

**RESEARCH ARTICLE****SPECTROPHOTOMETRIC STUDY OF 2, 4- DICHLOROPHEMOXY ACETIC ACID (2,4-D) IN WATER, GRAIN AND PLANT MATERIALS****Raj Kumar Singh<sup>1,\*</sup>, Jitendra kumar<sup>2</sup>, Anand Mohan<sup>3</sup> and Zamiruddin Ansari<sup>4</sup>**<sup>1</sup>Dept. of Zoology, Patna University, Patna, BiIndihar, a<sup>2</sup>SHIATS, (Formely - Allahabad Agricultural Institute- Deemed University), U.P, India<sup>3</sup>Dept. of Biotechnology, College of commerce Arts and Science, Patna, Bihar, India<sup>4</sup>Dept. of Biochemistry, Patna University, Patna, Bihar, India**ARTICLE INFO****Article History:**Received 27<sup>th</sup> July, 2020  
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Published online 30<sup>th</sup> October, 2020**Key words:**Animal health, Herbicides,  
2, 4- dichlorophemoxy acetic acid,  
Spectrophotometric study, Plant health.**ABSTRACT**

2, 4- dichlorophemoxy acetic acid (2, 4-D) is a selective herbicides. It is widely used as a weed killer. The half-life of 2, 4-D in the environment is relatively short, averaging 10 days in soils and less than 10 days in water. Its low concentrations can also stimulate RNA, DNA and Protein Synthesis leading to uncontrolled cell division and growth. On the other hand, high concentration of 2, 4-D can inhibit cell division and growth. Plant death typically within 3-5 weeks for Reagents 2, 4-D solutions are prepared, for this, stock solutions of 1mg/ml of 2, 4-D was prepared in concentrated H<sub>2</sub>SO<sub>4</sub>. A working standard of 40 µg/ml also prepared. 6-Amino-1-naphtol-3 Sulfonic acid (J-acid) solution (Wilson) was prepared by 0.4% solution in concentrated sulphuric acid. A 50% ammonium acetate solution was prepared in Distilled water lowing applications. 2, 4-D shows acute and non-acute effects. Determination of 2, 4-D effluent in water sample, grains like Maize, Rice and plants parts was taken for experiments and its effect were recorded. It affects badly on animal health and plants also. So, the limited amount like 0.7ppm can be allowed.

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**INTRODUCTION**

2, 4- dichlorophemoxy acetic acid (2, 4-D) is a selective herbicides as the acids, esters and several salts which vary in their chemical properties. It is widely used as a weed killer. The 2, 4-D dimethylamine is one of the salts of this group. 2, 4-D is a selective herbicides that kill & dicots (but not grasses) by mimicking the growth hormone auxin which causes uncontrolled growth and eventually death in susptible plants. The half-life of 2, 4-D in the environment is relatively short, averaging 10 days in soils and less than 10 days in water. 2, 4-D is a synthetic auxin. Its low concentrations can also stimulate RNA, DNA and Protein Synthesis leading to uncontrolled cell division and growth. On the other hand, high concentration of 2, 4-D can inhibit cell division and growth. Plant death typically within 3-5 weeks following applications. 2, 4-D shows acute and non-acute effects . That is in tabular forms.

Sl.No.	Organ (or) Organ System.	Types of effeccts
1	Central Nervous System	(i) Unconsciousness (ii)Electroencephalograph Changes
2.	Peripheral Nervous System	(i) Partial Paralysis (ii) Poly neuritis
3	Skeletal system	(i) Myotomia (ii) Kyotkymia (iii) Stiffness
4	Digestive System	(i) Vomiting (ii) Diarrhoea (iii)Viruious functional disorder
5	Respiratory System	(i) Irritation (ii) Coughing
6	Circulatory System	(i) Cardiac involvement (ii) Vascular involvement
7	Liver, Kidney	(i) Functional abnormalities
8	Skin	(i) Irritation (ii) Allergic reaction

