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Phytotoxicity of Selected Different Natural Plant Dyes Study on *Phaseolus Aureus*(Green Gram)

G. Athinarayanan[1], R. Mariselvam[2], A. J. A. Ranjitsingh[3], A. Usha Raja Nanthini[4]

[1]Department of Zoology Sri Paramakalyani College, Alwarkurichi 627412, M.S.University, Tirunelveli.

[2]Department of Chemistry, Sri Paramakalyani College, Alwarkurichi.

[3]The Principal, JP College of Arts and Science, Ayikudy, M.S.University.

[4]Department of Biotechnology, Mother Teresa University, Kodaikanal.

Abstract: ***The natural dyes effluent are not toxic to the growth of plants and also to aquatic plants. In this present study, it is understood that the effluent of plant dye did not interfere with seed germination and growth rateof many in selected plant species. The magnitude of toxicity if any depends upon the nature of the plant and chemicals present in the natural dyes effluent. In this study efforts were taken to evaluate the different concentrations of different plant dye effluent on seed germination and growth rate of green gram seeds. The untreated (control) and treated (plant dye effluent) green grams were almost equal in their germination and growth rate. The results help to studies the dye extract was found to be non-toxic.***

Keywords: Phaseolus aureus, C.sappan, R.cordifolia, A.catechu, M.champaca, N.arbor tristis, Green gram.

1. Introduction

India has an abundance of dye yielding plant species in different parts of the country. Traditionally, materials from leaves, roots, flowers and bark of the plants, mostly by boiling to get the desired colour. Natural dyes/colorants derived from flower, leaf, stem, root, fruit and bark are believed to be safe because of their non-toxic, non-carcinogenic and waste water problems and represent a more environmentally friendly alternative to synthetic dyes. Recently, the dye industry is more and more forced to reduce toxic effluents and to stop the production of potentially dangerous dyes or pigments. The use of non-toxic and eco-friendly natural dyes on textiles has become a matter of significant importance because of the increased environmental awareness in order to avoid some hazardous synthetic dyes. However, worldwide the use of natural dyes for the colouration of textiles has mainly been confined to craftsman, small scale dyers and printers as well as small scale exporters and producers dealing with high valued eco-friendly textile production and sales (Samanta,A.K and Agarwal,P., 2009; Bechtold, T et al.,2009 ; Vankar,P.S, 2007).

There are a small number of companies that are known to produce natural dyes commercially. For example, de la Robbia, which began in 1992 in Milan, produces water extracts of natural dyes such as weld, chlorophyll, logwood, and cochineal under the Eco-Tex certifying system, and supplies the textile industry. In USA, Allegro Natural Dyes produces natural dyes under the colour label for textile industry (Hwang, E et al., 2008). Aware of the Toxic Substance Act and the Environmental Protection Agency, they claim to have developed a mordant using a non-toxic aluminium formulation and biodegradable auxiliary substance. In Germany, Livos Pflanzenchemie Forschungs and Entwicklungs GmbH marked numerous natural products. In France, Bleu de Pastel sold an extract of woad leaves. Rubia Pigmenta Naturalia is The Netherlands company, which manufactures and sells vegetable dyes. There are several small textile companies using natural dyes. India is still a major producer of most natural dyed textiles (Vankar, P.S., 2007).

Research has shown that synthetic dyes are suspected to release harmful chemicals that are allergic, carcinogenic and detrimental to human health. On the other hand, natural dyes are environment-friendly; for example, turmeric, the brightest of naturally occurring yellow dyes is a powerful antiseptic which revitalizes the skin, while indigo gives a cooling sensation. Though, dyes have been discovered accidentally, their use has become so much a part of man's customs that it is difficult to imagine a modern world without dyes. The art of dyeing spread widely as civilization advanced (Krishnamurthy K.V, *et al*., 2002).

2. Materials and Methods

Collection and Storage of Sample
The effluent was collected from our lab experimental dyed natural solution selected plants in Sri ParamakalyaniCollege, Alwarkurichi in Tamilnadu. The effluent was collected in sterile plastic container. After collection, immediately the water sample was brought to stored at 4^0C in a refrigerator.

