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RESEARCH ARTICLE

EFFECT OF ORGANIC FERTILIZERS AND *TRICHODERMA* ON CHLOROPHYLL CONTENT OF *LAGENARIASICERARIA* (MOLINA) STANDL

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ABSTRACT

Microorganisms are the profound group of organisms found in the soil to facilitate nutrient transfer from soil pool to the plants. The organic fertilizers are rich source of carbon for the multiplication of microbes in the soil. The experiments were carried at Shri Gavisiddeshwar Arts, Science and commerce College Koppal. The present study was undertaken to assess the effect of organic fertilizers like city compost, vermicompost and *Trichoderma* chlorophyll content of *Lagenariasiceraria* (Molina) Standl.It was observed that, the plants treated with organic fertilizers and *Trichoderma* have shown increased chlorophyll content compared to control plants.It was notice during the experiment that, the plants treated with 9.8 gm of city compost, 8.0 gm of vermicompost and 10.5 gm of *Trichoderma*, the chlorophyll a, chlorophyll b and total chlorophyll concentration in young leaves were 12.57mg/ml, 9.24 mg/ml and 2.27 mg/ml respectively.When organic fertilizer used alone, can't show the much impact on the physiological parameters of the plants. Hence, the use of organic fertilizers in 2 or 3 combinations may be useful in getting the good results. Rather using only one fertilizer alone at a same quantity.

INTRODUCTION

Organic fertilizers offer a well-balanced blend of nutrient sources that are essential for the important microorganisms and earthworms living in the soil. These subterranean organisms or microorganisms are one of the many reasons for a fruitful and healthy soil structure. Soil health can be measured on the basis of number of microorganisms per unit area at unit time. Microorganisms are the profound group of organisms found in the soil to facilitate nutrient transfer from soil pool to the plants. The organic fertilizers are rich source of carbon for the multiplication of microbes in the soil. They help the plants to absorb nutrients effectively. The fertilizer provides major nutrients like Nitrogen (N), Phosphorus (P), Potassium (K), Manganese (Mn), Calcium (Ca), Magnesium (Mg) and others. It also enriches soil with micro nutrients like Boron (B), Zinc (ZN), Molybdenum (Mo), Iron (Fe), etc. Vermicompost is the stable fine, granular organic manure which enriches soil gravity by improving its physicochemical and biological properties. The fertilizers provide major nutrients like N,P, K, Ca, Mn, Fe, Mg, Zn etc.*Trichoderma* is an effective bio-fungicide. . It kills pathogenic fungi and competing with pathogenic fungi for space and nutrients. The city compost and green manurepromotes the growth of plants, has it contains higher amount of mineral nutrients, such Ca, Mg, Fe, Zn and Cu, NPK and also contains phytohormones such as indole-3-acetic acid (IAA), auxins, gibberellic acid. Therefore, these organic fertilizers and Bio-fungicide may affect positively on the growth and development of plants. Hence, the present study was undertaken to assess the effect of organic fertilizers like city compost, vermicompost and *Trichoderma* chlorophyll content of *Lagenariasiceraria* (Molina) Standl.

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MATERIALS AND METHODS

Study Area

The present study was carried at Botanical Garden, Shri Gavisiddeshwar Arts, Science and commerce College Koppal (15°19'46.4"N 76°09'09.6"E). Koppal is the district headquarters located in Karnataka state of India. Plants were grown under normal conditions temperature ranging from 28°C to 31°C during the day time to the temperature of 23°C to 25°C during night time.

Growing of Plants

Seed Collection and surface sterilization

Seeds of calabash/bottle gourd (*Lagenariasiceraria*) were collected from Krishi Nursery. The seeds are surface sterilized

by using 0.1% mercury chloride under laboratory conditions and distilled water to ensure the seeds free from contaminants. The surface sterilized seeds were sown in earthen pots of 25x22 cm containing growth media. The growth media is prepared by using red soil, sand and coco-peat in the ratio of 2:1:1. Plants were irrigated every fortnight.

