



# Response of Plant Growth Substances and Bio-enhancer on the Enhancement of Bulblet Growth under the *In-vivo* Condition of *Lilium*

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Lilies are one of the most extensively sold cut flowers due to their quantity and variety of colours [1]. This study aims at development of bulbs from microbulblets by the use of different bio-enhancers and growth substances to decrease the time for bulblet growth to bulb and ontogenic development. The experiment was carried out in the Department of Horticulture, Sikkim University in the year 2016-2017 to find out the effect of growth substances and bio enhancers on the enhancement of microbulblets *in vivo* condition of *Lilium* spp. This experiment was conducted using completely randomized block design (CRD) which was replicated thrice and the details of the treatments were as follows: T<sub>1</sub>-Paclobutrazol @10ppm, T<sub>2</sub>-Paclobutrazol @20ppm, T<sub>3</sub>-Paclobutrazol @ 30ppm, T<sub>4</sub>-Thiourea @10ppm, T<sub>5</sub>-Thiourea @20ppm, T<sub>6</sub>-Thiourea @30ppm, T<sub>7</sub>-Cow urine @10%, T<sub>8</sub>- Vermiwash @100%, T<sub>9</sub>- Vermiwash @50% and T<sub>10</sub>- Control. The treatments were sprayed at 15, 30 and 45 days intervals. Among all treatments used for the development of

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bulbs from microbulblets, the performance of the growth substance was found more effective. The treatment thiourea @30ppm (T<sub>6</sub>) and paclobutrazol 20ppm (T<sub>2</sub>) recorded maximum bulb weight (1.38g and 1.27g respectively while paclobutrazol @20ppm observed maximum bulb length (20.01 cm) and breadth (15.46 cm) after 90 days of the spray of treatment under. However, organic bio enhancers like vermiwash 100% (T<sub>7</sub>) and vermiwash 50% (T<sub>8</sub>) were found at par with the treatments of thiourea @30ppm and paclobutrazol @20ppm. The maximum root length (7.70 cm) was found in vermiwash 50% (T<sub>8</sub>) while the maximum shoot length (17.38 cm) was given by thiourea @30ppm (T<sub>6</sub>). The maximum amount of carbohydrate accumulated (76.31 mg/g) was observed in thiourea 10ppm (T<sub>4</sub>) after 60 days of the spray of treatments, which was at par with vermiwash 100% (T<sub>7</sub>) and vermiwash 50% (T<sub>8</sub>). After 90 days of the spray of treatments, the concentration of carbohydrates in the bulbs did not differ significantly where the maximum value of 78.97 mg/g was observed in cow urine @10% (T<sub>7</sub>). Maximum moisture content after 90 days of spray of treatments was observed in thiourea @10ppm (T<sub>4</sub>) (65.76%). Keeping in view of the results obtained in the study for the regeneration of microbulblets from scale and development of bulbs from microbulblets, bio enhancers like vermiwash and cow urine can be used as substitutes for the chemical growth substances like paclobutrazol and thiourea in organic farming and can be recommended to the farmers.

**Keywords:** *Lilium spp.*; microbulblet; growth substances; bio-enhancers.

## 1. INTRODUCTION

Lilies are one of the most extensively sold cut flowers due to their quantity and variety of colours [1]. The genus *Lilium* is among the top-cut flowers in the world. Lilies typically reproduce sexually through seeds and asexually through the growth of daughter bulbs, axillary bulblets which form in the axils of leaves, stem bulblets which form underground or above ground, and scales [2]. Lily bulb production is a highly complex process, which cannot be fully understood by analysis at any one specific method (i.e., conventional, *in vitro*). In general, 'scaling' is used in conventional bulb production. However, the scaling process is extremely slow and takes a lot longer to complete than the bulblet expansion (size) and ontogenic development. According to the findings of an experiment by [3], the conventional pathway required 3 to 4 growing seasons for the course of bulblet growth to bulb and ontogenic development to reach the adult flowering phase. During the growth of a lily bulb, three ontogenic phases, namely the juvenile, vegetative adult, and flowering phases, occur [4,5]. Park [6] also stated that commercial-size bulbs are obtained only after 3-4 years. The demand for high-quality planting materials is one of the major obstacles to the expansion of horticulture crops, especially in the floriculture sector. Additionally, most of the bulbs used in *Lilium*'s commercial manufacturing are expensive, imported bulbs. Currently, India imports *Lilium* bulbs from other countries to utilise in the production of flowers. According to Bala et al. [1], the Netherlands is the top exporter

