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EVALUATION OF EFFICACY OF SELECTED PLANT EXTRACTS ON SEED GERMINATION OF GREEN GRAM

Marhoob Banu[1], Prathibha K. Y.[1], Nalini T. J.[1], Geetanjali R.[1], Suresh Kumar C.[1], Khamer Ferzana Banu[1] and Keshamma E.[2*]

[1]Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India.

[2]Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India.

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***Corresponding Author**
Keshamma E.
Department of Biochemistry, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India.

ABSTRACT

The current study was conducted with the main purpose to assess four plant extracts *viz. Azadirachta indica* (Neem), *Costus pictus* (Insulin plant), *Ocimum tenuiflorum* (Tulsi) and *Trigonella foenum* (Fenugreek) at concentration 20%, 40%, 60%, 80%, 100% and 0% (untreated seeds were used as control) treated for seed germination on green gram seeds. Plant extract was prepared by grinding 200 grams of fresh and healthy leaves in 100 ml of distilled water and these leaf extracts were used to evaluate germination percentage of mung bean seeds. Green gram seeds were soaked for 24 hours in a distilled water. After 24 hours the seeds were treated in the leaf extracts of different concentrations for 2 hours. After 2 hours of seed treatment the seeds were transferred to the petriplates in the following manner- 2 in the centre, 8 in the 1st circle and 15 in the 2nd circle (Standard blotting method). The petriplates were kept at normal day light and at room temperature. Drops of water were added to the plates twice a day and the seed germination was observed for 7 days. Experiments were conducted in the closed petriplates and in triplicates. Germination of seeds was observed on daily basis for 7 days. The mean root length, shoot length, leaf length, wet weight and dry weight were recorded after 7 days. The seeds with full growth of radical and plumule were considered normal. The germination percentages were recorded. Results revealed that the *Trigonella foenum* extract at 100% concentration showed increase in the mean root, shoot and leaf length. The shoot length and root length of the seedlings of Green gram was inhibited by the plants extracts of

Ocimum tenuiflorum. *Azadirachta indica* and *Costus pictus*.

KEYWORDS: Seed germination, Plant extracts, Green gram, Seedlings, Standard blotting method.

INTRODUCTION

Pulses have been recognized as a major source of proteins (20-35%) with required minerals and vitamins. The mung bean (*Vigna radiata*) is a member of the legume family (Fabaceae). This family is a wide spread family as it occupies the third largest family of flowering plants, with approximately 650 genera and nearly 20,000 species.[1] Mung bean has many local names "mung bean, mash, golden gram or green gram". The species ranges from large tropical canopy trees to small herbs found in temperate zones, humid tropics, arid zones, high lands, savannas, and low lands.[2] The crop is of ancient cultivation in India and the plant is not found in a wild state. It is probably derived from *Phaselus radiatus* L., which occurs wild throughout India and Burma, and which is occasionally cultivated.[3]

Vigna radiata (Green gram) is an important crop in India, where it is considered as the most important among the pulses, free from the heaviness and tendency to flatulence which is associated with other pulses. As a legume plant, mung bean is in symbiotic association with Rhizobia which enables it to fix atmospheric nitrogen (58–109 kg per ha mung bean). It can provide large amounts of biomass (7.16 t biomass/ha) and nitrogen to the soil (ranging from 30 to 251 kg/ha).[4]

The nitrogen fixation ability not only enables it to meet its own nitrogen requirement, but also benefit the succeeding crops. It can be used as a cover crop before or after cereal crops in rotation, which makes a good green manure.[4] Mung beans are recognized for their high nutritive value. Mung beans contain about 55%-65% carbohydrate (equal to 630 g/kg dry weight) and are rich in protein, fat, vitamins and minerals.[5] It is composed of about 20%-50% protein of total dry weight, among which globulin (60%) and albumin (25%) are the primary storage proteins. Mung bean is considered to be a substantive source of dietary proteins. The proteolytic cleavage of these proteins is even higher during sprouting. Mung bean carbohydrates are easily digestible, which causes less flatulence in humans compared to other forms of legumes. Both seeds and sprouts of mung bean produce lower calories compared to other cereals, which makes it more attractive to obese and diabetic individuals.[6]

