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Research Article

The Effects of Compost Based Biofertilizer on Eggplant (*Solanum melongena* L.) Growth

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- Rapid composting process; Cellulolytic nitrogen-fixing bacteria; Peat carrier; Biofertilizer; Eggplant

Abstract

The research work comprises two parts. The first part deals with rapid composting process utilizing cellulolytic nitrogen-fixing bacteria, including *Azotobacter beijerinckii*, *Azotobacter vinelandii* and *Lysobacter* sp. on different types of composting materials. In Control, straw was used as composting material. The second Treatment 1 (T-1), straw, water hyacinth and cow dung were used as composting materials, while Treatment 2 (T-2), straw, water hyacinth and cow dung were used as composting materials and treated with cellulolytic nitrogen-fixing bacteria for rapid composting. The process was completed in 16 weeks (four months), 8 weeks (two months) and four weeks (one month) respectively which showed T-2 took a short duration of one month. During the process, variation in temperature, pH, volume, moisture content, C:N ratio were observed in different treatments. In the second part, the finishing products of T-2 was used as a peat carrier for formulation of biofertilizer named as "Test" and the effectiveness of this formulated biofertilizer on the growth and yield of eggplant (*Solanum melongena* L.) was conducted. In this formulated biofertilizer, the three cellulolytic nitrogen-fixing bacteria, *Sacchromyces cervisiae* and *Bacillus megaterium* were used. The "Test" biofertilizer showed its effectiveness in the yield of eggplant fruits compared with the yield using formerly formulated biofertilizer with highly significant difference level ($p < 0.2$).

INTRODUCTION

Myanmar is an agricultural country, with a total of 18 million hectares of cultivable land, and out of which about nine million hectares are under crop cultivation [1]. The demand for fertilizer is therefore very high. The currently used agricultural inputs are mostly chemicals. Long-term use of chemical fertilizer has been affected the environment by pollution and soil degradation. Organic farming in Myanmar is going to increase. The use of biofertilizer is an alternative to improve the structure of soil. Biofertilizers do not contaminate the soil and atmosphere; however, it helps to produce healthy foods [2]. The compost materials supply the requirements of plants for better growth and production. For biofertilizer preparation, compost also plays an important role as peat carrier system.

Crop residues are generated in large quantities and constitute an abundant but underutilized source of renewable biomass in agriculture [3]. Half the quantity of agricultural crop residues thus produced is used as roofing material, animal feed, fuel and packing material, while the other half is disposed of by burning in the field. Burning agro-residues in the field is considered a cheap and easy method of disposal of excess residues. This practice appends to air pollution, increases soil erosion and decreases the efficacy of soil-applied herbicides like isoproturon [4]. Moreover, it also causes health problems and increases the fog incidences even in distant cities [5]. Direct incorporation of agro-residues like rice straw in field solves the problem of air pollution, but it is not feasible due to the short time gap between harvesting of rice and sowing of wheat, besides the additional cost of labor, irrigation and extra tillage [6]. Observations of long term experiments indicated that though incorporation of agro-residues in soil significantly was improved soil fertility [6-9]. Moreover, it decreases the subsequent crop yields due to production of microbial phytotoxins and allelochemicals [10], and immobilization of the available nitrogen [11]. Incorporation of agro-residues like paddy straw increases the CH_4 emission from field [12,13], especially in irrigated soils, which in turn adds to the impact of global warming. Composting of agricultural residues through the action of lignocellulolytic microorganisms is easier to manage and it recycles the lignocellulosic waste with high economic efficiency. The recycled material when applied to soil, improves soil fertility and health. Composting is the biological degradation and stabilization of organic substrate under conditions that allow development of thermophilic temperature as a result of biologically produced heat [14].

The objectives of this research are (1) to produce finished products of compost within a short duration using soil microbes and (2) to observe the effectiveness of newly formulated biofertilizer based on the compost on the growth and yield of eggplant (*Solanum melongena* L.).

MATERIALS AND METHODS

Location of site and availability of materials

Tested area was located in Biotechnology Research

Department, Kyaukse, Mandalay Division, Myanmar. The tested area was 0.02 hectare. Materials for composting were paddy straw, water hyacinth and cow dung. *Azotobacter beijerinckii*, *Azotobacter vinelandii, Lysobacter* sp., *Bacillus megaterium* and *Sacchromyces cerevisiae* which were used in this research were provided by the Microbiology Laboratory, Biotechnology Research Department, Kyaukse.

