Print - ISSN No. 2395-1605
Online-ISSN No. 2395-7395

International Journal of Science, Technology and Society

Volume 3 | Number 2 | December 2017

Babasaheb Bhimrao Ambedkar University
Vidya Vihar, Rae Bareilly Road, Lucknow - 226025, INDIA
www.bbau.ac.in

Print-ISSN No. 2395-1605
Online-ISSN No. 2395-7395

International Journal of Science, Technology & Society

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International Journal of Science Technology and Society

Volume 3, Issue 2

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Babasahab Bhimrao Ambedkar University, Lucknow.
Printed at : Laxmi Offset, Sanjaygandhipuram, Faizabad Road, Lucknow

REVIEW ARTICLE

International Journal of Science, Technology and Society
Vol 3, No. 2 (2017) : 01-10

A review on QM/MM studies of nucleic bases interactions with graphene and carbon nanotubes

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Publication Info

Article history:
Received : 10.12.2017
Accepted : 22.12.2017
DOI: https://doi.org/10.18091/ijsts.v3i02.11404

Key words:
Nucleic Acid (NA), Carbon nanotube (CNT), Graphene, Binding Energy, Initial Configurations (IC), QM (Quantum Mechanics) / MM (Molecular Mechanics).

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ABSTRACT

Nucleic bases interaction with carbonaceous materials finds significant attention due to their application in various fields such as DNA sequencing, DNA sensing and drug delivery. Nucleic bases, building blocks of nucleic acids interact with carbon nanotube and contribute significantly to the stability of the nucleic bases, carbon nanotube hybrids and their properties. In the present work, a thorough review of previous studies on the binding of nucleic bases with graphene and CNT is presented, with a focus on the simulation works that attempted to evaluate the structure and strength of binding. Dissimilitude among these works is noticed and factors that might contribute to such discrepancies are discussed in detail.

INTRODUCTION

Graphene and carbon-nanotubes have different applications for many reasons, but the differences can be ultimately attributed to the difference between one-dimensional materials and two dimensional materials. For example, a single walled carbon nanotube can be regarded as a single crystal with a high length–diameter ratio. However, the current synthesis and assembly technology cannot prepare the carbon nanotube crystals on a macroscopic scale, which limits their applications. While, graphene may be considered as a two-dimensional crystal structure, and its strength, conductivity and thermal conductivity are seen to be the best in two-dimensional crystal materials. It has a broad range of applications because of its ability to have a large area of continuous growth.

Graphene, is a planar form of carbon atoms designed in a two-dimensional hexagonal lattice fashion. It has emerged as the most dominating allotropes of carbon during the last few years. Its extended honeycomb network is the basic building block of other important allotropes such as 3D graphite formed by the stacking of several layers of graphene; 1D nanotube, obtained by rolling the graphene and the 0D fullerene prepared by wrapped graphenes (J. Allen Matthew *et al.,* 2010). Graphene is being used in the designing of new nanomaterials for energy storage devices, fuel cells and biosensors owing to its high stability, elasticity and electromechanical modulation (Stoller Meryl D *et al.*, 2008, Si Yongchao and T Samulski Edward, 2008, Pumera Martin *et al.,* 2010). Also, graphene exhibits extraordinary electronic properties in comparison to many of the conventional materials; the highly conductive graphene becomes an insulator after hydrogenation. This hydrogenation of graphene is highly reversible; the intrinsic conductivity as well as the structure of graphene can be restored on annealing (Chen Liang *et al., 2007;* Denis Pablo A *et al.,* 2009; Rubes Miroslav *et al.,* 2009). Graphene is also an important material in nanoscale electronics due to its compatibility with industry standard lithographic processing. The electron mobilities is up to 150 times greater than Si, and the thermal conductivity is approximately twice that of diamond (Ritter Kyle A *et al.,* 2009). Thus one can say that graphene has revolutionalized the technology.

Graphene sensors have emerged as another area of recent interest. The chemical and physical properties of graphene make it a promising candidate that can be used as a sensor to detect different gases such as H_2, NO_2, and NH_3. Schedin *et* al., in their experimental results, illustrated that graphene based sensors allow the sensitivity levels such that the adsorption of individual gas molecules could be detected accurately (Rao, C. N. R. *et al.,* 2009; Schedin F *et al.,* 2007). Graphene-polyaniline nanocomposite is found to be a good sensor for H_2 gas while nitrogen doped graphene find its application in electrochemical biosensing (Al-Mashat Laith *et al.,* 2010). It is also shown that, through functionalization, properties of graphene can be modified. The functionalization of graphene with hydrogen, oxygen, or other chemical groups is of prime importance as a way to engineer the different properties of graphene. A recent study reveals that with controlled epoxide functionalization, graphene can be used as a starting material for diverse chemical functionalization by chemical modification of the epoxide group. The functionalization of graphene and single-walled carbon nanotubes with individual 3d transition metal atoms were also modeled using density functional theory calculations.(Lee Geunsik *et al.,* 2009; Hubert Valencia *et al.,* 2010; Ghaderi Nahid *et al.,* 2010;Park Sungjin *et al.,*2008; Wang Donghai *et al.,2010;* Quintana Mildred *et al.*, 2010; Tachikawa Hirotoet *al.,* 2010; Al-Aqtash Nabil *et al.,* 2009).

The capability to detect single bio-molecules with high accuracy and efficiency is of prime importance in many areas of environmental science, biology, and chemistry (Lim Sung H. *et al.,* 2009; Bano Fouzia *et al.,* 2009; Zwolak Michael *et al.,* 2008; Fredlake P. Christopher *et al.,* 2006; Jonkheijm Pascal *et al.,* 2008; Patolsky Fernando *et al.,* 2006; Vidic Jasmina *et al.,* 2006). Efficient bio-sensors are expected to contribute to the improvement of medicine and medical treatment (Zwolak Michael, 2008). It is, quite uncertain whether traditional chemical techniques can be simultaneously fast and inexpensive which is another very important aspect that needs to be taken care of (Fredlake P. Christopher *et al.,* 2006). Nano-materials, due to their extreme sensitivity of the electron-transport properties in confined materials to external perturbations, form an excellent technological platform for single-molecule recognition (Zhang Guangyu *et al.,* 2006; Meyer Jannik C *et al.,* 2007; Shapir Errez *et al.,* 2008; N Kang *et al.,* 2007). Recently, graphene nano-ribbon (GNR) has emerged as a suitable candidate for making sensors for single small molecules, such as H_2, H_2O, and NO. This concept is based on measuring a variation in the source-drain current of a GNR based field-effect transistor originating from the covalent bond formed between the molecule to be detected and a defect (or an edge) of GNR. However, few reports exist on the use of GNRs as bio-sensors. One of the main reasons is that the biomolecules do not usually bind GNR via covalent bonds as a result of which the electrical perturbation induced by a biomolecule on a GNR is too weak to be detected. However the GNR is proposed to be used for the DNA sequence via π-π stacking in many reports.

Relevance of DNA bases and graphene/carbon nanotube interaction

The interaction of the biomolecules such as nucleic bases on the surface of GNR and CNT has attracted many researchers. In particular, the DNA-CNT interaction has cast its spell in the research community due to its application in various fields such as DNA sensing, DNA sequencing, and drug delivery (Zhao Xiongce 2011; Paul Ambarish 2010; Liu Zhuang *et al.,* 2011; Yarotski Dzmitry A *et al.,* 2009). It has also been found that the determination of a patient's DNA sequence can even reveal his risk of falling ill with particular diseases and it also helps to design "personalized medicine", and it is therefore the DNA sequencing that appears to be one of the most potential applications for the carbon nanostructures (Sanchez Jimenez Gerardo *et al.,* 2001; Nelson Tammie *et al.,* 2010; Prasongkit Jariyanee *et al.,* 2011). Sensors for amplified detection methods based on CNT-biomolecule composites is an area of recent interest, and such sensors can be efficiently used to detect various carbon nanostructures as well as different biomaterials such as DNA, protein, and so on (Barone Paul W *et al.*, 2005). Also, DNA-functionalized carbon nanotubes form the basis for not only a new class of chemical sensors but also for the molecular electronic devices. An ultrasensitive graphene-embedded nano channel device which effectively controls the motion of nucleobases via π-π interaction was also reported (Min Seung Kyu *et al.,* 2011).Weizmann *et al.,* 2011, recently reported that DNA-CNT nanowire networks can be used for DNA detection and Zheng Y*et al.,* 2009, constructed a carbon nanotube-based DNA biosensor for sensing the phenolic pollutants. Beside biomedical applications, comprehending the DNA-CNT interaction can also be used in the separation of carbon nanotubes as it has been shown that single-stranded DNA can be effectively used for the dispersion and separation of single-walled carbon nanotubes (Zheng M *et al.,* 2003). Some of the important conclusions can also be drawn from the studies (Wang X et al., (2011); Lu G et al.,(2009) ; Li J et al.,(2003)). Many research groups have focused on

determining the DNA-CNT interaction and tried to explore the strength of binding of different nucleosides, nucleobases, and nucleobases pairs on the carbon nanotubes and graphene, in both experimental and computational studies (Chen Robert J. *et al.,* 2003; Stepanian S.G. *et al.,* 2008; Shtogun Yaroslav V. *et al.,* 2007; Wang Hongming *et al.,* 2009; Wang Po *et al.,* 2011). The different binding energy orders for different studies are found in many experimental studies and it is understood that this may be due to the different experimental conditions applied. For most cases, in computational studies, the order is G > A > T > C > U, and in some cases, were found to be in the order as G~A~T~C > U. There are a number of theoretical and experimental studies on the nucleobases interaction with carbon nanotube and graphene surfaces as shown below:

Table 1. BE (kJ/mol) of nucleobases with SWCNT and graphene in theoretical and experimental studies.

Nucleic bases interaction with different Carbon nanomaterials			
Type	**Order**	**Method**	**Ref.**
SWCNT	T A ~C	Exp.	100
CNT(5,0)	G A T C U	Comp.	23
CNT(7,0)	G A ~T ~C U	Comp.	65
CNT (5,5), CNT(10,0)	G A T C	Comp.	86
GNR	G A ~T ~C U	Comp.	22

The binding energy of all the considered complexes illustrates that the binding energy increases as the curvature of SWCNT (single walled carbon nanotube) decreases and reaches the maximum for graphene. In general, the nucleobases tend to have π-π stacking (Zheng Y *et al.,* 2009) type of interaction with the carbon nanostructures. Hence, as the curvature of SWCNT decreases, there will be more efficient stacking between the carbon nanotube and the nucleobases surface, resulting in an increase in the binding energy of the complexes. The effect of size and curvature generally plays an important role in the non-bonded interactions (Zheng Ming *et al.,* 2003; Chen Robert J. *et al.,* 2003).

METHODS

First-Principles Methodology

In the QM methods, mostly DFT has been used in the computational chemistry and quantum physics due to their relatively low computational cost compared to high level *ab initio* methods and high accuracy in comparison to the semi- empirical methods.

The dispersion forces in the dispersion interaction are the most important interactions in molecular systems that are not addressed well in several DFT approaches. Efforts were made by the several research groups (Rutledge *et al.*, 2009; Rutledge and Wetmore, 2010; Johnson *et al.*, 2004, 2009; Dion *et al.*, 2004a; Zhao and Truhlar, 2005, 2011; Meijer and Sprik, 1996; Tkatchenko and Scheffler, 2009; Grimme, 2004, 2006; Grimme *et al.*, 2010, etc) to precisely incorporate dispersion in the correlation term of DFT. There were several studies of interaction of nucleic bases with CNT or graphene which however did not consider the dispersion interaction into account. Some early works based on LDA scheme of DFT, also lack dispersion correction. However the recent studies adopted either classical FF (force field) or dispersion corrected DFTs to consider the dispersion factor. It is believed that the π-π stacking plays a key role in the binding of nucleobase to graphene or CNT, dispersion therefore can play a significant role in determining the binding structure and BE (Binding Energy). So, one can precisely say that different approaches lead to different results. Therefore the past studies can be broadly classified into two categories: those performed with methods that consider dispersion, and those which do not consider dispersion-corrected methods.

Among the QM studies, there are also various methods with different levels of complexity and accuracy, including *ab initio* methods (HF, MP2 and CCSD (T)), DFT and semi-empirical methods. HF, originally named SCF method, is the first *ab initio* method and forms the basis of post- HF methods. Despite of having the correct description for the exchange energy, HF does not address the electron correlation precisely. Post-HF methods include MP2 (Møller and Plesset, 1934; Head- Gordon *et al.*, 1988), CI and CCSD (T) that were proposed to properly describe the correlation energy. These *ab initio* methods are usually employed for very small atomic systems due to their high computational cost. Semi-empirical QM methods are based on *ab initio* methods but include empirical parameters to speed up the calculations, examples include AM1 (Dewar *et al.*, 1985), PM3 (Stewart, 1989a,b, 1991) and PM6 (Stewart, 2007). In computational quantum chemistry and physics, DFT has been widely used, due to its relatively low computational cost compared with high level *ab initio* methods and high accuracy compared with semi-empirical methods. In 1964, Kohn and Hohenberg published the first paper on DFT in which they substituted the many electron wavefunction with the electron density and reduced the number of

variables. One year later, Kohn and Sham in 1965 improved the Hohenberg and Kohn's theory by introducing effective potential that included external potential, exchange and correlation interactions.

