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Evaluation and Effect of Vermiwash on Growth, Yield and Chemical Analysis of Seeds in Cluster beans (*Cyamopsis tetragonoloba* (L.) Taub)

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A B S T R A C T

In the present investigation, a pot level experiment was carried out during *Kharif* 2021 from September to November at SRR and CVR Government Degree College; Department of Botany and Microbiology, Vijayawada on Cluster beans (*Cyamopsis tetragonoloba* (L.) Taub Var Pusa Navbahar) to access and calculate the effects of different concentrations of organic biofertilizer-Vermiwash. Earthworms such as *Eisenia foetida* are introduced and physico-chemical analysis of vermiwash is done at the laboratory level. The results revealed that at T4 (30% of Bagasse) there is extreme significant increase in higher growth and yield parameters such as number of leaves, total leaf area, number of branches, length of pod, number of pods per plant, number of clusters per plant, number of pods per cluster, number of seeds per pod, fresh seed weight, dry seed weight and total harvest. On the other hand, the chemical analysis of seed had shown an increase value in T4 when compared to T1 treatment. Therefore, application of 30% of vermiwash (Bagasse) as both foliar and soil application had showed a positive impact on various growth parameters.

Introduction

Cluster beans (*Cyamopsis tetragonoloba* (L.) Taub) is an important annual legume crop that belongs to order fabales and family Fabaceae. It is commonly called as guar, chavli kayi in some parts of the world. It grows in well drained sandy loam soil at pH of around 7.5 ± 0.5. These grow around 60cm in height, carry out self-pollination and leaves are trifoliate pinnately compound. It can be grown in arid and semi-arid regions of the world. Gaur is the most important vegetable, on the other hand it is grown for industrial purpose because of its endospermic gum (Shabarish rai *et al.*, 2012). It has deep rooted system and can grow well in both summer and rainy seasons for its young tender green pods. Pusa Navbahar is considered as the most popular variety of all types of cluster beans (Dholariya *et al.*, 2018). According to (Selvarani *et al.*, 2021) Pusa Navbahar is cross between the local cultivator of North India Pusa Mausami and local cultivator of Rajasthan Pusa Sadabahar. It can grow,

sustain in high salinity of soil and in drought regions. The pods are usually longer of 15cm and of better quality. The pods that are grown consist of low source of energy for every 100gm of edible portion i.e., energy (16kcal) protein (3.2g) fat, (1.4g) carbohydrate, (10.8g) vitamin A (65.3IU), vitamin C (49mg), calcium (57mg) and iron (4.5g) (DodlaRajashekar Reddy *et al.,* 2017) The crop is usually grown in *Kharif* which leads to the annual production of around 2 million metric tons.

In addition to this it contains high fiber content and ultimately low level of cholesterol and fats. It has low glycemic index in which, when eaten by diabetic patients will be benefitted. Taking of cluster beans in our daily diet can reduce Alzheimer's disease due to the high presence of flavonoids, tannis, saponins, and triterpenoids (Lalitha *et al.,* 2021). Growing of guar crop is not only used for vegetable but also gum is extracted from its seeds and is used in many Industrial activities such as textiles, food cosmetics, paper and pharmaceutical Industries (Abidi *et al.,* 2015; Mudgil *et al.,* 2014; Vaughn *et al.,* 2011 and Lubbe *et al.,* 2011).

Apart from this, Biofertilizers play an important role in increasing the availability of nitrogen and phosphorous besides increase in biological nitrogen fixation (Rana *et al.,* 2006). Vermiwash is a rich active biofertilizer that was often obtained from the fecal casts of earthworms. The excreta of earthworms is a enriched nutritive organic biofertilizer that is rich in humus, nitrogen fixing bacteria and many growth hormones such as auxins and cytokinin's (Sinha *et al.,* 2010). It is a liquid fertilizer that was obtained by passing a layer of soil and is enriched with micro and macro nutrients. (Ismail SA. Vermicology., 2005).

According to (Ismail SA. Vermicology, 2005) it is a clear pale yellow colored fluid. Moreover, according to (Kale *et al.,* 1986) it is considered as tonic and the plant is beneficial in having huge number of macronutrients, enzymes and hormones. According to previous studies conducted by (Tiwari and Sinha, 2016) they reported that different combinations of vermiwash obtained from cow dung with the biopesticides, neem oil, aqueous extract of leaf, bark and vermiwash alone caused the significant growth and started early flowering on yield of tomato crop. Hence vermiwash can employ productively for the crop production at low input (Edwards *et al.,* 2004)

To consider all things the main objective of the present study was to evaluate the physical and chemical properties of Vermiwash, along with different dilutions and to understand the growth yield and chemical seed analysis of cluster beans.