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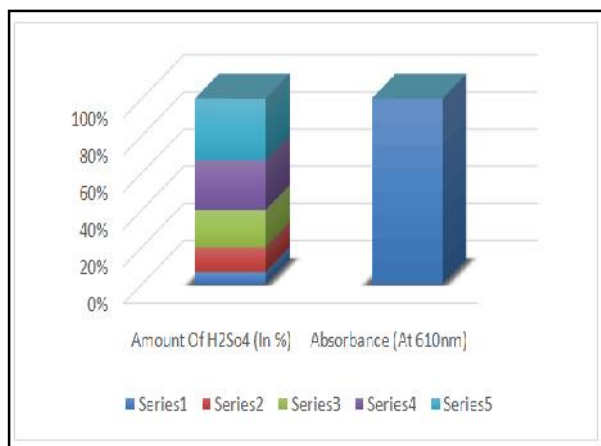
### MATERIALS AND METHODS

**Materials:-** A cart zesis specol with 1cm matched silica cells was used for all spectral measurements. Reagents 2, 4-D solutions are prepared, for this, stock solutions of 1mg/ml of 2, 4-D was prepared in concentrated H<sub>2</sub>SO<sub>4</sub>. A working standard of 40 µg/ml also prepared. 6-Amino-1-naphtol-3 Sulfonic acid (J-acid) solution (Wilson) was prepared by 0.4% solution in concentrated sulphuric acid. A 50% ammonium acetate solution was prepared in Distilled water

**Methods: -** A standard solutions containing 20-60 µg of 2, 4-D dissolved in concentrated sulphuric acid. Now 1ml J-acid solutions. Test tube was kept in hot air over at 130°C for 22 minutes after this reaction time, test tube was transferred at room temperature and then slowly poured 50% ammonium acetate solution, drop by drop .It was allowed in ice water bath. It was seen a blue coloured appear in their solution. The absorbance of this dye was measured at 610nm against a reagent blank using a spectrophotometer. For determination of 2, 4-D effluent in water sample, 50µl, 75µl and 100µl of 2, 4-D were added to 500 ml of polluted pond water.

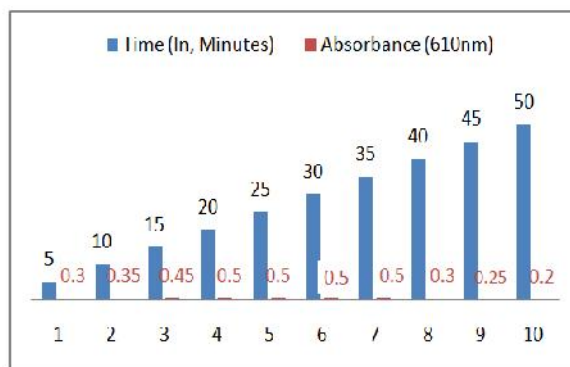
**Table no 01. Wavelength of 2, 4-D at different concentration of (80 µg & 120 µg per 25ml) by spectrophotometer**

Wavelength ( ) (in nm)	Concentration of 2,4-D		
	80µg/25ml	120 µg/25ml	Reagent Blank
500	0.08	0.01	0
520	0.01	0.05	0
540	0.15	0.1	0
560	0.25	0.13	0
580	0.30	0.2	0
600	0.39	0.34	0
620	0.60	0.38	0
640	0.55	0.25	0
660	0.45	0.15	0
680	0.35	0.05	0
700	0.25	0.04	0

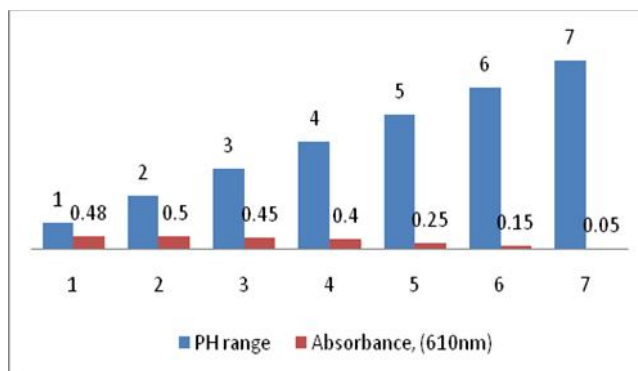


**Fig- 01. Concentration of H<sub>2</sub>SO<sub>4</sub> on colour development of fluorescent dye (Conc. of 2, 4-D was 100 µg/25ml)**

