ISSN 2349 - 2732

Annual International Journal

GYAN VIGYAN

A JOURNAL OF SCIENCE

Vol. 4 May 2017

JOURNAL

IN COLLABORATION WITH UP SELF FINANCE COLLEGES' ASSOCIATION

GYAN VIGYAN

ISSN 2349 – 2732
A Journal of Science

Annual International Journal
Volume – 4 May 2017

Gyan Vigyan : A Journal of Science is a Annual International Journal published every year in May by Gyan Mahavidyalaya, Aligarh.

The Journal aims to provide Science Teachers and Researchers a forum to present their work to community through original and critical thoughts in Science.

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GYAN VIGYAN : JOURNAL OF SCIENCE
VOL. 04 MAY, 2017 ISSN 2349-2732

Removal of metal ions from wastewater by using Chelating resin

Asma Khatoon*, Akil Ahmad**, Ajij Golandaj***, David Lokhat****

Abstract

With increasing population, urbanization and industrialization, most of the developing countries are facing drinking water problems and the conditions are very severe in present scenario. Therefore, the demand for new and novel purification and separation techniques of heavy metal ions have become increasingly worldwide. Various separation and purification methodology have been studied for metal removal such as adsorption, advanced oxidation process, ozonation, electrochemical removal process, nanofiltration and membrane bioreactor. In all these techniques, chelation has been exhibited to be a very simple, effective, easy and economical process used for the removal of metal ions from wastewater.

Keywords : Toxicity; heavy metals;separation; chelation;wastewater

1. Introduction

Demand of water is increasing day by day due to the increasing population, rapid urbanization and industrialization, which is the huge concern for developing countries like India. Presence of heavy metal ions in the water bodies have a severe impact on the animal and human health. Apart from that some of metal ions such as copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), magnesium (Mg), zinc (Zn) and nickel (Ni), are essential nutrients that are required in trace amount for various physiological and biochemical functions. Some non-essentials heavy metals such as Pb, Cd and Hg are also present in water bodies which affects the human body even in ppb level concentration[1,2,3]. Higher concentration of these metal ions in water can cause various disease such as eye irritation, headache, dizziness and diarrhoea and fragile bones and carcinogen.

Major contributors of heavy metal ions in the environment are industrial, geogenic, pharmaceutical, domestic effluents, agricultural, anthropogenic sources and atmospheric sources. With regard to heavy metal ions removal from the water and wastewater, there have been growing interest to develop analytical method which are capable to remove these pollutants. Various methods include sorption, chelation, adsorption, ion exchange, precipitation, membrane technology, photo degradation have been employed for the removal of metal ions[4-17]. In all these technology, new chelating resin are capable to remove such pollutants even in the trace amounts.

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In this review, we discussed and compiled the literature on various chelating resin which are used for highly toxic metal ions removal from water and wastewater.Chelating resin has been widely adopted for the removal of trace metal ions from wastewater.

2. Use of chelating resin for metal ions extraction :

Now a days chelating reagents are emerging as a popular and efficient agents to remove the heavy metal ions from the wastewater. Supported chelating agents embedded on materials like polystyrene has displayed a high regeneration capacity. In addition, these supported chelating agents are easy to synthesise in laboratory as well as on industrial scale, which makes it more popular. Some of chelating resins are presented in Table 1. Ferreira et al. 2000 developed a chelating resin, Amberlite XAD-2 resin loaded with calmagite reagent, in order to determine trace amounts of Cu(II) in seawater and biological samples by flame atomic absorption spectrometry (FAAS). The detection limit (3 σ) was 0.15 mg L^{-1} and the precision (relative standard deviation) reached values within the range of 2.7–6.0% for Cu(II) varying from 0.0 to 20 mg L^{-1} concentrations, respectively. The accuracy of the developed procedure was confirmed by applying it on biological certified reference materials. The recoveries of Cu(II) from seawater and biological samples, measured by standard addition technique, also favoured good accuracy of the proposed method[18]. Later on, Ferreira et al. 2001 prepared a chelating resin, Amberlite XAD-2 loaded with 2-(2-benzothiazolylazo)-2-p-cresol (BTAC), through a complexation process. An enrichment factor of 30 was achieved, for a sample volume of 7.0 mL, at a preconcentration time of 1 min. Detection limit (3σ) was found to be 1.1 µg L^{-1} and the precision (relative standard deviation, R.S.D.), for seven independent determinations, was about 5.0–0.9% for Ni(II) solutions of 5.0–250.0 µg L^{-1}[19]. Lemos et al. 2003a) reported an Amberlite XAD-2- Nitroso- R salt functionalized resin that was used for the preconcentration and determination of Co(II) in an online system using FAAS. The enrichment factors were found to be 79 and 223, with detection limits (3σ) of 1.25 and 0.39 g_ L^{-1}, for respective preconcentration periods of 60 and 180 s. The accuracy of the method was evaluated by analysing biological certified material and subsequently applied successfully for the determination of Co(II) in natural water samples[20]. Lemos et al. (2005) synthesized a chelating resin (Amberlite XAD-2 with 4,5-dihydroxy-1,3-benzenedisulfonic acid) and applied it on an online system for Ni(II) preconcentration. Using the experimental conditions defined in the optimization, detection limit (3s) of 2 µg L^{-1} and precision (assessed as the relative standard deviation) of 8.2–2.6% were achieved. The enrichment factor was found to be 46, for preconcentration time of 120 s. The proposed method was successfully applied for the determination of Ni(II) in food samples[21]. A new sorbent was synthesized (Amberlite XAD-2 functionalized with 3,4-dihydroxybenzoic acid) in on-line system for Cu(II) preconcentration and determination in food samples by Lemos et al. (2003b). The detection limit (DL) (3 s) was found to be 0.27 µg L^{-1} and the precision (assessed as the relative standard deviation) reached values of 5.7-1.1% for Cu(II) solutions of 5.00 to 50.00 µg L^{-1} concentrations. The accuracy of the method was confirmed by using certified reference materials (NIST 1568a-Rice Flour and NIST 1572-Citrus Leaves) and recoveries were achieved up to 90.0-110.0%. These results proved also that the procedure is not affected by matrix interference and can be applied satisfactorily for Cu(II) determination in rice flour and starch samples[22]. A column solid-phase extraction (SPE) preconcentration method was developed by Liu et al. (2005) for the determination of Cd(II), Co(II), Cu(II), Ni(II) and Zn(II) ions in natural water samples by FAAS. This method was based

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on the retention of analytes in the form of 2-acetylmercaptophenyldiazoaminoazobenzene (AMPDAA) complexes on a short column of AMPDAA-XAD-4 resin from buffered sample solution and subsequent elution with a mixture of hydrochloric acid and sodium chloride. The detection limit for Cd(II), Co(II), Cu(II), Ni(II) and Zn(II)was 0.028, 0.064, 0.042, 0.023 and 0.16 mgL^{-1}, respectively, and the quantification limit was 0.043, 0.11, 0.099, 0.044 and 0.29 mgL^{-1}, respectively. The method was validated by analysing a standard reference material (GBW 08301).The developed method was applied to the determination of trace metal ions in tap water and river water samples with satisfactory results[23]. Liu et al. (2007) developed rapid and sensitive flow injection online preconcentration method for determination of Cd(II), Co(II), Cu(II), and Zn(II)in natural water samples by FAAS. The proposed method allowed the determination of Cd(II), Co(II), Cu(II), and Zn(II)with detection limits of 0.1, 0.5, 0.3, and 0.2 mgL^{-1}, respectively. The selectivity of the XAD-4-DHDAA resin for Cd(II), Co(II), Cu(II), and Zn(II)over several electrolytes was also investigated. This method was validated by analysis of a standard reference material (GBW 08301). The developed method was applied to the determination of trace Cd(II), Co(II), Cu(II), and Zn(II)in tap water, ground water and river water samples with satisfactory results[24].Islam et al. (2010a) introduced Amberlite XAD-4 (AXAD-4) AXAD-4-HBA, which was synthesized through azo linking of p-hydroxybenzoic acid to AXAD-4, to study the sorption behavior for several metal ions which were subsequently determined by FAAS. The limit of preconcentration was in the range of 4.3–7.6 μgL^{-1}. The detection limit for Co(II), Ni(II), Cu(II), Zn(II) and Pb(II) were found to be 0.47, 0.45, 0.50, 0.80, and 1.37μgL^{-1}, respectively. The AXAD-4-HBA has been successfully applied for the analysis of natural water, multivitamin formulation, infant milk substitute, hydrogenated oil and fish[25]. In another work, Islam et al. (2010b) came up with a functionalised Amberlite XAD-4 resin (AXAD-4), by coupling it through an sNdNs group to 1-(2-pyridylazo)-2-naphthol (PAN), for the preconcentration of trace metal ions in some real matrices. The detection limits were found to be (0.65, 0.80, 0.85, 0.95, and 1.40) L^{-1} for Zn(II), Co(II), Ni(II), Cu(II), and Pb(II), respectively[26]. A selective FAAS method was developed by Islam et al. (2012a) for the determination of trace amount of metal ions. Here, AXAD-4 was functionalized with salicylic acid using azo spacer as the bridging group. The detection limits were found to be 0.42, 0.57, 0.63, 0.77, 0.94, 0.96, and 1.41 $\mu g\ L^{-1}$, respectively[27]. In a new study, Islam et al. (2012b) introduced a novel chelating resin, amberlite XAD-4 functionalized with o-Hydroxybenzamide (AXAD-4-HBAM), for the preconcentration of trace metal ions. The detection limit for Cu(II), Cr(III), Ni(II), Co(II), Zn(II), and Pb(II) were found to be 0.39, 0.49, 0.42, 0.59, 0.71, and 1.10 ng mL^{-1}, respectively. The AXAD-4-HBAM has been successfully applied in the analysis of natural water, multivitamin formulation, infant milk substitute, hydrogenated oil, urine, and fish28. Venkatesh and Singh (2005) synthesized a chelating resin, 2-{[1-(3,4-Dihydroxyphenyl)methylidene] amino}benzoic acid immobilized on Amberlite XAD-16, using azo linker and subsequently applied for the determination of Zn(II), Mn(II), Ni(II), Pb(II), Cd(II), Cu(II), Fe(III) and Co(II). High sorption capacities (in the range of 97 - 515 µmol g−1) and preconcentration factors (in the range of 100 - 450) were achieved. The half time to reach saturation, t1/2, is ~ 5 min and a regeneration of 50 cycles of sorption–desorption, without any significant change (<1.5%), was achieved. The values of limit of detection (blank +3 s) are 1.12, 1.38, 1.76, 0.67, 0.77, 2.52, 5.92 and 1.08μgL^{-1} for Zn(II), Mn(II), Ni(II), Pb(II), Cd(II), Cu(II), Fe(III) and Co(II), respectively. The chelating resin was successfully applied to determine all the studied metal ions in river and synthetic water samples, Co(II) in vitamin tablets and Zn(II) in milk sample[29]. Later on, a chelating resin developed by chemically modifying

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Amberlite XAD-16 with 4-{[(2-hydroxyphenyl) imino]methyl}- 1, 2-benzenediol (HIMB), via azo spacer, with the objective of determining Zn(II), Mn(II), Ni(II), Pb(II), Cd(II), Cu(II), Fe(III) and Co(II) at pH range 5.0–8.0 was reported by Venkatesh and Singh (2007)30. Sorption capacity was found between 56 and 415 µmol g−1 and the preconcentration factors from 150 to 300. Detection limit (blank + 3s) was found to be 1.72, 1.30, 2.56, 2.10, 0.44, 2.93, 2.45 and 3.23 µg L^{-1} for Zn(II), Mn(II), Ni(II), Pb(II), Cd(II), Cu(II), Fe(III) and Co(II), respectively. Soylak et al. (1995) developed a method for the preconcentration of trace amounts of tungsten as its thiocyanate complex, using a column filled with Amberlite XAD-1180 resin31. The analyte was determined spectrophotometrically and the detection limit for tungsten was found to be 12 µg L^{-1}. A simple and sensitive SPE procedure on Amberlite XAD-1180 resin, as reported by Soylak et al. (2003) 32, was used for the determination of Cr, Co(II), Mn(II) and Ni(II) at trace levels by atomic absorption spectrometry. The detection limits for Cr, Co(II), Mn(II) and Ni(II) were 0.27 µgg-1, 0.11 µgg-1, 0.13 µgg-1 and 0.086 µgg-1, respectively.