Azadirachta indica (neem) is a tree (Meliaceae) and it is growing in tropical and semi tropical regions. Neem is fast growing tree that can reach a height of 15 to 20 m, rarely to 35-40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. There are hundreds of secondary metabolites in the plant kingdom and many are known to be phytotoxic. Allelopathic effects of these compounds are often observed to occur early in the plant life cycle, causing inhibition of seed germination and seedling growth. The compounds exhibit a wide range of mechanisms of action, from effects on DNA, photosynthetic and mitochondrial function, phytohormone activity, ion uptake and water balance. Interpretations of mechanisms of action are complicated by the fact that individual compound can have multiple phytotoxic effects. One of the most studied aspects of allelopathy is the role of allelopathy in agriculture. This study was carried out to evaluate the allelopathic effect of leaves aqueous extract of *Azadirachta indica* on germination and seedling growth of *Vigna radiata*. Germination percentage and seedling growth of *V. radiata* significantly decreased gradually as the concentration of the aqueous leaves extracts of *A. indica* increased in comparison with control. Severe toxicity was observed at high concentrations and moderate toxicity at low concentrations in comparison with control.[12]

Costus igneus Nak (syn. *Costus pictus* D. Don, *Costus mexicanus* Liebm ex Petersen or *Costus congenitus* Rowle), commonly known as fiery costus, Step ladder or Spiral flag or Insulin plant, is native to South and Central America. This is a recent introduction to India from America as an herbal cure for diabetes and hence commonly called as 'insulin plant.[13] It is used in India to control diabetes and it is known that diabetic people eat one leaf daily to keep their blood glucose low.[14] The plant belongs to the family Costaceae.[15] This study was carried out to evaluate the allelopathic effect of aqueous leaves extract of *Costus pictus* on germination and seedling growth of *Vigna radiata*. *Costus pictus* showed severe inhibition of seed germination due to presence of secondary metabolites such as phenolic compounds. *Costus* pictus completely inhibited the seed germination because of more secondary metabolites compared to other plants.[21]

The genus Ocimum, of the family Lamiaceae (Labiatae), includes at least 60 species and numerous varieties.[16] Some of the Ocimum sp. is used in the traditional medicine for different ailments, especially in many Asian and African countries.[17] Ocimum has long been used as a medicinal plant, although little information is available about its bioactive ingredients and the influence of soil properties on modulation of secondary metabolites in

Ocimum has yet to be ascertained. Plant secondary metabolites comprise a plethora of compound. This study was carried out to evaluate the allelopathic effect of leaves aqueous extract of *Ocimum tenuiflorum* on germination and seedling growth of *Vigna radiata*.[18] Previous Studies reveal that aqueous leaves extract of some species contains phenolics or other toxic substances. These phenolics inhibit the germination and seedling growth of tested plants.[18,19] Same result was observed in this study where aqueous leaves extract of *Ocimum tenuiflorum* inhibited the germination and growth of seedlings of green gram.

Fenugreek (*Trigonella foenum-graecum L.*) is a medicinal plant that has been used as a food condiment as well as for its multiple therapeutic characteristics since ancient times. Fenugreek plant grows up to 60 cm in height and its seeds are golden-yellow rhomboidal-shaped. Though fenugreek is more commonly known for its seeds, the leaves and stem have also been reported to have medicinal uses. These properties exhibited are due to the content of the secondary metabolites, also known as phytochemicals in the fenugreek plant. Such metabolites are alkaloids, saponins, tannins, phenols, and many others. Fenugreek has been used traditionally for numerous indications, such as aid in labour, lactation stimulant, and laxatives.[20] This study was done to evaluate the Allelopathic effect of *Trigonella foenum* on seed germination and seedling growth of Green gram using aqueous leaves extract. The results showed that aqueous leaves extract of *Trigonella foenum* promoted the growth and germination of seedlings of Green gram.

Hence, in the present study we aimed to assess four plant extracts *viz. Azadirachta indica* (Neem), *Costus pictus* (Insulin plant), *Ocimum tenuiflorum* (Tulsi) and *Trigonella foenum* (Fenugreek) at concentration 20%, 40%, 60%, 80% and 100% treated for seed germination on green gram seeds and 0% was used as control.