Germination Studies
The seeds were taken for germination studies, Phaseolus aureus, (Green gram) from the family Fabaceae. The healthy and uniform sized seeds were selected and surface-sterilized with 0.1% HgCl2 for 3 minutes double distilled water for 5 times in 1minutes.

Toxicity Assay
A extract of weld (1g/100ml) was prepared and, from this stock, different concentrations (50, 75 and 100 ppm) were prepared for testing and were finally applied to sterile 9cm

diameter whatman No.1 filter paper disks in Petri dishes. Then 10 surface-disinfected green grams (moong beans) were placed on the wetted paper. After 14 days of incubation at 27^{0}C, total root growth (germination) was measured and compared with the control (untreated sample) and expressed as root growth inhibition percentage (Nagia and EL-Mohamedy, 2007).

Preparation of Dilution

The effluent of different concentration of 50 %, 75 % and 100 % were prepared with distilled water.
50 % = 50 ml of the effluent + 50 ml of distilled water
75 %= 75 ml of the effluent + 25 ml of distilled water.
100 %= 100 ml of raw effluent.

1) The seeds of Green gram were surface sterilized with 0.1 % of mercuric chloride for 2-3 minutes, washed in running tap water for 3 minutes and in distilled water for 2 minutes.
2) Ten seeds of green gram were placed in sterilized glass petri plate of lined with two Whatman filter paper discs.
3) The filter papers were wetted with 5 ml of distilled water (control) and same (5 ml) of various concentration of textile effluent.
4) After seed inoculation the plates were incubated at room temperature, in the laboratory.
5) The preparation was moistened with 5 ml of effluent every 12 hrs and observed for radicle emergence. Triplicates were maintained, the results were averaged.
6) Germination percentage, relative root length, shoot length, dry weight, fresh weight were determined.

$$\text{Relative germination} = \frac{\text{Number of germination in the extract}}{\text{Number of germination in the control}} \times 100$$

Phytotoxicity Study: The procedure was followed using dyes to test its toxicity level. The percentage of germination was also calculated by the following formula

$$\text{Phytotoxicity \%} = \frac{\text{Radicle length of control} - \text{Radicle length of test}}{\text{Radicle length of control}} \times 100$$

Measurement of Shoot, Root length, Fresh weight and Dry weight: The root, shoot length were measured in cm/plant. On the 12th day the germinated seeds were weighed to record the fresh weight using electrical Single Pan Balance. After the fresh weight of germinated seed it was dried at 65^o C in hot air oven for 24 hrs and dry weight was recorded.

3. Results and Discussion

Germination study

Relative germination percentage values also very less different respect of different concentration of different effluent compared to the control.In the case of green gram the germination percentage showed 98 % in control.The percentage of germinated growth rate of sample experiement is 2-4 % less than compared with control. The highest 98 percentage is observed of R.cordifolia, A.catechu and Turmeric aqueous dye effluent, the 97% observed of N.arbortristis and M.Champaca aqueous dye effluent and the 96 % percentage observed of C.sappan in 50 % effluent. In 100 % effluent, the 97 % germinated growth observed of A.catechu,N.arbortristis and M.champaca effluent, 96 % germinated growth rate observed of C.sappan and 95 % germinated growth rate observed of R.cordifolia.There is no changing in colour and shape of the experiment plant compared to the control.(Table 1 and Figure 1-6).

Growth rate of phaseolus aureus (Green gram)

Among these seven different concentrations of effluent 50 % recorded the maximum length of 20.6 cm. In control length was found to be 20.2 cm. From this the effluent at 50 % showed a positive effect on growth rate. The growth is just decreased by increase in concentration compared to the control. In three different concentrations are the appearance of length from 19.3- 20 cm (Table 2 and Figure 7-12).

The fresh weight maximum of 3.9 gram in turmeric 50 % concentration effluent, the less decrease the weight of fresh plant weight compared with control (4.2g). The minimum fresh plant weight is observed in C.sappan 100 % concentration effluent(Figure 13). In dry weight the maximum 0.981 gram in *M.champaca* 50% Concentration effluent,the minimum dry weight is observed in *C.sappan* 0.779 gram 100 % concentration effluent. In 1.02 gram dry weight observed in control. (Figure 14).

