ijar
ISSN NO. 2320-5407

INTERNATIONAL JOURNAL
OF ADVANCED RESEARCH

RESEARCH ARTICLE

Study on Effect of Caffeine on Growth of Vigna radiata L.

Machindra Jadhav, Nitin Taur, Sagar Sapkal, Shubham Tathe, Farha Quadri*
Department of Biotechnology, Shivchhtrapati College, Aurangabad.

Manuscript Info	*Abstract*
	Caffeine is a bitter crystalline alkaloid. It is in family of chemicals called xanthenes, which are often used as mild stimulants, and has a chemical formula of $C_8H_{10}N_4O_2$. The Mung bean (*Vigna radiata* L.) is a legume cultivated for its edible seeds and sprouts across Asia. Mung similar to other pulses is grown primarily for its protein rich seeds. Mung contains 20-25% protein. In present investigation *Vigna radiata* L. seeds were treated with different concentration of caffeine to check its effect on plant growth. Height of plant was measured as a daily parameter to check effect on growth. On twelve day of germination the plants were removed to evaluate fresh/dry weight and spectrophotometric measurements for chlorophyll content. It is found that 0.5-1.25 % caffeine gives best results which can be reconfirmed by height, weight,Chl a, b, Carotenoids estimation. The experimental errors are avoided by performing statistical analysis. The results indicating that caffeine have positive impacts on *Vigna rdiata* L. growth.

Introduction:-
The Mung bean (*Vigna radiata*) alternatively known as the moong bean or green gram, is commonly known as *Mung* in India.

Origin:-
Mungbean has been considered to have been domesticated in India (Vavilov 1926). His theory has been supported by other authors based on the morphological diversity (Singh et al. 1974), existence of wild and weedy types (Chandel 1984, Paroda and Thomas 1988), and archaeological remains (Jain and Mehra 1980) of mungbean in India.

Discription:-
The sprouted seeds ("bean sprouts" in English, and incorrectly called "germes de soja" or "pousses de soja" in French) are relished raw or cooked throughout the world. The immature pods and young leaves are eaten as a vegetable (Mogotsi, 2006). Several mung bean products are useful for livestock feeding (Vaidya, 2001).Mung bean is sometimes grown for fodders hay, straw or silage (Mogotsi, 2006). It is particularly valued as early forage as it outcompetes other summer growing legumes such as cowpea or velvet bean in their early stages (Lambrides et al., 2006).The mung bean plant makes valuable green manure and can be used as a cover crop (Mogotsi, 2006).

EconomicImportance:-
Mung, similar to other pulses, is grown primarily for its protein rich seeds. Mung contains 20-25% protein. The plants are sometimes cut and ploughed into the soil to enrich soil nitrogen.

Seasons and cultivation:-
Green gram is a warm weather crop and comes up in areas receiving an annual rainfall ranging from 50 to 70 cm. It is mainly cultivated in a cereal-pulse cropping system primarily to conserve soil nutrients and utilize the left over soil moisture particularly, after rice cultivation. Hence, although it can be grown in all the seasons, majority of green

gram cultivation falls in either Rabi or late Rabi seasons particularly in peninsular India. Optimum temperature range for growth is 27-30°C. A dry harvest period is desirable as this forces the crop to mature and reduces the risk of weather damage. Green gram is less tolerant to water logging.

Domestic Scenario:-
India is the world's largest producer as well as consumer of green gram. It produces about 1.5 to 2.0 million tons of Mung annually from about 3 to 4 million hectares of area, with an average productivity of 500kg per hectare. Green gram output accounts for about 10-12% of total pulse production in the country.

Caffeine:-
Caffeine is a bitter, white crystalline purine, a methylxanthine alkaloid. Caffeine is a substance present in coffee, chocolate, and many more foods. While it is known to experiment monitored the germination of growth of *vigna radiata* plant. Caffeine interferes with a cell's calcium regulation. Adenosine receptors play an important role in controlling calcium levels, and when they are inhibited by caffeine, cells release large amounts of their internal calcium. Calcium is extremely important in plant cells, and decreasing its normal concentrations in the cells could have many effects on plants. First, calcium controls the structure and permeability of cell membranes. It is also extremely important in proper cell division and cell wall development. Furthermore, calcium must be present for the proper functioning of alpha-amylase, an enzyme that breaks down starch. When caffeine causes the cell to release internal calcium, it could greatly hamper all of these functions of calcium. (Saheb D. 2009).

