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Editorial Note

Science and Technology for Inclusive growth and development of the Society

Science is often seen as technology by the common people and most of the scientific applications are available as technology in the print and electronic media. The principle and methods of scientific discoveries, which are mother of the technology, innovations and modern social order, are equally important, and hence required to be adopted by the society at large for the better benefits of the science and technology. The society is a complex identity and the impact of science; technology and innovation on different strata of people are not uniform. Therefore, such impact studies involving the authentic tools and techniques of social studies are also important for holistic understanding of science and its uses. This journal intends to cover these four different faces of science i.e. principle, methodology, technology and society.

This issue contains new scientific literature that show progress and technological innovations in field of sustainable agriculture and environment, health, artificial intelligence, advances in astro-physics, etc. whereas social communications reflects thinking, temperament and belief of people of the society towards the status of female, social justice, ethics and responsibilities.

We believe that this journal will not be just like another journal but will aid to fill the gaps of interdisciplinarism in the existing knowledge in area of science, technology and social studies relevant to the application of science and technology for the new world order.

Rana Pratap Singh
Editor-in-Chief

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About Babasaheb Bhimrao Ambedkar University

Babasaheb Bhimrao Ambedkar (BBA) University, Lucknow is one of the premier central Universities in the country. The jurisdiction of this residential University is over the entire state of Uttar Pradesh. Babasaheb Bhimrao Ambedkar University was established on 10th January 1996. The 250 acres' University campus named 'Vidya Vihar' is located off the Raebareli road, about 10 Km. south of Charbagh, the main railway station of Lucknow. One of the major objectives of the University is to cater and promote the educational needs of the marginalized people, particularly the SC/ST and women. The University is committed to develop as a center of excellence in the field of higher education to promote advanced knowledge by providing instructional and research facilities for integrated courses in Science and key frontier areas of Technology and other allied disciplines.

About the Journal

The journal (International Journal of Science, Technology and Society), which is a peer reviewed research journal is planned to publish the debate, commentary, reviews and original research papers of high quality primarily on the issues and knowledge regarding interdisciplinary approach in science and technology and inclusive policies by which ST can help marginalized sections of the society to become an integral part of main stream. The journal covers new knowledge and new insights in all disciplines of science including its interface with one another and with societies. it is unique in the sense, it makes a bridge between the science, technology and society which is an emerging thrust throughout the world to make an equal, peaceful and sustainable world order. There is no page charge and no direct or indirect expenditure involved, but for a meager amount charged, only if the authors intend to buy hard copy of reprints of their paper. The authors will be provided a PDF file of his/her paper and a copy of the issue in which they have contributed. We invite all the colleagues to contribute their work in this journal in benefit of science and society.

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Optimized Formulation of Colon Targeted Microsheres for Prednisolone Sodium Phosphate : Application of Taguchi Experimental Design

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Abstract

Microspheres of ethyl cellulose containing prednisolone sodium phosphate were prepared by double emulsion solvent diffusion method. A statistical design was used to study and optimize the variables that affect the preparation of microspheres. The experimental results showed that the drug: polymer ratio, stirring speed, concentration of surfactant, and volume of processing media played an important role in the formulation of microspheres. The prepared microspheres were characterized on the basis of particle size, scanning electron microscopy, entrapment efficiency and *in-vitro* release. Taguchi experimental design helped to reduce the number of experiments. Optimized formulation exhibited Higuchi square root kinetics displaying diffusion from the microspheres as the main mechanism for drug release.

Keywords: Taguchi experimental design, microspheres, double-emulsion solvent diffusion method, ethylcellulose, Prednisolone sodium phosphate, colon-targeted drug delivery systems

1. Introduction

Prednisolone sodium phosphate (PSP) is a corticosteroid often used in conjunction with aminosalicylates to bring about remission of ulcerative colitis (Berthold *et al.,* 1996) and also used in treatment of severe inflammatory conditions including allergies, arthritis, asthma, skin reactions etc (Adinarayana *et al., 2011*). Conventional formulations of PSP (suppositories, enemas) used to treat ulcerative colitis generally lead to patient incompliance, increased dosing, decreased bioavailability and inconvenience (Prantera and Marconi, 2013). These drawbacks create a potential need to modify the formulation so that PSP can be administered by oral route thus reducing dosing frequency and improving patient compliance.

Development of a formulation which can deliver PSP near the colon is a challenging task. Microencapsulation is used to modify and retard drug release. Microspheres, due to its small particle size, are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa (Li *et al.,* 1998).

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Microsphere systems also minimize the possible intestinal retention of undigested polymer materials in chronic dosing (Kramer and Blume, 1994).

Ethylcellulose (EC) is a water-insoluble and pH-independent polymer and has been widely used in the preparation of controlled release drug delivery system for active pharmaceutical ingredients (Wu *et al.,* 2003; Ana Rita *et al.,* 2006). The use of w/o/o double emulsion solvent diffusion method to microencapsulate the drugs was reported by various authors (Maravajhala *et al.,* 2009; Das and Rao, 2006; Kanoujia *et al*., 2010).

To design a new formulation in the field of pharmaceutical dosage forms, it is very important to identify the parameters and variables in the preparation method that may affect the properties of new dosage form. Statistical designs can be used for analyzing the influence of different factors on the properties of system being studied (Prantera and Marconi, 2013). The method of studying one variable at a time, while keeping all others at a predetermined level is very inefficient in many cases and also a time consuming technique (Venkata *et al.,* 2003). Taguchi's method has been used widely in design experiments and to investigate how different parameters affect the mean and variance of a process performance characteristic. The experimental design proposed by Taguchi involves orthogonal arrays to organize the parameters affecting the process and the levels at which they should be varied. Instead of having to test all possible combinations as in factorial design, the Taguchi method tests pairs of combinations (Senthil kumar *et al.,* 2010). This allows for the collection of the necessary data to determine which factors most affect product quality with a minimum amount of experimentation, thus saving time and resources (Dobrzanski *et al.,* 2007). A few reports are available on the application of Taguchi's method in the field of pharmacy (Asghari and Esmaeilzadeh, 2012; Gonzalez-Rodriguez *et al.,* 2012; Jahanshahi *et al.,* 2008; Mehravar *et al.,* 2011).

This study was undertaken to develop and optimize PSP loaded ethyl cellulose (EC) microspheres by the Taguchi design method which can be incorporated in enteric coated capsule thus acting as a sustained release oral formulation to treat ulcerative colitis.

2 Materials and Methods

2.1 Materials

Prednisolone sodium phosphate (PSP) was received as a gift sample from Syntho Pharmaceuticals, Lucknow, India. EC (14 cps viscosity grade) was procured from Central Drug House, Mumbai, India. Dichloromethane, light liquid paraffin and n-hexane were procured from SD Fine Chemicals, India. All other chemicals, reagents and solvents used were of analytical grade.

2.2 Preparation of PSP microspheres

Microspheres were prepared by the w/o/o double emulsion solvent diffusion method (Wu *et al.,* 2003). Weighed amounts of PSP and EC in varying ratios (1:1, 1:2, 1:3) were dissolved in 5 ml of a 1:1 methanol and dichloromethane mixture. The initial w/o emulsion was formed by adding 2 ml of

deionized water to the drug-polymer solution with constant stirring at 500 rpm for 5 min. The w/o primary emulsion was then slowly added to light liquid paraffin (variable volume of 50 ml, 100 ml and 150 ml) containing Span 80 (variable concentrations of 0.25%, 0.5% and 0.75% w/v) as a surfactant with constant stirring at 500 rpm for 2 h. n-hexane (10 ml) was added to harden the formed microspheres and the stirring was further continued for 1 h. The resulting microspheres were separated by decantation, freed from liquid paraffin by repeated washing with n-hexane (3 × 50 ml) and finally air dried. The parameters were chosen as per Taguchi's experimental design (Das and Rao, 2006; Jelvehgari *et al.,* 2010; Lee *et al.,* 2000).

2.3 Design of Experiment by Taguchi's Approach

In the present investigation, an L-9 orthogonal array of Taguchi experimental design was used to study the influence of independent process/formulation variables and establish the relationship between variables and operational conditions. Taguchi's orthogonal array table was used to carry out experiments by choosing four parameters (drug: polymer ratio, stirring speed, concentration of surfactant and volume of processing media) at three levels (low, medium high). Table 1 shows the parameters and levels used in this experiment. The Taguchi's design reduced the number of experiments to nine (Table 2) instead of other methods such as factorial experimental design (3^4 = 81 Run). Therefore, it is very time-consuming when there are many factors.

Orthogonal arrays provide a set of well balanced experiments and Taguchi's signal-to-noise ratios (S/N), which are log functions of desired output, serve as objective functions for optimization and help in data analysis and prediction of optimum results (Dobrzanski *et al.,* 2007).

Table 1: Variables and their levels

Independent Variables	Levels		
	Low (1)	Medium (2)	High (3)
A. Drug: Polymer (mg)	1:1	1:2	1:3
B. Stirring Speed (rpm)	500	1000	1500
C. Vol. processing media (ml)	100	150	200

In Taguchi method, the variables under consideration are divided in to two groups; those that can be controlled (control factors) and those that cannot be controlled or are too expensive to control (noise factors). The greater the effect of noise, greater is the inconsistency in the results. Although noise cannot be eliminated, its effect can be minimized. The Taguchi method employs a generic signal to noise (S/N) ratio to quantify the present variation. These ratios are meant to be used as measures of the effect of noise factors on the performance characteristics. S/N ratios take in to account both amount of variability in response data and closeness of the average response to the target. There are several S/N ratios available depending upon the type of characteristics: smaller the better as is the case with particle size and polydispersity, larger the better as is the case with encapsulation efficiency. In some cases, a nominal S/N ratio is the best. The following equations were used to calculate S/N ratio in Taguchi experimental design (Syrcos, 2003; Ballal *et al.,* 2012).

Table 2: Variables and levels in Taguchi's L9 orthogonal array design

Batch	Independent Variables					
	Drug: Polymer ratio (mg)	Stirring Speed (rpm)	Vol. of processing media (ml)	Particle size (µm)	Encapsulation Efficiency (%)	*In-vitro* drug release (%)
PSP 1	1 (1:1)	1 (500)	1 (100)	367.30	58.45	48.73
PSP 2	1 (1:1)	2 (1000)	2 (150)	258.30	64.67	44.06
PSP 3	1 (1:1)	3 (1500)	3 (200)	274.30	72.11	52.83
PSP 4	2 (1:2)	1 (500)	2 (150)	164.60	62.99	71.76
PSP 5	2 (1:2)	2 (1000)	3 (200)	137.86	67.96	72.76
PSP 6	2 (1:2)	3 (1500)	1 (100)	125.63	74.42	74.56
PSP 7	3 (1:3)	1 (500)	3 (200)	111.33	59.45	68.46
PSP 8	3 (1:3)	2 (1000)	1 (100)	96.00	57.44	69.86
PSP 9	3 (1:3)	3 (1500)	2 (150)	102.10	61.45	72.23

Low (1), Medium (2), High (3)

For S/N Ratio (Smaller the better)

$n = -10 \text{ Log}_{10}$ [mean of sum of squares of measured data]……(2.1)

For S/N Ratio (Larger the better)

$n = -10 \text{ Log}_{10}$ [mean of sum squares of reciprocal of measured data]……...(2.2)

3. Characterization of microspheres

3.1 Morphological characterization of microspheres

The shape and surface morphology of the microspheres was studied by scanning electron microscopy (SEM). The samples for SEM were prepared by lightly sprinkling the powder on a double-sided adhesive tape stuck to an aluminum stub then coated with gold to a thickness of ~300 Å under an argon atmosphere using a gold sputter module in high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with a scanning electron microscope (LEO-430, Cambridge, UK).

3.2 Particle size analysis

The particle size and size distribution was determined by microscopic method. For each batch of the microsphere, 100 particles were randomly selected using an optical microscope (Malvern Instruments Ltd. Malvern, UK) fitted with a camera (Yoko CDD camera, Taiwan) and Medical Pro software (Version 3.0).

3.3 Determination of Encapsulation Efficiency

Weighed amount of microspheres were triturated with 100 ml of phosphate buffer (pH 7.4). The resulting mixture was stirred by magnetic stirrer for 2h. The solution was filtered through a membrane filter (0.45 mm pore size). 1 ml of the filtrate was suitably diluted using phosphate buffer (pH 7.4) and analyzed spectrophotometrically at 247 nm using UV-1700 Pharmaspec, Shimadzu UV-Visible spectrophotometer. The EE was calculated using the formula.

Drug entrapment efficiency = Experimental drug content / Theoretical drug content × 100….(2.3)

3.4 In-vitro drug release study without rat cecal material

The United States Pharmacopoeia Dissolution Test Apparatus II (Type II, Veego DA, 6 DR Japan) was used for *in-vitro* release studies. A weighed quantity (30mg) of the microspheres was placed in a size 1 hard gelatin capsule shell pretreated with HPMC and Eudragit S100. In order to simulate the pH changes along the GI tract, dissolution media (900 ml) with pH 1.2, 7.4

and 6.8 were used. During experiments, pH 1.2 medium was first used for 2 h (average gastric emptying time being 2 h); it was replaced with the fresh pH 7.4 phosphate buffer saline. After 3 h (average small intestinal transit time is 3 h), the medium was replaced with phosphate buffer pH 6.8 maintained at 37±1°C and 75 rpm and the release was observed up to 24 hours. The 5 ml samples were withdrawn at predetermined time intervals and filtered through Whatman filter paper, diluted suitably and analyzed spectrophotometrically with UV-Visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan) at 247.2 nm. An equal volume of fresh dissolution medium was replaced in the jar. The concentrations of drug in respective samples was calculated using regression equation ($y = 0.096x - 0.006$, $R^2 = 0.998$).

3.5 Preparation of 2% rat cecal material

To overcome the limitations of conventional dissolution testing for evaluating the performance of colon specific drug delivery systems triggered by colon specific bacteria, rat caecal contents has been utilized as an alternative dissolution medium, called rat caecal content medium or simulated colonic fluid. This medium was prepared by the method as reported by *Van den* Mooter *et al.* (1994) (Dashora and Jain, 2009).

The experiment was performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by Institutional Animal Ethical Committee (IAEC) (IAEC/BBD/10-11/Clear/17). Two Wistar albino rats of uniform body weight (150-200 g) with no prior drug treatment were maintained on normal diet. The procedure involved oral treatment of rats with 1 ml of 1% w/v pectin dispersion for 7 days to initiate microbial enzyme induction. Thirty minutes prior to the study, each rat was humanly killed (Euthanasia) and the abdomen opened. The caecum was traced and dissected. The caecum was isolated, ligated at both the ends, cut loose and immediately transferred into simulated colonic fluid of pH 6.8 previously bubbled with carbon dioxide. The caecal contents were individually weighed, pooled, and suspended in buffer to provide final caecal concentration of 2% w/v (Pandey *et al.,* 2012).

3.6 In vitro drug release study in the presence of colonic fluid containing 2% rat cecal material

The United States Pharmacopoeia Dissolution Test Apparatus II (Type II, Veego DA, 6 DR Japan) was used for *in-vitro* release studies. A weighed quantity (30mg) of the microspheres was placed in a size 1 hard gelatin capsule shell pretreated with HPMC and Eudragit S100. In Drug release rate studies were performed as described in the previous section using simulated colonic fluid containing 2% rat caecal contents maintained at 37±1°C and 75 rpm and the release was observed up to 24 hours. The experiment was performed with a continuous supply of carbon dioxide into the dissolution media. Samples were analyzed spectrophotometrically with UV-

Visible spectrophotometer (UV-1700 Pharmaspec, Shimadzu, Japan) at 247.2 nm. An equal volume of fresh dissolution medium was replaced.

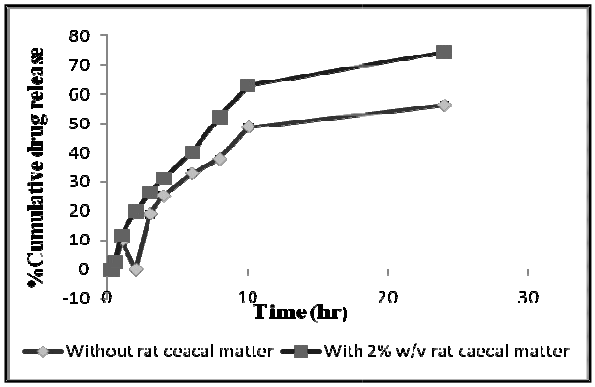

Figure 1: Comparative percent drug release in Simulated Colonic Fluid (pH 6.8) from PSP microspheres with and without rat caecal matter

3.7 Mechanism of drug release

The drug release data of optimized formulation was plotted using various kinetics models i.e. zero order, first order, Higuchi's kinetics and Korsmeyer's equation to evaluate the drug release mechanism. The regression analysis was performed.

3.8 Stability studies

Six batches of optimized formulation PSP 6 were stored in amber colored screw capped glass vials in stability chamber at 40±1 ^{o}C and 75%±5 relative humidity, room temperature and 4±0.5 ^{o}C (refrigerator) for 3 months. Samples were analyzed for physical appearance, residual drug content after a period of 0, 7, 15, 30, 60 and 90 days. Initial drug content was taken as 100 % for each formulation. Observations are recorded in Table 3. The log % residual drug content vs. time graph was also plotted for the optimized formulation in order to evaluate half-life and shelf life of formulations.

Table 3: Stability parameters for optimized formulation (PSP 6)

S. No	Parameters	Storage Conditions		
		4±0.5 °C	Room Temperature	40±0.5 °C /75%± 5 RH
1	K (day^{-1})	8.55 x 10^{-4}	1.0528 x 10^{-3}	3.08 x 10^{-3}
2	$t_{1/2}$ (days)	810.5	658	225
3	$T_{10\%}$ (days)	121.63	98.85	33.76

4. Results And Discussion

Microspheres of PSP have been successfully prepared using EC by w/o/o double-emulsion solvent diffusion method due to high entrapment efficiency and suitable for water soluble drug (PSP) likely to preferentially partition out into the aqueous medium, leading to low entrapment efficiency. The various variables (drug: polymer ratio, stirring speed, concentration of surfactant, volume of processing media) play an important role in the formulation of microspheres and their characteristics.

4.1 Morphological characterization of microspheres

SEM revealed that the optimized formulation of microspheres (PSP 6) were spherical and homogeneous smooth surface (Figure 2).

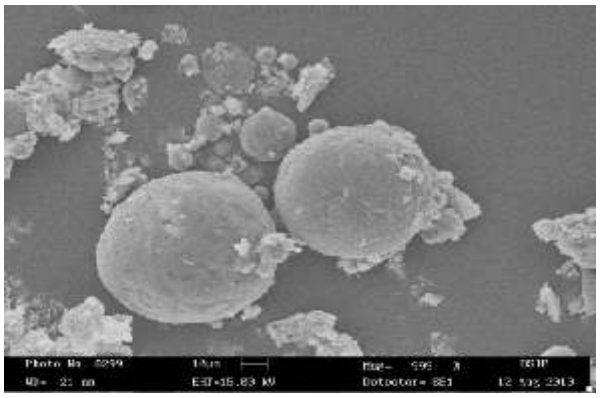

Figure 2: SEM photomicrograph of EC microspheres of predinisolone sodium phosphate (PSP 6)

4.2 Particle size analysis

The particle size of microspheres was distributed in the size range of 124.57 to 138.16 µm. The average particle size of optimized batch (PSP 6) was found to be 124.57µm (Table 2). As the volume of medium increased, the size of prepared microspheres decreased.

4.3 Drug Entrapment Efficiency

The entrapment efficiency of the drug depended on solubility of the drug in the solvents and continuous phase and physicochemical properties of the drug and polymer. The entrapment efficiency of optimized batch (PSP 6) was found to be 74.42±0.78%. Entrapment efficiency of microspheres was found to depend on surfactant concentration and volume of processing media. The high entrapment efficiency resulted from poor solubility of the drug in continuous phase and high solubility in internal water.

4.4 In-vitro Drug Release Study

The *in-vitro* drug release studies were performed in simulated colonic fluid (pH 6.8) with and without using rat caecal contents. *In-vitro* drug release in simulated colonic fluid without rat cecal material was 55.24± 0.77%, but drug release in simulated colonic fluid with 2% rat cecal material after 24 h was 74.50±0.92 %. An enhanced drug release was observed (Figure 1) due to presence of rat cecal material in the dissolution medium which increased the rate of drug release (degradation of polymer by various anaerobic bacteria present in the caecum) from the formulation compared with the control.

4.5 Mechanism of Drug release

The drug release data of optimized batch was plotted using various kinetic models i.e. zero order, first order, Higuchi's kinetics and Korsmeyer's equation to evaluate the drug release mechanism. The calculated regression coefficients for zero order, first order, Korsmeyer-Peppas and Higuchi models were 0.791, 0.466, 0.906 and 0.942 respectively. It was found that the *in vitro* drug release of prednisolone microspheres follows Higuchi's model as the plot showed the highest linearity (Figure 3).

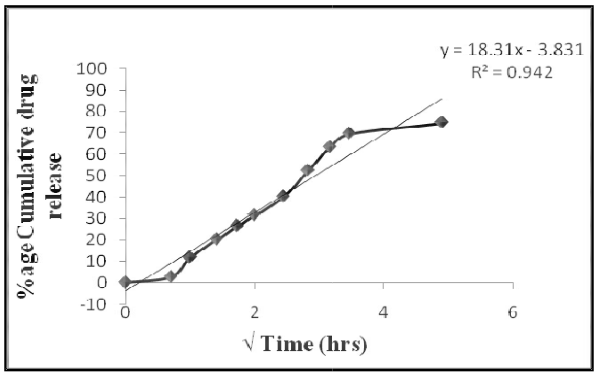

Figure 3: Higuchi plot of drug release from optimized formulation (PSP 6)

4.6 Stability studies

The PSP content of microspheres fell by a marginal 1.91% at 4°C, 3.67% at room temperature and 3.90% at 40°C. There was no significant difference in percent of residual drug content (Table 4) upon aging.

Table 4: Stability data for optimized formulation (PSP 6)

S.No.	Sampling interval (days)	% Residual Drug Content Mean ± S.D. (n=3)		
		4±0.5 °C	Room Temperature	40±0.5 °C 75%±5 RH
1	0	100	100	100
2	7	99.78±0.10	99.44±0.21	99.26±0.25
3	15	99.35±0.06	98.81±0.09	98.65±0.01
4	30	99.21±0.09	98.45±0.12	98.38±0.17
5	60	98.69±0.08	97.78±0.07	97.46±0.15
6	90	98.09±0.05	96.33±0.17	96.10±0.37

4.7 Optimization of Process Variables via Taguchi approach

Particle size

A Taguchi's L9 array (Table 1) was used to determine the optimized parameters combination influences the particle size of microspheres, nine different batches were prepared. Three formulations were prepared for each of the parameter combination. The completed response table for the data appears in Table 2. The average response of particle size was calculated to determine the effect of independent variables, on the formulation characteristics.

Table 5: Results of ANOVA for particle size

Factors	Degree of freedom	Sum of squares	Variance	F-Ratio	Percentage Contribution (%)
Drug:Polymer Ratio	2	46.328	23.164	1.045	0.713
Stirring Speed	2	191.094	95.547	4.313	51.69
Vol. of processing media	2	2.251	1.125	0.053	0
Error	2	44.302	22.151		47.596
Total	8	283.976			100

Table 6: Results of ANOVA for entrapment efficiency

Factors	Degree of freedom	Sumof squares	Variance	F-Ratio	Percentage Contribution (%)
Drug:Polymer Ratio	2	2.243	1.121	7.54	38.577
Stirring Speed	2	2.129	1.064	7.157	36.315
Vol. of processing media	2	0.373	0.186	1.256	1.514
Error	2	0.296	0.148	-	23.594
Total	8	5.043	-	-	100

Table 7: Results of ANOVA for *in-vitro* release studies

Factors	Degree of freedom	Sum of squares	Variance	F-Ratio	Percentage Contribution
Drug:Polymer Ratio	2	23.294	11.6	58.19	92.739
Stirring Speed	2	0.734	0.368	1.843	1.367
Vol. of processing media	2	0.253	0.126	0.634	0
Error	2	0.399	0.199	-	5.894
Total	8	24.686	-	-	100

Three observed response at level 1, 2, 3 of variable A, B, C, D were summed together and sum was divided by 3 to obtain average response. From Table 1 and Table 3, for variable A at level 2 is $Avg_{\text{variable A, level 2}} = Avg_4 + Avg_5 + Avg_6 / 3$ i.e. 135.90 + 31.16 + 24.57/ 3 = 63.87. Similarly the values for other variables at all three levels were calculated. The response of all the variables on particle size is represented graphically in Fi ure 4.

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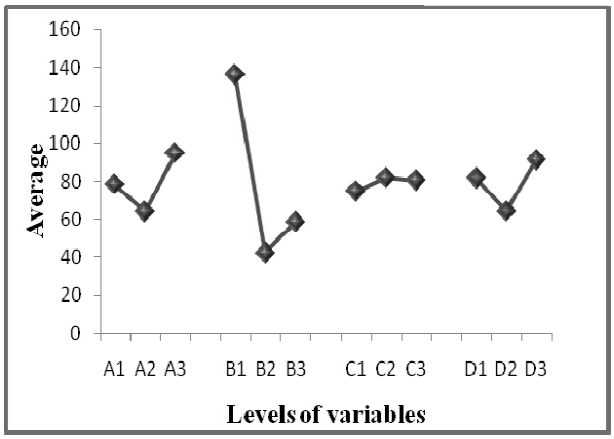

Figure 4: Response graph of average value for smaller-the-better analysis of particle size.

The smaller the particle size, better the formulation characteristics hence lower response is taken into consideration. Since there should be minimum variation between the batches, optimized values of level were taken as follows: A2, B2, C1, and D2.

Similarly, response graphs were plotted for signal to noise ratio (S/N ratio), and logarithm of standard deviation. For reproducible results in terms of small particle size, these values follow the rule, smaller-the-better.

4.8 Final optimized parameters:

Based on the S/N, average, log s, the significant optimal parameters for microspheres has been identified and the levels that maximize these parameters have been selected. Drug: polymer was 1:2, stirring speed 1500 rpm, concentration of surfactant 0.25% w/v and volume of processing media was 150 ml have been taken as optimum.

5. Conclusion

Stable microspheres of PSP have been prepared using EC by double-emulsion solvent diffusion method. The use of Taguchi design has helped to optimize the independent variables involved in preparation of microspheres and reduce the number of experiments. The experimental results showed that the drug: polymer ratio, stirring speed, concentration of surfactant, volume of processing media played an important role in the formulation of microspheres and their contributions were almost equal. The prepared microspheres were stable spherical particles and exhibited favorable release profiles in simulated colonic fluid.

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Differetial Increase in Efficacy of Azotobacter Chroococcum and Bacillus Subtilis Co-Immobilized with Different Organic Manuares in Relation to Plant Growth, Nutritional Status and Grain Yield of Wheat (Triticum aestivum L.)

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Abstract

Present study indicates that the growth, productivity and grain yield of wheat can be enhanced significantly by amending higher dose of *Azotobacter chroococcum* and *Bacillus subtilis* co-immobilized in 4-5mm diameter granules composed of different organic materials. The biofertilizers were co-immobilized in cow dung/ Farm Yard Manure (FYM) or vermicompost using acacia gum as binder, neem leaf powder and clay soil and applied to the earthen pots in forms of granules of 4-5 mm diameter. The vermicompost containing granules provided higher plant biomass (e.g. shoot height, root length, number of leaves, number of roots, tiller numbers, fresh weight and dry weight of shoot and root than others. FYM and cow dung based granules also enhanced the plant growth with a lower magnitude. The granules released NH_4^+, NO_3^-, NO_2^- and PO_4^{3-} in the plant's rhizosphere and enhanced availability of essential nutrients to the plants. The co-immobilized form of biofertilizers with vermicompost also increased yield of wheat (*Triticum aestivum* L. cv 502) in terms of grain weight per plant by 13.79%, with FYM by 11.65% and that with cow dung by 10.15% over free form of the chemical fertilizers (Recommended dose of urea; 150 kg/ha and DAP; 75 kg/ha). The studies indicate that the vermicompost is a better carrier for co-immobilization of the microbial biofertilizers *A. chroococcum* and *B. subtilis* to provide the better shelter and nutrient to the microbes and for higher nutrient availability during their application in the earthen pots.

Keywords: Organic matrix, biofertilizers, acacia gum, super granules, wheat (*Triticum aestivum* L.)

1. Introduction

The excessive use of inorganic fertilizers is unsustainable for agricultural ecosystems as it leads to the leaching, volatilization, emission losses and consequently water pollution and built up of the greenhouse gases resulting in global warming on one hand and wastage of capital and energy consumed in its manufacture and transport (Adesemoye *et al.*, 2009; Densilin *et al.*, 2011; Wang *et al.*, 2010; Kumar *et al.*, 2013a, b; 2014a, b; 2015b). The present agricultural practices are also one of the reasons for climate change related global warming. The high external input e.g. heavy doses of agrochemicals though increase crop productivity, contaminate the different spheres of the environment, when get disposed in soil, water, and atmosphere, affects the soil properties and cause serious environmental and health hazards (Abedi *et al.*, 2010; Sharma *et al.*, 2011; Sing *et al.*, 2010; Rawat *et al.*, 2012; Ryan *et al.*, *2012;* Kong *et al.,* 2013; Laik *et al.,* 2014).