Table 1: Percentage of Relative germinated growth rate

Dye	Seed	Percentage			
		50 %	75 %	100 %	**0 %**
C.sappan	Green gram	96	96	96	**98**
R.cordifolia		98	97	95	
A.catechu		98	97	97	
N.arbortris-tis		97	98	97	
M.champaca		97	95	97	
Turmeric		98	98	96	

Table 2: Length of Germinated Root, shoot and leaf

Dye	*Seed*	*Length of Germinated growth (Root, Shoot and leaf) cm*			
		50 %	*75 %*	*100 %*	*0 %*
C.sappan	Green gram	19.6	19.8	19.6	**20.2**
R.cordifolia		19.9	19.8	19.7	
A.catechu		18.6	18.5	18.5	
N.arbortris-tis		19.3	19.3	19.5	
M.champaca		20.1	19.8	19,9	
Turmeric		20.6	20.4	20.4	

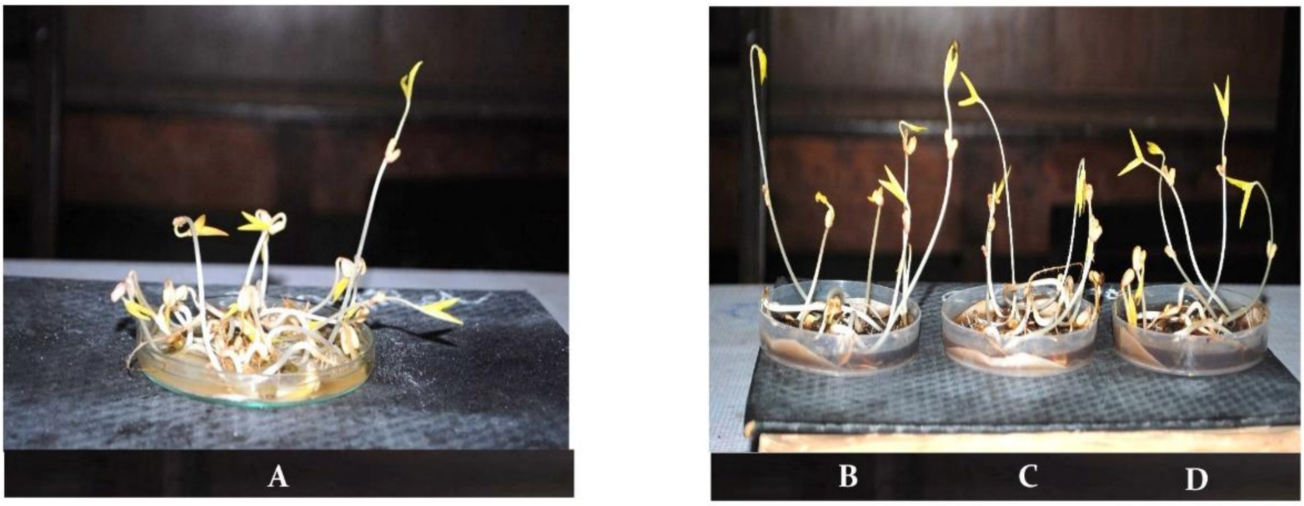


Figure 1 The seedling growth of C.sappan.

A- Growth of seed in 0 % effluent conentration, B-Growth of seed in 50 % effluent concentration, C-75 % effluent concentration, D-100 % effluent concentration