MATERIALS AND METHODS

Plant extracts used

1. *Azadirachta indica* (Neem) hu
2. *Costus pictus* (Insulin plant)
3. *Ocimum tenuiflorum* (Tulsi)
4. *Trigonella foenum* (Fenugreek)

Plant extract preparation

Plant extract was prepared by grinding 200 grams of fresh and healthy leaves in 100 ml of

distilled with the help of pestle and motor and the extract of leaves were extracted.

Concentrations of plant extract used for seed treatment

1. 0% = Control (Untreated seeds)
2. 20% concentration = 20 ml of extract was added to 80 ml of water
3. 40% concentration = 40 ml of extract was added to 60 ml of water
4. 60% concentration = 60 ml of extract was added to 40 ml of water
5. 80% concentration = 80ml extract + 20ml water
6. 100% concentration = 100 ml extract

Seed treatment with plant extracts

Green gram seed were soaked for 24 hours in a distilled water. After 24 hours the seeds were treated in the leaf extract of different concentrations for 2 hours. Glass petri plates were sterilized using 70% alcohol and blotting papers of the same size were cut and added to petriplates. After 2 hours of seed treatment the seeds were transferred to the petriplates in the following manner- 2 in the centre, 8 in the 1st circle and 15 in the 2nd circle (Standard blotting method). The petriplates were kept at normal day light and room temperature. Drops of water were added to the plates twice a day and the seed germination was observed for 7 days. Experiments were conducted in the closed petriplates and in triplicates. Germination of seeds was observed on daily basis for 7 days. The mean root length, shoot length, leaf length, wet weight and dry weight was recorded after 7 days. The seeds with full growth of radical and plumule were considered normal. The germination percentage was recorded.

RESULTS AND DISCUSSION

Assessment of effect of plant extracts on Green gram seed germination was observed. The seed treatment with the leaf extract of *Trigonella foenum* at 100% was found most effective with increase in mean root, shoot length and leaf length to some extent (Table 1 and Figure 1), followed by *Ocimum tenuiflorum* extract at 20% was found to be effective in the growth of mean root, shoot length and leaf length to some extent (Table 2 and Figure 2). The *Trigonella foenum* extract showed to increase in the root, shoot and leaf length compared to control (0%) and *Ocimum tenuiflorum* extract. The dry (0.316 g) and wet weight (0.357 g) of green gram treated with *Trigonella foenum* extract was more at 100% concentration compared to the dry weight (0.245 g) and wet weight (0.267 g) of *Ocimum tenuiflorum* extract at 100% concentration (Table 3 and Table 4). *Azadirachta indica* and *Costus pictus* plant extracts inhibited the growth of seedlings. In *Costus pictus* extract treated seeds, it

inhibited the complete germination (Figure 3). From the findings of Miah et al., it was revealed that there were no significant effects of *Azadirachta indica* (Neem) oil in reducing the viability of green gram seeds.[7] Besides, Haque et al., also found that there was no adverse effect on the viability of seeds treated with plant oils.[8] This is in contrast to our study results. Rajeswari et al., reported that *T. harzianum* as seed treatment and soil application as most effective in reducing the disease by 95.3% and increased seed germination by 96%, plant height by 35.5 cm and total biomass by 2.53 g plant^{-1} as against control (58%, 84%, 23.5 cm and 1.53 g plant^{-1}) of dry root rot caused by *Macrophomina phaseolina* of green gram.[9] Rahman et al., in chilli studied antifungal activity of the plant extracts *viz.*, Neem leaf, Ginger rhizome and Garlic clove, which increased the seed germination and reduced seed mycoflora.[10] Sahu and Kar reported that seed borne infection of *M. Phaseolina* reduced seed germination and seedling length in green gram varieties.[11] This study was carried out to evaluate the allelopathic effect of leaves aqueous extract of *Costus pictus* on germination and seedling growth of *Vigna radiata*. *Costus pictus* showed severe inhibition of seed germination due to presence of secondary metabolites such as phenolic compounds. *Costus* pictus completely inhibited the seed germination because of more secondary metabolites compared to other plants.[18,19] This study was also carried out to evaluate the allelopathic effect of leaves aqueous extract of *Ocimum tenuiflorum* on germination and seedling growth of *Vigna radiata*.[18] Previous Studies reveal that aqueous leaves extract of some species contains phenolics or other toxic substances. These phenolics inhibit the germination and seedling growth of these plant.[18,19] Same result was observed in this study where aqueous extract of leaves inhibition the germination and growth of seedlings of Green gram. Present work was done to evaluate the Allelopathic effect of Trigonella foenum on seed germination and seedling growth of Green gram using aqueous leaves extract. The results showed that aqueous leaves extract of *Trigonella foenum* promoted the growth and germination of seedlings of Green gram.[20]