Biofertilizers are live microbial cells such as N_2 fixing microbes e.g. *Cyanobacteria,*

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Rhizobium, Azotobacter, Azospirillum etc. and phosphate solubilizing bacteria e.g., *Bacillus* etc. (Tripathy and Ayyappan, 2005; Joshi *et al.*, 2007; Cheng *et al.*, 2009; Kundu *et al.*, 2009; Naher *et al.*, 2009; Oliveira *et al.*, 2009; Kumar *et al.*, 2012; Swarnalalkshmi *et al.*, 2013). These microbes are able to transform inert or insoluble forms of the nutrients to an accessible, usable soluble form, contributing to plant nutrition as plant growth promoting microorganisms (PGPMs) (Oliveira *et al.*, 2009; Sharma *et al.*, 2011; Kumar *et al.*, 2015a,b). They play an important role in various ways and increase in soil organic carbon (Pradhan and Shukla, 2005; Cheng *et al.*, 2009; Naher *et al.*, 2009; Mamta *et al.*, 2010; Ardakani *et al.*, 2011; Araujo *et al.*, 2013; Minaxi *et al.*, 2013). The microbial biofertilizers are known to be more effective, when applied with vermicompost generated from organic wastes, as it has high nutrient value to enhance microbial population and activity and subsequently yield to the soil fertility (Adil *et al.*, 2005; Jadia and Fulekar, 2008; Lazcano *et al.*, 2011; Singh *et al.*, 2013).

However, the dose optimization of microbial biofertilizers for different cropping systems in different agro climatic conditions are not adequately studied and hence the potential efficacy of these biofertilizers has not been realised yet as that of synthetic chemical fertilizers urea and DAP. Present study is planned to increase the efficacy of microbial biofertilizers by selecting a more suitable organic carrier system for its storage and application to the agricultural fields.

2. Materials and Methods

2.1 Experimental Site

The experiments were conducted during Rabi season at the Environmental field station of Babasaheb Bhimrao Ambedkar University, Lucknow, India. It is situated on 123 M above the sea level and lies between 26.30^0 and 27.10^0 north latitude and 80.30^0 and 81.13^0 east longitudes.

2.2 Experimental design

The certified seeds of wheat (*Triticum aestivum* L. cv. PBW-502) were obtained from a local dealer of Lucknow. Charcoal based *Azotobacter chroococcum* and *Bacillus subtilis* (Hindustan Bioenergy Ltd.) were obtained from Bio-tech Park, Lucknow and the experiments were conducted twice in 2011-12 and 2012-13, during Rabi (winter) season (December to April). The charcoal-based biofertilizers were applied in free as well as co-immobilized (as described below) forms, after 30 days of sowing of the seeds. The Charcoal based biofertilizers i.e., *A. chroococcum* (0.93 mg/10kg soil) *and B. subtilis* (0.93 mg/10 kg soil) were co-immobilized in an organic matrix (769.24 mg/ 10 kg soil) and the biofertilizer super granules were prepared and applied to the experimental earthen pots. Organic matrix such as cow dung or vermicompost or FYM, neem leaves (*Azadirachta indica*) and clay soil (diameter of particles <0.002 mm) were used to entrap biofertilizers.

All the materials were dried powdered and mixed in ratio of 1:1:1. The optimized dose of charcoal based *A. chroococcum* and *B. subtilis* were mixed in this powdery mixture. Acacia gum was added in organic mixtures by (15% w/w) to make thick paste to prepare granules. Spherical granules of about 4-5 mm diameter were prepared manually and dried on room temperature.

Cow Dung | Farm Yard Manure | Vermicompost

Figure 1 : Manually made organic supergranules made with the different organic matrix based coimmobilized biofertilizers

Table 1: Details of nutrient combinations used under in this study are as follows:

Treatments	Organic matrix used to co-immobilize with *A. chroococcum* and *B. subtilis* to prepare organic matrix based super granules in its optimized dose (1800 g/ha)
NF	No Fertilizer (Control -1)
CF	Soluble chemical fertilizers free form: Urea-384.62 mg/10 kg soil + DAP-192.31 mg/10 kg soil (Control -2)
BF	Charcoal based Biofertilizers; BF- *A. chroococcum* - 0.93 mg/10 kg soil *and B. subtilis* - 0.93 mg/ 10 kg soil previously optimized dose (Control -3).
IBC	BF co-immobilized with cow dung, clay soil, neem leaves and acacia gum (saresh) (769.24 mg/ 10 kg soil).
IBV	BF co-immobilized with vermicompost, clay soil, neem leaves and saresh (769.24 mg/ 10 kg soil).
IBFY	BF co-immobilized with FYM, clay soil, neem leaves and saresh (769.24 mg/ 10 kg soil).

2.3 Measurement of shoot height, root length, number of leaves, roots and tiller numbers and fresh and dry weight of shoot and roots

Measurement of shoot and root length, number of leaves, number of roots, fresh and dry weights of root and shoot was done for wheat at age of 30, 60, 90 and 120 DAS. Shoot and root lengths were measured using a metric scale. Number of leaves and primary roots were counted at regular intervals. The fresh weight of roots and shoots was determined using single pan electrical balance. The tissues were oven dried at 70°C, till constant dry weight was recorded and averaged (n=6).

2.4 Estimation of ammonium, nitrate, nitrite and phosphate contents in the soil and plant tissue

Ammonium [NH_4^+] content in the soil and root samples was estimated using Weatherburn method (1967) based on Nessler's reagent [K_2HgI_4+ NaOH]. Nitrate [NO_3^-] content in soil and root was estimated by method of Cataladu *et al.,* (1975). Nitrite [NO_2^-] was estimated by the method described by Steven and Oaks (1973), using sulphanilamide and N-(1-Naphthyl)-ethylene diamine-di-hydrochloride. Phosphate [PO_4^{3-}] was estimated by stannous chloride method (APHA, 2005). Absorbance (optical

density) in each case was recorded using UV-visible spectrophotometer (Varian, carry 100 Bio) at the wavelength of 420, 410, 540 and 680 nm respectively. The values of ammonium, nitrate, nitrite and phosphate were calculated using standard curve of known concentration for each nutrient.

2.5 Statistical analysis

All treatments were replicated for three times with two determinations of each (n=6). The data were analysed using one way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was applied to compare the means within the treatments at $p<0.05$.

3. Results

3.1 Soil physico-chemical characteristics before the seed sowing

Before seed sowing pH, electrical conductivity (EC), organic carbon, organic matter, bulk density, water holding capacity, ammonium, nitrate, nitrite and phosphate of soil were recorded as 8.7, 0.03 ds m^{-1}, 0.52%, 0.89%, 1.36 mg g^{-3}, 35.49%, 0.41 µg g^{-1}, 20.24 µg g^{-1}, 0.66 µg g^{-1} and 0.97 µg g^{-1}, respectively.

3.2 Grain yield and straw yield of wheat applied with charcoal based and organic matrix co-immobilized biofertilizers and straw yield

Organic matrix based super granules co-immobilized *A. chroococcum* and *B. subtilis* as biofertilizers increased the grain yield (grain weight per plant) of wheat over no fertilizer and charcoal based commercial free form of biofertilizers (Fig. 2). The percentage increase was 80.80, 21.96 and 65.90%, by the application of co-immobilized biofertilizers with vermicompost in granular matrix (IBV) over No fertilizer (NF), chemical fertilizer (CF) and a charcoal based biofertilizers (BF). The application of co-immobilized biofertilizers with FYM as granular matrix (IBFY) showed 78.88, 14.16 and 62.5% and by the co-immobilized biofertilizers with cow dung as granular matrix (IBC) was increased 73.70 and 53.28% grain yields over the controls of NF and BF respectively at 120 DAS. Application of IBV in the organic granules as carrier matrix increased the 9.09% and 27.02% increase in wheat grains per plants over IBFY and IBC. The straw yield was also increased by 71.09, 16.30 and 43.23% over the NF, CF and BF by the application of IBV. The IBFY increased 60.07% straw yield over NF and 1.76% over CF, whereas IBC increased it by 65.18 and 31.61% over NF and BF at crop maturity (Fig. 2).

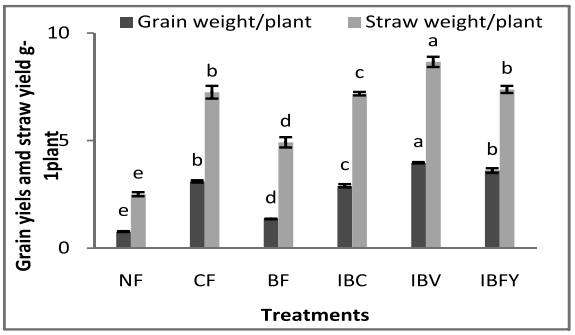

Figure 2: The yield of grain and straw at 120 days after sowing (DAS) the seeds. The straw weight of plant with the application of IBV recorded significantly higher followed by IBFF and IBCS. One way ANOVA was performed to compare the means of different treatments at $p<0.05$. Values by different letters are significant differences between the treatments at 5% level.

3.3 Growth parameters

The plant growth parameters were significantly enhanced when added with different kind of organic matrix based super granular biofertilizers in soil. All the treatments enhanced the shoot and root

Table 2: Effect of organic matrix co-immobilized *A. chroococcum* and *B. subtilis* biofertilizers on shoot growth of wheat (*Triticum aestivum* L. cv. PBW-502) on 30, 60, 90 and 120 DAS.

	Treatments	30 DAS	60 DAS	90 DAS	120 DAS
Shoot Height (cm)	NF	22.96±1.59^{a}	38.40±3.60^{e}	47.65±1.86^{c}	52.68±3.28^{d}
	CF	23.15±1.67^{a}	54.26±2.62^{bc}	61.92±5.8^{a}	62.73±2.26^{b}
	BF	21.70±1.37^{a}	44.71±1.70^{d}	49.25±1.44^{c}	55.51±2.31^{d}
	IBC	22.08±1.11^{a}	51.65±3.33^{c}	55.95±2.02^{b}	61.36±1.64^{c}
	IBV	23.06±1.76^{a}	59.15±3.41^{a}	66.68±4.84^{a}	74.31±2.44^{a}
	IBFY	23.01±1.13^{a}	56.48±3.97^{ab}	63.25±5.60^{a}	70.21±3.57^{b}
Number of leaves	NF	3.33±0.51^{a}	4.33±0.51^{a}	5.66±0.81^{a}	7.00±1.54^{d}
	CF	3.16±0.40^{a}	10±2.09^{ab}	16.66±1.63^{a}	17.50±1.51^{b}
	BF	3.16±0.40^{a}	7.16±2.13^{b}	10.83±2.22^{b}	11.83±2.56^{b}
	IBC	3.00±0.00^{a}	8.33±2.33^{b}	11.50±3.08^{b}	13.66±2.94^{c}
	IBV	3.16±0.40^{a}	11.50±2.81^{a}	17.00±3.74^{a}	21.66±5.12^{a}
	IBFY	3.00±0.00^{a}	8.66±2.73^{b}	15.50±2.51^{a}	17.33±2.42^{b}
Number of tillers	NF	1.00±0.00^{a}	1.00±0.00^{c}	1.16±0.40^{a}	1.00±0.00^{a}
	CF	1.00±0.00^{a}	2.33±0.51^{a}	3.16±0.40^{c}	3.00±0.63^{c}
	BF	1.00±0.00^{a}	1.66±0.51^{b}	2.33±0.51^{b}	2.16±0.40^{b}
	IBC	1.00±0.00^{a}	2.00±0.63^{c}	2.83±0.98^{bc}	2.83±0.40^{c}
	IBV	1.00±0.00^{a}	2.50±0.54^{c}	2.83±0.40^{c}	3.33±0.05^{d}
	IBFY	1.00±0.00^{a}	1.66±0.51^{b}	2.33±0.51^{b}	3.00±0.63^{b}
Fresh weight of shoot (g)	NF	0.17±0.00^{a}	1.15±0.02^{e}	1.37±0.09^{f}	3.27±0.24^{e}
	CF	0.17±0.00^{a}	2.99±0.21^{bc}	4.26±0.17^{c}	10.34±0.84^{c}
	BF	0.17±0.00^{a}	2.08±0.08^{d}	2.68±0.24^{e}	6.27±0.61^{d}
	IBC	0.17±0.01^{a}	2.82±0.22^{c}	3.53±0.18^{d}	10.07±0.36^{c}
	IBV	0.17±0.00^{a}	3.93±0.21^{a}	5.46±0.28^{a}	12.62±0.61^{a}
	IBFY	0.17±0.01^{a}	3.13±0.13^{b}	4.63±0.24^{b}	10.98±0.24^{b}
Dry weight of shoot (g)	NF	0.03±0.00^{a}	0.28±0.01^{f}	0.36±0.02^{f}	1.10±0.15^{e}
	CF	0.03±0.00^{a}	0.79±0.02^{c}	1.10±0.10^{c}	3.33±.18^{c}
	BF	0.03±0.00^{a}	0.53±0.03^{e}	0.70±0.05^{e}	1.84±0.11^{d}
	IBC	0.03±0.00^{a}	0.71±0.06^{d}	0.97±0.05^{d}	3.25±0.12^{c}
	IBV	0.03±0.00^{a}	0.97±0.07^{a}	1.40±0.09^{a}	3.89±0.12^{a}
	IBFY	0.03±0.00^{a}	0.85±0.03^{b}	1.20±0.09^{b}	3.52±0.22^{b}

Data are represented by mean of three individual measurement ± SE. One way analysis of variance was used to compare the means. Values followed by different letters are significantly different at P < 0.05.

Table 3: Effect of organic matrix co-immobilized biofertilizers on root growth of wheat on 30, 60, 90 and 120 DAS.

	Treatments	30 DAS	60 DAS	90 DAS	120 DAS
Root length (cm)	NF	2.33±0.27 [a]	3.20±0.21 [d]	4.65±0.44 [d]	5.73±0.48 [c]
	CF	2.45±0.36 [a]	5.78±0.17 [c]	7.66±0.60 [c]	8.68±0.22 [b]
	BF	2.51±0.34 [a]	6.95±0.57 [b]	7.43±0.49 [c]	8.45±0.27 [b]
	IBC	2.31±0.28 [a]	7.49±0.52 [ab]	9.30±0.99 [b]	10.06±0.73 [a]
	IBV	2.41±0.38 [a]	7.96±0.55 [a]	10.61±0.53 [a]	10.28±0.85 [a]
	IBFY	2.43±0.27 [a]	7.85±0.81 [a]	9.43±0.66 [b]	10.06±0.68 [a]
Number of root	NF	3.66±0.51 [a]	7.33±1.81 [d]	8.83±0.75 [d]	10.00±0.89 [d]
	CF	3.83±0.75 [a]	14.33±1.36 [b]	19.66±1.63 [b]	22.83±2.04 [b]
	BF	3.83±0.40 [a]	11.33±1.21 [c]	13.66±1.21 [c]	17.66±1.63 [c]
	IBC	4.00±0.63 [a]	13.66±2.94 [b]	19.00±3.28 [b]	20.33±3.26 [b]
	IBV	3.83±0.75 [a]	18.83±1.32 [a]	25.66±2.65 [a]	30.16±2.99 [a]
	IBFY	3.66±0.51 [a]	15.16±2.99 [c]	20.33±3.14 [b]	21.50±1.76 [b]
Fresh weight of root (g)	NF	0.13±0.03 [b]	0.21±0.02 [e]	0.29±0.02 [f]	0.35±0.01 [e]
	CF	0.15±0.00 [a]	0.45±0.04 [c]	0.59±0.02 [c]	0.67±0.03 [c]
	BF	0.12±0.01 [b]	0.28±0.00 [d]	0.32±0.03 [e]	0.38±0.01 [d]
	IBC	0.13±0.01 [b]	0.43±0.05 [c]	0.56±0.02 [d]	0.65±0.05 [c]
	IBV	0.15±0.01 [a]	0.58±0.02 [a]	0.68±0.02 [a]	0.76±0.02 [a]
	IBFY	0.14±0.00 [ab]	0.52±0.01 [b]	0.64±0.03 [b]	0.72±0.03 [b]
Dry weight of root (g)	NF	0.03±0.00 [a]	0.05±0.01 [d]	0.06±0.00 [d]	0.08±0.01 [e]
	CF	0.04±0.00 [a]	0.11±0.01 [b]	0.15±0.00 [b]	0.16±0.01 [b]
	BF	0.03±0.00 [a]	0.06±0.00 [d]	0.07±0.00 [c]	0.10±0.01 [d]
	IBC	0.03±0.00 [a]	0.09±0.01 [c]	0.14±0.01 [b]	0.14±0.02 [c]
	IBV	0.03±0.00 [a]	0.14±0.00 [a]	0.17±0.00 [a]	0.18±0.01 [a]
	IBFY	0.04±0.00 [a]	0.12±0.01 [b]	0.16±0.01 [a]	0.16±0.02 [b]

Same as table 2, All the values of this data are replicate with three replications and two determinations (n=6) ± S.E. One way ANOVA was perform to compare the values at $p<0.05$.

length, number of leaves and root, shoot and root biomass (Table 2 and 3). The shoot length was increased on 120 DAS by 30.02, 7.50 and 25.29% when vermicompost was used as organic matrix (IBV) and by 25.93, 2.10 and 20.93% when vermicompost was replaced with FYM (IBFY) over NF, CF and BF, respectively. This increase was, however, 14.14 and 9.53% over the NF and BF when the cow dung was used as organic matrix in supergranules (IBC). The increase in root length, numbers of roots, fresh and dry weight of root were similar on application of co-immobilized form of the biofertilizers. Fresh weight of shoot increased in the presence of IBV by 74.08, 18.06 and 50.31% and IBFY by 70.21, 5.82 and 42.89% over the NF, CF and BF, respectively, at 120 DAS, whereas IBC

showed increase by 67.52, and 37.73% over the NF and BF only. The IBV significantly increased the fresh and dry weight of plants over the CF. The dry weight of plant increased on application of IBV by 71.72, 14.39, 52.69 and IBFY by 68.75, 5.39 and 47.72% over NF, CF and BF at 120 DAS respectively. Application with IBC supergranules increased plant dry weight by 66.15 and 43.38% over the NF and BF at 120 DAS during cultivation of wheat crop. It appears that the biofertilizers in the organic matrix based granules produced higher biomass and increased wheat productivity and yield over the same amount of commercially available biofertilizers.

3.4 Availability of ammonium content in the soil and plant parts on application of super granules of biofertilizers co-immobilized with organic matrix

The organic matrix co-immobilized biofertilizers increased soil ammonium content in plant rhizosphere at the depth of 0-15 cm and was recorded maximum when co-immobilized biofertilizers applied with vermicompost used as a carrier (IBV) by 66.23, 71.27%, 9.40, 10.49% and 46.58, 35.35% over no fertilizer (NF), chemical fertilizers (CF) and commercialized charcoal based biofertilizers (BF) during 60-90 DAS. Similarly, application of co-immobilized biofertilizers with FYM used as a carrier (IBFY) increased ammonium content by 63.31, 69.50%, 2.30, 4.98% and 42.39, 31.37% over the NF, CF and BF and application of co-immobilized biofertilizers with cow dung as a carrier (IBC) by 60.59, 67.19 and 38.11, 26.18% significantly increased over the NF and BF respectively after 60 and 90 DAS (Fig. 3a).

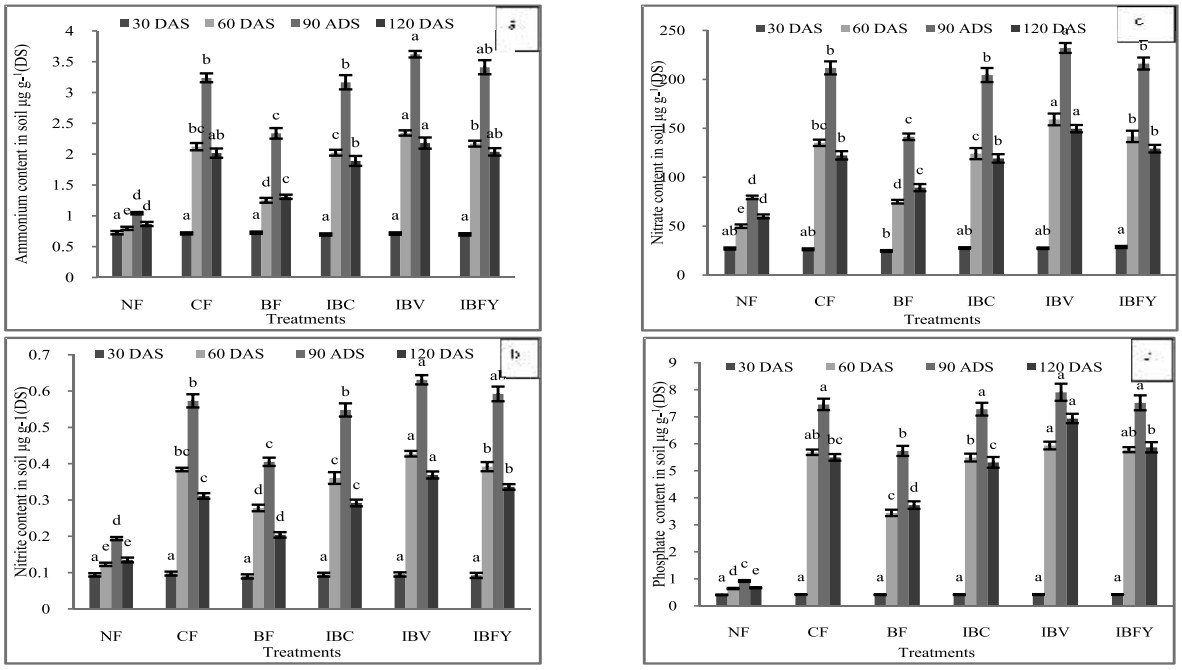

Figure 3a-d: Effect of biofertilizers co-immobilized with different organic matrix on the availability of a- ammonium, b-nitrite, c- nitrate, d- phosphate in the soil on 30, 60, 90 and 120 DAS. One way ANOVA was performed to compare the means of different treatments at $p<0.05$. Values by different letters are significantly differences between the treatments at 5% level.

The ammonium content was found assimilated significantly in roots of wheat and was recorded highest by the application of co-immobilized biofertilizers with vermicompost as a carrier (IBV) which was observed to be increased by 79.54, 13.02% and 53.73% over NF, CF and BF after 90 DAS, respectively. Application of co-immobilized biofertilizers with FYM as a carrier (IBFY) increased ammonium content by 77.23, 3.16 and 48.48% at 90 DAS, over controls including NF, CF and BF, respectively (Fig. 4a).

In leaves the ammonium content assimilation was increased significantly by the application of the co-immobilized biofertilizers with vermicompost as a carrier (IBV) and was increased by 74.56, 9.05 and 47.37% after 90 DAS over controls of NF, CF and BF. By applying the co-immobilized biofertilizers with FYM as a carrier (IBFY) increased ammonium 73.70 and 45.57 % followed by co-immobilized biofertilizers with cow dung as carrier (IBC) 71.41 and 40.85% over NF and BF after 90 DAS (Fig. 5a).

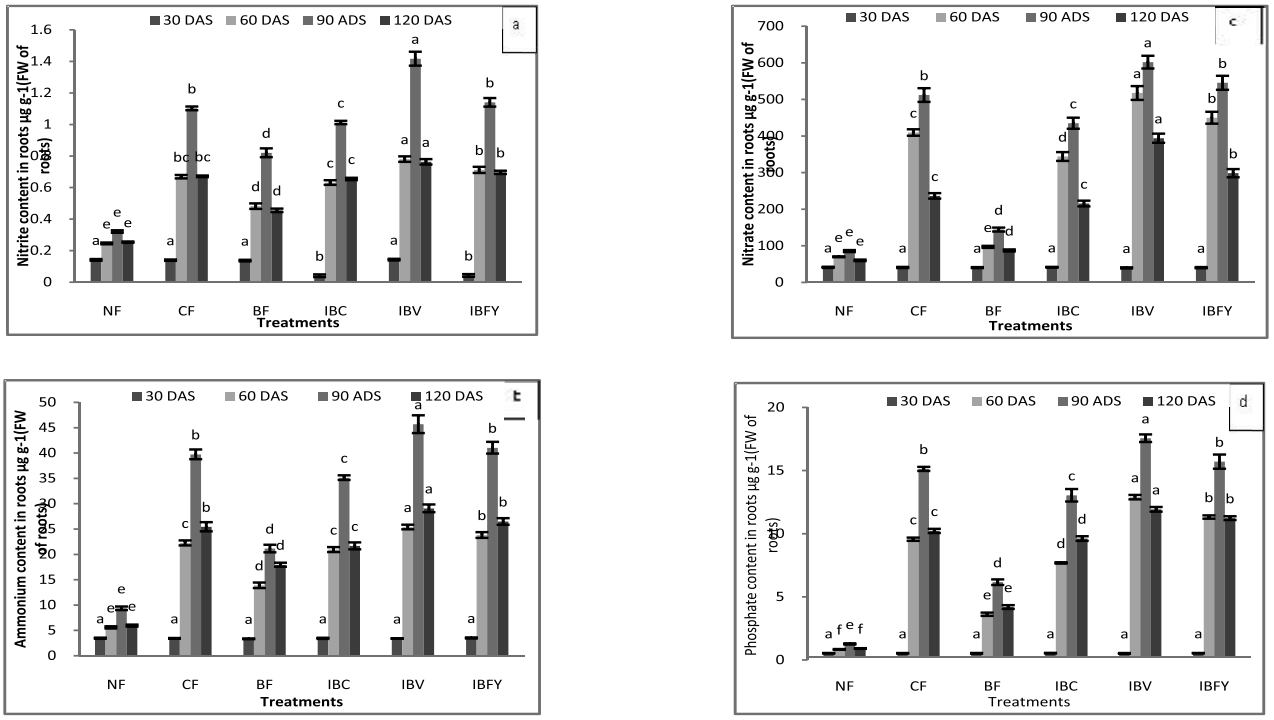

Figure 4a-d: Effect of biofertilizers co- immobilized with different organic matrix on the availability of a- nitrite, b- ammonium, c- nitrate, d- phosphate in the roots on 30, 60, 90 and 120 DAS. One way ANOVA was performed to compare the means of different treatments at $p<0.05$. Values by different letters are significantly differences between the treatments at 5% level.

3.5 Availability of nitrate content in the soil and plant parts on application of super granules of biofertilizers co-immobilized with organic matrix

Significant nitrate content in soil was found on applications of biofertilizers co-immobilized with vermicompost (IBV) during cultivation of wheat. Increased nitrate percentage by IBV was 65.81, 8.77 and 39.11% over controls of NF, CF and FB. Similar result was observed when biofertilizers co-immobilized with FYM (IBFY) was applied and nitrate percentage was reported to be increased by 63.30, 2.06 and 34.64% over different controls (NF, CF and BF) 90 DAS (Fig. 3c) providing nitrogen to the plants for better growth.

Assimilation of nitrate in roots on application of supergranules of biofertilizers co-immobilized with vermicompost (IBV) was found to increased by 85.78, 14.93, 76.01% whereas with FYM (IBFY) was recorded to be increased by 84.30, 6.13 and 73.53% over the controls of NF, CF and BF, 90 DAS. Application of biofertilizers co-immobilized with cow dung (IBC) increased nitrate content by 80.32 and 66.81 % over controls of NF and BF after 90 DAS (Fig. 4c). Similarly trend in increase in nitrate content in plant roots was recorded at different time intervals i.e., 30, 60, 90 and 120 DAS.

The increase percentage of nitrate in plant leaves on application of supergranules of biofertilizer co-immobilized with vermicompost (IBV) and FYM (IBFY) over NF, CF and BF was recorded as 77.43 and 75.26, 21.83 and 14.29, 57.41 and 53.30%, respectively, 90 DAS and when cow dung used as a carrier (IBC) nitrate content increased by 68.13 and 39.84% significantly over NF and BF, 90 DAS. The co-immobilized formulations enhance the nitrate availability in soil and nutrient uptake by plant roots and it significantly translocate into the plants leaves as well (Fig. 5c).

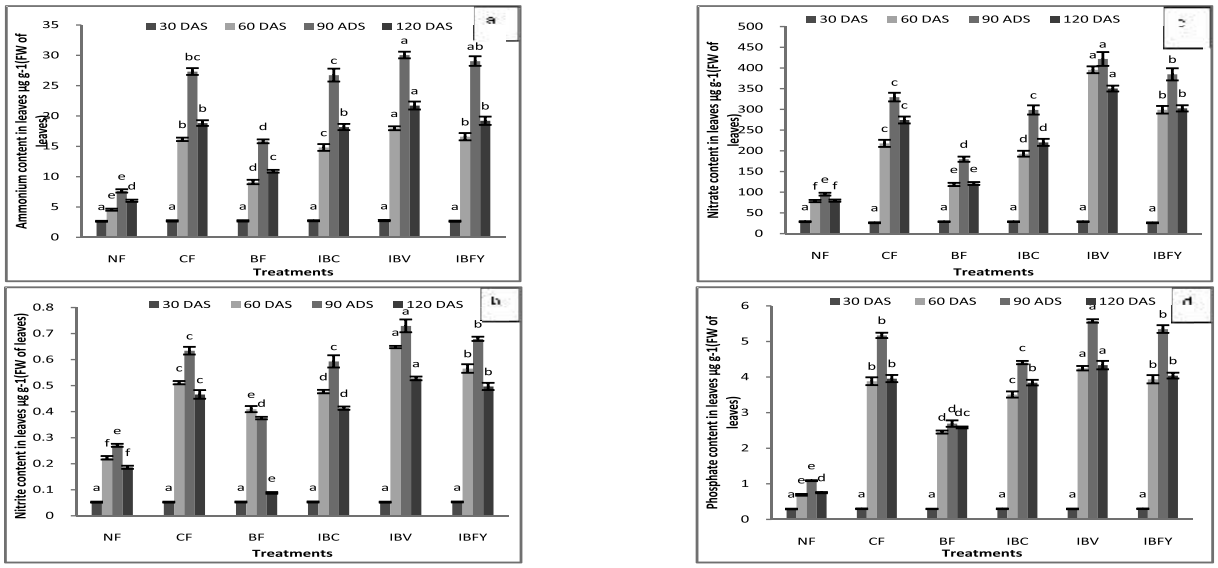

Figure 5a-d: Effect of biofertilizers co-immobilized with different organic matrix on the availability of a- ammonium, b-nitrite, c- nitrate, d- phosphate in leaves on 30, 60, 90 and 120 DAS. One way ANOVA was performed to compare the means of different treatments at $p<0.05$. Values by different letters are significantly differences between the treatments at 5% level.