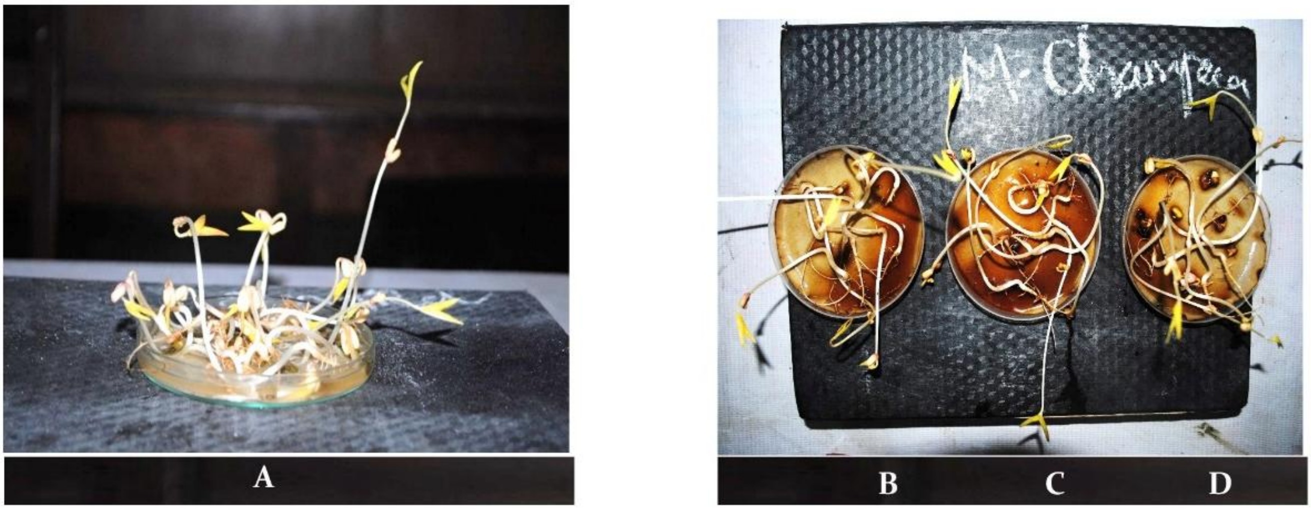


Figure 2 The seedling growth of M.champaca
A- Growth of seed in 0 % effluent conentration, B-Growth of seed in 50 % effluent concentration, C-75 % effluent concentration, D-100 % effluent concentration.

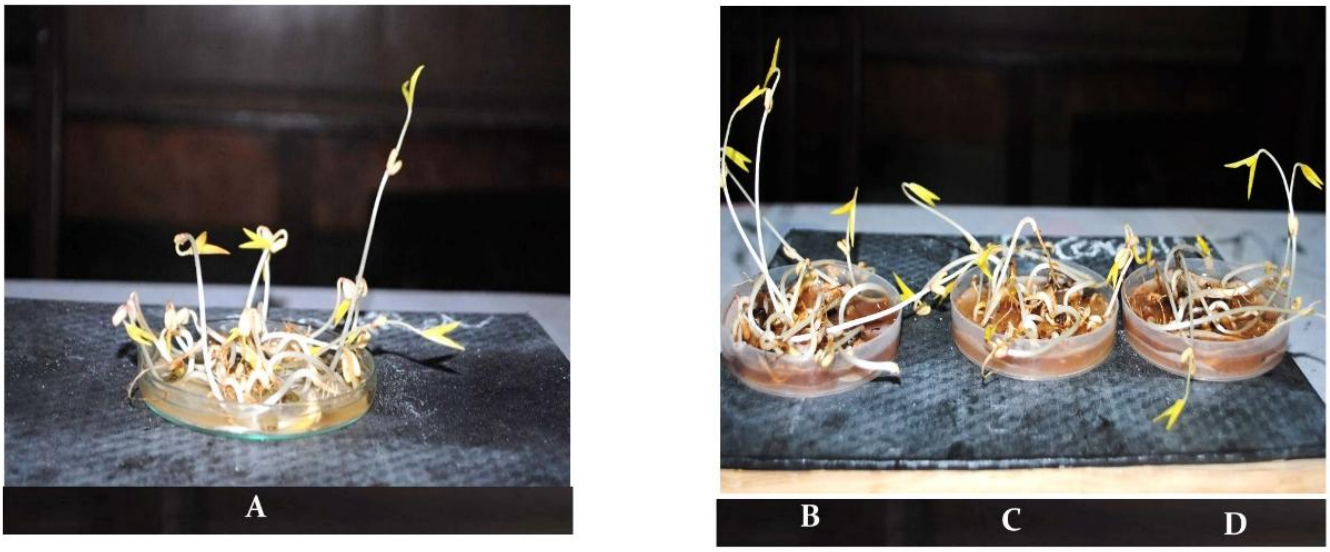


Figure 3 The seedling growth of R.cordifolia
A- Growth of seed in 0 % effluent conentration, B-Growth of seed in 50 % effluent concentration, C-75 % effluent concentration, D-100 % effluent concentration