Table 1

Modified Chelating resin for metal removal

Reagent	pH	Metals	Sorption Capacity (mg.g-1)	Ref.
Salicylic acid	5, 4-6	Zn^{2+}, Pb^{2+}	1.146, 0.461	[8]
Chromotropic acid	5.0–6.0, 4.0–4.5, 4.5–5.5, 4.0–5.0, 5.0–6.5, 5.5–7.0	Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+}	9.35, 3.84, 8.50, 3.24, 6.07, 9.65	[9]
1-(2-thiazolylazo)-2-naphthol (TAN)	5.7, 8.3	Cu^{2+}, Zn^{2+}	-	[10]
1-(2-pyridylazo)-2-naphthol (PAN)	6-11.5	Ni^{2+}	0.1097	[11]
Pyrocatechol Violet	5, 5-7, 4, 3	Zn^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}	1.41, 1.27, 0.62, 1.36	[12]
Alizarin Red-S	5-6, 5-6, 3-4, 3-4	Zn^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+}	0.511, 0.124, 0.139, 0.306	[13]
o-vanillinthiosemicarbazone	2.5-4, 5.5-6.5, 6.0-7.5	Cu^{2+}, Zn^{2+}, Pb^{2+}	0.85, 1.50, 2.00	[14]
Tropolone	--	Sn	0.60	[15]
N-(dithiocarboxy)-sarcosine	9.0	Co^{2+}	---	[16]
Chloro complex	2.0	Au^{3+}	108	[17]

3. Mechanism of Chelation

Various amberlite resins such as Amberlite XAD, Dowex-50, IR-120 etc. have been used for the removal of metal ions. These resins have been modified by various organic moieties with the aid

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of its ligating sites. Generally, these ligating sites are composed of electron donating groups such as $-NH_2$, -OH, -SH and –COOH, which readily donates their lone pair of electron forbinding with the metal ions. Binding mechanism of metal ions with chelating sites are presented in Figure : 1.

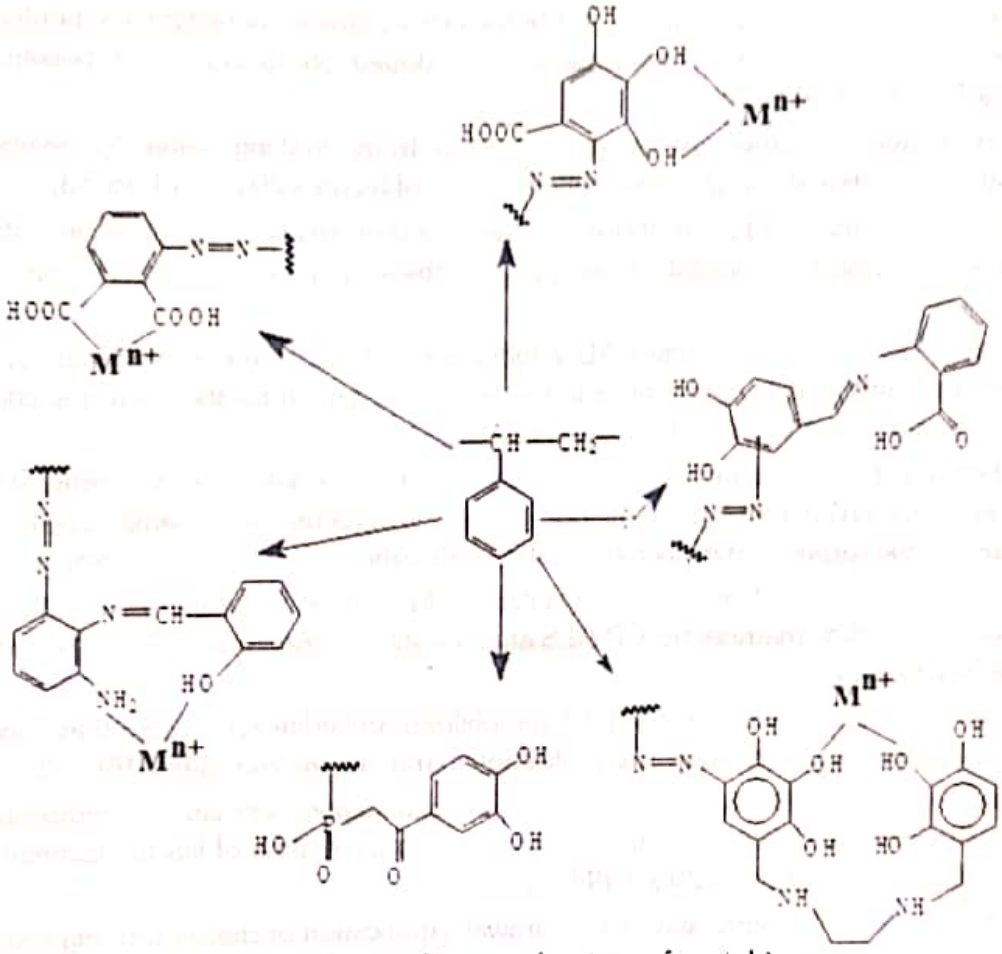

Figure 1. Binding mechanism of metal ions

4. Conclusion :

This article has attempted to cover various chelating resins as metal extractant are used for the removal of metal ions from wastewater. These materials are considered as very efficient, technical feasibility and cost effective. Most of the research work was carried out in batch and column method for the removal of metal ions. These chelating resins are found to be very effective for the removal of toxic heavy metals from water bodies. These materials should be considered as next generation materials that will encompass a wide variety of applications.

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VOL. 04 MAY, 2017
ISSN 2349-2732

Seasonal Variations of copulatory organ of *Gyrodactylus aculeati* Malmberg, 1956 on the gills of *Labeo rohita* and *Carassius auratus*.in river Ganges near Chandpur Distt. Meerut (U. P.), India.

*Pragati Rastogi and ** Jyoti Singh

Abstract

There are several abiotic factors including temperature, oxygen and pH etc. that affect the phenotypic plasticity of parasites. During present study, the authors concentrated on seasonal variation in measurements of copulatory organ of *Gyrodactylus aculeati*, Malmberg, 1956 from skin and fins of *Labeo rohita and Carassius auratus*[1]. Worms were collected from *Labeo rohita* at different water temperature. Samples were taken at monthly intervals of about 30 (± 5) days during the period from January 2009 to March 2012. Main results indicate that the difference between winter and spring conditions cause significant changes in morphometric variables of measurements of cirrus (copulatory organ).

Keywords : Monogenea, *Gyrodactylus aculeati*, copulatory apparatus, seasonality, morphometric variables.

1. Introduction :

Malmberg, 1956 described *Gyrodactylus aculeati* from skin and fins of *Gasterosteus aculeatus (Linn.)* at Namdo. This is second report of *G. aculeati* Malmberg, 1956 from Indian region[4]. Worms were found parasitizing *Labeo rohita and Carassius auratus*. Highest prevalence of infection was recorded in period from January 2009 to March 2012. There are several morphological and anatomical characteristics of monogenea that are used for species determination. Main morphological parameters are morphometric characteristics of attachment apparatus and copulatory organ. Species definition using only the shape and measurement of attachment apparatus and copulatory organ is difficult in similar species, because some measurements often overlap, while shape is variable. Because the mistaken description, of an already existing species as newly discovered occurs, it is important to know which factors affect morphometrical variation to avoid incorrect determination of species. The aim of this study is to investigate the differences of measurements of copulatory organs of *Gyrodactylus aculeati* at different temperatures. It is expected to detect metrical variation in morphology of *Gyrodactylus aculeati* considering seasonal changes in water temperature.

2. Materials and Methods :

Fishes for present investigation were collected from river Ganges at Chandpur, distt. Meerut (UP). Total 50 specimens were examined. All specimens were used to study seasonal variations. Mizelle's (1936 and 1938) freezing technique was employed for collecting parasites. Parasites thus collected, were processed for morphometric studies[5,6].

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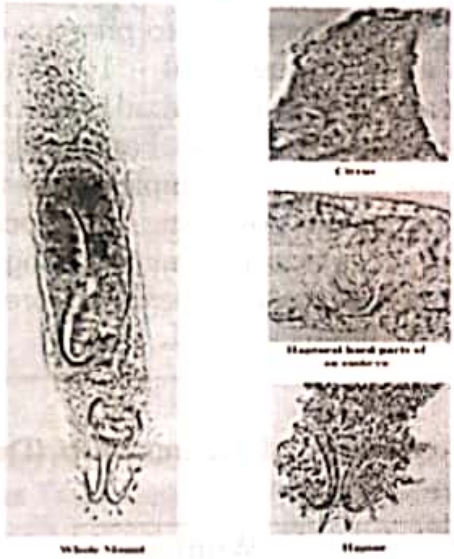
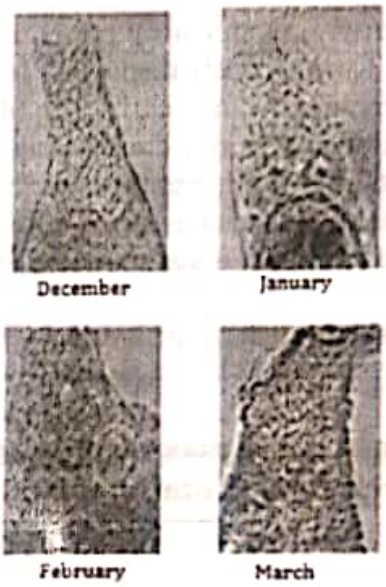
 ISSN 2349-2732

3. Morphometric Analysis :

Worms were washed thoroughly several times with chilled distilled water, to remove any mucous or debris adhering to the parasites. These worms were fixed in hot 4% neutral formaldehyde for at least 8 hours. Worms were washed thoroughly with distilled water. For study of hard parts worms were mounted in glycerol.



Photomicrographs of *Gyrodactylus aculeati* Malmberg, 1956



Photomcrograph of cirrus of *G. aculeati*, Malmberg, 1956

These worms were observed under microscopes and photographs of hard parts were taken. Parameters that were measured for morphometric analysis and study of seasonal variation as suggested by Mo (1991) include 13 morphometric parameters of the attachment apparatus and two of the copulatory organ[7]. Out of them copulatory organ's parameters are: length of cirrus tube, and width of cirrus tube. A total 50 specimens of *G. aculeati* were measured and photographed with help of calibration tool of Motic DMB1 Microscope. It was not always possible to measure all specimens because some prepared specimens were destroyed during the preparation or because with time had deteriorated. Sometimes copulatory organs had inconvenient position or were unsuccessfully compressed between the coverslip and the slide therefore, have not been recorded, all envisaged measurements. Unequal number of measurements and low number of specimens in autumn period was taken into account during statistical analysis. All data was processed using SPSS version 11 and a computer for calculating the mean and standard error. This data was also processed for probability coefficient and regression analysis. All results obtained were graphically presented. All the measurements are in microns (μ) in table 1. All temperature readings are in degree Celsius (°C).