Methods lacking the dispersion correction

Gowtham *et al*., studied the adsorption of nucleobases (A, C, G, T and U) on graphene using MP2 and LDA (Gowtham *et al.*, 2007). Nucleobases in their work were attached to a methyl group. Plane wave basis set was used in the LDA calculations (Supercell approach), while in the MP2 calculations, 6-311++G(d,p) basis set was used with the graphene containing 28 carbon atoms terminated by hydrogen atoms at the edges. For each configuration of the nucleobase on the graphene, they initially performed a force relaxation to determine the preferred orientation and kept the bases at optimum separation distance. This was followed by a scan of the potential energy surface (PES) where the nucleobases were kept parallel to the graphene surface at a fixed distance. For each configuration, single point energy calculations were also performed and the minimum potential energy was determined. This configuration was subjected to a further optimization step in which all atoms were free to move and the final optimized structure was identified. Thereafter, BE was then calculated for the optimized structure using both MP2 and LDA. Table 2 shows the values of the obtained BEs. Among these two, MP2 predicted BE values that were almost doubled the LDA values. The BEs with respect to the different nucleobases almost remained in the same order: it was G>A=T=C>U using LDA and G>A>T>C>U using MP2. The final optimized nucleobases were found to be parallel to the graphene sheet with the separation distance being 3.5 Å.

Table 2. BE (kJ/mol) between nucleobases and graphene [Gowtham *et al.*, (2007)].

Nucleobase	LDA	MP2
G	58.86	103.24
A	47.28	90.70
T	47.28	80.08
C	47.28	77.19
U	42.25	71.40

In a later work, Gowtham *et al.,* also studied the adsorption of the same nucleobases on a (5,0) CNT (Gowtham *et al.,* 2008), using the same approach except that the BE calculation was only done with LDA only, and not with MP2. The order of the BE was found to be the same, i.e., G>A>T>C>U with the values being 47.28, 37.63, 32.81, 27.98 and 27.02 kJ/mol, respectively. Their results confirmed that the BEs for CNT were much smaller than those for graphene, that was attributed to the larger curvature of the CNT and resulting smaller area of contact.

Meng *et al.,* (Meng *et al.*, 2007a) first optimized the structures using CHARMM FF which includes an empirical description of dispersion interaction, but this dispersion was neglected again during the re-optimization step using LDA.

Meng *et al*., used a different approach (time-dependent LDA method) to study the binding between DNA nucleosides and a CNT (10,0) (Meng *et al.*, 2007b). From these simulations, the optical absorbance spectrum for DNA nucleosides were obtained, which were used to determine the preferred orientation of the nucleosides on the CNT. Optimized binding structures were also obtained using MM (CHARMM) calculations, and were found in good agreement between the MM results and LDA results. According to MM calculations, the order of the BE for the most stable structures was G>A>T>C with the BE values of 82.01, 78.15, 74.29 and 67.54 kJ/mol, respectively.

The dependence of BE on CNT chirality was studied by Wang and Ceulemans (Wang *et al.*, 2009) using LDA. They considered two connected adenosine-monophosphates with the phosphate groups terminated by H atoms. The resulting molecule was neutral and was taken to interact with different CNTs, including five (m,0) zigzag tubes with m = 7,8,9,10,17 and four (n,n) armchair tubes with n= 4,5,6,7. Periodic boundary condition using supercell approach and the linear combination of numerical atomic orbitals (LCAO) basis set with double-zeta polarizations were used.

In another work, Wang considered all four DNA nucleobases interacting with two types of CNTs: (5,5) and (10,0) (Wang, 2008). Same as his first work (Wang *et al.*, 2007), for each type of CNT, only a small part (C24H12) was made to interact with the nucleobases. Both DFT and MP2 methods were adopted in the simulations. The geometry optimization was carried out at MPWB1K/cc-pVDZ level where carbon and hydrogen atoms were kept frozen in the C24H12 fragments. The optimized structures were then subjected to a single point energy calculation at MP2/6-311++G(d,p) level. The BSSE-corrected BE for the C(5,5) CNT hybrid in vacuum was 46.46 kJ/mol which is quite different from Wang's former study (Wang *et al.*, 2007) in which the BE for the same system was determined to be 32.76 kJ/mol. The order of the BE between nucleobase and

CNT in the gas phase was found to be G>A>T>C for both CNTs. This is in agreement with the DFT studies of Gowtham *et al.,* on the interaction of nucleobases with graphene and (5,0) CNT (Gowtham *et al.*, 2007, 2008), and also with the MM results of Meng *et al.*, for the interaction of nucleosides with a (10,0) CNT.

The simulation works reviewed above are all based on methods that lack correction for dispersion interaction. With the pace of time and advancement in computational chemistry, more accurate dispersion corrected methods have been introduced.

Methods with dispersion-corrected methods

Recent works using dispersion-corrected DFT also gave rise to different results, possibly due to the difference in ways of incorporating dispersion interaction in these methods. The choice of basis sets can affect the BE evaluation, even with the same method (Shukla *et al.*, 2009). In addition, it is also found that BSSE can be large and has to be taken into account (Tournus *et al.*, 2005). Performance of simulation methods and basis set are still being widely evaluated in the computational chemistry community.

Though a large number of dispersion-corrected methods exist in literature however benchmarking has been performed by some of them (Johnson *et al.*, 2004; Dion *et al.*, 2004a; Hohenstein *et al*., 2008; Zhao *et al.*, 2008; Johnson *et al.*, 2009; Rutledge *et al.*, 2010; Zhao *et al.*, 2011; Grimme, 2011; Ehrlich *et al.*, 2013). Among these methods, Minnesota density functional developed by Truhlar's group, e.g., M05, M05-2X, M06, M06-L, M06-2X and M06-HF, are based on meta-GGA approximations (Zhao *et al.*, 2005, 2006; Zhao *et al.*, 2008, 2006 a,b). The exchange-correlation term in all Minnesota functionals depend on kinetic energy.

In the M06 family, M06-2X has shown good performance in several studies where vdW interaction played an important role (Rutledge *et al.*, 2010). Panigrahi *et al.*, employed dispersion-corrected DFT using wB97XD functional to study nucleobase-graphene binding (Panigrahi *et al.*, 2012). Nucleobases in their work were attached to a methyl group, similar to the study by Gowtham *et al.,* (Gowtham *et al.*, 2007). Each nucleobase was placed above a square graphene sheet with eight carbon rings in each direction and H atoms at the edges. The IC (initial configuration) of the base plane was parallel to the graphene surface with a separation distance of 4 Å, which was subjected to a full optimization at wB97XD/6-31G(d,p) level. The separation distance in the optimized structures was found to be around 3.5 Å. BSSE corrected BE was calculated at the same level and found to be 94.16, 85.03, 79.30, 77.04 and 68.41 kJ/mol respectively for G, A, C, T and U, i.e., G>A>C>T>U. Such order is identical to what was observed by Gowtham *et al.*, (Gowtham *et al.*, 2007) on the same system using LDA optimization accompanied by MP2 energy calculation. The BE values are also close to the MP2 results (Gowtham *et al.*, 2007) but almost double to those obtained using LDA alone.

Swathi and Chandra Shekar (with wB97XD functional) examined physisorption of nucleobases on coronene (C24H12) as a model of graphene (Chandra Shekar *et al.*, 2014). Different ICs were considered while the separation distance was considered to 3Å in all ICs. Geometry optimization was carried out at wB97XD/6-31G(d,p) level followed by a single point energy calculation at wB97XD/6-311+G (d,p). The order of the BSSE corrected BEs was determined to be G>T>A>C>U with the values of 75.73, 66.53, 65.27, 64.43 and 56.48 kJ/mol, respectively. BE values in this work were less than the ones obtained by Panigrahi *et al*., (Panigrahi *et al.*, 2012), which may be attributed to the smaller size of graphene in this study compared to that in Panigrahi *et al*. The separation distance in the optimized structures was found to be 3.24, 3.25, 3.30, 3.22 and 3.20 Å, respectively for G, T, A, C and U. These separation distances were also smaller than the ones obtained by Panigrahi *et al.,* (Panigrahi *et al.*, 2012).

Antony and Grimme studied the interaction of nucleobases with graphene in which four different sizes of graphene were considered, with 24 (C24H12), 54 (C54H18), 96 (C96H24) and 150 (C150H30) carbon atoms respectively (Antony *et al.*, 2010). Hybrids were fully optimized at B97-D/TZV(d,p) level. A three dimensional PES scan was also performed for the interaction of nucleobases with the C96H24 fragment, and no other minima was found except the one obtained from optimization. Nucleobases were attached to a methyl group and PBC was applied in their study. Full geometry optimization for the hybrid structures was also performed but no detailed explanations were given for the ICs.

Vovusha *et al.,* studied the interaction of nucleobases with graphene using M05-2X and M06-2X functional. Vovusha *et al.*, 2013 in their study of graphene model included 54 carbons with 18 hydrogen atoms capping the edge carbons. Geometry optimizations were all performed at M05-2X/6-31G(d) level and BEs were evaluated using both M05-2X and M06-2X methods with 6-31+G(d,p) and 6311++G(d,p) basis sets. The separation distance between nucleobases and graphene in the optimized structures was determined to be 3.2-3.5 Å that is close to previously reported results. Results obtained using M06-2X were considerably

larger than the ones obtained using M05-2X method. The order of the BE using M05- 2X was determined to be G>C=T>A>U and G>C>T>A>U respectively with 6-31+G(d,p) and 6-311++G(d,p) basis sets. When M06-2X was used for the BE calculation, the order was changed to G>T>A>C>U and G>T>C>A>U respectively using 6-31+G(d,p) and 6-311++G(d,p) basis sets. This demonstrates the great effect of method and basis set on the value and order of the BE. In most of the previous results on the BE between nucleobases and graphene, BE of A was only second to G, while this was not obtained by Vovusha *et al.*

Studies on semi-empirical and force-field methods

The studies of the binding of nucleobases with graphene or CNT at lower level methods involve classical MM or semi-empirical QM approaches. AM1 (Dewar *et al.*, 1985), PM3 (Stewart, 1989 a,b, 1991) and PM6 (Stewart, 2007) are the widely used semi-empirical methods. Non-bonded interactions including electrostatic and vdW forces that are implemented in classical FFs such as Amber (Cornell *et al.*, 1995) and CHARMM (MacKerel Jr. *et al.*, 1998). It has been shown that Amber FF can even be more accurate than some of the semi-empirical QM methods when evaluating the BE for biological systems (Rutledge *et al.*, 2009; Rutledge *et al.*, 2010).

Optimization process also plays a key role in BE calculation for these weakly bound systems where PES is expected to be near local minima. Direct optimization may lead system to nearby local minima, but not near the global minima. So, optimization is very sensitive to the IC chosen. The different IC result in different BE values and can even change the order of BE for different nucleobases (NB).

Umadevi *et al.*, brought to light the dependence of the curvature by considering the binding of nucleobases with graphene and a series of armchair (n,n) CNTs where n=3, 4 and 5 (Umadevi *et al.*, 2011). The graphene and CNTs were made using the Gaussian software package with H atoms at the edges was used to saturate the dangling bonds at the boundaries. Each system was optimized using ONIOM method at the (M06-2X/6-31G(d):AM1) level. Atoms of the nucleobases and the "reacting atoms" of CNTs were modeled as the high layer using M06-2X/6-31G(d). The atoms in CNT were considered as the low layer using semi-empirical AM1. Single point energy calculations were performed for the optimized structures using the dispersion-corrected B3LYP method (B3LYP-D) with the 6-31G(d) basis set. The BE was found to be graphene>CNT(5,5)> CNT(4,4)> CNT(3,3) for all nucleobases except T, for which the order was graphene>CNT(5,5)> CNT(3,3)>CNT(4,4). The BSSE-corrected BE was 30-51 kJ/mol for CNTs and 50-73 kJ/mol for graphene. The order of the BE with respect to different nucleobases was determined to be G>T>A>C>U for the CNTs and G>A>T>C>U for the graphene. In another work, using the M06-2X/6-311G**, Umadevi *et al*., found the order of binding for nucleobases with graphane in the order G > A > C > T > U (Umadevi *et al.*, 2015).