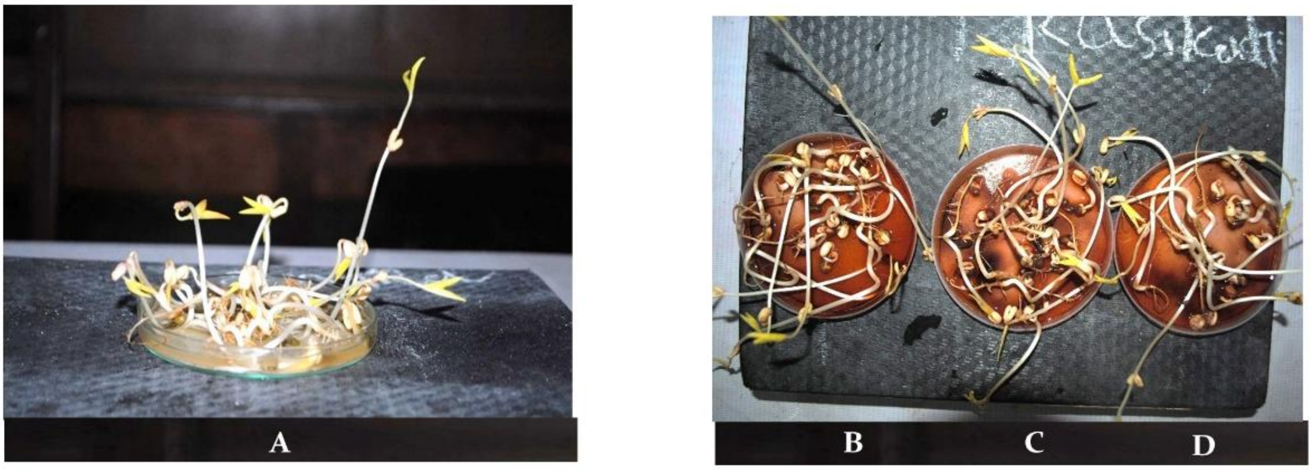


Figure 4 The seedling growth of A.catehu
A- Growth of seed in 0 % effluent conentration, B-Growth of seed in 50 % effluent concentration, C-75 % effluent concentration, D-100 % effluent concentratio

Figure 5 The seedling growth of N.arbortris-tis
A- Growth of seed in 0 % effluent conentration, B-Growth of seed in 50 % effluent concentration, C-75 % effluent concentration, D-100 % effluent concentration