4. Results :

Site of infection in host :Gills of *Labeo rohita* (Ham) No. of hosts examined : 20, No. of hosts found infected : 15, No. of total worms collected : 145-150. Locality : Chandpur distt. Meerut (UP) India . Worms are small and elliptical in shape measuring 210.12 - 223.93 μ in length with a distinct

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prohaptor and haptor. Minimum and maximum width of parasite is 35.72 - 58.47 µ and 27.47 - 69.31 µ (at position of anterior end and uterus respectively).Male reproductive organ comprises of testis, vas deferens, seminal vesicle and cirrus. Testis single, fusiform and post equatorial. A fine vas deferens arises from anterior border of testis. Vas deferens runs anteriorly, overlapping uterus and opens into seminal vesicle. It is situated near bifurcation of intestinal crura posterior to pharynx. Seminal vesicle opens at base of cirrus by a small duct, vasa efferentia. Male copulatory organ consists of cirrus pouch. Cirrus pouch is small and oval, situated posterior to pharynx at level of oesophagus. Cirrus pouch measures 16.15 – 24.90 µ in length and 6.04 – 14.05 µ in inner diameter. Armature of cirrus comprises of a single large conical spine with a broad base and a row of five spinelets arranged in a circle, while seventh spinelet lies outside circle. Female reproductive organ consists of ovarian complex, ootype complex and uterus. Ovarian complex consists of eight post-equatorial ovarian lobes, with diffused vitellaria, both pre-and post-testicular in location. An oval, pre-testicular, post-equatorial ootype complex and / or receptaculum seminis, having one large developing egg, is present anterior to ovarian lobes. Uterus is large and occupies entire pre-testicular intercaecal field. Length of cirrus during December to March is given in Table 1.

Table 1

Mean minimum and maximum length and width of cirrus of *G. aculeati* (December 2009 – March 2012) Total length (µ) Width (µ)

	Total length (µ)	Width (µ)
December	9.57, 11.12 (10.1)	6.53, 9.45 (7.72)
January	14.53, 15.01 (14.75)	9.01, 12.48 (10.66)
February	13.90, 15.80 (15.19)	7.58, 8.25 (7.98)
March	11.70, 12.01 (11.89)	7.00, 7.78 (7.32)

Table 2

Significant Pearson correlation of length and width of *G. aculeati*

Hard parts having bivariate correlation	*G. aculeati*
Total length of cirrus - width of cirrus	0.564

5. Correlation :

Descriptive Statistics

	Mean	Std. Deviation	N
VAR00001	12.9825	2.41519	4
VAR00002	8.4200	1.51781	4

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Correlations

		VAR00001	VAR00002
VAR00001	Pearson Correlation	1	.564
	Sig. (2-tailed)		.436
	Sum of Squares and Cross-products	17.499	6.207
	Covariance	5.833	2.069
	N	4	4
VAR00002	Pearson Correlation	.436	1
	Sig. (2-tailed)		.436
	Sum of Squares and Cross-products	6.207	6.911
	Covariance	2.069	2.304
	N	4	4

Table 3

Spss parameters and their outputs of G. aculeati (December 2009- March 2012), Output as Total length - Width of Cirrus (l-w) showing Regression values :

Variables Entered/Removed

Model	Variables Entered	Variables Removed	Method
1	VAR00002		Enter

a. All requested variables entered.
b. Dependent Variable : VAR00001

Model Summary

Mode	R	R Square	Adjusted R Square	Std. Error or the estimated	Square change	Change Statistics			
						F change	df1	df2	Sig. F Change
1	.564	.319	-.022	2.44174	.319	.935	1	2	.436

a. Predictors : (Constant), VAR00002
b. Dependent Variable VAR00001

ANOVA

Model		Sum of Square	df	Mean Square	F	Sig.
1.	Regression	5.575	1	5.575	.935	.436
	Residual	11.924	2	5.962		
	Total	**17.499**	**3**			

a. Predictors : (Constant), VAR00002
b. Dependent Variable VAR00001

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Coefficient Correlations

Model			
1.	Correlations	VAR00002	1.000
	Covariance	VAR00002	.863

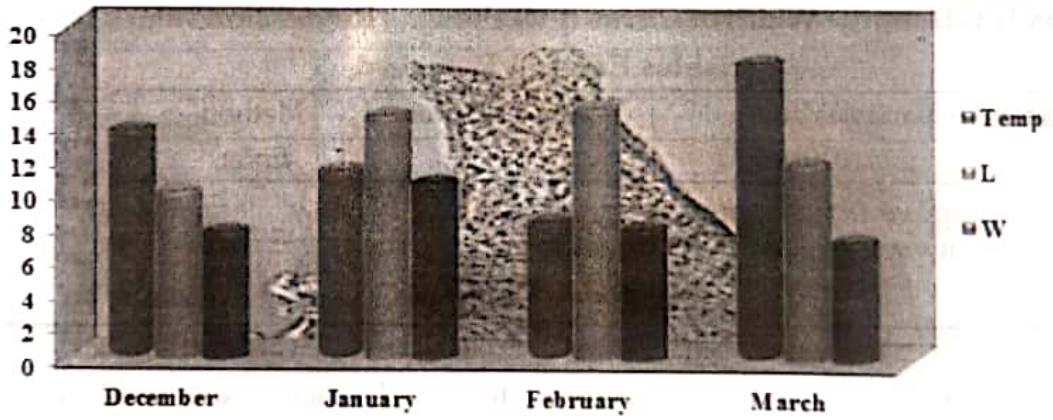
a. Dependent Variable : VAR00001

Table 4

Mean variations of cirrus of G. aculeati with temperature (Dec., 2009 - March 2012)

	Temp	Total length (µ)	Width (µ)
December	13.8	10.10	7.72
January	7.0	14.75	10.66
February	9.0	15.19	7.98
March	16.1	11.89	7.32

Line Graph 1: Mean variations in size of cirrus of G. aculeati with temperature (°C) from December 2009 – March 2012.



Graph 1: Mean variations in size of cirrus of *G. aculeati* with temperature (December 2009 – March 2012)

6. Discussion :

All characters (of cirrus) measured on each of the 20 samples were normally distributed. Copulatory organ has shown positive correlations. Length and width of cirrus is inversely related to water temperature. When temperature falls from January to February, length of cirrus shows slight increase. While, as temperature doubles in March length of cirrus decreases significantly. During present investigation worms were obtained from hosts *viz. Labeo rohita* and *Carassius auratus*. Worms at disposal of author exhibit variations in measurements of cirrus and various haptoral hard parts. These variations in measurements are due to increase or decrease in water temperature during summer and winter respectively. Table 1 shows variations in size of cirrus (minimum and maximum length) of *G. aculeati (Malmberg, 1956)*[1]. Cirrus of *G. aculeati* is oval in outline. Its length and width

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are inversely related to water temperature. As water temperature decreases from December to January length and width of cirrus increases. There is a slight rise in temperature during February length of cirrus increases slightly with a decrease in width. As temperature rises further in March length and width of cirrus decreases considerably.

Thus, cirrus appears more elliptical in December and January. While in February and March it appears a bit stout (more broad). Cirrus appears broadest in February.

7. Pearson correlations :

Pearson correlations were used to determine if hard armature of *G. aculeati* is significantly correlated or not. On the basis of these spss outputs the authors have drawn data Table 2 showing correlation between variables. Results of data obtained are significantly correlated to each other. Pearson correlations were used to determine if the hard armature of *G. aculeati* is significantly correlated or not. In case of total length and width of cirrus there is no significant correlation, r = 0.564. Results of this data obtained were significantly correlated to each other. Table 3 shows all spss outputs of cirrus in *G. aculeati*. In case of length - width of cirrus there is positive correlation in January and March, r = 0.789, r = 0.891 respectively. Obtained correlation value is significant at 0.05 and 0.01 level respectively. In February Length of cirrus with width shows negative correlation. r = -0.384.

8. Regression analysis :

Values of regression analysis of *G. aculeati* helps to find out a linear mathematical relationship between two variables or characters of hard parts of cirrus. Length of cirrus has chosen as independent variable because they can be measured easily. And total width of cirrus is dependent variables whose values have to be determined.

9. Line graphs :

The line graph of cirrus were obtained and represented in Graph 1 of three months. Length and width of cirrus is inversely related to water temperature. When temperature falls from January to February by approximately 1.5°C, length of cirrus shows a slight increase. While, as temperature doubles in March length of cirrus decreases significantly. Most line graphs show a wide range of variations in size and shape of cirrus and haptoral hard parts of *G. aculeati* with temperature (°C).

10. Histogram :

The graphical representation of different hard parts were obtained and represented in form of Table 4- ; Graph 1.Histogram diagram shows a wide range of variations in size and shape of cirrus of *G. aculeati* with temperature (°C). It is found that total length and width of cirrus have changed. In December temperature is higher as compared to January the width of cirrus is less. Width of cirrus increases in January when temperature falls by approximately 6° - 7°C. Width of cirrus is lower in February while the temperature is low. Further width decreases in March showing that it is independent on temperature. Length of cirrus increases in January as temperature falls. However despite a slight increase in temperature in February length of cirrus increases. It decreases with a rise in temperature in March.

Thus, length and width of cirrus is more or less temperature dependant.

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The author agrees with Mo (1991)[8] who suggested that when temperature increased during spring, size of hard parts decreased rapidly. The drop in water temperature during autumn did not cause a corresponding rapid increase in size. The size however, gradually increased during cold period of year. Kulemina (1977)[3] made similar observations on species of *Gyrodactylus* from the skin of crucial carp *Carassius carassius* (L.) and gold fish *Carassius auratus* (L.).
She stated that phenomenon could depend upon the curtailment of embryogenesis in uterus at higher water temperature and subsequent production of new born individuals with functional, although not yet fully developed hard parts (Ergens 1965 and 1965)[1,2]. Furthermore, a rapid increase in water temperature could increase the mortality among old specimens, resulting in a rapid replacement of old specimens by young ones. Inversely, a decrease in water temperature would prolong embryogenesis in uterus, resulting in new born individuals with more completely developed hard parts. Furthermore, a lower temperature would prolong the life span, resulting in a slow replacement of old specimens by young specimens.

11. Conclusion :

From this study it is concluded that anchors show a slim and long appearance in colder months of January and February. Whereas, in warm spring month, anchors appear short, stout and curved. Dorsal transverse bar also appears long and slender in colder months and short and stout in warm month. Parasites belonging to different genera also differ in their hard parts. Present study also describes that size of copulatory hard parts (cirrus) of a parasite depend upon seasonal temperature. These seasonal variations increase our knowledge and are very important for improving species descriptions. It helps in resolving some disparity in taxonomy of monogeneans. This study concluded that correlations between hard parts of haptor cannot be used as characteristic feature to differentiate between two genera. In addition regression analysis is carried out to find out a mathematical linear relationship between two variables (hard parts). One variable is independent variable i.e. its value is known. Other variable is dependent i.e. whose value is to be determined. Obtained equations help to find out unknown values of different hard parts of haptor with known value. Results from present statistical study of correlation and regression analysis indicate towards common purpose of degree and direction of relationship between different hard parts of cirrus and haptor. And it also indicates to differentiate among monogeneans genera.