Materials and Methods

Soil preparation and Collection of Seeds

A pot level experiment was carried out during *Kharif* season from September to November 2021. The enriched soil was collected from the local nursery, thoroughly mixed and filled into the pots up to the brim. The seeds that were used to carry out the experiment were collected from Taiyo Gold Agri Biotech India Pvt. Ltd (Hyderabad).

Experimental design and Seed sowing

The present experiment was carried out in Factorial Randomized Complete Block Design (FRCBD) with three factors as Bagasse, Cow dung and controlled and is replicated four times respectively. The various compositions and treatments that were carried out during this experiment is tabulated in Table 1. The seeds that are collected were soaked overnight for the better germination and were sown 1 inch each. Moreover, seed to seed spacing is about 25cm*15cm.Two Healthy plants were tagged from each pot and the data was recorded successively.

Collection of Wastes and Earthworms

The animal waste (cow dung) was collected freshly every week from Desi cow at NTR College of Veterinary Sciences-Gannavaram. On the other hand the kitchen wastes was collected from the daily usage of household vegetable and fruit waste for

about 2 months. Most importantly Earthworms that were used in this experiment are *Eisenia foetida* and were collected from Haritha organic vermicompost – Nuzividu and were directly placed in Vermiwash units of 100 each.

Vermiwash units

The experimental setup of vermiwash units was carried out by usage of four columns. A hole was drilled for the container and a plastic tap was fixed at the lower side of the container. The unit is kept on a bucket at certain height for the easy collection of vermiwash. At the base of the container i.e., about 20cm a handful of pebbles were placed. On top of that coarse sand was placed of about 25cm.thereafter water was allowed to pass through the layers to enable the setting of the unit.

A 20-30cm thick loamy soil bed was prepared for filtration. Cow dung and Bagasse that was collected from specified areas were placed on the top of soil.

Earthworms were then introduced into the soil in all the containers and a layer of mulch is placed on top of it and is splashed with water for every two days to keep them moist. After about six weeks worm castings were formed in the soil, and were collected by slow passage of water through the Vermiwash units. Vermiwash units and the collected vermiwash were displayed in (plate 2).

Physico-chemical Analysis of Vermiwash

To determine the Physico-Chemical analysis, vermiwash is analyzed with different compositions. The physical components such as pH, colour, odour electrical conductivity was analyzed according to the procedure followed by (Garg *et al.,* 2006).

A double water suspension of vermiwash was agitated in the ratio of 1:10 (w/v) for about 30 minutes and filtered through whatman No.1 filter paper. The chemical parameters of vermiwash with different compositions were observed and analyzed. The component such as the Total organic carbon was analyzed according to Nelson and Sommers (1982). Nitrogen was determined according to Bremmer and Mulvaney (1982). Total available phosphorus was determined by colorimetric method (Bansal and Kapoor, 2000). Total Potassium and all the essential micronutrients were determined by flame photometer (Bansal and Kapoor, 2000). The physico-chemical analysis of vermiwash was tabulated in Table 2.

Chemical analysis of Cluster beans seeds

The mature seeds from different treatments at the time of harvest were chemically analyzed in Concentrated Sulphuric acid and perchloric acid to examine different macronutrients. The chemical components such as the total Nitrogen was examined according to Bremmer and Mulvaney (1982) procedure. Total Phosphorous was determined by using a colorimetric method. Total potassium was assumed by an Atomic Absorption Spectrometer (ASA).

Calcium was analyzed by using an emulsion flame spectroscopy. Magnesium is analyzed by absorption flame spectroscopy. On the other hand, the seeds that were analyzed for micronutrients was digested in a complete mixture of nitric acid and perchloric acid. All the micro nutrients that were considered were analyzed by means absorption flame spectroscopy. The chemical analysis of cluster beans seeds that were analyzed are presented in Table 3.

Morpho-Physiological Parameters

Plant height(cm)

Height of the plant was measured from base to the tip of the main shoot with the help of measuring scale and the average height was expressed in centimeters.

Number of leaves

The leaves that were big in size and fully matured were taken and average was tabulated.

