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The study on the survival rate of UV exposed *Pisum sativum* saplings and treated with *Arnica montana* 30c and *Hypericum perforatum* 30c

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Abstract
Ultraviolet radiation, a non ionising radiation that is emitted by the sun. There are three types of rays UV-A, UV -B, UV- C 6. Out of which UV B radiation affects the plants easily because of its sessility. This study is put forward to describe the effects of UV radiation on *Pisum sativum* saplings and to compare and to identify which remedy among *Arnica montana* and *Hypericum perforatum* acts the best on UV exposed *Pisum sativum* sapling. From this study, the survival rate of the plant is decreased after UV exposure and by treating it became clear that *Hypericum perforatum* increases plant tolerance to the stress created by UV radiation than Arnica, thereby produces morphological improvements. But both the medicine acted really well to increase the survival rate of the plant by increasing the stress protein.As ozone level is decreasing, it may produce serious effects in both plants and humans. Hence as a futuristic approach this study has done.

Keywords: *Pisum sativum, saplings, Arnica montana, Hypericum perforatum*

Introduction
Ultraviolet radiation is a non ionising radiation that is emitted by the sun [1]. The entry of UV rays into our planet earth is protected by the layer called ozone. Ozone is a colorless gas that is found mostly in the stratospheric layer of the atmosphere [2]. The Ozone layer acts as a sunscreen for our planet earth [3].

Sequelae of ozone layer depletion
A slight reduction in ozone level may lead to increased entry of UV radiation. This is due to human activities such as release of Chlorofluorocarbon (CFC) and nitrogen oxides that act as the Ozone depletors. Ozone depletors are stable molecules that reach the stratospheric ozone layer and when they come in contact with UV rays, chemical reaction takes place and leads to depletion of the ozone layer [4]. The quantity of the radiation entering the ozone column mainly depends on the elevation above the sea level and angle of earth's surface along with concentration in the atmosphere. Therefore, the increase in surface level of ultraviolet radiation is due to depletion of the ozone layer [5]. The ozone hole is not literally a hole but where there is decreased amount of ozone [6].

Consequences of UV radiation in plants
UV radiation emits three types of rays UV-A [400-315nm], UV-B [315-280nm], UV-C [280-100nm] [7]. Out of which, UV-B affects the plants easily because of its sessility. A reduction in the rubisco activity is markedly seen without much due to prolonged UV-B radiation [8]. UV-B radiation inhibits photosynthesis effect on PSII photochemistry in plants. Flavonoids are the substance secreted by plants against UV-B radiation. It acts as a defense mechanism providing UV absorbing sunscreen to the plants [9]. In future there are chances that terrestrial plants will experience increased levels of UV exposure. UV radiation particularly damages the plant morphology [10]. The projected increase of UV radiation in future can alter the plant growth and development [11] and it can also affect human beings leading to skin cancer by mutating the DNA.
Pisum sativum is used in this study as they were one of the first plants where many evolutionary and genetic studies are done. *Arnica montana* and *Hypericum perforatum* are mentioned in homoeopathic literature to have an effect against internal shock and injury[12]

Hypericum has the main action on sentinel nerves [13]. It has the ability to protect against toxicity either by neuroprotective mechanisms or by antioxidant property [14]. We therefore selected both the remedies in this study.

Need For the Study

- This study mainly focuses on showing the serious effects of UV radiation on plants.
- As the ozone layer is depleting, it leads to the entry of harmful UV radiation. These entry of harmful radiation affects not only plants but also humans by mutating the gene and leading to many diseases.
- There are many experiments done with UV radiation in plants but homoeopathic application is minimal.
- This study shows the effectiveness of homoeopathic medicine in UV exposed sapling and to show the withholding capacity of the UV exposed sapling treated by homoeopathic medicine.