Experiment 1 - Rapid composting process:

a) Process of the compost preparation: The basic model of compost bin has the standardized size of one cubic-meter-built with bamboo fencing screens. The inner layer of bin was lined with plastic sheet to conceal the compost materials and protect from wind which tends to dry the materials. The rice straw and water hyacinth were chopped into small pieces (5 cm-size) to enable fast and efficient process. The chopped straw was heaped into a compost bin and named as control (T-0). For the treatment 1 (T-1) the chopped straw, water hyacinth and cow dung that it is sterilized by using autoclave with a ratio of 3:1:1 were thoroughly mixed on a plastic ground sheet and evenly spread into bamboo-fenced bin. For treatment 2 (T-2), chopped straw, water hyacinth and sterilized cow dung using autoclave were prepared as T-1. The mixed materials were equally divided into four quarters. Firstly, a quarter of mixed materials were evenly spread into a compost bin. Then cellulolytic nitrogen-fixing bacteria, *Azotobacter beijerinckii*, *Azotobacter vinelandii* and *Lysobacter* sp. Broth was sprinkled in the ratio of 15 ml/kg and covered with another quarter. This pattern of mixed materials and a single sprinkled broth was repeated until all materials were filled in the bin.

Composting bins were normally made to be damp but not soggy. The moisture content of compost for all treatments was prepared at 75 % after organic wastes have been mixed. Thermometer was inserted into the heap for each treatment and bins were covered with black plastic sheet to retain moisture and heat. Bins of different treatments were left in the same environmental conditions and same maintenance of piles was given for all treatments. Three replications for each treatment were made for statistical confirmation. Turning the heaps up and down for different treatments and water sprinkling were done to supply the need of aeration and moisture.

b) Physical and chemical analyses: Analyses of composting materials dealing with physical and chemical were made regularly. Physical analysis deal with temperature, volume and moisture changes for each treatment were made and recorded at five days intervals. Weekly chemical analysis of compost materials such as Carbon Nitrogen ratio (C: N), nitrogen, phosphorus and potassium (NPK), moisture and pH were made once in every week to determine the rate of composting for different treatments. The nitrogen content in the peat soil was determined by Kjedahl Method. The organic carbon content of soil was determined by dry combustion (loss on ignition (LOI)), [15]. The examination for persistence of cellulolytic nitrogen-fixing bacteria throughout composting time in each treatment was made once per two weeks for biological analysis.

c) Preparation of peat: The composted materials were made into peat. For peat package, the composted material and burned paddy husks were ground into powder and mixed thoroughly in 1:1 ratio. Sugar, T-super phosphate and slaked lime were added into the mixed powder in the ratio of 10, 2 and 10 grams respectively per kilogram package. Then, they were packed into plastic bags and autoclaved at 121°C for 30 minutes to wipeout unnecessary microbes. The net weight for one peat package was 1 Kg.

d) Preparation of biofertilizer: The optimum growth of each bacterium (such as *Azotobacter beijerinckii*, *Azotobacter vinelandii, Lysobacter* sp and Sacchromycese cerevisiae) broth culture was collected and injected into respective named packages of peat carrier in 1:25 ratio (i.e. 40 ml of broth culture to 1 Kg of peat). Moisture adjustment for 40% was made carefully and packages were stored in a cool and dry place (20-27°C) for further experiments.

Experiment 2 - Field trial for study on the effect of compost-based biofertilizer :

a) Experimental design: To find the effect of the present compost and formulated biofertilizer "Test", field trial was conducted on eggplant at cultivating plot. A total of four- furrowed experimental plots were established manually. Each furrow was 5.48 m long and 0.61 m wide, with 0.91 m in between furrows. Each furrow contained six seedlings and the space between seedlings was 0.91 m. Manual elimination of weed control was done daily throughout research period. The experiment was conducted for three months. Four treatments with three replications conducted in which biofertilizer were as follows; Plants without fertilizer treatment were designated as Tr-0 (Control) while Tr-1 was treated with compost only. Tr-2 and Tr-3 were treated with biofertilizers of presently formulated "Test" and formerly formulated "A+10" (which contains four nitrogen-fixing bacteria (*Azotobacter chroococcum, Azotobacter beijerinckii, Azosperillum* sp. and *Acetobacter* sp.), four phosphate-solubilizing bacteria (two strains of *Bacilluas megaterium* and two strains of *Bacillus polymyxa*) , one photosynthetic bacteria (*Rhodo pseudomonas* sp.) and *Sacchromycese cerevisiae*), respectively.