Spectrophotometric Evaluation:-
The determination of leaf pigment content is an important analytical tool in the field of plant physiology. It can be used to classify plant material, establish appropriate treatments and to understand the interactions between plants and environmental factors. Given this importance, reliable assays for chlorophylls (Chls) are required. The amount of solar radiation absorbed by a leaf is largely dependent on the foliar concentrations of its photosynthetic pigments. Therefore, low Chl concentration can limit photosynthetic potential and primary production (DaMatta et al. 2008). In addition, a variety of environmental stresses, such as water shortage, temperature and light extremes, UV-B radiation and mineral deficiencies, reduce chlorophyll levels (Campos 2012, Pompelli 2010). This reduction may be related to the photobleaching of chlorophyll (Campos et al. 2012). Therefore, the Chl level is an accurate indicator of plant vigor and is routinely measured in physiological research. In higher plants, Chl a and Chl b are the most important forms of chlorophyll and are essential for the oxygenic conversion of light energy into the stored chemical energy that powers the biosphere (Richardson 2002). Chl c and Chl d are other common forms of chlorophylls, but are only found in the photosynthetic members of the Chromista and Dinoflagellates (Ceulemans & Saugier 1993). In all the above mentioned cases, carotenoids (Car) are accessory pigments that protect the photosystem when excitation energy is high. To accurately determine Car by measuring A470, one needs to know the exact level of Chl b, which, in contrast to Chl a, absorbs considerable energy at 470 nm wavelength (Lichtenthaler & Buschmann 2001). If Chl b is underestimated, the level of total will be overestimated. The accuracy of any Chl extraction method will vary according to the solvent's nature, polarity (López 2004), and temperature and incubation time (Ritchie 2008, Tait & Hik 2003). Methods of extraction and conservation of chlorophyll have to be adjusted for different plant species due to variations in Chl levels (Porra 2002). Absolute ethanol or acetone is often used in basic research for Chl and Car extraction from leaves (Lichtenthaler & Buschmann 2001).

Methodology:-

- ▪ **Test seed sample was collected**.
  From at Post Pisadevi Tq. Dist.Aurangabad.

- ▪ **Sterilization of soil.**
  Soil was collected from Shivchhatrapati College N-3 Cidco Aurangabad, Botanical garden. The soil was prepared sterilization before use to avoid contamination during experiment.

- ▪ **Concentration range of caffeine was prepared(Saheb D, 2009)**
  Foundation, healthy seeds of *Vigna radiata* were pre-soaked in distilled water for 12hours and were then subjected to nine different concentration.(0.05%,0.25%,0.50%,0.75%,1.00%,1.25%,1.50%,1.75% and 2.00%) of caffeine solution was prepared(60ml). One set of seed was kept untreated to act as control.

- ▪ **Culturing of plant with respect to selected caffeine solution.**
  The seeds were socked is a distilled water 12hours.After incubation the seeds were sown in soil pots containing 147gm soil each and every pots was sown with equal amount of seeds viz. 5seeds of in 60ml of

the selected caffeine solution.(0.05%,0.25%,0.50%,0.75%,1.00%,1.25%,1.50%,1.75% and 2.00%) was added in each pots containing seed.

- **Screening height of plant as a parameter for growth**.
  The cultured were screened for seedling weight as growth parameters. The height was daily examined with geometric scale.

- **Measuring dry /wet weight of plant shoots /roots as growth indicator.**
  The weight of plant is considered as a indicator of growth
  (Pooter H. 2011).The dry weight weigh of plant roots and shoot was performed.

- **Statistical analysis of data for result interpretation.**
  The experimental data were statistically analyzed using the analysis of variance techniques according to Gomez and Gomez (1984).

- **Spectrophotometric determination for chlorophyll pigments.**
  For the Chl extraction using acetone, the samples were ground with 2ml of 80% acetone in glass beads to prevent chlorophyllase activities. After grinding the sample were filtered and the final volume (6ml) was transferred to graduate tubes and characterized using a dual-beam spectrophotometer at $A_{663}, A_{646}, A_{470}$ as described by Lichtenthaler (1987).

Result and Discussion:-

The purpose of this experiment was to observe how caffeine would affect germination and growth of plant seed. For this purpose test sample were collected from at post Pisadevi Tq. Dist. Aurangabad.