DISCUSSION AND FUTURE PERSPECTIVES

This paper presents a comprehensive review of past computational work, where three categories of methods have been used: (1) first-principles studies based on methods lacking dispersion correction, (2) first-principles studies based on dispersion corrected methods and (3) studies based on semi-empirical and FF methods. In nearly all studies reviewed above, the nucleobases were found to be parallel to the graphene or CNT with the separation distance being around 3 Å, which confirms the π-π stacking nature of the interaction. On the other hand, drastically different results have been reported for the BE.

Previous QM calculations on BE already illustrates some effects of CNT chirality (Akdim *et al.,* 2012), however it is not yet clear whether such effects are correlated with the electronic structure of the CNT. Finally, it can be noted that BE has been used as the main parameter for comparisons made in this review. Other properties such as charge transfer and density of states have only been reported in some

Table 3. BE (kJ/mol) between nucleobases and graphene [Vovusha *et al.*, (2013)].

	DFT level			
	M05-2X		M06-2X	
Nucleobase	6-31+G(d,p)	6-311++G(d,p)	6-31+G(d,p)	6-311++G(d,p
G	37.62	27.23	65.08	57.46
A	27.01	16.70	52.19	44.23
T	27.98	19.64	52.93	46.23
C	27.98	20.50	51.02	45.10
U	22.19	13.93	46.36	35.00

(<50%) of the cited works and hence are not suitable for systematic comparison. Also, the calculation of charge transfer does not only depend on the QM method but also on the charge distribution scheme (e.g., Mulliken, ESP, RESP, etc.). This makes the comparison among different studies more complicated.

CONCLUSION

In the present work, a thorough review on the theoretical studies, mainly at the QM level, on the binding of nucleobases (and in a few cases, nucleosides or nucleotides) with graphene or CNT has been performed. BE, as an indicator for the stability of the binding, is used to compare different studies. Due to the different simulated systems and procedure considered for the study, a large range of binding energy values were reported, and considerable discrepancies exist among the past investigations. So, the importance of using dispersion-corrected method and proper design of the optimization procedure plays a crucial role in understanding the interaction of the nucleobases with the CNTs or graphene.

ACKNOWLEDGEMENT

AK would like to acknowledge the UGC for the financial support.

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REVIEW ARTICLE

International Journal of Science, Technology and Society
Vol 3, No. 2 (2017) : 11-27

A review on theoretical studies of various types of Drug-DNA Interaction

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Publication Info

Article history:
Received : 17.10.2017
Accepted : 20.12.2017
DOI: https://doi.org/10.18091/ijsts.v3i02.11408

Key words:
Minor Groove Binders, Intercalators, Major Groove Binders, Molecular Docking, Molecular Dynamics, MMPBSA/ MMGBSA, QM/MM.

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ABSTRACT

A large number of the currently used chemotherapeutic anticancer agents fall into the category of DNA-binding drugs. Study of interactions of various drugs with DNA plays a key role in pharmacology. Due to the potential application of such drugs to cancer and beyond, further discovery and characterization of such compounds are of considerable interest. The combinations of distinct binding modes will improve the stability of recognition and enhance target specificity with respect to both DNA structure as well as sequence. The current review gives an overview of the recently used computational chemical techniques to understand mechanism of drug-DNA interaction. The discussions will provide a theoretical protocol for complementing experimental techniques, generation of database for structure activity/property relationship in drug-DNA complexes. This will be helpful for the improvement of existing drugs, design of new drugs etc.

INTRODUCTION

Deoxyribonucleic acid (DNA) was first discovered by Friedrich Miescher, when he was working with white blood cells obtained from the pus drained out from surgical bandages and determined that the DNA was rich in phosphorous and acidic in nature. However the role of DNA to store heredity information was not reported before 1940s until Avery and co-workers published that the nucleic acids are the genetic information carriers and not proteins (Avery *et al.*, 1944). In 1950 Chargaff recognized that the composition of DNA is unique for each and every species. Chargaff also found that when DNA is broken into its components, the amount of guanine fluctuated from one organism to another is always equal to the cytosine and the amount of cytosine is equal to the amount of thymine (Chargaff, 1951). Rosalind Frankllin elucidates basic helical structure of DNA on the basis of X-ray crystallography technique. In 1953, Watson and Crick scooped Franklin's and Chargaff's information and cracked the code of DNA structure (Chargaff, 1950; Watson, 1953a; Watson, 1953b). They recognized that the relationship between the nitrogenous bases suggested by Chargaff may be due to the complementary base pairing between adenine-thymine and guanine-cytosine and due to this type of base pairing they discovered the hydrogen bonding between these bases, which is currently known as Watson-Crick hydrogen bonding. With this information they modeled a right-handed double helical structure of DNA in which phosphate backbone lied outside the helix and the bases are held together by hydrogen bonding pointed towards the center of helix.

In 1979, a first crystal structure of left-handed double helical DNA $d(CGCGCG)_2$ at atomic resolution was reported, known as Z-DNA (Wang, 1979). After a year, the single-crystal structure analysis of right-handed B-DNA, with the self-complementary dodecamer sequence $d(CGCGAATTCGCG)_2$ was discovered by Dickerson and co-workers. This dodecamer is one of the most studied DNA fragments (Wing, 1980). These discoveries revealed that how the genetic information passes from one to next generation. The most common conformations of DNA are B-, A-, Z-DNA and B-form DNA is the most common occurring conformation. In this type of DNA, the base pairs are perpendicular to the helix axis and twisted by 36^0 with respect to each other. A single turn in the double helix consists of 10 base pairs (Table 1). The two strands of the

Table 1. Structural properties of A-, B-, Z-form DNA.

Conformation	Helix Sense	Twist/bp (Å)	Rise/bp (Å)	Residues /turn	Sugar pucker	Groove Width (Å)		Groove Depth (Å)	
						Minor	Major	Minor	Major
A-DNA	right	32.7	2.56	11	C3'-endo	11	2.7	2.8	13.5
B-DNA	right	36	3.4	10	C2'-endo	5.7	11.7	7.5	8.8
Z-DNA	left	-9,-51	3.8	12	C3'-endo (Syn)	-	8.8	3.7	3.7

double helix are separated by two different grooves minor groove and major groove. Specific recognition of DNA sequences by small molecules is achieved by the combination of hydrogen bond acceptor/donor sites available on the major groove or minor groove of DNA.

DNA is the pharmacological target of many anticancer drugs which are currently under clinical trials. Transcription and replication are the vital processes essential for the survival of the living system. In transcription, information is fetched from DNA to RNA and has recourse to synthesize protein in the body. In replication, DNA yield self-replication process and reconstruct two identical strands. DNA starts these processes only after receiving the signal which is usually in the form of regulatory protein to a specific region of DNA. If this regulatory protein is mimicked by a drug molecule (mainly heterocyclic aromatic molecule), then the functions of DNA can be artificially modulated, inhibited or activated by this small molecule to cure or control a disease. DNA involved in vital processes such as replication, transcription etc. are of particular interest as target for wide range of anticancer and antibiotic drugs (Chaires, 1998; Chaires, 1997; Chaires, 2008; Hurley, 2002).

Molecular interaction between drugs and DNA is a field of current research and also plays an important role in its biological activity. Many anticancer therapies depend on the interaction of drug molecule with DNA. These interactions may cause damage of DNA in cancerous cells by inhibiting the process of replication or transcription, which inhibits the growth of cancer cells. To design efficient chemotherapeutic agents and better anticancer drugs, it is important to inspect the interaction of drug with DNA. The number of known DNA-based drug targets is very limited in comparison to the protein based drug targets and also the number of available structures of DNA-drug complexes is also small relative to protein-drug complexes deposited in the PDB (Berman *et al.,* 2000).

Different Modes of DNA binding with Drug

There are many binding modes in which drug molecule can interact with DNA such as surface binding to their minor or major grooves, intercalation between adjacent base pairs, covalent attachments to the double helix, or electrostatic binding. Thus both covalent as well as non-covalent interactions were found between drug molecules and DNA. DNA interacting drug molecules are given in Table 1.

Covalent Binding

Many chemotherapeutic drug molecules which are in clinical use bind with DNA not only non-covalently but also by covalent binding. Covalent binding in DNA is irretrievable and regularly points to complete inhibition of DNA processes and subsequent cell death. Drug molecule covalently binds with DNA via inter- and intra-strand cross linking or alkylation. Covalent binders of DNA are the high binding strength (Paul and Bhattacharya, 2012; Liu and Sadler, 2011). The covalent binders are also known as alkylating agents because they can attach an alkyl group to DNA and are also used in the treatment of Cancer. Alkylating agents are the important class of anticancer drugs, they play crucial role in the cure of several types of cancers. Alkylating agents have methyl or other alkyl groups (C_nH_{2n+1}) onto molecules. Chemical structure of some important alkylating agents is shown in Figure 1. Alkylating agents are involved in reaction with the preferential N-7 position of guanine and N-3 of adenine in DNA. Thus the base pairing of the DNA could be inhibited and this leads to miscoding of DNA. Alkylating agents can interact to DNA via three mechanisms. In first mechanism an alkylating agent attaches alkyl group to the nucleic acid bases, this results in the DNA being fragmented by repair enzymes in their attempts to replace the alkylated bases. In second mechanism alkylating agent leads to DNA damage due to formation of cross-links and bonds between atoms in the DNA. In this process, two bases are linked together by alkylating agents that has two DNA-binding sites. Cross-linking prevents DNA from being separated for synthesis or transcription. In third type of mechanism, alkylating agents causes the mispairing of the nucleotides leading to mutations (Silvestri and Brodbelt, 2013; Kondo *et al.,* 2010). The nitrogen

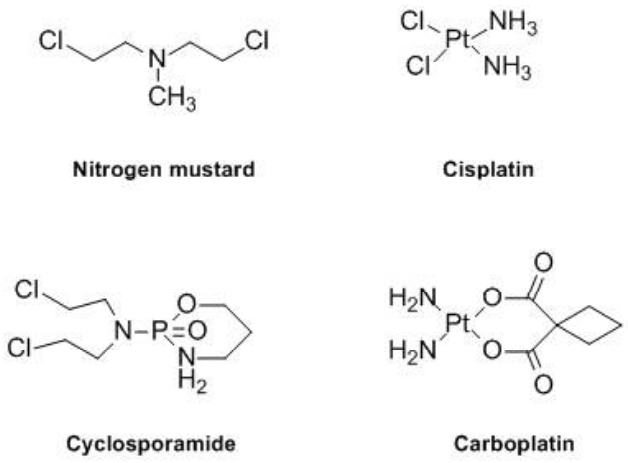


Fig. 1. Chemical structure of some DNA alkylating agents.

mustards were the first alkylating agent used medically, as well as the first modern cancer chemotherapies (Bauer and Povrik, 1997). Cis-platin is one of the anticancer antibiotics, which covalently binds to DNA, which makes an intra/ interstrand cross-link with nitrogens on the DNA bases and is used in the treatment of testicular, ovarian, head and neck cancers. Most alkylating drugs are mono functional methylating agents (e.g. temozolomide[TMZ], N-methyl-N'-nitro-N-nitrosoguanidine [MNNG], and dacarbazine), bifunctional alkylating agents such as nitrogen mustards (e.g. chlorambucil and cyclophosphamide), or chloroethylating agents (e.g. nimustine [ACNU], carmustine [BCNU], lomustine [CCNU], and fotemustine) (Kondo *et al.,* 2010; Rajski and Williams, 1998; Park and Hurley, 1997).

Non-covalent Binding

Non-covalent binding drug may change DNA torsional tension, interrupt protein-DNA interactions, and potentially lead to the breaking of DNA strands. All of these can have substantial effects on gene expression. Non-covalent interactions, in particular hydrogen bonding and stacking interactions, determine the structure of biomolecules (such as nucleic acids and proteins). While it is understood that hydrogen bonding is essential for the specificity of base pairing, ð-ð stacking interactions between planar aromatic rings of nucleobases are equally important contributions to the final stability of nucleic acid structures. Although individually weak, the additive power of these interactions has large cooperative stabilizing effects. Non-covalently binding of drug with DNA is mainly classified into two category viz. groove binders and intercalators. Groove binders are of two types: minor groove binders and major groove binders. Groove binders are highly sequence-specific. The two types of grooves in nucleic acid differ in hydrogen-bonding, electrostatic potential and in degree of hydration. Mainly the large protein molecules binds to the major groove of DNA while small molecules generally bind to the minor groove of DNA which are long elongated structures with a curvature that acquires the shape of the minor groove.