Present study discovers that the aqueous leaves extract of *Trigonella foenum* have beneficial role for improving the growth and germination of seedlings of Green gram and this study will be helpful in agriculture where aqueous leaves extract of *Trigonella foenum* can be used as manure. This study also revealed the phytotoxic effects of aqueous leaves extract of Ocimum tenuiflorum, Azadirachta indica, costus pictus. The aqueous leaves extract of these three can also be used for antibacterial activity.

Table 1: Mean root, shoot and leaf length of Green gram treated with *Trigonella foenum* (Fenugreek) extract.

Concentration	Mean Root length (cm)	Mean shoot length (cm)	Mean leaf length (cm)
0%	5.90 cm	9.70 cm	1.60 cm
20%	6.00 cm	9.80 cm	1.40 cm
40%	6.20 cm	9.90 cm	1.40 cm
60%	6.40 cm	10.30 cm	1.40 cm
80%	6.70 cm	10.70 cm	1.40 cm
100%	7.10 cm	11.20 cm	1.40 cm

Table 2: Mean root, shoot and leaf length of Green gram treated with *Ocimum tenuiflorum* (Tulsi) extract.

Concentration	Mean Root length (cm)	Mean shoot length (cm)	Mean leaf length (cm)
0%	5.90cm	9.70 cm	1.60 cm
20%	5.80 cm	9.10 cm	1.62 cm
40%	5.20 cm	8.70 cm	1.63 cm
60%	5.00 cm	8.40 cm	1.65 cm
80%	4.80 cm	8.00 cm	1.66 cm
100%	4.10 cm	7.90 cm	1.67 cm

Table 3: Mean root, shoot and leaf length of Green gram treated with *Azadirachta indica* (Neem) extract.

Concentration	Mean Root length (cm)	Mean shoot length (cm)	Mean leaf length (cm)
0%	5.90cm	9.70 cm	1.60 cm
20%	5.78 cm	9.00 cm	1.59 cm
40%	5.10 cm	8.50 cm	1.58 cm
60%	4.98 cm	8.37 cm	1.56 cm
80%	4.68 cm	7.79 cm	1.56 cm
100%	4.08 cm	7.76 cm	1.56 cm

Table 4: Dry and wet weight of Green gram treated with *Trigonella foenum* extract.

Concentration	0%	20%	40%	60%	80%	100%
Dry weight (grams)	0.261	0.271	0.282	0.301	0.313	0.316
Wet Weight (grams)	0.288	0.298	0.301	0.341	0.351	0.357

Table 5: Dry and wet weight of Green gram treated with *Ocimum tenuiflorum* extract.

Concentration	0%	20%	40%	60%	80%	100%
Dry weight (grams)	0.261	0.260	0.258	0.250	0.248	0.245
Wet Weight (grams)	0.288	0.288	0.273	0.270	0.269	0.267

Table 6: Dry and wet weight of Green gram treated with *Azadirachta indica* extract.

Concentration	0%	20%	40%	60%	80%	100%
Dry weight (grams)	0.261	0.258	0.258	0.249	0.248	0.245
Wet Weight (grams)	0.288	0.286	0.273	0.269	0.269	0.267