Review of literature

Related to effects of UV radiation

1. A review by Mpoloka shows the genetic effects of long-term UV-B exposure in plants. Both direct and indirect effects of UV-B on plants are discussed [15].
2. The study by V.G. Kakani, K.R. Reddy, Dzho and A.R. Mohammed. Was the first step in a larger USDA-UVB monitoring programme to note the effects of enhanced UVB radiation on cotton. The objective of this study is to examine the effects of enhanced UVB radiation on whole plant morphology, vegetative and reproductive characters with specific focus on the changes in leaf surface ultrastructure and anatomy [16].
3. A review by F. Hollosy summarizes the main aspects of ultraviolet radiation on plants at physiological and biochemical level, with particular emphasis on protective structures and mechanism [5].

Related to UV radiation on pea plant

1. The article by R. Mepsted, Nigel D Paul, J. Stephen, J. E Corlett, Salvador Nogues, N. R Baker, Hamlyn G Jones, P.G. Ayres shows that enhanced UV-B resulted in small reductions in the number of stems and total stem length per plant. There were also significant decreases in the dry weight of peas, pods and stems per plant. UV-B treatment had no effect on the number of peas per pod or average pea weight but it significantly reduced (12.1%) the number per plant and suggest the reduction in yield may be due to direct effects of UV-B on plant growth rather than a decrease in photosynthetic capacity per unit leaf area [17]
2. The article by Jie Hie, L.K. Huang, Wah Soon Chow, M.I. Whitecross, Jan M Anderson done an experiment to compare the effects of supplementary UV-B radiation on a tropical /subtropical and a temperate plant, two Indica rice cultivars and peas were exposed to supplementary UV-B radiation for 8 days. This study confirms the multiplicity of photosynthetic responses and of different protective strategies that may account for the differential sensitivity of plants to supplementary UV-B radiation [18].
3. The Journal by J. Stephen, R. Woodfin, J.E. Corlett, N.D. Paul, H.G. Jones and P.G. Ayres indicates that yields of pea, and probably barley, would not be markedly affected by the increase in UV-B associated with a 15% reduction in stratospheric ozone. However, given uncertainty, such as the possible interactions between the effect of UV-B and those of other environmental factors, the possibility of significant responses to stratospheric ozone depletion cannot be excluded [19].

Related to action of *Arnica montana* in DNA damage

1. The Journal by Anisur Rahman Khuda Bukhsh revealed DNA damage and generation of oxidative stress in E. coli as a result of exposure to UV radiation more in quantity at the longer and higher exposure. The consequences of UV irradiation included generation of ROS, DNA damage and decrease in levels of SOD, CAT and GSH. This explains the molecular mechanism of action of potentised homoeopathic drugs by their ability to trigger selective and relevant gene expression as revealed also in the lower primitive form of unicellular organisms like E. coli with a simple genetic system [20].
2. The article by Marta Marzotto, Clara Bonafini and Paolo Bellavite tested *Arnica montana.* effects on gene expression using an *in vitro* model of macrophages polarized towards a wound healing phenotype. The results of this work, taken together, provide new insights into the action of *Arnica m.* in tissue healing and repair, and identify the extracellular matrix regulation by macrophages as a therapeutic target [21].

Related to action of *Hypericum perforatum*

1. The review by Al Oliveira demonstrates that *Hypericum* extracts and several of its components have the ability to protect against toxic insults either directly through neuroprotective mechanisms or indirectly through its antioxidant properties [13].
2. A Journal by O. Tusevski shows the detailed phenolic profile of *Hypericum perforatum* roots. Roots produced significant quantities of xanthones and flavan-3-ols. Roots exhibited strong antimicrobial, antidepressant and antidiabetic effects [22].

Aim and Objectives

- To check the effects of UV radiation on *Pisum sativum* saplings.
- To check the survival rate of the *Pisum sativum* saplings after UV exposure.
- To verify the effects of *Arnica montana* 30C, *Hypericum perforatum* 30c on UV exposed *Pisum sativum* sapling.
- To compare and to identify which remedy among these acts the best on UV exposed *Pisum sativum* sapling.