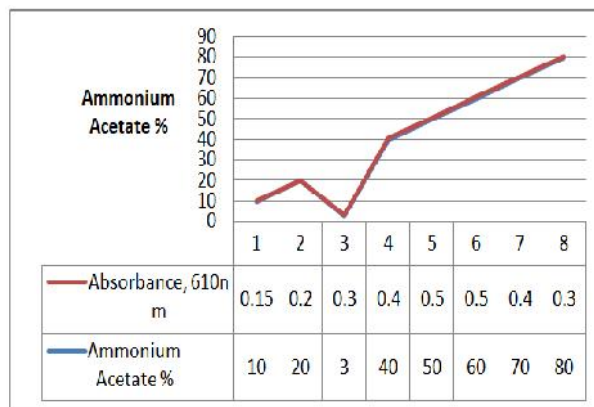
Now, added 20 ml 2% CCl<sub>4</sub> was evaporated off and then colorimetric determination was performed. For determination of 2, 4-D in grains (Maize and Rice), 25µg ,50µg and 100µg amounts of 2,4 –D was added to the sample and kept for same time and then extracted with 2 x 10ml ethanol. After same time, ethanol was evaporated off and 2, 4-D was determined. Plants materials was crushed and treated with 50µg, 75µg and 100µg amounts of 2, 4-D then to stand for few hours.



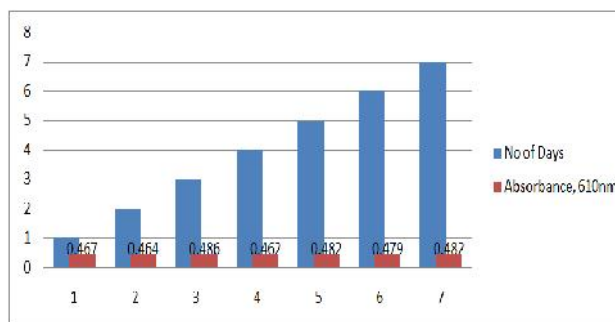
**Fig- 03. Effect of time on colour development of fluorescent dye, Conc. of 2,4-D=100 µg/25ml )0))was)100µg/25ml)**



**Fig- 04. Effect of P<sup>H</sup> on final Absorbance, Concentration 2,4-D= 100 µg/25ml.**



**Fig. 05. Effect of Concentration of ammonium acetate (2, 4-D was 100 µg/25ml)**



**Fig. 06. Effect of Concentration of ammonium acetate (2, 4-D was 100 µg/25ml)**

These were washed with 250ml of deionised distilled water. 2, 4-D now present in water was extracted with 2 x 20ml portion of CCl<sub>4</sub>. The CCl<sub>4</sub> layer was greenish yellow due to the presence of organic matter from plants. The CCl<sub>4</sub> layer was then passed through silica gel column (10 x1cm) to remove chlorophyll and other interfering plant materials. The column was washed with 10ml and CCl<sub>4</sub> it was later evaporated and the 2, 4-D obtained was analysed.

## RESULT AND DISCUSSION

### In colour reaction, four steps are involved.

- ) In the first step, cleavage of 2, 4-D in concentrated sulphuric acid takes place at 130°C and forms glycolic acid I.
- ) The glycolic acid produced in I is cleaved by concentrated sulphuric acid functioning as a dehydrant with formation of formaldehyde-II
- ) Formaldehyde reacts with J-acid to give a fluorescent dye-III.
- ) The fluorescent dye is converted into blue coloured dye- IV when added to 50% ammonium acetate solution.