b) Collection and analysis of the soil samples: The conditions and chemical characteristics of the soil were determined before and after cultivation period. Soil samples were obtained, and analyses were determined at the Myanmar Science and Technological Research Department.

c) Treatment with biofertilizer: Respective biofertilizers were applied to designate field plots. Ten grams per plant of respective biofertilizer was applied to treatment plots at two weeks interval regularly up to the time of harvest. Respective peat carrier was evenly spread to the base of plant.

d) Determination of plant growth parameters: Regular measuring for different treatments was done at every two weeks interval initiating day after transplant (DAT) till the day after harvest (DAH). Weekly harvested weight of fruits for different treatments were recorded and interpolated up to final harvest. Data for actual mean weights of total fruits for different treatments were also recorded.

e) Data analysis: Data of growth parameter was analyzed statistically using the method of Analysis of Variance (ANOVA). Yield was calculated in kilogram per acre for each treatment.

RESULTS AND DISCUSSION

Experiment 1

Rapid Composting Process: To prepare the qualified broth, the optimum growth of specific microbes within respective selected media needs to be known. The approximate optimum incubation period of cellulolytic microbes for *Azotobacter beijerinckii*, *Azotobacter vinelandii* and *Lysobacter* sp. were 48 hours in Cellulose nitrogen free mineral medium, while *Bacillus megaterium* in mc inimal medium and *Sacchromyces cerevisiae* in Peptone Yeast Glucose (PYG) medium with 48 and 42 hours respectively in their respective culture medium (and was ready for composting process (Figure 1). The composting process was completed in 4 weeks (1 month) for T-2, 8 weeks (2 months) for T-1, 16 weeks (4 months) for control respectively. The result obtained from T-2 showed complete compost in process within 4 weeks. This may be due to the dual actions of cellulolytic and nitrogen-fixing bacteria used in the experiment. Control treatment which lacks cellulolytic microbes took 16 weeks whereas T-1 took 8 weeks. The composting processes to be completed showed that the process has occurred only by the action of naturally occurred microbes. Control experiment lacks nitrogen source (absence of water hyacinth and cow dung) and this factor may delay the activities of microbes. The rapid composting in T-2 could be due to these dual actions.

Moisture is necessary to support the metabolic activity of the microorganisms. Sharma et al., stated that "Composting materials should maintain moisture content of 40–65% if the pile is too dry, composting occurs more slowly, while moisture content in excess of 65 percent develops anaerobic conditions [16]. In practice, it is advisable to start the pile with moisture content of 50–60%, finishing at about 30 percent". Data obtained from present research showed that moisture content was within the limit as stated by Sharma et al. The moisture content in T-2 was 75% at the commencement of the process and the finishing content being 50% (Figure 2). The temperature in control varied from 25-38°C, in T-1 from 30-48°C, and in T2-2 from 31-50°C. High temperature in T-1 and T-2 may be due to emergence of heat generated from the compost by the action of bacteria. The peak temperature of T-2 reached earlier than other treatments, which referred to its faster decomposing. The highest temperature was of 50°C in T-2 treatment in the present study (Figure 2).

Sharma et al., stated that although the natural buffering effect of the composting process lends itself to accepting material with a wide range of pH, the pH level should not exceed eight. At higher pH levels, more ammonia gas is generated and may be lost to the atmosphere. The pH of compost varied between 6.5-7.7 in control, 6.0- 7.5 in T-1 and 6.0-7.3 in T-2 respectively (Figure 3).

Decomposition is the fastest at the thermophilic stage. While composting high C:N ratio material like paddy straw, wheat straw etc, it was observed at the Indian Agricultural Research Institute (IARI) that temperature did not go beyond 52°C which indicated that temperature rise depends upon the type of material used for composting. The present result of maximum temperature was in agreement with Mathur's and this cause of rise in temperature may be due to activities by bacteria [17].

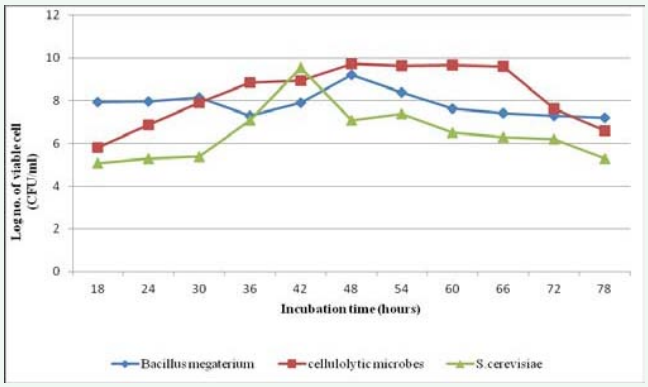

Figure 1 Optimum Growth Rate of Supplemented Microbes (CFU/ml) in their Respective Culture Medium.