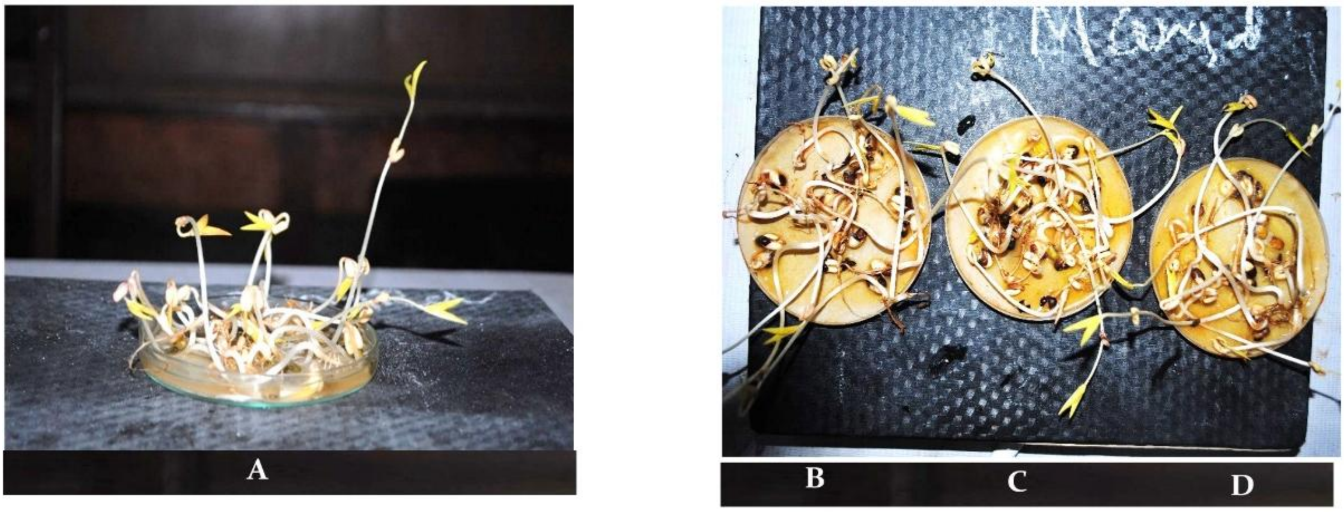


Figure 6 The seedling growth of Turmeric
A- Growth of seed in 0 % effluent conentration, B-Growth of seed in 50 % effluent concentration, C-75 % effluent concentration, D-100 % effluent concentration

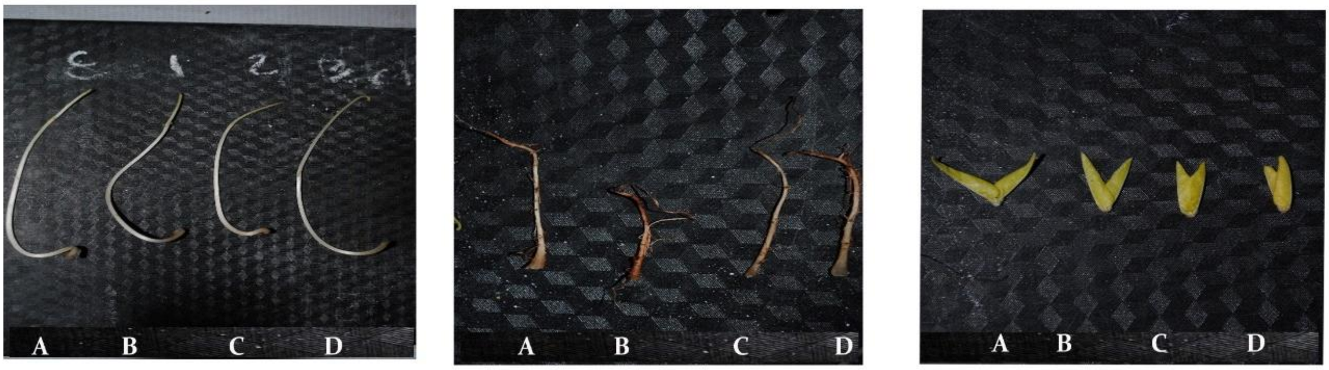


Fig 7. The Root, Shoot and leaf length of Phaseolus aureus in C.sappa

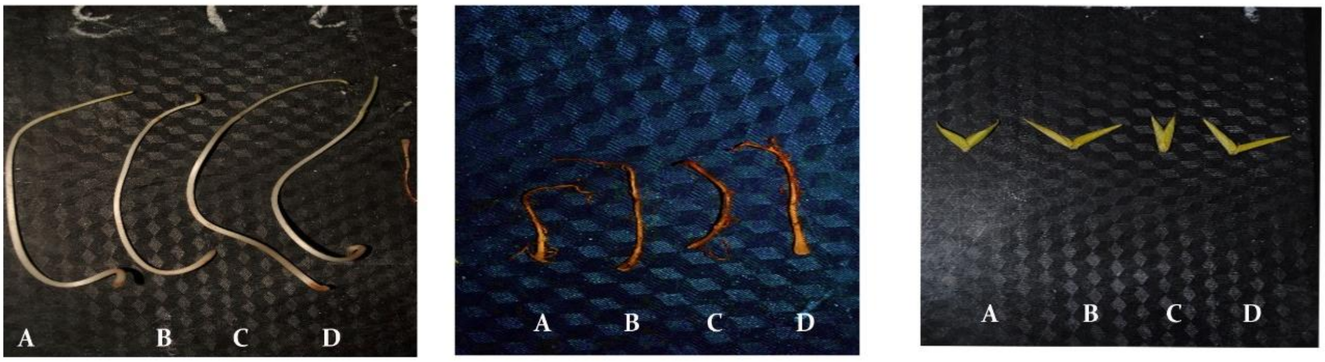


Fig 8 The shoot, root and leaf length of Phaseolus aureus (green gram) in M.champaca

Fig 9 The shoot, root and leaf length of Phaseolus aureus (green gram) in R.cordifolia

Fig 10 The shoot, root and leaf length of Phaseolus aureus (green gram) in A.catechu

A-Germinated seed from 0 % effluent concentration, B-Germinated seed from 50% effluent concentration
C- Germinated seed from 75 % effluent concentration, D- Germinated 100 % effluent concentration.