Table no 01. Wavelength of 2, 4-D at different concentration of (80 µg & 120 µg per 25ml) by spectrophotometer

Wavelength ( ) (in nm)	Concentration of 2,4-D		
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640	0.55	0.25	0
660	0.45	0.15	0
680	0.35	0.05	0
700	0.25	0.04	0

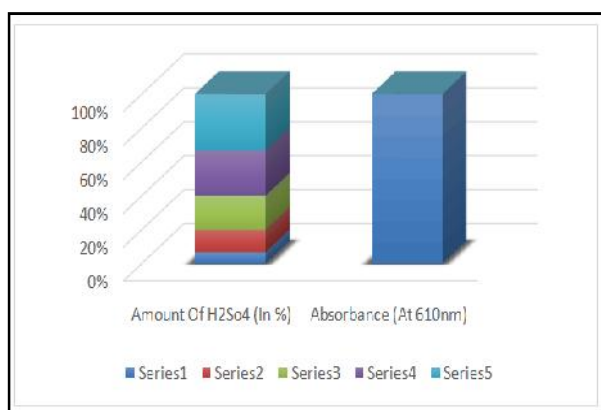


Fig- 01. Concentration of H<sub>2</sub>SO<sub>4</sub> on colour development of fluorescent dye (Conc. of 2, 4-D was 100 µg/25ml)

The effect of concentration of H<sub>2</sub>SO<sub>4</sub> on colour development was studied and it was found that concentrated H<sub>2</sub>SO<sub>4</sub> was necessary for the development of fluorescent yellow coloured dye which was responsible for the final blue coloured dye. The effect of different amount of J-acid was studied and it was observed that 1ml of 0.4% J-acid was sufficient for all colour development.

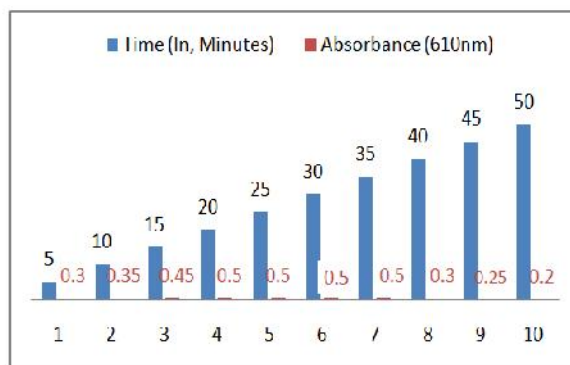


Fig- 02: Effect of time on colour development of fluorescent dye, Conc. of 2,4-D=100 µg/25ml (0)))was)100µg/25ml)

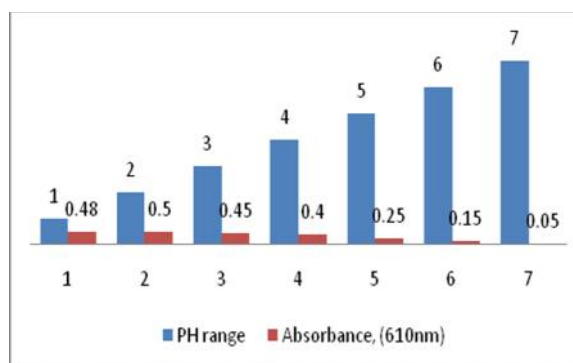


Fig- 03: Effect of P<sup>H</sup> on final Absorbance, Concentration 2,4-D= 100 µg/25ml.

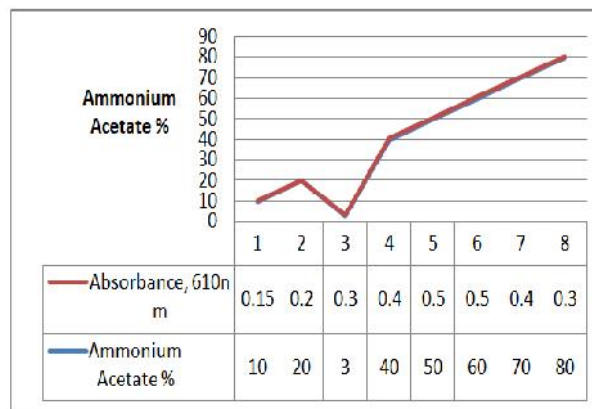


Fig. 04. Effect of Concentration of ammonium acetate (2, 4-D was 100 µg/25ml)