Minor Groove Binders

Minor groove binders usually consist of aromatic rings covalently linked by sigma bonds. Small molecules can form hydrogen bond to the nucleic bases, generally N3 of adenine and O2 of thymine. Minor groove binders generally bind with A-T rich region of the DNA. This preference in addition to the designed propensity for the electro negative pockets of AT sequences is probably due to better vander Waals contacts between the ligand and groove regions and also because of the steric hindrance in the latter, presented by the C2 amino group of the guanine base (Nelson *et al.,* 2007; Khan *et al.,* 2012; Privalov *et. al,* 2007). Sequence specific DNA-binding proteins commonly binds with the major groove because of numerous possibilities for hydrogen bonds with donors and acceptors on the nucleic bases, which provides complex stability and sequence specificity. Proteins and small molecule binding to the minor groove of DNA; depends upon the hydration properties of minor grooves, the latter binding to that AT-rich regions in which water ordering is most prevalent. Thus the minor groove binding is normally driven by the very large entropy of releasing the ordered water, despite an unfavorable enthalpy (Wemmer and Dervan, 1997; Sterkowski and Wilson, 2007; Gilbert and Feigon, 1991). Minor-groove binding usually involves greater binding affinity and higher sequence specificity than that of intercalator binding. Minor-groove has been demonstrated for neutral, mono-charged and multicharged ligands (Baily and Chaires, 1998). Generally, minor-groove binders show AT-rich region selectivity, several factors are responsible for this preference. The electrostatic potential of AT-rich region is greater than that of GC-rich region. On the other hand, the dimensions of the minor groove at AT sites are narrower and deeper than at the GC sites. This difference is due to the differences in the ionic interactions in the two types of base pairs (Hamelberg *et al.,* 2001). The cationic minor-groove binders include the lexitropsins and their conjugates, analogues of Hoechst 33258, DAPI and diarylamidines, Berenil, SN series, and pentamidines (Bhattacharya and Thomas, 2000; Erikson *et al.,* 1993; Brown *et al.* 1979).

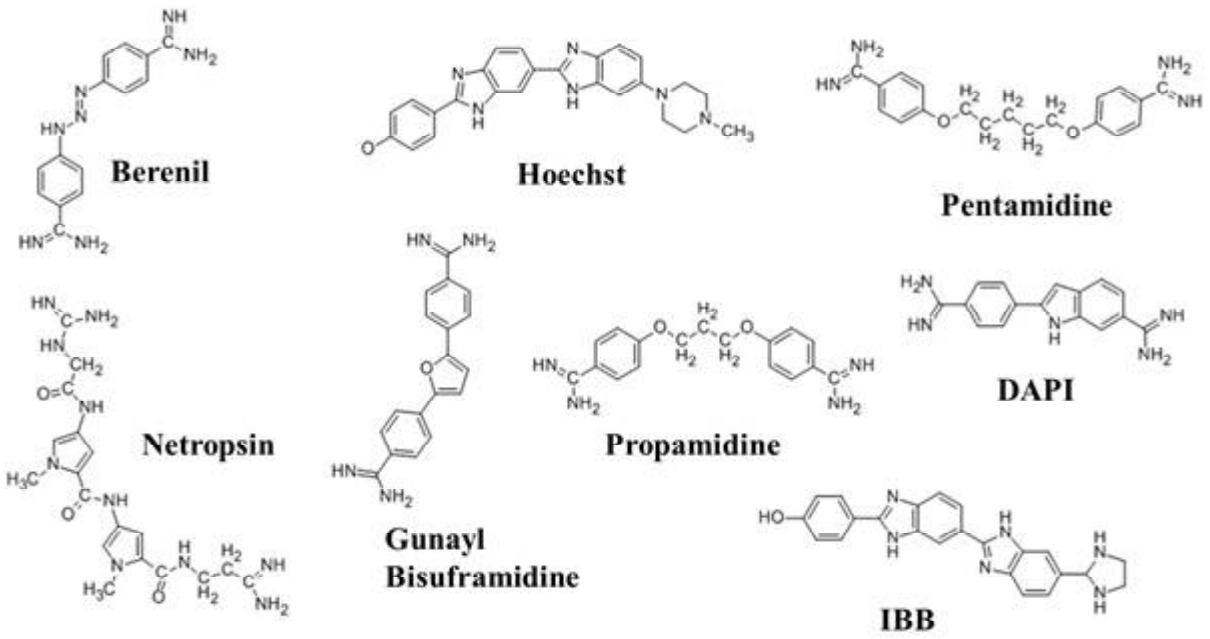


Fig. 2. Chemical Structure of some DNA minor groove binders.

Intercalators

Another type of non-covalently binding drugs is intercalators which are generally planar heterocyclic molecule which stacks between the two adjacent nucleic acid base pairs. The complex remains stabilized because of ð-ð stacking between the drug molecule and DNA bases. Intercalation was first explained by Lerman, in which the drug molecule held rigidly perpendicular to the DNA backbone without breaking up the hydrogen bonding between the nucleic bases (Lerman, 1963). This may be distorting the sugar phosphate backbone and also leads to decrease in the pitch (Williams *et al.,* 1990). The driving forces for the stability of DNA-intercalator complex are vanderWaals, hydrophobic, stacking or charge transfer forces and hydrogen bonding and electrostatic forces also become important for the stabilization of complexes (Wang, 1992). DNA intercalation results in conformational changes in DNA structure, causing lengthening, stiffing and unwinding of DNA helix. Intercalation needs changes in the torsional angles of sugar-phosphate backbone to adjust the incoming aromatic compound, which causes separation between the base pairs with a lengthening of the DNA approximately 3.4Å and decrease in helical twist, unwinding the DNA in the vicinity of the binding site to less than 36° base pair (Neto and Lapis, 2009). Intercalation preferentially occurs at GC-rich sequences because these sequences get unstacked easily. Intercalators generally cause more significant distortion to the conformation of DNA. Echinomycin, noglamycin, triostin A, acridine, cis-Platin, adriamycin, ethidium, propidium, actinomycn D, adriamycin are some examples of the DNA intercalating agents (Pigram *et al.,* 1992).

There are few major binding modes for reversible binding of molecule to the DNA: (i) electrostatic interactions with the anionic sugar phosphate backbone of DNA (ii) interaction with DNA minor groove (iii) interaction with DNA major groove (iv) intercalation between DNA base pairs via DNA minor groove (v) intercalation between DNA base pairs via DNA major groove and (vi) threading intercalation mode. After the intercalation of a structure, the access of another intercalator to binding site next to neighboring intercalation pocket is hindered. This phenomenon is referred as the "neighbor exclusion principle" and could be explained considering that due to intercalation the significant structural changes in DNA with deep alterations in the nucleotide secondary structure (Neto and Lapis, 2009; Yen *et al.,* 1982; Tanious *et al.,* 1991). Intercalating compounds without bulky substituents can intercalate without having significant part of molecule in either minor or major groove, this type of molecules (DACA, proflavin etc.) are called classical intercalators (Pigram *et al.,* 1972). Some of the intercalating molecules have bulky substituents, and these bulky substituents are placed in the major or minor groove along with intercalating moiety. These types

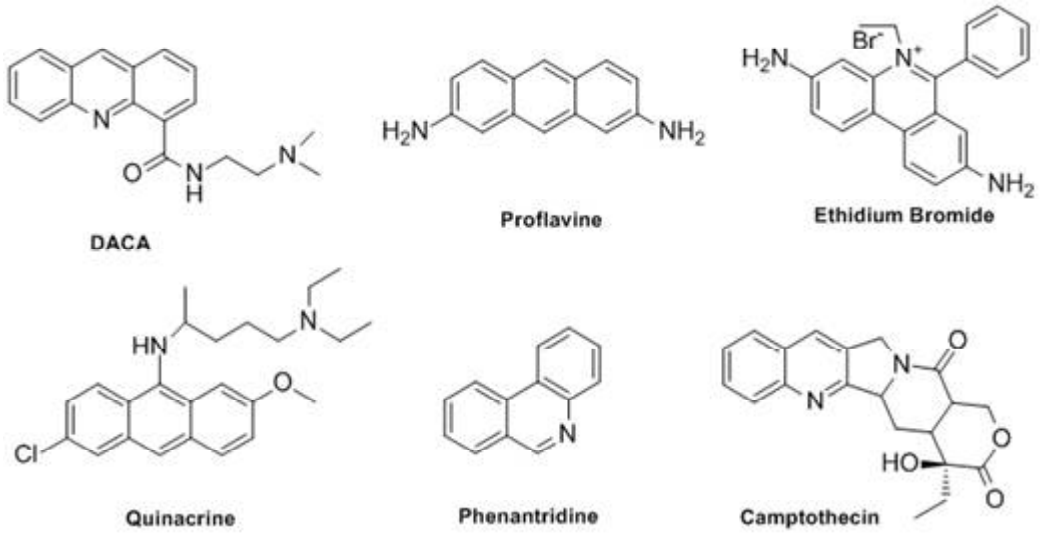


Fig. 3. Chemical Structure of some DNA intercalators.

of intercalators are called threading intercalators (Martinez and Chacon-Garica, 2005). In threading intercalation complexes, an aromatic system inserted between base pairs, while bulky substituent binds strongly with both major and minor groove (Neto and Lapis, 2009; Yen *et al.,* 1982). Other important intercalating drugs are the anthracyclin, daunorubicin, adriamycin, quinacrine and actinomycin have bulky substituents that must be in one groove or the other after the planar aromatic ring of the drugs is bound by intercalation (Tanious *et al.,* 1991; Rao and Kollman, 1987; Bond *et al.,* 1975; Wilson *et al.,* 1998; Martinez and Chacon-Garica, 2005; Denny, 2002; Nakamoto *et al.,* 2008; Wheate *et al.,* 2007; Baraldi *et al.,* 1999; Reddy *et al.,* 1999).

Major Groove Binders

The major groove is wider than the minor groove, the groove width values for B-form of DNA are 11.6 Å and 6.0 Å respectively. Due to this difference in dimension, the major groove is the target for many DNA-interacting proteins. Many biological macromolecules such as proteins interact by the variety of hydrogen bond acceptor and donor supplied in the major groove (Simonsson *et al.,* 1998; Singh and Lambowitz, 2001; Mamoon *et al.,* 2002). It is important for a major groove binding molecule that it could block access to proteins that recognize the same groove. This can be achieved by sequence affinity and sequence selectivity (Scheilf, 1988). DNA duplexes which are made up of polypurine–polypyrimidine sequences can be read by oligomers and bind to the major groove and form hydrogen bond with nucleic bases of the purine strand. These are called triplex-forming oligonucleotides (TFOs) (Thoung and Helene, 1993; Jain and Bhattacharya, 2010). Another form of major-groove recognition could be achieved by peptide nucleic acids (PNAs) (Neilsen, 1999; Ganesh and Kumar, 2005).

DNA interacting organometallic compounds

Many coordination complexes possess an intercalating ligand in their coordination sphere; the study of such complexes reveals the preferential geometry of the metal center, the nature of the intercalating ligand and the number and the position of the substituents over the intercalating ligand in the capacity and selectivity of the coordination complexes to intercalate with DNA. These coordination compounds bind to DNA via two interaction modes: irreversible (covalent or coordination binding) and reversible (intermolecular association). The later binding mode can be further classified into electrostatic interactions, groove binding and intercalation. However, these coordination complexes may exhibit a preference for a particular binding mode or a nucleotide sequence depending upon the size and the shape of the molecule (Han *et al.,* 2004; Hazarika *et al.,* 2012; Juan *et al.,* 2013; Rodrigo 2015).

