF; and in T₁ and T₂ 3kg/pit of BOF. Only mode of application of BOF was different. In treatments T₂, T₃ and T₄, the total amount of BOF was applied at planting time. Conversely, in treatments T₁, T₅ and T₆, 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 T₃ and T₄ 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.44 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.51,42

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/cfug³) of soil | Fungi | Bacteria |
|-----------|-----------------------------------------|-------|----------|
|           |                                        | 1     | 5        |
| T₁        | 8.50×10⁶d                               | 2.47×10⁶d |          |
| T₂        | 14.61×10⁹c                              | 3.06×10⁹c |          |
| T₃        | 17.33×10⁹abc                            | 5.13×10⁹b |          |
| T₄        | 15.66×10⁹bc                             | 4.55×10⁹bc |         |
| T₅        | 18.33×10⁹ab                             | 6.94×10⁹a  |         |
| T₆        | 18.33×10⁹ab                             | 7.36×10⁹a  |         |
| T₇        | 18.00×10⁹abc                            | 6.96×10⁹a  |         |
| T₈        | 20.00×10⁹abc                            | 7.17×10⁹a  |         |
| 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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