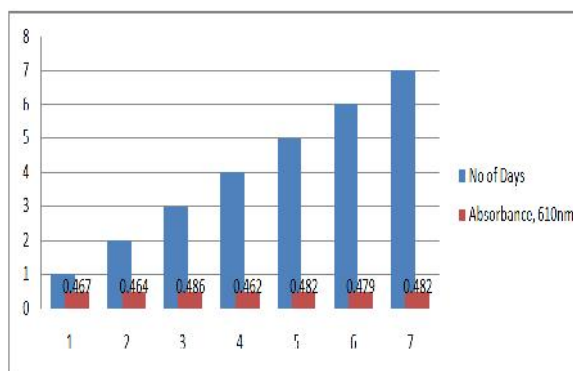


Fig. 05. Effect of Concentration of ammonium acetate (2, 4-D was 100 µg/25ml)

More than this concentration did not cause any adverse effect. There was optimum temperature was 130-140°C for development of dye colour and the  $P^H=2$  of solution for full colour development was found.

**Effect of Concentration of Ammonium acetate:** - The effect of Concentration of ammonium buffer was studied, The 50% ammonium acetate solution was used for adjusting the  $P^H$  of final solution. Below and above this concentration range, absorbance of final colored solution was found.

**Reproductivity of the method:-** The reproducibility of the method was checked by analyzing 1µg/25ml of 2,4-D for a period of 7 days.

**Determination of 2,4-D effluent water sample:-** After adding 50µg and 100µg of 2,4-D, it was found 48.8µg, 73.0µg, 98.0 µg respectively and its percentage of recovery was 97.0%, 97.3% and 98.0% respectively.

**Determination of 2,4-D in Grains (Maize and Rice sample):-** After adding 25µg, 50µg & 100µg of 2,4-D in Maize, 2,4-D was found 23.90 µg, 46.25 µg and 96.48 µg respectively and its percentage of recovery was 95.97%, 93.20%, 96.47% respectively. It was also observed in rice grains after adding 25 µg, 50 µg and 100 µg of 2,4-D it was found 24.30 µg, 48.10 µg & 97.10 µg respectively. Its % of recovery was 98.10%, 96.25% and 93.97%.

**Determination of 2,4-D in plant materials :-** In plant materials, by adding 50 µg, 75 µg and 100 µg of 2,4-D, it was found 95.78%, 71.92% and 94.10% respectively and its % recovery was 92.10%, 95.93%, 94.10%.

## Conclusion

2,4-D which is widely used a herbicide. Its effect on plants and animals had recorded. It affect badly on animals health and plants also.

So, the limited amount like 0.7 ppm can be allowed. 2, 4-D drift has long been a known problem to off-site locations, endangered species and non-target crops, as well as to people who live near its application sites.

## REFERENCES

- American Conference of government Industrial Hygienists. Am. Ind. Hyg. Asso. J. (1961), 22:325.
- Marthin. H. Pesticide manual. British Crop protection Council (1968).117.
- Avilla, L., Hirteta, P., Taylor K.S. and Jun, J.H. 1986. J. Agric. Food chem. 34:530.
- Ground R,B. and J.E. Forrette, J. Assoe off. Anal. Chem (1983). 66(5): 1220
- Milan P. and V. zdena, vide. C.A.(1988). 108 (7):145285e
- Brandberry, S.M., Proudfoot, A.T., Vale, J.A.(2004) poisoning due to Chlorophenoxy herbicides. Toxicol Rev. 23(2):65-73.
- Charles, J.M., Hanley, T.R., Wilson, R.D., Van Ravenzwaay, B., Bus, J.s. Developmantal toxicity studies in Rats and Rabbits on 2,4-D and its form. TOxicol. Sci. (2001).60:121-131.
- Vogue, P.A., kerle, E.A., Jenkins, J.J. 2004. 2, 4-D Technical facts sheet. Osu extension pesticide properties database. Oregon state university.
- Garacet, D., and philbert, M. Review of 2,4-dichlorophenoxyacetic acid epidemiology and Toxicology (2002). 32(4). 233-257.
- Gandhi, R., Wandji, S.A., Snedeker, S. 2000. Critical evaluation of cancer risks from 2, 4-D. Rev. Environment contain. Toxicol.. 167:1-33.

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