Treatments

The present study was conducted under controlled condition with organic fertilizers such as city compost, vermicompost and *Trichoderma*. The fertilizers are added to plants in their 21st day (i.e., after the 3 leaves are developed). The fertilizers

Table 1: The Optical density of young and older leaf recorded for all the experimental treatments

Sl No	Samples	Optical Density-1	Optical Density-2	Optical Density-3	Mean
	Control plant Young leaves				
1	A at 663	0.79	0.79	0.79	0.79
	A at 645	0.43	0.43	0.43	0.43
	A at 652	0.53	0.58	0.58	0.55
	Control plant Older leaves				
2	A at 663	0.73	0.73	0.73	0.73
	A at 645	0.4	0.41	0.4	0.4
	A at 652	0.55	0.54	0.55	0.55
	***Trichoderma* Young plant leaves**				
3	A at 663	0.74	0.75	0.74	0.74
	A at 645	0.43	0.43	0.43	0.43
	A at 652	0.51	0.51	0.51	0.51
	***Trichoderma* Older plant leaves**				
4	A at 663	0.99	0.99	0.99	0.99
	A at 645	0.5	0.5	0.5	0.5
	A at 652	0.62	0.62	0.6	0.62
	City compost Young plant leaves				
5	A at 663	0.59	0.59	0.59	0.59
	A at 645	0.38	0.73	0.37	0.37
	A at 652	0.42	0.42	0.42	0.42
	City compost Older plant leaves				
6	A at 663	0.21	0.21	0.21	0.21
	A at 645	0.19	0.18	0.19	0.19
	A at 652	0.19	0.19	0.19	0.19
	Vermicompost Young plant leaves				
7	A at 663	0.59	0.59	0.59	0.59
	A at 645	0.35	0.35	0.34	0.35
	A at 652	0.41	0.41	0.41	0.41
	Vermicompost Older plant leaves				
8	A at 663	0.99	0.99	0.99	0.94
	A at 645	0.5	0.5	0.5	0.5
	A at 652	0.62	0.62	0.6	0.62
	T+CC Young plant leaves				
9	A at 663	0.89	0.89	0.89	0.89
	A at 645	0.41	0.41	0.41	0.41
	A at 652	0.63	0.63	0.63	0.63
	T+CC Older plant leaves				
10	A at 663	0.84	0.85	0.84	0.84
	A at 645	0.53	0.53	0.53	0.53
	A at 652	0.61	0.61	0.61	0.61

	T+VC Young plant leaves				
11	A at 663	0.99	0.99	0.99	0.99
	A at 645	0.48	0.48	0.48	0.48
	A at 652	0.72	0.72	0.72	0.72
	T+VC Older plant leaves				
12	A at 663	0.99	0.99	0.99	0.99
	A at 645	0.52	0.52	0.52	0.52
	A at 652	0.68	0.68	0.68	0.68
	CC+VC Young plant leaves				
13	A at 663	0.89	0.89	0.89	0.89
	A at 645	0.55	0.55	0.55	0.55
	A at 652	0.61	0.61	0.61	0.61
	CC+VC Older plant leaves				
14	A at 663	0.86	0.86	0.86	0.86
	A at 645	0.56	0.56	0.56	0.56
	A at 652	0.71	0.71	0.71	0.71
	T+CC+VC Young plant leaves				
15	A at 663	0.99	0.99	0.99	0.99
	A at 645	0.42	0.42	0.42	0.42
	A at 652	0.78	0.78	0.78	0.78
	T+CC+VC Older plant leaves				
16	A at 663	0.99	0.99	0.99	0.99
	A at 645	0.51	0.51	0.51	0.51
	A at 652	0.73	0.73	0.73	0.73

of quantity 28.3 gm (nearly 2 tablespoons) of each fertilizer are added to 8 different pots, only one type or on combination with two or three types of fertilizers, where as follows,