The soil was pre-sterilised at 15lbs for 20 minutes. Pre-sterilization of soil to avoid contamination was referred by Brandan H. (2005). Steam sterilization increases amount of extractable soil nutrients about four to eight fold (Williams G. 1984).
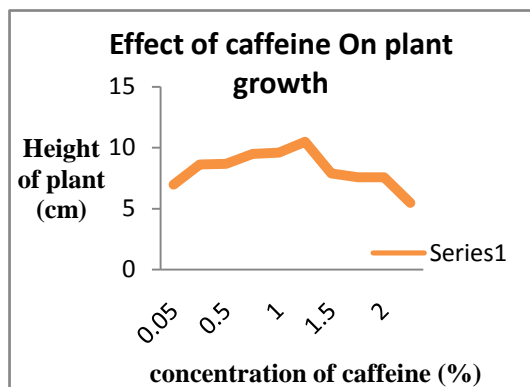

Caffeine solution was prepared in range (0.05%, 0.25%, 0.50%, 0.75%, 1.00%, 1.25%, 1.50%, 1.75% and 2.00%) as explained by Saheb D. (2009). The seeds were pre-soaked for distilled water for 12 hrs. (Saheb D. 2009).

The seed were sown in soil pots containing147gm soil. Five seeds per pot were added. A control was prepared and flourished with distilled water. The experimental data statically analyzed using the analysis of variance techniques according to Gomez and Gomez (1984).
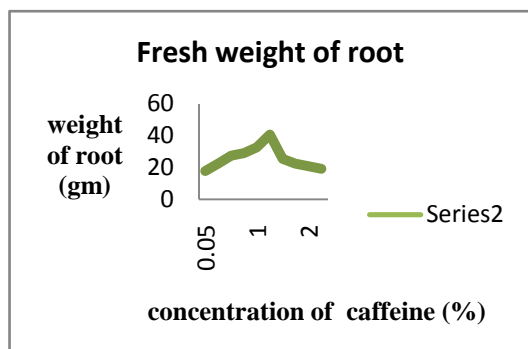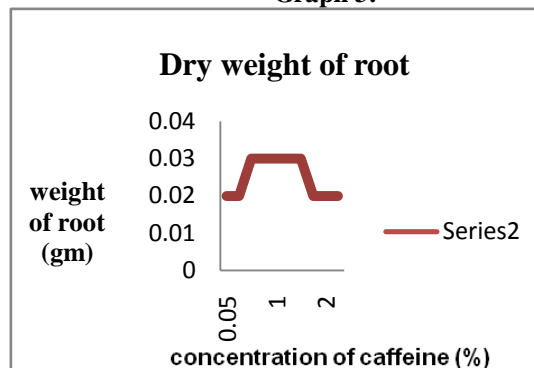
The height is defined as to which anything rises about that on which it stand the measure upward from as surface as a floor of on 12 day of germination show increases in height as compare to test plants with 10.5cm ± 2.7(Refer Graph 1).
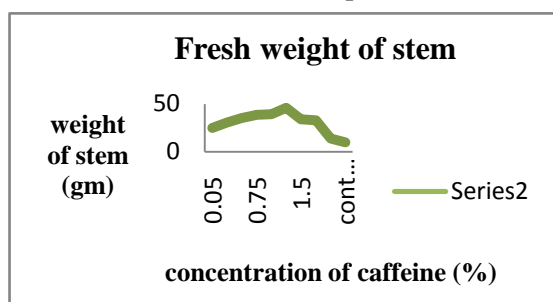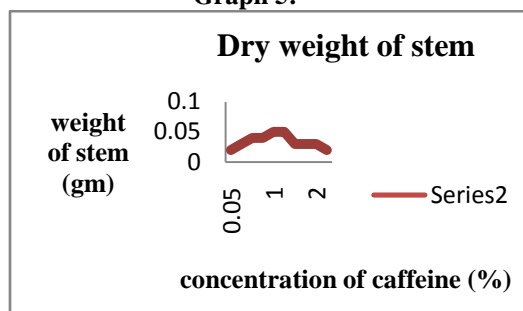
Graph 1:

          Caffeine treatment in the enhancement of seedling growth at lower concentration is quite similar to that of lower doses of DES and 2, 4-D as observed by Siddiqui et al. (1980).This results indicating caffeine helps in plant growth (Helper Piter, 2005, Harald F., 1957).

    Plant biomass measurement was done by weighing weight and dry weight of Vigna *radiata* root/shoot. The fresh weight was taken by removing plant from soil measure weight. The samples were dried in oven at 60^0c for 24 to 48 hrs (Pooter H., 2011). Adjustment in root and shoot growth are often assume fundamental facet of plant phenotypic plasticity in response to its environment (Robinson D.2010). This phenomenon of "Balanced Growth" was also supported by. Fresh of young stem was measured and it was found that 1.25% caffeine solution gives more values as 46±7.12. Fresh weight of root gives yield 40.66 ±3.88. The controlled plant were found to be giving less promising result as compare to test plants (Refer Graph 2 and 3) respectively.