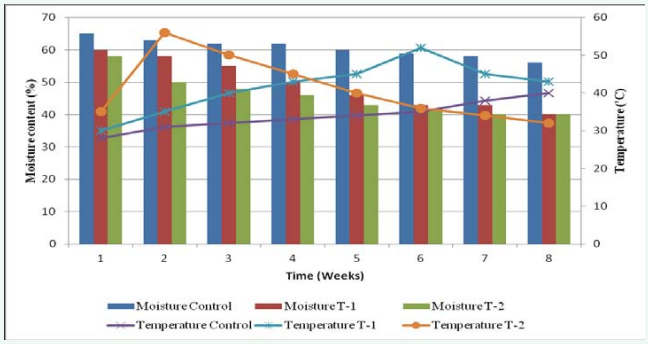

Figure 2 Graph Showing Variation in Moisture content (%) and Temperature (°C) in Different Treatments.

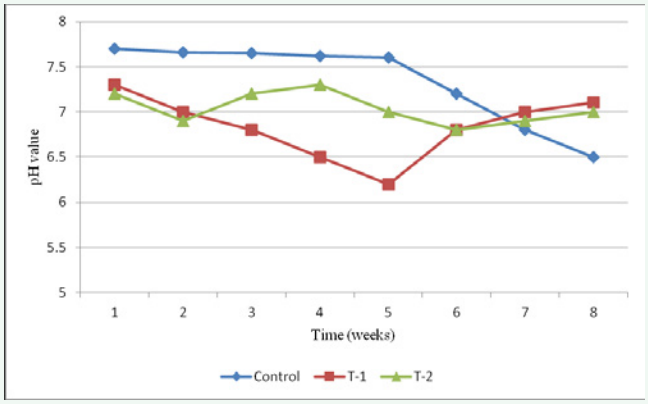

Figure 3 Graph Showing Variation in pH during composting process.

Volume changes among composting treatments was the visible parameter, in which initial volume was considered as 100%. Gaur [18], stated that "The composting involves decrease in the organic carbon content of the raw substrates, because with the decomposition of organic matter carbon is evolved as CO_2 and the biomass/volume of the compost is decreased, as a consequence of the decomposition the C:N ratio of organics is lowered down". In the present work the volume in control commenced with 100% and dropped to 60%, T-1 dropped

from 100% to 26% whereas T-2 dropped from 100% to 19% respectively. Among the treatments volume in reducing of T-2 was markedly seen from third week and was earlier than those of control and T-1.The initial ratio of C:N in T-2 was 70:1 and within 4 weeks it was reached 13.4:1. In T-1 it was reached 15.3:1 in 8 weeks and in control it was reached 21.47:1 in 16 weeks (Figure 4) and (Table 1).

The effectiveness of cellulolytic nitrogen-fixing microbes on C:N ratios of composting materials was determined and analyzed weekly throughout the research period time. Field experiments showed that the utilization of cellulolytic nitrogen-fixing microbes caused the composting materials to decompose rapidly. The composting time of T-2 was more rapid, four times faster than that of control and two times to that of T-1. The initiated ratio C:N of T-2, 70:1 reached 13:4 within 4 weeks (one month) while in control lasted for 16 weeks (4 months) to reach 21.47, and in T-1 it reached at 15:3 in 8 weeks (2 months). A compost is considered as complete when the C:N ratio has the value reaching to 20:1 [19]. In many instances it can be reduced to 15:1 or in extreme cases it may be as low 10:1 comparable to C/N ratio of soil humus [18]. In agreement to that, it was suggested that the supplemented microbes were in coordination with microbes of cow dung resulting in rapid composting process. Microorganisms require C, N, P and K as the primary nutrients. Of particular importance is the C:N ratio of raw materials. The optimal C:N ratio of raw materials is between 25:1 and 30:1 although ratios between 20:1 and 40:1 are also acceptable. Where the ratio is higher than 40:1, the growth of microorganisms is limited, resulting in a longer composting time. A C:N ratio of less than 20:1 lead to underutilization of N and the excess may be lost to the atmosphere as ammonia or nitrous oxide, and odor can be a problem. The C:N ratio of the final product should be between about 10:1 and 15:1 [16]. In the present research though the initial C:N ratio was nearly three times higher than that of Sharma's statement, the final product achieved within four weeks was within the suggested range. This factor was clearly shown that microbes used in this study actually described its effectiveness on decomposition.