Methodology

Materials and Methods

Experimental site: Sarada krishna homoeopathic medical college, Kuleshekharam.

Materials required

- Pea seeds
- Loam soil
- Biodegradable cups
- Spray bottle
- Supplementary UV lamp
- **Medicine:** *Arnica montana* 30c and *Hypericum perforatum* 30c

Methodology in detail

- Medicine used – *Arnica montana* 30c, *Hypericum perforatum* 30c [Schwabe pvt. ltd].
- Homoeopathic medicines are generally used by diluting

them in water. Hence throughout this research study, 5 drops of *Arnica* and *Hypericum* have been distilled in 100 ml of water to prepare medicated water for using it in experiments.

Preparation of seeds for the experiment

- Seeds of the best variety were collected from the Farm House, Chenthitta, Trivandrum, Kerala.
- The seeds were soaked on 16.05.2022 to accelerate germination. It is allowed to soak in water for 24 hours
- The next day the seeds are taken and tied in a dry cotton cloth. After two days, the sprouting of seeds is noted.
- On the same day, the seeds were planted in biodegradable cups on 20.05.2022 which is filled with Loam soil at 3/4th level.
- A number of 24 biodegradable cups are taken and divided into 4 sets which contain 6 cups each.
- The seeds are sowed in the moist soil and are placed 1 to 1 ½ inches deep and gently closed it with soil. The watering of the plant is done every day.

Preparation of soil

Locally available 1kg of sand is taken and mixed with ½ kg of silt and ¼ kg of clay. The mixture was well aerated by thorough mixing. This mixture of soil is then filled in each cup in equal amounts. Aged compost is also added to this soil to absorb the excess water in the cup after watering the plant.

Biodegradable cups

- The biodegradable cups are easily breakable by natural microorganisms which gives an end product that is least toxic to the environment. Therefore, they are more ecofriendly which are far better than ordinary plastics. Biodegradable cups are used in this study to create awareness regarding plastics [32].
- A number of 24 biodegradable cups are taken and divided into 4 sets named SET I SET II, SET III and SET IV which contain 6 cups each.

Temperature and pH

The favourable pH of pea plant growth is 6-7.5. The optimum temperature is 18- 22 degree celsius. Peas are sensitive to drought [32]. So, the plants are kept in a shaded area. Direct sunlight is exposed to the plant only in the morning and the evening time.

Watering

Equal amount of water is poured into each sapling every day. Soak the soil to a depth of at least 1 to 2 inches while watering. The water mainly poured into the root and sprayed in the leaves. Peas can't tolerate wet soil as it may lead to root rot.

Sunlight

The minimum ideal amount of sunlight (6 hours per day). Peas can able to tolerate full sun conditions i.e., 8 hours per day. They grow well in cooler temperature also. Higher temperature (75 degree) produces stress injuries in plants.

UV Exposure

- Radiation exposure to pea plants – UV radiation [supplementary UV lamp -Laminar Airflow]
- After 30 days, the first, second and the third sets are exposed to UV supplementary lamp.
- The duration of exposure of the saplings to UV radiation is 10 mins.
- In this study supplementary UV lamp is used which is kept in a laminar air flow chamber. The plants are placed at a distance approximately 30 to 35 cm from the supplementary UV lamp.
- Plants are mainly affected by UV-B type of rays. As UV-B lamps are expensive, supplementary UV lamp is used in this study.

Method of medicinal application

Foliar spray method

Medication of the plant has started on the next day of UV exposure -*Arnica montana* 30c dilution – 5 drops in 100 ml of water is taken in a spray bottle and sprayed over the leaves of SET I.

-*Hypericum perforatum* 30c dilution – 5 drops in 100ml of water is taken in a spray bottle and sprayed over the leaves of SET II.

The medication is given for the plants for 15 days continuously after UV exposure and observed.