All mononuclear platinum complexes could form intrastrand and interstrand adducts with DNA. When interstrand lesion is formed, massive distortions of the B-DNA are observed. Similarly, intrastrand lesion, while it forms much more readily than the interstrand lesion, it induces mutational events via the distortion of its nucleic acid target. Binuclear platinum (II) complexes were designed and synthesized and their interactions were studied with calf thymus DNA and a small 49 base pair

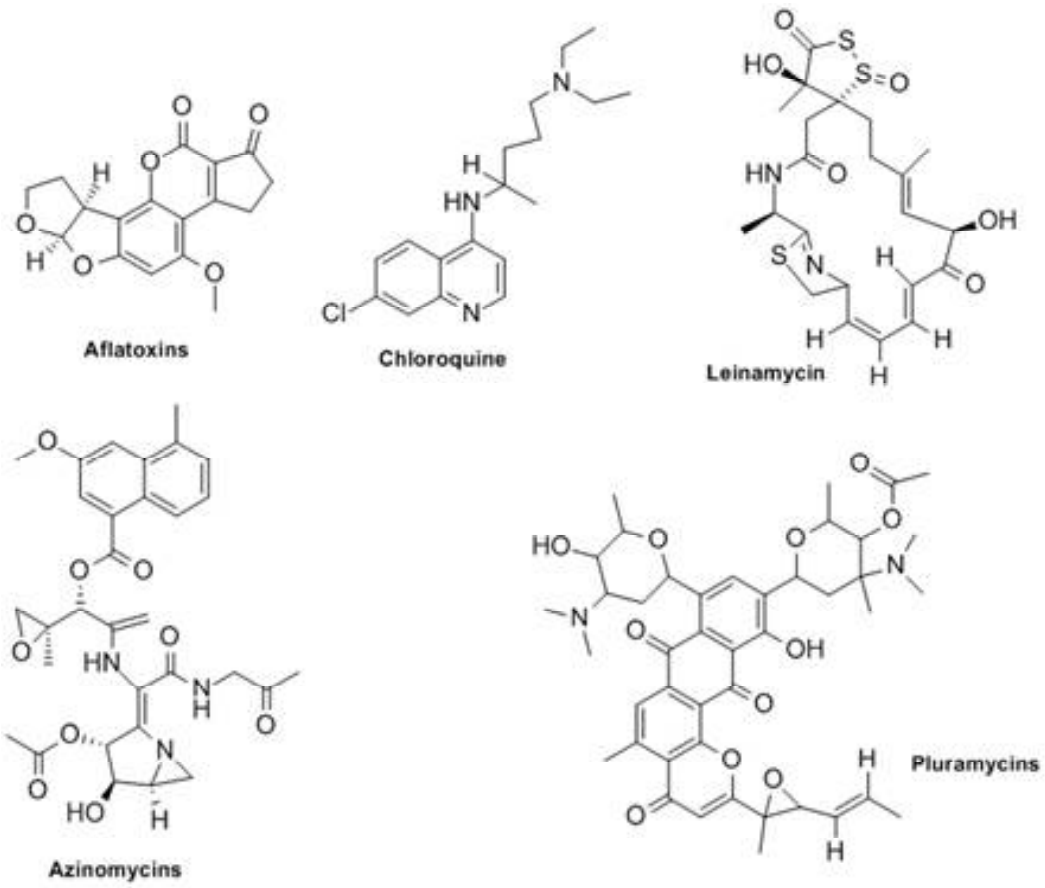


Fig. 4. Chemical Structure of some Major Groove Binders.

oligodeoxyribonucleotide. Owing to the presence of the pyridyl ligands, this compound induces a much higher degree of DNA unwinding than that seen with the either of the ammonia bound complexes, as well as the mononuclear trans-[$PtCl_2$-$(py)_2$]. Similarly, to the mononuclear compound [(Pt(trans)-$(py)_2Cl_2$-µ-(diaminobutane)]$^{2+}$. These alterations likely involve [(Pt(trans)-$(py)_2Cl_2$-µ-(diaminobutane)]$^{2+}$ to undergo ð-stacking interactions upon DNA association which in turn, disfavors the Z-DNA conformations. Importantly, interstrand-cross links formation is very efficient for all three complexes. The directionality is dependent upon the nature of the cross link. Interestingly, this is a unique example of anti-cancer drugs behaving in this manner. Molecules normally reach DNA through one of the grooves and react to either the backbone or the nucleobases. Electrostatic binding occurs due to the interaction between cations with the negatively charged phosphate backbone at the exterior surface of the DNA helix (Konstantinos *et al.,* 2013; Decatris *et al.,* 2004).

The use of transition metal complexes gives a strong tool to the drug chemists to develop and study molecules capable of obtaining specific DNA-drug interactions considering the multiple options of d-block metals from the periodic table. Transition metals are dynamic in geometry, electron affinity and reactivity, making them excellent choices to feed the ongoing field of antineoplastic drug discovery.

The fundamental factors of these interactions still possess greatest gaps in as much as the results provided by experimental designs that do not involve expensive protocols and equipment that are extremely poor to identify the specific interactions due the lower energetic changes involved, making clear the use of methodologies such as computational chemistry to help solve these problems (Ge and Sun, 2007; Wang and Lippard, 2005; Umezawa, 1976; Wong and Giandomenico, 1999; Bonnet, 1995).

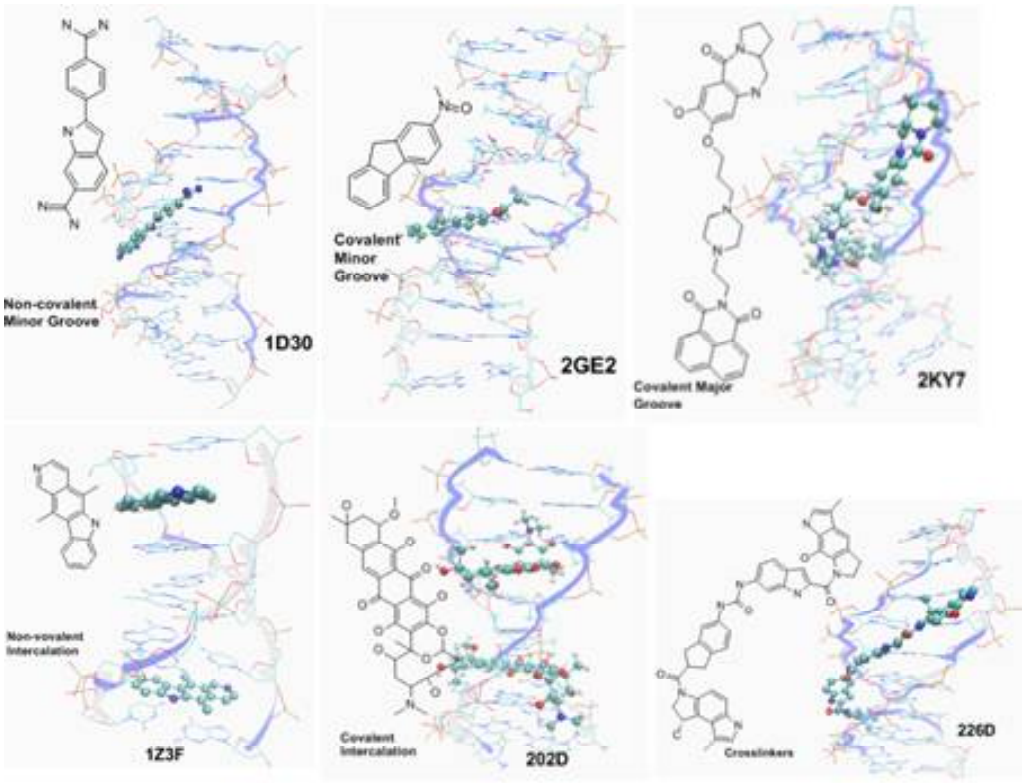


Fig. 5. Different modes of Drug-DNA Binding.

Experimental studies used in drug-DNA interaction

Experimental Studies play crucial role to explore the drug-DNA interaction. Thermodynamic studies provide the necessary information of free energy, enthalpy, entropy, heat capacity and also about the binding constant changes during complex formation. Various experimental techniques which are used to understand the interaction of drug molecule with nucleic acid holds Infrared (IR), Raman, Circular dichorism, UV-visible, Nuclear magnetic resonance (NMR) spectropies, Atomic Force Microscopy (AFM), Electrophoresis, Mass spectrometry, Viscosity measurements, Thermal denutration studies, Cyclic square wave and Differential pulse voltammetry, etc. The above techniques have been used as a primary tool to characterize the behavior of drug-DNA binding and the consequences of such type interaction on the structure of nucleic acid. The commonly used experimental techniques are UV-visible, fluorescence spectroscopies and cyclic voltammetry (Sirajuddin *et al,* 2013). Chaires also provided the change in experimental values of thermodynamic properties during the drug-DNA complex formation (Garbett and Chaires, 2012). This information may be helpful for the theoretical prediction and structural analysis. In the binding of drug molecule with bimolecular system, the solvent water plays important role. In the case of DNA-focused drug approaches there is a need to understand how water take part in the reorganization (CheathamIII *et al.,* 1995; Bellissent-Funel *et al.,* 2016; Yu, 2008; Chalikian and Breslauer, 1998).

Molecular Modelling Studies for drug-DNA interaction

Molecular Docking

Molecular docking method is used to predict the structure (or structures) of the intermolecular complex formed between two or more molecules. The docking program generates large number of possible structures, and so it is required to rank them according to their score to identify those of most interest (Blaney and Dixon, 1995; Abagyan and Totrov, 2001; Kuntz, 1992; Lengauer and Rarey, 1996). The three important components of docking are:

(1) Representation of the system.

(2) Conformational space search via a search algorithm.

(3) Ranking of potential solutions using the scoring function.

Table 2. DNA interacting drug molecules.

Non-covalent Binding drug molecules			Covalent binding drug molecules
Groove Binding		Intercalators	
Minor groove Binders	Major Groove Binders		
Berenil	Chloroquine	Daunomycin	Nitrogen mustard
Netropsin	Netamycine	Nogalamycin	PBDs
Hoechst 33258	Cis-{$Pt(NH_3)_2$(pyridine)}$_2$+	Ethidium bromide	CC1065
Distamycin A	Aminoglycoside (NB33)	Proflavine	Cis-platinum
GunaylBisuframidine	Chlorambucil	Ellipticine	Menogril
SN6999	Nimustein	Diplamine	Clomesone
SN7176	Pluramycins	Chlorpheniramine	Cyclodisone
Pentamidine	Aflatoxins	Bis-napthalimide	
MithramycinPilcamycin	Azinomycins	Doxorubcin	
Chromomycin A3	Leinamycin	Aminoacridines	
Diamidine-2-phenylindole	Ditercalinium	Arylaminoalcohals	
Bisbenzimadoles		Coumarines	
Bleomycin		Cystodytin	
Mitomycin		Diplamine	
FR66979		YO and YOYO-1	
Duocarmycins		QuinolinesQuinoxalines	
CC-1065		Echnomycin	
Yatakemycin		Methapyrilene	
Neocarzinostatin		Tamoxifen	
Calicheamicins		M-AMSA	
Retrorsine		Indoles	
Anthramycins		Aclarubicin	
Saframycins		Idarubicin	
Ecteinascidin 743		Epirubcin	
Isochrysohermidin		Pirarubcin	
		Valrubicin	
		Amrubicin	
		Actinomycin D	
		Camptothecin	
		Topotecan	
		Irinotecan	
		Rebeccamycin	
		Podophyllotoxin	
		Etoposide	
		Teniposide	
		Elsamicin	
		Dynemicin	
		Triostin A	
		Luzopeptins	
		Sandramycin	

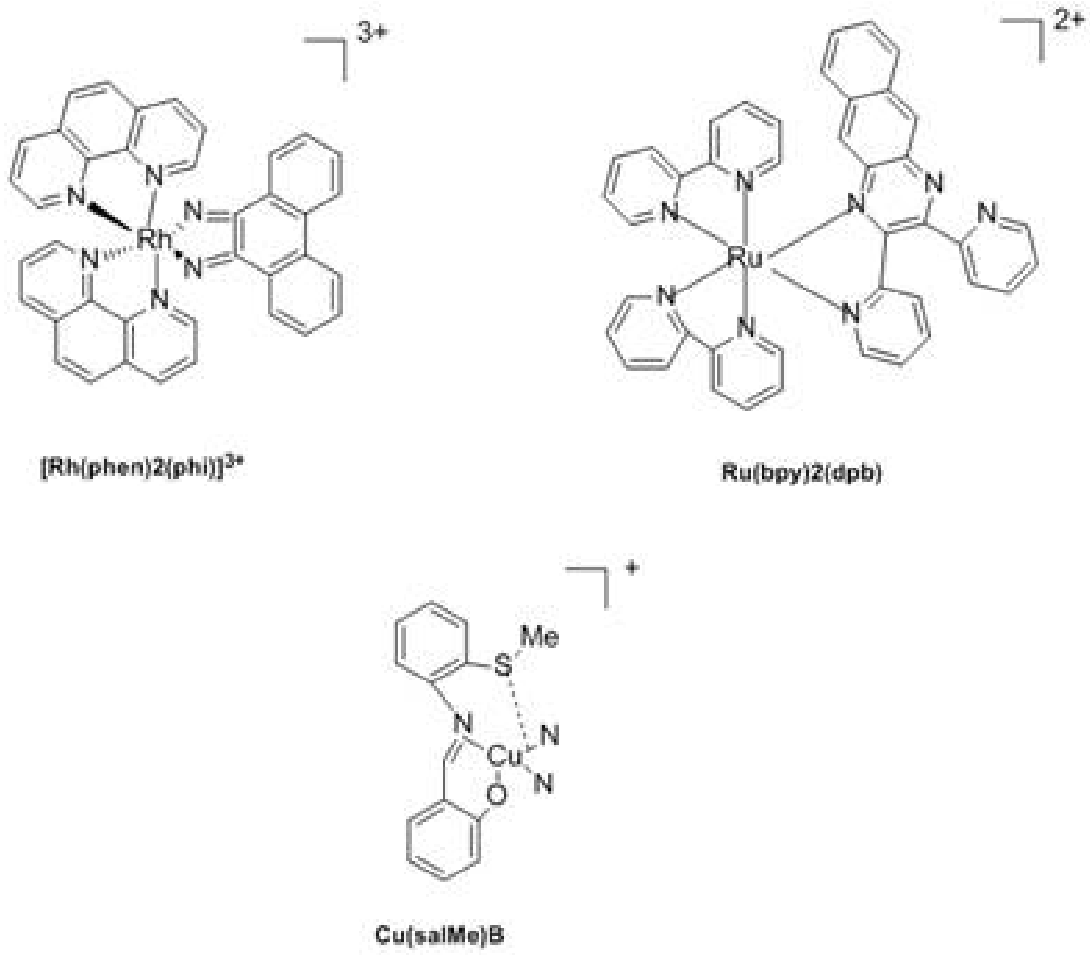


Fig. 6. Chemical Structure of some organometallic compounds.

