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The effect of caffeine and nicotine on different plant growth

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ABSTRACT

This science fair project was performed to find out the effect on plant growth of adding caffeine to the soil. This experiment was done by using Mung bean (Vigna radiata L.) plants, and watering them daily with normal water, water mixed with caffeine bean and with a coffee mixture. Height of plant was measured as a daily parameter to check effect on growth. On fifteen day of germination the plant was removed to evaluate dry weight and spectrophotometric measurement for chlorophyll content. The result of the experiment found that caffeine with low amount gives best results which can be reconfirmed by height, Chlorophyll a, b, carotenoid estimation. The result indicate that mung bean grows faster in soil with caffeine. Another experiment was carried out to show that the effect of nicotine on plant growth fenugreek seeds (Trigonella foenum-graecum) by using cigarette and chewing tobacco. Nicotine is an addictive drug that is found mostly in cigarettes, cigar, chewing snuff. Cigarette and chewing tobacco contain thousands of chemical compounds but nicotine is a major constituent of cigarette and tobacco, kills insect pests and used in many organic insecticides to protect plants. Our study investigated the impact of pure tobacco (chewing tobacco) and cigarette on seed germination of fenugreek. We had three plants of same type and plant them in the same soil and give them same amount of water. One had just water with no nicotine making this plant the control. Second plant had pure tobacco mixed with soil, third plant had cigarette contents mixes with soil. The result showed that if we use cigarette, the plant will grow taller" as compare to the normal plant. But there was significant decrease in germination rate at pure tobacco or at high concentration of tobacco.

Keyword**:** *Mung bean (Vigna radiata), fenugreek seeds (Trigonella foenum- graecum), coffee, coffee bean crushed (caffeine bean), cigarette, chewing tobacco.*

1. INTRODUCTION

This study was carried out to show the effect of caffeine and nicotine on different plants. From many experiments it shows that plant contain minimum amount of nicotine it may come from human use or nicotine-based pesticides. As nicotine is harmful for human beings it's important to know its effect on growth of plant.

1.1 Caffeine

In today's world, caffeine is more popular than ever before. In fact, more than 120,000 tons of caffeine is consumed worldwide each year. This goes towards drinks such as coffee and tea, as well as popular foods such as chocolate. Caffeine is complex compound and probably one of the most researched components of the diet. This experiment was performed to find out the effect of adding caffeine to soil and its effect on plant growth. Some plants seem to benefit and grow faster when caffeine is added to the soil, while other seems to become stunted or grow slower. There are also some plants that are not affected by presence of caffeine in the soil. Caffeine can be introduced to the soil by sprinkling grounded coffee over the soil, adding leftover coffee to the pot or watering with a caffeine solution made by dissolving a caffeine bean powder in water. The grounded coffee is actually organic matter and will help in adding nutrients to the soil. It will also attract worms that feed on grounded coffee and at same time help to aerate the soil.

1.2 Nicotine

Nicotine, the active ingredient in tobacco is second only to caffeine as the most widely used central nervous systems stimulant. In combination with the tars and carbon monoxide in cigarette smoke and it is represent a serious health risk but however, is approved by FDA as an insecticide. Another experiment was carried out to show that effect of nicotine on plant growth and nicotine substance have found it actually have a positive effect on plant growth.

2. EXPERIMENTAL WORK

2.1 Aim

To study the effect of caffeine and nicotine on growth of plants.

2.2 Objectives

- Three pots are used for Vigna radiata & three for Trigonella foenum-graecum. In pot number 1 added only seeds of Trigonella foenum-graecum seeds and in pot number 2 added seeds with cigarette and pot 3 contain seeds with chewing tobacco. Three pots for vigna radiata, in pot number 1 added only seeds and in pot number 2 added seeds, watered with coffee and pot number 3 added seeds, watered with caffeine bean powder solution.
- For concentration: Coffee and cigarette, tobacco was used at different concentration for vigana radiata and Trigonella foenum-graecum respectively.
- Qualitative test for Carbohydrate & Protein.
- Protein estimation by Folin lowry's method.
- Absorption spectrum of plant pigments

3. MATERIALS AND METHODS USE

3.1 Requirements

3.1.1 Cultivation: Soil, water, pots, *Vigna radiata L, Trigonella foenum-graecum* seeds, coffee (Nescafe sunrise) and coffee bean powder, cigarette and chewing tobacco (local tobacco packet) both are nicotine source.