12. Acknowledgments :

The authors are thankful to the Head Department of Zoology, Meerut College, Meerut for providing laboratory facilities.

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VOL. 04 MAY, 2017
ISSN 2349-2732

GENETIC VARIABILITY INDUCED BY HZ AND MMS IN CHICKPEA

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Abstract

Chick pea production has immensely expanded in the last decade due to its diverse utility in crude, cooked and processed forms. The variability in seed quality and crop yield is less due to self-fertilized nature of corp. Attention has been paid for the advancement of genotypes having higher yield and wide adaptability. The present research has been conducted to study the comparative effects of HZ, a base analogue and MMS, an alkylating agent for the improvement of yield and yield attributing traits of chick pea. Dry and healthy seeds of chickpea were treated with different doses of chemical mutagens. Results showed variation in the mean values of bio-physiological parameters in M_1 and quantitative traits in M_2 generation. Different morphological variations such as bushy, tall and dwarf as well as chlorophyll variants were also observed. Tall mutants were found to be in highest frequency followed by dwarf and bushy mutants.

Keywords : Genetic variability, Hydrazine hydrate (HZ), Methyl methane sulphonate (MMS), Chick pea

1. Introduction :

Like many leguminous crops, pulses play a key role in crop rotation due to their ability to fix nitrogen. The term "pulse", as used by the "food and agricultural organization", is reserved for crops harvested for the dry seed. India is world's largest producer and consumer of pulses. Chickpea, a pulse crop, commonly known as gram is cultivated as a winter crop in India particularly in North India. Chickpea is considered to have originated in the tract lying between the caucuses and the Himalayas from where it spread into south Europe, Iran, Egypt and India. There are two main groups of chickpea Desi (wrinkled seeded) which constitute about 85 percent of the total production and Kabuli (round seeded) which form the remaining 15 percent of the seed produced. The diploid number of chromosome in chickpea, in desi type with small brown seed is 14, while in the case of Kabuli type with large white seed, it is 16. Average yield in India over the past two decades have fluctuated between 550 and 650 kg/hectares.*Chickpea (Cicer arietinum L.), is an important source of dietary proteins, is an autogamous crop with natural cross pollination ranging between 0–1 %, therefore, there is a lack of sufficient natural variation. Induce mutation is efficient tool to create new genetic variability in chickpea.* Considering the economic importance and the limited work on induced mutagenesis, the present study was undertaken to assess the comparative response of chemical mutagens viz., Hydrazine hydrate (HZ) and Methyl methane sulphonate (MMS) on chickpea variety.

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 ISSN 2349-2732

2. Materials and methods :

Seeds of chickpea variety Avrodhi were treated with four concentrations (0.01,0.02,0.03 and 0.04%) of HZ and (0.02,0.04,0.06 and 0.08%) of MMS to raise M_1 generation. Three replications of 10 seeds were sown for each treatment of mutagens and control in 11 inch earthen pots filled with soil manure. The pots were kept in the net house of the Department of Botany, Aligarh Muslim University, Aligarh. 20 seeds were grown on moist cotton in petri plates in each treatment and control to determine percentage of seed germination and the seedling height i.e., root and shoot lengths. M_1 plants were harvested individually and their seeds were sown in next crop season (2015-16) to raise M_2 generation. Data on quantitative traits were collected from M_2 population.

3. Experimental results :

The mutagenic effects of HZ andMMS were studied on biological parameters (seed germination, seedling height) in M_1 and morphological changes in leaves, plants and certain quantitative traits ofchickpea in M_2 generation.

4. Biological damages :

Germination started second day after sowing in control and in the mutagen treated population of chickpea. The lower concentrations of both the mutagen viz. HZ and MMS enhanced the seed germination, whereas the higher concentrations decreased the seed germination. In the control, seed germination was recorded 100%. The percentage seed germination was 90% and 30% at 0.01% and 0.04% of HZ treatment, respectively. It was 90% (0.02%) and 60% (0.08%) of MMS treated plants (Table 1; Plate I). The height of seedlings in petri plates, measured after 11 days of sowing, showed that with the increasing concentrations of both the mutagens, the seedling height decreased considerably. Higher biological damages were observed in HZ treated plants(40%) as compared to MMS treated plants.

5. Morphological mutants :

Different morphological mutants (bushy plant type, leaf shape, chlorophyll) were observed. MMS produced more such mutants than HZ treatments. Bushy mutants in both HZ and MMS treated population in M_2 generation(Plate II) may be used in hybridization programme to develop short stature varieties of chickpea.

6. Quantitative traits :

In MMS treated plants the plant height increased with the increasing concentrations of mutagen (50.9-57cm) as compared to the control (38.6cm), while in HZ treated plants, the plant height showed irregularities in height (Table II). The height was maximum 57cm at 0.06% MMS and it was maximum (54.1cm) at 0.02% HZ treatment. However dwarf mutants were isolated at higher concentrations of both the mutagens (Table II; Plate III). Numbers of pods per plant were counted at the time of plants maturity. In control the mean number of pods per plant were observed as 3.7, while in MMS treated plants the number of pods per plant ranged from 3.4-4.3, the maximum number of pods per plant were observed in 0.02% concentration (4.3), while in HZ treated plants the number of pods per plant ranged between 2.9-5.4, the maximum number of pods were observed in 0.02% concentration (5.4) (Table II).Weight of 50 seeds per plant was taken at the time of harvesting of plants. In control the average weight of 50 seeds per plant was 14.77 g, while in MMS treated plants the seed weight ranged between 13.30-14.19g, the maximum seed weight per plant was observed in 0.04% MMS concentration (14.19g), while in HZ treated plants the seed weight ranged between 12.47-14.45g, the maximum seed weight per plant was observed in 0.02% concentration (14.45g) (Table II).

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7. Discussion

In plant breeding programmes, generally the criteria such as germination, seedling injury, sterility and chromosomal aberrations are used to assess the effectiveness of mutagen. Mutations affecting growth habit, flower colour and plant type have been reported earlier (Ahmad and Godward 1993, Kharkwal 1999, Gaur and Gour 2001 and Khursheed and Khan 2015)[1,10,6,11]. Data showed that seed germination was reduced with the increasing concentration of both the mutagens. Reduction in seed germination has been explained due to delay or inhibition in physiological and biological processes necessary for seed germination which also include hormonal imbalance (Ananthaswamy *et al.*, 1971), imbibitional chilling injury and consequent infection by soil borne pathogens (Chen *et al.*, 1983, Balasubramanian *et al.*, 1998)[3,5,4]. Seedling height was also affected adversely at different doses of both the mutagens used. Stimulatory effect of lower concentrations on plant height was noticed but higher concentrations of the mutagens produced more dwarf plants. Occurrence of dwarf mutants in chickpea was also reported by khan *et al.*, (2011)[9]. Dwarfism may be due to reduced internode length or internode number or both (Sjodin 1971)[14]. In this study reduction in internode length was mainly responsible for dwarfness. Results showed that the plants were more sensitive to the MMS mutagen than HZ. Bushy plant type with excessive branching, increased number of flowers and narrow leaves were noticed. In chickpea, erect or bushy growth habit is a characteristic controlled by oligogene where erectness is dominant and bushy is recessive (Khan *et al.*, 2011)[9]. Variation in leaf morphology, chlorophyll and other yield attributing traits, number of pods per plant were observed after mutagen treatment. The increase in yield contributing characters like pods per plant was reported in *Vicia faba* (Ismail *et al.*, 1977), *Cicer arietinum* (Sharma *et al.*, 1990 and Raina *et al.*, 2017)[7,13,12], while the decrease in the number of pods was reported in *Vicia faba* (Amer and Farah 1976) by carbamate pesticide. Khan (1990) is of the opinion that various chromosomal abnormalities are related to pollen fertility and ultimately the seed set[2,5]. These reports show that chemical mutagenesis is a potential tool to be employed for crop improvement.

Table 01

Seed germination and seedling height (cm) in mutagens treated population of chickpea.

Treatment	Germination (%)	Inhibition (%)	Seedling height (cm)		Total (cm)
			Root length	Shoot length	
Control	100	---	8.0	8.0	16.0
0.01% HZ	90	10	2.0	6.4	8.4
0.02% MMS	90	10	6.6	6.3	12.9
0.02% HZ	70	30	1.7	0.5	2.2
0.04% MMS	80	20	6.0	8.9	14.9
0.03% HZ	60	40	1.0	---	1.0
0.06% MMS	70	30	5.5	8.6	14.1
0.04% HZ	30	70	1.5	---	1.5
0.08% MMS	60	40	0.4	0.6	1.0

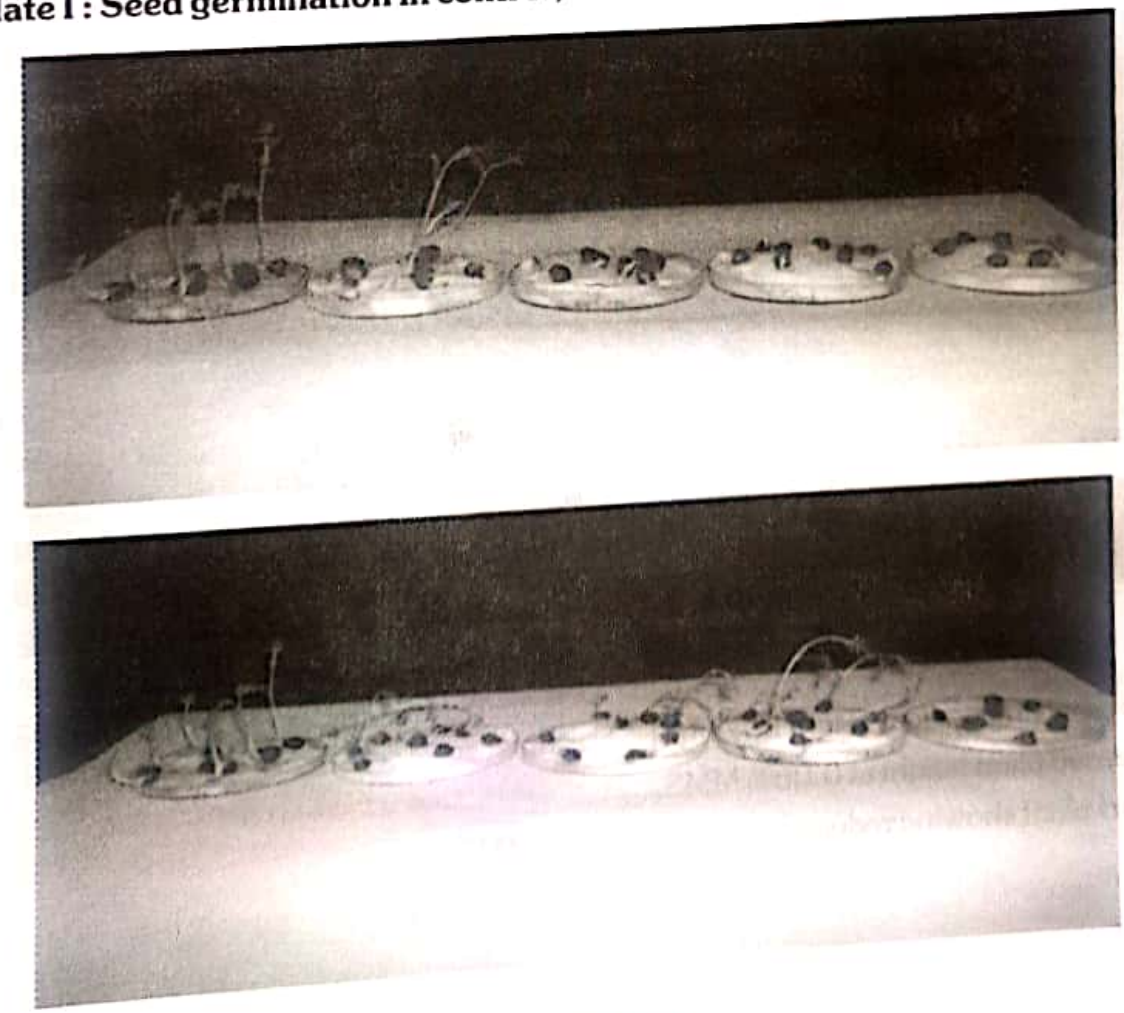
ISSN 2349-2732

Table 2

Estimates of Mean values and coefficient of variation (C.V. %) for quantitative traits in M_2 generation of chickpea.