Chemical analysis

Estimation of chlorophyll

1. Weighed 0.1g of finely cut and well mixed representative sample of leaf into a clean mortar.
2. Grinded the tissue to a fine pulp with the addition of 2ml of 80% acetone.
3. Centrifuged (5000 rpm for 5 min) and transferred the supernatant to a new tube.
4. Grinded the residue with 2ml of 80% acetone, centrifuged and transfer the supernatant to the same tube.
5. Repeated this procedure until the residue was colorless. Made up the sample to 10 ml with 80% acetone.
6. Read the absorbance of the solution at 645, 663 nm against the solvent 80% acetone blank.

Calculation

The amount of chlorophyll presents in the extract mg chlorophyll per gram tissue using the following equations.

mg chlorophyll a/g tissue = 12.7 (A663) – 2.69 (A645) ×(V/1000×W) mg chlorophyll b/g tissue = 22.9 (A645) – 4.68 (A663) × (V/1000×W) Total chlorophyll content =20.2(A645) +8.02(A663) ×(V/1000×W) Where A = absorbance at specific wavelengths, V = final volume of chlorophyll extract in 80% acetone, W = fresh weight of tissue extracted.

Estimation of protein by biuret test

Aim

To perform the estimation of protein by Biuret method.

Principle

Biuret method is the simplest method for protein estimation. This method is sensitive to the amino acid composition of the protein. Its sensitivity is moderately constant from protein to protein and because of its simple trials procedure and quick result, it is used to estimate protein in crude extract over a large range of concentration. The method can also be used to monitor the concentration of protein during purification. This assay is based on copper ions binding to peptide bonds of protein under alkaline conditions to give violet or purple colour. The intensity of the charge transfer absorption bond resulting from the Cu-protein complex linearly proportional to the mass of protein present in the solution. The chromophore or light-absorbing center seems to be a complex between the peptide backbone and cupric ions.

Materials Required
- Biuret reagent Dissolve 1.5 gm of CuSO4 and 4.5 gms of Na-K tartrate in 250 nil 0.2 N NaOH solution.
- Add 2.5 gms of Kl and make up the volume to 500 mL with 0.2 N NaOH

Protein standard
Bovine serum albumin at a concentration of 1 mG/ml in distilled water is used as a stock solution.

Procedure
- Pipette out standard protein solution into a series of tubes (0.0, 0.2, 1 mL) and make up the total volume 4ml by adding water (Use IML of Unknown sample). The blank tube will have only 4 ml of water.
- Add 6 mL of biuret reagent to each tube and mix well. Keep the tubes at 37 °C for 10 minutes during which a purple colour will develop.
- Measure the optical density of each tube at 520 mm (Green filter). Draw the standard graph to the known concentration of a protein and calculate unknown/ test sample protein concentrations.

Estimation of reducing sugar by glucose pap sl method
Method
- Enzymatic –calorimetric GOD-PAP
- Trinder end point.

Principle
Enzymatic determination of glucose according to the following reactions.
- Glucose + O_2 in the presence of glucose oxidase gives gluconic acid +H_2O.
- $2H_2O$ + Phenol +4AAP in the presence of perfoxidase gives Quinonimine + $4H_2O$.

Reagent composition
Reagent R
- Phosphate buffer, pH 7.4 13.8 mmol/L
- Phenol 10 mmol/L
- Amino 4 antipyrine 0.3 mmol/L
- Glucose oxidase ≥10, 000U/L
- Peroxidase ≥700 U/L

Standard
- D glucose 100 mg/dL
- 5.55 mmol/L

Procedure
This reagent can be used in most analyzers, semi-automatic analyzers, and manual methods.