3.1.2 Lab work

- **Chlorophyll extraction:** Pestle and mortar, leaf, separating funnel and stands, beakers. 80% Acetone, Petroleum Ether, Methanol, Diethyl Ether, 30% KOH Methanol, Distilled water.
- **Test for carbohydrates:** Plant extract, Molisch reagents, concentrated sulphuric acid.
- **Test for protein:** Plant extract, 0.2% Ninhydrin, Water bath.
- **Estimation of protein by Lowry's method:** Plant extract, Phosphate buffer, Alkaline sodium carbonate reagent, Folin's reagent, 20% TCA, Acetone, 0.1N NaoH , Bovine serum albumin (BSA) , Alkaline copper sulphate, Sodium potassium tartarate, Distilled water.

3.2 Method

3.2.1 For Cultivation of plants

a) Six pots are taken equal amount of soil is added on all pots.
b) Three pots are used for *Vigna radiata* & three pots for *Trigonella foenum-graecum.*
c) Three pots for *Vigna radiata:* Fill the 3 pots with equal amounts of soil. Placed fifteen mug beans in each
d) pot and additional seeds placed in the pots in case some of the seeds do not germinate; the additional plants can be removed later.
e) Prepared the caffeine solution by dissolving 10g of caffeine beans powder in 100ml of water in a beaker. Labelled the beaker 'caffeine'. Similarly, added 10g of coffee to 100ml of water in another beaker and labelled it 'coffee'.
f) Labelled the 3 pots 'water', 'caffeine' or 'coffee' and water the pots once a day with 100ml water, caffeine solution or coffee mixture, according to the labels on the pots.
g) Three pots for *Trigonella foenum-graecum:* In pot number 1 added only seeds and in pot number 2 added seeds with cigarette content and pot 3 contain seeds with chewing tobacco (local packet).
h) Equal amount of water is given on daily basis as well as same environmental condition is provided.
i) All plant is placed at distance so that there's no competition between the plants for sunlight.
j) After one-week growth were observed and average length of each pot is measured.

3.2.2 Absorption spectrum of plant pigment

a) Grind properly the leave bits with 80% acetone in pestle and mortar.
b) Filter the extract with funnel.
c) Add 30ml of petroleum ether and mix thoroughly, then add 35ml of distilled water.
d) Allow for the separation in a separating funnel.
e) The upper is petroleum ether containing the pigment. Discard the lower acetone-water layer.
f) To the petroleum ether layer add 25ml of methanol and mix thoroughly. Allow to separate in separating funnel.
g) The petroleum ether fraction will contain the Chlorophyll b and carotenoids. The methanol fraction will have the Chlorophyll a and xanthophyll.
h) Collect the two fractions in two different beakers.
i) To the petroleum ether fraction add 30ml of KOH methanol and 15ml of water and mix thoroughly in separating funnel. Collect the two layer.one is the Chlorophyll b and the other carotenoids.
j) To the methanol layer add 25ml of diethyl ether and 25ml of distilled water. The xanthophyll and chlorophyll a layer separate. Collect it in two different beakers.
k) Observe the different fraction in the spectra photometer.

3.2.3 Qualitative test for protein: Take 2 ml of plant extract, 4 drops of ninhydrin were added and heated to 100 degree Celsius. Formation of blue color indicates presence of protein.

3.2.4 Qualitative test for carbohydrate: Take 2ml of plant extract, 1ml of Molisch reagent & 4 drops of conc. sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrates.