Number of branches

The total number of branches were counted and tabulated.

Total Leaf area of the plant(cm^2)

The leaves were collected and kept in polythene bags to avoid wilting and immediately brought to the lab. The total leaf area per plant was measured by using *LICOR 3100* leaf area meter. The total leaf area was expressed in terms centimetres per square meter (cm^2).

Yield parameters

Length of the pod

Pod length was measured form the successively tagged plants and tabulated.

Pod number

The fully matured pods from the plants were counted and tabulated.

Clusters per plant

The total number of pods bearing cluster per plant were counted at the time of harvest and tabulated.

Number of seeds per pod

The total number of seeds per pod were counted from the mature pod at the time of harvest and tabulated.

Fresh seed weight (mg)

The seed of the fresh mature pods was measured and weight was recorded in grams and tabulated.

Dry seed weight (mg)

The seeds that were obtained after the harvest were autoclaved for 36 hours and the weight was tabulated.

Yield/hectare (Kg/h)

Yield per hectare was measured by using the following formula.

$$\text{Yield/hectare} = \frac{\text{Plot yield (kg)} \times 10000}{\text{Plot size } (m^2)}$$

Statistical Analysis

The following data was completely analyzed by using analysis of variance (ANOVA) by following complete randomized block design (Gomez Gomez, 1989). The differences were considered significant at 5% level of probability

Results and Discussion

The Physico-chemical properties of Vermiwash was analyzed and the results were tabulated in table 2. The colour of vermiwash when different mixture such as bagasse is golden yellow and light yellow in cow dung. The physical parameters such as the pH is neutral having a range of 7.0 ± 0.3 and the Electrical conductivity is 1.1 ± 0.3 in both. Organic carbon is comparatively higher in bagasse when compared to cow dung with a value of 227ppm. Vermiwash was collected from an average of 30-40 days and when applied to plants as foliar spray and soil application had no harmful effects on plants.

The chemical parameters of vermiwash such as the nitrogen and potassium concentration had shown a good number of values and were almost higher in cow dung with a value of 49.1 and 61.25. On the other hand, the macronutrient such as the phosphorus with a value of 3.82 is higher in bagasse when compared to cow dung. All the other micronutrients that were analyzed had a permissible range and is considered as best tonic for plants. The results of chemical analysis of vermiwash have been tabulated in table 2.

The chemical analysis of cluster bean seeds was analyzed following the standard procedures. The

macronutrients such as NPK is having highest range in T4 (2.01,0.42,1.58). Moreover, the nutrients such as the phosphorous and potassium is highest in T4 followed by T3(0.38,1.44). Calcium is highest in T1(1.37) followed by T2(1.26). Magnesium is having its highest value in T4 (0.37) and the least is observed in T5(0.30). All the micronutrients that were analyzed had a high significant value in T4. The morpho-physiological growth parameters of cluster beans have been reported in Table 3. The different concentrations of vermiwash were measured successively at 15, 30, 45 and 60 days.

Plant height (cm)

Height of the plant had a drastic effect in T5 (92.2cm) at 60 days and the mean value ranged from 5.9 to 84.2cm which was successively followed by T4 (86.2cm) and the least was recorded in T3 (78.5cm) than the control plants.

Number of leaves

The different concentrations of vermiwash had a significant increase in number of leaves. It was varied from 3 to 56 with an average mean of 4.6 to 50.2. Apart from all the treatments the highest number of leaves was recorded in T4 (60). And on the other hand, the least number of leaves is recorded in T1 (42). On the contrary the number of leaves were almost similar in the initial days (Table 3)

Total Leaf Area per plant (cm^2)

The average leaf area is calculated from control and all other treatments and was presented in table 3.it was varied from 10.4 to 66.8 cm^2. Among all the treatments maximum leaf area was recorded in T4 (72.6cm^2) followed by T5 (60cm), T2 (57 cm). Moreover, the mean value ranged from 11.5 to 60.1. The minimum leaf area is recorded in T3 (48cm).

Number of branches

The total number of branches were counted and tabulated in table 3. The data showed a vast increase among different treatments and it was ranged from 1 to 18 of which T4 (20) has maximum number of branches while T1 (11) has minimum number of branches. The maximum number of branches might be due to the supplement of essential nutrients that is utilized by the plant.