3.6 Availability of nitrite content in the soil and plant parts on application of super granules of biofertilizers co-immobilized with organic matrix

Co-immobilization of biofertilizers with vermicompost in form of super granules (IBV) increased the nitrite content in soil. The nitrite content was recorded increased significantly by 71.26, 10.28, 35.04% and 69.25, 9.19, 35.81% and 63.41, 15.71, 44.71% after 60, 90 and 120 DAS respectively over controls of NF, CF and BF. Similarly the applications of co-immobilized biofertilizers with FYM (IBFY) increased nitrite content by 68.62, 67.22% and with cow dung (IBC) increased by 65.92 and 64.59% after 60 and 90 DAS over control of NF. Application with super granules of IBFY increased nitrite content by 29.08, 31.57% and IBC by 22.99 and 26.09% after 60 and 90 DAS respectively over control of charcoal based commercialized biofertilizers (BF) during cultivation (Fig. 3b).

The percentage increase of nitrite content in plant roots by the application of co-immobilized biofertilizers with vermicompost used as a carrier (IBV) was followed by FYM as a carrier (IBFY) and cow dung as a carrier (IBC) over controls of NF, CF and BF at different days of intervals. Application of IBV supergranules increased nitrite content by 8.97, 19.14% over the supergranules of IBFY and 19.23, 28.36% over the supergranule of IBC after 60 and 90 DAS respectively (Fig. 4a).

The increased nitrite content in leaves by application of co-immobilized biofertilizers with vermicompost as organic carrier (IBV) after 90 DAS was increased by 62.96, 13.16 and 48.55% and with FYM (IBFY) was 60.23, 6.77 and 44.77% over controls of NF, CF and BF. Application of co-immobilized biofertilizers with FYM as a carrier (IBC) also increased percentage of nitrite content by 54.39 and 36.65% over controls of NF and BF after 90 DAS (Fig. 5b).

3.7 Availability of phosphate content in the soil and plant parts on application of super granules of biofertilizers co-immobilized with organic matrix

In rhizosphere the phosphate content was recorded highest on application of co-immobilized biofertilizers with vermicompost as a carrier (IBV) and was increased by 88.35, 5.56 and 27.46%, and on application of biofertilizers co-immobilized with FYM (IBFY) increased by 87.74, .66 and 23.70% over controls of NF, CF and BF after 90 DAS respectively (Fig. 3d). The applied supergranules enhanced phosphate concentration in rhizosphere making them available for various living activities.

The available phosphate content from rhizospheric soil was uptake by the roots on application of vermicompost co-immobilized with biofertilizers. The increased percentage of phosphate content was 92.76, 13.84 and 64.90% by supergranule of IBV over controls of NF, CF and BF at the 90 DAS. The increased phosphate content percentage also increased on application of co-immobilized biofertilizers with FYM (IBFY) by 91.91, 3.37 and 60.76%, 90 DAS over controls of NF, CF and BF. Similarly the application with co-immobilized biofertilizers with cow dung (IBC) increased the phosphate content over controls of NF and BF (Fig. 4d).

Phosphate content in plant leaves was recorded increased by 83.77, 80.61% and 8.49, 7.36% and 42.21, 51.70% over controls of NF, CF and BF after 60 and 90 DAS respectively when IBV i.e. vermicompost based supergranules of biofertilizers applied and enhanced the nutrient availability in soil. Similar results were observed on application of biofertilizers co-immobilized with FYM (IBFY) and with cow dung (IBC) that increased the phosphate content in plant leaves over NF, CF and BF at different days of intervals (Fig. 5d).

4. Discussion

The experiment demonstrated that co-immobilization of commercial biofertilizers *A. chroococcum* and B. *subtilis* with organic matrix to formulate supergranules provided suitable carrier for storage and application in soil that subsequently increased the efficacy of these biofertilizers as well as growth, nutrient availability, biomass generation and grain yield of wheat (*Triticum aestivum.* L. c.v. 502) over the conventional non-immobilized commercial form of the biofertilizers. The biofertilizers co-immobilized with vermicompost, used as organic matrix in the supergranules showed maximum increase in growth and yield of wheat in comparison to FYM and cow dung. The vermicompost has been reported to be a good source of nutrients for the growth of plants (Channabasanagowda *et al.,* 2008). Organic matrix based immobilized biofertilizers enhance the productivity and grain yield of wheat in comparison to the conventional chemical fertilizers (Gopinath *et al.,* 2008; Patra *et al.,* 2009; Zaman *et al.,* 2011; Yang *et al.,* 2011; Kumar *et al.,* 2014a; 2015a, b). An increased nutrient levels have been found in soil and plant leaves in our experiments too, which demonstrates increase in nutrient status in plants rhizosphere and wheat leaves.

The use of biofertilizers and organic matrix to maintain soil fertility in crop field have been studied for wheat to a great extent (Al-Amoudi and Moujahed, 2006; Crecchio *et al.,* 2007; Gopinath *et al.,* 2008; Sarwar *et al.,* 2008; Shah *et al.,* 2009; Sharma *et al.,* 2011; Kumar *et al.,* 2015a, b). The organic amendments enhanced the microbial biomass, microbial activity and nutrient availability significantly for all nutrients in comparison to the chemical fertilizers (Hasanuzzamau *et al.,* 2010; Sharma *et al.,* 2011; Singh *et al.,* 2011; Kumar *et al.,* 2011a 12b). Kizilkaya *et al.* (2008) have proved that *A. chroococcum* showed good efficiency to increase the production of grain yield in wheat as it has the ability to fix atmospheric free nitrogen into available form i.e. NH_4^+ for plant uptake (Cocking, 2003 Kristensen *et al.,* 2009; Ahmed *et al.,* 2011; Ardakani *et al.,* 2011; Yadav *et al.,* 2011; Sharma *et al.,* 2012; Sapkota *et al.,* 2014). The PSB solubilised the phosphorus content and provided essential nutrient for plants growth (Cakmakic, 2006; Browne *et al.,* 2009; Chang *et al.,* 2009; Panhwar *et al.,* 2012).

Developmental strategies found to improve beneficial microbes functioning by the use of organic matrix carriers such as cow dung, vermicompost and FYM (Fig.-1). Organic matrix provides the suitable substrate for nutrient availability, maintain the microbial population and a better shelter (Acevedo and Pire, 2004; Dominguez, 2004; Ferreras *et al.,* 2006; Gopinath *et al.,* 2008; Lazcano *et al.,* 2008; Dominguez *et al.,* 2010). There are organic matrixes such as cow dung, FYM, poultry manure, kitchen waste, agro-waste, green manure etc. (Szczech and Smolinska, 2001; Atiyeh *et al.,* 2002; Tilman *et al.,* 2002; Reddy and Ohkura, 2004; Anwar *et al.,* 2005; Lores *et al.,* 2006; Wang *et al.,* 2010; Warman and Anglopez, 2010). But the vermicompost is selected by many researchers as it is obtained from decomposed organic waste by earthworms by the process of vermicomposting technology that improves the quality of organic matrix (Canellas *et al.,* 2002; Dominguez, 2004;

Hidalgo *et al.,* 2006; Aira and Dominguez, 2007; Bachman and Metzger, 2007; Arancon *et al.,* 2008; Vivas *et al.,* 2009; Dominguez *et al.,* 2010; Lazcano and Dominguez, 2010). Vermicompost, especially rich in nitrogen, phosphorus and potassium (NPK) and micronutrients, provides beneficial nutrient profile for growth of soil microbes and a good shelter too (Bhattacharjee *et al.,* 2001; Bulluck *et al.,* 2002; Arancon *et al.,* 2006; Channabasanagowda *et al.,* 2007; Campitelli and Ceppi, 2008; Vivas *et al.,* 2009; Kizilkaya *et al.,* 2012). Vermicompost increases the population of beneficial microorganisms such as N-fixers, P-solubilizers and increases the nitrogenase and urease enzyme activity and influence the growth and yield of wheat (Arancon *et al.,* 2006; Channabasanagowda *at el.,* 2008; Kijilkaya *et al.,* 2012; Kumar *et al.,* 2015a, b; Sharma *et al.,* 2012).

Studies have shown that the addition of vermicompost to soil significantly reduces the incidence of protozoans, insect, pests, bugs, aphids, beetles, and spider mites (Rao, 2002; Edwards *et al.,* 2004, 2006; Yardim *et al.,* 2006; Arancon *et al.,* 2007). Vermicompost reduces not only the degree of plant infestation, but also the populations of plant parasitic insects in soils (Arancon *et al.,* 2005b, 2007). Vermicompost may also have significant effects on both the incidence and abundance of plant parasitic nematodes in soil and reported to result in significant decrease in the egg mass of *Meloidogyne javanica* after the application to the growth medium (Ribeiro *et al.,* 1998; Arancon et al., 2003). The amendment of biofertilizers with vermicompost in the soil rhizosphere can increase the bacterial population considerably due to nutrient availabiliy. It has been demonstrated that *A. chroococcum* are metabolically versatile and can grow vigorously in the presence of nitrogenous compounds. Vermicompost increase microbial biomass in soil and changes the diversity and abundance of soil fauna (Gunadi *et al.,* 2002; Arancon *et al.,* 2006; Prabha *et al.,* 2007) and thus a broader range of organisms may act as biocontrol agents (Bulluck *et al.,* 2002; Brussaard *et al.,* 2007). Reductions in disease incidence are sometimes accompanied by an increase in the production of defence substances by the plant (Singh *et al.,* 2003; Edwards *et al.,* 2004, 2006; Yardim *et al.,* 2006; Arancon *et al.,* 2007), thus suggesting the induction of plant systemic resistance by vermicompost (Dominguez *et al.,* 2010).

Application of microbiologically active organic substrate may have important effects on soil enzyme activity in comparison to other organic fertilizers (Marinari *et al.,* 2000; Arancon *et al.,* 2005; Arancon *et al.,* 2006; Prabha *et al.,* 2007; Saha *et al.,* 2008; Lazcano *et al.,* 2011). Vermicompost therefore has a rather different microbial community structure than the parent waste, with lower biomass and activity but with enhanced metabolic diversity (Lores *et al.,* 2006; Aira *et al.,* 2007; Lazcano *et al.,* 2008). Co-immobilized form of biofertilizers with organic matrix i.e. vermicompost enhanced the nutrient availability to the plants for better growth and grain yield (Gajalakshmi and Abbasi, 2004; Shaharoona *et al.,* 2008; Boonsiri *et al.,* 2009; Singh *et al.,* 2011; Kumar *et al.,* 2012; Minaxi *et al.,* 2013; Ashok *et al.,* 2014; Kumar *et al.,* 2014a, b; 2015a, b).

5. Conclusions

The nutrient availability and plant growth parameters were significantly affected by

the use of different kind of organic matrix to co-immobilize biofertilizers and applied in the form of supergranules. The organic inputs tested in this experiment performed well as compared to the inorganic fertilizers in terms of nutrient availability, plant growth and grain yield. However, performances of the commercially available inorganic fertilizers namely DAP and urea with the natural organic manures/composts was well studied. The organic formulations applied in combination with different organic matrix were found to produce higher grain yield compared to inorganic fertilizers. The co-immobilized form of biofertilizer was able to produce highest nutrient availability for plant growth. IBV significantly shown the highest plant growth production in comparison with others applied fertilizers. Hence, these can be recommended as an option for indiscriminate use of inorganic fertilisers.

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Methane Hydroxylation by Axially Ligated Iron (IV)-oxo Porphyrin Cation Radical Models

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Abstract

Methane hydroxylation is a thermochemically difficult process due to the strength of the C–H bond that needs to be broken in the process. In Nature only the methane monoxygenases have a catalytic center that is active enough to perform this task. Other metalloenzymes, such as, mononuclear iron monoxygenases and dioxygenases, including the cytochromes P450, are not known to catalyze methane hydroxylation. The cytochromes P450 contain an iron heme group that in a catalytic cycle is converted into an iron(IV)-oxo heme cation radical (Compound I). To gain insight into the features that affect methane hydroxylation by Compound I and synthetic model complexes, we have done a detailed computational study. Thus, we investigated the chemical properties of iron(IV)-oxo porphyrins with varying axial ligands, including SH^-, F^-, OH^-, CN^-, CF_3COO^- and CH_3COO^-. In addition, we calculated the methane hydroxylation pathways for a selection of these oxidants and rationalize the obtained trends with thermochemical cycles and valence bond schemes. In general, the rate determining hydrogen atom abstraction barrier is dependent on the π_{xz}/π^*_{xz} energy splitting along the Fe–O bond, the excitation energy from π_{xz} to a_{2u}, as well as the bond dissociation energies of the methane C–H bond and the newly formed O–H bond. Our studies predict that iron(IV)-oxo porphyrin cation radical models with hydroxide as axial ligand should be efficient oxidants of substrate hydroxylation reactions and able to activate methane at room temperature. However, changing the axial ligand to a weaker electron donating group decreases its activity and raises the hydrogen atom abstraction barriers dramatically. These studies show that subtle modifications to the oxidant can have a great impact on the catalytic ability of the active center.

Keywords: cytochrome P450, Compound I, hydroxylation, hydrogen abstraction, valence bond, density functional theory.

1. Introduction

Methane hydroxylation is a challenging chemical process that few enzymatic systems are able to perform due to the strength of the methyl C–H bond that needs to be broken in the process. Nature has developed only a single class of enzymes that perform this task efficiently, namely the methane monoxygenases (Balasubramanian *et al.,* 2007; Shiota *et al.,.,* 2009; Himes *et al.,* 2010). These are generally dicopper enzymes that utilize molecular oxygen and convert methane to methanol. Nature utilizes a large range of metalloenzymes with a broad functional scope, yet most of these enzymes cannot activate methane. For instance, non-heme iron dioxygenases are efficient oxidants of hydrogen atom abstraction and oxygen atom transfer reactions and therefore are well studied. In particular, the non-heme iron enzymes taurine/α-ketoglutarate dioxygenase (de Visser,

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2006a,b; de Visser, 2007a; de Visser, 2009), prolyl-4-hydroxylase (Karamzadeh *et al.,* 2010) and cysteine dioxygenase (Aluri *et al.,* 2007; de Visser *et al.,* 2009a; Kumar *et al.,* 2011a; Kumar *et al.,* 2012a; Sallmann *et al.,* 2015) are known to react with substrates efficiently with large turnover numbers. Our computational modelling predicted relatively small hydrogen atom abstraction barriers of methane by the high-valent iron(IV)-oxo intermediate of taurine/α-ketoglutarate dioxygenase (Latifi *et al.,*., 2009), but so far no experimental support of methane hydroxylation by non-heme iron enzymes exists. Similarly, heme monoxygenases, which are considered as highly efficient in hydrogen atom abstraction processes, do not naturally utilize methane during their activity either (Meunier *et al.,* 2004; Abu-Omar *et al.,* 2005; Kryatov *et al.,* 2005; Shaik *et al.,* 2005; de Visser *et al.,* 2011a).

Cytochrome P450 enzymes are heme-based monoxygenases found in all forms of life and have important biochemical functions that essentially include the chemical modification of substrates. Thus, they are involved in the biosynthesis of hormones, such as estrogen.In addition, the P450s catalyze the metabolism of drugs (Ji *et al.,* 2015) and the detoxification of xenobiotics in the liver (Munro *et al.,* 2007). The P450s have a characteristic structure with a central protoporphyrin IX group (heme) that is attached to the protein via an Fe–S linkage using a conserved cysteinate residue of the protein; the axial ligand. Figure 1 displays the active site structure of the mammalian P450 isozyme $P450_{2C5}$ with the drug molecule diclofenac bound, as taken from the crystal structure coordinates (Wester *et al.,* 2003). The substrate is located in a tight binding pocket and is positioned in such a way that promotes a regioselective and stereospecific oxidation. The heme is linked to the protein via an interaction of the metal with a cysteinate group in the axial position. In addition, the heme is placed in position through a number of hydrogen bonding interactions, for instance, toward the propionate side chains of the heme. Molecular oxygen binds *trans* to the cysteinate moiety, i.e. on the distal site.Prior to substrate hydroxylation, however, the heme needs to be activated and an active species is generated. This process includes the binding of molecular oxygen, two reduction and two protonation steps and ultimately leads to the formation of a high-valent iron(IV)-oxo heme cation radical species, called Compound I (Cpd I) (Meunier *et al.,* 2004; Shaik *et al.,* 2005; de Visser *et al.,* 2003). The P450s, therefore, have an intricate hydrogen bonding network surrounding the heme active site that delivers protons (Kumar *et al.,* 2005b). Interestingly, hydrogen bonding interactions, for instance through water molecules, were found to affect also reaction barriers of substrate activation, and, hence they are often non-innocent (Sharma *et al.,* 2003b; Sharma *et al.,* 2004; Kumar *et al.,* 2005c). Moreover, the spin state ordering and relative energies often gives a dependence on environmental effects, such as hydrogen bonding (Sahoo *et al.,* 2015) or a point charge (de Visser, 2005).

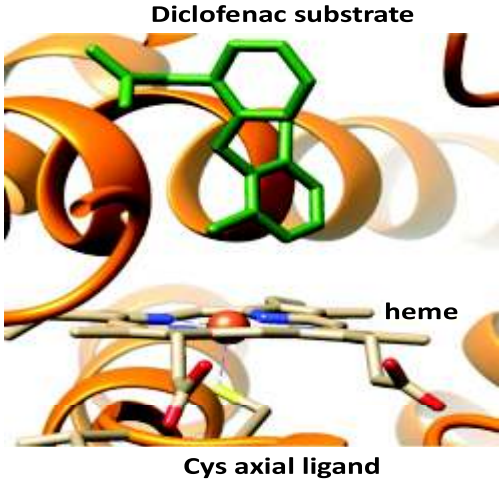


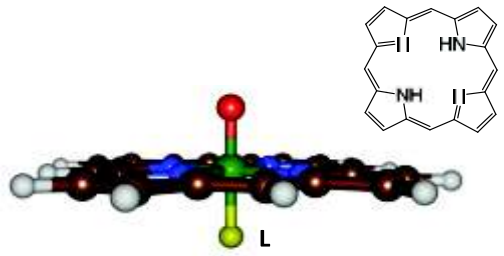
Figure 1: Extract of the active site of $P450_{2C5}$ with substrate diclofenac bound.

P450s are efficient oxidants in nature and react with substrates via aliphatic hydroxylation (Shaik *et al.,* 2005), aromatic hydroxylation (de Visser *et al.,* 2003b; de Visser 2006e; de Visser *et al.,*

2007; Hazan*et al.,* 2007; de Visser *et al.,* 2009b; Kumar *et al.,* 2012b; Latifi *et al.,* 2012a; Sahu *et al.,* 2013), olefin epoxidation (de Visser*et al.,* 2001a,b,c; Ogliaro *et al.,* 2002b; de Visser *et al.,* 2002a,b; de Visser *et al.,* 2003c; de Visser *et al.,* 2004b; Kumar *et al.,* 2005a,d; de Visser 2006a,b,c,d,g; de Visser 2008; de Visser *et al.,* 2008b; Kumar *et al.,* 2010; Kumar *et al.,* 2013;Sainna*et al.,* 2015a)) and sulfoxidation (Sharma *et al.,* 2003b; Kumar *et al.,* 2005e; Kumar *et al.,* 2011b; Prokop *et al.,* 2011).

Enzymes with a structure closely resembling the P450 active site features, are, for instance, the peroxidases and catalases, but these systems lack the cysteinate axial ligand of P450.Instead, the peroxidases and catalases contain either a histidine or a tyrosinate axial ligand, respectively (Veitch *et al.,* 2000; Vidossich *et al.,* 2012). The difference in chemical structure of the P450s, on the one hand, and peroxidases and catalases, on the other hand, has led to speculation that the axial ligand is responsible for the change in reactivity and the chemical properties of these enzymes. Indeed, quantum chemical calculations of Cpd I with either a cysteinate, a histidine or a tyrosinate axial ligand as mimics of Cpd I of P450, horseradish peroxidase and catalase, respectively, showed dramatic differences in their electron affinities, and, consequently their ability to pick up electrons from substrates (de Visser *et al.,* 2003a; de Visser 2005; de Visser 2006f). This was further highlighted through a push-effect of electron density from the axial ligand upon the heme and, in particular, the mixing of a lone pair of the axial ligand with the heme a_{2u} orbital (Ogliaro *et al.,* 2002a).

In order to understand spectroscopic and reactivity differences of these enzyme classes, synthetic model complexes have been developed: the so-called biomimetic models (Bruijnincx *et al.,* 2008; Costas 2011). Thus, Gross *et al.,* (Gross *et al.,* 1994) showed that for synthetic iron(III)-porphyrins, there was an axial ligand effect on the reactivity differences of the various complexes but there also were spectroscopic differences as a result of binding an alternative axial ligand. Theoretical modelling can assist in understanding the fundamental differences in reactivities and establish the key factors that determine the reaction mechanism. To gain insight into the axial ligand effect of iron-porphyrins, we investigated the properties and reactivities of a range of iron(IV)-oxo porphyrin cation radical models with varying axial ligand description:**1**$_X$, X = F^-, Cl^-, CN^-, OH^-, CH_3COO^-, CF_3COO^- and SH^-, see Figure 2. We then studied the reaction of a selection of these oxidants with methane on the doublet and quartet spin state surfaces and compare reactivity differences and how this is affected by the axial ligand of the oxidant.



L = F^-, Cl^-, CN^-, OH^-, CH_3COO^-, CF_3COO^-, SH^-

Figure 2: Chemical structure of the reactants studied in this work.

2. Methods

The studies presented in this work use density functional theory methods as implemented in the *Gaussian*-03 program package (Frisch *et al.,* 2013). Following previous experience in the field (Quesne *et al.,* 2014; de Visser *et al.,* 2014; Sainna *et al.,* 2015b), we use the unrestricted hybrid density functional method UB3LYP (Becke, 1993; Lee *et al.,* 1988) in combination with a double-ζ quality LACVP basis set on iron and 6-31G on the rest of the atoms: basis set BS1 (Hay *et al.,* 1985). We performed a full geometry optimization (without constraints) followed by an analytical frequency. All reactant structures were confirmed as local minima and had no imaginary

frequencies. Single point calculations using a triple-ζ quality LACV3P+ basis set on iron and 6-311+G* on the rest of the atoms energies were used throughout: basis set BS2. All data reported here include zero-point corrections and solvent corrections using an acetonitrile solvent.

Subsequently, we investigated methane hydroxylation by several iron (IV)-oxo oxidants. We optimized a reactant complex, radical intermediate and product complex at UB3LYP/BS1 on the doublet and quartet spin state surfaces. Extensive geometry scans linking reactants with radical intermediates and of radical intermediates with products were performed. The maxima of these scans were used as starting points for the transition state searches and established first order saddle points characterized by a single imaginary frequency for the correct mode.

Previously, we tested the effect of substituents to the periphery of the porphyrin scaffold and found few changes to the chemical structures and reactivities (Kumar *et al.,* 2009; Neu *et al.,* 2014). Furthermore, full geometry optimization with basis set BS2 reproduced all calculations well. The methods and procedures used here were extensively tested and benchmarked and, for instance, were found to reproduce experimental rate constants within about 4 kcal mol^{-1} (Vardhaman *et al.,* 2011; Vardhaman *et al.,* 2013a; Jastrzebski *et al.,* 2014) but also reproduce experimental infrared spectra (de Visser 2008,) and resonance Raman spectra (Draksharapu *et al.,* 2015).

3. Results and Discussion

Before going through the reactivity studies, let us first discuss one of the reactant complexes, namely ($Fe^{IV}(O)(Por^{+\bullet})F$) or $\mathbf{1}_F$, in more detail as an example of a Compound I model. Figure 3 displays the high-lying occupied and low-energy virtual orbitals that are relevant for the electronic description and the subsequent reactivity with methane. The molecular orbitals follow close analogy to those found for P450 Compound I (Ogliaro *et al.,* 2000b; Ogliaro *et al.,* 2001a,b; Sainna *et al.,* 2015a). The metal orbitals form bonding and antibonding pairs of orbitals, e.g. there is a pair of π_{xz}/π^*_{xz} set of orbitals for the interaction of the $3d_{xz}$(Fe) with $2p_x$(O). Similarly, the atomic $3d_{yz}$(Fe) and $2p_y$(O) mix to form the π_{yz}/π^*_{yz} pair of orbitals. In addition, there is a nonbonding orbital in the plane of the porphyrin ring: δ_{x2-y2}. Finally, two virtual orbitals for the σ* interactions along the O–Fe axis (σ^*_{z2}) and in the plane of the porphyrin ring (σ^*_{xy}) complete the set of iron-type orbitals. In addition to the metal-type orbitals there are two π-orbitals for the conjugated porphyrin system high-lying, which under D_{4h} symmetry have the labels a_{1u} and a_{2u}. The latter orbital strongly mixes with a lone-pair orbital on the axial ligand and influences the electron affinity of the oxidant strongly, see below.

The set of orbitals displayed in Figure 3 is occupied with the following configuration: ^{4,2}Compound I = $\pi_{xz}^2\pi_{yz}^2\delta_{x2-y2}^2\pi^*_{xz}{}^1\pi^*_{yz}{}^1\sigma^*_{z2}{}^0\sigma^*_{xy}{}^0a_{1u}^2$ a_{2u}^1. The three unpaired electrons in the system are either ferromagnetically coupled into a quartet spin state ($^4A_{2u}$ state) or the two π* electrons are up-spin and the a_{2u} electron down-spin to give it an overall doublet spin state ($^2A_{2u}$ state). These two spin states are close in energy and for all systems considered here they fall within 1 kcal mol^{-1}.

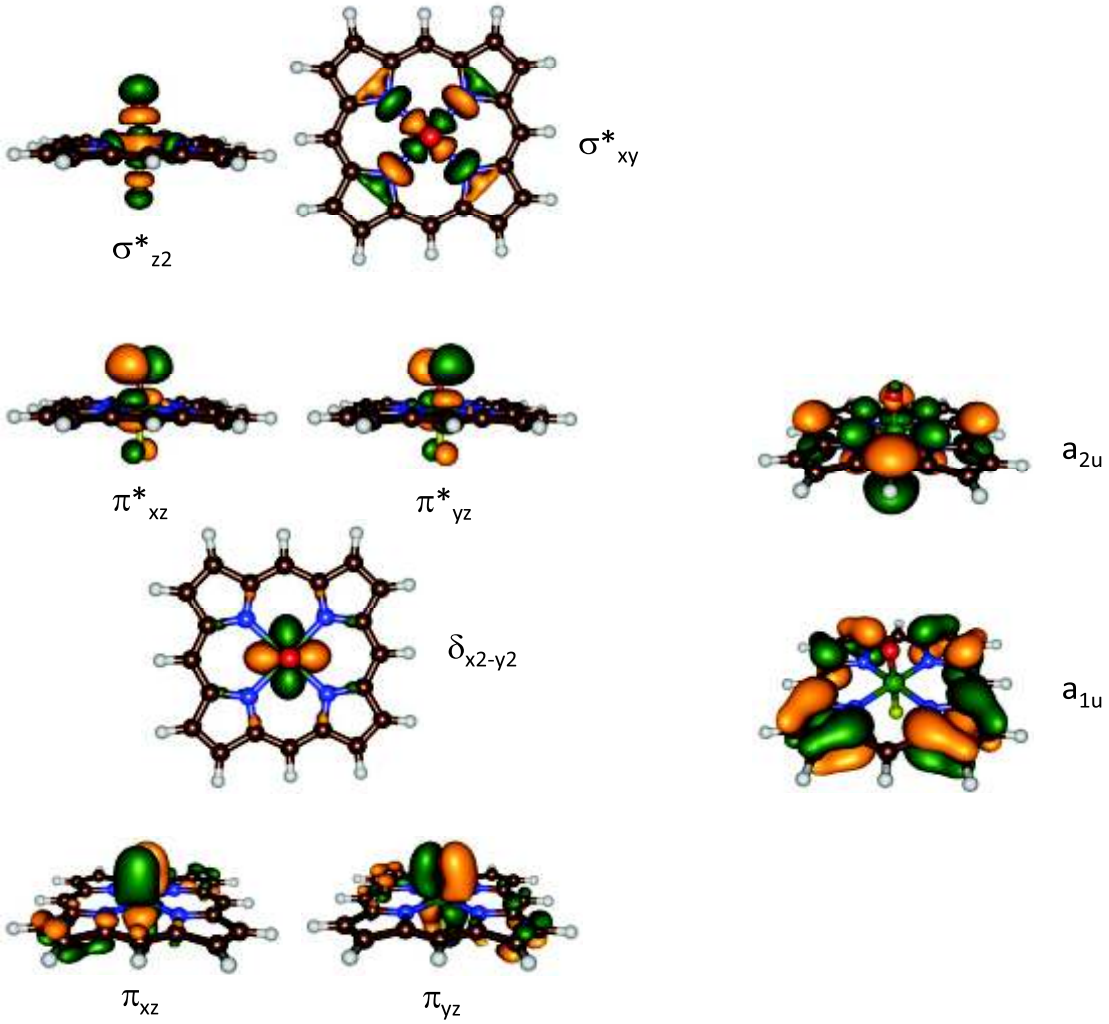


Figure 3: Molecular orbitals of $\mathbf{1}_F$.

Next, we did a geometry optimization of doublet and quartet iron(IV)-oxo porphyrin cation radical models with varying axial ligands, namely, $^{2,4}\mathbf{1}_X$ with X = CF_3COO^-, CH_3COO^-, Cl^-, F^-, ImH (imidazole), acetonitrile, OH^- and SH^-.Key geometric and vibrational parameters obtained for the doublet spin state structures are given in Figure 4. As follows from the data shown in Figure 4, the Fe–O distances vary strongly dependent on the axial ligand bound to the iron-porphyrin system. Thus, an axially ligated fluoride ligand gives a relatively long Fe–O distance of 1.681 Å, whereas a neutral acetonitrile molecule bound gives a much shorted Fe–O bond of 1.645 Å. This is further reduced to 1.630 Å when the axial ligand is removed altogether (Sainna *et al.,* 2015a). The opposite trends are found for the Fe–O stretch vibration (n_{FeO}), whereby an axial thiolate ligand gives the highest mode at 870.6 cm^{-1}. Strong electron-donating axial ligands, such as F^- and OH^-, by contrast, give dramatically reduced Fe–O stretch vibrations of the order of 817.7 and 816.6 cm^{-1}, respectively. Note that without an axial ligand the Fe–O vibration is located at 920.0 cm^{-1}. Consequently, the axial ligand affects the strength of the Fe–O bond and as a result the Fe–O frequency is shifted by about 100 cm^{-1}. This will have major effects on the oxygen atom transfer reaction with substrates.