and supplier (2019). As stated by Panda & Mohanty [7], there has been a lot of interest in *Lilium* bulb production *in vivo* for commercial production. However, adequate information about growth substances, bio-enhancers, and *in vivo* conditions is still being determined. So different growth substances and bio-enhancers *in vivo* conditions can be utilised for the development of bulbs from bulblets and thereby decreasing the period for bulb production. Therefore, to reduce the bulb cost and to meet the increasing demand for flowers throughout the year by providing sufficient planting material, there is an urgent need to develop cheaper multiplication techniques indigenously to increase bulblet production. So this study aims the development of bulbs from microbulblets by the use of different bio-enhancers and growth substances.

## 2. MATERIALS AND METHODS

For the development of microbulblets into the bulb, a study was conducted under the medium cost poly house of the Department of Horticulture, Sikkim University. The microbulblets were of local variety and were collected from farmer's fields. They were adequately cleaned, treated with fungicide, dried, and then planted in disposable plastic cups which was filled with coco peat, perlite, and compost (1:1:1 ratio) as growing media. A completely randomized design (CRD) was used to conduct the experiment which was replicated thrice and the details of the treatments were as follows: T<sub>1</sub>- Paclobutrazol@10ppm,

T<sub>2</sub>-Paclobutrazol @20ppm, T<sub>3</sub>-Paclobutrazol@30ppm, T<sub>4</sub>-Thiourea@10ppm, T<sub>5</sub>-Thiourea@20ppm, T<sub>6</sub>-Thiourea@30ppm, T<sub>7</sub>-Cow urine@10%, T<sub>8</sub>- Vermiwash@100%, T<sub>9</sub>- Vermiwash @50% and T<sub>10</sub>- Control. The treatments were sprayed at 15, 30 and 45 days intervals. Development of bulbs from microbulblets, fresh weight of the bulb, size of bulb, root, and shoot development of bulb was recorded. Also, chlorophyll content from leaves and moisture and carbohydrate content from bulbs was calculated after 30, 60, and 90 days of the spray of treatments.

Irrigation was done in both experiments periodically, and *Artemisia* sp. spray was used as per requirement for protection from fungal infection [8]. The data were analyzed statistically using a completely randomized design. The critical difference at 5 per cent of significance level for each character was worked out to compare the significance among the treatment mean.

### 3. RESULTS AND DISCUSSION

#### 3.1 Effect of Different Treatments on the Average Value of Carbohydrate Content of the Bulb after 30, 60 and 90 days after Spraying of Treatments

The carbohydrate content of the bulb after 30 and 90 days of the spray of treatments had non significant response while on 60 days after the spray of treatments, the data showed a significant difference.

It was evident from Table 1. That the maximum carbohydrate content after 30 days was recorded in treatment T<sub>6</sub> (62.57) under thiourea @30ppm and the minimum carbohydrate content was recorded in T<sub>9</sub> (16.94) under vermiwash @50%.

The maximum average amount of carbohydrate content after 60 days of the spray of treatments was recorded in treatment T<sub>4</sub> (thiourea@10ppm) i.e., 76.31, which was found statistically at par with T<sub>5</sub> (thiourea @20ppm), i.e., 72.77, T<sub>8</sub> (vermiwash@100%) i.e., 71.44, T<sub>7</sub> (cow urine @10%), i.e., 65.23 and T<sub>6</sub> (thiourea @30ppm) i.e., 56.81, and the minimum average amount of carbohydrate content was recorded in treatment T<sub>10</sub> (22.69) under control (Table 2).

A study of the data presented in Table 3 revealed that the maximum average carbohydrate content was found in treatment T<sub>7</sub> (cow urine @10%) i.e.,

78.97 after 90 days of the spray of treatments Treatment T<sub>10</sub> (47.95) under control showed the minimum average carbohydrate concentration.

Thiourea was the most effective in the enhancement of bulblets. Nitrogenous compounds increase the protein level in the explants during differentiation resulting in a higher number of bulblets [9]. Therefore the presence of nitrogenous compounds in cow urine and vermiwash could have aided in increasing the size of the microbulblet and also the carbohydrate accumulation in the bulb. In their experiment, Zheng et al. [10] confirmed that plant growth retardants CCC and PBZ could stimulate the formation of new scales of lily bulbs and promote starch and sucrose contents in an in vitro environment in bulbs during and after the blossoming period.