Yield Parameters

Length of the pod

The length of the pod was highest in T4 (15.25cm) and least was observed in T1 (12cm). Length of the pod values ranged from 12.1 to 15.2 with an average mean value of 13.76. The comparative analyses of different treatments of vermiwash were placed in plate 7.

Number of pods per plant

The number of pods per plant was measured at the time of harvest and the data that was depicted (table3).

In the present study the highest number of pods per plant was recorded in T4 (64) and the least number of pods per plant was recorded in T2 (58) with average mean value of 51.2. But in contrary equal number of pods has been recorded in control and T3 treatment.

Number of clusters per plant

The number of clusters measured in the present study was tabulated in Table 3. The values ranged from 9 to 16. When compared to rest of the treatments T4 showed the highest number of clusters per plant followed by T5 and the least number of clusters per plant was recorded in T1.

Number of pods per cluster

The total number of pods per cluster was counted and tabulated in table 4. The activity of vermiwash with different treatments had shown a positive

impact with having highest number of pods per cluster in T4 with an average mean value of 4.6 and the least number of pods were recorded in T1. Organic formulations like vermiwash is accepted to have increased microbial activity and enzymatic production.

Number of seeds per pod

This character was ranged from 4 in control plants (T1) to 10 in T4 with an average mean value of 6.8. Data showed a vast significant difference in having highest number of seeds per pod in T4 (10), successively by T2 (8) and the least number of seeds per pod at the time of harvest was recorded in T1 (4).

Fresh seed weight (mg)

Total fresh seed weight is weighed immediately at the time of harvest. Among all the treatments the highest fresh seed weight was recorded in T4 (0.13mg), followed by T2 (0.10mg) with an average mean value of 0.09. Moreover, the least fresh seed weight was recorded in T1 (0.05mg).

Dry seed weight (mg)

The total dry seed weight had a significant difference among the treatments. The highest dry seed weight was found in T4 (0.12) followed by T2 (0.09) and the least was observed in T1(0.04) with an average mean value of 0.72.

Yield/hectare (Kg/h)

The total yield that was measured at the time of harvest was tabulated in table 3. Off all the treatments studied the harvest had an immense increase with different concentrations of vermiwash in T4 (1.12kg), followed by T2 (1.04kg) and the least was recorded in T1 (865gm) at 60 days. (Plate 6)Maximum pod yield might be due to vigorous vegetative growth with accelerated photosynthetic activities thereby increasing the supply of carbohydrates to the plants.

Globally, now a days excess use of chemical fertilizers and pesticides are causing an environmental hazard which are initially destroying the soil and many more. Vermiwash acts as a plant tonic, because it contains number of microorganisms, actinomycetes, enzymes, hormones and other nutrients. It increases disease resistance of plants against bacterial, viral and fungal diseases (Kale *et al.,* 1986). The epigenic earthworm *Eisenia foetida* is a suitable species for management of wastes which are utilized successfully in vermicomposting (Gunadi and Edwards, 2003; Chaudhari and Battacharjee, 2002; Garg *et al.,* 2003; 2004).

Hand *et al.,* (1988) have been already reported that Eisenia foetida in cow dung slurry increased the nitrate-nitrogen content. According to Lee (1992) the passage of organic residue through the gut of earthworm to the plant the released of phosphorus in available form is performed partly by earthworm gut phosphatases and further released of phosphorus might be attribute to phosphorus solubilizing microorganism present in worm cast. Benitez *et al.,* (1999) studied that the leachates collected during vermicomposting process had higher K concentrations. Similar kinds of results were obtained by Bawankar (2008) and Gorakh Nath and Keshav Singh in 2012 supporting our findings of present investigation.

Nitrogen content is seeds plays a vital role in plant growth and development. The increase of phosphorus concentration in the seeds improves phosphorus supplying for plants in early developing stages (Grant *et al.,* 2001). Potassium is predominantly found in seeds as free or adsorbed cation, and it may be easily translocated along cells or tissues in the plant (Lindhauer, 1985). Magnesium is the activator of enzymes that are related to the synthesis of carbohydrates, nucleic acids, and active enzymes. Moreover, determining the concentration of each and every nutrient for seed development is important for improving the fertility management and fertilization recommendations in seed production areas.