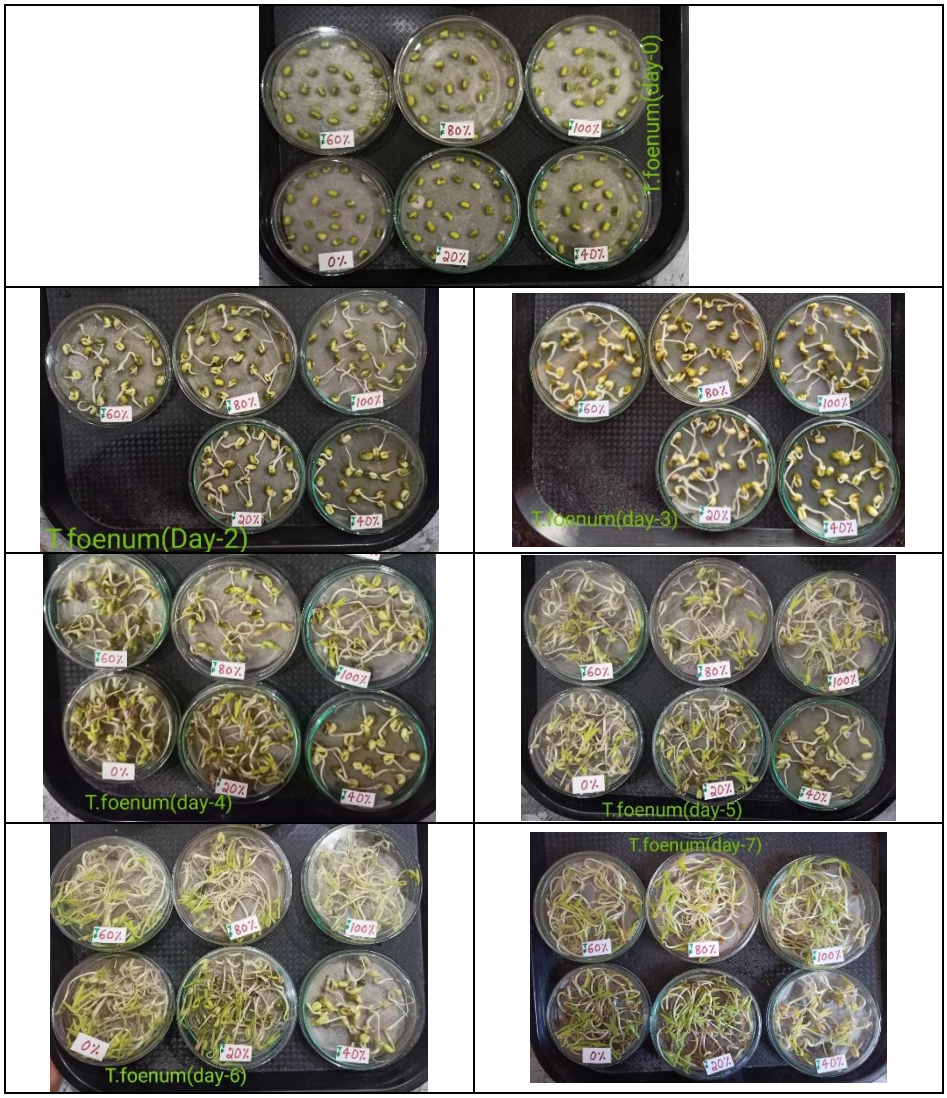
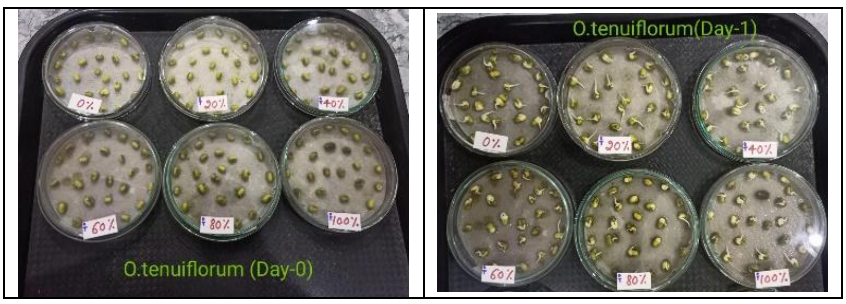


Figure 1: Green gram seeds treated with *Trigonella foenum* (Fenugreek).