1. Control plant.
2. Plant treated with 28.3 gm of *Trichoderma*(T)
3. Plant treated with 28.3 gm of city compost (CC).
4. Plant treated with 28.3 gm of vermicompost (VC).
5. Plant treated with 28.3 gm of *Trichoderma*(T)+ 28.3gm of city compost (CC).
6. Plant treated with 28.3 gm of *Trichoderma* (T) + 28.3 gm of Vermicompost (VC).
7. Plant treated with 28.3 gm of city compost (CC)+ 14.0 gm of Vermicompost (VC).
8. Plant is treated with 28.3 gm of city compost (CC)+ 28.3 gm of Vermicompost (VC) + 28.3 gm of *Trichoderma*(T).

Extraction of Chlorophyll (Arnon, 1949)

A 250 mg of fresh calabash leaf material excluding midribs from a mixed representative sample was (accurately) weighed on an electronic balance and thoroughly macerated in a mortar with a pestle in 10 ml of 80% acetone. The homogenate was filtered through a sheet of Whatman filter paper No.1 and the filtrate was collected in a volumetric flask. The volume of the filtrate was adjusted to 25 ml with 80% acetone.

Estimation of Chlorophyll Content

The acetone extract of different samples was subjected for estimation of chlorophyll a, chlorophyll b and total chlorophyll by using UV - viable spectrometric at Avishkar research laboratory, SJGAM college Koppal. The absorbance for the estimation of chlorophyll was noted at wavelength of 645nm, 652nm and 663nm against the blank i.e., acetone solvent. The O.D for all the samples was recorded for the further calculation by using the following formula as follow.

- Chlorophyll a = 12.7(A at 663) - 2.69(A at 645) $\times \frac{V}{1000 \times W}$
- Chlorophyll b = 22.9(A at 645) - 4.69(A at 663) $\times \frac{V}{1000 \times W}$
- Total Chlorophyll = $\frac{A \text{ at } 652}{34.5} \times \frac{1000 \times V}{1000 \times W}$

Where:

A- Absorbance.

V- Final volume of supernatant (25 ml).
W- Fresh weight of sample taken grams (0.25 g).
The chlorophyll content of the leaves is expressed as mg/g fresh leaf.

Observations

Plant where grown under controlled conditions temperature ranging from 25-30^0 C day time to 15-20^0 C night time, (6:8, light: dark photo period) and a relative humidity 75-85 %. The plants where irrigated manually with tap waters as and when required during the experiment. The chlorophyll content of the plants was estimated for all the treatments by recording the optical density (OD) of both young and adult plants. The OD for all the treatments is mentioned as follows (Table 1)

RESULT AND DISCUSSION

The experimental plants showed varied response to different

treatments. It was observed that, the plants treated with organic fertilizers and *Trichoderma* have shown increased chlorophyll content compared to control plants. The chlorophyll content of plants was estimated by using leaves at different age i.e. young leaves and older leaves. This was done to understand effect of leaf age on chlorophyll content along with different treatments used during the experiment.

Thecontrol plants young leaves of showed the concentration of Chl a., and Chl b., content were 2.69 mg/ml and 4.31 mg/ml respectively. The total chlorophyll content in control plant young leaves is 0.56mg/ml. In control plants young leaves, the Chl.b was high in concentration. In older leaves of control plant. The Chl.a and Chl.b contents were 7.12mg/ml and 8.13mg/ml respectively. The total chlorophyll content in control plant older leaves is 1.20mg/ml. The concentration of Chl.b was high in control plant older leaves. However, when it comes to control plants, the concentration of chlorophyll was more. When plants alone treated with *Trichoderma*, plants showed high concentration with thick green pigmentation, the concentration of Chl.a and Chl.b in young leaves of plants are treated with *Trichoderma* were 9.39mg/ml. respectively and the total chlorophyll concentration was 1.50mg/ml. The concentration of Chl. a, Chl.b and total chlorophyll in older leaves plants treated with *Trichoderma* were 12.54mg/ml, 11.12mg/ml and 1.79mg/ml. Respectively the concentration of chl.a in *Trichoderma* treated older leaves were more, when compared to Chl.b and total chlorophyll.