Table.1 Compositions and Treatments

Sl. No.	Compositions	Treatments
1	Control (Distilled Water)	T1
2	30% of Cow Dung (300 gm of Cow Dung +Earthworms of 100 each	T2
3	60% of Cow Dung (600 gm of Cow Dung + Earthworms of 100 each)	T3
4	30% of Bagasse (300 gm of kitchen waste + Earthworms of 100 each)	T4
5	60% of Bagasse (600 gm of kitchen waste + Earthworms of 100 each)	T5

Table.2 Physico-chemical analysis of Vermiwash

Sl. No.	Parameters	Units	Values	
			Cow Dung	**Bagasse**
1.	pH		7.32	7.15
2.	Colour		Golden yellow	Light yellow
3.	Odour		Odourless	Odourless
4.	Electrical conductivity		1.12	1.14
5.	Organic Carbon	ppm	215	227
6.	Nitrogen	ppm	49.1	47.2
7.	Phosphorous	ppm	3.54	3.82
8.	Potassium	ppm	61.25	53.67
9.	Calcium	ppm	20.91	25.42
10.	Magnesium	ppm	14.39	14.84
11.	Zinc	ppm	0.03	0.03
12.	Iron	ppm	0.82	0.56
13.	Copper	ppm	0.56	0.61
14.	Manganese	ppm	28.36	32.97
15.	Boron	ppm	0.15	0.17
16.	Molybdenum	ppm	0.006	0.02

Table.3 Chemical analysis of Cluster bean seeds

Sl. No	Parameters	Units	Values				
			T1	T2	T3	T4	T5
1	Nitrogen(N)	%	1.87	1.92	1.62	2.01	1.76
2	Phosphorous	%	0.37	0.35	0.38	0.42	0.32
3	Potassium	%	1.08	1.41	1.44	1.58	1.12
4	Calcium	%	1.37	1.26	1.19	1.24	1.18
5	Magnesium	%	0.33	0.35	0.32	0.37	0.3
6	Zinc	ppm	18.17	25.9	19.9	28.24	20.37
7	Iron	ppm	163.3	181.2	161.2	186.3	165.3
8	Copper	ppm	16.24	15.32	14.3	18.32	15.27
9	Manganese	ppm	46.31	50.21	49.5	67.54	52.31
10	Boron	ppm	37.32	59.31	48.6	58.32	38.07
11	Molybdenum	ppm	18.67	23.21	26.8	27.79	19.47

Fig.1

Plate 1 : Collection of Earthworms

Plate 2 : Vermiwash units and Vermiwash

Table.4 Effect of Vermiwash on Morph physiological parameters of Cluster beans (*Cyamopsis tetragonoloba* (L.) Taub)

Sl. No		Plant Height (cm)				Number of Leaves				Total Leaf Area (cm^2)				Number of Branches			
		15 Days	30 Days	45 Days	60 Days	15 Days	30 Days	45 Days	60 Days	15 Days	30 Days	45 Days	60 Days	15 Days	30 Days	45 Days	60 Days
1	T1	6.03	16.1	60.3	81.6	3	12	38	42	10.4	17.4	40.1	55.5	1	3	10	14
2	T2	6.09	18.3	60.7	82.4	4	14	46	48	11.5	21.1	42.4	57.1	1	9	15	16
3	T3	5.4	17.2	58.5	78.5	3	16	42	45	10.3	18.3	41.1	48.3	1	8	14	14
4	T4	6.22	22.4	68.3	86.2	7	18	48	60	12.3	56.4	66.4	72.6	2	10	17	20
5	T5	6.19	23.8	75.1	92.2	6	21	51	56	13.1	52.2	60.3	66.8	2	9	16	18
	MEAN	5.98	19.56	64.58	84.18	4.6	16.2	45	50.2	11.52	33.08	50.06	60.06	1.4	7.8	14.4	16.4
	SD	0.3	3.4	7.0	5.3	1.8	3.5	5.1	7.6	1.2	19.5	12.3	9.6	0.5	2.8	2.7	2.6
	CV	5.6	17.2	10.8	6.2	39.5	21.6	11.3	15.1	10.5	58.9	24.7	16.0	39.1	35.6	18.8	15.9

Table.5 Effect of Vermiwash on Yield parameters of Cluster beans (*Cyamopsis tetragonoloba* (L.) Taub)