#### 3.2 Effect of Different Treatments on the Average value of Chlorophyll Content (ug/ml) in the Leaves after 30, 60 and 90 days after Spraying of Treatments

The chlorophyll content highly varied significantly among different treatments after 30 days of spraying treatments (Table 1). Maximum average chlorophyll content was observed in treatment T<sub>9</sub> (9.34 ug/ml) i.e., vermiwash @50%, which was at par with T<sub>6</sub> (8.44 ug/ml) i.e., thiourea @30ppm and T<sub>5</sub> (7.03 ug/ml) i.e., thiourea @20ppm. While the minimum average chlorophyll content was observed in T<sub>3</sub> (4.58 ug/ml) i.e., paclobutrazol@30ppm, which was at par with T<sub>10</sub> (6.00 ug/ml) i.e., control and T<sub>8</sub> (5.48 ug/ml) i.e., vermiwash@100%.

Different treatments significantly influenced chlorophyll content after 60 days of the spray of treatments (Table 2). Here too, treatment T<sub>9</sub> (10.12ug/ml) i.e., vermiwash @50%, showed the maximum average value of chlorophyll content, which was at par with treatment T<sub>4</sub> (8.96 ug/ml) i.e., thiourea@10ppm. And treatment T<sub>2</sub> (3.82ug/ml) i.e., paclobutrazol @20ppm showed the minimum average value of chlorophyll content, which was at par with T<sub>7</sub> (4.66 ug/ml) i.e., cow urine @10%, T<sub>8</sub> (4.57 ug/ml) i.e., vermiwash@100%, T<sub>3</sub> (4.18 ug/ml) i.e., paclobutrazol@30ppm and T<sub>1</sub> (4.14 ug/ml) i.e., paclobutrazol@10ppm.

**Table 1. Effect of different treatments on the average value of carbohydrate content (mg/g), chlorophyll content (ug/ml), moisture content (%), bulb weight (g), bulb length (mm), bulb breadth (mm), root length (cm) and shoot length (cm)h after 30 days of the spray of treatments**

Treatments	Carbohydrate content (mg/g)	Chlorophyll Content (ug/ml)	Moisture Content (%)	Bulb Weight (gm)	Bulb Length (mm)	Bulb Breadth (mm)	Root Length (cm)	Shoot Length (cm)
T <sub>1</sub>	27.57	4.62	86.97	0.67	16.19	11.41	3.50	3.60
T <sub>2</sub>	44.85	4.87	88.50	0.47	12.82	9.78	2.00	3.94
T <sub>3</sub>	25.35	4.58	79.56	0.58	14.04	10.44	2.50	3.33
T <sub>4</sub>	45.74	8.48	83.67	0.40	12.07	9.20	4.25	4.87
T <sub>5</sub>	43.96	7.03	81.21	0.28	11.46	8.55	4.75	4.88
T <sub>6</sub>	62.57	8.44	80.37	0.25	9.95	7.49	4.87	5.19
T <sub>7</sub>	50.61	6.06	76.33	0.36	11.18	8.69	4.23	5.22
T <sub>8</sub>	49.73	5.48	82.37	0.29	11.27	8.43	4.48	3.76
T <sub>9</sub>	16.94	9.34	79.50	0.46	11.05	9.42	3.88	5.02
T <sub>10</sub>	24.89	6.00	85.76	0.16	9.15	6.47	3.58	4.34
<b>SE(m)±</b>	11.007	0.495	2.925	0.974	1.117	0.596	0.952	0.399
<b>CD@5%</b>	NS	**1.462	NS	*0.287	*3.295	** 1.760	NS	* 1.179

NS – Non-significant, \* - Significant and \*\* - Highly significant

**Table 2. Effect of different treatments on the average value of carbohydrate content (mg/g), chlorophyll content(ug/ml), moisture content(%), bulb weight(gm), bulb length(mm), bulb breadth(mm), root length(cm) and shoot length(cm) after 60 days of a spray of treatments**