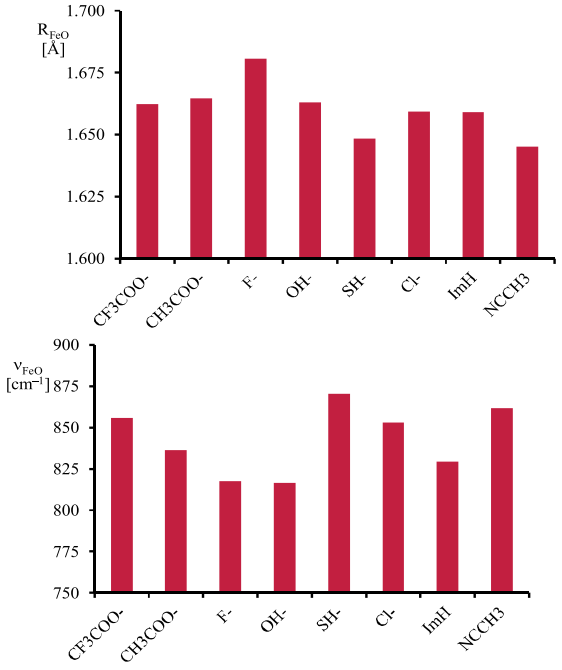


Figure 4: (a) Fe–O bond distance (R_{FeO}) for various complexes $^{2}\mathbf{1}_{X}$. (b) Fe–O stretch vibration ($_{NuFeO}$) for various complexes $^{2}\mathbf{1}_{X}$.

We subsequently investigated the methane hydroxylation mechanism by $^{2,4}\mathbf{1}_{X}$ with X = OH^{-}, F^{-} and CN^{-}. As an example, the potential energy profile of methane hydroxylation by $^{2,4}\mathbf{1}_{OH}$ is shown in Figure 5, the results for $^{2,4}\mathbf{1}_{F}$ and $^{2,4}\mathbf{1}_{CN}$ are very similar. The reaction is stepwise with an initial hydrogen atom abstraction via transition state $\mathbf{TS}_{HA}$ leading to an iron(IV)-hydroxo complex with a radical, **IM**. The OH and radical are combined via a rebound transition state $\mathbf{TS}_{reb}$ to form the alcohol products **P**. This mechanism is analogous to earlier hydrogen atom abstraction reactions calculated for similar complexes and substrates (Ogliaro *et al.,* 2000; de Visser *et al.,* 2002a,b; Sharma *et al.,* 2003a; Kumar *et al.,* 2003; Kumar *et al.,* 2004a,b; de Visser *et al.,* 2004a; Shaik *et al.,* 2004b; Kumar *et al.,* 2005a; de Visser 2006a,b,c,d,e; Wang *et al.,* 2007; de Visser, 2007b; de Visser *et al.,* 2008a; Godfrey *et al.,* 2008; Shaik *et al.,* 2008; Heyes *et al.,* 2009; Kim *et al.,* 2009; de Visser *et al.,* 2009; Latifi*et al.,* 2009; Tahsini *et al.,* 2009; Kumar *et al.,* 2009; de Visser, 2010; Prokop *et al.,* 2010; Karamzadeh *et al.,* 2010; de Visser *et al.,* 2011b; Latifi *et al.,* 2011a,b; de Visser 2012; Latifi *et al.,* 2012a,b; de Visser, 2013; de Visser *et al.,* 2013; Latifi *et al.,* 2013; Pratter *et al.,* 2013; Vardhaman *et al.,* 2013b; Kumar *et al.,* 2014; Karamzadeh *et al.,* 2014; Ji *et al.,* 2015). As described before, the rebound barriers are small on the high-spin surface and negligible on the low-spin surface, which is also seen here (Shaik *et al.,* 2004a).

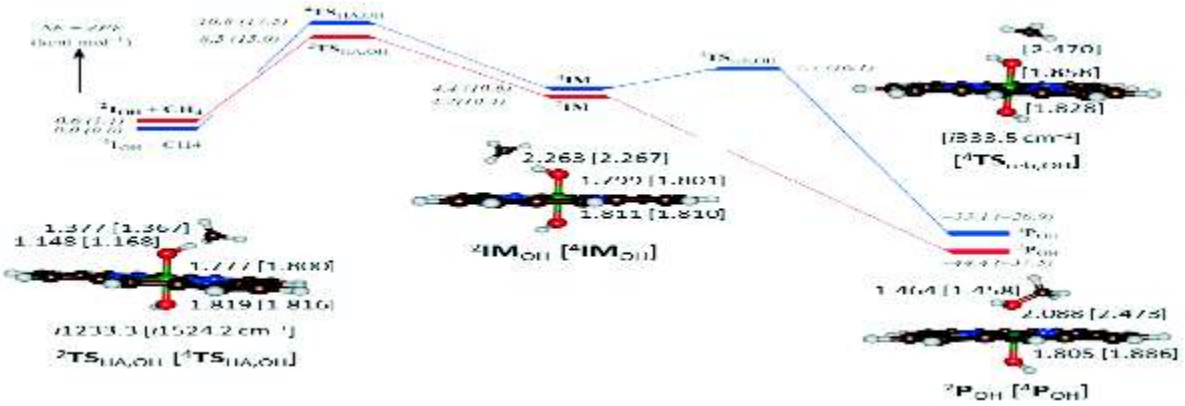


Figure 5: Potential energy landscape of methane hydroxylation by $^{2,4}\mathbf{1}_{OH}$ with energies in kcal mol^{-1}. All energies obtained with UB3LYP/BS2//UB3LYP/BS1 and contain ZPE corrections. Values given out of parenthesis refer to gas-phase data and those inside parenthesis include solvent corrections. Optimized geometries of local minima and transition states give bond lengths in angstroms and the imaginary frequency in the transition state in wave numbers.

For $^{2,4}\mathbf{1}_{OH}$, small hydrogen atom abstraction barriers of 8.5 (10.8) kcal mol^{-1} on the doublet (quartet) spin states are found in the gas-phase, whereas those with a dielectric continuum included with Σ = 4.7 are raised to 15.0 (17.5) kcal mol^{-1}. Nevertheless, these barriers are accessible at room temperature and imply that $^{2,4}\mathbf{1}_{OH}$ should be able to hydroxylate methane efficiently. The hydrogen atom abstraction barriers are narrow with a large imaginary frequency of *i*1233.3 (*i*1524.2) cm^{-1}, which implies they will proceed with a large kinetic isotope effect for replacing the transferring hydrogen atom by deuterium (Kumar *et al.,* 2004a; de Visser, 2006e). Geometrically, the barriers are late with long C–H bonds and much shorter O–H bonds, similarly to H-atom abstraction barriers calculated before for methane by (Fe^{IV}(O)($Por^{+\bullet}$)SH), (de Visser *et al.,* 2004).

After H-atom abstraction a radical intermediate is formed representing an (Fe^{IV}(OH)(Por)OH) complexed to $CH_3^{\bullet}$ with a small exothermicity of 4.2 (4.4) kcal mol^{-1} for $^{2}\mathbf{IM}_{OH}$ ($^{4}\mathbf{IM}_{OH}$), respectively. The rebound barrier is negligible on the low-spin surface, whereas a OH rebound barrier of 3.5 kcal mol^{-1} is obtained in the high-spin state. The overall mechanism to form alcohols is strongly exothermic as expected.

Subsequently, we replaced the OH axial ligand of $^{2,4}\mathbf{1}_{L}$ by alternative ligands, namely L = F^- and CN^- and the results are given in Table 1. In general, the potential energy profile for all complexes follows the same pattern as that seen in Figure 5, with a rate determining hydrogen atom abstraction barrier and negligible rebound barrier at the low-spin pathway. The only differences obtained are due to the axial ligand interactions with the metal and oxygen atom.

Table 1: Relative energies (ΔE+ZPE, in kcal mol^{-1}) of local minima and transition states for methane hydroxylation by $^{2,4}\mathbf{1}_{L}$, L = OH^-, F^-, and CN^-. Values in the gas-phase and in solvent are in given.

	L = OH^-		L = F^-		L = CN^-	
	gas	solvent	gas	solvent	gas	solvent
$^{4}\mathbf{1}_{L}$	0.0	0.0	0.0	0.0	0.0	0.0
$^{2}\mathbf{1}_{L}$	0.6	1.1	2.1	2.6	0.6	1.1
$^{4}\mathbf{TS}_{HA}$	10.8	17.5	15.5	22.2	18.4	25.1
$^{4}\mathbf{IM}$	4.4	10.6	9.2	15.4	13.0	19.2
$^{4}\mathbf{TS}_{reb}$	7.7	16.1	ND	ND	ND	ND
$^{4}\mathbf{P}_{L}$	-33.1	-26.9	ND	ND	ND	ND
$^{2}\mathbf{TS}_{HA}$	8.5	15.0	26.2	32.7	16.3	22.8
$^{2}\mathbf{IM}$	4.2	10.4	9.0	15.2	13.2	19.4
$^{2}\mathbf{P}_{L}$	-44.4	-37.5	ND	ND	ND	ND

ND = not determined.

The studies presented in Table 1 implicate that the axial ligand has a profound effect on the catalytic efficiency and performance of the active oxidant. This is not surprising and has been seen before for the reactivity of iron (IV)-oxo porphyrin radical cation models with substrates (de Visser, 2006d; Wang *et al.,* 2007; de Visser *et al.,* 2009b; de Visser 2010; Kumar *et al.,* 2013). To understand the electronic features of the reaction mechanism and find factors that determine the rate constant we set up a valence bond (VB) curve crossing diagram, see Figure 6. Previously, we used the VB technique to rationalize the hydrogen atom abstraction reactions of heme and non-heme iron(IV)-oxo complexes (Shaik *et al.,* 2008; Latifi *et al.,* (2009); de Visser, 2010; Ji *et al.,* 2015) as well as double bond epoxidation reactions (Kumar *et al.,* 2010; Sainna*et al.,* 2015a) and sulfoxidation reactions (Kumar *et al.,* 2011b).

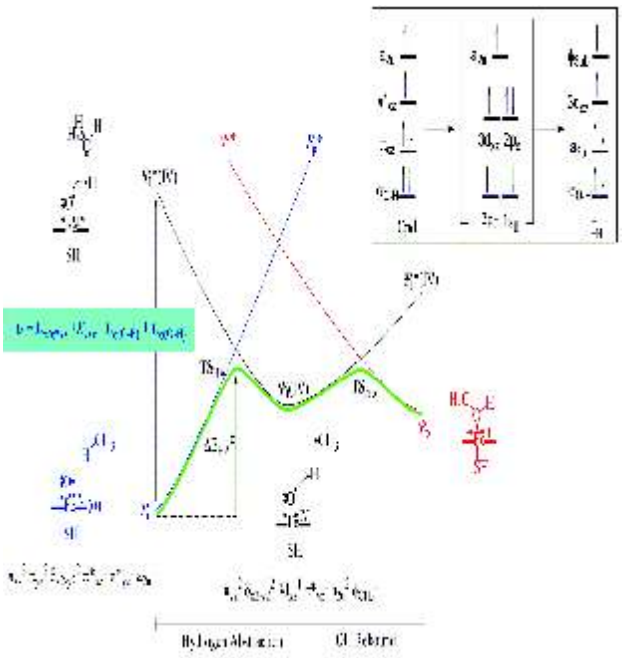


Figure 6: Valence bond curve crossing diagram rationalizing the methane hydroxylation reaction by P450 Cpd I. Dots represent valence electrons and lines (curved or straight) are chemical bonds.

Thus, in VB one starts on the left-hand-side with the reactant state Ψ'_r, which in our case is Compound I and substrate (methane). The reactant state is connected to an excited state in the radical intermediate as well as in the product geometry. Similarly, the product state Ψ'_p, although the ground state in the product geometry, is an excited state in the radical intermediate and reactant geometries. The radical intermediate state (Ψ'_I) bisects the reactant and product wave functions and creates a stepwise hydrogen atom abstraction-radical rebound mechanism by creating a stable radical intermediate. The height of the barrier for hydrogen atom abstraction ($\Delta E_{HA}^{\ddagger}$) is equal to the energy where the two curves cross, e.g. crossing point of the blue and black lines, minus the geometric distortion (resonance energy *B*) upon moving from reactants to the transition state. It has been shown previously (Shaik *et al.,* 2008) that the curve crossing energy is a fraction (usually with value of 0.3) of the excitation energy (*G*) from the reactant state (Ψ'_r) to the radical intermediate state (Ψ'_I*) in the geometry of the reactants. The analysis of the VB structures for the reactant and excited states enables one to determine the factors that influence the barrier. The VB structures give dots for key valence electrons. For instance, in Compound I the π_{xz}/π^*_{xz} set of orbitals has three electrons, which is given on the right-hand-side of the Fe–O bond with three dots. Similarly, the π_{yz}/π^*_{yz} pair of orbitals have three electrons and these are given with three dots on the left-hand-side of the Fe–O bond. In addition, there is of course a radical on the porphyrin ring for single occupation of the a_{2u} orbital. Two electrons occupy the C–H bond of the substrate.

A look at the VB structure of the radical intermediate shows the same π_{yz}/π^*_{yz} occupation with three electrons, however, the π_{xz}/π^*_{xz} set of orbitals has ceased to exist and has reverted back into atomic orbitals ($3d_{xz}$ and $2p_x$). The $3d_{xz}$ orbitals remains as a nonbonding orbital and singly occupied, while the $2p_x$ orbital on the oxo group pairs up with the 1s orbital of the incoming hydrogen atom to form the new O–H bond (σ_{OH}). As the π_{xz}/π^*_{xz} set of orbitals had three electrons, this implies that one of these electrons will have to move to another orbital and indeed fills the a_{2u} orbital with a second electron. This change in orbitals is summarized and highlighted in the inset of Figure 6.

As a result, the hydrogen atom abstraction barrier will depend on the energy to split the π_{xz}/π^*_{xz} orbitals back into atomic orbitals ($E\pi/\pi^*_{xz}$) and an electron excitation from π_{xz} to a_{2u} (E_{exc}). In addition, the excitation energy refers to the breaking of the C–H bond and the formation of the O–H bond. The bond dissociation energy (BDE_{CH}) of the C–H bond

of methane is ΔE+ZPE = 101.6 kcal mol^{-1} (Shaik *et al.,* 2008). We calculated the O–H bond strength from Eq 1 for $\mathbf{1}_L$ with L = F^-, Cl^-, OH^-, CH_3COO^-, CF_3COO^- and SH^-, see Table 2. As follows the strength of the O–H bond that is formed varies by as much as 14 kcal mol^{-1} between L = CF_3COO^- and L = OH^-.

$(Fe^{IV}(OH)(Por)L) \rightarrow (Fe^{IV}(O)(Por^{+\bullet})L) + H^{\bullet} + BDE_{OH}$ (1)

Table 2: Calculated thermochemical properties (ΔE+ZPE) for selected $\mathbf{1}_L$ species (L = CF_3COO^-, Cl^-, SH^-, CH_3COO^-, F^- and OH^-). All energies are in kcal mol^{-1}.

	BDE_{OH}	$EA(1_L)$	ΔH_{acid}	BDE_{FeL}	$E\pi/\pi^*_{xz}$	E_{exc}
CF_3COO^-	85.4	85.8	314.3	90.7	ND	ND
Cl^-	87.6	79.2	305.5	ND	ND	ND
SH^-	89.3	71.6	296.3	112.2	81.2	88.2
CH_3COO^-	89.4	80.5	305.0	103.6	75.5	84.8
F^-	94.0	78.9	298.8	128.4	86.3	86.0
OH^-	99.4	76.0	290.5	137.5	75.2	79.4

In addition to the BDE_{OH} values for the oxidants, we calculated several other thermochemical properties. Firstly, we calculated the electron affinity (EA) of all complexes $\mathbf{1}_L$ from the difference in energy of $^4\mathbf{1}_L$ and its one-electron reduced species ($^3\mathbf{1}_L^-$). Technically, a hydrogen atom transfer is the sum of an electron and a proton transfer, so that the BDE_{OH} can be split into a the electron affinity of the oxidant, the acidity of the iron(IV)-hydroxo complex (ΔH_{acid}) and the ionization potential of a hydrogen atom (IE_H), Eq 2. The latter we took from the NIST database (Linstrom *et al.,* 2014) and has a value IE_H = 313.9 kcal mol^{-1}.

$BDE_{OH} = EA(\mathbf{1}_L) - \Delta H_{acid} + IE_H$ (2)

As discussed above, the electron affinity of $\mathbf{1}_L$ is dependent on the amount of mixing of the axial ligand orbitals with the a_{2u} orbital on the porphyrin. With L = SH^- this interaction is strong and as a consequence $\mathbf{1}_{SH}$ has a very low EA of 71.6 kcal mol^{-1}. A weak anionic ligand like CF_3COO^- gives weak interactions with the a_{2u} orbital and has a considerably higher EA of 85.8 kcal mol^{-1}. Neutral axial ligands, such as a solvent molecule or a imidazole group of a histidine give a further rise in electron affinity and removal of the axial ligand altogether gives a maximum electron affinity of about 8.2 eV (Sainna *et al.,* 2015a) as determined by mass spectrometry.

The BDE_{OH} value of the various complexes depends on the electron affinity and ΔH_{acid} differences of the complex. As mentioned the electron affinity differences depend on the mixing of axial ligand and a_{2u} orbitals. The ΔH_{acid} value, however, contains an electronic component for the splitting of the π_{xz}/π^*_{xz} orbitals back into atomic orbitals and formation of the O–H bond. We analyzed the orbitals of the complexes in detail and determined the $E\pi/\pi^*_{xz}$ and E_{exc} energy gaps and report those in the last two columns of Table 2. Both parameters vary by as much as 10 kcal mol^{-1} for the four iron (IV)-oxo species studied. The smaller the π_{xz}/π^*_{xz} energy splitting and the smaller the excitation energy from π_{xz} to a_{2u}, the lower in energy the hydrogen atom abstraction barrier will be.

4. Conclusions

In this work a series of computational studies are presented on the methane hydroxylation mechanism of models of Compound I of cytochrome P450, whereby the axial ligand is replaced by alternative anionic ligands. The work shows that an axial ligand at a distance well over 4 Å from the reaction center has a profound effect on the catalytic properties of the oxidant. We identified several key components. Firstly, there are thermochemical impacts on the electron affinity and iron (IV)-hydroxo acidity due to differences in orbital

occupation. In addition, during the rate determining hydrogen atom abstraction the π-bond of the iron-oxo group needs to be broken and the π_{xz}/π^*_{xz} energy splitting affect the hydrogen atom abstraction. Finally, during the hydrogen atom abstraction step an electron migrates from the oxo group to the heme. In general, a hydroxide axial ligand gives small reaction barriers, and should be the best oxidant.

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Biocontrol of Ear Rot Fungi By Plant Growth Promoting Fluorescent Pseudomonads

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Abstract

In India, maize is the third most important food crop after rice and wheat but the productivity is severely affected as the plant is also prone to a number of diseases caused by fungi and bacteria. Ear rot caused by *Fusarium moniliforme* is one of the economically important soil and seed borne disease of maize and not easily controlled by chemical methods. Antagonistic bacteria may constitute an alternative for improving the crop productivity. In this study 13 fluorescent pseudomonads were isolated from rhizosphere of different plants and screened for plant growth promoting activities and biocontrol potential against *F. moniliforme* ITCC No. 2193. Among them a potential isolate, W3Gr-6(b) not only inhibited growth of *F. moniliforme* under in-vitro conditions but was also capable of maize growth promotion in presence of pathogen.

Key words: Maize, Ear rot, Pseudomonas, Bioformulation

1. Introduction

Maize *(Zea mays L.)* is one of the most versatile emerging crops having wider adaptability under varied agro-climatic conditions. It is cultivated on nearly 150-million-hectare land in about 160 countries having wide diversity of soil, climate, biota and contributes 36% that is 782 metric tons (MT) in the global grain production (Plessis, 2003). In India, about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (for example starch and oil production) and 1% as seed (Zaidi, 2010). Maize is often prone to several fungal pathogens. Among them *Fusarium* ear rot is the most damaging disease of corn (Davis, 1989; Nelson, 1992). *Fusarium* spp. cause diseases in corn such as ear rot *(F. moniliforme, F. verticillioides and F. proliferatum),* ear and stalk rot *(F. graminearum),* stalk rot *(F. verticillioides),* root rot (*F. graminearum* and *F. verticillioides*) and seedling blight (*F. graminearum* and *F. verticillioides*) (Munkvold, 2003). In India, *F. moniliforme* and *F. semitectum* are reported to be widespread in Uttar Pradesh, Punjab and Rajasthan (Lal and Dwivedi, 1982). For controlling the disease, farmers usually apply synthetic fungicides to the plants (Tagne, 2013). But reports reveal their post health and environmental issues which are very severe (Jacobsen and Backman, 1993). They can also increase the development of fungicide resistance to pathogens (Tarlochan, 2012). Now the application of microbes to control these phytopathogens is being explored as an alternative way to reduce the load of chemical fungicides and regarded as eco-friendly and cost-effective (Che and Jacob, 1994; Pal and Gardener, 2006; Khare and Arora, 2015). Among the bacteria applied in biocontrol of phyto-pathogenic fungi, fluorescent pseudomonads gained much attention of workers (Hass and Défago 2007; Mishra and Arora 2012; Tewari and Arora, 2014). They are major constituents of rhizospheric microbial

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flora and also show potential of plant growth promotion and biological control of different phytopathogens (Khare *et al.*, 2011; Bakthavatchalu *et. al.*, 2012; Tewari and Arora 2014). Present study was performed to isolate some fluorescent pseudomonads having plant growth promoting and biocontrol potential against phytopathogen *F. moniliforme* and to evaluate their effect on maize growth enhancement and disease suppression.

2. Material and Methods

2.1 Bacterial isolates

Fluorescent pseudomonads were isolated from the rhizospheric soil of corn plants growing in farmers fields in vicinity of Kanpur (6.5°N, 80.3°E, 142 m above mean sea level), UP (India) on King's B (KB) medium. Colonies showing fluorescence under ultra violet exposure were further characterized by their cultural, morphological and biochemical characteristics (Garrity, 2005). Isolates were stored on KB slant for further use.

2.2 Phytopathogen fungus

Phytopathogen fungi *Fusarium moniliforme* ITCC No. 2193 was procured from IARI, Plant Pathology Division, New Delhi (India) and maintained on Potato Dextrose Agar (PDA) (HiMedia, Mumbai) for further use.

2.3 In-vitro screening of bacterial isolates for plant growth promoting (PGP) and biocontrol activities

Indole acetic acid (IAA) production was detected by the modified method as described by Brick *et al.*, (1991). Bacterial strains were grown without any shaking for 48 h on minimal medium amended with 0·2g/l L-tryptophan at 28°C. Fully grown cultures were centrifuged at 10000 rpm for 10 min at 4°C. The supernatant (1 ml) was mixed with two drops of orthophosphoric acid and 2ml of the Salkowski reagent. Development of pink color indicates IAA production. All isolates were screened on Pikovskaya's agar plates for phosphate solubilization. Determination of solubilization index (S.I.) was performed by measuring the halo (clear zone) diameter and the colony diameter, using the formula described by Edi-Premono *et al.*, (1996). Siderophore activity of the isolates was determined on Chrome-Azural S (CAS) medium. CAS plates were spot inoculated with bacterial strains and observed for development of orange halo against dark blue background around the colonies after 48 h of incubation at 28±2°C (Pérez-Miranda *et al.*, 2007).

All the isolates were also screened for the production of hydrogen cyanide by method described by Bakker and Schippers (1987). HCN induction media was prepared by adding 4.4 g glycine/l in King's B medium and bacteria were streaked on modified medium. A Whatman filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed inside the upper lid of petri plates and sealed with parafilm and incubated at 28±2°C for 4 days. After incubation color change (yellow to brown) in filter paper was visually assessed against control.

2.4 In vitro antifungal activity

A 10 mm disk of a pure culture of *F. moniliforme* was placed at the centre of a petri dish containing PDA. A loopful of bacterial isolate was streaked on PDA, 1.5 cm from the edge of each plate. Plate was cultured for 72h at 28°C and percent inhibition of radial growth (PIRG) was recorded by the following formula

(Naureen *et al.*, 2010).

PIRG = (R1-R2/ R1) ×100
R1 = Radial growth of *F. moniliforme* in control plate
R2 = Radial growth of *F. moniliforme* interacting with antagonistic bacteria

2.5 Preparation of bacterial inoculum

For the preparation of bacterial inoculum, isolate W3Gr-6(b) with highest biocontrol activity was selected. This isolate was grown in 100 ml King's B broth on shaking incubator at 150 rpm for 48 h at 28ºC ± 2ºC followed by addition of one percent carboxy methyl cellulose (CMC) as adhesive in aseptic conditions (Vidhyasekaran and Muthamilan, 1999).

2.6 Preparation of fungal inoculum

For this 8 mm disc from 14 days old culture of fungal pathogen, actively grown on a PDA plate, was transferred into potato dextrose broth aseptically and incubated on a shaking incubator for 5 days at 28± 2ºC.

2.7 Bacterial treatment in pot experiment

The W3Gr-6(b) isolate was tested for efficiency in promoting maize growth and controlling disease in pot experiment. For this maize seeds (Suvarna-589) were surface sterilized with 1 % sodium hypochlorite and washed 3 times with sterilized distilled water. Seeds were dried and coated with bacterial suspension and left whole night at 28 ± 2ºC for proper mixing and coating with the bacterial suspension was drained off and the seeds were dried in shade for 30 min. For control untreated surface sterilized seeds were used (Vidhyasekaran and Muthamilan, 1999).

Pot experiments were conducted in BBAU campus, Lucknow (India) during the month of April-May. Earthen pot (30 x 24 x 16 cm) was filled with 5 kg of sterilized soil. Pots were mixed with 1% (w/w) of inoculum of *F. moniliforme*. Enough moisture was maintained by watering the pots regularly. Each pot was sown equidistance with 3 seeds. The treatments were: (1) Control (without any inoculation) (2) Negative control (with *F. moniliforme* inoculation) (3)W3Gr-6(b) (4) *F. moniliforme* + W3Gr-6(b). Each treatment was replicated three times.

After 14 days of sowing maize seedlings were observed for appearance of lesions and disease caused by *F. moniliforme* ITCC No. 2193. The number of dead or unemerged plants were also counted. On completion of 30 days plants were observed for growth enhancement by measuring root length, shoot length, fresh weight and dry weight.

3. Results

3.1 Bacterial isolates

On the basis of cultural, morphological and biochemical characteristics all the isolates were found to be fluorescent pseudomonads as described in Bergey's Manual of Systematic Bacteriology (Garrity, 2005) (Table 1).

3.2 In vitro screening of bacterial isolates for their plant growth promoting (PGP) and biocontrol activities

The production of IAA was highest by W3Gr-6(b) isolate followed by TS-42 and TS-43. Rest of the isolates showed very less amount of IAA production. All isolates were visually observed for determination of solubilization index (SI) by measuring the halo (clear zone) diameter and

the colony diameter. Amongst all W3Gr-6(b) showed maximum amount of phosphate solubilization and their P.S.I. was calculated to be 4.3. TS-42 and TS-43 also solubilized phosphate with P.S.I., 3.2 and 3.1, respectively. Production of siderophore was only detected in W3Gr-6(b) and TS-5. Amongst all isolates, TS-5 showed maximum HCN production, whereas, W3Gr- 6 (b) showed low cyanide production as it was evident by light yellow color. Rest of the isolates did not show cyanide production (table 2).

3.3 In- vitro antifungal activity

Amongst all isolates only six showed antifungal activity. W3Gr-6(b) showed maximum (57%) inhibition of radial growth of *F. moniliforme* ITCC No. 2193. Besides this GS-5, TS-41, TS-42, TS-43 and TS-44 showed 45, 38, 47, 42 and 41% growth inhibition respectively.

3.4 Bacterial treatment in pot experiment

Among the different treatments, maize seeds treated with W3Gr-6(b) bacterial suspension showed best results of plant growth parameters over untreated seeds (Table3). W3Gr-6(b) isolate showed 118% increase in mean fresh weight, 179% dry weight, 32% root length and 42% shoot length compared to untreated control. Even in presence of pathogen, isolate W3Gr-6(b) showed 91% increase in mean fresh weight, 34% dry weight, 11% root length and 11% shoot length which was significantly higher than control. Treatment with pathogen only resulted in drastic decrease in all the parameters. The plants showed clear symptoms of ear rot.