Graph 2:

Fresh weight of root

Graph 3:

Dry weight of root

Dry weights of both root and stem of treated plants gives results as 0.05±0.004,0.03±0.015. The controlled plant were found to be giving less promising result as compare to test plants. (Refer Graph 4 and 5) respectively.

Graph 4:

Fresh weight of stem

Graph 5:

Dry weight of stem

The determination of leaf pigment content is an important analytical tool in the field of plant physiology. It can be used to classify plant material, establish appropriate treatments and to understand the interactions between plants and environmental factors. Given this importance, reliable assays for chlorophylls (Chls) are required. The amount of solar radiation absorbed by a leaf is largely dependent on the foliar concentrations of its photosynthetic pigments. Therefore, low Chl concentration can limit photosynthetic potential and primary production (DaMatta , 2008). In addition , a variety of environmental stresses, such as water shortage, temperature and light extremes, UV-B radiation and mineral deficiencies, reduced chlorophyll levels (Campose, 2012, Pompalli, 2010).This reduction may be related to the photo bleaching of chlorophyll (Campose, 2012). Therefore, the Chl level is an accurate indicator of plant vigor and is routinely measured in physiological research. In higher plants, Chl a and Chl b are the most important forms of chlorophyll and are essential for the oxygenic conversion of light energy in to the stored chemical energy that powers the biosphere (Richardson, 2002). Chl c and Chl d are other common forms of chlorophylls, but are only found in the photosynthetic members of the Chromista and Dinoflagellates (Ceullemans and saugier 1993). In all the above mentioned cases, carotenoides (Car) are accessory pigments that protect the ecosystem when excitation energy is high. To accurately determine Car bu measuring A470, one needs to know the level of Chlb, which, in contrast to Chla, absorbs considerable energy at 470 nm wavelength (Lichtenthaler and Buschmann 2001). If Chl b,underestimated, the level of total Car will be over estimated. The accuracy of any Chl extraction method will vary according to the solvent's nature, polarity (Lopez et al.2004), temperature and incubation time (Ritchie 2008), Methods of extraction and conservation of Chlorophyll have to be adjusted for different plant species due to variations in Chl levels (Porra2002). In this study acetone is found to be good extract of chlorophyll as described by Lichtenthaler (1987) and Porra (1989). Spectrophotometer Chorophyll can easily be quantified with a spectrophotometer based on the Beer-Lambert Law and the extinction coefficient for chlorophyll (Arnon, 1949).
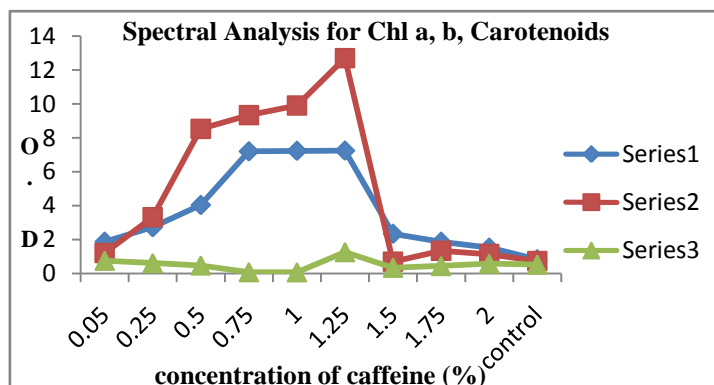
Eq (1) Chl a= $(12.25A_{663.2})$-$(2.79A_{646.8})$

Eq (2) Chl b= $(21.50A_{646.8})$-$(5.10A_{663.2})$

Eq (3) Car" $[(1000A_{470})$-$(1.82Chl"a")$-$(85.02 Chl"b")]/198$.

In this study, none of the acetone combination was considered good extractiors of chlorophylls. (Popelli, 2012).Under present investigation Chl a Chl b carotenoids gives positive values indicating assuring results of

selected caffeine range. Worth upshots are obtained for concentration 0.5- 1.25 % (Refer Graph 6). Pompelli (2012) have reported similar results with *Jatropha*.