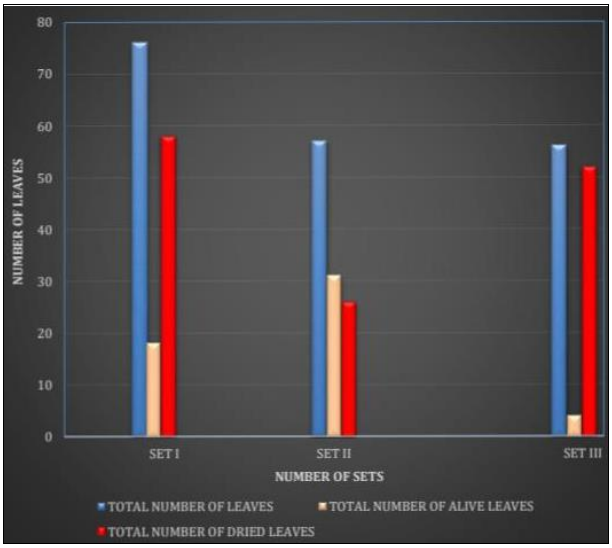
Observation
At the day of UV exposure, the changes taken place in the plants are noted and the changes in plants after application of *Arnica montana* and *Hypericum perforatum* is also noted.

Results

Table 1: Assessment of alive and dried leaves after uv exposure

Sets	Total number of leaves	Total number of alive leaves	Total number of Dried leaves
Set I	76	18	58
Set II	57	31	26
Set III	56	4	52

Total number of leaves in a set = Number of leaves present in each plant of that particular set. The tabulation shows that SET II (*Hypericum)* has more alive leaves than the other two sets while SET I (*Arnica)* has alive leaves better than SET III (control).



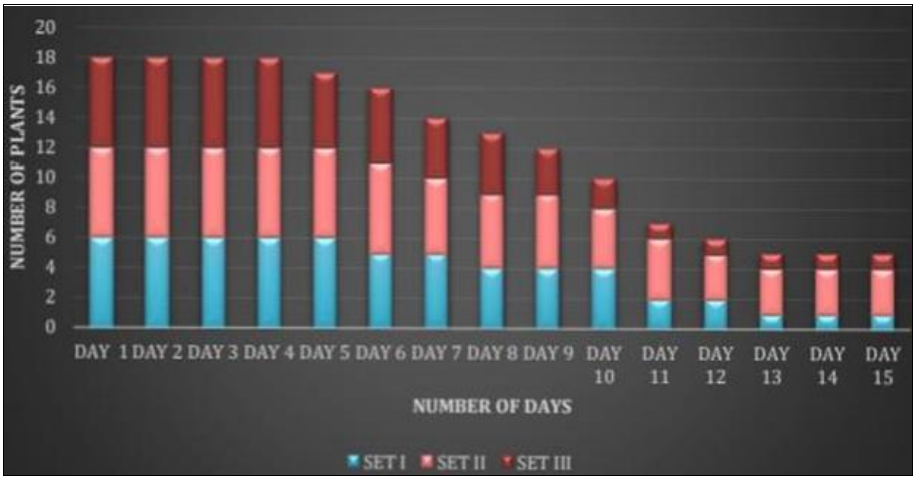
Graph 1: Number of alive and dried leaves

On comparing the number of alive and dried leaves in each set, a graph was plotted which shows SET II has more alive leaves than the other two sets whereas SET 1 has better alive leaves than Set III

Table 2: Assessment of survival rate of plant after uv exposure

Treatment days	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15
SET I	6	6	6	6	6	5	5	4	4	4	2	2	1	1	1
SET II	6	6	6	6	6	6	5	5	5	4	4	3	3	3	3
SET III	6	6	6	6	5	5	4	4	3	2	1	1	1	1	1

The tabulation shows the survival rate of the plants after UV exposure in each set where Set II (*Hypericum*) shows the high survival rate whereas Set I (*Arnica*) is slightly better than Set III (control**)**