Table 1: Biochemical characterization of the test isolates

Biochemical characters		GS-5	TS-4	TS-5	TS-6	TS-41	TS-42	TS-43	TS-44	W3Gr-4	W3Gr-6 (a)	W3Gr-6 (b)	W4H5
Fluorescent diffusible pigment	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram reaction, cell shape	- rods	- rods	- rods	- rods	- rods	- rods	- rods	- rods	- rods	- rods	- rods	- rods	- rods
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
Casease	+	+	–		+	–	–	–	–	+	-	-	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+

MR test	−	−	−	−	−	−	−	−	−	−	−	−	−
VP test	−	−	−	−	−	−	−	−	−	−	−	−	−
Urease	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+
Hydrolysis													
Starch	-	+	-	+	-	-	-	+	+	-	+	+	-
Lipid	-	+		+	-	+	+	+	-	-	+	+	-
Gelatin	-	+	-	+	-	+	+	-	+	+	+	+	-
Arginine hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate utilization													
Glucose	-	+	+	-	-	+	+	+	-	-	+	+	-
Lactose	-	+	-	+	-	-	+	-	+	+	+	+	+
Sucrose	+	+	-	+	-	-	+	-	+	-	+	+	-

Abbreviations: +, positive; -, negative

Table 2: Biocontrol activity of test

S.N.	Name of Strain	HCN Production	Percent of radial growth inhibition (PIRG)*
1.	GS-4	-	Nd
2.	GS-5	-	45.00±.034
3.	TS-4	-	Nd
4.	TS-5	+++	Nd
5.	TS-6	-	Nd
6.	TS-41	-	38.33±0.42
7.	TS-42	-	46.66±0.32
8.	TS-43	-	Nd
9.	TS-44	-	41.66±0.43
10.	W3Gr-4	-	41.35±0.39
11.	W3Gr-6(a)	-	Nd
12.	W3Gr-6(b)	++	56.89±0.28
13.	W4H5	-	Nd

*Values are mean of three replicates; ± SD
+++ (Excellent), ++ (Moderate), - Negative for traits, Nd Not

Table 3: Effect of different treatments on growth parameters of maize after 30 days

Types of treatment	Dry weight (g)	Fresh weight (g)	Root length (cm)	Shoot length (cm)
Control	2.90±0.38^{b}	18.67±0.34^{b}	8.6±0.47^{b}	15.00±0.62^{b}
F. moniliformae	0.83±0.05^{a}	5.96±0.18^{a}	7.00±0.81^{a}	10.83±0.23^{a}
W3Gr-6(b)	8.10±0.14^{d}	40.87±0.15^{d}	11.33±0.95^{d}	21.33±1.2^{d}
F. moniliformae+ W3Gr-6(b)	5.53±0.68^{c}	25.03±1.2^{c}	9.60±1.20^{c}	17.83±0.62^{c}

Values are mean of three replicates.±SD. Means in the columns followed by same letters indicates no significant difference (P = 0.05) by Duncan's Multiple Range Test.

4. Discussion

Microbial inoculants play an important role in supplementing the essential plant nutrients for sustainable agriculture, economic and eco-friendly environment (Khare and Arora, 2014). (Maheshwari and Pandey, 2007; Arora *et al.*, 2014; Tewari and Arora, 2014). A wide variety of microbes have been utilized to enhance maize crop production (Gholami *et al.*, 2012) and efforts have been made to identify potential PGP and biocontrol bacteria. In the present study 13 isolates of fluorescent pseudomonads were screened *in vitro* for PGP and biocontrol activities against *F. monilifomae* causal organism of ear rot in maize. Ear rot results in great economic loses to farmers in India (Sharma *et al.*, 1993) and it is estimated to be causing damage of up to Use of fluorescent pseudomonads as bioinoculant has been reported (Weller, 2007; Gao, 2012). Studies confirmed their potential in P solubilization, siderophores production, secretion of lytic enzymes, and phytohormones, directly involved in plant growth promotion (Ardakani *et al.*, 2010). In this study isolate W3Gr-6(b) showed *in-vitro* production of IAA, solubilzation of insoluble phosphate and siderophore production. These attributes probably played a key role in maize growth promotion in *in vivo* conditions when seeds were coated with bacterial suspension. Our result showed that W3Gr-6(b) isolate increased growth parameters of maize to a significant level over untreated control. Earlier study by Chabot *et al.*, (1993) reported that *Pseudomonas* sp. caused a significant increase in maize plant height after 60 days. Isolate W3Gr-6(b) is a very promising IAA producer and contributed to 32% root and 42% shoot length increment in comparison to control. In a similar study Patten and Glick (2002) confirmed that the production of IAA by *Pseudomonas putida* GR12-2 plays a major role in the root development of canola (*Brassica rapa*) root system. Pan *et al.*, (1999) also reported that isolates of *Pseudomonas* (fluorescent) produced IAA that stimulates maize plant growth. In a study Khare and Arora (2009) reported dual effect of IAA in growth promotion and charcoal rot suppression of chickpea by *Pseudomonas aeruginosa*. Another PGP activity of W3Gr-6(b) isolate was P solubilzation, that influences plant growth by providing soluble form of phosphate (Khan and Joergesen, 2009). W3Gr-6(b) also showed siderophore production. A number of workers have reported the production and role of siderophore produced by pseudomonads in controlling phytopathogens (Bouizgarne, 2013).

Crop loss due to ear rot caused by *F. moniliforme* is globally reported (Scauflaire *et al.*, 2011; Wang *et al.*, 2014) and current practice used in eradication involves chemical pesticides. Fluorescent pseudomonads produce metabolites such as antibiotics, siderophores and hydrogen cyanide (HCN) as mechanism of biocontrol (Weller, 2007) and commercially adopted in controlling several plant pathogens. Studies indicate that fluorescent and non-fluorescent *Pseudomonas sp*. directly or indirectly control soil borne pathogen including *F. moniliforme* (Hebbar *et al.*, 1992). In a study Pal *et al.*, (2001) reported suppression of maize root diseases caused by *Macrophomina phaseolina* and *F. graminearum* by PGP isolate of a fluorescent *Pseudomonas sp.* EM85. Recently Yasmin *et al.*, (2014) also confirmed that the *P. aeruginosa* Z5 has biocontrol activity against *F. oxysporum, Fusarium sp.* In our investigation W3Gr-6(b) also inhibited the growth of *F. moniliforme* ITCC No. 2193 both *in vitro* and *in vivo* conditions.

In this way it can be recommended that use of fluorescence pseudomonads in the form of bioinoculants may be done in stimulating yield and growth of maize crop in field conditions. Besides this, further studies on its field performance in eradication of fusarium ear rot will be done before developing the biopesticides targeting crop and phytopathogen in field conditions.

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A Review on The Quantum Mechanical Calculation of Rotational Constants for Interstellar Organic Molecules

Khemendra Shukla, Devendra Singh and Devesh Kumar*

Abstract

Interstellar medium consists of gas and dust, even no part of galaxy is completely empty and interstellar dust is most crucial constituent of our Galaxy. ISM has extremely low density, 90% of it contains gas mainly atomic or molecular hydrogen, 9% is helium, and the remaining 1% consists of heavier elements. The spectroscopic observations both in absorption and emission in ISM, along with laboratory studies of sample materials, have increased interest in the study of organic molecules in ISM. Dust grains in ISM absorb and emit energy in the microwave and far-IR part of the spectrum. Here we are presenting the comparison between computed and observed rotational constant for interstellar organic molecules like CN, CO, HCN, OCS and so many other identified interstellar molecules.

Keywords: ISM- Interstellar medium, LCAO-linear combination of atomic orbitals, MOs-Molecular Orbitals

1. Introduction

Star and planets are not the only denizens of our Galaxy. The void between stars and planets is filled with gas and dust, so called interstellar medium, this gas and dust originates when new stars are born and old star expel their matter when they die. There is nearly as much mass in the voids among the stars as there is in the stars themselves. The composition of interstellar gas is understood by spectroscopic studies of absorption lines formed when light from distant star interacts with gas along the observer's line of sight. In most of cases, the elemental abundances detected in interstellar gas mirror those found in other astronomical objects, such as the Sun, the stars, and the Jovian planets. The abundances of some heavy elements, such as carbon, oxygen, silicon, magnesium, and iron, are much lower in interstellar gas than in our solar system or in stars.

The events of interstellar dust may be observed in normal conditions such as dark patches can be observed in the Milky Way galaxy. The very effective obscuration of starlight by irregular clusters of small particles results into the formation of dark clouds in our Galaxy. Sometimes the gas and dust between the stars is detected only in infrared. Dust grains absorb visible and ultraviolet light which causes them to heat up and reradiate in the infrared. In the Figure.1 the bright regions in this in large are congregations of innumerable unresolved stars, merging together into a continuous blur at the resolution of the telescope. However, the dark areas are not simply "holes" in the stellar distribution (Chaisson and McMillan). These are regions of space where interstellar matter

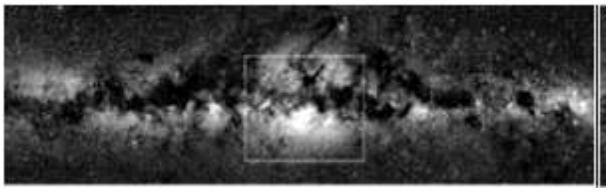

Figure 1: The Milky Way Galaxy contains high concentrations of stars and interstellar gas and dust. (Chaisson and McMillan)

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obscures (blocks) the light from stars beyond, blocking from our view what would otherwise be a rather smooth distribution of bright starlight. Their very darkness means that they cannot easily be studied by the optical methods used to examine stellar matter.

2. Interstellar Extinction and Emission from Dust

2.1 Extinction (absorption and scattering) is by far best studied property of diffuse dust and outer cloud dust because it can be determined accurately over a wide range of wavelengths. Interstellar extinction was first observed by Trumpler (1930), who discovered that distant star clusters appeared dimmer than expected.

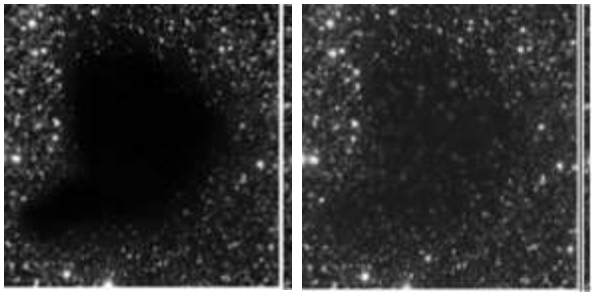


Figure 2: The effect of reddening. (Chaisson and McMillan)

When light from a distant star passes through the cosmos and arrives on Earth, the intensity of the light is reduced. This is referred as optical extinction. It occurs because of the absorption and scattering of the light from the interstellar dust lying in the pathway of the light on its way to earth. Study of this extinction is done by measuring the intensity of light coming from the stars at different wavelengths, that is, with different colours. The general increase in absorption toward shorter wavelengths gives rise to the effect of reddening. This can be easily gleaned from multi-wavelength images of the sky toward regions of interstellar dust.

2.2 Interstellar dust emissions

The European Infrared Satellite Observatory (ISO) discovered emission lines from interstellar water vapour in a variety of sources, including star forming regions, planetary nebulae and near formed stars. Neufeld et al. (1999), Neufeld *et al.* (2000), Cernicharo and Crovisier (2005). Also, using ISO astronomers discovered for the first time hydrogen cyanide ice molecules in a dusty cloud surrounding a newly forming star. Many of the most important spectral lines produced by interstellar gas fall within infrared wavelengths.

A study conducted by the Infrared Astronomical Satellite (IRAS) shows that ISM also consists of faint tufts of dust, which can't be seen in visible light and given the name "infrared cirrus" because it resembles the cirrus clouds in the Earth's atmosphere. Infrared cirrus is very cold (15-30 K) and can only be detected in the

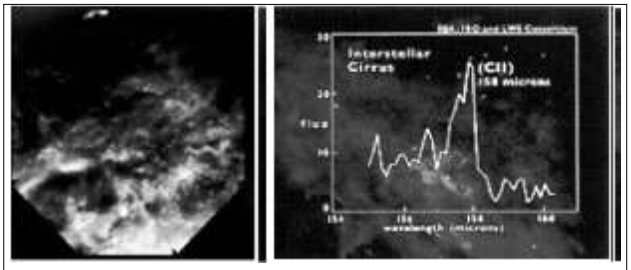


Figure 3: IRAS image of cirrus at the south celestial pole and ISO-LWS spectra of infrared cirrus.

infrared. Its temperature is due to dust grains being slightly heated by starlight.

3. Organic Molecules in Interstellar Medium

Molecules were predicted over 60 years ago, and soon after CH, CN and CH^+ were detected at optical wavelengths. Weinreb *et al.* (1963), made the first radio wavelength detection of the rotational lines of the interstellar hydroxyl

radical (OH). In ISM, gas phase molecules produce rotational transitions which fall in the radio and sub-millimetre portions of the electromagnetic spectrum. Allowed rotational transitions, which result in a change in dipole moment of molecule produces narrow characteristic bands at these frequencies. A large fraction of heavier elements is incorporated as solid phase molecules in interstellar grains and ices. These molecules are not free to rotate, but their vibrational transitions may be observed in the infrared portion of the spectrum. There are also gas phase lines that fall in the IR part of the spectrum which are due to rotational-vibrational transitions.

Infrared wavelengths are ideally suited to the study of the composition of interstellar dust, especially in the range between 2 and 30μm (5000 to 330 cm^{-1}), because this spans the energy range associated with the fundamental or ground state interatomic vibrations of many molecular bonds associated with the cosmogenically most abundant species. Now it is possible to calculate the rotational constant for organic molecules which possesses ground state transition (rotational and vibrational) in the ISM.

Since ground-based infrared observations are constrained by the atmospheric windows, astronomers have been unable to observe the complete 2-30μm region. This has inhibited unique identifications of the organic materials present in the ISM.

The organic material responsible for the 3.4μm feature seen in the diffuse interstellar medium most likely formed in icy grain mantles inside dense molecular clouds. The relatively short cycling times between the dense and diffuse clouds would suggest the organics could be dispersed and incorporated into the next generation of molecular clouds efficiently. There are currently two observational problems with this scenario, however, and they raise questions about both the production site of the organic material observed in the DISM, and the cycling efficiency that occurs. The aliphatic hydrocarbon absorption bands, so readily seen in the diffuse interstellar medium, do not appear in the spectra of objects seen through the dense cloud material and another possible organic signature, observed near 4.62μm in dense clouds and thought to be the result of energetically processed interstellar ices, does not appear in the diffuse interstellar medium. There are some linear/planar organic molecules detected in the interstellar medium.

3.1 Cyano radical (CN)

Cyano Radical CN is one of the most widely distributed and best known species. The Cyano radical was just the second interstellar molecule to be identified. The first rotational transition from J=0 to J=1 was observed In the Orion Nebula and W51 at 113492 MHz. Both of these studies detected features in the near UV or UV region of the electromagnetic spectrum due to electronic transitions, and both found CN in absorption toward the bright star Zeta Ophiuchi. A microwave detection in the Orion nebula and W51 by Jefferts *et al.* (1970). Turner and Gammon (1974) provided confirmation several years later and found CN in many other sources.

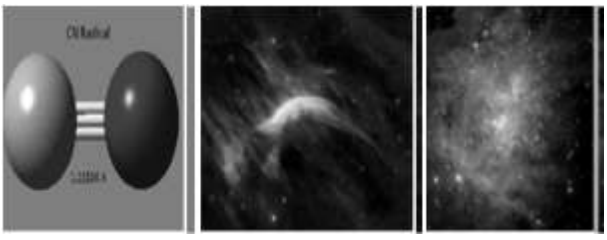


Figure 4. Optimized structure of Cyano Radical (Left), Zeta Ophiuchi (Middle), and Orion Nebula (Right).

CN was also one of the earliest molecules to be detected in other galaxies, beginning with the 1988 work of Henkel *et al.* (1988) First transition observed for rotational level J=0 to J=1 at 113492 MHz. Therefore 2B = 113492 MHz which gives rotational constant B = 61.1335 GHz.

3.2 Carbon monoxide (CO)

Carbon monoxide (CO) is the most abundant C-bearing molecule in the universe. It is the second most common molecule in dense interstellar clouds, after H_2. It is found in comets and the atmospheres of several planets. It has also been detected in brown dwarfs such as Gliese 229b and Epsilon Indi B. Since 1952, it has been known that CO is present in the atmosphere of our own sun, residing in the chromospheres. CO is observed in the

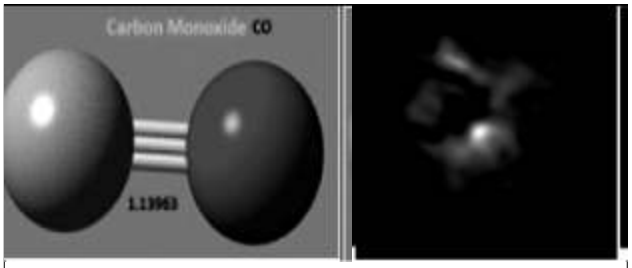


Figure 5: The Optimized structure of Carbón monoxide And Carbonstar IRC +10216.

interstellar media by means of its characteristic rotational spectrum. It is used as a substitute for detecting molecular hydrogen and is consequently used extensively for mapping molecular clouds in our own galaxy and elsewhere. The by-far most abundant interstellar molecule is molecular hydrogen, low rotational transitions of the CO molecule, although about by 10^{-4} less abundant than H_2, have become the most important tool to study the large scale distribution of the molecular gas in the interstellar medium. Carbon Monoxide (CO) in the Orion Nebula Wilson *et al.* (1970), dense envelope of the carbon star IRC+10216 first rotational transition observed for J=1 to J=0 at 115271.2 MHz. Thus the rotational constant B is 57.6356 GHz.

3.3 Hydrogen Cyanide (HCN)

Hydrogen cyanide (HCN) is one of the most common astromolecules very poisonous, found in almost every astrophysical environment. HCN is close kin to potassium cyanide, a

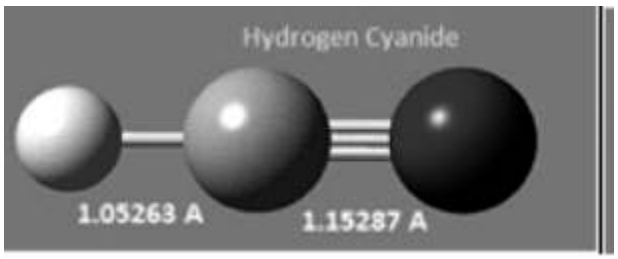


Figure 6: The Optimized structure of Hydrogen Cyanide.

familiar agent of death in murder mysteries, sometimes assuming the title role as in the case of Agatha Christie's, Sparkling Cyanide. The presence of cyanide is often detected by the "smell of bitter almonds". Hydrogen cyanide detected in W3 (OH), Orion A (which includes an O-type star, proto-stars, and an H II region) at 88 GHz.HCN has also been detected in a comet

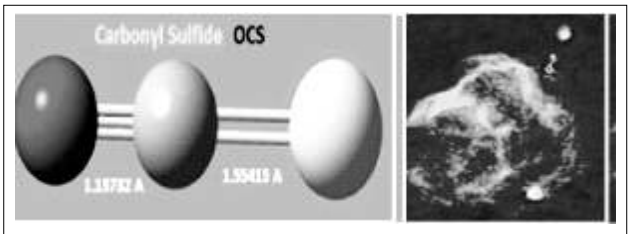


Figure 7: Optimized structure of Carbonyl Sulfide. And Sagittarius B2 (North).

Kohoutek in 1973 by Huebner *et al.* (1974). Among solar system planets and their satellites, HCN has been found in Jupiter, Neptune, and

Titan. Another related species, protonated hydrogen cyanide ($HCNH^+$), was also reported by Ziurys and Turner (1986).

3.4 Carbonyl Sulfide (OCS)

Carbonyl sulfide (OCS) is one of the principal and most long lived reservoirs of sulfur in the Earth's troposphere and has been detected in different astrophysical objects (like the Orion molecular clouds, the star burst galaxies). Observed microwave spectrum of OCS J=1 at 0.8109 cm^{-1}. Jefferts *et al.* (1971)

3.5 Hydrogen isocyanide (HNC)

Hydrogen isocyanide (HNC) was detected via its J=1-0 rotational line at 90.7 GHz by Snyder and Buhland (1972), Zuckerman *et al.* (1972) in W51 and NGC 2264, respectively. While Snyder and Buhland assigned the line to HNC, definitive experimental data to confirm the

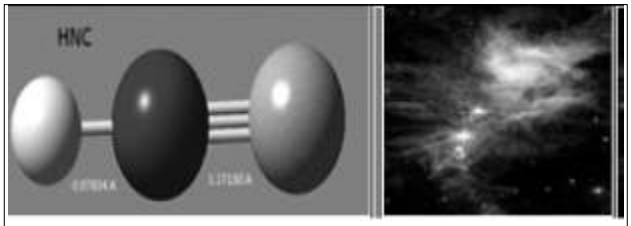


Figure 8: The Optimized structure of Hydrogen Isocyanide. And NGC 2264Cone Nebula (right).

assignment was not available until the laboratory study of Blackman *et al.* (1976) was published. HNC was first detected in an extragalactic source.

3.6 Isocyanic Acid (HNCO)

The detection of isocyanic acid (HNCO) in the interstellar medium was first reported by Snyber and Buhl (1972). It has since been observed in extragalatic sources. In comets, it

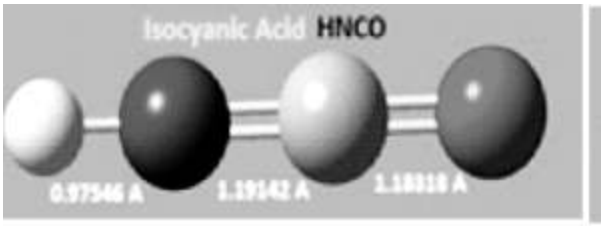


Figure 9: Optimized structure of Isocyanic acid.

was first observed in Hyakutake and then in Hale-Bopp. Isocyanic acid (HNCO) was one of the first polyatomic molecules identified in the interstellar gas. Rotational transitions of HNCO identified in Sagittarius B2 (North) at 21.982 GHz for $J= 1_{01-00.}$

3.7 Formyl Radical (HCO)

The formyl radical (HCO) was detected in four molecular clouds as reported in by Snyder *et al.* (1976).

It was observed toward the giant molecular clouds W3, W51, K3-50, and NGC 2024 (the Flame nebula). Radio Detection of the Interstellar Formyl Radical (HCO) in NGC 2024 observed at 86670.65 MHz. The Flame Nebula, (NGC 2024), is an emission nebula in

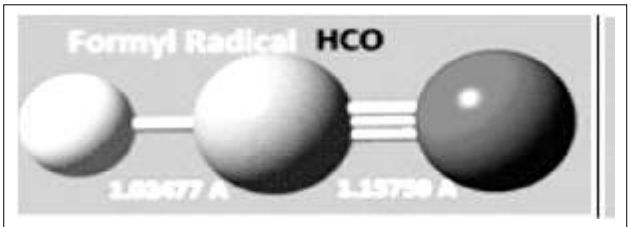


Figure 10: The Optimized structure of Formyl Radical. And The Flame Nebula; NGC 2024

the constellation Orion. It is about 900 to 1,500 light-years away.NGC 2024, the Flame Nebula, is an emission nebula in the constellation Orion. The Flame Nebula is visible through small telescopes. It is locatedin the constellation of Orion near the star known as Alnitak.

3.8 Nitrosyl Hydride (HNO)

A single rotational line for HNO observed in SgrB2(OH) was reported as early as by Snyder *et al.* (1977)The Nitroxyl (HNO) is astronomically important specie because it helps to detect the interstellar HNO. Transition

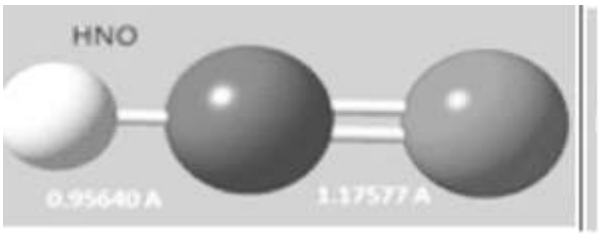


Figure 11: The Optimized structure of Nitrosyl Hydride.

observed at 81477.49 MHz in Sgr B2, NGC 2024.

3.9 Cyanoformaldehyde (HCOCN)

The detection of cyanoformaldehyde (HCOCN) was reported by Remijan *et al.* (2008) in SgrB2(N) on the basis of observations made with the Green Bank Telescope.

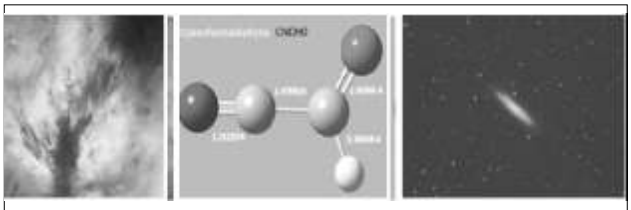


Figure 12: The Optimized structure of Cyanoformaldehyde. And Sagittarius B2 (North)

Confirming observations have not yet been reported. Gerin *et al.* had searched for HCOCN in the late 1980s and failed to find any of its various rotational lines from about 86 to 106 GHz as well as one feature near 217 GHz.

3.10 Hydro magnesium Isocyanide

The detection of hydro magnesium isocyanide (HMgNC) in the carbon-rich evolved star

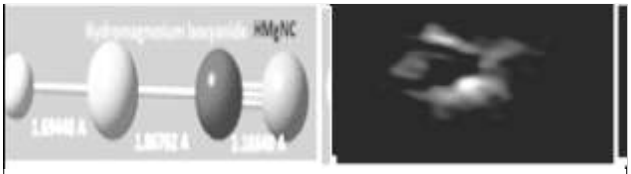


Figure 13: The Optimized structure of Hydromagnesium Isocyanide. And IRC +10216 or CW Leonis

IRC+10216 (Cabezas *et al.*, 2013).
The rotational constants derived from space frequencies are B_0 = 5481.49 (3) MHz.

4. Methods and system Data

Geometry optimizations as well as frequency calculations and determination of rotational constant were performed with Gaussian 09 program. Gauss view 05 is a graphical user interface designed to help prepare input for submission to Gaussian and to examine the output that Gaussian 09 produces. Geometry optimization is the name of the procedure that attempts to find the configuration of minimum energy of the molecule. Geometry optimization calculations are done by starting with an input structure that is believed to resemble (the closer the better) and submitting to a computer.

The B3LYP hybrid density functional method with the 6-31G basis set were used. Methods that are a combination of a Hartree-Fock approximation to the exchange energy and a DFT approximation to the exchange energy, all combined with a functional that includes electron correlation. These methods are known as hybrid methods, and are currently (fall of 2006) the most common and popular DFT method used in practice. A basis set is a set of mathematical functions (basis functions), linear

combinations of which yield molecular orbitals. The functions are usually, but not invariably, centered on atomic nuclei. Approximating molecular orbitals as linear combinations of basis functions is usually called the LCAO approach, although the functions are not necessarily conventional atomic orbitals, they can be any set of mathematical functions that are convenient to manipulate and which in linear combination give useful representations of MOs. Physically, several (usually) basis functions describe the electron distribution around an atom and combining atomic basis functions yields the electron distribution in the molecule as a whole.

Density Functional Theory (DFT) is a computational method that derives properties of the molecule based on a determination of the electron density of the molecule. Unlike the wave function, which is not a physical reality but a mathematical construct, electron density is a physical characteristic of all molecules. The most significant advantage to DFT methods is a significant increase in computational accuracy without the additional increase in computing time. There are a large number of approximations that attempt to calculate the electron exchange-correlation energy. The electron correlation aspect addresses how an electron in an atom or molecule interacts with another electron. The electron exchange aspect describes a quantum mechanical property of electrons that is related to their exchange between a fermion and a boson (a fermionicor bosonic electron).This exchange is well beyond the scope of this Guide, but suffice it to say that it is related to the Pauli Exclusion Principle, which states that no two electrons can occupy the same energy state.

Available Data: Interstellar organic molecules detected in interstellar medium are listed below. These molecules show ground state transition. The following table represents the detected molecules and the site from they were detected. (Table.1)

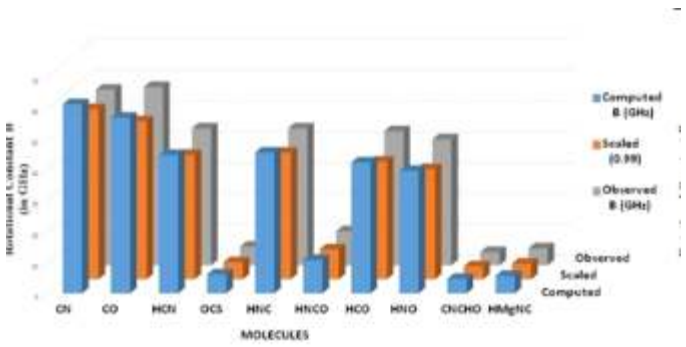
5. Results and Discussion

Interstellar organic molecules (linear & planar) with observed first transitions and their source/origin are presented in Table 1. Rotational constants B (in GHz) have been evaluated from these observed transition frequencies and used for comparison with theoretical values. The quantum mechanically calculated rotational constants B (in GHz) for studied interstellar organic molecules vis-à-vis experimental data are presented in Table 2. In Graph 1, bars of each molecule corresponding to computed, scaled and observed caption clearly represents the close agreement of observed rotational constants to calculated rotational constants. Interstellar compounds can also be predicted by estimation of rotational constants of those molecules who fall in microwave-IR region of spectrum. The computational method used appears to be accurate enough for such type of molecules.

6. Conclusion

i) The computed rotational constants are in close agreement with observed experimental values.

ii) For interstellar organic molecules absorption spectrum, Infra-red, Raman spectrum can be obtained easily by using B3LYP hybrid density functional method with a 6-31G basis set.



Graph 1: Comparison between computed and observed value.