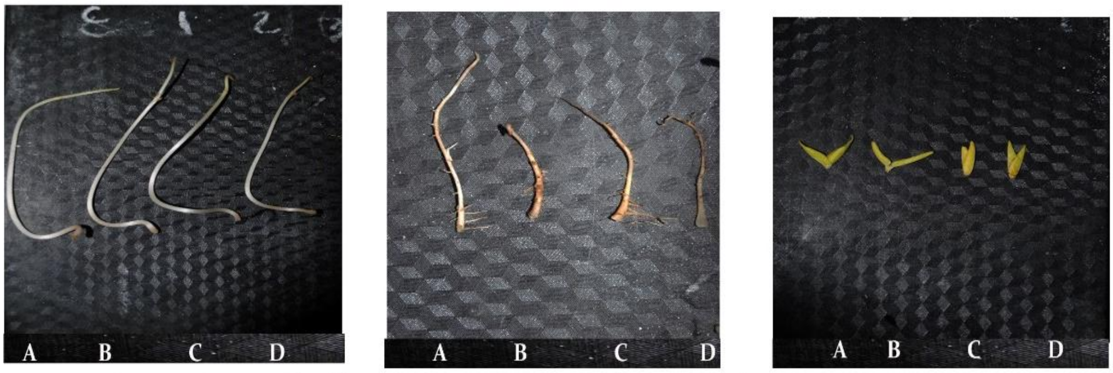


Figure 11 The root, shoot and leaf lengthof Phaseolus aureus (Green gram) N.arbortristis

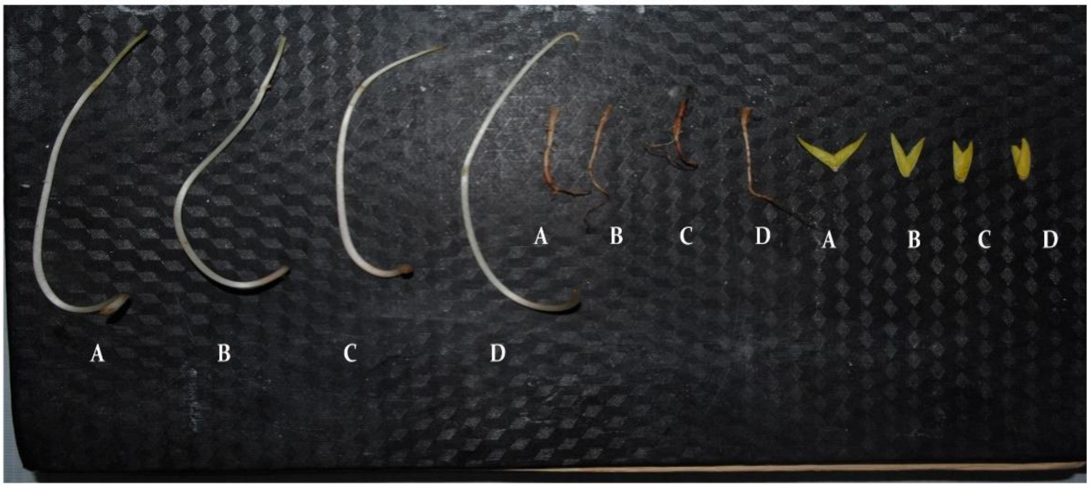


Figure 12 The root, shoot and leaf lengthof Phaseolus aureus (Green gram) Turmeric

A-Germinated seed from 0 % effluent concentration, B-Germinated seed from 50% effluent concentration
C- Germinated seed from 75 % effluent concentration, D- Germinated sees from 100 % effluent concentration.

