

Effect of Sugar Mill Effluent on Mitotic chromosomes of *Pisum sativum*

Narendra Prasad Roy¹, Dr. Anil Kumar Singh², Vishal Patel³

*Dr. Shabana Karim⁴ and Dr. Rajesh Kumar^{5*}

¹Faculty, Department of EWM, A.N. College, Patna – 800013

E-mail : drnarendrapdroy@hotmail.com, anilkr Singh11@gmail.com, vshlptl45@gmail.com
shabana.hamid4@gmail.com, rajeshkumar1952@gmail.com

Manuscript received online 19 December 2019, accepted on 12 March 2020

Abstract : Sugar mill is a sulphination factory and its effluent is known to be acidic in nature. These factories are discharging their effluents in aquatic system that has led to the disturbance in growth and reproductive behavior of the Pea plants i. e. *Pisum sativum*. The area of the North Bihar, where this work is carried out is known for cultivation of Pea plants on large scale. Over the years decrease in the yield of the crop has been noticed. The effect of sugar mill effluent on Pea plant has not been studied so an attempt has been taken to know the mitotic chromosome behavior on Pea plant and their effect on productivity. Somatic chromosome count of *Pisum sativum* has been recorded as $2n = 14$ and in meiotic studies $n = 7$ (Base number) has been reported. Effluents of sugar mills of North Bihar contain both organic and inorganic hazardous materials and non-biodegradable materials. During the course of production it has been dumping effluents in water body, which affected human health as well as plant productivity. Water pollutants of this area can be broadly divided into two categories i.e. Toxic and Nontoxic, the former may include heavy metals, phenols, pesticides etc. and the later may comprise wholly or partly of degradable organic materials and minerals. Sugar mills effluents is a major problem, as polluted water is used for irrigation of crop plants like *Pisum sativum* (Pea). Following physico-chemical analysis of sugar mill effluents have been done during study. They include pH, Temperature ($^{\circ}\text{C}$), Conductivity, Total Alkalinity, Free CO_2 , Turbidity (NTU), TS, TDS, TSS, DO, BOD, COD, Nitrogen, Phosphorous, Potassium and Oil & Grease.

It was noticed that due to sugar mills effluents several types of irregularities like - breaking of chromosome,

clumping, stickiness, unequal separation, formation of micronuclei and laggards formation have been recorded. It was also noted that biological materials such as seeds and roots of *Pisum sativum* when come in contact with such polluted water, a series of reaction occurs between chemical compound and active tissue. Chemical compounds reach to react with the genetic targets such as chromosomes, and DNA. As a result of such reaction several types of irregularities in cell division occur. It can be safely stated that "the presence of chemicals in specific proportion may be responsible for damaging the chromosome and ultimately the decrease in productivity of Pea Plant i.e. *Pisum sativum*."

(Keywords : Sugar mills effluents, Mitotic chromosome, Pea)

Introduction

Pisum sativum (Pea Plant) is a major food crop which belongs to family Leguminosae. It is the second largest family of dicots with 600 genera and 12000 species (Ref. - 1). In India 925 species are recorded (Ref. – 2). Edible seeds of *Pisum sativum* are known as Pulses or Beans and they have more protein than Cereals and are next only to cereals as main source of Human food. In view of the above observations, the present investigations aim to study the effects on cytological behavior of Chromosomes. Two variety of *Pisum sativum* have been selected named as Var-Arkel and Var Azad-I of the family

²Associate Professor, Department of Chemistry, A. N. College, Patna

³Project Associate, Centre for Fluorosis Research, A. N. College, Patna

⁴Associate Professor, P.G. Department of Botany, A. N. College, Patna

⁵Project Supervisor, Centre for Fluorosis Research, A. N. College, Patna

Leguminosae and tribe – Viciae for cytological mitotic observation having $2n = 14$ and $n = 7$ chromosome number. In course of present investigation mitotic drive and effects of sugar mill effluents have been studied in detail. Trace has been given to know the role of chromosome in decreasing productivity of pea plants in North Bihar. Keeping this in view mitotic investigation, karyotypic analysis, and Idiogram of both species have been made along the following lines.

Experimental

Materials and methods

The standard seeds of the *Pisum sativum* var ARKEL and AZAD - I were purchased from private seeds retailer of Jamal Road Patna for the experimental purpose.