Treatment	Carbohydrate concentration (mg/g)	Chlorophyll Content (ug/ml)	Moisture Content (%)	Bulb Weight (gm)	Bulb Length (mm)	Bulb Breadth(mm)	Root Length (cm)	Shoot Length (cm)
T <sub>1</sub>	62.57	4.14	71.8	0.88	18.13	12.77	3.65	4.62
T <sub>2</sub>	24.47	3.82	75.74	0.59	14.75	10.76	3.32	5.63
T <sub>3</sub>	43.08	4.18	65.49	0.67	14.07	11.54	3.07	7.55
T <sub>4</sub>	76.31	8.96	71.69	0.31	11.72	8.65	4.00	9.81
T <sub>5</sub>	72.77	6.09	72.95	0.35	11.19	9.20	6.63	11.63
T <sub>6</sub>	56.81	8.39	70.45	0.59	14.40	11.3	4.73	12.85
T <sub>7</sub>	65.23	4.66	66.79	0.41	13.09	9.52	5.55	14.26
T <sub>8</sub>	71.44	4.57	51.78	0.40	12.16	9.20	5.38	10.90
T <sub>9</sub>	40.86	10.12	64.56	0.36	12.08	9.06	5.13	15.44
T <sub>10</sub>	22.69	6.99	71.21	0.23	10.16	7.34	5.49	7.23
<b>SE(m)±</b>	10.669	0.686	3.890	0.759	0.926	0.635	0.892	1.329
<b>CD@5%</b>	*31.475	**2.026	*11.476	**0.223	**2.734	**1.875	NS	**3.920

NS – Non-significant, \* - Significant and \*\* - Highly significant

**Table 3. Effect of different treatments on average value of carbohydrate content (mg/g) , chlorophyll content(ug/ml), moisture content(%), bulb weight(gm), bulb length(mm), bulb breadth(mm), root length(cm)and shoot length(cm) after 90 days of spray of treatments**

Treatments	Carbohydrate content (mg/g)	Chlorophyll Content(ug/ml)	Moisture Content (%)	Bulb Weight (g)	Bulb Length (mm)	Bulb Breadth (mm)	Root Length (cm)	Shoot Length (cm)
T <sub>1</sub>	62.13	3.06	55.88	0.43	13.21	10.66	2.80	7.45
T <sub>2</sub>	60.36	3.42	60.28	1.27	20.01	15.46	3.78	9.00
T <sub>3</sub>	58.14	3.33	59.58	0.86	14.13	12.47	3.38	12.33
T <sub>4</sub>	70.11	2.55	65.76	0.97	16.63	12.71	4.83	13.00
T <sub>5</sub>	61.24	2.67	64.09	0.69	15.80	12.20	2.43	14.70
T <sub>6</sub>	72.77	3.01	58.10	1.38	17.45	14.82	4.80	17.38
T <sub>7</sub>	78.97	3.37	59.58	0.94	15.25	13.35	6.08	15.56
T <sub>8</sub>	73.21	3.12	58.09	0.96	16.60	13.63	7.70	15.42
T <sub>9</sub>	65.23	3.16	63.91	0.92	15.88	11.19	6.33	13.48
T <sub>10</sub>	47.95	4.00	43.20	0.19	7.20	6.76	2.80	8.84
<b>SE(m)±</b>	7.986	0.384	3.554	0.163	1.500	0.944	0.990	1.856
<b>CD@5%</b>	NS	NS	*10.486	**0.481	**4.427	**2.785	*2.920	*5.477

NS – Non-significant, \* - Significant and \*\* - Highly significant

A non-significant difference was observed concerning average chlorophyll content after 90 days of the spray of treatments as per the data presented in Table 3. The maximum and minimum average chlorophyll content was recorded in control (T<sub>10</sub>) i.e., 4.00 ug/ml and thiourea@30ppm (T<sub>6</sub>) i.e., 3.01 ug/ml respectively.

According to the data, vermiwash @50% gave maximum chlorophyll content in the leaves, which was at par with the use of thiourea. However, using vermiwash 100% reduced the average chlorophyll content. Nitrogenous compounds in vermiwash and thiourea could have increased the chlorophyll content. As Bojovic and Markovic [11] discussed, nitrogen in cow urine is a growth booster as there was a close link between chlorophyll and nitrogen in the leaf. Similar findings have been found by Tucker [12] and Daughtry et al. [13], as nitrogen is the structural element of chlorophyll and protein molecules and thereby affects the formation of chloroplast and the accumulation of chlorophyll in them.