The chlorophyll concentration in plants treated with city compost, showed some valid impact, the results as follows, the Chl. a, Chlb and total chlorophyll concentration in young leaves of plants treated with city compost was 7.49mg/ml, 8.38mg/ml and 1.23mg/ml. The Chl.b concentration was high compared to chl.a and total chlorophyll. In plants treated with city compost older leaves Chl.a,Chl.b and total chlorophyll concentration was 10.02mg/ml, 9.51mg/ml and 1.55mg/ml respectively. This shows that chlorophyll concentration content was high in older leaves, in comparison to young leaves. Plants treated with vermicompost showed least concentration in compared to other treatments. The results are as follows, the chlorophyll concentration of chlorophyll a, chlorophyll b, and total Chlorophyll in young leaves treated with vermicompost is 7.51 mg/ml, 7.80 mg/ml, and 1.21 mg/ml respectively. The chlorophyll b concentration was more in compared to chlorophyll a and total chlorophyll.

The chlorophyll concentration of chlorophyll a, chlorophyll b and total chlorophyll in older leaves treated with vermicompost is 9.20 mg/ml, 8.97 mg/ml and 1.59 mg/ml. The chlorophyll a concentration was more in compared to chlorophyll b and total chlorophyll. This shows that chlorophyll concentration content was high in older leaves was more in comparison to young leaves are treated with two or more organic fertilizers. The above results are obtained when plant is treated with only one of the organic fertilizers and showed some good chlorophyll concentration compared with controlled plant which is not treated with any organic fertilizer. To get some impulsive impact, the plants are treated with two fertilizers of different composition and measurements showed the following results.

Sl. No.	Treatments	Young Leaves		Total chl. content in young leaves	Older Leaves		Total chl. content in older leaves
		Chl. a	Chl. b		Chl. a	Chl. b	
1	Control Plant	2.69±0.05	4.31±0.12	0.56±0.08	7.12±0.01	8.13±0.01	1.20±0.01
2	*Trichoderma* (T)	9.39±0.11	9.53±0.04	1.50±0.09	12.54±0.09	11.12±0.08	1.79±0.06
3	City Compost (CC)	7.49±0.03	8.38±0.02	1.23±0.02	10.02±0.06	9.51±0.10	1.55±0.07
4	Vermicompost (VC)	7.51±0.02	7.80±0.07	1.21±0.06	9.20±0.02	8.97±0.06	1.59±0.01
5	T+CC	11.01±0.01	9.16±0.08	1.84±0.11	10.63±0.05	11.78±0.01	1.79±0.01
6	T+VC	9.11±0.10	10.81±0.05	1.52±0.06	11.64±0.07	9.24±0.03	1.98±0.04
7	CC+VC	11.27±0.11	12.37±0.03	1.79±0.02	10.87±0.09	12.59±0.05	2.08±0.08
8	CC+VC+T	12.57±0.03	9.24±0.04	2.27±0.11	12.55±0.10	11.40±0.03	2.13±0.10

When plant treated with 14.3 gm of *Trichoderma*and 14 grams of city compost, the chlorophyll a, chlorophyll b, and total chlorophyll concentration in young leaves is 11.01 mg/ml, 9.16 mg/ml and 1.84 mg/ml respectively. The chlorophyll a concentration was more compared to chlorophyll b and total chlorophyll. The chlorophyll a, chlorophyll b and total chlorophyll concentration in older leaves is 10.63 mg/ml, 11.78 mg/ml and 1.79 mg/ml respectively. The chlorophyll b concentration was more when compared to chlorophyll a and total chlorophyll. This shows that chlorophyll concentration was high in older leaves compared to young leaves which are treated with *Trichoderma* and city compost.