Effluents of the sugar mills of North Bihar Narkatiyaganj were diluted to 100 %, 75%, 50%, 25% and 10% by adding required value of distilled water. Firstly the seeds of *P. sativum* were allowed to germinate and grow in tap water. When roots become 1-3 cm long, they are placed in different concentration of effluents for 12 hours. The time of fixation was constant in each treatment hour as well each concentration.

The root tips were cut at 10:00 AM and pretreated with help of Para-dichlorobenzene for 03:30 hrs. Fixation of root tips was done in 1:3 Acetoalcohol for 24 hours. Each series of experiments is followed by a control with tap water treated in an identical way.

All the treatment and experiments have been carried out at room temperature that varies between 20-30°C Squashes were prepared according to the method of 15% Aceto-carmin squash was used in the present study as it was proved to be the best method of micro technique to observe all abnormalities caused by effluent upon meristematic cells of *Pisum sativum* root tips (Ref. – 3). Karyotypic preparations along with

Idiogram were made. Root tips after hydrolysis were thoroughly washed in distilled water, stained in 15% acetocarmine and then squashed in 45% acetic acid. 10 well spread and well counted chromosome of metaphase plates were used for following parameters. The microphotographs were taken from temporary slide preparation. The slides were made permanent after passing through the following stages-

- i. Slides were placed in 45% Acetic acid for 2 minutes to separate the coverslip from the slide.
- ii. 1:1 solution of n-butanol and acetic acid are used for 2 minutes to separate slides and coverslip.
- iii. Then slide and coverslip are placed in n-butanol for 2 minutes.
- iv. Finally the slides are mounted in Euparal.

Observations

(Effect of effluent on chromosome)

Pisum sativum is chosen as test material because of its good size chromosome, well understood genetic system, short life cycle and easy cultivation. Two varieties of *Pisum sativum* named AZAD-I and ARKEL were widely been used in studying the cytological, genotypic potential of various purative and mitotoxic effect. During Mitotic study, the effects of sugar mills effluents were clearly noticed on chromosomes. In AZAD-I variety of *Pisum sativum* the diploid chromosome number was recorded as $2n = 14$.

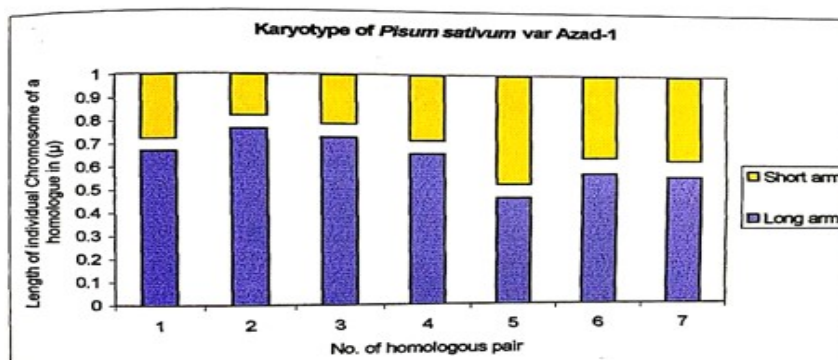
The number of submetacentric (Sm) chromosomes has been recorded more in number as compared to metacentric (M) or sub-tetocentric (St) chromosome. Out of 7 pairs - 4 pairs of Sm, 1 pair of M and 2 pairs of St chromosomes were recorded. The total chromatin length of diploid genome was 65.4 μ . The minimum size of chromosome was 3.90 μ in length and maximum 5.35 μ in length. The detailed karyotypic analysis and Idiogram has been illustrated in Table 1 and Graph 1 respectively.

Table -1
Karyotypic Analysis of *Pisum sativum* (var Azad – I)
2n = 14 (7 – Homologous Pair)

No. of Homo pair	Length of individual chromosome of a homologue in μ			Long arm / short arm	Position of Centromere
	Long arm	Short arm	Total length in μ		
1	3.790	1.560	5.350	2.429	Sub-metacentric
2	4.060	0.930	4.990	4.365	Telocentric
3	3.790	1.090	4.880	3.477	Telocentric
4	3.400	1.440	4.840	2.365	Sub-metacentric
5	2.340	2.340	4.680	1.000	Metacentric
6	2.500	1.560	4.060	1.602	Sub-metacentric
7	2.340	1.560	3.900	1.500	Sub-metacentric
Total	22.120	10.580	32.700		