The maintenance of five species in peat carrier, the initial colony forming unit (CFU)/g of 2.08 x 10^9 slowly dropped to 4.4 x 10^8 at 8^{th} week and rapidly dropped to 4.4 x 10^6 at 10^{th} week of storage. Again, a rise of 1.6 x 10^7 was observed on 18^{th} week and later deteriorated (Figure 5).

The ability to preserve a sufficient population of inoculated microorganisms until using is the most important quality of the carrier system. This means that the quality and shelf life of a biofertilizer should be determined by how many viable cells are in carrier and how well they survive during storage. It is a common perception and thus the viability of inoculated microbes was determined. The initial was 2.08x10^9 and a rapid loss of viability to 4.4 x 10^8 beyond 8^{th} week of storage. However, at that time of 18^{th} week of storage viable cell rose up again to 1.6 x 10^7 and rapidly dropped to 4.8 x 10^5 at 20^{th} week. The writer assumed that for effective use of presently formulated biofertilizer should be applied within 8 weeks of manufacturing date.

Experiment 2

Field trial for study on the effect of compost-based biofertilizer: The Nitrogen content of soil sample for Tr-2

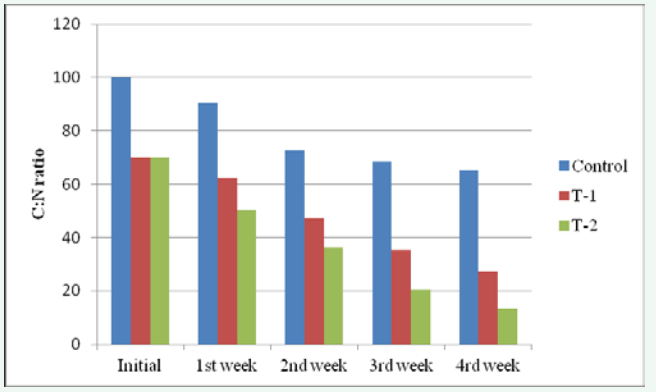

Figure 4 C: N Ratio of Various Treatments.

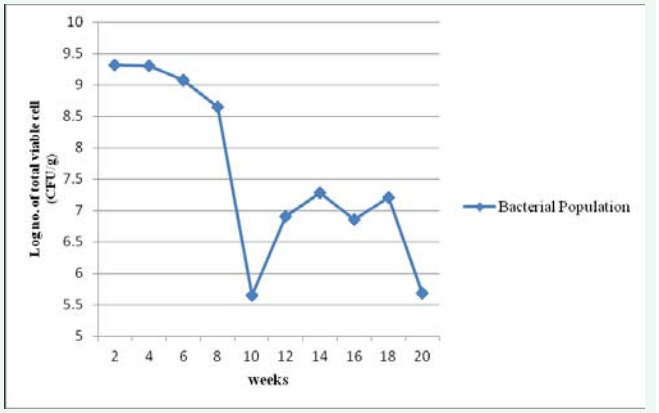

Figure 5 Persistence of Total Microbial Population in Formulated Biofertilizer.

showed the highest (0.41%) among the other treatments whereas the amount of K showed almost the same value. The bacterial growth parameter of soil (8.4 x 10^7) was shown the best among the treatments.

The initial soil analysis together changes in total nitrogen, potassium and phosphorus contents of cultivated soils were determined before and after cultivation. Results indicated that the cultivation plot has a very low organic carbon, nitrogen and potassium contents. It has been observed that the pH of all treated soils showed pH of 6.0 – 6.5 beyond cultivation when compared with the control one. The preferred pH for eggplant is 5.5-6.0 and strongly acidic soils (pH<5.0) are unsuitable [20]. Nitrogen content of soil sample from plants treated with the new formulated "Test" (Tr-2) was higher than other treatments. The effectiveness of this formulated biofertilizer was also observed to be the best in growth and yield of eggplant compared to other treatments.