When plant is treated with 15.3 grams of *Trichoderma* and 13.3 gm of vermicompost, the chlorophyll a, chlorophyll b and total chlorophyll concentration in young leaves were 9.11 mg/ml, 10.81 mg/ml and 1.52 mg/ml respectively. The chlorophyll b concentration was more in young leaves when compared to chlorophyll a and total chlorophyll. The chlorophyll a, chlorophyll b and total chlorophyll concentration in older leaves were 11.64 mg/ml, 9.24 mg/ml and 1.98 mg/ml. The chlorophyll a concentration was high when compared to chlorophyll b and total chlorophyll. This shows that chlorophyll concentration was more in older leaves compared to young leaves.

When plant is treated with 14.4 gm of city compost and 14.0 gm of vermicompost the chlorophyll a, chlorophyll b and total

chlorophyll concentration in young leaves were 11.27 mg/ml, 12.37 mg/ml, 1.79 mg/ml respectively. The chlorophyll b concentration was high in comparison to chlorophyll a and total chlorophyll. The chlorophyll a, chlorophyll b and total chlorophyll concentration in older leaves were 10.87 mg/ml, 12.59 mg/ml and 2.08 mg/ml. The chlorophyll b concentration was more in compared to chlorophyll a and total chlorophyll. This shows that chlorophyll concentration in older leaves treated with city compost and vermicompost is more compared to young leaves. The above results are obtained when plants are treated with two organic fertilizers and the results showed were very much effective compared to plants treated alone.

To get more effective results, the plant is treated with all the three fertilizers. When a plant is treated with 9.8 gm of city compost, 8.0 grams of vermicompost and 10.5 gm of *Trichoderma*, the chlorophyll a, chlorophyll b and total chlorophyll concentration in young leaves were 12.57mg/ml, 9.24 mg/ml and 2.27 mg/ml respectively. The chlorophyll a concentration was more when compared to chlorophyll b and total chlorophyll. The chlorophyll a, chlorophyll b and total chlorophyll concentration in older leaves were 12.55 mg/ml, 11.40 mg/ml and 2.13 mg/ml. The chlorophyll a concentration was more when compared to chlorophyll a and total chlorophyll. This shows that the chlorophyll content concentration is more in older leaves when compared to young leaves when treated with all three fertilizers such city compost, vermicompost and *Trichoderma*. The highest chlorophyll concentration in young leaves was found in plants treated with all the three fertilizers i.e., city compost, vermicompost and *Trichoderma*. The concentration was at 12.55 mg/ml which is followed by 11.27 mg/ml that is observed in plants treated with city compost and Vermicompost.

The concentration of chlorophyll b in young leaves was found more in plants treated with two fertilizers i.e., city compost and vermicompost, the concentration was found at 12.37 mg/ml peaks. This was followed by plants treated with city compos, vermicompost and *Trichoderma* and peak is at 11.40 mg/ml.

The total chlorophyll content in young leaves was high in plants treated with city compost, vermicompost and *Trichoderma* that is 2.13 mg/ml. This is followed by plants treated with city compost and *Trichoderma* and peak is at 1.84 mg/ml. The highest chlorophyll a concentration in older leaves is 12.57 mg/ml which is observed in plants alone treated with *Trichoderma* the peak is at 12.54 mg/ml.

The highest concentration of chlorophyll b in older leaves was found in plants treated with city compost and vermicompost, the peak is observed at 12.59 mg/ml which is followed by plants treated with city compost and *Trichoderma*, peak is observed at 11.78 mg/ml and 11.12 mg/ml respectively. The total chlorophyll content in older leaves was high in plants treated with all the three fertilizers, peak is observed at 2.27 mg/ml which is followed by plants treated with city compost and vermicompost, the peak is at 2.08 mg/ml. In almost all the treated plants, the chlorophyll content was high in older leaves that to, plant older leaves which are treated with all the three fertilizers i.e., city compost, vermicompost, and *Trichoderma* which is followed by plants treated with only two fertilizers i.e., city compost and vermicompost. This shows that chlorophyll concentration was high in older leaves of plants that are treated with all the three organic fertilizers.