Total Length in diploid genome = 65.44 μ

Graph - Q - Karyotype of *Pisum sativum* var Azad-1



The Arkel variety of *Pisum sativum* was also analyzed cytologically to know the karyotypic analysis and their total diploid genome. Diploid chromosome number was found to be 2n = 14 like Azad – I variety. Out of the 7 (Seven number of homologous chromosomes – 5 were of Sm (Sub metacentric), only one Metacentric (M), and one Telocentric (T) chromosome were counted. The

minimum size of chromosome was recorded as 3.170 μ in length and maximum size – 6.357 μ in length. The total chromosome length in diploid genome of Arkel was 62.148 μ

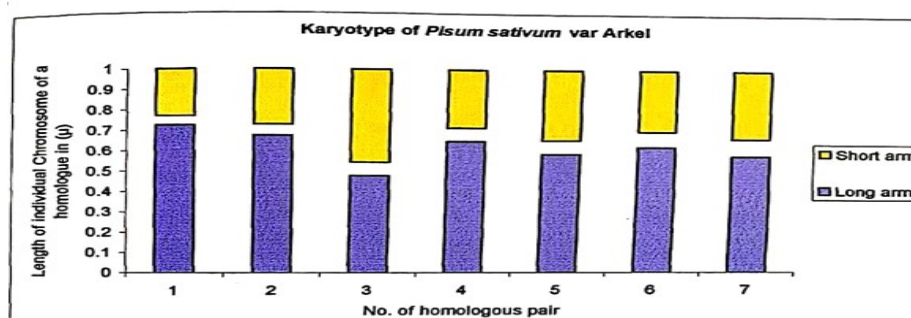
The detailed Karyotypic analysis is given in Table No – 2 and Idiogram is represented in graph – 2

Table - 2
Karyotypic Analysis of *Pisum sativum* (var Azad - I)
 $2n = 14$ (7 – Homologous Pair)

No. of Homologous pair	Length of individual chromosome of a homologue in μ			Long arm / short arm	Position of Centromere
	Long arm	Short arm	Total length in μ		
1	4.830	1.527	6.357	3.163	Telocentric
2	3.720	1.490	5.210	2.502	Sub-metacentric
3	2.227	2.090	4.317	1.065	Metacentric
4	3.000	1.310	4.310	2.292	Sub-metacentric
5	2.570	1.510	4.080	1.754	Sub-metacentric
6	2.440	1.190	3.630	2.053	Sub-metacentric
7	2.000	1.170	3.170	1.709	Sub-metacentric
Total	21.787	9.287	31.074	14.538	

Total length of diploid genome = 62.148 μ

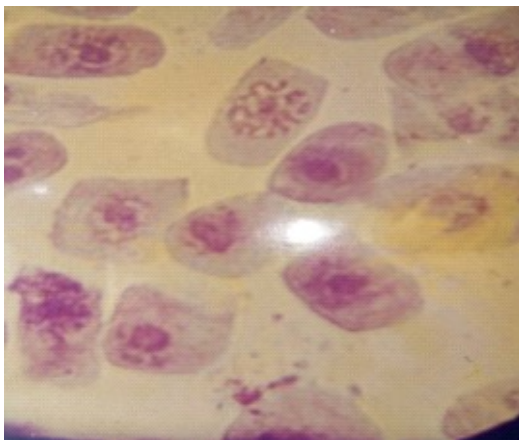
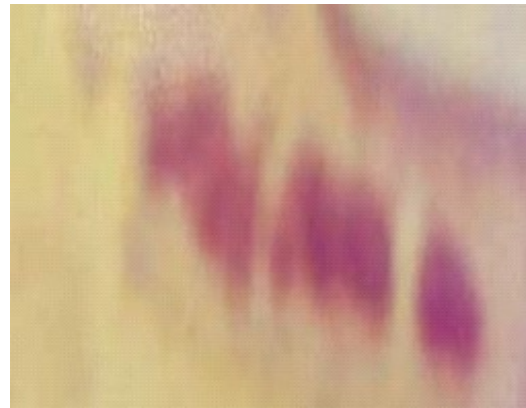
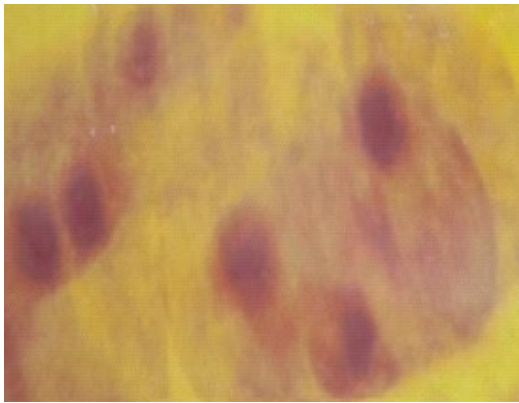
Graph - R - Karyotype of *Pisum sativum* var Arkel