3.2.5 Estimation of protein by Folin-lowry's method

- Sample extract: weigh 1 gm sample (leaves) macerate the sample in pestle mortar in 5 ml of phosphate buffer & transfer the

material to centrifuge tubes. Centrifuge the homogenate it 8000 rpm for 20 min. collect the supernatant and repeat the extraction 4- 5 times. Combine the supernatants and make the volume to 50ml with phosphatebuffer.

- Take 1ml of above extract and add 1 ml of 20%TCA. Keep it for half an hour and centrifuge at 8000 rpm for 20 min. Wash the pellet with acetone twice and again centrifuge it. Discard the supernatant.
- Dissolve the pellet in 5 ml of 0.1 N NaoH and mix well till it gets dissolved.
- Take suitable aliquot 1 ml of above solution and add to it freshly prepared alkaline copper sulphate reagent. Mix properly and after 10 min add 0.5 ml Folin's reagent. Mix the contents instantaneously. Allow the color to develop for 30 min.

Table 1

Tube	Protein conc (micro-gram)	Aliquot of protein (mL)	Amount of D/W (mL)	Total volume= 1.0 ml	Alkaline Cu solution= 5 ml	Mix well, incubate at room temperature for 15mins	Folin ciocaltean reagent 0.5 ml	All it to react at RT for 30 min & take O.D at 750nm
Blank	0	-	1.0					
1.	40	0.2	0.8					
2.	80	0.4	0.6					
3.	120	0.6	0.4					
4.	160	0.8	0.2					
5.	200	1.0	-					
Sample 1								
Sample 2								
Sample3								

- Record the absorbance at 660 nm after setting the instruments with reagent blank which contains 1 ml of 0.1 N NaoH & develop the colour as described in steps 4 and 5.

Table 2

Tube	**Protein conc. (Aliquot)**	**O.D At 750nm**
Blank	-	
1.	0.2	
2.	0.4	
3.	0.6	
4.	0.8	
5.	1.0	
Sample1M(Normal)		
Sample2M(Coffee)		
Sample3M(Caffeine)		
Sample1F(Normal)		
Sample2F(Cigarette)		
Sample3F(Tobacco)		

4. RESULTS

4.1. Cultivation of plant

4.1.1 Sprouting (germinating seeds)

Fig. 1: Normal water

Fig. 2: Coffee solution

Fig. 3: Soaked beans for sprouts

Fig. 4: Select 10 mung bean

Fig. 5: Bean sprouts (normal)

Table 3

Normal bean Length/swell (cm)	Caffeine bean Length/swell (cm)
0.7/0.4	0.7/0.5
0.7/0.5	0.7/0.4
0.8/0.5	0.6/0.4
0.8/0.5	0.7/0.4
0.7/0.4	0.7/0.4
0.7/0.5	0.7/0.5
0.7/0.5	0.7/0.4
0.6/0.4	0.5/0.4
0.6/0.3	0.5/0.4
0.8/0.4	0.5/0.4

Normal bean average = Total ÷ 10 =7.1÷10 = 0.71
Caffeine bean average = Total ÷10 = 6.3 ÷10 = 0.63

4.1.2 Seeds planted

Fig. 6: Caffeine solution plants Coffee solution plant Normal plant

4.1.3 Observation
Day 5th observation
Fenugreek *(Trigonella foenum-graecum)*

Fig. 7: Pot contain tobacco Pot cigarette Normal soil

Table 4

Normal-stem/root (cm)	Cigarette (cm)	Tobacco (cm)
5/2.4	**5.6/4.2**	**4.5/8**
6.2/7.5	**5.7/7.5**	**4.3/3.8**
5.2/5.5	**5.2/6.8**	**3.8/8**
5.6/4.1	**6.2/5.2**	**3.6/4**
6.3/2	**6.1/6.7**	**4/7**
5.5/7	**5.9/7.2**	**4.5/3**
6.3/3	**6.3/7.9**	**4.3/7.5**
5.6/8	**5.6/7**	**4.4/2.8**
6.8/7	**5.4/2**	**3.5/2**
5.3/7.8	**6.4/4.7**	**4.2/2.8**