Treatment	Plant height (cm)		Pods per plant		50 seed weight (g)	
	$\bar{X}$ ± S.E.	C.V. (%)	$\bar{X}$ ± S.E.	C.V. (%)	$\bar{X}$ ±S.E.	C.V. (%)
Control	38.6±0.92	7.59	3.7 ±0.14	38.91	14.77±0.11	2.46
0.01% HZ	47.1±1.22	8.23	2.9±0.30	33.17	10.55±0.11	3.30
0.02% MMS	50.9±5.21	7.97	4.3±0.42	30.93	14.45±0.09	2.94
0.02% HZ	54.1±0.63	3.73	5.4±0.55	32.59	14.45±0.09	2.94
0.04% MMS	54.2±1.00	5.86	3.4±0.34	32.35	13.55±0.30	7.15
0.03% HZ	36.2±0.75	4.14	4.4±0.44	31.81	12.47±0.12	3.27
0.06% MMS	57.0±0.97	5.36	3.6±0.48	42.50	14.19±0.12	2.86
0.04% HZ	31.4±0.42	3.51	5.2±0.40	24.80	13.77±0.41	9.44
0.08% MMS	29.3±0.84	4.91	4.1±0.62	48.00	13.81±0.10	2.47

Plate I : Seed germination in control, HZ and MMS treatments of chickpea.



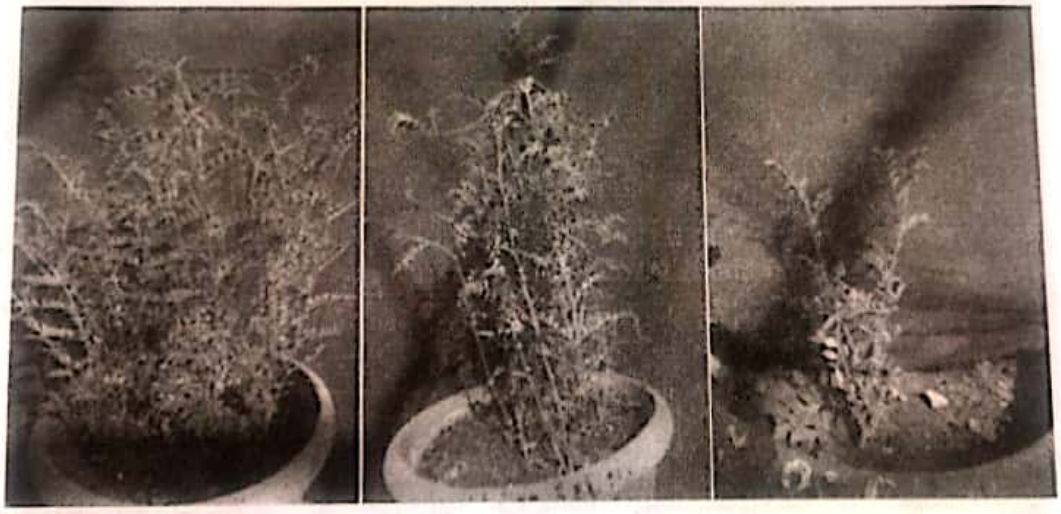
 ISSN 2349-2732

Plate II : Morphological mutants in mutagen treated population of chickpea.

a b c d

a) Leaf size in control and 0.08% MMS-showingbroad leaflets.
b) Leaf size in control and 0.04% of HZ-showing narrow leaflets.
c) Chlorina mutant at higher concentration of mutagens.
d) Bushy plant type with excessive branching and increased number of flowers at 0.08% MMS.

Plate III : Plant height mutants of chickpea.



a b c

a) Plant showing normal height in control.
b) Increased plant height at 0.06% MMS.
c) Dwarf plant showing reduced growth at 0.08% MMS.

 ISSN 2349-2732

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GYAN VIGYAN : JOURNAL OF SCIENCE
VOL. 04 MAY, 2017 ISSN 2349-2732

Assessment of Morphological and Quantitative Variability in Linseed Induced by Caffeine and MMS

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Abstract

The availability of accessible genetic variability is highly important for initiating crop improvement programme and the induced mutagenesis is considered to be the most effective technique for induction of genetic variability. In the present study, the assessments were made on the biological damages, morphological variations in M_1 generation and quantitative traits in M_2 generation of linseed to determine mutagenic potency of chemical mutagens viz. Caffeine and MMS (Methylmethane sulphonate). The seeds of *Linum usitatissimum* L were treated with five different concentrations (0.1%, 0.25%, 0.50%, 0.75%, and 1.0%) of Caffiene and MMS. The indexes of biological damages showed linear increase with increasing concentration of both the mutagens, while MMS was found to be more damaging compared to caffeine in the respective concentrations. Morphological variations affecting different agro-morphological parts of plant were induced and documented. Statistics of different quantitative traits revealed that useful variations on the yield attributing traits were induced by lower concentrations of mutagens, while caffeine was relatively more efficient in inducing yield traits then MMMS. Overall, the lower doses of caffeine and MMS were found suitable for inducing wide array of desirable mutations for genetic improvement of linseed.

Key Words : *Linum usitatissimum* L., Caffeine, MMS, genetic variability, quantitative traits.

1. Introduction :

Linum usitatissimum L, 2n=30 (Muravenko *et. al.*, 2003) is a member of family linaceae commonly known as flax, linseed[10]. The genome size of cultivated flax is 686 Mbp (Bennett and Leitch, 2004)[3]. The genus linum is a large group with ~230 species (Heywood, 1993)[6]. The origin of flax leis in southern Europe, the near East, or central Asia (Helback, 1959)[5] It is native to Mediterranean region or south western Asia. All species of linum are self-pollinated (Zohary and Hopf, 1993) and cross-pollination occurs via, honey bees (Williams, 1988) or by artificially[21,20]. Linseed occupies a greater importance among oilseeds. Flax seed is a good source of dietary fiber and omega-3 fatty acids. The fiber in flaxseed is found primarily in the seed coat. Flax fibers are used to make linen. Overall, flaxseed's effects on cholesterol and blood clotting may lower the risk of "hardening of the arteries"(atherosclerosis). In flaxseed a chemicals called "lignans" are found which is similar to female hormone estrogens. Many researchers believe that lignans helps to slow down the progress of certain breast cancers and other types of cancers that need estrogens to develop well. Linseed oil was used to preserve the bodies of deceased pharaohs (Dewilde, 1983)[4]. Linseed oil has

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 ISSN 2349-2732

high linolenic fatty acid content (45-60%) which is used to making dry agent. Although the oil is edible, it is used primarily for industrial purposes, such as the production of paints and oil-based coverings and the manufacture of linoleum flooring (Rowland, 1998)[11]. In most of the agricultural crops commercial breeding of flax started at the end of 19th century. From mutation breeding we can induce desirable characters as our wish. Nowadays, several breeding methods are available for flax breeding, but the pedigree method is the most common one used in flax breeding (Salas and Friedt, 1995)[12]. Caffeine is a purine 1, 3, 7 tri methyl xanthine. Because of purine nature it has a mutagenic potential for plant and animals. It has been observed that caffeine also has the ability to act synergistically in inducing chromosomal aberrations in plant cells. Caffeine has remarkable possibilities of improving plants with regard to their quantitative and qualitative characters and mutagenic potential of caffeine has been tasted in several plants by many workers (Anis and Wani, 1997, Kumar and Tripathi.,2004)[13]. MMS (methyl methane sulphonate) is a mutagenic compound ($C_2H_6O_3S$). It produces random mutation in genetic material by nucleotide substitution particularly guanine alkylation. In alkylating groups, MMS has been found to be very effective chemical mutagens like any other alkylating agent. The methyl group of MMS reacts with guanine in DNA forming abnormal base which cause mutation.

2. Materials and methods :

The seeds of *linum usitatissimum* L, are obtained from NBPGR (National Bureau of Plant Genetic Resource) new Delhi, India. Fresh, dried, and healthy seeds of *linum usitatissimum* L, were pre-soaked in double distilled water for 12 hrs at room temperature (25±2). After pre-soaking, the seeds of *linum usitatissimum* L, were treated with five different concentrations (0.1%, 0.25%, 0.50%, 0.75% and 1.0%) of Caffeine and MMS (Methyl methane sulphonate) for 24 hrs. The treated seeds were thoroughly washed with running tap water for 45 minutes to remove the residual effects of mutagens sticking to the seed coat before sowing. One set of seeds was kept as control for comparison which was untreated. In all set of seeds, taking 50 seeds (including control which was no treated to any chemical mutagens) was sown in pots. Mutagens treated seeds were sown in pots to raise M_1 generation. Seeds of M_1 plants harvested treatment wise and mixed together. From each treatment 30 seeds were selected randomly and sown in pots to raise M_2 generation. Three replications were made for each treatment. Data on quantitative traits were collected on 15-20 plants.

3. Results :

The present work was undertaken to assess the mutagenic effect of Caffeine and MMS on seed germination, plant survival, plant height, pollen fertility and various quantitative characters in *Linum usitatisimum* L. These results are observed on the basis of Morphological parameters.

4. Biological damages

Germination rate are maximum (90%) in control plant. It decreased with increasing concentrations in both the mutagens i.e. from 86.25-66.23% and 84.59- 66.35% in 0.10-1.0% caffeine and MMS respectively (Table-1; Figure-1). In general, germination was affected in all the treatments in both the mutagens; however, MMS was more effective than caffeine. Inhibition in germination was found to be increase from 4.16- 26.41% and 6.01-26.27% in 0.10-1.0%, [caffeine and MMS] respectively with the simultaneous decrease in germination (Table-1; Figure-1). The survival of seedlings decreased with an increase in dose/concentration in almost all the mutagenic

ISSN 2349-2732

treatments. The survival of plants at maturity was 83.33% in control, while decreased from 86.25-59.92% in caffeine and from 80.12- 57.49% in MMS (Table-1; Figure-1). The average pollen fertility was 86.42% in control, while it decreased from 84.59-59.54% and 79.35-57.97% in 0.10%-1.0% caffeine and MMS respectively (Table-1; Figure-1).