The aim of docking process is to computationally simulate the molecular identification process and accomplish an optimized conformation so that the free energy of the overall complex is minimized. The docking method involves many degrees of freedom. With six degrees of translational and rotational freedom along with the conformational degrees of freedom of each molecule results large number of possible binding modes between the two molecules. Computationally it would be too expensive to generate all the possible conformations. Various algorithms have been developed to tackle the docking problem. These algorithms can be characterized according to the number of degrees of freedom that they ignore. The search algorithm generates number of conformations for a particular ligand, and scoring functions are then applied in order to identify the energetically most favorable pose (Gane and Dean, 2000; Schneider and Bohm, 2002; Sthal and Rarey, 2001; Koremer, 2003; Gohlke and Klebe, 2002).

Surflex (Surflex, 2007), Autodock (Morris *et al.,* 1998), DOCK (Rarey*et al.,* 1996), GOLD (Jones *et al.,* 1997), Glide, Flex (Rarey *et al.,* 1996), CDOCKER (Wu *et al.,* 2003) are the docking programs which are used for the Molecular Docking of drug and DNA. A study shows that the GOLD and GLIDE docking protocols seem to be very reliable in modelling of nucleic acid ligand complexes (Srivastava *et al.,* 2011). Molecular docking studies show that the intercalators generally bind to the CG –rich region of DNA and minor groove binders to the AT-rich region of DNA (Srivastava *et al.,* 2011; Rashidaa and Ahsen, 2015). ð-ð interaction dominates in case of intercalators. In case of minor groove binders, hydrogen bonds are mainly formed between minor groove binders and the functional groups on the bases are exposed in the grooves via their end groups and also their amide or other linker groups. Other studies shows that if the nature of ligand with DNA is not known, the exact mode of binding of ligand to DNA cannot be predicted on the

basis of molecular docking as a result other molecular modelling techniques such as molecular dynamics simulation and thermodynamics integration will be required to further resolve the problem (Mariya and Ahsen, 2015).

Molecular Dynamics Simulation

Molecular Dynamics (MD) is the most important computational approach for the study of flexible nucleic acids. In MD, the motion of a biomolecular system under the effect of a "force" (i.e a specified force field) is simulated by following its molecular configurations in time, according to Newton's equation of motion (second law). A MD calculation starts with a set of initial co-ordinates and velocities. The force-field calculates the potential energy and the forces acting on the system, and Newton's second law is used to determine the accelerations on each particle. Numerical integration of these accelerations provides a set of new velocities and positions, which are used to build up a trajectory. MD protocols include algorithms to fix the temperature and the pressure, allowing the simulation of nucleic acids under conditions close the physiological ones. MD simulations of nucleic acid are performed using explicit solvent representations including thousands of water molecules and periodic boundary conditions (PBC). Explicit water models used in bio molecular simulations include TIP3P, TIP4P, SPC, extended SPC/E, and F3C models among these TIP3P is the most commonly used model (Jorgensen *et al.,* 1983; Berendsen *et al.,* 1987; Levitt *et al.,* 1997). Ions (generally Na^+ and Cl^-) are introduced to neutralize the system and simulate the given ionic strength. The evolution of trajectories shows the movement from one stable state to a stable one.

In mid 1990s several groups performed successful MD simulations of DNA and RNA with an explicit representation of solvent using the AMBER, CHARMM nucleic acid, or GROMOS force field. Various force fields used in the nucleic acid simulation includes, CHARMM, AMBER, GROMOS, OPLS, ENCAD and BMS26. AMBER (Case *et al.,* 2012), GROMACS (Hess *et al.,* 2008), CHARMM (Brooks *et al.,* 2009) and NAMD (Nelson *et al.,* 1996) are the popular software packages for the simulation of nucleic acid ligand complexes. The three latest force-fields (AMBER-99, CHARMM-27 and BMS) provide accurate representations of standard DNA and RNA structures (York *et al.,* 1995; Cheatham *et al.,* 1995; Weerasinghe *et al.,* 1995; Weiner and Kollman, 1981; Nilsson and Karplus, 1986; Gunsteren and Berendsen, 1986). The root-mean square deviation (RMSD) of the simulated nucleic acids with respect to experimental structures is small, the dihedral distributions are correct, and the average helical parameters are also reasonably close to the accepted experimental values. Among the variety of available force fields, CHARMM and AMBER are the most popular force fields (Cheatham and Young, 2001; Cheatham *et al.,* 1999; Cornell *et al.,* 1995; Foloppe and Mackerell, 2000; Mackerell and Banavali, 2000; Langley, 1998; Modesto *et al.,* 2003). In a number of studies for the minor groove binders and intercalators, computational methods have shown good agreement with the experimental results (Kamal *et al.,* 2007, 2009, 2010a, 2010b).

MMPBSA/MMGBSA Method

The molecular mechanics energies combined with the Poisson-Boltzmann or generalized Born and surface area continuum solvation (MMPBSA/MMGBSA) methods are popular approaches to calculate the free energy difference between two states, generally the bound and unbound state of two solvated molecules, or ultimately to compare free energy of two different solvated conformations of the same molecule (Gohlke and Klebe, 2002; Kolman *et al.,* 2000; Srinivasan *et al.,* 1998; Hou *et al.,* 2011; Homeyer and Gohlke, 2012). Snapshots obtained from MD simulation are used for the calculation, yielding an average of the energies. The free energy of binding is calculated by the equations mentioned below-

$$\Delta G_{bind} = \Delta H - T\Delta S$$

$$\Delta G_{bind} = (\Delta E_{MM} + \Delta G_{SOL}) - T\Delta S$$

Where,

$$\Delta E_{MM} = (E_{MM}{}^{complex} - E_{MM}{}^{receptor} - E_{MM}{}^{ligand})$$

$$\Delta G_{SOL} = (\Delta G_{SOL}{}^{complex} - \Delta G_{SOL}{}^{receptor} - \Delta G_{SOl}{}^{ligand})$$

$$\Delta S = (S^{complex} - S^{receptor} - S^{ligand})$$

where ΔH is the enthalpic contribution to binding energy, ΔE_{MM} is the average difference in molecular mechanics energy, while ΔG_{SOL} term accounts for the solvation free energy(including both polar and non-polar component); T is the temperature and ΔS is a change in entropy.

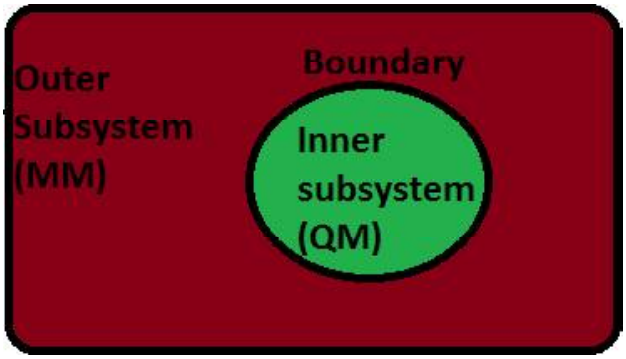
Thus the net binding free energy of complex system is equal to the sum of an intermolecular energy (calculated using MM force field), a solvation free energy term and an

entropic term. Polar solvation free energies is calculated either solving the linear Poisson Boltzmann (PB) equation or more approximate, or computationally effective Generalized Born (GB) model, whereas the non-polar contribution is estimated from a linear relation to the solvent accessible surface area (SASA). Entropy contributions to the free energy are estimated either by quasi-harmonic analysis or by using normal mode analysis (Schwarzl *et al.,* 2002; Rastelli *et al.,* 2010).

The MMPBSA calculations are based on single minimized structures, rather than on a large number of MD snapshots. This method will save much computational time but ignores dynamical effects. It has been observed that the more time can be saved by performing the minimizations in a GB continuum model. Hou *et al.* reveals that the MMGBSA results varied with the length of simulation, but there is no gain of using simulation longer than 4ns (Hou *et al.,* 2011). H.K. Srivastava *et. al.* proves that the MM-PBSA based interaction energies calculated from the MD Simulations are in good agreement with the experimental values for the DNA-ligand complexes (Srivastava *et al.,* 2011; Spackova *et al.,* 2003). The MMPBSA approach was originally developed for the AMBER software but recently some automatic scripts have also been presented for the freely available GROMACS, NAMD and APBS software. A study reveals that the energies calculated using g_mmpbsa (GROMACS) and the AMBER MM-PBSA package is approximately similar and the difference of 1-3 kcal/mol has been observed due to the difference in ΔG_{polar} (Kumari *et al.,* 2014). The difference in ΔG_{polar} is observed because of different algorithms, implemented in APBS and PBSA (Mishra *et al.,* 2015).

Quantum Mechanics/Molecular Mechanics (QM/MM) method

The QM/MM concept was introduced, as early as 1976, by Warshel and Levitt, who presented a semi empirical QM/MM treatment for a chemical reaction in lysozyme (Warshel and Levitt, 1976). Combined QM/MM theory has emerged as the method of choice for modeling local electronic events in large bimolecular systems.The basic idea is to describe the active site (where chemical reactions or electronic excitations occur) by quantum mechanics, as accurately as needed, while capturing the effects of the bimolecular environment by molecular mechanics, i.e., at the classical force field level. The accuracy of QM and speed of MM, combined QM/MM methods enable the modelling of reactive bimolecular systems at reasonable computational cost with the necessary accuracy. Due to the potential uses of this method, this field gained the Nobel Prize in chemistry in 2013. There are two schemes to calculate the total energy of the system the additive and the subtractive scheme (Sherwood *et al.,* 2008, Senn and Theil, 2009, Sherwood *et al.,* 2003). Regarding this boundary schemes, the labeling conventions given in **Figure** that apply to covalent bonds across the QM-MM boundary.



Subtractive schemes:

$$E_{QM/MM}(system) = E_{MM}(system) + E_{QM}(QM) - E_{MM}(QM)$$

Where $E_{QM/MM}$(system) is the total energy, E_{MM}(system) is the MM energy of the entire system, E_{QM}(QM) the QM energy of the QM region and E_{MM}(QM) the energy of the QM region.

The scheme encounters shortcomings due to the treatment of interactions between QM and MM region only at MM level which is inaccurate. This scheme needs the MM parameters for the QM region. Parameters are not usually available for these systems which are present in excited electronic states or contain transition metals.

Additive schemes:

$$E_{QM/MM}(system) = E_{MM}(system) + E_{QM}(QM) - E_{QM-MM}(QM, MM)$$

In this scheme, the total energy of the system $E_{QM/MM}$ (system) comprises of only three components viz., E_{MM} (MM) theMM energy of the MM region only, E_{QM} (QM) the QM energy of the QM region andthe $E_{QM^{\circ}MM}$(QM, MM) a term which interfaces between the QM/MM through the inclusion of bonded and non-bonded interactions. The bonded interactions account for bond stretching, bending and torsion while the non-bonded account for the vander Waals and electrostatic interactions.

The key to such QM/MM methods is the coupling between the electric field from the surrounding and the QM Hamiltonian in the active-site region. This requires careful treatment of the boundary between the QM and MM

regions.

The most important part of QM/MM is partitioning of the system. The basic considerations for QM/MM partitioning are:

(a) The choice of the QM region is usually made by consideration of the chemical problem; chemical arguments normally suggest a minimum-size QM region which can then be enlarged to check the sensitivity of the QM/MM results with regard to such an extension.

(b) If a QM/MM division through covalent bonds cannot be avoided, cut only unconjugated single bonds, preferably without electronically demanding substituent's (e.g., cut unpolar C–C bonds).

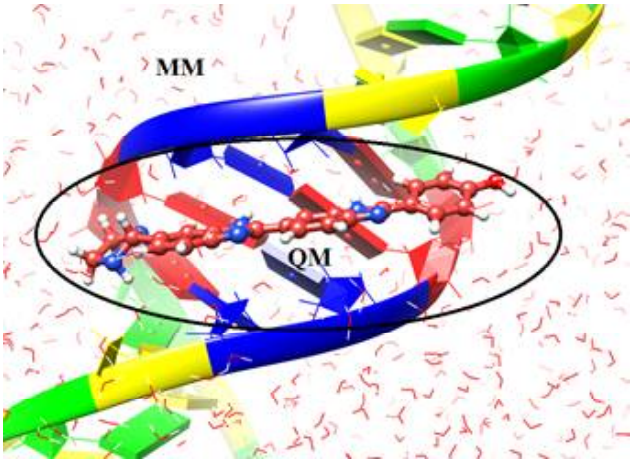


Fig. 7. QM/MM partitioning: *drug* and associated bases (QM region), remaining bases and water molecules and ions (MM region).