Highest growth were observed on cigarette contain plant as compare with normal then tobacco

Day 7th observation
Mung bean *(Vigna radiate)*:

Fig. 8: Caffeine solution Coffee solution Normal water

Table 5

Plant name	DAY 2	DAY 5	DAY 10
***Vigna radiata L.* (Normal) (cm)**	No growth	3.6	8.2
***Vigna radiata L.* (Coffee) (cm)**	No growth	4.6	7.4
***Vigna radiata L.* (Coffee bean solution) (cm)**	No growth	2.0	3.8

Highest growth of mung bean were observed in plant which containing coffee solution as compare with normal. But continuous watering with coffee solution, as result their growth is stunted.

Day 10th observation

Fig. 9: Mung bean *(vigna radiata)*

Fig. 10: Fenugreek (Trigonella foenum-graecum)

Day 15-20th observation

Fig. 11: Vigna radiata & Trigonella fornum-graecu

Observation of mung bean and fenugreek plant was carried out till Day 15 TO 20

4.2. Lab work

4.2.1 Absorption spectrum of plant pigment

Fig. 12: Trigonella foenum-graecum & Vigna radiate

For mung bean *(Vigna radiata)*:

Vigna radiate	Normal (O.D)	Coffee (O.D)	Caffeine (O.D)
Chlorophyll a	0.52	0.26	0.25
Chlorophyll b	0.99	0.36	0.30
Carotenoids	0.21	0.14	0.12
Xanthophyll	0.26	0.24	0.19

For fenugreek *(Trigonella foenum-graecum)*:

Trigonella foenum- graecum	Normal (O.D)	Cigarette (O.D)	Tobacco (O.D)
Chlorophyll a	0.82	0.70	0.12
Chlorophyll b	0.50	0.45	0.08
Carotenoids	0.28	0.23	0.18
Xanthophyll	0.47	0.08	0.09