The above observations on both varieties of *Pisum sativum* clearly indicate that normally the Karyotype is symmetrical. The fact is that sugar mill is a sulphination factory and its effluents is known to be acidic in nature. The physicochemical characteristic of the effluent also affects the cytological and genetical behavior of the chromosomes which have been well observed during the cell division of *Pisum sativum* in both varieties. The common anomalies which appear and well-marked during cytological studies are – Spindle disturbances, Formation of fragments, clumping of chromosomes, chromosome stickiness,

breakage, formation of binucleate and multinucleate condition, formation of laggards and micronuclei.

All such irregularities are due to toxic effect of sugar mills effluent, which will be discussed during later stage of discussion. However, one thing is clear that all such anomalies recorded during cell division in *Pisum sativum* (Pea) plant will lead towards the sterility and ultimately they are major cause for decreased level of Pea production in North Bihar area, where the study is carried out.



Results and Discussion

Modern technology has spread to plenty of chemical compound like pesticides, cosmetics, water and air pollutant, Industrial discharge, house production and in last five years method has been developed to access the relevance of their genetic risk for individuals and for the whole population. The process of chemically induced genetical damage has begun to alarms both the scientific communities and general populations.

Effluent from sugar mills have also been found to cause chromosomal aberrations besides interfering with the mitotic division. Physico-chemical analysis of sugar mills effluent suggest their toxic nature. The induction of short arm, clumping, fragments, spindle disturbances, nuclear disintegration, micronuclei and binucleate cells following treatment of *Pisum sativum* root tip have also been earlier reported (Ref. – 4), (Ref. – 5) and (Ref. - 6). In view of the induction of chromosomal damages caused by the industrial effluents and the presence of chemical constituent in the sugar mill effluent necessitate the study of the influence of sugar mill effluent in root tip of *Pisum sativum*. The effect of sugar mill effluent has been studied for various duration of time on root tip that shows the presence of chromosomal stickiness, clumping and unequal separation of chromatids. It is noticed and also recorded earlier (Ref. – 6), (Ref. – 7) and (Ref. – 8) that when biological material are treated then initially a series of reaction occurs between chemical and the active tissue. Chemical compounds reach to react with the genetic targets such as chromosomes and DNA. As a result of such reactions, potency of formaldehyde in causing chromosome stickiness in *Vicia faba* chromosomes has been reported (Ref. – 9) and (Ref. - 10) in paint and plywood factory effluent. In contrast sugar mill effluent induced slight stickiness. The frequency of which has been found to be inversely proportional to the concentration of the sugar of mill effluent and it may be attributed to chemical reaction between the effluent and chromosome resulting into stickiness of the chromosome.

Breakage is another type of irregularity reported during this study (Ref. – 11) as chromosomal aberration and inhibition of mitosis in root tip cells of *Pisum sativum*, *Allium cepa* and *Vicia faba* treated with adenine. A large number of plant products are known to induce chromosomal breakage in plant (Ref. – 12). A number of dyes cause breakage in chromosomes (Ref. - 13). X-rays are also major cause for breakage in chromosome (Ref. – 14). Although different authors have attributed different reactions for induction of break, however the common fact emerging out from these discussions is that the upset in nucleic acid metabolism is ultimately responsible for break. The effluent present in investigation, produced significant effect on cell division. They induced chromosome breakage and also alternation in divisional frequency. The chromosomal aberration took various forms like breakage, stickiness, clumping, unequal distribution of chromosomes and ultimately formation of micronuclei etc.