A common procedure for QM/MM calculations is to take a crystal structure from protein data bank as a starting point for the calculations and add hydrogen, missing atoms etc. and water molecules (soak the complete system). Thereafter, the system is equilibrated using MM followed by molecular dynamics production run and low-energy snap-shots are studied. These snapshots will eventually be taken for QM/MM calculations.These structures contain the bimolecular system in a droplet of water (20000-30000 atoms) and this setup requires a lot of prior work to avoid errors and wrong choices for the actual QM/MM calculations. Further study of the reaction mechanism is similar to the typical methods for the study of gas-phase reaction (Senn and Theil, 2007, Frieesner and Guallar, 2005).

The computational chemistry techniques, especially the hybrid methods (ONIOM) based on combination of several theoretical approaches, have been developed by Morokuma and co-workers for large biomolecular systems (Svensson *et al.,* 1996, Morokuma *et al.,* 2001, Morokuma, 2003, Kuno *et al.,* 2003). In many studies, the ONIOM method is used for the study of DNA binding drugs (Rebeca *et al.,* 2009, Ahmadia *et al.,* 2011, Robertazzi and Platts, 2006).

CONCLUSION

In this review the different types of small organic molecules which targeted DNA have been discussed. The array of available computational approaches and molecular modelling methods are being used for complementing the experimental efforts to improve the existing drugs and also in designing novel drug candidates which can act as good DNA inhibitor. Advances in computational resources over the last years have made screening of large chemical libraries and application of molecular dynamics and quantum chemical calculations feasible. This review seeks to highlight some recent molecular modelling studies performed at electronic structure level to study the mechanism of drug interaction to DNA which can give an insight in designing inhibitors for the treatment of cancer and AIDS.

ACKNOWLEDGEMENTS

AK like to acknowledge the UGC for financial support. The authors are thankful to Dr G. Narahari Sastry for his support and discussions.

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REVIEW ARTICLE

International Journal of Science, Technology and Society
Vol 3, No. 2 (2017) : 28-35

Redefining Sustainability Through Digital India: A Preview

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Publication Info

Article history:
Received : 13.11.2017
Accepted : 23.12.2017
DOI: https://doi.org/10.18091/ijsts.v3i02.11407

Key words:
Digitization, Digital India, Sustainability.

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ABSTRACT

Digital describes electronic technology that generates, stores and processes data so that it is stored in a virtual central repository and is easy to access anywhere anytime in addition to creating a transparent environment. Digital India is a dream project of the Indian Government to remodel India as a front-runner in this knowledge based society and empower them through good governance by synchronizing and coordinating in public accountability, digitally connecting individuals and organizations and delivering the government programmes to mobilize the capability of information technology across government departments. This programme will help to make India smart. Today, every nation wants to be highly digitized and this programme strives to provide equal opportunities and benefits to both the user and service provider. The current paper tries to project how digital services will make India smart and more sustainable besides impacting the common citizen.

INTRODUCTION

In our day and age, technology is omnipresent and an integral part of our lives. Technology is being implemented in almost every section of our lives and business structures. From smart phones to lightning-fast laptops to GPS devices, it's hard to imagine life without technology. In the twenty-first century, one of the most important technologies is the power of the digitization. The system, which allows individuals to communicate globally. Digital India is a campaign run by the government of India to make this country digitally empowered. The aim of launching this campaign is to provide Indian citizens electronic government services by reducing the paperwork. It is very effective and efficient technique which will save time and man power to a great extent. This initiative was started on 1st of July in 2015 to connect people of rural areas with the high-speed internet networks to access any information needed. Digital India is an umbrella programme that covers multiple Government Ministries and Departments. It weaves together a large number of ideas and thoughts into a single, comprehensive vision so that each of them can be implemented as part of a larger goal.

DIGITAL READINESS OF INDIA

Immediately with the introduction of this campaign, many organizations came forward to lend their hands for achieving India a digitally equipped country. Organizations like BSNL, Reliance Ltd. are coming forward to spread digitalization among rural areas. And over 42000 villages all over India will be having seamless mobile connectivity by 2018. The Internet *Saathi* initiative aims to cover 4,500 villages over the next 18 months, starting with Gujarat, Rajasthan and Jharkhand. India is aiming to achieve universal digital literacy across the country. The prime importance is to make sure every individual can be able to leverage the potential of Digital India. The focus is at least one person in a household should transform into an e-literate. This can be achieved by BBNL which is planning to connect 2, 50,000 panchayats under the scheme. This will ensure the digitization and connectivity of local institutions like panchayats offices, schools, other government offices and libraries etc. India is reforming its government through technology in the name of E-Governance with the advancement of technology and digitalization. Under the e-governance programme, out of 252 schemes planned, 222 services have been provided in short span of time. The nine pillars of Digital India

programme clearly confirms that India as a nation is at its nascent stage. One can easily assure that India will be digitally ready in the next three years. The vision of Digital India programme is to transform India into a digitally empowered society and knowledge economy. The Digital India programme is centered on three key vision areas:

1. Digital Infrastructure as Utility to Every Citizen

- Availability of high speed internet as a core utility for delivery of services to citizens.
- Cradle to grave digital identity that is unique, lifelong, online and authenticable to every citizen.
- Mobile phone and Bank account enabling citizen participation in digital and financial space.
- Easy access to a Common Service Centre.
- Shareable private space on a public Cloud.
- Safe and secure Cyber-space.

2. Governance and Services on Demand

- Seamlessly integrated across departments or jurisdictions.
- Services availability in real time from online and mobile platforms.
- All citizen entitlements to be available on the Cloud to ensure easy access.
- Government services digitally transformed for improving Ease of Doing Business.
- Making financial transactions above a threshold, electronic and cashless.
- Leveraging GIS for decision support systems and development.

3. Digital Empowerment of Citizens:

- Universal digital literacy.
- All digital resources universally accessible.
- All Government documents/ certificates to be available on the Cloud.
- Availability of digital resources / services in Indian languages.
- Collaborative digital platforms for participative governance.
- Portability of all entitlements for individuals through the Cloud.

The overall scope of this programme is to prepare India for a knowledge future, on being transformative that is to realize :

IT (Indian Talent) + IT (Information Technology) = IT (India Tomorrow)

making technology central to enabling change, on being an Umbrella Programme – covering many departments. The programme weaves together a large number of ideas and thoughts into a single, comprehensive vision, so that each of them is seen as part of a larger goal. Each individual element stands on its own, but is also part of the larger picture. The weaving together makes the Mission transformative in totality. The Digital India Programme will pull together many existing schemes which would be restructured and re-focused and implemented in a synchronized manner.

Our Prime Minister wants India on the cloud. What that means is that every individual's personal record is stored in a virtual central repository or *Digital Locker*, which can be accessed by government officials anywhere anytime, through established protocols. For example, an individual's certificates from school or college, birth certificates, medical records etc are all stored in the individual's folder and these can be accessed by any government official to confirm or verify the individual's claims without the need for the individual to present the hard copy. The purpose is to free the individual from the need to physically present the hard copy for verification in any government office. This will save a lot of time for the individual and the government officials, as also reduce the opportunity for bribes being demanded.

The government has invited suggestions from the technical talent pool in India to submit suggestions through the site MyGov.in, on how to develop the communication protocol or the applications programming interface (API) for delivering this project. The plan is to create a digital platform to serve nine verticals; e-governance, e-Kranti, public internet access program, broadband highway, information access for all, mobile connectivity, early harvest programs, IT for jobs, and electronic manufacturing. The government plans to extend the project to the Clean India campaign as also, the Skills and Entrepreneurship project for job creation. The government will realign the National Informatics Centre to assist in integrating the above with delivering projects of various ministries, through direct or PPP mode.

DISCUSSION

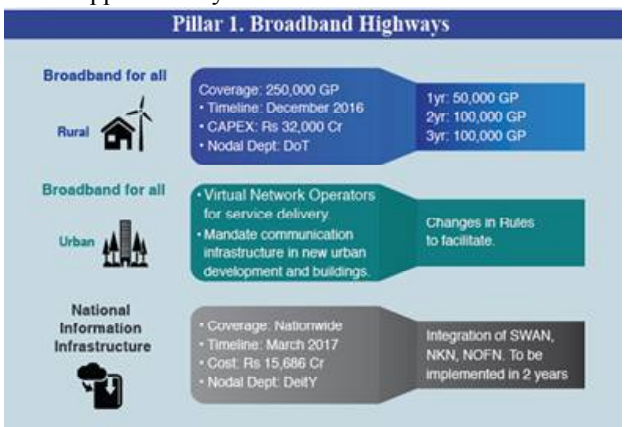
Nine pillars of Digital India

Digital India aims to provide the much needed thrust to the nine pillars of growth areas, namely

1. Broadband Highways

This covers three sub components, namely Broadband for All Rural, Broadband for All Urban and National Information Infrastructure.

- Under **Broadband for All Rural**, 250 thousand village Panchayats would be covered by December, 2016. **DoT** will be the nodal Department and the project cost is estimated to be approximately Rs. 32,000 Cr.
- Under **Broadband for All Urban**, Virtual Network Operators would be leveraged for service delivery and communication infrastructure in new urban development and buildings would be mandated.
- **National Information Infrastructure** would integrate the networks like SWAN, NKN and NOFN along with cloud enabled National and State Data Centres. It will also have provision for horizontal connectivity to 100, 50, 20 and 5 government offices/ service outlets at state, district, block and panchayat levels respectively. DeitY will be the nodal department and the project cost is estimated to be around Rs 15,686 Cr for implementation in 2 years and maintenance & support for 5 years.



2. Universal Access to Mobile Connectivity

- The initiative is to focus on network penetration and fill the gaps in connectivity in the country.
- All together 42,300 uncovered villages will be covered for providing universal mobile connectivity in the country.
- DoT will be the nodal department and project cost will be around Rs 16,000 Cr during FY 2014-18.

3. Public Internet Access Programme

- The two sub components of Public Internet Access Programme are Common Service Centres and Post Offices as multi-service centres.
- Common Service Centres would be strengthened and its number would be increased from approximately 135,000 operational at present to 250,000 i.e. one CSC in each Gram Panchayat. CSCs would be made viable, multi-functional end-points for delivery of government and business services. DeitY would be the nodal department to implement the scheme.
- A total of 150,000 Post Offices are proposed to be converted into multi service centres. Department of Posts would be the nodal department to implement this scheme.

4. e-Governance – Reforming Government through Technology

- Government Business Process Re-engineering using IT to improve transactions is the most critical for transformation across government and therefore needs to be implemented by all ministries/ departments.

The guiding principles for reforming government through technology are:

1. Form simplification and field reduction – Forms should be made simple and user friendly and only minimum and necessary information should be collected.

2. Online applications, tracking of their status and interface between departments should be provided.
3. Use of online repositories e.g. school certificates, voter ID cards, etc. should be mandated so that citizens are not required to submit these documents in physical form.
4. Integration of services and platforms, e.g. UIDAI, Payment Gateway, Mobile Platform, Electronic Data Interchange (EDI) etc. should be mandated to facilitate integrated and interoperable service delivery to citizens and businesses.

- **Electronic Databases** – all databases and information should be electronic and not manual.
- **Workflow Automation Inside Government** – The workflow inside government departments and agencies should be automated to enable efficient government processes and also to allow visibility of these processes to the citizens.
- **Public Grievance Redressal –** IT should be used to automate, respond and analyze data to identify and resolve persistent problems. These would be largely process improvements.

5. e-Kranti (NeGP 2.0) – Electronic delivery of services

- There are 31 Mission Mode Projects under different stages of e-governance project lifecycle. Further, 10 new MMPs have been added to e-Kranti by the Apex Committee on National e-Governance Plan (NeGP) headed by the Cabinet Secretary in its meeting held on 18th March 2014.

Technology for Education – e-Education

- All Schools will be connected with broadband. Free wifi will be provided in all secondary and higher secondary schools (coverage would be around 250,000 schools). A programme on digital literacy would be taken up at the national level. MOOCs – Massive Online Open Courses shall be developed and leveraged for e-Education.

Technology for Health – e-Healthcare

- E-Healthcare would cover online medical consultation, online medical records, online medicine supply, pan-India exchange for patient information. Pilots shall be undertaken in 2015 and full coverage would be provided in 3 years.