Sl. No		Length of the Pod (cm)	Pod Number	Clusters per plant	Pods per cluster	Seeds per pod	Fresh seed weight (mg)	Dry seed weight (mg)	Yield/hectare(Kg/h)
1	T1	12.1	42	9	3	4	0.05	0.04	9.65
2	T2	14.2	58	13	4	8	0.11	0.09	1.22
3	T3	13.4	42	11	5	5	0.09	0.06	1.14
4	T4	15.2	64	18	6	10	0.14	0.12	1.32
5	T5	13.9	50	16	5	7	0.08	0.05	1.04
	MEAN	13.76	51.2	13.4	4.6	6.8	0.094	0.72	2.874
	SD	1.13	9.75	3.64	1.14	2.38	0.03	0.03	3.78
	CV	8.26	19.05	27.2	24.7	35.1	35.7	45.4	131.8

Fig.2

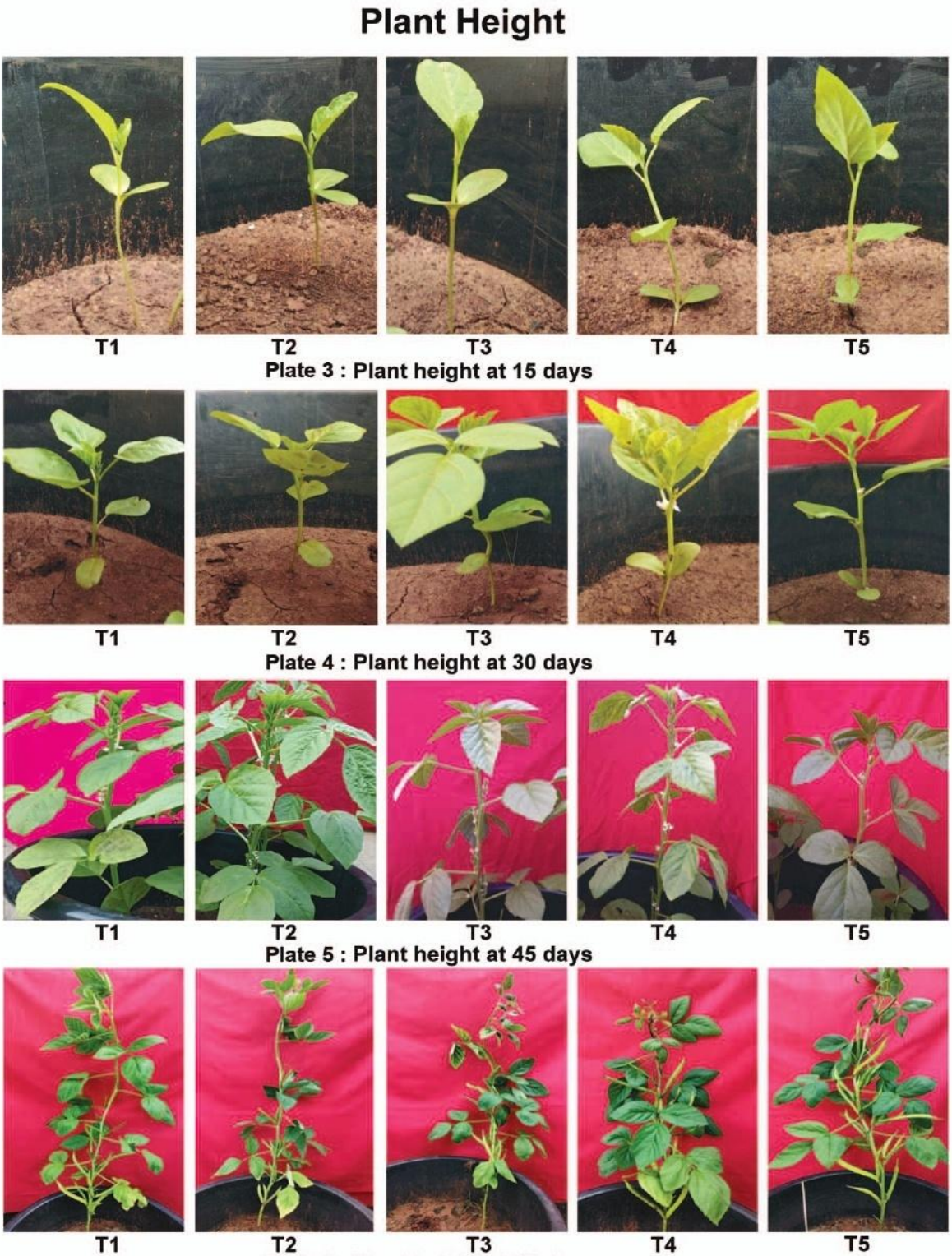
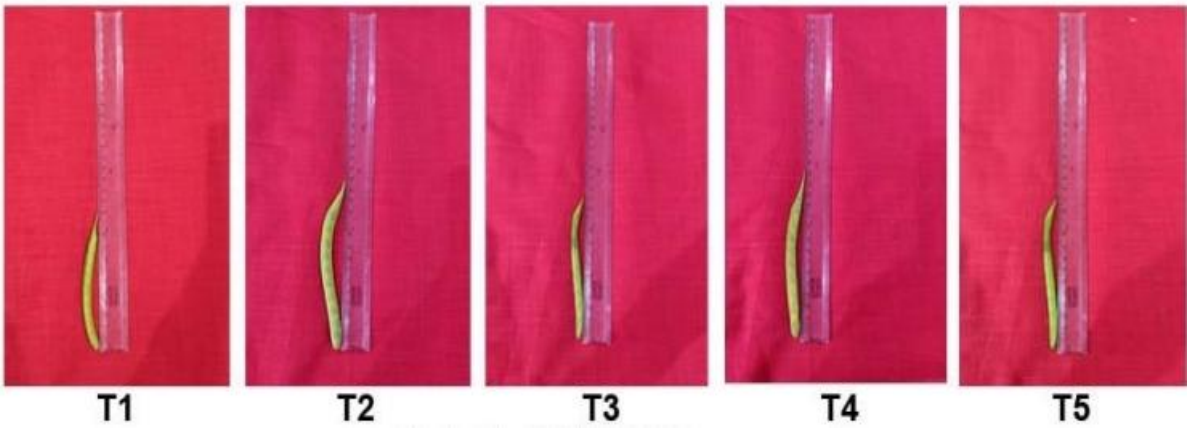