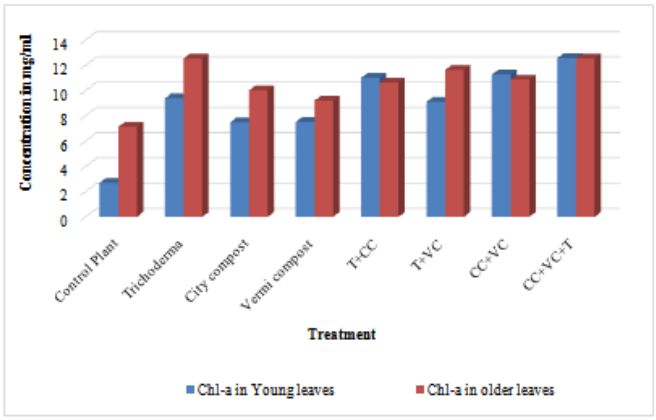


Fig. 1 Chlorophyll a content in younger and older leaves of the plant

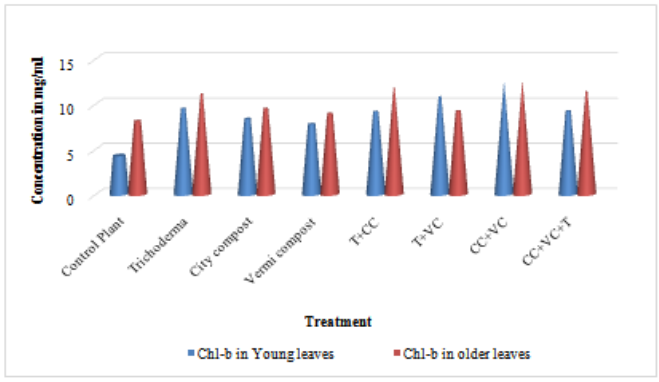


Fig. 2 Chlorophyllbcontent in younger and older leaves of the plant

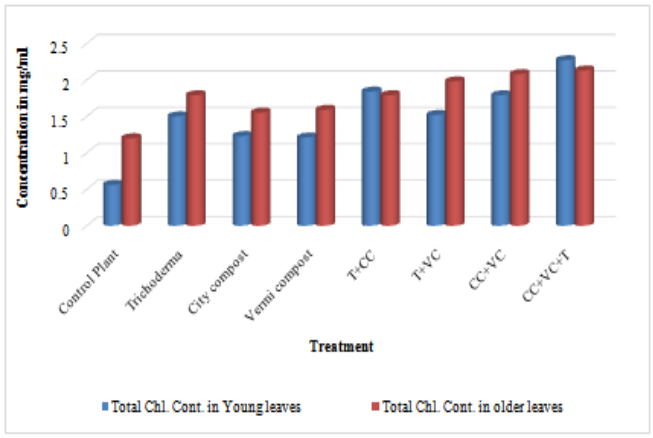


Fig.3 Total Chlorophyllcontent in younger and older leaves of the plant

In almost all the treated plants, the chlorophyll content was high in older leaves in comparison to young leaves, when organic fertilizers are used in combination of two or three. Therefore, the possible explanation could be that, use of fertilizers combinations CMP+CO+Nof could be recommended (Yanar et al., 2011) and also cow dung manure can be used in the absence of N:P: K fertilizers considering the cost and associated environmental effect of later. Moreover, Kamble et al.,(2015) showed that in most of the plants showed higher chlorophyll content in adult leaves as compared to young leaves. Siwach and Gill (2014); James et al., (1999) also explained that chlorophyll content was high in adult

Fig. 1 The Lagenariasaceraria (Molina) Standl., plants after 8 days of sowing seeds.