Table 1			
MOLECULE	**Origin/ Source**	**Transition Freq.**	**Observed B (GHz)**
CN	Orion Nebula	113492 MHz	56.7460
CO	Orion Nebula, IRC +10216	115271.2 MHz	57.6356
HCN	W3(OH), Orion A	88 MHz	44.3000
OCS	Orion molecular clouds	0.8109 cm^{-1}	06.0610
HNC	W51 and NGC 2264	90.7 GHz	44.3000
HNCO	Sagittarius B2 (North)	21.982 GHz	10.9910
HCO	W3, NGC 2024	86670.65 MHz	43.3353
HNO	Sgr B2, NGC 2024	81477.49 MHz	40.7387
CNCHO	Sagittarius B2(North)	8.6 GHz	04.2870
HMgNC	IRC+10216	87697.5 MHz	05.4814

Table 2: Comparison between computed and observed value

MOLECULE	Computed B (GHz)	Scaled (0.99)	Observed B (GHz)
CN	61.1335	60.522165	56.7460
CO	56.7626	56.194974	57.6356
HCN	44.6996	44.252604	44.3000
OCS	06.1304	06.069096	06.0610
HNC	45.5132	45.058068	44.3000
HNCO	10.7525	10.644970	10.9910
HCO	42.2855	41.862645	43.3353
HNO	39.5868	39.190932	40.7387
CNCHO	04.6913	04.644387	04.2870
HMgNC	05.6413	05.584887	05.4814

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Analysis on Pattern Classification using Artificial Neural Networks

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Abstract

Artificial neural networks (ANN) is one of the most dynamic research and application areas for pattern classification. ANN is the branch of Artificial Intelligence (AI). The network is trained by 'n' number of algorithm like back propagation algorithm. The different combinations of performance functions are used for training the ANN. The back propagation neural network (BPNN) can be used as a highly successful algorithm for pattern classification with suitable combination of performance functions while training and learning ANN. When the maximum likelihood algorithm was compared with back propagation neural network method, the BPNN was more accurate than other algorithms. A Multilayer feed-forward neural network algorithm is also used for pattern classification. However BPNN gives more effective results than other pattern classification algorithms.

Handwriting Recognition (or HWR) is the ability of a machine to receive and interpret handwritten input from different sources like paper documents, photographs, touch-screens and other input devices. Various performance functions is examined in this paper so as to get to a conclusion that which function would be better for usage in the network to produce an efficient and effective system. The training of back propagation neural network is done with the application of Offline Handwritten Character Recognition.

Keywords: Back propagation algorithm, Multilayer feed-forward neural network, Performance functions, Mean square error.

1. Introduction

A neural network model which is the branch of artificial intelligence is generally referred to as artificial neural networks (ANNs). ANN teaches the system to execute task, instead of programming computational system to do definite tasks. To perform such tasks, Artificial Intelligence System (AI) is generated. It is a pragmatic model which can quickly and precisely find the patterns buried in data that replicate useful knowledge. One case of these AI models is neural networks. AI systems should discover from data on a constant basis. In the areas of medical diagnosis relationships with dissimilar data, the most available techniques are the Artificial Intelligence techniques.

An artificial neural network is made up of many artificial neurons which are correlated together in accordance with explicit network architecture. The objective of the neural network is to convert the inputs into significant outputs. The teaching mode can be supervised or unsupervised. Neural

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Networks learn in the presence of noise. ANNs found their usage in many areas such as,

- Speech recognition
- Product inspection
- Fault detection
- Character recognition

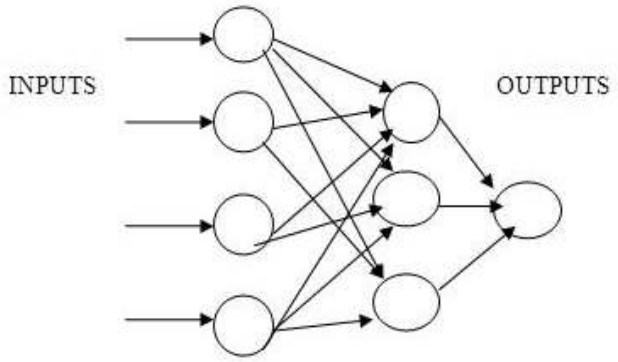

Fig 1: Architecture of Artificial Neural Network

2. Existing System

2.1 Back propagation Neural Network

Artificial neural networks (ANN) consider classification as one of the most dynamic research and application areas. The major disadvantage in using ANN is to find the most appropriate grouping of training, learning and transfer function for classifying the data sets with growing number of features and classified sets. The different combinations of functions and its effect while using ANN as a classifier is studied and the correctness of these functions are analyzed for various kinds of datasets (Saravanan *et al.,* 2014).

The real world problems which are represented by multidimensional datasets are taken from medical background. The classification and clustering of these data sets are significant. The data set is divided into training set and testing set and it has no usage in the training process. The results are produced with the help of these datasets and it is used for testing. The training set is taken from 2/3rd of the dataset and the remaining has been taken as test set. This is made through the assessment of the accuracy achieved through testing against these data sets. Then the network is simulated with the same data.

The back propagation algorithm trains the neural network. Gradient descent method (GDM) was used to decrease the mean squared error between network output and the actual error rate. The following parameters are considered to measure the efficiency of the network,

- Rate of convergence
- No of epochs taken to converge the network.
- The calculated Mean Square Error (MSE).

With the appropriate combination of training, learning and transfer functions the dataset classification uses the most successful tool called back propagation neural network

Aria *et al.,* (2003) proposed a method for Classifying IRS-1D Satellite Images.

The fitness of Back Propagation Neural Network (BPNN) for classification of remote sensing images based on three steps is proposed. As an initial step, from the measures of first order histogram measures the features are extracted. In the second step, feature classification based on BPNN is

done, and in the third step the outcomes are compared with the maximum likelihood classification (MLC) method. The statistical features in this paper depend on the first-order distribution measure such as mean, standard-deviation, skewness, kurtosis, energy, and entropy. The network contains 3 layers. The input layer is fed with extracted features which contains 18 neurons. In the classification of IRS-1D satellite images six classes were used and the back propagation neural network was trained on these classes. The whole image was classified using this trained network. The regions of Iran are taken for testing.

The IRS-1D satellite images uses Artificial neural network for the classification of images. The major problem with the classification of IRS data is to choose a better method for training. TrainLM method has been implemented on using back propagation neural networks algorithm on IRS images.

There are various training algorithms for feed forward networks The gradient of the performance function is used by all the algorithms to find out how to fiddle with the weights to decrease the performance. The back propagation technique determines the gradient. This gradient performs computational backwards through the network. When the maximum likelihood method was compared with back propagation neural network method, the BPNN was more accurate than maximum likelihood method. The overall preciseness in MLC method is 75.00% whereas in BPNN method is 85.19%.

Grip and Fredrik (2003) proposed Whiplash-Associated Disorders by classifying of neck movement patterns.

A novel method for the classification of neck movement patterns related to Whiplash-associated disorders (WAD) using a flexible back propagation neural network (BPNN) is studied. WAD is a common diagnosis after neck trauma, mainly caused by rear-ends car accidents. Since physical injuries cannot be detected with the current imaging techniques, the diagnosis can be complex to make. The dynamic range of the neck is often visually detected in patients with neck pain, but this is a biased measure, and a more intentional decision support system, that gives a consistent and more complete analysis of neck movement pattern, is needed. The estimation of the prognostic ability of a BPNN, using neck movement variables as input is the main objective of the paper. The collection of three-dimensional (3-D) neck movement data from 59 subjects with WAD and 56 control subjects is made with a proReflex system. Rotation angle and angular velocity were measured using the direct helical axis method and motion variables and the results are extracted. To increase the performance of BPNN a principal component analysis was performed which reduces data. BPNNs with six hidden nodes had a yield of 0.89, a sensitivity of 0.90 and a specificity of 0.88, which are very hopeful results. The results were predicted from the neck movement analysis. The result was combined with a neural network where the origin of decision support system is constructed, which classifies the suspected WAD.

The flexible back propagation neural

network (BPNN) resulted in a correct calculation for 84 percent of the control subjects and 89 percent of the WAD, showing that a BPNN could be appropriate for predicting motion characteristics. The presented method is very hopeful as an aid to determine whether a patient with suspected WAD has a neck movement pattern that deviates from that found in control subjects. A few perceptive variables seem to amplify the efficiency on abbreviating the results. A high predictive ability with stable and well-functioning BPNN is presented using early stopping method.

Wan and Li (2010) proposed classification of Remote sensing images using artificial neural network

Artificial neural network (ANN) is a significant part of artificial intelligence, It has been extensively used in the research field of remote sensing classification. The wetlands remote sensing classification based on ANN is complicated, because of the intricate feature of wetlands areas. The remote sensing image supervised classification is carried out on the training samples. The clarity is examined and it was found that it is hard to guarantee because it will have an effect on the classification results. This article proposed a method for sample purification to filter the training samples based on statistical analysis theory for enhanced wetlands remote sensing classification based on ANN. The BP ANN with a nonlinear mapping function gives better classification results for intricate areas. The TM image of Honghe Wetlands National Nature Reserve is chosen for classification. First, the statistical analysis theory to eradicate noise in training samples is used. Then the original samples and purified samples are used to train the BP ANN individually and created two classification maps of TM image based on two trained BP ANN. At last, the classification accuracy between the two maps is compared. The statistical analysis method for purifying training samples for remote sensing classification based on BP ANN is performed. The experiments showed that it was an efficient method to develop image classification.

Zhang (2014) proposed the use of harmony search and back propagation based ANN to classify breast cancer data.

Breast cancer is one of the most commonly seen cancer types. Analysis of breast cells characteristics has great importance in diagnosis, treatment and following of this disease. Through two different types of ANN algorithm 699 instances of breast cancer data which is present in UCI are classified. In this study, abundant algorithms are used for training of artificial neural network. In this study, two algorithms namely harmony search and back propagation algorithms are used to train feed forward artificial neural network. While classifying the performance values of classification are found by means of Accuracy/SSE/Regression parameters. The performance values of back propagation are achieved as 94.1/0.007/0.92 whereas the results obtained by the harmony search algorithm are 97.57/0.005/0.96 respectively. With the help of harmony search based

ANN algorithm breast cancer data are classified for the first time with this study.

2.2 Multilayer Feedforward Network

Zhang (2000) proposed Neural Networks for Classification.

Classification is one of the most dynamic exploratory and application areas of neural network. The issues of posterior probability estimation, link between neural and conventional classifiers, and learning and generalization tradeoff in classification, feature variable selection, effect of misclassification costs are studied.

Multilayer feed-forward network is used for classification. Although many procedures are available in traditional statistical classification, the usefulness depends on assumptions and circumstances. In order to overcome the above disadvantage, neural networks are used since they are,

- self-adaptive methods,
- universal functional approximators and
- non-linear models.

Many problems still stay unsettled even though neural networks have grown rapidly. As represented prior, many researches should be dedicated to develop more successful and proficient methods in neural model identification, feature variable selection, classifier combination, and uneven misclassification treatment. As a practical decision making tool, systematic evaluation and comparison of neural networks with other traditional classifiers need to be done. The lacks of comparisons between other classifying methods and neural network have been proposed by some authors in the existing literature. Due to the lack of comparisons, mixed results are produced and reported in pragmatic studies.

There are other topics which are related to neural classification. The topics include sample size issues, Bayesian analysis, wavelet network and network training model design and selection. Some applications of neural network have been reviewed formerly and the problems mentioned above are general to all applications. Abundant research opportunities are available in neural classifiers. More research actions are generated by neural network classification because of its multidisciplinary nature and bring about more productive outcomes in the future.

Ali *et al.,* (2009) proposed Neural Network Based Pattern Recognition Technique which detects the misfire in gasoline engines.

The application of ANN to misfire detection in gasoline engines is studied. A feed-forward multiple layer neural network is used for the classification of firing and misfiring events. Importance is given on the transaction between performance, computational cost and implement ability and the system is applied on a production electronic control unit (ECU). The technique is used to identify misfire events over the whole range of operation defined by official on-board diagnosis (OBD) regulations and it is applied to a six-cylinder gasoline engine. Experimental results on a passenger car are presented.

Misfire occurs because of some error in the combustion process. This error occurs

because of some insufficiency in ignition, injection or compression system of the engine or due to a substandard fuel. In addition to their disagreeable effects on the drive quality, misfires in gasoline engines amplify pollutants in exhaust gases and causes harm to the components. Misfire due to some defect in ignition system will enlarge unburned mixture of hydrocarbons and oxygen in the raw exhausts causing an increased exothermic reaction in the catalyst and increased HC and CO emissions.

The investigation of realistic application of a neural network based pattern recognition technique to misfire detection in gasoline engines is done. The difficulty of misfire detection is framed as a pattern recognition problem. The classification of firing and misfiring events is done with the help of feed-forward multiple-layer neural network. To detect the misfire events in six-cylinder gasoline engines, the developed technique is applied over a wide range of operation. The results are produced in this section. The results show that the developed technique detects all patters of misfire.

Furferi et al., (2011) proposed the classification of car seat fabrics.

Car seat fabrics are exclusively shaped textiles. A first-rate car seat fabric needs a little quantity of spots and discolored areas. There are large and low number of spots in the fabrics which characterizes them. The above mentioned fabric types are considered to be of poor quality. Most of them is branded by a sponged-like appearance, characterized by spots and slightly discolored areas. The human experts with the help of manual inspection and classification perform sponged-like fabric grading. Though this manual classification and inspection proves to be valuable in fabric grading, the procedure is skewed and its results may differ depending on the operator skills.

The computer based tool which classifies sponged-like fabrics similar to the classification performed by skilled operators is shown here. Such a tool which is developed by an oppositely devised machine vision system is able to extract a lot of numerical parameters characterizing the fabric veins and discolored areas. Since the Artificial Neural Network has to be trained, the parameters are used which classifies the fabrics based on quality. At last, a relationship between the ANN-based classification and the classification provided by fabric inspectors is done. The validation set composed by 65 sponged-like fabrics is tested and it shows that it is able to classify the fabrics into the correct quality class in 93.8% of the cases, similar to the selection provided by human operators.

With the aim of training an ANN able to correctly classify the fabrics, a series of parameters describing their appearance have been extracted by means of image processing-based algorithms; such data are used as training, validation and testing set for the ANN. In detail, as described below, three kinds of parameters are extracted: colorimetric data, entropy curve and area subtended to the entropy curve.

Most of the methods evaluate the classification performance by defining a dimensionless parameter. A comparison between those methods and the proposed

method was provided. For instance, the correlation between measured and forecasted classifications of colored textiles is stated to be in the range 0.85 – 0.98. The classification error of textured objects is less than 10%. These results are comparable with the ones provided by the present work, thus allowing to state that a satisfactory performance has been obtained.

3. Problems in the Existing system

The major disadvantage in using ANN is to find the most appropriate grouping of training, learning and transfer function for classifying the data sets with growing number of features and classified sets. The problem in existing system is o fine moat appropriate performance function that will give me better result while training the data over back propagation neural network

4. Performance Metrics

Artificial Neural Network is a representation of human brain that tries to learn and simulate its training input patterns by a predefined set of example patterns. The network is trained with particular specifications. The obtained output after training the network is compared with the desired target value and error is calculated based upon these values (Kumar *et al.,* 2015).

For training an input pattern and measuring its performance, a function must be defined. The various functions being included in neural network are:

1.) Sum Of Squared error (SSE)

The firstbasic cost evaluation function. The Sum of Squared error is defined as

$$\mathbf{SSE}=\sum_{p-1}^{p}\sum_{i=1}^{N}(t_{pi}-y_{pi})^2$$

Where, tpi= Predicted value for data point i;

y_{pi} =Actual value for the data point i;

N = Total number of data points

2.) Mean squared error (MSE)

The most widely used and efficient performance function. The *Mean Squared error* is defined as

$$\mathbf{MSE}=\frac{1}{N}\sum_{p=1}^{p}\sum_{i=1}^{N}(t_{pi}-y_{pi})^2$$

Where, t_{pi}= Predicted value for data point i

y_{pi} =Actual value for the data point i;

N = Total number of data points

3.) Root Mean Squared error(RMSE):

The *root Mean squared error* value for finite set of data is defined as

$$\mathbf{RMSE}=\sqrt{\frac{1}{N}\sum_{p=1}^{p}\sum_{i=1}^{N}(t_{pi}-y_{pi})^2}$$

Where,t_{pi}= Predicted value for data point i;

y_{pi} =Actual value for the data point i;

N= Total number of data points

4.) Mean Magnitude of Relative Error (MMRE) (Srinivasan *et al.,* 1995):

The *mean magnitude relative error* is defined as

$$\mathbf{MMRE}=\frac{1}{N}\sum_{p=1}^{p}\sum_{i=1}^{N}\frac{|t_{pi}-y_{pi}|}{y_{pi}}$$

Where, t_{pi}= Predicted value for data point i;

y_{pi} =Actual value for the data point i;

N = Total number of data points

5.) Relative Absolute Error (RAE):

The *Relative absolute error* is defined as the summation of the difference between predictive value and given value for the sample case j to that divide it by the summation of the difference between the given value and average of the given value. The relative absolute error of individual data set j is defined as

$$\mathbf{RAE}=\frac{\sum_{i=1}^{N}|t_{ij}-y_{ij}|}{\sum_{i=1}^{N}|y_i-y_m|}$$

Where, tij= Predicted value by the individual dataset j for data point in i;

y_i =Actual value for the data point i;

N = Total number of data points

y_m = Mean of all y_{pi}

6.) Root Relative Squared Error (RRSE):

The *root relative squared error* of individual data set j is defined as

$$\mathbf{RRSE}=\sqrt{\frac{\sum_{i=1}^{N}(t_{ij}-y_{ij})^2}{\sum_{i=1}^{N}(y_i-y_m)^2}}$$

Where, t_{ij}= Predicted value by the individual data set j for data point in i;

y_i =Actual value for the data point i;

N = Total number of data points

y_m = Mean of all y_{pi}

7.) Mean Absolute Error (MAE)

The *mean absolute error* measures of how far the estimates are from actual values. It could be applied to any two pairs of numbers, where one set is "actual" and the other is an estimate prediction.

$$\mathbf{E}=\frac{1}{N}\sum_{p=1}^{p}\sum_{i=1}^{N}|t_{pi}-y_{pi}|$$

Where, t_{pi}= Predicted value for data point i;

y_{pi} =Actual value for the data point i;

N = Total number of data points.

The above equation represents the output nodes, $\mathbf{t_{pi}}$ **and** $\mathbf{y_{pi}}$ which are target and actual network output unit on the p^{th} pattern, respectively.

8.) Mean Square Error with Regularization (MSEREG):

The *standard Mean square error with Regularization* value can be evaluated by the formula

$$\mathbf{MSE}=\frac{1}{N}\sum_{p=1}^{p}\sum_{i=1}^{N}(t_{pi}-y_{pi})^2$$

MSEREG= α***MSE + (1-**α**)***$\sum_i(W_i)^2$

Where, t_{pi}= Predicted value for data point i;

y_{pi} =Actual value for the data point i;

N = Total number of data points

α = Performance Ratio

W_i= Weight of the network

The network learns by adjusting weights. The process of adjusting the weights to make the neural network learn the relationship between the input and targets is known as learning or training. There are several techniques for training a network gradient descent method which is the most common.

5. Performance Comparison

The various performance functions are being calculated for the training input patterns over back propagation neural network. Then an estimate of which would be better for a neural network.

The calculated error value is being shown through the table:

	MSE	SSE	MSEREG	MMRE	RMSE	MAE
Error	0.00095	0.00152	0.00082	0.0254	0.0309	0.0565

Table 1: Comparative analysis of various Performance functions

The readings are being obtained by training the Back propagation neural network by the various error functions. As being concluded from the readings above that the functions shows positive results as compared to the other evaluated functions. The result is obtained from 100 pixel values through binarization of input data. The lesser the value of error signifies the more efficient and cost reduced the network is being devised.

6. Conclusion

The Back propagation training algorithm has its advantage over training patterns as it increases the accuracy of the simulated data. The mean square error Regularization value is beneficial for having no prior knowledge for class distributions. Through this paper it is being proposed that by reducing the error value, accuracy of a particular network can be increased. Further work is under process for reducing the training time and increasing the accuracy for a network using Back propagation training algorithm with its application on hand written character recognition. This concept can be further applied to various applications related to classification and pattern recognition where larger dataset is being processed. Work can also be done to improve the accuracy and increasing recognition speed as its area of application is wide.

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Comparison of Drying Characteristics of Green and Black Seedless Grapes using Hot Air Dryer

S.P. Singh[1*], K.S. Jairaj[2] and K. Srikant[2]

Abstract

Drying characteristics of Thompson seedless (Green) and Sharad seedless (Black) grapes were obtained using a simple laboratory scale hot air dryer. Grapes were dipped for three minutes in a solution prepared by adding 25 g Potassium carbonate and 15 mL Ethyl oleate to 1 liter of distilled water maintained at a temperature of 40°C. Grapes were dried using hot air at a temperature of 60°C and a flow rate of 0.82 m/s created by chimney effect. Green grapes with an initial moisture content of 79.94 % required 19 hrs while Black grapes with an initial moisture content of 81.38 % required 27 hrs to reach a final moisture content of 18 % (wet basis). Drying rate constant value for Green grapes was 0.036505 h^{-1} while that for Black grapes was 0.024793 h^{-1}. Raisins produced from both variety grapes possessed all the required quality parameters acceptable in the international market.

Keywords: Grape drying, Pretreatment, Hot air drying, Drying rate constant.

1. Introduction

Drying is one of the easily accessible and the most widespread food processing technology (Jairaj *et al.,* 2009). Improved drying methods adopted for a wide range of agricultural products have enhanced their quality. Food processing has become a dire need specifically for fruits and vegetables due to their reduced shelf life and seasonal availability. Water, a major constituent of fruits, is important in controlling rates of deteriorative reactions, including those resulting in nutrient losses (Saguy and Karel, 1980). Drying is one of the methods used to preserve fruits by lowering moisture levels below which microorganisms cannot grow and reaction rates slow down (Mahmutoglu *et al.,* 1996).

Grape is a good source of vitamin C, vitamin A, vitamin K, carotenes, β-complex vitamins such as pyridoxine, riboflavin, and thiamine. It also contains folate, calcium, chlorine, copper, fluorine, iron, magnesium, manganese, phosphorus, potassium, silicon and sulfur in abundance (Narang and Pandey, 2013). Grape berry has an outer protective skin with different layers and pulp inside. Drying process has to remove water from the berries without affecting outer skin structure and arrangement of wax plates. This waxy layer protects the berries from getting dried in fresh form (Adsule *et al.,* 2012).Waxy layer on the berry skin is the main target during pretreatment of grapes for hastening the drying process. The pretreatment of grape berry only helps in loosening the waxy plates on the outer skin and not removing the wax on the skin (Sharma and Adsule, 2007). Though the basic idea of adopting pretreatment initially was to reduce the drying time, importance was also given to enhance the quality of raisins. Some experimental results reported on treatment of grapes

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before drying are available in the reviewed literature (Lutz *et al.,* 1987; Tiris *et al.,*1994; Halak *et al.,* 1996; Mahmutoglu *et al.,* 1996; Karathanos and Belessiotis,1997; Yaldiz *et al.,* 2001; Pangavhane and Sawhney, 2002; Pangavhane *et al.,* 2002; El-Sebaii *et al.,* 2002; Togrul and Pehlivan, 2004; Fadhel *et al.,* 2005; Telis *et al.,* 2006; Doymaz, 2006 ; Al-Juamily *et al.,* 2007; Togrul, 2010 ; Sharma *et al.,* 2013 ; Singh *et al.,* 2014a ; Singh *et al.,* 2014b).

Farmers in most of the countries all around the globe in different climatic zones are into cultivation of vineyards. In most of the countries, harvesting of grapes happens round the year with a brief gap usually during the rainy season. Seasonal variations in most of the grape growing countries can create varying ambient temperatures during the raisin-making period. Even the ambient temperature during a particular day will be different early in the morning compared to that in the afternoon or in the evening. Considering these aspects, it was decided to investigate the effect of dipping solution temperature of 40°C on drying time as well as drying rate constant of grapes.

Green and Black grapes, after dipping treatment were dried in a simple laboratory scale hot air dryer working in the natural circulation mode with drying air temperature of 60°C. The effect of pretreatment on drying time, drying rate constant, as well as quality of raisins produced is investigated in this paper.

2. Materials and Methods

2.1 Material preparation

Fresh, mature and ripe Thompson seedless as well as Sharad seedless grapes procured from the vineyards of Tikota, Bijapur district, Karnataka, India, were used in drying experiments. The fruits were stored in cool enclosures prior to their use. Uniform sized grape berries were selected and cut out from bunches along with their pedicels (cap stem) for each set of experiment. Potassium carbonate and ethyl oleate required for the experiment was procured from local chemical suppliers. Dipping solution was prepared by adding 25 g potassium carbonate and 15 mL ethyl oleate to 1 liter of distilled water and maintained at a temperature of 40°C. Fresh grapes were initially washed with tap water and rolled on a tissue paper to remove surface water. Grape berries of both varieties weighing around 100 g each were dipped for three minutes in the solution maintained at a temperature of 40°C. Grape berries were later rolled on a tissue paper to remove excess solution from their surfaces. Ambient temperature inside the laboratory ranged from 32 to 36°C during experimentation.

2.2 Instrumentation

Figure 1 shows Laboratory scale hot air dryer fabricated in our departmental workshop. It was designed to operate in the natural convection mode by chimney effect. Drying experiments were conducted inside the laboratory using a simple hot air dryer. A wire mesh tray was hung in the drying chamber from the top. A heater of 250 W fixed to an aluminum plate was placed at the bottom part of the dryer. Pt-100 temperature sensors were used along with a 16-channel data logger supplied by Ambetronics Engineers, Mumbai, India. Airflow rate was measured using

KUSUM-MECO 909 make Thermo-anemometer. Weight loss of the drying grapes was measured using OHAUS - PAG 214 analytical weighing scale. All the above-mentioned equipments were calibrated before conducting the experiment. LAB TECH make water bath was used for maintaining the dipping solution temperature at 40°C during the pretreatment process.

2.3 Experimental Procedure

In order to determine the initial moisture content of grapes, nine uniform sized grape berries of both varieties were weighed initially and then placed in a hot air oven at a constant temperature of 110°C till they attained nearly constant weight. The grapes after removal from the oven were placed in a desiccator to cool them down before weighing. The initial moisture content of Thompson seedless was 79.94% (wet basis), while that of Sharad seedless was 81.38 % (wet basis). The dipping solution temperature was maintained at 40°C by using a water bath. Electrical supply to the heater was switched ON and grapes were then spread uniformly on the mesh tray as a thin layer, after the drying tray attained the required drying temperature of 60°C.

Grape samples of 100 g each of both varieties were considered for the drying experiment. Weight loss was noted for three specifically selected grape berry samples of both varieties at every one hour interval. Drying experiment was continued until a final moisture content of 18 % (wet basis) was reached. Drying air velocity measured at the outlet of chimney was 0.82 m/s.

To describe the drying kinetics of agricultural products though different models have been proposed, Singh *et al.,* (2012) have shown that the exponential model given by:

$$MR = a \exp(-kt) \quad \text{----} \quad (1)$$

provides a good prediction of the drying characteristics of seedless grapes. In Equation 1, '*k*' is called the drying rate constant. Drying rate constant describes the mechanisms of heat and mass transport phenomena and investigates the influence that certain process variables exert on moisture removal processes.

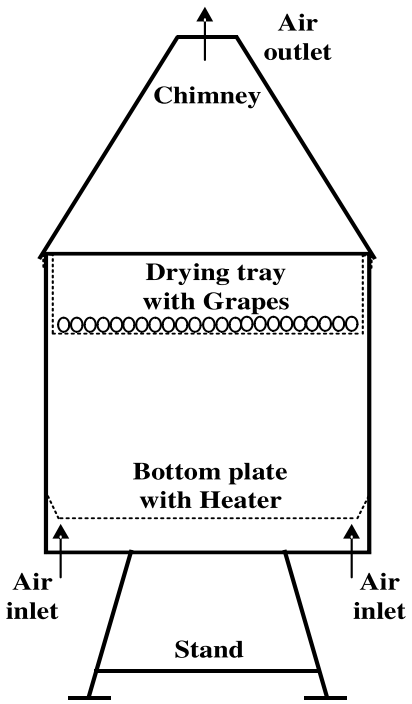

Figure 1: Schematic diagram of laboratory scale hot air dryer used for drying grapes

3. Results and Discussion

3.1 Drying time and drying rate constant

The moisture ratio is found to decrease continuously with increase in drying time. The entire drying process occurred in the range of falling rate period. Drying time recorded were 19 and 27 hours respectively for Thompson seedless and Sharad seedless grapes. After comparing the drying time of both the grape samples, it was observed that Thompson seedless required 42 % lesser drying time than Sharad seedless grapes. Reduction in drying time of Thompson seedless is due to higher skin permeability and more porosity being created resulting in higher mass transfer. As skin permeability and porosity created is less in Sharad seedless the mass transfer is low. As the drying time required for Thompson seedless was less, the value of drying rate constant was high. As the drying time required for Sharad seedless was high, the value of drying rate constant was low.

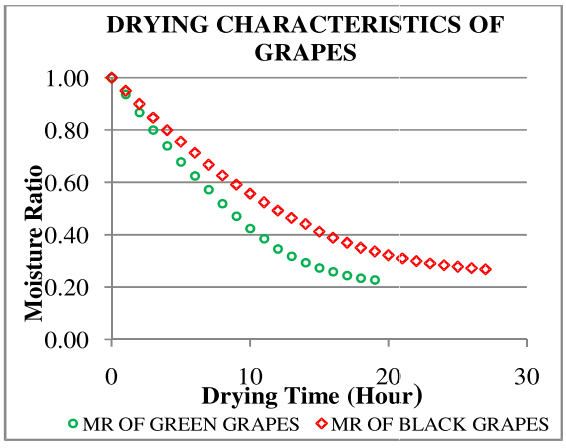

Figure 2: Drying characteristics of grapes dipped for 3 minutes in a solution maintained at 40°C and dried at 60°C in a Laboratory scale hot air dryer

3.2 Appearance and quality of raisins

One of the most important criteria of foods is colour. Undesirable changes in colour of food may lead to a decrease in its quality and marketing value (Doymaz and Pala, 2002). Shiny golden yellow coloured raisins with greenish tinge are in much demand. Important quality parameter requirements for raisins are soft texture, good skin integrity, small wrinkles, better collapsed structure of outer skin and non-sticky. The most distinctive feature observed while conducting experiments was the quality parameters of the raisins produced. No cracks were observed on the outer skin of both the raisin samples. Outer surface of the raisin samples had a soft texture and were neither sticky nor damaged. Thompson seedless had an attractive shiny golden yellow colour while Shard seedless had a black shiny appearance

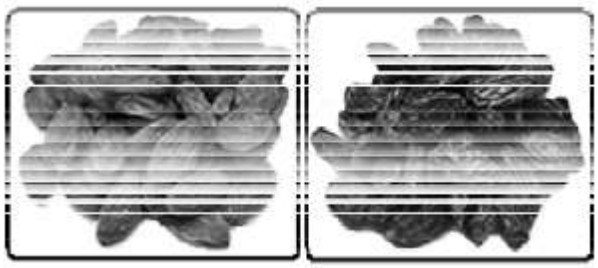

Figure 3: Photographs of raisins obtained with grapes dipped for 3 minutes in a solution maintained at 40°C and dried at 60°C in a Laboratory scale hot air dryer

3.3 Drying curves fitted into Exponential model

Values of moisture ratio and drying time in hours obtained from the experimental results were fitted into the exponential model y = a exp (kt) using Curve Expert Version 1.3. Non-linear regression analysis was carried out and estimates of the model parameters 'a', 'k' as well as correlation coefficient (R^2) and standard error (χ^2) obtained are tabulated in Table 1.