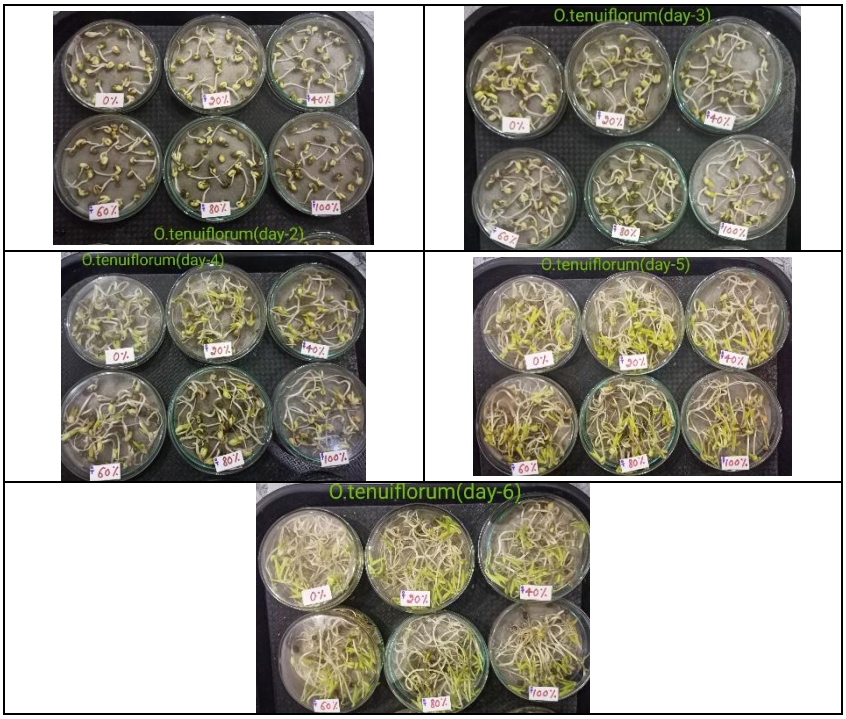


Figure 2: Green gram seeds treated with *Ocimum tenuiflorum* (Tulsi).