Using paclobutrazol and cow urine reduced the average chlorophyll content in the leaves. This may have occurred due to the reduced size of leaves because of the use of growth retardant. However, this result contradicted the findings of Zheng et al. [10]. According to Zheng et al. [10], CCC and PBZ treatments enhanced chlorophyll a and b contents and inhibited the reduction that usually occurred during vegetative growth. Furthermore, the findings of Bojovic and Markovic [11] have been mentioned above.

### 3.3 Effect of Different Treatments on an Average Value of Moisture Content (%) in the Bulb after 30, 60 and 90 days after Spraying of Treatments

After 30 days of the spray of treatments, the result in Table 1 revealed a non-significant difference among different treatments. The value ranged from 88.50% to 76.33% in paclobutrazol@20ppm (T<sub>2</sub>) and Cow urine @10%(T<sub>7</sub>) respectively.

Data presented in Table 2 for the average moisture content of bulbs under different treatments was recorded as significantly variant after 60 days of the spray of treatments. The maximum moisture content (75.74%), was observed when treated with paclobutrazol@20ppm (T<sub>2</sub>) which was found

statistically at par with treatments T<sub>5</sub> (72.95%) i.e., thiourea @20ppm, T<sub>4</sub> (71.69%) i.e., thiourea @10ppm, T<sub>10</sub> (71.21%) i.e., control, T<sub>1</sub> (71.8%) i.e., paclobutrazol @ 10ppm, T<sub>6</sub> (70.45%) i.e., thiourea @30ppm, T<sub>7</sub> (66.79%) i.e., cow urine @10%, and T<sub>3</sub> (65.49%) i.e., paclobutrazol @30ppm. The minimum moisture content was observed in treatment T<sub>8</sub> (51.78%) i.e., vermiwash @100%.

Different treatments showed a significant influence on average moisture content after 90 days of the spray of treatments (Table 3). Among the treatments, treatment T<sub>4</sub> (65.76%) i.e., thiourea @10ppm showed maximum average moisture content, which was at par with T<sub>5</sub> (64.09%) i.e., thiourea @20ppm, T<sub>9</sub>(63.91%) i.e., vermiwash @50% and T<sub>2</sub>(60.28%) i.e., paclobutrazol @20ppm and the minimum average moisture content was in T<sub>10</sub> (43.20%) i.e., control.

Moisture content is inversely related to carbohydrate content. If there is an increase in carbohydrate content, there will be a decrease in the moisture content of the bulb. Using different growth substances and bio enhancers like paclobutrazol, thiourea, vermiwash etc., increases carbohydrate content, decreasing moisture content. In their experiment, Zheng et al. [10] confirmed that plant growth retardants CCC and PBZ could stimulate the formation of new scales of lily bulbs and promote starch and sucrose contents in an in vitro environment but also bulbs during and after the blossoming period.

### 3.4 Effect of Different Treatments on an Average Value of Bulb Weight (g) after 30, 60 and 90 days after Spraying of Treatments

The observation made in Table 3, the effect of different treatments on the average weight of the bulb, was found to be significant after 30 days of the spray of treatments. The minimum average weight of the bulb was recorded in treatment T<sub>10</sub> (0.16g) i.e., control which was at par with T<sub>4</sub> (0.40g) i.e., thiourea @10ppm, T<sub>7</sub> (0.36g) i.e., cow urine @10%, T<sub>8</sub> (0.29g) i.e., vermiwash @100%, T<sub>5</sub> (0.28g) i.e., thiourea @20ppm and T<sub>6</sub> (0.25g) i.e., thiourea @30ppm. The maximum average weight was recorded in T<sub>1</sub> (0.67g) i.e., paclobutrazol @10ppm, which was at par with T<sub>2</sub> (0.47g) i.e., paclobutrazol @20ppm and T<sub>9</sub> (0.46g) i.e., vermiwash @50%.

A glance at the data presented in Table 2 analyzed that the effect of different treatments on average bulb weight after 60 days of the spray of treatments was found highly significant. Paclobutrazol @10ppm (T<sub>1</sub>) recorded the maximum average weight of the bulb (0.88gm) which was statistically at par with treatment T<sub>3</sub> (0.67g) i.e., paclobutrazol @30ppm. Control (T<sub>10</sub>) recorded minimum weight of the bulb (0.23g), which was at par with treatments T<sub>7</sub>(0.41g) i.e., cow urine @10%, T<sub>8</sub>(0.40g), i.e., vermiwash @100%, T<sub>9</sub>(0.36g) i.e., vermiwash @100%, T<sub>5</sub>(0.35g) i.e., thiourea @20ppm and T<sub>4</sub>(0.31g) i.e., thiourea @10ppm.