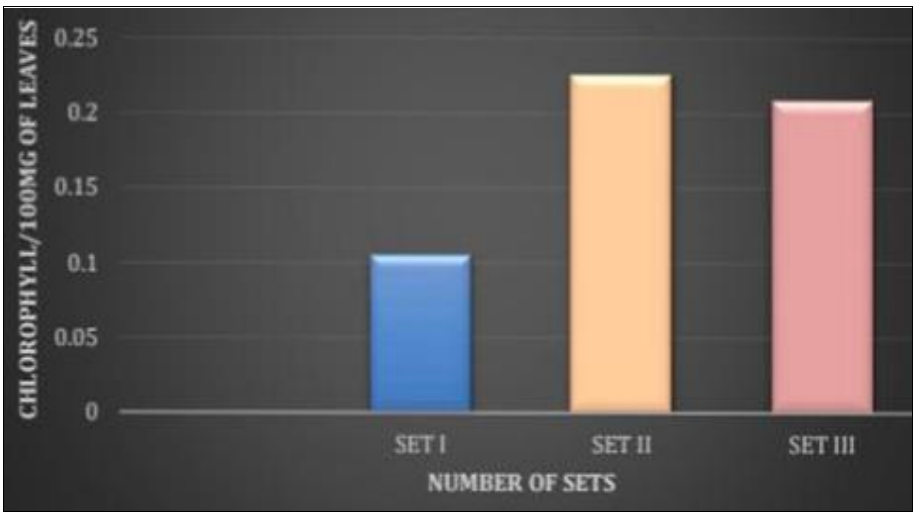


Graph 2: Survival rate of UV exposed saplings

On comparing the survival rate of each set in days after UV exposure, A graph was plotted which shows SET II survived for longer days whereas set I is slightly better than set III.

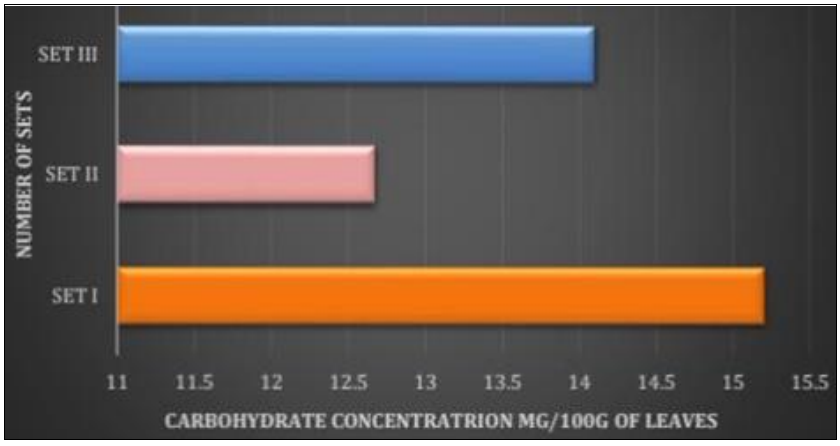
Table 3: Results of chlorophyll estimation

Samples	OD 645 nm	OD 663 nm	Chlorophyll a 100 mg/tissue	Chlorophyll b 100 mg/tissue	Total chlorophyll 10 mg/tissue
SET I	0.172	0.001	0.0124	0.1180	0.1044
SET II	0.335	0.095	0.0292	0.2167	0.2258
SET III	0.325	0.046	0.0087	0.2168	0.2080



Graph 3: Total chlorophyll 100 mg/tissue

The tabulation shows that SET II (*Hypericum*) has high chlorophyll content than the other two sets. SET III (*Arnica*) has slightly better chlorophyll content than SET 1 (control). The graph shows high chlorophyll content in set II whereas set I has the lowest chlorophyll.



Graph 4: Carbohydrate content in each set

The graph shows high chlorophyll content in set II whereas set I has the lowest chlorophyll.