The occurrence of binucleate and multinucleate cells in the treated material shows that the effluent inhibits cell plate formation. Binucleate and multinucleate conditions due to inhibition of cytokinesis are formed by several types of cyclic organic compounds such as hydrazine compounds. A number of pesticides such as bromacil are known to induce binucleate and multinucleate conditions (Ref. – 15 and Ref. - 16). The induction of such conditions in root tips of *Allium cepa* by paper mill effluent have also been reported (Ref. 17). However, our findings of the binucleate and multinucleate conditions due to effluent of sugar mills in the treated root tip of *Pisum sativum* is almost similar to the above observations.

Considerable literatures are available regarding the formation of laggards treated with both physical and chemical mutagens (Ref. - 14) have indicated the induction of Laggards with pesticides. Sugar mill effluents in the present investigation have been found to induce laggards

which might constitute micronuclei in later stage of cell division suggesting genetic damage. Fragments both at metaphase and telophase have been produced in *Pisum sativum* of both varieties Azad- I and Arkel root tips as a consequence of effluent treatment. The occurrences of fragment may be attributed to the failure of rejoining between the broken ends. It means sugar mills effluent besides inducing fragments in chromosomes also inhibit reunion of the fragments. Failure of inclusion of acentric fragments into either of the two polar nuclei results into the formation of micronuclei. This suggests the genotoxic potential of sugar mill effluent.

Many chemical mutagens are known but a few compounds have been tested for synergistic or antagonistic action. Physico chemical analysis of effluent simply indicate that since it is a mixture

of several organic and inorganic compound, its action on the genetic system may be considered as a synergistic action. In view of the combined nature of effluent, it is difficult to correlate the magnitude of Chromosome damage by a specific chemical.

Moreover in present study, we may conclude that the presence of chemicals in the specific proportion may be responsible for damaging the chromosome. Further studies are needed to find out the exact cause for chromosomal irregularities in *Pisum sativum* by sugar mill effluent. It is true that the effluent is mutagenic and clastogenic in nature and they are responsible for decrease in productivity of *P. sativum*.

References

1. J. D. Hooker, : The flora of British India. L. Reeve and Co. Ltd. (1982)
2. G. L. Stebbins : Chromosomal evolution of higher plants. *Edward Arnold Ltd*; London (1971)
3. R.C Mathur, Systematic Botany, Agra Book Store, Agra P. 426 (1974)
4. Y. Huziwara, ; Karyotype evolution in some genera of compositae. *Amer.J.Bot.***49**: 116-119. (1962)
5. R.N Trivedi and R. Kumar Cytomorphological studies in natural populations of *Elicta alba* (L). Hassk. *Cytologia* 49 :731 (1984)
6. M. P. Mishra, Effects of Calcium Salts on *Allium cepa* chromosomes. *Cytologia*. **47**: 47-51 (1982)
7. R. Kumar and Tiwary : Effect of X-ray treatment of somatic chromosomes ; of *Eclipta alba* (L.) Hassk. *Cytologia*. **79(1)**:63 (2014)
8. B. Singh. : Chromosomes number in some flowering plants. *Curr. Sci.* **20**, 1105 (1951)
9. A.K. Ghosh, P. Kumar : Effects of plywood industry effluent on seed germination of *C. arietinum*. *Geobios*, vol. **27**, 77 (2000)
10. A.K Ghosh, P. Kumar and N. P. Roy, : Effect of distillery effluents on seed germination and seedling growth of some Legumes. *Neo Botanica*, Vol. **7**, 27 (2000)
11. A. K Sharma, and N. K. Das, : A study of Karyotypes and their alteration in Aroidae. *Agron-Lusit* 16 : 23 (1954)
12. Ravi, R. and M.M. Srivastva, : Effect of distillery effluents on seed germination of wheat. *Environment and Ecology*, 6 (2) : 214 (1985)
13. Ravindra and Ravindran : Cytological irregularities induced by water pollution with factory effluents. *Cytologia*, **43** : 565 – 68 (1978)
14. D. Doxey, The effect of Isopropyl phenyl censoltae on mitosis in Rye and Onion. *Hann. Bond. IS* : 329-36. (1949)

15. W.F. Grant, : Cytogenetical studies of Agricultural perspective. R.A. Fleck and Hollander (ed) : 353 (1982)
16. S. Md. and Farah : Cytogenetic effects of Pesticides on mitosis of O.R. *Vicia faba*. Cytologia, 37 : 507 (1974)
17. A. R. Bhatnagar and P. Dodein : Effect of sugar mill effluent on germination and growth of Rice. Environment and Ecology 4 (2) : 218–20 (1986).