5. Morphological studies :

Various type of morphological variants with altered characters i.e.,leaf variation, plant height, growth habit (bushy and dwarf) and yield parameters were isolated in *Linum usitassimum* L. of both the mutagens. Notched and kidney shaped leaves are observed in 0.50% caffiene mutagens. Unequal cotyledonary leaves, one large and another one is small and broad in 0.75% MMS and Notched, curved and spatulate leaves in 0.50% MMS are observed. Average height of mature plants reduced with increasing concentration of both the mutagens except in lower concentrations where plant height was insignificantly increased over control. The plant survival decreased with the increasing concentration of both mutagens. These variation included changes in plant height (tall, dwarf), growth habit (bushy), yield parameters (increased number of branches and pods per plant).

6. Quantitative traits :

In M_2 generation, the effect of Caffeine and MMS treatments was studied on various quantitative characters viz., plant height (cm), number of branches per plant, number of capsule per plant, number of seeds per capsule, 100-seed weight (g), total yield per plant (g), in *Linum usitatissimum L.* are summarized in the Tables-2.The average plant height in control population was 54.1 cm (maximum), whereas in caffeine it first increased 57.65 to 55.39 in (0.1-0.25%) concentration and then decreased from 53.71 to 50.45 cm in (0.50-1.0%) concentration while in MMS plant height decreased from 53.41 to 48.13 cm in (0.1-1.0%) respectively. The coefficient of variations were high than the control (Table- 2).The average number of branches per plant was 3.50, while it increase 3.99-3.52 in 0.1-0.75% caffeine and decrease 2.93 at 1.0%, whereas in MMS it decreased 3.35-2.63 from 0.1-1.0% concentration respectively. The coefficient of variations were high than the control (Table- 2).The average number of pods per plant in control population was 36.62 (maximum), while in caffeine it first increased 41.2 to 38.28 from 0.1-0.50% concentration and then decreased from 36.53 to 35.25 in (0.75-1.0%) concentration, whereas in MMS it decreased from 35.39 to 32.15 in 0.1-1.0% concentration respectively, (Table- 2), while the coefficient of variation increased in all concentration than the control. The average number of seeds per pod in control population was 7.9 (maximum), firstly increased 8.23 to 8.00 in caffeine in 0.1-0.25%, while decreased from 7.7 to 6.9 in 0.50%-1.0% caffeine, whereas in MMS it decreased from 7.86 to 6.9 in (0.1-1.0%) concentration respectively, (Table- 2), while the coefficient of variation increased in all concentration than the control. The average 100- seeds weight (g) in control population was 0.606 (maximum), firstly increased 0.704 to 0.626 in caffeine in 0.1-0.50%, while decreased from 0.591 to 0.574 in 0.75% to 1.0%, whereas in MMS it decreased from 0.585 to 0.492 in (0.1-1.0%) concentration respectively, (Table- 2), coefficient of variation continuous increase according to dose than control. The total seed yield (g) in control population was 16.23 (maximum), first increased at 0.10% (17.09), while decreased from 14.25 to 9.92 in 0.25%-1.0% caffeine, whereas in MMS it decreased from 15.0 to11.60 in (0.1-1.0%) concentration respectively, (Table- 2).

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7. Discussion :

Mutation breeding technique is the best method to enlarge the genetically conditioned variability of a species within a short duration of time and has played a significant role in the development of many crop varieties. In the present investigation seed germination, plant survival and pollen fertility decreased with the increased in the mutagenic treatments. These results are in confirmation with (Wani *et al.*, 2004) in lentil, (Banu *et al.*, 2004) in cowpea, (Kumar, 2005) in *Coriandrum sativum* and (Shahwar *et al.*, 2016) in *Vicia faba*[19,2,8,13]. In general, caffiene treatment was found to more effective than MMS treatment in *Linum usitatissimum* L. The adverse effects of physical and chemical mutagens on various biological parameters have been reported by many workers (Jafri *et al.*, 2011)[7]. Most of these workers have observed the dose dependent reduction in the above mentioned biological parameters. Decrease in seedling survival may be attributed to the series of events occurring to the cellular level which affects the macromolecules and bring about a physiological imbalance in the cells as a consequence of exposure to ionizing radiations and chemical mutagens. Varying degree of pollen sterility was induced in all mutagenic treatments in the present investigation.Very high pollen sterility was recorded at the higher doses of treatments. These results are in agreement with many workers who also reported a dose dependent pollen sterility following mutagenic treatments (Jafri *et al.*, 2011)[7].

The present investigation proved fruitful in inducing a range of morphological variations by mutagenesis. Although most of them proved uneconomical but some variations recorded in present in the present study may be used as a source of many beneficial genes in cross breeding programmed or for improvement of many components of yield. In the present study, the morphological variations affecting different parts of the plants were isolated by careful screening of M_1 population. The frequency of these morphological variations differed among different mutagenic treatments. These results are contradictory with earlier reports of Wani and Anis (2008) in *Cicer arietinum*. Differences in the frequency of various morphological mutations have been reported in lentil (Solanki and Sharma, 1999) and (Wani and Anis, 2008) in *Cicer aritenum*[15,18]. The tall, dwarf and bushy variants as noticed in the present investigation have also been reported by Solanki and Sharma (2003) in lentil and Singh *et al.* (2004) urd bean following mutagenic treatments[16,14].

The mutagenic effects of various doses/ concentrations of caffiene and MMS was studied on plant height (cm), number of branches/plant, number of leaves/plant, number of capsule/plant, seed weight (g) total yield/plant (g) . Assessment of mean, S.D., coefficient of variation (CV %) in control and treated population indicated that mutagenic treatment had induced wider magnitude of variability in M_2 generation for all the quantitative characters. Plant height decreased considerably in the treated population similar findings in this respect has been reported by (Banu *et al.*, 2004) reduction in the plant height may be due the chromosomal damage and/or inhibition of cell division (Sparrow *and Even*, 1961)[17]. Therefore, reduction in plant height might have occurred due to inhibitory effect of mutagens on growth regulating substances responsible for cell division and elongation. In the present investigation quantitative characters like mean number of branches /plant, number of leaves/plants, number of pods/plant, 100 seed weight (g) and total yield/plant showed significant increase in mean values at the lower concentrations of the mutagens, while higher concentration showed inhibitory effect in the present investigation increase in mean number of leave/plant had positive co relation with in number of pods/plants. In the same way the mean length

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of pods had positive correlation with 100 seed weight and consequently the pods per plant. These traits play an important role in boosting the yield of a plant to a considerable extent. Relation of yield with other quantitative characters has also been studied. The possible cause of increased values of these yield contributing traits in the present study may be due to some use full gene mutations. In brief the present finding have revealed that lower and moderate concentration of chemical mutagens proved to be efficient in increasing the genetic variability for yield oriented selection in *Linum* and isolated variant possess desirable characters associated with higher yield, may be evaluated in future generations for promising performance. Thus the genetic variability induced by mutagenesis may be effectively exploited for the improvement of *Linum usitatissimum* L.

8. Conclusion :

It is concluded that the genotype of *Linum usiitatissimum* L. can be improved by using lower doses of MMS and caffeine, as it is quite clear from results of present investigation that lower doses showed effective shift in the mean in desired direction.

9. Acknowledgments :

The authors are thankful to chairman, Department of Botany, Aligarh Muslim University, Aligarh for providing all the facilities for completing the work.

Figure 1. Graph showing effects of Caffiene and MMS on germination, plant survival and pollen fertility of Linum usitatissimum L. in M_1 generation.