Plant Height
T1
T2
T3
T4
T5
Plate 3 : Plant height at 15 days
T1
T2
T3
T4
T5
Plate 4 : Plant height at 30 days
T1
T2
T3
T4
T5
Plate 5 : Plant height at 45 days
T1
T2
T3
T4
T5

Fig.3



Therefore, the morphophysiological parameters of cluster beans were tabulated in Table 3. To summarize according to (Sinha *et al.,* 2010) growth parameters showed an excellent result in increase of crop productivity and boost the fertility of soil. The morphological parameters such as the shoot length was found maximum in T5 with 92.2cm and the least was observed in T3 with 78.5cm. Our results coincide with (Cook *et al.,* 1980 and Tiwari *et al.,* 1989) that maximum shoot length is may be due to the increased availability of more exchangeable nutrients in the soil by the application of Vermiwash. Maximum number of leaves has been found in T4 and minimum was found in T1. Leaf area was found highest in T4 and on the other hand our observations with leaf area from the other studies on abelmoschus (Lalitha *et al.,* 1999) showed the similar increase in leaf surface area. Number of branches had shown a maximum number in T4. The yield attributes of cluster beans were tabulated in Table 4. According to (Zambare *et al.,* 2008) the application of vermiwash has shown a feasible growth in terms of cost, availability, time consuming, ecofriendly in nature and reliability. The usage of vermiwash that was extracted from different combinations and treatments from agro and kitchen wastes is an effective liquid biofertilizer for growth and productivity. The highest number of pods has been recorded in T4 as it probably shows the application of vermiwash absolutely increases the uptake and availability of nutrients and ultimately for the biosynthesis of protein. All the yield parameters were significantly higher in T4 such as number of pods per plant (64) number of clusters per plant(T4), number of pods per cluster (6), number of seeds per pod (10), fresh seed weight (0.14) and total yield(1.32kg). Therefore, our results coincide with the yield attributes of the present study (Ansari, 2008).

According to our findings, vermiwash at 30% of bagasse acts as an excellent enriched Biofertilizer to many farmers as it enhances the crop quality and ultimately the yield attributes. The analysis of vermiwash has shown a excellent mineral content for seeds and for the good growth of the plant. On the other hand, the essential elements such as macro and micro nutrients are present in abundant due to the epigenic earthworm *E. foetida* and this is utilized successfully due to deposition of fecal casts of earthworms in which it leaded to healthier plant growth and can gain momentum in all crops in the best way possible and leads us to an imperishable agricultural organic farming.

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