Statistical results such as correlation coefficient had values greater than the acceptable value of 0.99 and standard error values were very low. High values of correlation coefficient and low values of standard error indicate that the exponential model satisfactorily describes the drying characteristics of both variety grapes.

3.4 Realisation of drying rate constant values- by Curve fitting

Equation MR = a exp (– kt) can be written as log MR = – kt + a. The plot of log MR versus time is a straight line and slope of the straight line yields drying rate constant '*k*'. The values of log MR and drying time 't' were fitted into the linear model using Curve Expert Version 1.3, the parameter '*k*', correlation coefficient (R^2) and standard error (χ^2) values were obtained and are tabulated in Table 2. High values of correlation coefficient and low values of standard error obtained indicate the suitability of the linear model in estimating the value of drying rate constant. Drying rate constant value for Thompson seedless grapes was 0.036505 h^{-1} while that of Sharad seedless grapes was 0.024793 h^{-1}.

Table1: Parameters of Exponential model obtained by non-linear regression analysis for thin layer drying of grapes using CURVE EXPERT VERSION 1.3

Grape variety	Value of *a*	Value of *k*	Value of R^2	Value χ^2
Thompson seedless	1.0232422	- 0.085793	0.998406	0.014680
Sharad seedless	1.0110655	- 0.058235	0.999133	0.010193

Table 2: Drying rate constant of grapes and statistical parameters obtained by fitting Log MR Vs Time in a linear model using CURVE EXPERT VERSION 1.3

Grape variety	Value of *a*	Value of *k* (h^{-1})	Value of R^2	Value χ^2
Thompson seedless	- 0.00395181	0.036505	0.996893	0.018133
Sharad Seedless	0.00018227	0.024793	0.998093	0.012855

4. Conclusion

The dipping treatment in same solution for both variety grapes shows that their outer skin structures are not similar.

In order to enhance the drying rate of Sharad seedless the quantity of potassium carbonate may be increased or the dipping time duration may be increased or the dipping solution temperature may be increased.

- Thompson seedless required 19 hours while Shard seedless required 27 hours.
- Reduction in drying time of Thompson seedless is due to higher skin permeability and more porosity which resulted in higher mass transfer.
- Drying rate constant value for Thompson seedless grapes was 0.036505 h^{-1} while that of Sharad seedless grapes was 0.024793 h^{-1}
- Results obtained from this investigation, support the claim that Sharad seedless grapes have thicker outer skin when compared to Thompson seedless grapes.
- Pretreatment of grapes improved the appearance and enhanced softness of raisins.
- Exponential model satisfactorily describes drying characteristics of grapes.

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A Sociological Study of Determinates and Attitude of The People Toward Female Foeticide in Rohtak City of Haryana

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Abstract

The present study was undertaken to study the social determinant and attitude of people towards female foeticide in Rohtak city, Haryana. The study highlighted that socio-economic and cultural reasons were predominant in study area for female foeticide rather than economic reasons even among educated people, suggesting that people living in urban areas are first generation migrants of villages, carrying forward the rural ethos and backward tradition to have son for old age security and continuity of lineage of the family. Therefore, to curb problem of foeticides there is a need for amending legislation which is foolproof and bring socio and cultural reforms to make society a better place for women, where females have equal right to live as males.

Keywords: Female foeticide, Rohtak, socio and cultural reforms.

1. Introduction

Female foeticide is the selective abortion/ elimination of the girl child in the womb itself, done deliberately by the family after the detection of the child's gender through medical means. The mothers suffer usually under pressure from the husband and in-laws or even parents. Getting more than one girl child is generally the reason behind abortion in wait of son and pressure to maintain small family and fear of increasing maintenance and educational expenditure over children (Koradia, 2013).

Like many societies around the word, India too is patriarchal in nature. A hierarchical system prevails in all tiers of the social order. The fanatic obsession with the male sex, through, instances of growing sentiment for son as the key to continue the family lineage a women psychologically and socially forced to undergo multiple pregnancies and abortion, until she fulfills her lifelong goal to produce a male off spring.

It has age old lame reasoning, which is used as genuine justification by its staunch supporters, the root cause for female foeticide lies within the cultural norms as well as the socio-economic policies and practices in the society.

It has probably originated from the Asian countries like China, India, Vietnam, Korea etc., from where it has mushroomed today to the western nations like USA and Canada even (Satyamev Jayate, 2012). The female infanticide is not uncommon in Indian society and is still prevalent in certain parts of the country, due to poverty, malnutrition and lack of adequate health care system. With the advancement of modern technology and its practices for sex determination, however the problems has taken a different shape, as it is possible to detect the sex of the foetus, when it is still in the womb of the mother.

This has made it possible to know about the sex of the unborn child well in advance and hence abort the foetus, it is not desired. The most commonly used sex determination test is amniocentesis, discovered primarily for the detection of foetal malformations, over the years it has been used to determine sex of the foetus.

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In India, since 1978, the tests are being used as sex determination or more so as a sex selection test. Since then the test has become very popular and has led to mushrooming of private clinics of ultrasound sonography which perform the test, all over the country. The seriousness and social implications of this practice were realized only in 1986 (Part *et al.*, 1995).

In 1975, amniocentesis techniques for detecting foetal abnormalities began to be developed in India, at All India Institute of Medical Sciences, New Delhi. It was soon known that sex of the foetus can also be detected and doctors at the institute noted that most of the mother wanted to know the sex of the child and are not interested in the possibility of genetic abnormalities. Most women who already had two or more daughters and who learnt that their expected child was female went on to have an abortion (Chhachhi and Satyamala, 1986).

Between 1977 and 1985 in an effort to check the misuse of technology, three circulars were sent to central and state government departments making the use of the prenatal sex determination for the purpose of abortion as a penal offense (Kulkarni, 1986).

As a result of these efforts, the state government of Maharashtra implemented the Maharashtra Regulation of the use of prenatal diagnostic technique, Regulation and Prevention of Misuse Act in 1994. The act States that determining and communicating the sex of foetus is illegal. It also stated that such ultrasound sonography that tests can be carried out only in registered facilities and only to meet certain medical criteria, such as being over age 35, having, a family history of genetic disorders etc (Arora, 1992; Menon, 1996; Das Gupta, 1992).

Continuing with its dubious distinction of killing its daughter Punjab made it to the bottom fine state in the country in the child sex ratio (0-6 years) at 895. The only solace that the state can derive is from the fact that Haryana is at the rock bottom with 879 girls for every 1000 boys, followed by Jammu and Kashmir at 889. Other states in the list are Uttar Pradesh, 912 and Bihar, 912. National average is 940 as per census, 2011 (Census of India, 2011; Menon and Sreeltha, 2011).

The main aim of the present study is to evaluate the opinion of the respondents regarding the causes and determining factor for the female foeticide in urban areas of Haryana:-

I. To know the Socio-cultural determinants of the female foeticide.
II. To know economic determinants of the female foeticide.
III. To know the attitude of people towards foeticide and health concerning aspects of women.

2. Methodology and Data Collection

We selected Rohtak city for our study purpose as no such study is available in urban area in Haryana, which reveal Social determinants and the attitude of people towards female foeticide. The data are obtained by the interview schedule, random sampling and observation.

3. Results and Discussion

3.1 Trends of Child Sex Ratio

The data in Table-1 indicate those sex ratios for girl child is declining in most of the Indian states through the economic parameters are considered to be getting better. In India a number of cases have highlighted from that

area, where economy is considered to be better developed and people are better educated. The sex ratio is low in fewer of female. Among those regions, Gujarat, western Uttar Pradesh, Punjab, Haryana, Delhi and Chandigarh are more prominent. (Census, 2011; Dhar, 2010-11; Menan, 2011).

3.2 Trends in Number of children

Table-2 shows relation between respondent's age group and number of children.

Table1: Girl-child sex ratio during 1991-2011, in certain Indian states [1000 boys per girls (0-6 Years)]

State	1991	2001	2011
Jammu & Kashmir	-	941	859
Himachal Pradesh	951	897	909
Punjab	875	793	846
Chandigarh	897	843	867
Haryana	879	820	830
Delhi	915	865	866
Rajasthan	916	926	883
Uttar Pradesh	972	916	899
Bihar	953	948	933
Karnataka	960	946	943
Maharashtra	946	918	883
Manipur	974	964	944
Nagaland	993	964	934
Goa	964	938	920
Gujarat	928	978	886
Andhra Pradesh	975	949	943
Orissa	967	950	943
Tamilnadu	948	939	946
West Bengal	967	963	950
Mizoram	967	975	953
Tripura	967	975	957
Assam	975	964	957
India	945	927	914

50% respondent's have one child in the age group of 26-35 yrs, 45.5 % respondent's are having two children and 4.5% respondent's are having three or more children in the age group of 36-45 yrs. About 16.6 % respondent's having three or more children are in the age group of 36-45 yrs.

Table-2: Distributions of the respondents on basis of number of children and their age group

Number of children	Age of Respondents				Total
	26-35 yrs	36-45 yrs	46-55 yrs	Above 56 yrs	
One child	11(50.0)	3(16.6)	1(6.2)	1(12.9)	16(25.0)
Two children	10(45.5)	12(66.6)	6(37.5)	2(25.0)	30(46.9)
Three or more Children	1(4.5)	3(16.6)	9(56.2)	5(62.9)	18(28.1)
Total	22(100.0)	18(99.8)	16(99.9)	8(100.8)	64(100.0)

(Figure in the brackets represents percentage)

3.3 Knowledge about foeticide tools

Table-3 shows that now a days the most commonly used sex determination test are ultrasound sonography and amniocentesis. Out of these two, ultrasound sonography as a technique of foetus sex determination is more common.

Table -3: Distribution of respondent's knowledge of foeticide tools and techniques

Knowledge of sex determination	Number of Respondent's		Total
	Male	Female	
Amniocentesis	13(40.6)	15(46.8)	28(43.8)
Ultrasound sonography	19(59.3)	17 (53.1)	36(56.2)
Total	32(99.9)	32(99.9)	64(100.0)

(Figure in the brackets represents percentage)

3.4 Causes of female foeticide

Table-4, indicates the opinion respondents about the causes of female foeticide. As per the respondents, the most effective cause of female foeticide (43.4% female and 34% male) the socio-culture reasons for the lineage. As per total respondents 17.1% dowry was a major causes. The male respondents were more wormed about the dowry to be given to groom's family. Maintain small family was found second most important cause of female feticide as per the respondents (31.2% Male and 25% female) as compound to 15.6 percent respondents said son preference as a cause. However the data indicate that the respondents were not honest on speaking about their preference for a son over the daughter which appeared as an obvious reason when they were selective for small family size, and wanted to convey that girls are born less than the boys, when small family size is maintained .

Table-4: Percentage distribution of Respondent's opinion about most effective Socio-cultural causes of female foeticide-

Causes of female of foeticide	Number of Respondents		Total
	Male	Female	
For the continuation of the lineage	11(34.3)	14(43.7)	25(39.0)
Dowry problem	6(18.8)	5(15.6)	11(71.1)
Son preference	5(15.6)	5(15.6)	10(15.6)
Small family	10(31.2)	8(25.0)	18(28.1)
Total	32(100)	32(100)	64(100)

(Figure in the brackets represents percentage)

It is interesting to note that the causes of the preference for the male child were different for the male and females (table 5). For 43.7% female respondents the necessity of male child was community of lineage in comparison for 34.3% female, whereas 40.2 % male were of opinion that old age security is the major reason for preference to son rather than only 28.1% female respondents assigned this for having a son. Only 28.1%female respondents and 25% male were of opinion that sons are required for performing rituals over the daughter.

Table-5: Opinion of the Respondent's regarding the causes of the Preference for the male child

Causes of Preference for the male child	Number of Respondents		Total
	Male	Female	
Continuity of lineage	11(34.3)	14(43.7)	25(39.0)
Old age security	13(40.2)	9(28.1)	22(34.4)
Rituals norms	8(25.0)	9(28.1)	17(26.5)
Total	32(100)	32(100)	64(100)

(Figure in the brackets represents percentage)

Table-6: Respondent's opinion on side-effects due to foeticide practice

Side effects on health of women	Number of Respondent's	Percentage
Fever	19	29.7
Infection	15	23.4
Weakness	18	28.1
Excessive bleeding	12	18.7
Total	64	100

3.5 Health awareness of mothers

Out of 64 respondents who were interviewed for their awareness about the health related problems related to mother's health on feoticide practice, the maximum about 29.7% were aware about the occurrence of fever, closely followed by 28.1% who were of opinion that weakness occurs to female go for the feticide practice. About 23.4% respondents felt that infections may occur during the practice;

whereas only 18.7 percent were of the opinion that it can cause an excessive bleeding to the women.

Social awareness and education are the basic windows through which life of a neglected girl child can be made better. An educated nation can make full utilization of all its resource, including the most precious human resources, for its development and growth. In particular there are a number of social and economic issues that demand attention and agenda in some of the backward societies and nations. India which is trying to march forward in curbing social evils, many of which relates to foeticides, infection, dowry system and neglect of girl child etc. need to identify it root cause for a betters planning and execution. Most of the evils pertain to the society as a whole and have their origins in the oppression of women by men.

A variety of unimaginable barbaric methods are used to sniff out the life of the new born or unborn girls. It is clearly not just poverty which drives parent to kill their daughters; these are many socio-economic reasons behind it which need to be identified. This study reveal that cultural issue like continuity of lineage of family is more prominent cause in women's mind, whereas the old age security with son family rather than a daughter's family is more concerned to men. High amount of dowry and many other culture and socio-economic factors which drive the male dominating patriotically hierarchy towards female foeticide in most of the case.

Culturally females get shifted to the male's family after the marriage and are not allowed socially to look after their old age parents. Dowry did not appear as a major reason in the effluent urban society and woman were less bothered for dowry than man. It also appear that people in urban Haryana who are mostly the first generation migrants from the village to cities, do not bother than about need of son for religious rituals, rather they are more concerned to their old age security. About 47% urban family in Rohtak (Haryana) are preferring two child norm, where as the only 28% respondent had more than two children and only 25% had single child. Almost all respondents were aware of amniocentesis and Ultrasound sonography but less than 30% respondents were aware about one or more side effects of female foeticide to the women goes for the unnatural termination of pregnancy.

Surviving girl children undergo chronic nutritional educational and emotional deprivations and out of the 13 million girls born every year about 25% do not live to see their 15 birthday. The more effluent families resort subjective to the pregnant women to the widely available and cheap sonography test before they go for the more expensive and painful amniocentesis. Once it has been ascertained that the foetus is female, it is aborted. One doctor who has been facilitating the killing of the girl child by transporting the diagnostic equipment from village to village in Haryana and Punjab actually appear in a documentary film to justify his action. In his explanation, he was helping in Indian's population control drive.

What then can be done to correct this blinkered view of the girl child and her worth? The progressive and gender sensitive policies have not been implemented successively by the state governments, which has brought down sex ratio from 927 in 1991 to 914 in 2011 girl child sex ratio in India .

The awareness of female foeticide will probably curb the problem. In sub-urban areas,

quacks also jump on the sex determination bandwagon. Even though teeth to bring the quit to the book. Further, the legislation is not a foolproof method of stopping the in human practices of female foeticide.

The study represented that son preference still prevalent in our society and the urban educated people are not exception of such a traditional and backward attitude however, old age security and continuity the lineage of the family are more pronounced region of the son preference. This occur due to one son and balanced families i.e. one son and one daughter, the need of the son to carry their hierarchy and socio-economic security dominates the mind of the people. In this study we found that socio-economic and cultural reasons are predominant in this urban area than the economic reasons, as the people living in these urban colonies are recently migrated from their rural basis and most of them are very vitally connected to their rural bases, though they have obtained some so called 'Modern Values' e.g. small family size, dowry as a bad practice and equal job opportunities for women. A practice to rural ethos and similarity in the socio-cultural traditions as a continuity of rural traditions, it's quite obvious as the township in Haryana, are not much old phenomenon, and many residents in the area are first generation migrants from the villages. The study indicates that socio and cultural reforms are needed to make the society truly modern and livable for the women.

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Role of Tele-Communication in Rural Development in India

Pradeep Kumar Tiwari

Abstract :

This paper presents the impact of mobile communication on livelihood of rural India. This revolution has brought a revolutionary change in lifestyle of rural people like, farmers, bricklayers. It has impacted the education, health services, community services and other allied services in rural and semi urban part of India. In the era of globalization it empowers the villages to market their products globally. Now
Key Words- Rural Development, Livelihood, Telecommunication revolutions, Mobile Communication, Internet.

1. Introduction

Economic reforms in India post 1991, impacted Indian economy radically. The maximum impact was of telecom sector. Advancement in communication technologies has changed the way of life in India. It has impacted changed the socio - economic structure of India. Expansion of telecommunication services influenced not only socio-economic structure but the cultural development as well. Communication services empowered them to utilize their socio-economic and cultural capabilities in their personal, social and professional development and they improved themselves respectively. Mobile telecommunication has supplied the information to human being who is deprived with basic facilities like health, safe drinking water, human right, fundamental rights etc. It is mobile communication through which government is approaching directly to the end beneficiaries and communicating them about their policies and schemes run for the rural development. Overall teledenisity in India was 82.30% as on December 2015. Rural teledenisity was 42.12% and urban Teledensity was 57.88% as on December 2015. Growth in Telecommunication has affected the total GDP of India. McKinsey study has reported that if Teledensity increase by 10% then it will affect GDP growth by o.6%. Overall teledenisity in India is 82.30% as on December 2015. Rural teledenisity was 42.12% and urban Teledensity was 57.88% as on December 2015.

This aeon is the era of globalization. We cannot limit ourselves only up to local things. The rural development is a very critical issue for government and they know that without development of rural Bharat, India cannot become as a developed country. Rural areas are big market and with increased transaction power, they can change the country's economic scenario effectively. Mobile communication would be a tool to achieve this target. The contribution or rural communities in country's GDP is considerably high. Only agriculture contributes around 23% in country's GDP. Even after the continuous declination of this contribution in GDP, it is still the largest contributor in Indian economy and capable to give the employment to 65% OF total available workforce.(Neeraj Dangi et al 2010). India is second largest country in the world having land area around 168 millions hectare. Total cultivating area is around 90 millions hectare.

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But this area is full of diversity in respect of climate, agro - ecosystem, water, soil, environment etc. Despite of this diversity it is also a fact a fact that a variety of crops and livestock has developed over few decades in India. (Menon 2007). India is a country having largest diversity in crops and livestocks. Indian formaers cultivate arount 166 crop species and 320 wild relative crops. (Kothari 1994, Menon 2007)

The handicraft industry in rural communities is also an important means of employment and income generation. Over six millions population is engaged in handicraft business and they belomgs from the weaker section of the Indian communities. The size of India rural economy is $113 bn and is supposed to be translated in to $208 bn. (ASSOCHAM Report 2011) The Indian rural Telecommunication Industry is expected to account for more than 65% of the entire telecom subscribers in India. In an attempt to envelop rural subscribers, many telecom giants in India have willingly invested in rural telecom industry to benefit from its massive potential. Mobile equipments manufacturing firms like Intex Technologies, Karbonn, etc have allocated USD 21.03 mn for its promotional activities for 2010. (CII Report2009) It is interesting that 65% of service industry has been contributed by rural communities. (NSSO Report 2007) One more fact has been observed that rural areas are becoming as a new centres of BPOs. The role of tele-communication revolution is main cause behind thesde changes taking place.

Telecom revolution made possible to access the information to rural communities that could be helpful to change their livelihood. There are many initiative have been taken by government, for an example e-Choupal. E-Choupal have been able successfully to benefited the internet to farmers and other rural communities. E- choupal is running in various part of India with help of central government, state government companies and NGO's. Like e-Choupal by ITC. This program provides to rural communities with now how product and services, accurate whether information at right time, accurate pricing and access to global market and their position of their in respect of competitive product, by the means of single device known as mobile covering a wider network. It has been proved with time that millions of farmers are managing the risk involved in agriculture and other professions very well. Same is applicable for cottage industries, fisheries, handicrafts etc. The idea of e-choupal was first tested in Madhya Pradesh among the Soya Farmers with a very small fund Rs.50 Lack only. Till date around 5 millions farmers has been benefited with e-choupal program.

It is a fact that if Indian rural population learns to use the internet based applications and search engines, then it will bring a radical change in our society, development and demography. Mobile applications would be a great way to fulfil this dream. Although it is possible through computers as well and Indian government and NGO's has established many computer centers in rural areas to serve to rural communities and are educating them to learn and use these facilities in their day to day life. But accessing the information through mobile application is an easy and handy means of communication and fetching the information on real time. Many mobile applications have been launched for formers, labours, and fishermen etc. to help them. Mobile application is actually software installed on mobile handset or any other communication device, enabling a subscriber to accomplish one or more tasks

which are not related directly with device function. For example fetching the information about whether, agriculture, pesticides etc. There are many applications which are too easy to use and run on low speed data network.

74% consumers are from the rural areas of the total consumers in India. In recent years the lifestyle of rural consumers in India is changing dramatically. Their buying behaviour affected by various factors like, environment, socio-economic conditions, their literacy level and foremost the information and perception they have.(Singh et al2014) . India perhaps has largest rural market. It has around 47,000 hats more that 35000 supermarkets in USA. .(Singh et al2014) . Things are changing due to development programmes introduced in rural areas tom promote agriculture, handicrafts, education, communication, rural electrification and other allied activities.

2. Research Methodology:

The survey has been carried out in rural areas of the Pratapgarh District of Uttar Pradesh State. The presented study is purely done on the basis of primary data. Data has been collected by interviewing the respondents through a structured questionnaire. Total 200 respondents were selected out of 20 villages. 10 Respondents selected from each village. The selection of the respondents has been done by using multistage systematic sampling method. Collected data has been analysed with help of ANOVA.

3. Hypothesis,

1) There is a significant influence of mobile telecommunication on agricultural development rural India.
2) There is a significant influence of mobile telecommunication on education in rural communities in India.
3) There is a significant influence of mobile telecommunication on exposure of greater market size to rural communities to sell their product and services.

4. Data Analysis

Table 1. Preference for Mobile Communication

Responses	Frequency	Percentage	Cumulative Percentage
Yes	120	60	60
No	80	40	100
Total	200	100	

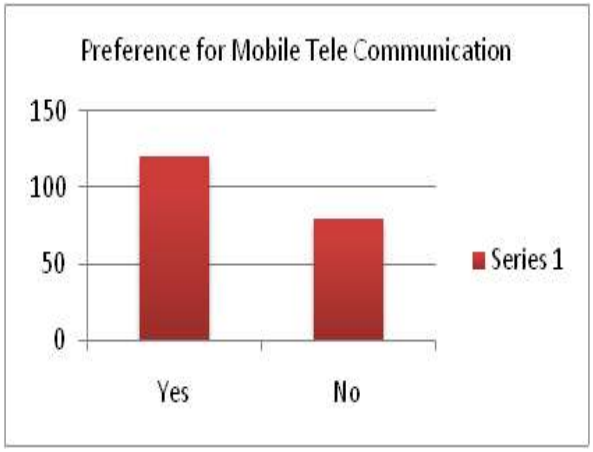

Figure 1

Table presents preference for mobile telecommunication of the respondents. 60% of the respondents prefer mobile communication and 40% respondents do not prefer mobile communication.

Table 2 - Impact of tale communication on Education in Rural areas.

Responses	Frequency	Percentage	Cumulative Percentage
Yes	140	70	70
No	60	30	100
Total	200	100	

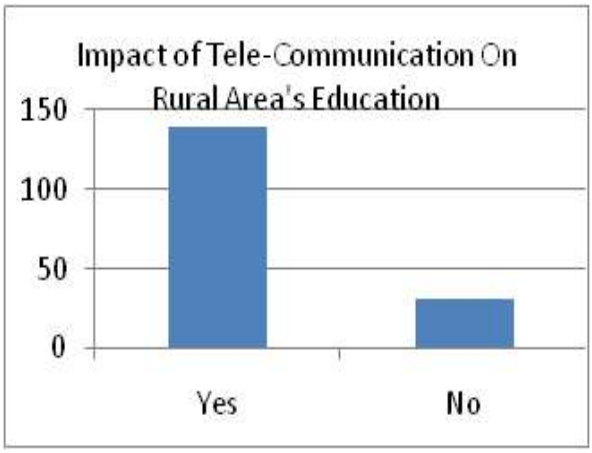

Table-2

presents impact of mobile telecommunication on education of rural communities. 70% of the respondents are favour that mobile communication has influenced the education in rural areas. and 30% respondents do not prefer mobile communication.

Table-3, Influence of Tele-Communication on Greater Exposure of Market for Rural Product and Services

Responses	Frequency	Percentage	Cumulative Percentage
Yes	110	55	55
No	90	45	100
Total	200	100	

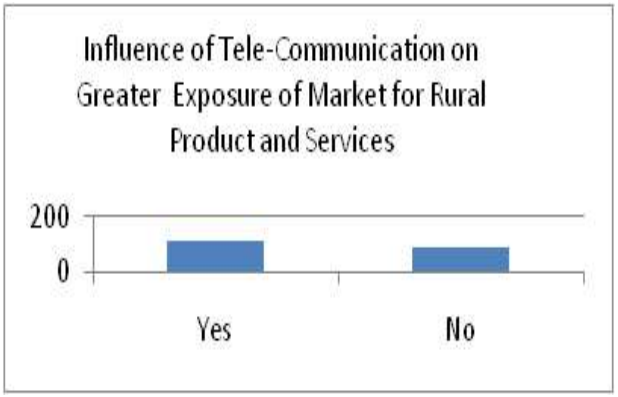

Table-3

presents preference for mobile telecommunication of the respondents. 55% of the respondents are of view that tale communication has opened new and greater market for rural industries and 45% respondents do not in the favour of statement.

Table-4, Influence of Tele-Communication on Agriculture Development

Responses	Frequency	Percentage	Cumulative Percentage
Yes	115	57.5	57.5
No	85	42.5	100
Total	200	100	

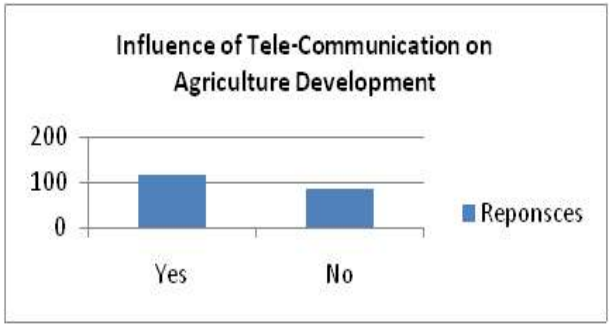

Figure -4

Table-4 presents influence of mobile telecommunication on agricultural development in rural India. 57.5% of the respondents are of view that tale communication has impacted the agricultural development and facilitated it in a better sense. While 42.5% respondents are against to this view.

5. Chi Square Test:-

We have tested the survey data by usibg Chi Squrae Test. We use the formulae mentioned as below,

$$X^2 = \sum_{i=1}^{r} \sum_{j=1}^{c} \frac{(O_{i,j} - E_{i,j})^2}{E_{i,j}}.$$

5.1. Hypothesis 1 :- 1

There is a significant influence of mobile telecommunication on agricultural development rural India..
Contingency table is as under

Agricultural Development	**Influence of Mobile Telecommunication**		
	YES	NO	Total
YES	100	40	140
NO	20	40	60
TOTAL	120	80	200

Calculated Value of Chi Square at 0.5 degree of Freedom is 25.3968, p-value at 5 degree of freedom is 0.0001 less than o.05. Thus relation is significant.

5.2. Hypothesis -2

There is a significant influence of mobile telecommunication on education, health and other allied services in rural communities in India
Contingency table is as under

Education in Rural Communities	**Influence of Mobile Telecommunication**		
	YES	NO	Total
YES	95	45	140
NO	25	35	60
TOTAL	120	80	200

Calculated Value of Chi Square at 0.5 degree of Freedom is 12, p-value at 5 degree of freedom is 0.0004 less than o.05. Thus relation s significant

5.3. Hypothesis 3

There is a significant influence of mobile telecommunication on exposure of greater market size to rural communities to sell their product and services.
Contingency table is as under;

Agricultural Development	Influence of Mobile Telecommunication		
	YES	NO	Total
YES	90	20	110
NO	30	60	90
TOTAL	120	80	200

Calculated Value of Chi Square at 0.5 degree of Freedom is 46.49, p-value at 5 degree of freedom is 0.0001less than 0.05. It implies that relation between both variables is significant.

There is a significant influence of mobile telecommunication on education in rural communities in India.

6. Result and Discussion

In last two decades telecom in India encountered with a substantial growth rate. In Ninth, tenth and eleventh five year plan, there was a strong determination and focus on providing tale-services not only on demand but at universal convergence equipped with world level services. Government has targeted and pass the regulation to telecom operators to strengthen mobile communication network in only metros and urban areas but to develop the infrastructure and provide the same services in semi urban and rural areas. The result is that the teledenisity in rural areas improved up to around 43% . The idea of shore up the rural mobile communication is an integral ingredient of well being and development of rural India. It has affected the various facets of life style and social structure. Few main effects are as under,

Mobile tele phony is playing a vital role in the establishment of strong

communication between government and rural people. Now government is using mobile tale-phony as a tool to aware the rural development about the policies and schemes for them. Not only awaking them but also ensuring that benefit must be reached to them. One application launched by Vodafone foundation named as WOSCA to track the livelihood of the people in tribal areas of Orissa.