Data in Table 3 shows that different treatments significantly affected the average bulb weight recorded on the 90th day. The maximum bulb weight observed in treatment treated with thiourea @30ppm (1.38g), which was at par with treatments T<sub>2</sub>(1.27g) i.e., paclobutrazol @20ppm, T<sub>4</sub>(0.97g) i.e., thiourea @10ppm, T<sub>8</sub> (0.96g) i.e., vermiwash @100% and T<sub>7</sub> (0.94g) i.e., cow urine @10%. In contrast, the minimum average bulb weight observed in control (T<sub>10</sub>) (0.19g) was at par with treatment T<sub>1</sub>(0.43g) i.e., paclobutrazol @10ppm.

Using different growth retardants and growth substances may have aided in the increase of bulb weight. Plant growth retardants exerted a long-term influence on bulb scale formation and carbohydrate accumulation, probably due to their manipulation of exogenous hormones. Using growth retardants like paclobutrazol decreases the GA content. The decrease in the GA content is necessary for bulb formation and leads to the accumulation of carbohydrates, thereby increasing bulb weight. Zheng et al. [10] noticed a similar result in the Liliun bulb in the Liliun Oriental hybrid 'Casa Blanca'. Urea and its derivatives also stimulate bulbs' weight, which Kumar et al. [9] found. In Gladiolus, the weight of cormels per plant was to be maximum with GA3 150 ppm, SA 150 ppm and thiourea 2%, as observed by Padmalatha et al. [14]. The use of Thiourea also increased the bulb size in garlic, as found by Chattopadhyay et al. [15].

### 3.5 Effect of Different Treatments on the Average Value of Bulb Length (mm) and Bulb Breadth (mm) after 30, 60 and 90 days after Spraying of Treatments

Table 1 represents the observation recorded for the average bulb length and average bulb

breadth and the data obtained was significant and highly significant, respectively. The maximum average length of the bulb was obtained at treatment T<sub>1</sub>(16.19mm) i.e., paclobutrazol @10ppm, which was found at par with T<sub>3</sub>(14.04 mm) i.e., paclobutrazol @30ppm and the minimum average length of bulb was obtained at treatment T<sub>10</sub>(9.15mm) i.e., control which was found at par with T<sub>4</sub>(12.04mm) i.e., thiourea @10ppm, T<sub>5</sub>(11.46mm) i.e., thiourea @20ppm, T<sub>8</sub>(11.27mm) i.e., vermiwash @100%, T<sub>7</sub>(11.18mm) i.e., cow urine @10%, T<sub>9</sub>(11.05mm) i.e., vermiwash @50% and T<sub>6</sub>(9.95mm) i.e., thiourea @30ppm. The maximum average breadth recorded in treatment T<sub>1</sub> (11.41mm) i.e., paclobutrazol @10ppm, which was at par with T<sub>3</sub> (10.44mm) i.e., paclobutrazol @30ppm and T<sub>2</sub> (9.78mm) i.e., paclobutrazol @20ppm and the minimum average breadth recorded in treatment T<sub>10</sub> (6.47mm) i.e., control which was to be at par with T<sub>6</sub> (7.49mm) i.e., thiourea @30ppm.

The result in Table 2 shows that the effect of different treatments on the average length and breadth of the bulb was highly significant after 60 days of spray. Treatment T<sub>1</sub> i.e., paclobutrazol @10ppm, showed the maximum average length of the bulb (18.13mm) and treatment T<sub>10</sub> i.e., control, showed the minimum average length of the bulb (10.16mm). Statistically at par values with the maximum value was recorded with treatments T<sub>8</sub>(12.16mm) i.e., vermiwash @100%, T<sub>9</sub> (12.08mm), i.e., vermiwash @50%, T<sub>4</sub> (11.72mm) i.e., thiourea @10ppm and T<sub>5</sub> (11.19mm) i.e., thiourea @20ppm. The maximum average bulb breadth was recorded in treatment T<sub>1</sub> (12.77mm) i.e., paclobutrazol @10ppm, which was found at par with treatments T<sub>3</sub> (11.54mm) i.e., paclobutrazol @30ppm and T<sub>6</sub> (11.3mm) i.e., thiourea @30ppm and the minimum bulb breadth was recorded in treatment T<sub>10</sub> (7.34mm) i.e., control which was at par with treatment T<sub>4</sub> (8.65mm) i.e., thiourea @10ppm.