4.2.2. Qualitative test for proteins

Fig. 13: Vigna radiata & Trigonella foenum-graeum

Fig. 14: For mung bean *(Vigna radiata)*

Fig. 15: For fenugreek *(Trigonella foenum-graecum)*

4.2.3 Qualitative test for carbohydrates

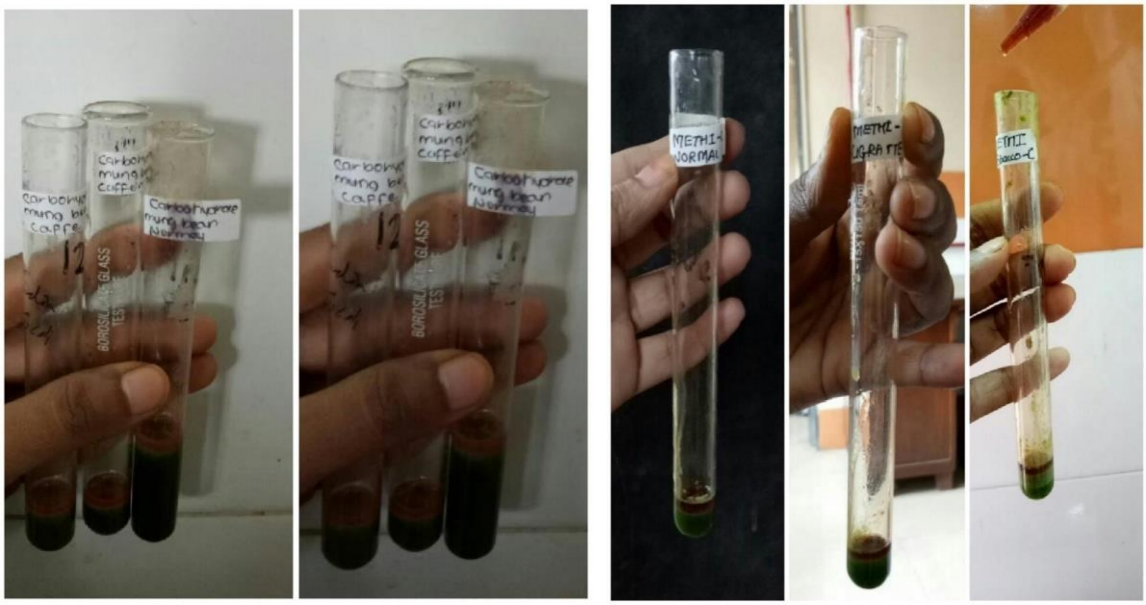


Fig. 16: For mung bean *(Vigna radiata)* **For fenugreek *(Trigonella foenum-graecum)***