Table 4: Results of carbohydrate estimation

Sets	Concentration (mg/dl)	Absorbance
Set I	15.20	0.0666
Set II	12.67	0.0615
Set III	14.10	0.0549

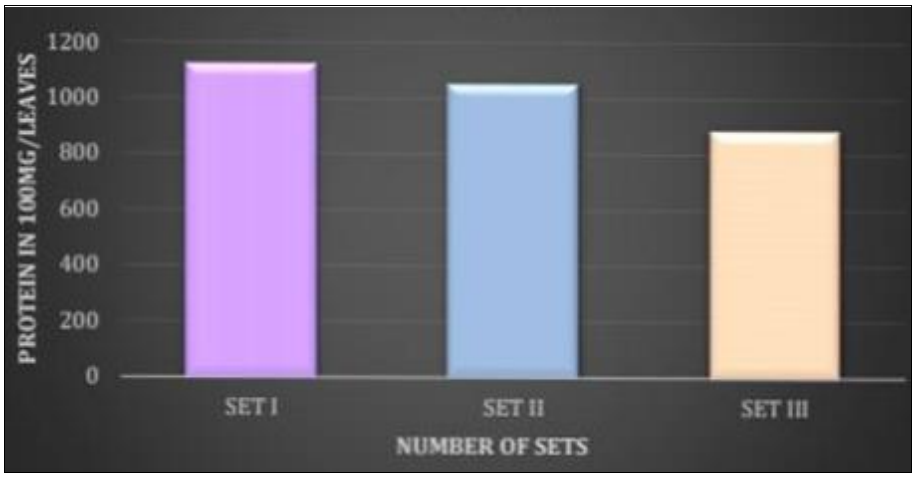
This tabulation shows SET I has high carbohydrate content than the other two sets and SET III is better than SET II.

This graph shows the carbohydrate content in each set where SET 1(Arnica) has high carbohydrate content than the other two sets while the least carbohydrate content is seen in SET II (Hypericum).

Table 5: Protein content in each set

Sets	Absorbance AT 760 nm	Concentration In mg/100 mg of leaves
Set I	0.255	1120
Set II	0.238	1052
Set III	0.196	884

This tabulation shows that set I has high protein content than the other two sets and the control group that is set III has low protein content.



Graph 5: Concentration in mg/100 mg of leaves

The graph shows set I has protein content which is slightly higher than set II and set III has low protein content.

Statically analysis of biochemical parameters

Source	DF	Sum of square	Mean square	F statistic	P Value
Treatment Groups (Set I,II,III)	2	2046882.25	1023441.125	208.4108(2,4)	0.00009035
Parameters (chlorophyll, Carbohydrates, Proteins)	2	9875.1289	4937.5644	1.0055 (2,4)	0.4428

Inference

The tabulation shows, the P value for treatment groups is highly significant whereas the P value for parameters such as chlorophyll, protein and carbohydrate are insignificant.

Discussion

The main objective of this work was to study changes happening in Pea plant after UV exposure. Many studies were conducted to evaluate the influences of UV light in plants [15, 16, 22, 23] Specifically, studies to evaluate the effects of UV radiation in pea has done [19, 20, 21, 25] but its homoeopathic application is minimal. Hence, this study was on UV exposed *Pisum sativum* sapling treated with *Arnica montana* 30c and *Hypericum perforatum* 30c. In this study, sensitivity of Pea plants to UV radiation resulted in appearance of chlorosis of the plant especially at the lower 7 cm of the plant. The appearance of chlorotic and necrotic patches after UV exposure has been widely reported in many studies. In this study the leaves that shows chlorotic changes turned into dried leaves as day passes. Researchers studying other plant species have reported changes in growth parameters on exposure to UV radiation [9, 16, 24]. Hence for a change, in this study growth parameters are not noted instead the survival rate of the sapling along with the tolerant capacity of plant to UV rays was noted. In this study, the plants exposed to UV is treated with *Arnica montana* 30c (SET 1) and *Hypericum perforatum* 30c (SET 2) by foliar spray method. The number of alive leaves is assessed and set 1 is lower than set 2 but it is higher than control group (set 3). The survival rate of the plants after UV exposure is also noted. Set B which is treated with *Hypericum* shows more tolerant effects and able to withstand the stress created by UV rays than the other 2 sets. Set 1 that is treated with *Arnica* doesn't produce results as much our expectation morphologically. A study was done by inducing UV radiation in mice which creates skin changes and that is treated by *Arnica* [20]. In this study, *Hypericum* shows the best results morphologically because *Hypericum* acts best on the nerve injuries and has the capacity to expel the foreign agents and improve the functionality while *Arnica* acts best on bruises and in wound healing. Set III which is exposed where no medication applied shows poor results. The biochemical analysis of chlorophyll shows high chlorophyll content in set II and decreased chlorophyll content in set I. Protein is seemed to be increased in set 1 because the sapling treated with Arnica after UV exposure struggled for its survival and creates stress proteins and the plants can't able to produce