Table 3 represents the observations for the average length and breadth of the bulb on the 90th day, and the observations were highly significant. Treatment T<sub>2</sub>(20.01mm) i.e., paclobutrazol @20ppm, recorded the maximum average length of the bulb, which was at par with treatments T<sub>6</sub> (17.45mm) i.e., thiourea @30ppm, T<sub>4</sub>(16.63mm) i.e., thiourea @10ppm, T<sub>8</sub>(16.60mm) i.e., vermiwash @100%, T<sub>9</sub>(15.88mm) i.e., vermiwash @50% and T<sub>5</sub>(15.80mm) i.e., thiourea @20ppm. Treatment



T<sub>10</sub> (7.20mm) i.e., control, recorded the minimum average bulb length. The maximum average breadth of the bulb recorded in treatment T<sub>2</sub> (15.46mm), i.e., thiourea @20ppm, which was at par with treatments T<sub>6</sub> (14.82mm) i.e., thiourea @30ppm, T<sub>8</sub> (13.63mm) i.e., vermiwash @100% and T<sub>7</sub> (13.35mm) i.e., cow urine @10%. In contrast, the minimum average length of the shoot recorded in treatment T<sub>10</sub> (6.76mm) i.e., control.

Seeing the data, the application of paclobutrazol resulted in the maximum length and breadth of bulbs, thiourea and vermiwash had at-par results with paclobutrazol. Applications of different growth substances and bio enhancers may have affected the bulb length and breadth. Using growth retardants like paclobutrazol increased the carbohydrate accumulation in the bulbs, increasing the bulb's length and breadth. Zheng et al. [10] supported these findings.

The differences may also be due to the use of thiourea, cow urine as urea, and its derivatives also stimulate the weight of bulbs found by Kumar et al. [9]. Similar findings were in *Gladiolus*, where the size of cormels per plant was maximum with GA3 150 ppm, SA 150 ppm and thiourea 2%, as observed by Padmalatha et al. [14]. Chattopadhyay et al. [15] also reported that using thiourea increased the bulb size in garlic.

### 3.6 Effect of Different Treatments on the Average Value of Root Length (cm) and Shoot Length (cm) after 30, 60 and 90 days after Spraying of Treatments

According to statistical analysis, data in Table 1 for the effect of different treatments on the average length of root and shoot of microbulblet after 30 days of the spray of treatments was non-significant and significant, respectively. The maximum and minimum average root length after 30 days was in treatments T<sub>6</sub> (4.87cm) i.e., thiourea @30ppm and T<sub>2</sub> (2.00cm) i.e., paclobutrazol @20ppm, respectively. Treatment T<sub>5</sub> (4.88cm), i.e., thiourea @20ppm, showed the maximum average length of the shoot, which was to be at par with treatments T<sub>10</sub>(4.34cm) i.e., control, T<sub>2</sub>(3.94cm) i.e., paclobutrazol @20ppm and T<sub>8</sub>(3.76cm) i.e., vermiwash @100%. Treatment T<sub>3</sub> (3.33cm) i.e., paclobutrazol @30ppm, showed the minimum average length

of the shoot, which was to be at par with T<sub>10</sub>(3.34cm) i.e., control, T<sub>2</sub>(3.94cm) i.e., paclobutrazol @20ppm, T<sub>8</sub>(3.76cm) i.e., vermiwash @100% and T<sub>1</sub>(3.60cm) i.e., paclobutrazol @10ppm.