Figure 13: Fresh weight of seed germinated growth (Root, shoot and leaf)

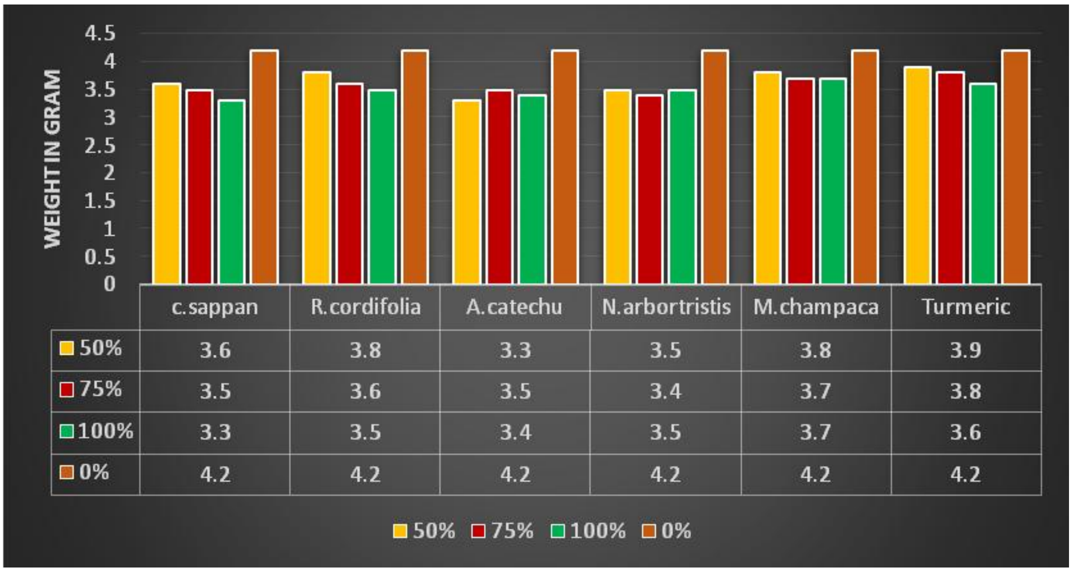
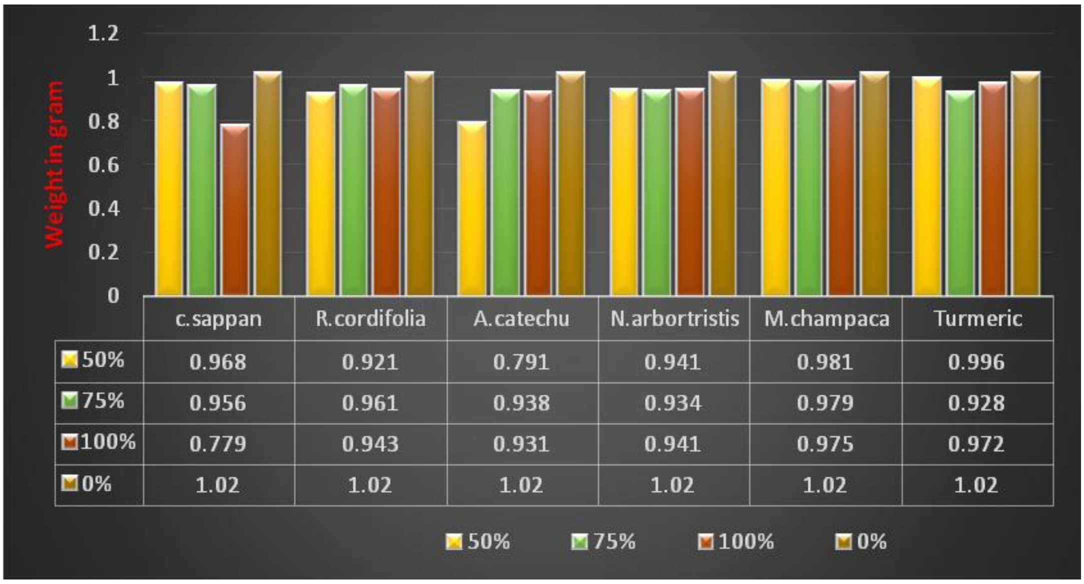


Figure 14: Dry weight of seed germinated growth (Root, shoot and leaf)



4. Acknowledgement

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