Mobile telephony has affected the way of doing agriculture. It has enables the farmers to fetch the more advanced, accurate and reliable information on whether, agriculture, alternative crops, more fertile and productive seeds and their availability. It is also empowering them to come up and compete with globalization. Government has launched various mobile applications to fulfil this purpose. On December 2015 ministry of Agriculture, Government of India has launched two mobile applications for farmers - Crop Insurance and Agri-Market Mobile KISAN SANCHAR is another mobile application helping the farmer in farming.

Mobile telephony has affected the quality and availability fundamental facilities like education, health, transportation, business and other allied services. It has enables them to communicate with each other more quickly and share their views, fetching the information through internet, reading e-content on mobile. Now they using the mobile apps to be up to date. Like read the newspaper by installing the news papers apps. . One another mobile application is MAHILA SHAKTI helping women to get better understanding of basic mathematics.

7. Recommendations

Affordability of mobile tele phony voice calls or data calls is a considerable issue and needed to address with an appropriate business model. It is recommended to encourage the usage of internet and mobile application. There is a need of more franchisees working in more transparent model. Government and corporate bodies should encourage the BPO business in rural areas. It will not only generate employment but operators will be obliged to development the telecom infrastructure in rural area with advanced technology and adequate capacity.

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The Ethics of Caring: A Study of Doctors in Hospital Settings

Ramesh Kumar Sangwan*

Abstract

Medical ethics are not merely a moral code but a legally sanctioned code of conduct acceptable and normal within the medical profession. This does not mean that morality or moral theories do not influence medical ethics, but medical ethics must be understood, analyzed and practiced from a rational standpoint. This rational component of professional conduct is legally codified under the code of medical ethics of the legally constituted medical council with which all qualified medical practitioners must be registered.

Professional ethics of a doctor suggest that a doctor is supposed to treat the patient irrespective of caste, class, ethnic, gender and other biasness. Forty one per cent doctors straight away replied that they can refuse treatment whereas majority of the doctors i.e. fifty nine per cent still maintain that they cannot refuse treatment to any patient.

Keywords: Health, Ethics, Doctor

1. Introduction

1.1 Medical Ethics

Most of our personal and professional attitudes stem from handful of values. There are four main sources from which we derive our values: experience, culture, science and religion. Our values are shaped by experience. Those who have felt the sting of discrimination may become champions of fair and just treatment. Health professionals who have been patients themselves can become highly sensitized to patient's rights. The values we hold as individuals and as groups are inseparable from the continual changing experiences of our lives. Much of our experience is vicarious, we learn by watching and listening to other people. What others have experienced or are experiencing are powerful influences in ordering our personal values.

Each one of us has a value system that is unique. Nonetheless, our individual values originate from the core value of our culture. Our culture offers variety of positions on these issues that can affect ones practice as a clinician. For example, ignoring a person's feelings of privacy or being insensitive to his or her religious beliefs, not being considerate of a person's reservation about confidentiality and informed consent, or disagreement about how much of a person's welfare check should be allocated to healthcare, can affect the type of relationship that can be established between a client and a helper (Coleman, 1974).

The practice of medicine has a special characteristic not found in so pronounced manner in other occupations. Doctors deal directly, and at a time when the person is most vulnerable, with the immediate issues of life and death, health and illness. The patient is heavily dependent upon the technical knowledge and integrity of the doctor. The doctor thus has a

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unique involvement with the patient, but this relationship, between doctor and patient, is not balanced. The patient's attitude is a complex of trust (which comes from perceived competence and integrity of doctor) and, paradoxically, also that of distrust which comes from the state of uncertainty and vulnerability (Jesani, 1995).

The feelings and expression of sympathy is an integral part of the approach to a patient. But sympathy has its pitfalls. The mere expression of sympathy, however genuine and heartfelt, is far from being therapeutic. Sympathy is an emotional response to be used under conscious intellectual control. It may act as a barrier to effective communication between doctor and patient, and secondly, it may be used by the patient for his own ends: the doctor may be maneuvered in to actions which may serve only to foster the patient's neurosis and be the reverse of therapeutic (Goel, 1990).

Health promotion initiatives and in particular health education programs, have two major ethical concerns: liberty and justice. With regard to liberty, they assert that health promotion programs should be carefully examined with reference to their degree of voluntariness. As they note, most programs are not simply informative but often involve some element of persuasion as well. They argue that programs should maintain, to the degree possible, options for voluntary behaviour and personal freedom on the part of the individual (Faden and Faden, 1978).

It is more likely that the classes must have overlapped and physicians must have shared the ideals and prohibitions of the hierarchical order. The place of palpation as an investigative tool is a case in point. Although palpation is an aspect of physical examination, it never becomes the instrument it could have been. The taboos against touching seem to have played a role, and even if fear of contamination is accounted for, the indigenous theory of purity and pollution must have played a major role. After all, the physician was inferior because he mingled with all sorts of people of inferior classes (Chattopadhyaya, 1977).

The process of helping involves confronting one's values with others. Often there is a gap between patient's or client's values and what the helper views as important, or there is a direct clash between client's goals and practitioner's values and culture. Clinical sociologists need to have a good understanding of their own values before they can understand the value systems of others. Hoff has pointed out that it is important for the helper to "attempt to understand" the patient or client and "to make the patient or client feel understood." This entails removing barriers to communication such as unwarranted assumptions and double messages that are value driven. We have personal and professional codes of ethics that spell out certain limits for what constitutes acceptable and unacceptable behaviour (Hoff, 1989).

When doctors enroll themselves in the profession, they are bound to observe certain professional ethics and decorum. That is not for sainthood but for the upkeep of a certain code of professional conduct which was practiced by seniors. This gives us the social recognition and dignity which is earned collectively. In India, no qualified doctor willing to practice ethically is going to starve. There is enough revenue generation from consultancy for everybody's need, but may be not enough to everybody's greed. The "greed" is often perceived and firmly believed as "need" when you actually embark upon certain ambitions of life for the sake of survival in cut-throat competition. One more

building block, one more operation theatre, a CT scan machine and similar sorts, for which banks are ready to advance loans fast but also quick to retract when EMIs are delayed. The stress is passed on to the middle class patients who might not have actually needed it at all (The Hindu, July 17, 2011).

It has been argued that mainstream medical ethics is biased by the assumption of a framework in which individuals are not simply free to contact with one another to provide whatever medical treatment is demanded, subject to the ability to pay. Because a high proportion of medical care is typically provided via the welfare state, and because there are legal restrictions on what treatment may be provided and by whom, an automatic divergence may exist between the wishes of patients and the preferences of medical practitioners and other parties. Tassano has questioned the idea that beneficence might in some cases have priority over autonomy. He argues that violations of autonomy more often reflect the interests of the state or of the supplier group than those of the patient (Tassano, 1995).

In the field of medicine, basic ethical conflicts are reflected in the struggle for control over knowledge, its use or misuse by professionals and the role of the state in ensuring equity in health. As the scope of medicine expanded from clinical practice to mass interventions and the realm of public health, ethical guidelines were initially built in to code of conduct for physicians, the rights of patients and informed consent (Leary, 1997).

Numerous indicators reflect a growing interest in ethical issues in medical and health care. Increasing media coverage includes discussion of ethical issues surrounding such developments as has been the case with reproductive technologies such as surrogate motherhood, cloning of human beings etc. Moreover, professional organizations are devoting more time at their meetings and more space in their publications to discussion of ethical issues (Gottlieb et al., 1987). Against this background one might suspect an equivalent development of sociological interest in medical ethics. This is so because many issues in medical ethics are sociological topics of major interest. For example, work in the area of medical care ethics, particularly work focusing on the rights and duties of medical professionals and patients, bears directly on the evolving nature of the doctor-patient relationship (Stacey, 1985). Ethical analysis of medical and health care public policy has significant implications for such macro sociological issues as equality in access to care and the role of the professions in determining the availability of medical and health care services (Mechanic, 1979).

The relationship between ethics in idealistic perspective and in the reality has been the subject of debate in Sociology of Health. The disease is experienced by the patient and the doctor along with providing the health care also bound by medical ethics. The manner in which the relation between sufferer's experience and ethics of doctors are articulated is a core aspect of Sociology of Health.

2. Sample

The purpose of our study is to understand the medical ethics; therefore hospital institutions are taken into account. Hospitals are comprised of doctors, patients and other para-medical staff. Out of the 21 districts, we randomly selected Bhiwani district, which is one of the largest districts in the state of Haryana. To select the hospitals for the study

purpose, we decided to study both government and private hospitals in Bhiwani city of Haryana. Bhiwani has one big govt. hospital and approximately 700-800 patients visit in a day in the Out Door Department (OPD) and 80-90 (per day) patients in indoor. On the other hand, there are 46 well established private clinics/nursing homes/hospitals.

Among 46 private hospitals, only 2 clinics are providing emergency ambulance services, and They are J.B. Gupta Hospital and Chugh Multispecialty Hospital. Three hospitals are providing free OPD service, which are newly established and providing free OPD facility for their advertisement and initial reputation. The oldest hospital is 'Ganpatrai Maternity & General Hospital (estb. 1954) which is run by trust. The per day indoor patients in Ganpatrai Maternal and General Hospital are less as compared to the second oldest 'Chugh Multispecialty Hospital (estb. 1979), therefore, we selected the hospital which is oldest in terms of year of its establishment and simultaneously has highest number of indoor patients. Thus, it becomes important to look into the nature of medical ethics and to what extent health professionals practice medical ethics in their professional life. After a presentation of the problems as it reveals itself in the analysis of medical ethics, the paper proceeds to examine the medical ethics in the processual form.

3. Medical Profession and Ethics

Medical ethics concerns how to handle moral problems arising out of the care of patients; offen clinical decisions must consider more than just the patients medical condition. Medical ethics must be understood, analyzed and practiced from a rational standpoint. This rational component of professional conduct is legally codified under the code of medical ethics of the legally constituted medical council with which all qualified medical practitioners must be registered.

The registrations as well as the conduct as per the code of ethics are essential because, "doctors use technical skills and expertise which the untrained person does not possess. Possessing these skills gives him great power over his patients who by the very fact of being patients are dependent, ill and vulnerable. In caring for his patients, a doctor makes a series of judgments and decisions which patients have the right to expect are made fairly in the light of the doctor's knowledge and experience" (British Medical Association, 1986). Thus, although the code of medical ethics is for the self-regulation of the profession, it is there in order to fulfill certain rights and expectations of the patient. In a nutshell, the code describes the doctor's duties towards the patient. If these rights are not fulfilled or duties are not performed, then the doctor in question loses his or her right to be part of the profession.

Table 1: Doctors and Ethical Elements in Profession

Doctors and Ethical Elements in their Profession	Government Hospital	Private Hospital	Total Response *
Respect for autonomy	11 (50)	5 (22.73)	16 (72.73)
Beneficence and Non-malfeasance	9 (40.91)	3 (13.64)	12 (54.54)
Justice	7 (31.82)	2 (9.09)	9 (40.91)
Informed consent	10 (45.45)	5 (22.73)	15 (68.18)
Truthfulness and confidentiality	5 (22.73)	2 (9.09)	7 (31.82)

(Figures in the bracket represent percentage)
**Multiple Responses*

The Medical Council of India under its Medical Council Act, 1956 has recently, i.e. in 2002, made the regulations relating to the professional Conduct, Etiquette and Ethics for registered medical practitioners. Based on fieldwork with doctors' practice in government and private hospital settings, the basic elements of medical ethics in the profession of medicine are there, however, their priority to practice them in doctor's professional life depends upon the individual doctor.

There are five major elements of ethics in the medical profession. They are respect of autonomy of medical professionals, beneficence and non-malfeasance, justice to all patients, informed consent and truthfulness and confidentiality in the medical profession. We presented these above ethical elements to the doctors and ask them to provide their preferences in order of priority for the medical ethics. Nearly seventy three per cent of the doctors mention that autonomy of the doctor is very important in the medical profession. Another fifteen (68.18 per cent) give priority to informed consent in their profession. 12 (54.54 per cent) doctor maintain that medical profession is basically a profession of beneficence and non-malfeasance. To provide justice to all irrespective of their strata is another element of ethic in the medical profession (see table-1).

4. Nature of Medical Ethics

Borrowing from feminist discourse and social psychology (Gilligan, 1982) this provides the notion of moving away from the ideal of a clinician who 'objectively' keeps his distance from the patient, who must not get emotionally concerned. In addition, it requires involving patients in decisions about their treatment and fulfilling subjective 'felt needs' to achieve patient satisfaction. Similarly, the community must be involved in deciding its health activities. Those viewed as the main 'beneficiaries' of the health services are to get both autonomy and justice, and therefore their voices must be acknowledge as valid and critical.

We asked doctors very pertinent question as to what conditions medical ethics. The responses collected from the doctors are varied. Fifteen (68.18 per cent) of the doctor maintain that they respect the confidentiality of the patient in terms of disease as well as about treatment. Nearly sixty four per cent of the doctors view medical ethics in terms of health and life of patient as the prime concern. Exactly fifty per cent doctor feel that it is unfair to use medical knowledge contrary to the laws of humanity. 16 (72.73 per cent) feel that doctor should use their medical power and knowledge for the benefit of the patient. Majority of the doctors i.e. 81.82 per cent maintain that medical profession is the profession of conscience and dignity (see table- 2).

Table 3 shows the doctors immediate response towards the person who is sick or injured outside the hospital. Fifty per cent of the doctors mention diplomatically that it depends upon the situation of the person. However, nearly forty six per cent of the doctors reply that they will immediately provide first aid to the patient irrespective of any situation. 8 (36.36 per cent) doctors mention that they will immediately inform their relatives.

5. Ethics in Practice

Ethics has been suggested as an additional window to ascertain the criteria for quality of health care and draw attention to some of the less-attended dimensions (Kass,

Table 2: Nature of Medical Ethics

Nature of Medical Ethics	Government Hospital	Private Hospital	Total Response*
Doctor should always use 'medical power' for patient's benefit	11 (50)	5 (22.73)	16 (72.73)
Profession with conscience and dignity	12 (54.54)	6 (27.27)	18 (81.82)
Humanity or to save to mankind	8 (36.36)	4 (18.18)	12 (54.54)
Respect and gratitude	7 (31.81)	3 (13.64)	10 (45.45)
Maintaining good medical practice	8 (36.36)	3 (13.64)	11 (50)
The health and life of my patient will be my first consideration	9 (40.91)	5 (22.73)	14 (63.64)
Do not use medical knowledge contrary to the laws of humanity	8 (36.36)	3 (13.64)	11 (50)
Respect the secrets which are confided to me	11 (50)	4 (18.18)	15 (68.18)

(Figures in the bracket represent percentage)
**Multiple Responses*

Table 3: Response of Doctor toward Sick or Injured Outside the Hospital

Response	Government	Private	Total
To provide him/her first Aid	7 (31.82)	3 (13.64)	10 (45.45)
To admit him/her in nearby health centre	3 (13.64)	2 (9.09)	5 (22.73)
To inform his/her relative	5 (22.73)	3 (13.64)	8 (36.36)
Depend on situation	7 (31.82)	4 (18.18)	11 (50)

(Figures in the bracket represent percentage)
**Multiple Responses*

Table 4: Can a Doctor Refuse Treatment?

Can a doctor Refuse Treatment?	Government Hospital	Private Hospital	Total
Yes	6 (27.27)	3 (13.64)	9 (40.91)
No	7 (31.82)	6 (27.27)	13 (59.09)
Total	13 (59.09)	9 (40.91)	22 (100)

(Figures in the bracket represent percentage)

2001). However it has been difficult to develop operational definitions for this seemingly consistent set of principles of bioethics due to their contrary implications in many instances, such as the conflict between the individual versus the collective good and agency of patients as against responsibility of the care provider. The following outline of components of a philosophy of ethics for health care system has been proposed to include the motivations and attitudes of providers necessary to ensure improved 'quality of health care' (Priya and Singh, 2003).

6. Professional Ethics and Treatment to Patient

Professional ethic of a doctor suggest that doctor is suppose to treat the patient irrespective of caste, class, ethnic, gender and other biasness. We simply asked our respondents if they could refuse treatment to the patient. As many as 9 (40.91 per cent) doctors straight away replied that, they can refuse treatment whereas majority of the doctors i.e. 59.09 per cent still maintain that they cannot refuse treatment to any patient (see table- 4).

The doctors who refuse to treat the patient have shared their reasons for it. We received responses in the multiple forms. Nearly eighty nine per cent of the doctors say that there is no legal binding on them to treat patient after their duty hours. Furthermore, seventy per cent of the doctors share that they treat patient only when they think that it is an emergency. Some had a bad experience in the past for treating patients after their duty hours; they also refuse to treat the patient when there are no facilities available facilities to treat

patients. Some time doctors also refuse to treat the patient, if they are already occupy with the treatment process of some others patient or if the they themselves are not well. Some doctors also refuse to treat patient in odd hours (see table- 5).

7. Conclusion

There are five major elements of ethics in the medical profession. They are respect of autonomy of medical professionals, beneficence and non-malfeasance, justice to all patients, informed consent and truthfulness and confidentiality in the medical profession. Nearly seventy three per cent of the doctors mention that autonomy of the doctor is very important in the medical profession. Another, nearly sixty nine per cent doctors gives priority to informed consent in their profession. To provide justice to all irrespective of their strata is another element of ethic in the medical profession.

Sixty eight per cent of the doctors maintain that they respect the confidentiality of the patient in terms of disease as well as about treatment. Nearly sixty four per cent of the doctors view medical ethics in terms of health and life of patient as the prime concern of doctors. Majority of the doctors, i.e. eighty two per cent maintain that medical profession is the profession of conscience and dignity. Fifty per cent of the doctors mention diplomatically that it depends upon the condition of the person. However, nearly forty six per cent of the doctors reply that they will immediately provide first aid to the patient irrespective of any situation. Professional ethics of a doctor suggest that a doctor is supposed to treat the patient irrespective of caste, class, ethnicity, gender and other biasness. Forty one per cent doctors straight away replied that they can refuse treatment whereas majority of the doctors, i.e. fifty nine per cent still maintain that they cannot refuse treatment to any patient.

The doctors who refuse to treat the patient, have shared their reasons for it. Nearly, eighty nine per cent of the doctors say that there is no legal binding on them to treat patient after their duty hours. Furthermore, seventy per cent of the doctors share that they treat patient only when they think that it is an emergency Sometimes doctors also refuse to treat the patient if they are already occupied with the treatment process of some other patient or if they themselves are not well. Some doctors also refuse to treat patients in odd hours.

Hospital is part of a sophisticated health service. It is connected in thought to a wide network of people and in actions which in one way or another is concerned with maintaining and improving his health for the rest of his life- as for the rest of the population. Doctors, policy makers, and now a days, financers for big corporate hospitals and private hospitals together form a health system. Health systems have played a part in the dramatic rise in life expectancy, diagnosis of diseases, treatment of number of diseases, decreasing percentage of mortality with diseases are some of the manifestations in the 21st century. Medical science has contributed enormously to better health and influenced the lives and well-being of billions of men, women and children around the world. Their role has become increasingly important. With the increasing level of education, knowledge and awareness of people about their health responsibilities, their health behaviour, their process of seeking treatment and of course awareness about health right has also contributed to the wellness of human society.

Table 5: Reasons for Refusing Treatment

Reason for refusing Treatment	Governmen t Hospital	Private Hospita	Total Response
If the doctor himself is not well	3 (33.33)		5 (55.55)
If he has had a bad experience in the past	4 (44.44)		7 (77.78)
In odd hours	1 (11.11)		3 (33.33)
There is no legal obligation to treat patient after duty hours	5 (55.55)		8 (88.89)
Doctor treats a patient in emergency on ethical grounds	6 (66.67)		7 (77.78)
Due to non availability of certain facilities, instruments, medicines, staff etc	4 (44.44)		7 (77.78)
Already busy with other patients	3 (33.33)		4 (44.44)

(Figures in the bracket represent percentage)
**Multiple Responses*

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Regression Modeling of Occupational Stress Among Workers of Unorganized Sector

Kalpana devi[1] and U.V.Kiran[2*]

Abstract

The purpose of the study is to investigate the possible cause and effect relationships between occupational stress as dependent variables, and age, working experience, number of working hours daily as independent variables. With the help of linear regression model, the relationship has been explored. The present study was conducted on the workers working in unorganized sectors at Lucknow city, India. Unorganized sector comprises of construction, chikankari, brick kilns, sanitary and ornament workers. The study adopted survey research design and a sample of 500 workers were selected for the study. The data was collected with the help of modified version of occupational stress scale developed by the Srivastava AK (1976). The findings of the study revealed that age, gender, types of work are able to explain only 54% variance of the total variance in occupational stress.

Keywords: Linear regression model, Occupational stress, unorganized sector

1. Introduction

Today's it is a global economic completion where each country has to competes with others in terms of economy. The economic condition of the country purely reflects the status of industry, trade in that country. The whole of the economy moves around the formal and informal economy. The part of informal economy is important for a country as this part of the economy reflects the level of activity and its contribution in economy performed by the last person of the country.

The argument of informal economy was developed by the three different schools of thought. John. K. (2012) has reviewed these three schools of thoughts as the dualist school, popularized by the ILO in the 1970s, considers informal sector as distinct and not related to the formal sector. According to this school of thought, informal economy provides income for the poor at the time of crisis. The structuralized school was popularized by Caroline Moser and Alejandro Ports in the late 1970s and 1980s. This school establishes a connection between the formal sector and the informal sector. Informal sector increases the competitiveness among large firms by reducing labour and input costs. The legalist school was popularized by Hernando de Soto in 1980s and 1990s. According to this school, the informal sector consists of micro-entrepreneurs who choose to operate informally to avoid the costs, time and effort of formal registration (Chen, 2004).

The Occupational stress may be defined as the harmful physical and emotional responses arises inherent to an individual at the time when there is a mismatch among job demanded and the capabilities of the individual as well as the required resources used to complete the job or needs of the

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worker. Occupational stress is continual situation arised by the environmental and condition at work place which negatively affect an individual's capability of performing a job as well as the physical well being.

According to Selye (1956) stress is "any external event or internal drive which threatens to upset the organism equilibrium". Lazarus (1966) referred "stress a state of imbalance with in an organism that is elicited by an actual/perceived disparity between environmental demands and the organism's capacity to cope with these demands; and is manifested through variety of physiological, emotional and behavioral responses". Stress is injurious to not only to physical health but mental health also. Therefore it should be important task for an individual to manage the stress arised from jobs along with the other environmental factors. As stress is a negative response of organism, in order to cope with this, one have to learn relaxing their organism every day in his life. It is better to take precautions to prevent the stress, and try to reduce the level at all time for the betterment of life.

2. Literature Review

Occupational stress is a response of inner organism but affects the physical well being. In the past researchers have investigated several aspects of the occupational stress and influencing factors which have influences on the occupational stress. Majorly, the occupational stress is explored with the help of demographic variables, working environment factors, some of the important studies related to this are reviewed.

Occupational stress is influenced greatly by the demography of the respondents. Aadya and Kiran (2013) argued that the level of occupational stress i.e., physical stress and biomechanical stress among various types of work was significantly different and it was also revealed that highest amount of stress is perceived by the women involved in sanitary work. The occupational health is closely related to the occupational stress, in a study of women farm workers in lucknow city. Dwivedi and Kiran (2013) has showed that majority of the workers feels pain in upper arms and lower arms as they were exposed to high level of repetitive task and threshing. The physical discomfort leads to stress among the farm women as they have to perform different roles in the society.

The socioeconomic status of individuals may lead to work hard in informal economy; these factors hamper the level of physical well being and increase the level of occupational stress, poor socioeconomic status, poor working conditions, job insecurity or poor social support, poor working environment, work deadlines, too much work, lack of support and liberty for the work may play a significant role in causation of stress (Sharma and Mahajan, 2013).

Johnson et al. (2005) has investigated the relationship of occupational stress across a large and diverse set of 26 occupations, with the other stress related variables as psychological well-being, physical health and job satisfaction. The relationship between physical and psychological stress

and job satisfaction at an occupational level is also explored. Six occupations reported worse than average scores on each of the factors– physical health, psychological well-being and job satisfaction (ambulance workers, teachers, social services, customer services– call centers, prison officers and police). Differences across and within occupational groups, for example, teaching and policing, are detailed. The high emotional labor associated with the high stress jobs is discussed as a potential causal factor.

Niharika and Kiran (2014) have studied the relationship among the occupational stress and other variables, in hierarchy, work experience, type of bank. Their work revealed that private bank employees had high occupational stress due to strenuous working conditions, unreasonable group, and role conflict, under participation, peer relations and intrinsic impoverishment in comparison to nationalized bank employees because of their heavy workload and work pressure to achieve their target. A positive highly significant relationship was observed between occupational stress and independent variables. Positive relationship was also observed between types of bank with role overload. Highly significant negative relationship also is observed between independent variables and occupational stress.

Occupational stress is highly influenced by environmental factors, where a worker works, the variables related to the nature of job is also an important factor to study the occupational stress.

- Based on the literature review, it is very clear that occupational stress is a very common factor among the workers of organized and unorganized sector.
- Hence, a study was conducted to study and compare occupational stress among workers belonging to various works of unorganized sector.
- Many independent variables were considered under the study, hypothesized, to the effecting the occupational stress among the respondents the present paper is an attempt to fit and test the linear regression model to estimate the impact of identified independent variables on the dependent variable is occupational stress.

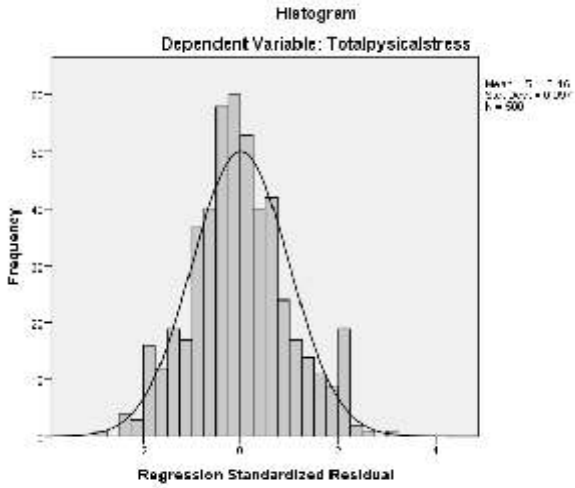
3. Data and Methodology

The data was collected from the workers of five different sectors construction, chikankari, sanitary, ornaments, brick kilns in Lucknow city, state capital of the Uttar Pradesh. The area for study was selected using multistage random sampling techniques. Data was collected using modified version Occupational Stress Scale developed by Srivastava AK (1976) consist of 26 statements, comprising 17 items of physical stress. The total score obtained by a respondent through the score for each item as recorded by using 5 point Likert scale, scores ranged from 5 for strongly agree to 1 for strongly disagree. The demographic variables and working pattern were recorded with the help of self developed instrument. The respondents were selected randomly and interviewed personally in order to get their responses by using the above interview

schedule, total, of 500 workers 100 from each of the sectors were interviewed.

4. Results and Discussion

Descriptive table shows that Average amount of physical stress among the workers is 47.27, average age of the respondents is 32 years, and average of types of works are 3.0, average of the gender is1.6 that means majority of the workers are females.



Descriptive statistics

	Mean	Std. Deviation	N
Total Physical Stress	47.27	8.099	500
Age of the respondent	32.40	9.116	500
Gender	1.60	.490	500
Types of work	3.00	1.416	500

Model summary

Model	R	R Square	Adjusted R Square	Std. error of the Estimate	Change Statistics				
					R Square	F Change	df1	df2	Sig. F Change
1	.224[2]	0.05	0.044	7.918	0.054	8.718	3	496	0

a. Predictors: (Constant), age of the respondent, gender, types of work
b. Dependent Variable: Total Physical stress

ANOVA

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1639.684	3	546.561	8.718	.000[b]
	Residual	31095.324	496	62.692		
	Total	32735.008	499			

a. Dependent Variable: Total Physical stress
b. Predictors: (Constant), age of the respondent, gender, types of work

Model		Un standardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	Constant	43.246	2.022		21.385	.000
	Age of the respondent	.065	.039	.073	1.672	.095
	Gender	-.910	.731	-.055	-1.245	.214
	Types of work	1.124	.253	.197	4.442	.000

ANOVA table reveals that significance value of the model fitted is 0.00 less than the 0.05 that means at the 95% confidence interval null hypothesis is rejected in specifying that the model is not good fit.

The above table shows that highest contribution in occupational stress is offered by the types of work by 1.12, followed by age 0.065, the contribution of gender is -0.9. Further analysis of t statistics, constant and types of works have the significance value, 0.00, 0.00, less than 0.05, which indicates that types of works and constant have significant importance in the explanation of occupational stress among the workers. The significance value for age and gender is more than the 0.05 which indicates that the null hypothesis that importance of these variables in explanation of the occupation stress cannot be rejected, i.e., age and gender do not have explanatory power to explain the occupational stress.

The regression expression can be written as:

Occupational stress = 43.62 + 1.13 Types of work + 0.06 Age -0.9 gender

5. Conclusion

Occupational stress has been thoroughly studied with the help of demographic variables, these variables are the important variables in order to understand the level of the occupational stress. The present study is an attempt to study the occupational stress in different manner, in order to understand the occupational stress with the help of the age, gender and types of work, a linear regression model considering occupational stress as dependent and age gender, types of work independent variables is fitted. The result of the present study revealed that occupational stress cannot be explained with the help of fitting linear regression models including the selected variables as it was not possible to capture the significant portion of the occupational stress, that means the occupational stress cannot be explained only with the help of age, gender, and types of work, even though the addition of the extra variables do not have any additional advantage in order to improve the explanation level of occupational stress.

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