4.2.4. Protein estimation by Lowry's method

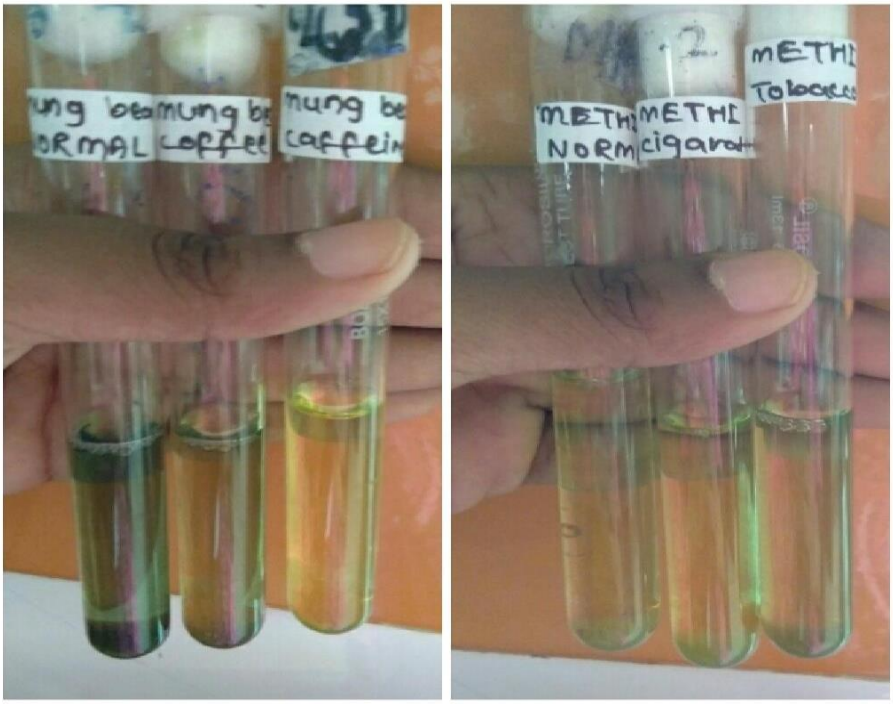


Fig. 17: For mung bean pant **For fenugreek plant**

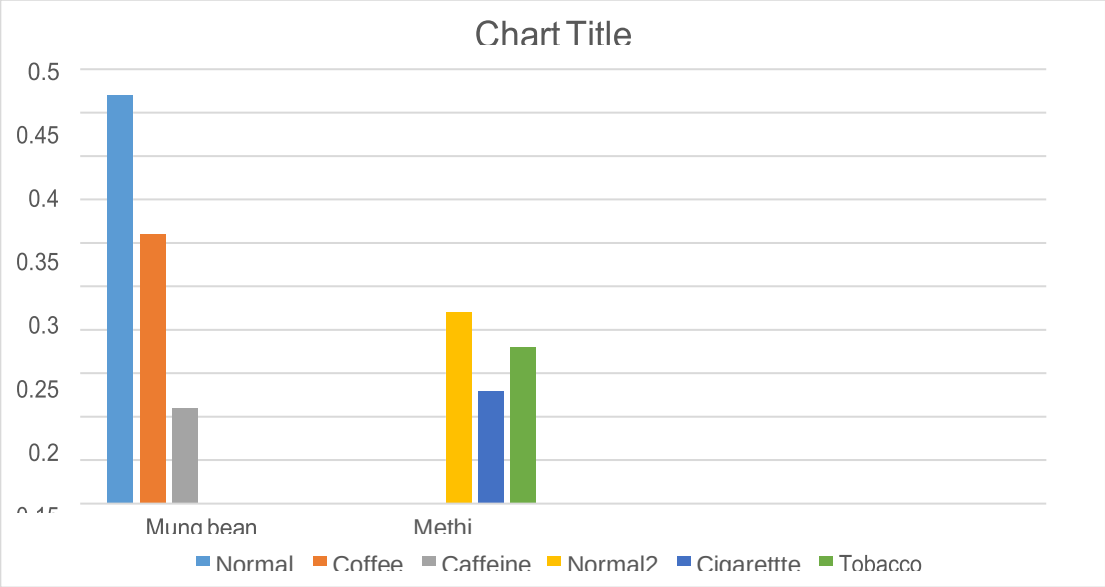
Fig. 18

Observation table:

Table 7

Tube	Protein conc. (aliquot)	O.D. at 750 nm
Blank	-	00
1.	0.2	0.10
2.	0.4	0.11
3.	0.6	0.12

4.	0.8	0.14
5.	1.0	0.16
Sample1 mung (normal)	-	0.42
Sample2 mung (coffee)	-	0.30
Sample3 mung (caffeine)	-	0.10
Sample1 fenugreek(normal)	-	0.22
Sample 2 fenugreek(cigarette)	-	0.13
Sample3 Fenugreek(tobacco)	-	0.18



Observation table: Sample concentration

Tube	Protein conc. (aliquot)	O.D. at 750 nm
Blank	-	00
1.	0.2	0.10
2.	0.4	0.11
3.	0.6	0.12
4.	0.8	0.14
5.	1.0	0.16
Sample1 mung (normal)	1.53	0.42
Sample2 mung (coffee)	1.1	0.30
Sample3 mung (caffeine)	0.36	0.10
Sample1 fenugreek (normal)	0.8	0.22
Sample 2 fenugreek (cigarette)	0.47	0.13
Sample3 fenugreek (tobacco)	0.65	0.18

4.3 Plant growth- at different concentration of compound:
Mung bean *(Vigna radiata)*:

Fig. 19

Fenugreek *(Trigonella foenum-graecum):*

Fig. 20

5. CONCLUSION

5.1 For cultivation of plant

The effect of caffeine on growth of plant was studied and from this experiment we can conclude that caffeine enhances the plant growth as compare with normal plant *(Vigna radiata)*. Caffeine source like coffee and coffee bean powder were used. From these two-caffeine source, coffee enhances the growth more efficiently than normal and then coffee bean powder. But also, coffee bean powder stunt growth of plant. And continuous watering with coffee solution also have shown that, initially cell growth rates are stable but soon the coffee begins to kill or distort these cells, resulting in a stunted plant. That means too much caffeine can have a detrimental effect on plant growth.
From this result we can conclude that caffeine at low amount such in coffee enhance the growth of plant but stunt growth of plant in high amount such in caffeine beans powder.

The effect of nicotine on growth of plant was studied and from this experiment we can conclude that nicotine enhances the plant growth as compare with normal plant *(Trigonella foenum-graecum)*. Nicotine source like cigarette and chewing tobacco was used. From these two-nicotine source, cigarette enhance the growth more efficiently than normal and the least growth on tobacco.

Three pots were used for *trigonella graceum foenum* pot no.1 containing normal seeds, pot no.2 containing seeds with cigarette & no. 3 seeds with tobacco. After day to day observation it showed that as compare to normal one the nicotine containing *Trigonella foenum-graecum* shows high length of root & shoot which may be due to hormone production. Abscisic Acid & cytokinin which is responsible for the root & shoot growth.