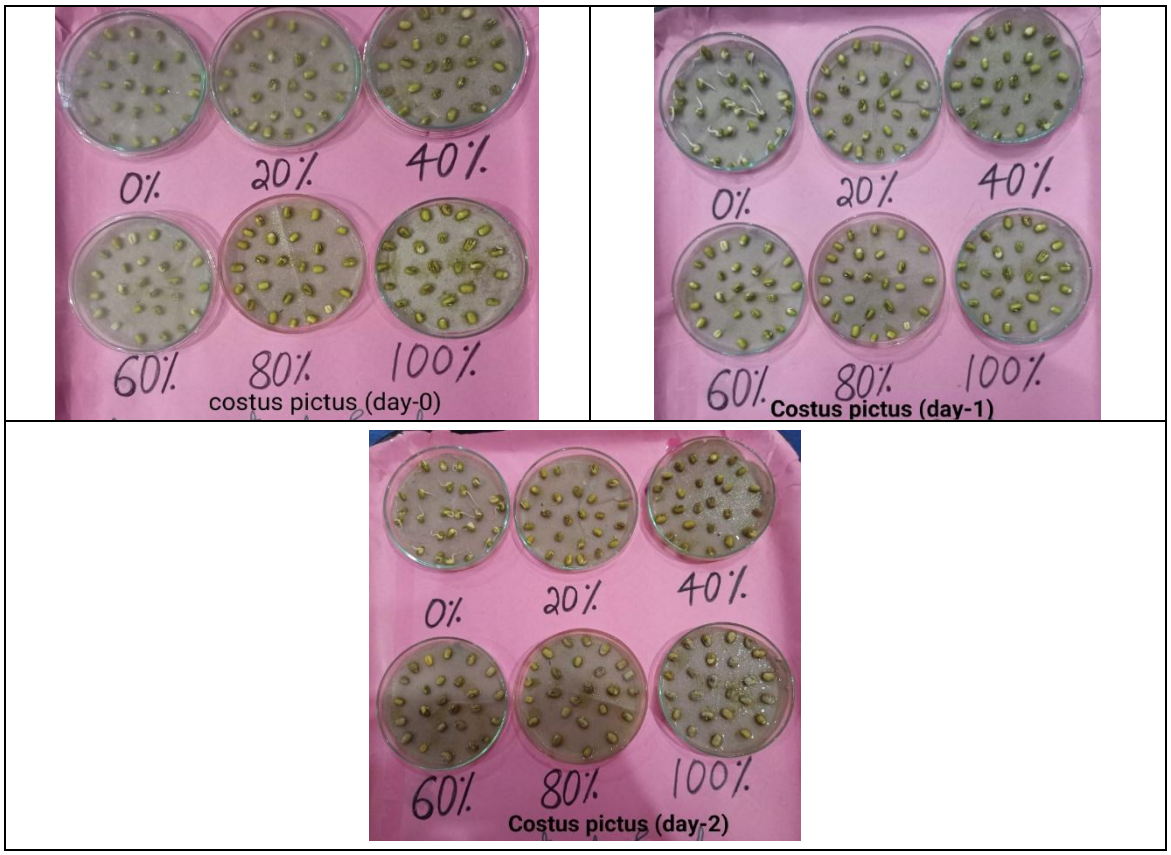


Figure 3: Green gram seeds treated with *Costus pictus* (Insulin plant) extract

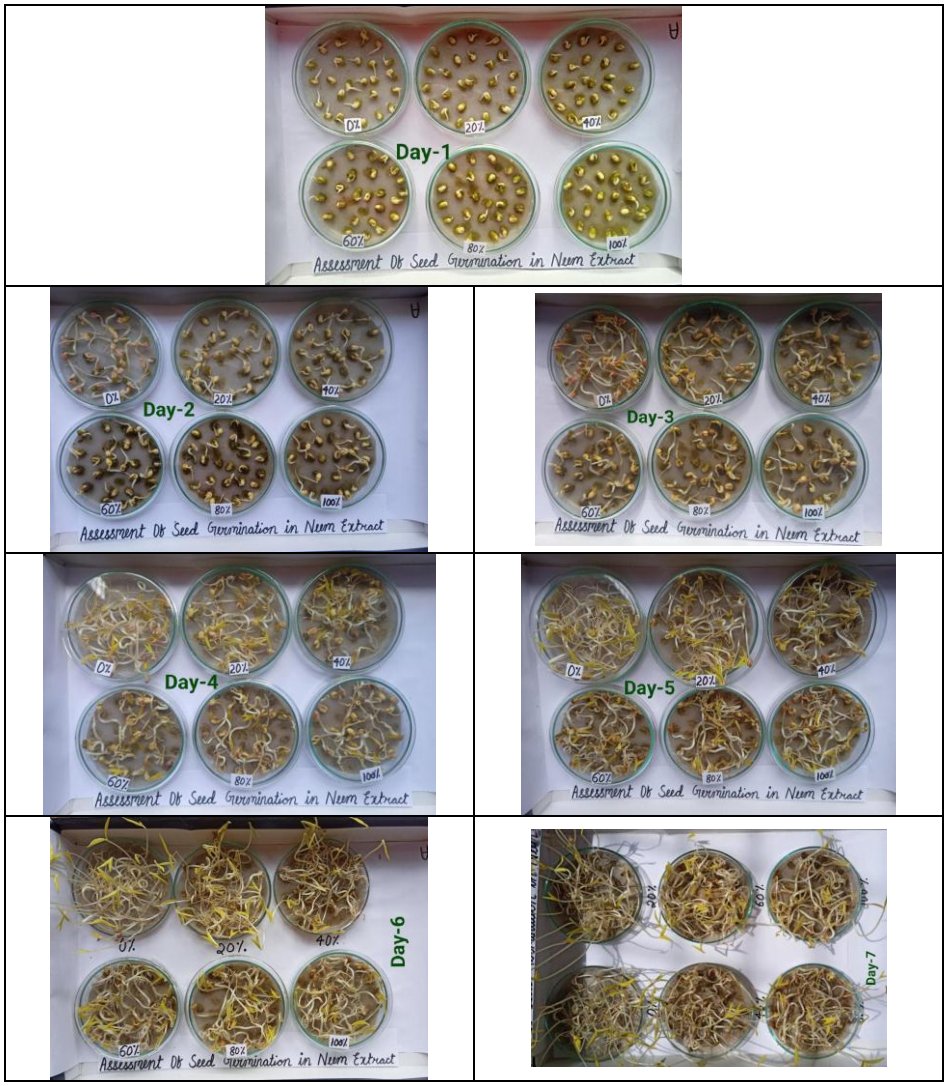


Figure 4: Green gram seeds treated with *Azadirachta indica* (neem) extract.

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