The mean plant height at the time of harvesting (10^{th} week of harvest) showed 49.66 cm in Tr-0, 73.1 cm in Tr-1, 77.8 cm in Tr-2, and 81.0 cm in Tr-3 respectively, in which Tr- 3 showed the best result (Figure 6). However, the total yield of eggplant fruits at the end of the harvest showed 14.55 kg in Tr-0, 41.35 kg in Tr-1, 49.32 kg in Tr-2 and 33.67 kg in Tr-3 respectively in which Tr-2 showed the best of all treatments (Figure 7).

Table 1: Changes in C:N Ratio During Composting Process.

Duration	Control	T-1	T-2
4rd week	65.4	27.3	13.4
5th week	60.1:1	25.2:1	
6th week	57.2:1	22.6:1	
7th week	53.4:1	18.4:1	
8th week	49.6:1	15.3:1	
4th month	21.47:1		

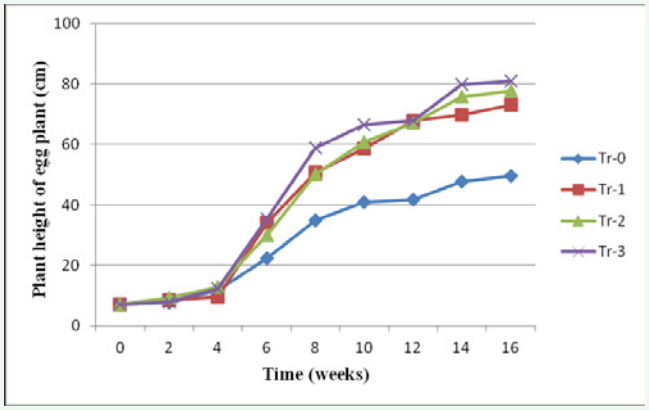

Figure 6 Mean Plant Height of Egg Plant in Different Treatments.

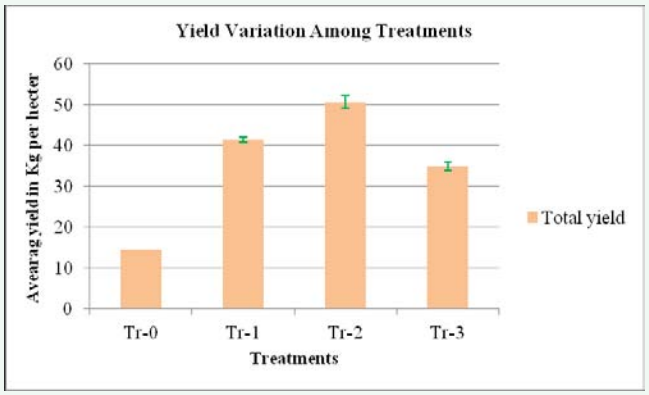

Figure 7 The Effectiveness of Biofertilizer on Eggplant Fruit.

The effect of inoculation was determined by counting how many viable cells are in inoculated soil after application. It has been observed that the best total soil bacterial growth parameter in the present research "Test" fertilizer-fed (Tr-2) soil sample showed the best bacterial growth parameter. Purely compost-fed soil (Tr-1) and A+10 treated soil (Tr-3) showed the second and third respectively.

Plant height of eggplant (*Solanum melongena* L.) usually grow 40 to 150 cm [20]. The maximum mean height of 77.8 cm was observed in this trial. Among inoculated treatments Tr-3 showed the best mean plant height compared with Tr-1 and Tr –2. The initial mean plant height was 7.2 cm. The plant height in Tr-0 reached 49.66 cm, Tr-1 73.1 cm, Tr-2 77.8 cm, and 81.0 cm in Tr-3 respectively. The mean plant height of Tr- 1, Tr-2, and Tr-3 were significantly higher at p-value < 0.2 when compared with Tr-0.

The total yield of eggplant at the end of 10th week of harvesting showed 14.55 kg in Tr-0, 41.35 in Tr-1, 49.32 in Tr-2 and 33.67 in Tr-3 respectively. The total mean weight in Tr-2 was significantly higher than in Control (p < 0.2), T-1 (p < 0.5) and Tr-3 (p = 0.5).

CONCLUSION

The cellulolytic nitrogen-fixing bacteria, namely *Azotobacter beijerinckii* and *Azotobacter vinelandii*, and *Lysobacter* sp. worked well in the rapid composting process with the duration of 4 weeks in T-2 compared to other two treatments which took 16 weeks in control, and 8 weeks in T-1. It is therefore suggested and recommended that the cellulolytic nitrogen-fixing bacteria used in present composting worked well and produced a finishing product in an environmental friendly way and is a suitable process need to be applied by farmers and agriculturists.

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