leaves in compared to young leaves, the reason is young leaves were not mature and adult leaves were fully matured. Another possible reason would be that young leaves appear in blue gray in colour showed high concentration of mesophyll, while the adult leaves were shown to have a low concentration of mesophyll because of they appear green in colour, (Johnson, 1926; Jacobs, 1955)

Environmental factors and temperature also play and important role in determining the chlorophyll content of the leaves, the justification would be that, the chlorophyll content of *Tridaxprocumbens* grown in normal and polluted region and reported that the chlorophyll content in normal and polluted regions is 2.99 mg/g and 2.56 mg/g respectively, (Indira Priyadarshini et al., 2015).In almost all treated plants, chlorophyll a concentration was high in compared to chlorophyll b in both young and adult leaves, except in few leaves treated with different fertilizers. As chlorophyll a was the main pigment, that converts light energy into relatively stable chemical energy via the photosynthetic process and chlorophyll b was the accessory pigment, absorbs light energy for the process of photosynthesis. The possible explanation could be that, in almost all cases the chlorophyll a concentration was higher in adult leaves than that of Chlorophyll b, the chlorophyll a us the primary pigment, while other pigments are accessory pigments (Srichaikuletal, 2011) and also reported that higher amount of chlorophyll a (0.91 ± 0.19 mg/g FW) and chlorophyll b (0.61 ± 0.09 mg/g FW) in micro propagated

Fig. 2: The *Lagenariasaceraria* (Molina) Standl., plants after 16 days of sowing seeds

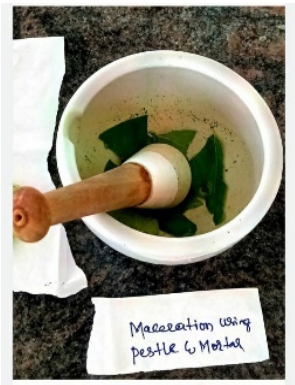
plants of *Psoralea corylifolia* compared to chlorophyll a (0.83 ± 0.31 mg/g FW) in micro propagated plants of *Psoralea corylifolia* compared to chlorophyll a (0.83 ± 0.31mg/g FW) and chlorophyll b (0.53 ± 0.14 mg/g FW) in seedlings , (Faisal and Anis*et al*., 2006). Smith and Nobel, (1978) also showed that in both young and adult leaves; the chlorophyll a/b ratio was higher in adult leaves then compared to young leaves.

Chlorophyll is a green pigment that can be used as indicators of plant health stress and nutritional deficiencies there by if a plant produces more chlorophyll, it can produce more starch which ultimately results in a higher yield.The amount of chlorophyll also affects the rate of photosynthesis. Chlorophyll absorbs the light required to convert carbon dioxide and water into glucose. The use of organic fertilizers has advantage of being cheap, improving soil structure, texture and aeration increasing the soil water retention abilities and stimulating healthy root development. When organic fertilizer used alone, can't show the impact, so the usage of organic fertilizers in 2 or 3 combinations may be useful in getting the good results. Rather using only one fertilizer alone at a same quantity, it's better to use combination of fertilizers in the same quantity. Here by we conclude that plant showed high concentration of chlorophyll, when we used in combination rather than using only one fertilizer.

Fig. 3: The *Lagenariasaceraria* (Molina) Standl., plants after 40 days of sowing seeds

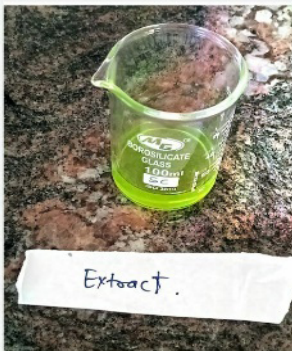
Fig. 4 The *Lagenariasaceraria* (Molina) Standl., plants after 40 days of sowing seeds

Leaf Sample



Maceration of Leaf sample with acetone

Filtration of macerated leaf sample



Chlorophyll extract

Fig. 5 Extraction of Chlorophyll

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