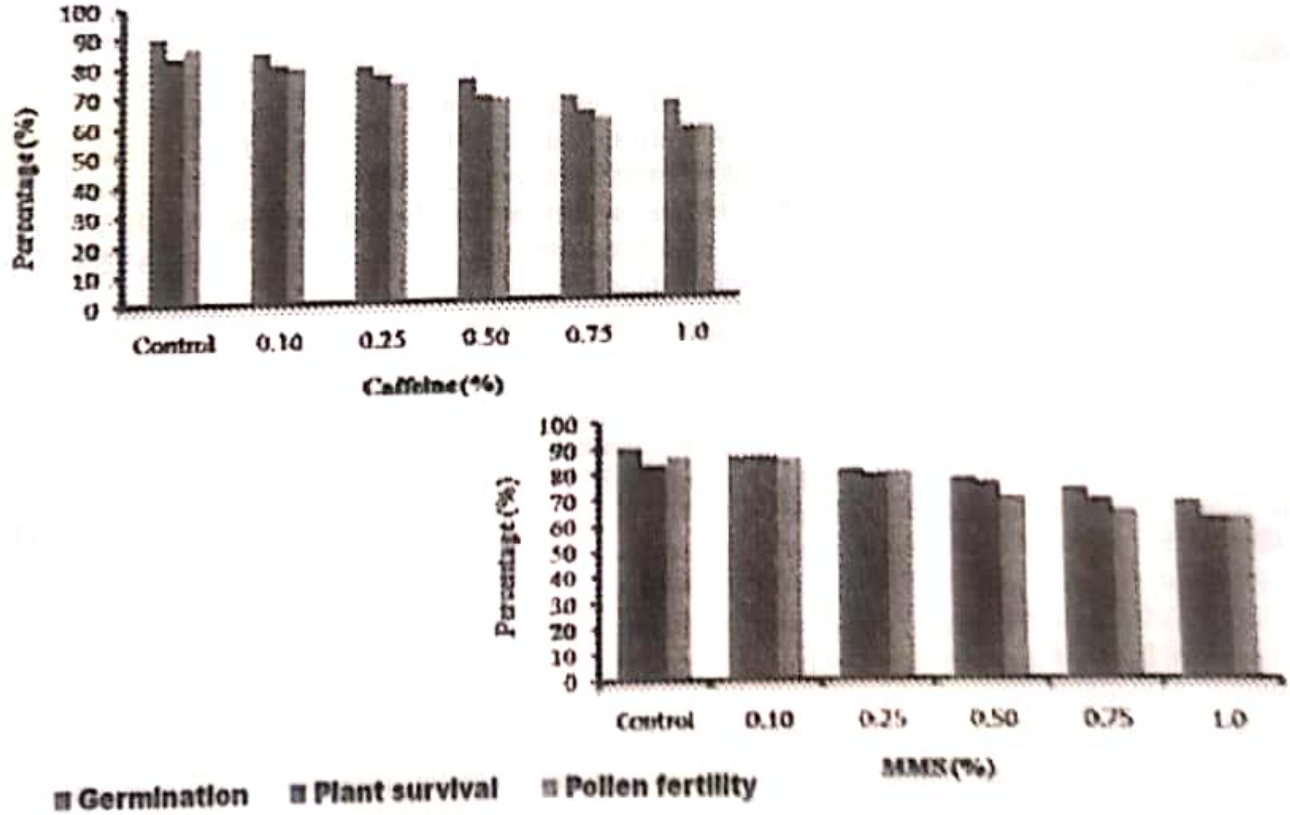

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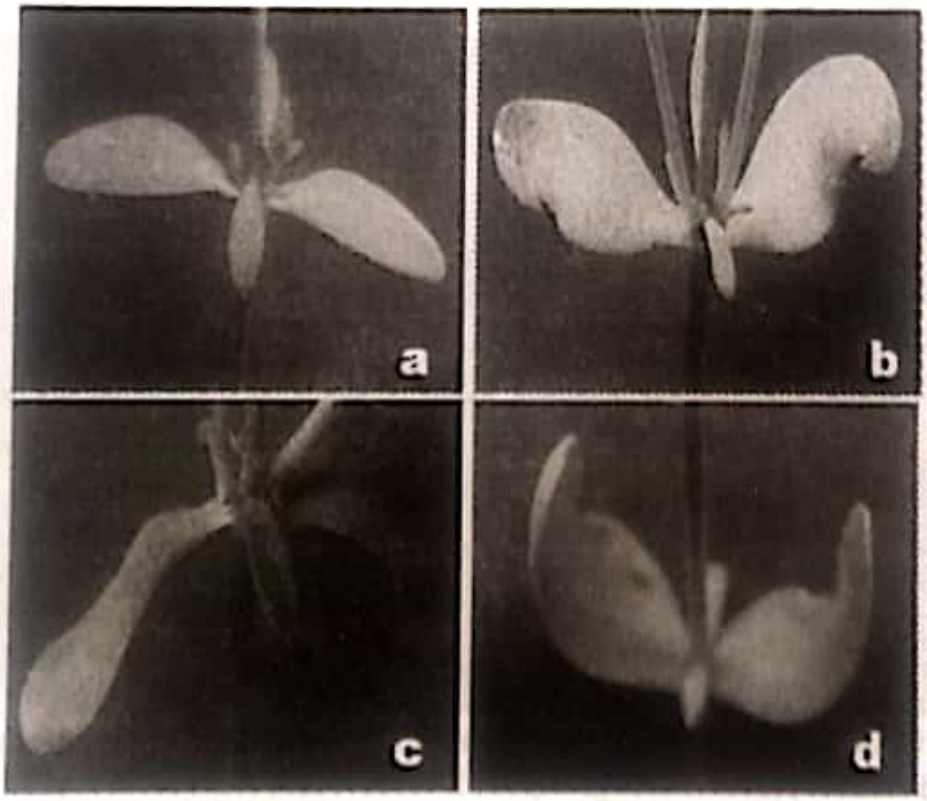
Table : 1

Effect of Caffeine & MMS on seed germination, plant survival and pollen fertility in *Linum usitatissimum* L. in M_1 generation.

Treatment	Germination (%)	Inhibition (%)	Plant survival (%)	Pollen fertility	Reduction (%)
Control	90.0	----	83.33	86.42	----
			Caffeine		
0.1%	86.25	4.16	86.25	84.59	2.11
0.25 %	80.39	10.67	78.46	78.75	8.87
0.50 %	76.19	15.34	74.47	68.58	20.64
0.75 %	71.45	20.61	67.73	63.11	26.97
1.0%	66.23	26.41	59.92	59.54	31.16
			MMS		
0.1%	84.59	6.01	80.12	79.35	8.18
0.25%	80.00	11.11	76.58	73.59	14.84
0.50%	74.69	17.01	69.13	68.23	21.04
0.75%	68.66	23.71	63.54	61.56	28.78
1.0%	66.35	26.27	57.49	57.97	32.92

Figure 2 : Morphological variations showing altered leaf achetecture in *Linum usitatissimum* L.



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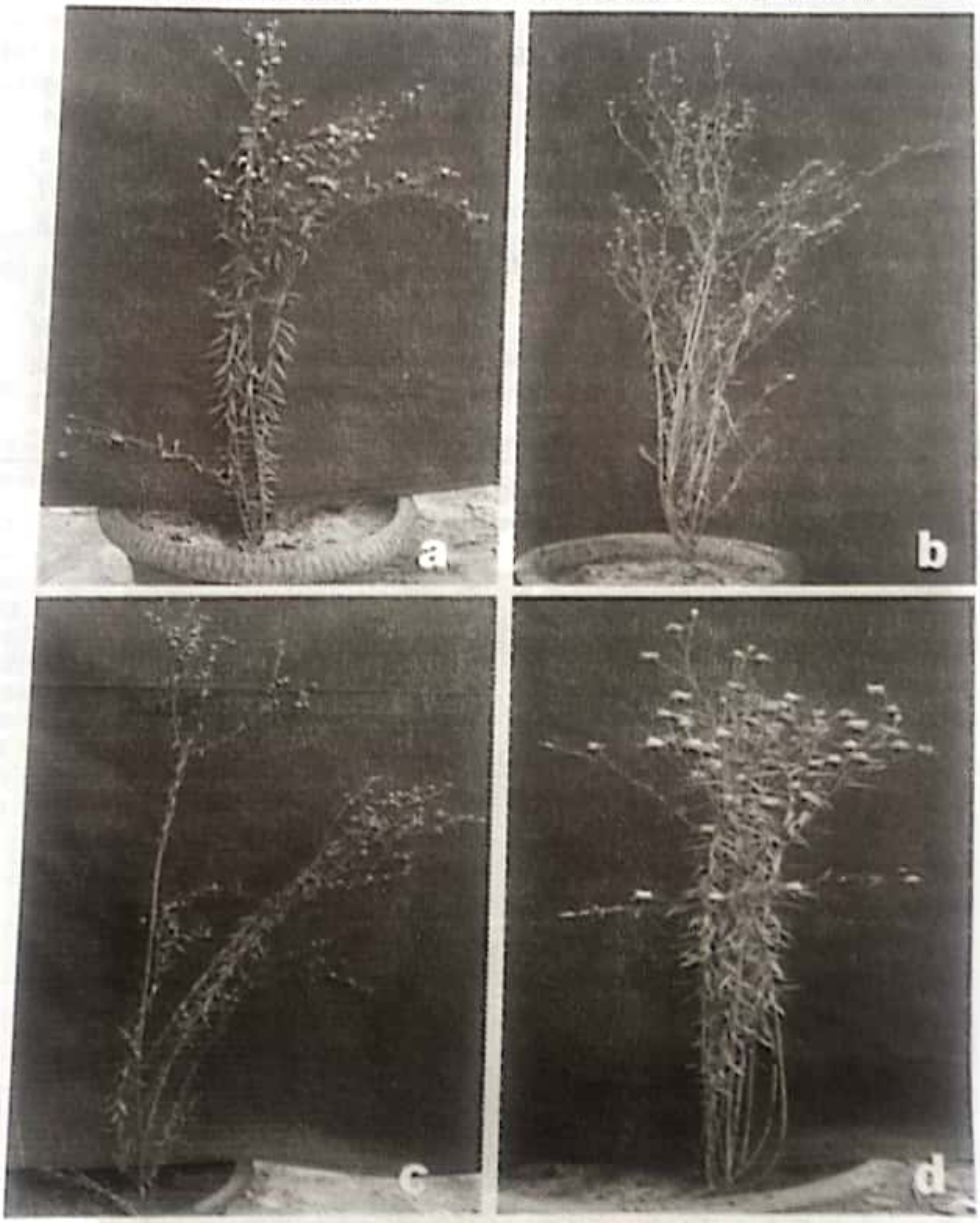
a. Control.
b. Notched and kidney shaped leaves in 0.50% caffiene.
c. Unequal cotyledonary leaves, one large and another one is small and broad in 0.75% MMS.
d. Notched, curved and spatulate leaves in 0.50% MMS.

Figure 3 : Altered growth habit in *Linum usitatissimum* L.



a. Control. **b.** High yielding with more branches in (0.50% MMS). **c.** Tall variant with high yield in (0.75% caffeine). **d.** Bushy plant with large capsule in (0.25% caffeine)

 ISSN 2349-2732

Table : 2

Effect of Caffeine and MMS on plant height, branches/plant, capsule/plant, seeds/capsule, weight of 100 seed and total yield/plant in *Linum usitatissimum* L. in M_2 generation.

Treatment		Plant height (cm) X̄ ± SD(CV)	Number of Branches Plant (cm) X̄ ± SD (CV)	Number of Capsule/plant X̄ ± SD (CV)	Number of Seeds/capsule X̄ ± SD (CV)	Weight of 100 Seeds (g) X̄ ± SD (CV)	Total yield/plant (g) X̄ ± SD (CV)
Caffeine	Control	54.1±5.2(39.66)	3.50±0.84 (24.00)	36.62±3.13(8.54)	7.9±1.13(14.30)	0.606±0.019(3.13)	16.23±0.65(4.00)
	0.1%	57.65±8.45(14.70)	3.99±0.74(8.54)	41.2±3.52(8.58)	8.23±1.19(14.45)	0.704±0.025(3.55)	17.09±0.69(4.03)
	0.25%	55.39±8.67(15.65)	3.80±0.75(19.73)	40.92±3.78(9.23)	8.00±1.23(15.37)	0.636±0.03(25.03)	14.25±0.72(5.05)
	0.50%	53.71±9.39(17.48)	3.71±0.79(21.29)	38.28±3.89(10.16)	7.7±1.31(16.88)	0.626±0.049(7.82)	12.09±0.76(6.28)
	0.75%	52.31±10.07(19.24)	3.52±0.81(23.01)	36.53±3.94(10.78)	7.2±1.39(19.30)	0.591±0.05(28.79)	10.17±0.79(7.76)
	1.0%	50.45±11.33(22.45)	2.93±0.83(28.32)	35.25±4.05(11.48)	6.9±1.42(20.57)	0.574±40.071(12.36)	9.92±0.83(8.36)
MMS	0.1%	53.41±8.95(16.75)	3.35±0.76(22.68)	35.39±3.67(10.25)	7.86±1.29(16.41)	0.585±0.020(3.41)	15.0±0.62(4.1)
	0.25%	52.15±9.21(17.66)	2.91±0.78(26.80)	34.33±3.86(11.24)	7.73±1.34(17.33)	0.572±0.023(4.02)	14.5±0.68(4.68)
	0.50%	50.39±9.86(19.54)	2.76±0.80(28.98)	33.51±3.91(11.81)	7.63±1.69(21.75)	0.562±0.054(9.89)	13.70±0.75(5.47)
	0.75%	49.82±10.72(21.15)	2.68±0.82(30.59)	33.02±3.99(12.08)	7.21±1.84(25.52)	0.546±0.066(12.08)	12.04±0.81(6.72)
	1.0%	48.13±11.49(23.87)	2.63±0.83(31.55)	32.15±4.25(13.21)	6.9±1.96(28.40)	0.492±0.073(14.83)	11.60±0.87(7.5)

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GYAN VIGYAN : JOURNAL OF SCIENCE
VOL. 04 MAY, 2017

ISSN 2349-2732

PDE based time dependent model for image restoration with free local constraints

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Abstract

A total variation based approach was developed by Rudin, Osher and Fatemi to over come the basic limitations algorithms. The L1 norm of total variation of the image is minimized subject to constraints involving the satatistics of the noise. In total variation image restoration Rudin and Osher, the solution is obtained by solving a time dependent nonlinear PDE on a manifold that satisfies the degradation constraints. In this paper, we propose a new time dependent model for solving total variation (TV) minimization problems in image deblurring and denoising. The main idea is to apply a priori smoothness on the solution image. 2D numerical experiment results by explicit numerical schemes are discussed.

Key words: Total variation norm, image restoration, Lagrange's multiplier

1. Introduction

The total variation (TV) deblurring and denoising models are based on a variational problem with constraints using the TV Norms as a nonlinear non differentiable functional. The formulation of these models was first given by Rudin, Osher and Fatemi for denoising case and Rudin and Osher for the denoising and deblurring case[8,9]. In spite of the fact that the variational problem is convex, the Euler-Lagrange's equation are non linear and ill-conditioned. Linear semi-implicit fixed point procedures devised by Vogel and Oman, and interior-point primal dual implicit quadratic methods by Chain, Golub and Mulet, were introduced to solve the models. Those models give good results when treating pure denoising problem, but the models become highly ill-conditioned for the deblurring and denoising case[3,13]

In this paper we present a new time dependent model constructed by evolving the Euler-Lagrange equations to the optimization problem. We propose to apply priori smoothness on the solution image and then denoise it by minimizing the total variation norm of the estimated solution. We have tested our algorithm on various types of signals and image and found our model (2.21) better than previously known model (2.20).