Conclusion:-

  In this research work we have estimated effect of caffeine on *Vigna radiata.* It is found that 0.5- 1.25 % caffeine gives best results which were reconfirmed by height, weight, Chl a, b, Carotenoids estimation. The experimental errors are avoided by performing statistical analysis. The results indicating that caffeine have positive impacts on *Vigna radiata* L.

Acknowledgement:-

References:-

1. Arnon.D.I. (1949). Analysis of Leaf Pigment.Plant Physiology.24.1.
2. Arora,P.K., K.P.Chandel and B.S.Joshi.(1973). Morphological diversity in *Phaseolus sublobatus* Roxb. Curr. Sci. 42: 359-361.
3. Brandan H. Anderson and Frederick R. Magdoff (2005) Autoclaving Soil Samples Affects Algal-Available Phosphorus.
4. Chandel,K.P.S., R.N.Lester and R.J.Starling.(1984).The wild ancestor of urid and mungbeans (*V.mungo* (L.)Hepper and *Vigna radiata* (L.)Wilczek) Bot. J. Linn. Soc. 89: 85-96.
5. DaMatta ,Cunha F.M., Antunes R. L,Martins W.C.(2008).In field grown coffee trees source –sink manipulation alters photosynthetic rates, independently of carbon metabolism, via alteration in stomatal function. New Phytol.178:348-357.
6. Gomez Meza J.I Uvalle Sauceda, Gonzalez Rodriguez, Ramirez Lozano R.G, Silva Cantu I (1984). Seasonl Trends of chlorophylls a and b Carotenoids in Native Trees and Sheubs of Northeasteran Mexico.
7. Harald F. (1957)**.** The Measurement and Value of Plant Height in the Study of HerbaceousVegetation. Ecology.Vol. 38, No. 2. p. 313-320.
8. Helper Piter, (2005). Calcium:A Central regulator of plant growth and Development. The Plant Cell. 17:2142-2155.
9. Jain,H.K. and K.L.Mehra. 1980. Evaluation, adaptation, relationship and cases of the species of *Vigna* cultivation in Asia. Summerfield,R.J. and A.H.Butnting (eds.) Advances in legume science. Royal Botanical Garden, Kew. pp. 459-468.
10. Lichtenthaler and Buschmann (2001).Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Method in enzymology. vol. 148:350-382.
11. Lopez et al. Lucena.J.M. (2004) Design of a flow injection method for chlorophyll determination in in-vitro plants. Talanta, 64:1304-1308.
12. Marcelo Francis Pompelli, Suele Camile Franca, Rafaela Carvalho Tigare, Marciel Teixeire De Olivera, Marco Sacilot and Eugenia Cristina Pereira. (2012) Spectrophotometric determination of chloplastidic pigments in acetone,ethanol and dimethylsulphoxide. Brazillan journal of Biosciences.V.11, n.1, p. 52-58.

13. Pooter H., Karl J. Niklas Peter, B. Reich Jacek Oleksyn, Pieter Poot and Liesje Mommer (2011). Biomass allocation to leaves stems and roots: meta-analyses of interspecific variation and environmental control. New Phytologist .193: 30–50.

14. Porra R.J., Thompson, Kriedemann P.E., (1989). Determination of accurate extinction coefficients and simultaneous equation for assaying chlorophyll a and b extracted with for different solvents verification of the concentration of chlorophyll standard by atomic absorbtion spectroscopy. Biochemical Biophysical Acta 975, 384-396.

15. Richardson A.D., Duigan S.P. and Verlyn G.P. (2002).An evaluation of non invasive methods of estimate foliar chlorophyll content. New Phythol. , 153:158-194.

16. Ritchie (2008). Universal chlorophyll equation for estimating chlorophyll a, b, c,and d total chlorophylls in natural   assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents .Photosyntheticaa.46(1):115-126.

17. Robinson D. (2010). An efficient regional energy – moisture balance model for stimulation of the green land ice sheet responce to climate change. 4, 129-144.

18. Saheb D, Khursheed, T, Ansari MYK, (2009) Studies on the effect of caffeine on growth and yields parameters in helianthus annnus. Variety modern. Biology and Medicine

19. 1 (2):56-60.

20. Siddiqui S. S, Von Ehrenstein,G. (1980) Biochemical genetics of Celegans. 1,285-312.

21. Vavilov N. I. Vladimir Filimonovich Dorofeev (1926). Origin and Geography of Cultivate Plants.

22. Williams G and Ewel J.J (1984), Effect of autoclave sterilization of a tropical andept on seed germination and seedling growth. Plant and Soil. 82,263-268.