Since the nicotine has a drastic effect on root & shoot length which can be concluded as nicotine enhances the production of various hormones in plant which in turn influences hormone production in plant. So, in future it can be used to increase the growth of plant where as the nicotine shows the effect on nitrogen deficiency this can be overcome by adding nitrogen source such as fertilizers or organic manure.

5.2 Plant growth at different concentration

5.2.1 Plant growth at different concentration of coffee

Different concentration of coffee like 1packet, 2packet, 3packet, 4packet, 5packet were used for mung bean plant. For mung bean used coffee 1pack, 2packet, 3packet, 4packet, 5packet were added on three pots and provided environmental condition. After 7 days result shows that highest growth appears on 4packet contain plant then 3, 2, 1, and least growth on 5packet.

5.2.2 Plant growth at different concentration of cigarette and tobacco

For Different concentration of nicotine like cigarette, 1cigarette, 2cigarette, 4cigarette and tobacco, low amount of tobacco, high amount of tobacco were used for fenugreek plant. Different concentration cigarette and tobacco were added in pots and provided equal amount of water as well as environmental condition. After 7day result shows that highest growth appears on 4 cigarette contain plant then 2cigarette and then 1cigarette. For tobacco, highest growth appears on high amount of tobacco contain plant than low amount of tobacco.

So, we can conclude that nicotine enhances the hormones (Abscisic Acid & cytokinin). Presence of white spots on leaves of plant were detected due to abiotic stress and fungal infection.

5.3 For Absorption spectrum of plant pigments

The amount of chlorophyll a, chlorophyll b, carotenoids, xanthophyll were determined. Normal *Vigna radiata & Trigonella foenum-graecum* shows highest amount of chlorophyll a, chlorophyll b, carotenoids, xanthophyll than caffeine containing *Vigna radiata* & nicotine containing *Trigonella foenum-graecum.* So, we can conclude that caffeine and nicotine lower the plant pigments like (chlorophyll a, chlorophyll b, carotenoids, Xanthophyll). It may be due to decrease nitrogen content. Since caffeine and nicotine effect on pigment content also which can be due to deficiency of Iron

5.4 For qualitative test for

a. Carbohydrate
b. Protein

Qualitative test for carbohydrate was performed by using Molisch reagent which showed positive result.
Qualitative test for protein was performed by using 0.2% Ninhydrin reagent which showed positive result.

5.5 Protein estimation by Folin Lowry's method:

Amount of protein of leaves of *Vigna radiata & Trigonella foenum-graecum* was estimated by using folin lowry's method. For both *Vigna radiata & Trigonella foenum- graecum* the result showed that highest protein content was on normal than the caffeine & nicotine containing leaves of *Vigna radiata and Trigonella foenum-graecum* respectively. Caffeine and nicotine containing leaves shows very less amount of protein than the normal leaves. So, we can conclude that caffeine and nicotine lower the protein content of plants. Since the deficiency of protein was detected as compared with normal which can be due to Nitrogen deficiency. The fungal infection may be creating hinderance in Nitrogen absorption of root and due to this protein content was found to be low.

Since plant were detected with spots on nicotine contain plant which is due to fungal infection. This infection may be creating hindrance in N2 absorption of root and due to this protein content was found to be low.

We concluded that the effect of nicotine and caffeine has a drastic effect on growth of plant but more prove to infection by external source and also abiotic stress, nicotine and caffeine increases the growth hormones in plants but at specific concentration, beyond the specific concentration it has a disadvantage over it.

6. FUTURE PROSPECT

As per the observation it was concluded that the nicotine and caffeine serve a source of growth hormones or fertilizer which gives a drastic increase in shoot and root length in plants but the plant is more prove to infection so the insecticides are needed in order to avoid so that fungus does not create any interference in absorption of N2 and various minerals.

In future this experiment can be carried out with addition of insecticides and manure to overcome the deficiency of nutrients and avoid any infection and abiotic stress also to be considered as a major factor in infection of plants so abiotic condition also to be monitor at proper environmental condition.

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