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ISSN 2349-2732

2. Total variation based restoration algorithms

Image restoration is a fundamental problem in both image processing and computer vision with numerous application. Given a blurry and noisy image $u_0 : \Omega \rightarrow \mathbf{R}$,

$$u_0 = k * u + n, \tag{1.1}$$

where Ω is a bounded open set in $\mathbf{R}^2$, u_0 is the observed image, u is the original image, k is the PSF (point spread function) and also called blur kernel (*stands for convolution) and n is additive white noise assumed to be close to Gaussian. The value n(i,j) of n at the pixels (i,j) are independent random variable, each with a Gaussian distribution of zero mean and variance σ^2.

In ref. [9] gave another models.

$$u_0 = [(k * u)(x, y)] + n(x,y), \tag{2.2}$$

and

$$u_0 = (k * u)(x,y) + (u(x,y))(n(x,y)), \tag{2.3}$$

where n is multiplicative noise. Equation (2.2) and (2.3) have been treated in [15] and [16] respectively, for example, using homomorphic filtering, i.e., basically taking the log of u_0 and treating it as a problem involving additive noise, then filtering and applying the exponential. This is not appropriate for equation (2.3). We present below our restoration algorithms for (2.2) and (2.3).

The total variation based image denoising model, which is based on the constrained minimization problem appeared in [8], is as follows:

Minimize $$\int_\Omega |\nabla u| \, dx\, dy = \int_\Omega \sqrt{u_x^2 + u_y^2}\, dx\, dy, \tag{2.4}$$

subject to constraints

$$\int_\Omega u \, dx\, dy = \int_\Omega u_0 \, dx\, dy \tag{2.5}$$

and

$$\int_\Omega 1/2\,(k * u - u_0)^2 \, dx\, dy = \sigma^2. \tag{2.6}$$

The first constraint corresponds to the assumption that the noise has zero mean, and the second constraint uses a priori information that the standard deviation of the noise n(x, y) is σ.

The Euler-Lagrange equation, see the references [8,14,16]

$$0 = -\nabla.(\nabla u/\nabla u) + \lambda k * (k * u - u_0). \tag{2.7}$$

in Ω, with $\partial u / \partial n = 0$ on the boundary of the domain.

Since (2.7) in not well defined at points where $\nabla u = 0$ due to the presence of the term $1/|\nabla u|$, it is common to slightly perturb the TV algorithm to become

$$\int_\Omega |\nabla u|_\beta \, dx\, dy = \int_\Omega \sqrt{u_x^2 + u_y^2 + \beta}\, dx\, dy, \tag{2.8}$$

where β is a small positive parameter [4].

Rudin and Osher [9] for the denoising and deblurring model is given by

$$u_t = \nabla.(\nabla u/|\nabla u|) - \lambda k * (k * u - u_0), \tag{2.9}$$

for $t>0, x,y \in \Omega$, $\partial u / \partial n = 0$ on the boundary of the domain.

The first constraint (2.5) is dropped because it is automatically enforced by the evolution procedure, i.e, the mean of u(x,y,0) is the same as that of $u_0(x,y)$, see reference [8,9]. As t increases, a

 ISSN 2349-2732

restoration version of image is realized.

The projection step is the gradient projection method [7] which just amounts to updating $\lambda(t)$ so that (2.6) remains true in time. This follows [14] if we define,

$$\lambda(t) = \frac{\int_\Omega \nabla.(\nabla u/|\nabla u|).k*(k*u-u_0)\,dx\,dy}{\int_\Omega (k*(k*u-u_0))^2\,dx\,dy} \tag{2.10}$$

We thus have a dynamic procedure for restoring the image. As $t \to \infty$, the steady state solution is the desired restoration.

Next we consider restoration involving multiplicative noise [16]. We are given an image $u_0(x, y)$ where

$$u_0 = un. \tag{2.11}$$

The unknown function u (x,y) is the image we wish to restore, and n is Gaussian white noise.

We are given the following information

$$\int_\Omega n = 1, \tag{2.12}$$

$$\int_\Omega (n-1)^2 = \sigma^2, \tag{2.13}$$

where the equation (2.12) has mean one and equation (2.13) given variance.

Thus the constrained optimization algorithm is (2.4) subject to the following constraints

$$\int \frac{u_0}{u} = 1 \tag{2.14}$$

$$\frac{1}{2}\int\left(\frac{u_0}{u}-1\right)^2 = \frac{\sigma^2}{2} = \frac{1}{2}\int\left(\left(\frac{u_0}{u}\right)^2 - 1\right) \tag{2.15}$$

The gradient projection method leads to

$$u_t = \nabla.(\nabla u/|\nabla u|) - \lambda\,(u_0^2/u^3) + \mu\,(u_0/u^2)\ . \tag{2.16}$$

Next we consider image which are both blurry and noisy. The model (2.2) is as follows. We are given an image $u_0(x, y,)$ for which

$$u_0 = (k*u)n. \tag{2.17}$$

The noise n is as above (2.12) and (2.14) are still satisfied.

The constraints are given by

$$\int \frac{u_0}{k*u} = 1 \tag{2.18}$$

$$\int\left(\frac{u_0}{k*u}-1\right)^2 = \frac{\sigma^2}{2} = \int\left(\frac{u_0}{k*u}\right)^2 - 1. \tag{2.19}$$

The gradient projection method leads to

$$u_t = \nabla.\left(\frac{\nabla u}{|\nabla u|}\right) - \lambda\ k*\left(\frac{u_0}{(k*u)^2}\right)\left(\frac{u_0}{(k*u)^2}-1\right) + \mu\ \kappa*\left(\frac{u_0}{(k*u)^2}\right). \tag{2.20}$$

with u (x,y,0) given as initial data (the original blurred and noisy image u_0 is taken as initial guess) and homogeneous Neumann boundary conditions $\partial u/\partial n = 0$.

Applying a priori smoothness on the solution image, our new time dependent model becomes.

 ISSN 2349-2732

$$u_t = \nabla \cdot \left(\frac{\nabla G_\sigma * u}{|\nabla G_\sigma * u|} \right) - \lambda k * \left(\frac{u_0}{k * G_\sigma * u^2} \right) \left(\frac{u_0}{k * G_\sigma * u^2} - 1 \right) + \mu \kappa * \left(\frac{u_0}{k * G_\sigma * u^2} \right). \quad (2.21)$$

with u (x,y,0) given as initial data (the original blurred and noisy image u0 is taken as initial guess) and homogeneous Neumann boundary conditions $\partial u / \partial n = 0$. It should be noticed that (2.21) only replaces u in (2.20) by its estimate $G_\sigma * u$.

With in [12] noticed that the convolution of the signal with Gaussians at each scale was equivalent to solving the heat equation with the signal as initial datum. The term $(G_\sigma * \nabla u)(x,y,t) = (\nabla G_\sigma * u)(x,y,t)$, which appears inside the divergence term of (2.21), is simply the gradient of the solution at time σ of the heat equation with u(x,y,0) as initial datum. Then the restoration analysis associated with u_0 consists in solving the problem

$$\partial u(x,y,0)/\partial t = \Delta u(x,y,t), \qquad u(x,y,0) = u_0(x,y).$$

The solution of this equation at time t is given by

$$u(x, y, t) = G_\sigma * u_0,$$

where G_σ is the Gaussian function.

In order to preserve the notion of scale in the gradient estimate, it is convenient that this kernel G_σ depends on a scale parameter [5]. In fact, the function G_σ can be considered as "low-pass filter" or any smoothing kernel, i.e., a denoising technique is used before solving the nonlinear diffusion problem [1,2].

We still write $G_\sigma * u$ as u. Let u_{ij}^n be the approximation to the value $u(x_i, y_j, t_n)$, where

$$x_i = i\Delta x,\ y_j = j\Delta x,\ i,j = 1,2,\ldots\ldots,N,$$
$$N\Delta x = 1,\ t_n = n\Delta t,\quad n \geq 1,$$

where Δx, Δy and Δt are the spatial step sizes and the time step size respectively.

In [10,11] the explicit partial derivatives of models (2.20) and (2.21) can be expressed as :

$$u_t = \frac{u_{xx}(u_y^2 + \beta) - 2u_{xy}u_x u_y + u_{yy}(u_x^2 + \beta)}{(u_x^2 + u_y^2 + \beta)^{3/2}} - \lambda k * \left(\frac{u_0}{(k*u)^2} \right) \left(\frac{u_0}{(k*u)^2} - 1 \right) + \mu \kappa * \left(\frac{u_0}{(k*u)^2} \right). \quad (2.23)$$

We define the derivative terms as,

$$u_{ij}^x = \frac{u_{i+1,j}^n - u_{i-1,j}^n}{2\Delta x} \quad ; \quad u_{ij}^y = \frac{u_{i,j+1}^n - u_{i,j-1}^n}{2\Delta x} \quad ;$$

$$u_{i,j}^{xx} = \frac{u_{i+1,j}^n - 2u_{i,j}^n + u_{i-1,j}^n}{\Delta x^2} \quad ; \quad u_{i,j}^{yy} = \frac{u_{i,j+1}^n - 2u_{i,j}^n + u_{i,j-1}^n}{\Delta x^2} \quad ;$$

$$u_{i,j}^{xy} = \frac{u_{i+1,j+1}^n - u_{i-1,j+1}^n - u_{i+1,j-1}^n + u_{i-1,j-1}^n}{4\Delta x \Delta x} \quad ; \quad u_{ij}^t = \frac{u_{i,j}^{n+1} - u_{i,j}^n}{\Delta t}$$

We let,

$$r_{i,j}^n = u_{i,j}^{xx}((u_{i,j}^y)^2 + \beta) - 2u_{i,j}^{xy} u_{i,j}^x u_{i,j}^y + u_{i,j}^{yy}((u_{i,j}^x)^2 + \beta), \quad (2.24)$$

and

 ISSN 2349-2732

$$p_{i,j}^{n} = \sqrt{(u_{i,j}^{x})^2+(u_{i,j}^{y})^2+\beta} \qquad (2.25)$$

Then (2.23) reads as follows:

$$u_{i,j}^{1} = \frac{r_{i,j}}{p_{i,j}^{n}} - \lambda k\bullet\left(\frac{u_0}{(\kappa\bullet u_{i,j}^{n})^2}\right)\left(\frac{u_0}{(\kappa\bullet u_{i,j}^{n})2}-1\right)+ \mu\ \kappa\bullet\left(\frac{u_0}{(\kappa\bullet u_{i,j}^{n})^2}\right) \qquad (2.26)$$

with homogeneous Neumann boundary conditions.

3. Concluding Remarks :

We have presented a new time dependent model (2.21) to solve the nonlinear total variation problem for image restoration. The main idea is to apply a priori smoothness on the solution image. Nonlinear explicit schemes are used to discretize models (2.21) and (2.20) have used in our experiment $(\Delta t/\Delta x) < 0.5$ and $\lambda = 0.85$ [4,6] The model (2.21) gives large Improvement Signal to Noise Ratio (ISNR) values than that of model (2.20), at the same iteration numbers.

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