The effects of different treatments after 60 days of the spray of treatments on the average root length and shoot length are depicted in Table 2. The observations were found non-significant for root length, and shoot length was recorded to be highly significant. The maximum and minimum average root length was given by treatments T<sub>5</sub> (6.63cm) i.e., thiourea @20ppm and T<sub>3</sub> (3.07cm) i.e., paclobutrazol @30ppm, respectively. The maximum average length of the shoot was recorded in treatment T<sub>9</sub> (15.44cm), i.e., vermiwash @50%, which was at par with treatments T<sub>7</sub>(14.26cm), i.e., cow urine @10%, T<sub>6</sub>(12.85cm) i.e., thiourea @30ppm and T<sub>5</sub>(11.63cm) i.e., thiourea @20ppm and the minimum average length of the shoot was recorded in treatment T<sub>1</sub> (4.62cm) i.e., paclobutrazol @10ppm which was at par with treatments T<sub>3</sub>(7.55cm) i.e., paclobutrazol @30ppm, T<sub>10</sub>(7.23cm) i.e., control and T<sub>2</sub>(5.63cm) i.e., paclobutrazol @20ppm.

Observation made in Table 3 on the effect of different treatments on the average length of root and shoot on the 90th day of the experiment was found significant. The minimum length (2.43 cm) was recorded in T<sub>5</sub> (thiourea - 20ppm), which was at par with T<sub>4</sub> (thiourea @10ppm) (4.83cm), T<sub>6</sub> (thiourea @30ppm) i.e., 4.80cm, T<sub>2</sub> (paclobutrazol 20ppm) i.e., 3.78cm, T<sub>3</sub> (paclobutrazol 30ppm) i.e., 3.38cm, T<sub>1</sub> (paclobutrazol @10ppm) i.e., 2.80cm and T<sub>10</sub> (control) i.e., 2.80cm. The maximum average length of the root was in T<sub>8</sub> (vermiwash @100%) i.e., 7.70cm, which was at par with treatments T<sub>9</sub> (vermiwash @50%,) i.e., 6.33cm, T<sub>7</sub> (cow urine @10%,) i.e., 6.08cm, T<sub>4</sub> (thiourea - 10ppm) i.e., 4.83cm and T<sub>6</sub> (thiourea @30ppm.) i.e., 4.80cm. The maximum average shoot length was recorded in treatment T<sub>6</sub> (thiourea @30ppm) i.e., 17.38cm which was found at par with treatments T<sub>7</sub>(cow urine @10%) i.e., 15.56cm, T<sub>8</sub>(vermiwash @100%) i.e., 15.42cm), T<sub>5</sub>(thiourea @20ppm) i.e., 14.70cm, T<sub>9</sub>(vermiwash @50%,) i.e., 13.38cm, T<sub>4</sub>(thiourea @10ppm) i.e., 13.00cm and T<sub>3</sub>(paclobutrazol @30ppm) i.e., 12.33cm and the minimum average shoot length was recorded in T<sub>1</sub>(paclobutrazol @10ppm) i.e., 7.45cm which was found to be at par with treatments T<sub>2</sub>(paclobutrazol @20ppm) i.e., 9.00cm and

T<sub>10</sub>(control) i.e., 8.84cm. The shortest root and shoot length recorded at treatments treated with paclobutrazol 10, 20 and 30 ppm.

The reason may be because of paclobutrazol, which is a growth retardant. In paclobutrazol-treated plants, stem length decreased, as found by Zheng et al. [10]. Francescangeli [16] got similar findings in Iris production. Treatments like

thiourea 10, 20 and 30 ppm and cow urine 10 ppm produced long root and shoot length, possibly because of nitrogenous substances, which is a growth booster. Also, according to Kumar et al. [9], all levels of urea and thiourea produced leafy bulblets. Treatments treated with vermiwash also gave good root and shoot length as auxin is present in vermiwash, promoting root development.



**Paclobutrazol @10%**



**Paclobutrazol @20%**



**Paclobutrazol @30ppm**



**Thiourea @10ppm**



**Thiourea @20ppm**



**Thiourea @30ppm**



**Vermiwash @50%**



**Vermiwash @100%**



**Cow urine 10 %**



**Control**

**Fig. 1. Enhancement of bulblets with effect of different treatments**

#### 4. CONCLUSION

From the findings, the effect of plant growth substances and bio-enhancers on the production of bulbs from micro bulblets showed great potential with treatment like paclobutrazol, leading to the enhancement of bulbs. However, using organic growth substances and bio-enhancers like vermiwash (100% and 50%) and cow urea (10%) showed at par results with chemical growth substances like paclobutrazol and thiourea.

From the study, we can conclude that the organic growth substances and bio-enhancers like vermiwash @50%, vermiwash @100% and cow urine @10% can be used as a substitute for chemical growth substances like paclobutrazol and thiourea in the production of bulbs in *Lilium* in vivo conditions.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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