functional improvement whereas set II treated with *Hypericum* can able to withstand the stress and able to produce functional improvement and increases the survival rate of the plant. A study conducted in water stressed seedling of cucumber shows increased stress protein after UV exposure and there are also increase protein content seen in the UV exposed leaves of Barley [26].The carbohydrate content is seemed to be more in set 1 than the other two sets.

Carbohydrates are rapidly and strongly influenced by UV exposure. However, in many studies, it is observed that there is accumulation and reduction of starch content in leaves depending on that particular plant which is exposed to UV [27].The statistical analysis of biochemical parameters is assessed and there is high significant among the treatment groups and insignificant among the parameters. The in Signiant values are seen due to the stress created by UV rays in the leaves of different treatment plants a study shows where UV radiation affects the cutaneous nerve fibres of skin [28]. As *Hypericum* acts well on nerve injuries [29], prolonged exposure to sunlight can be treated with *Hypericum.* If the skin lesion leads to bruise formation in skin *Arnica* can be given. Further additions to this study can be done by what type of stress protein is increased and to compare the DNA damage and improvements seen after the damage. The medication to increase the improvements in DNA damage can also be applied.

Conclusion

- The main aim of this study is to show the serious effects of UV rays in plants.
- The survical rate of the plant is decreased after UV exposure.
- From forgoing study, it became clear that *Hypericum* increases plant tolerance to the stress created by UV radiation than Arnica, thereby produces morphological improvements. But both the medicine acted really well to increase the survival rate of the plant by increasing the stress protein.
- This study can be applied for humans as well in treating skin diseases that occur due to harmful exposure to sunlight as Dr. Hahnemann states that “if the laws of nature I proclaimed are true, then they can be applied to all human beings”.
- As ozone level is decreasing, it may produce serious effects in both plants and humans.
- Hence as a futuristic approach, this study has done.

Summary

- *Pisum sativum* sapling is exposed to UV radiation for 10 minutes.
- The number of alive leaves is assessed and is higher in set II and lower in set III.
- The survival rate of the sapling is assessed and is high in set II and equal in set III.
- Biochemical components of leaves such as protein, carbohydrate and chlorophyll are studied. Protein content seemed to be more in set I than the other two sets as *Arnica* produces stress protein for the survival of the plant.
- Carbohydrate content is seemed to be increased in set I as the stored carbohydrates cannot be used up by the plant as UV rays destroyed the plant.
- Protein and carbohydrate seem to be low in set II as *Hypericum* increases the tolerant capacity of the plant and thereby produces functional improvement (new leaves and tendrils formation)
- Chlorophyll contents such as chlorophyll a, b and total chlorophyll count is studied where set II has higher chlorophyll content. Set II shows high chlorophyll content.
- Thus, the survival rate is studied along with the quality of the leaves exposed to UV and compared with unmedicated one.

Scope of the study

The success of this study will help us to understand about the serious effects of UV rays in plants and the action of homoeopathic medicines in UV exposure.

Conflict of interest
Not available
Financial support
Not available

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