Technology for Farmers

- This would facilitate farmers to get real time price information, online ordering of inputs and online cash, loan and relief payment with mobile banking.

Technology for Security

- Mobile based emergency services and disaster related services would be provided to citizens on real time basis so as to take precautionary measures well in time and minimize loss of lives and properties.

Technology for Financial Inclusion

- Financial Inclusion shall be strengthened using Mobile Banking, Micro-ATM program and CSCs/ Post Offices.

Technology for Justice

- Interoperable Criminal Justice System shall be strengthened by leveraging e-Courts, e-Police, e-Jails and e-Prosecution.

Technology for Planning

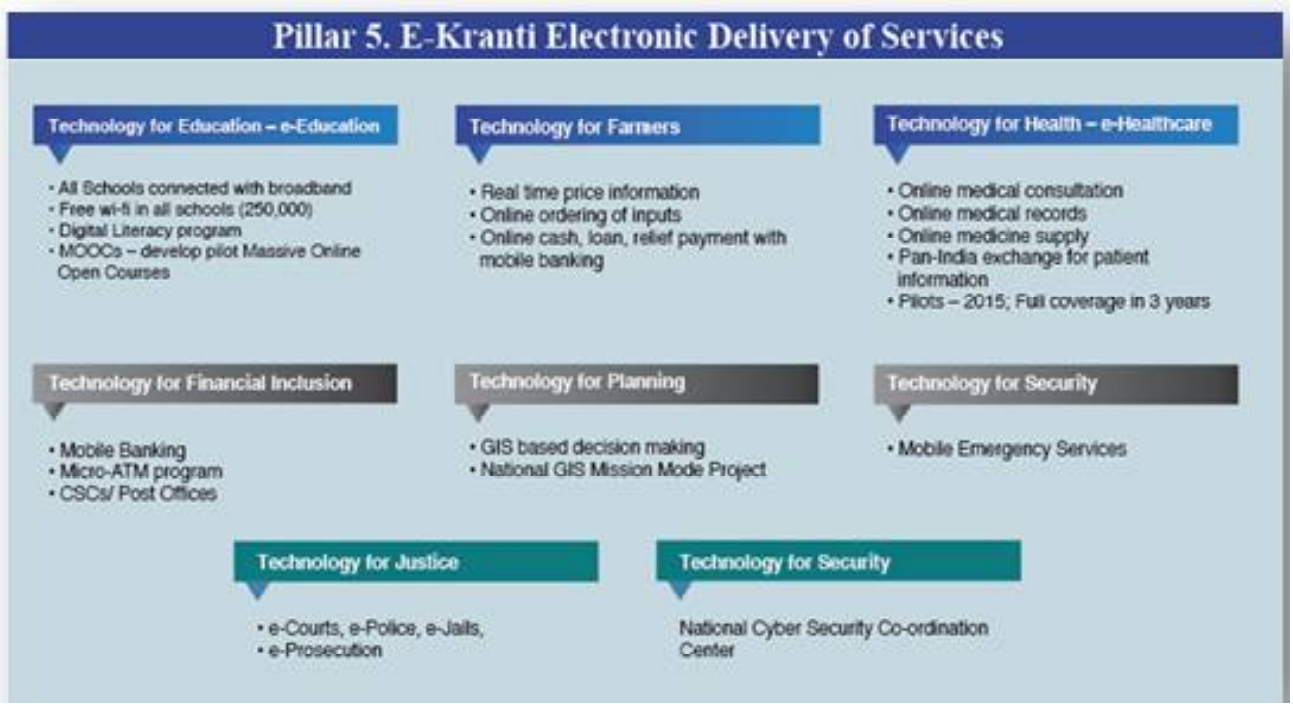
- National GIS Mission Mode Project would be implemented to facilitate GIS based decision making for project planning, conceptualization, design and development.

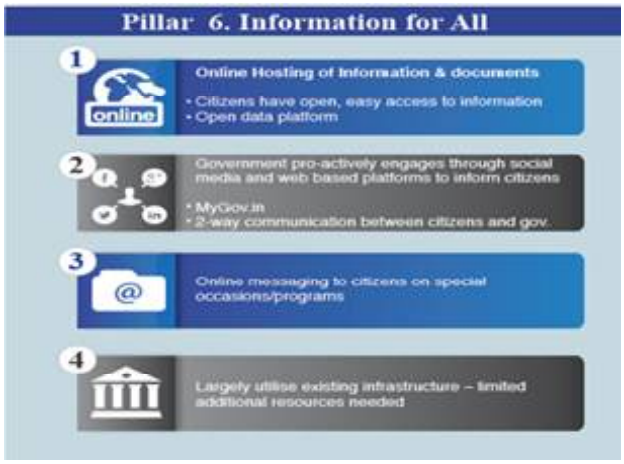
Technology for Cyber Security

- National Cyber Security Co-ordination Center would be set up to ensure safe and secure cyber-space within the country.

6. Information for All

- **Open Data platform and online hosting of information & documents** would facilitate open and easy access to information for citizens.
- **Government shall pro-actively engage through social media** and web based platforms to inform citizens.**MyGov.in** has already been launched as a medium to exchange ideas/ suggestions with Government. It will facilitate 2-way communication between citizens and government.
- **Online messaging** to citizens on special occasions/ programs would be facilitated through emails and SMSes.
- The above would largely utilise existing infrastructure and would need limited additional resources.





7. Electronics Manufacturing – Target NET ZERO Imports

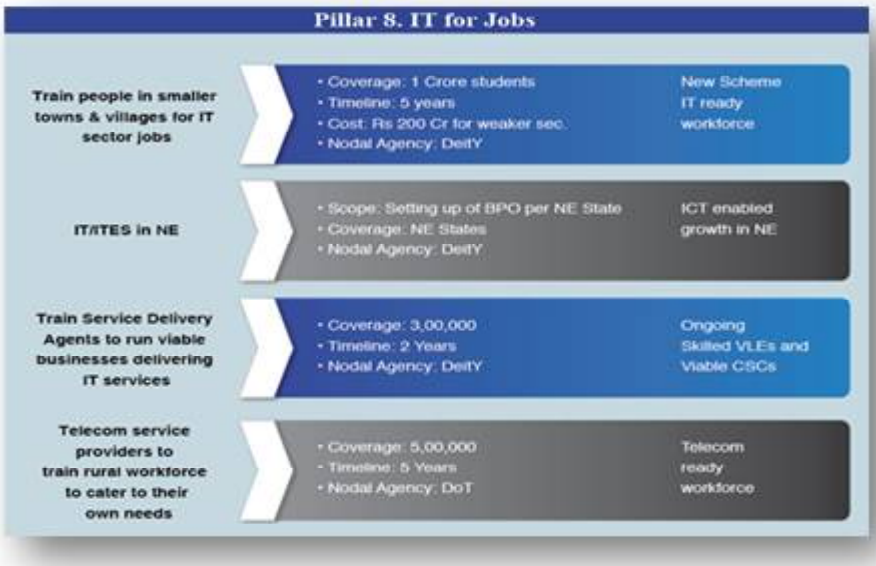
Target NET ZERO Imports is a striking demonstration of intent. This ambitious goal requires coordinated action on many fronts

- Taxation, incentives
- Economies of scale, eliminate cost disadvantages
- **Focus areas** – Big Ticket Items FABS, Fab-less design, Set top boxes, VSATs, Mobiles, Consumer & Medical Electronics, Smart Energy meters, Smart cards, micro-ATMs
- Incubators, clusters
- Skill development
- Government procurement

There are many ongoing programs which will be fine-tuned. Existing structures are inadequate to handle this goal and need strengthening**.**

8. IT for Jobs

- 1 Cr students from smaller towns & villages will be trained for IT sector jobs over 5 years. DeitY would be the nodal department for this scheme.
- BPOs would be set up in every north-eastern state to facilitate ICT enabled growth in these states. DeitY would be the nodal department for this scheme.
- 3 lakh service delivery agents would be trained as part of skill development to run viable businesses delivering IT services. DeitY would be the nodal department for this scheme.
- 5 lakh rural workforce would be trained by the Telecom Service Providers (TSPs) to cater to their own needs. Department of Telecom (DoT) would be the nodal department for this scheme.



9. Early Harvest Programmes

- **IT Platform for Messages**

A Mass Messaging Application has been developed by DeitY that will cover elected representatives and all Government employees. 1.36 Cr mobiles and 22 Lakh emails are part of the database.

- **Government Greetings to be e-Greetings**

Basket of e-Greetings templates have been made available. Crowd sourcing of e-Greetings through MyGov platform has been ensured. E-Greetings portal has been made live on 14th August 2014.

- **Biometric attendance**

It will cover all Central Govt. Offices in Delhi and is already operational in DeitY and has been initiated in the Department of Urban Development. On-boarding has also started in other departments.

- **Wi-Fi in All Universities**

All universities on the National Knowledge Network (NKN) shall be covered under this scheme. Ministry of HRD is the nodal ministry for implementing this scheme.

- **Secure Email within Government**

1. Email would be the primary mode of communication.
2. Phase-I upgradation for 10 lakh employees has been completed. In Phase II, infrastructure would be further upgraded to cover 50 lakh employees by March 2015 at a cost of Rs 98 Cr. DeitY is the nodal department for this scheme.

- **Standardize Government Email Design**

Standardised templates for Government email are under preparation and would be ready by October 2014. This would be implemented by DeitY.

- **Public Wi-fi hotspots**

Cities with population of over 1 million and tourist centres would be provided with public wi-fi hotspots to promote digital cities. The scheme would be implemented by DoT and MoUD.

- **School Books to be eBooks**

All books shall be converted into eBooks. Min. of HRD/ DeitY would be the nodal agencies for this scheme.

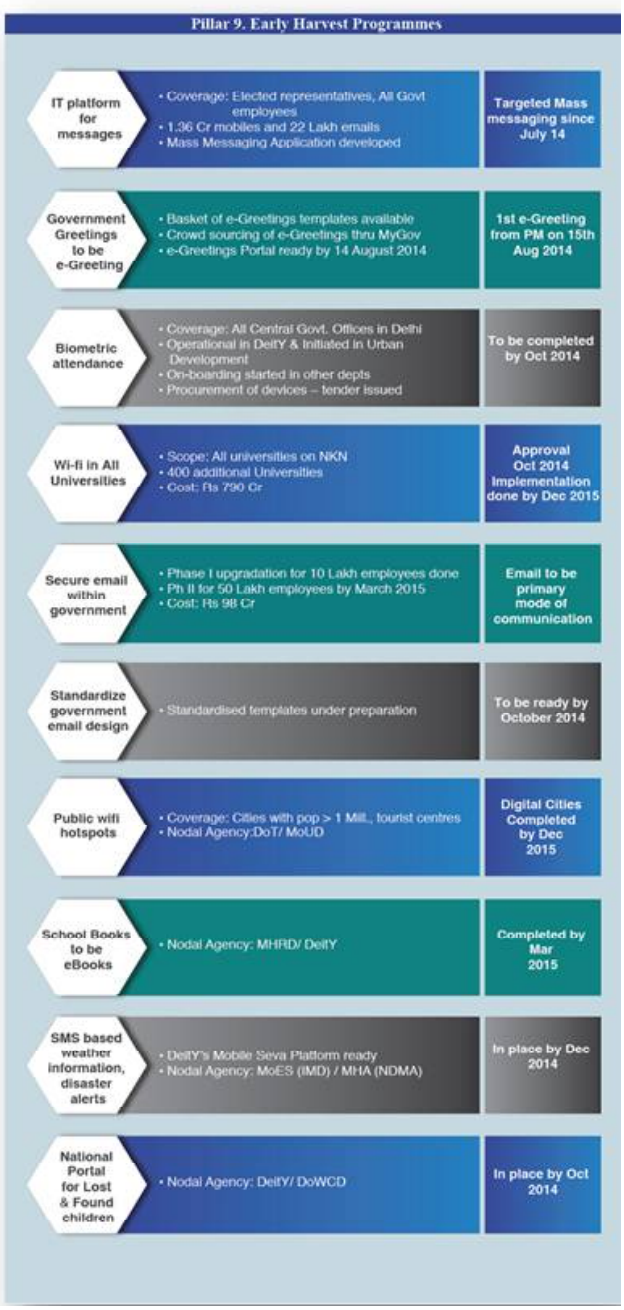
- **SMS based weather information, disaster alerts**

SMS based weather information and disaster alerts would be provided. DeitY's Mobile Seva Platform is already ready and available for this purpose. MoES (IMD) / MHA (NDMA) would be the nodal organizations for implementing this scheme.

- **National Portal for Lost & Found children**

1. This would facilitate real time information gathering and sharing on the lost and found children and would go a long way to check crime and improve timely response.
2. DeitY/ DoWCD would be the nodal departments for this project.

Some of the aforementioned projects are under various stages of implementation and may require some



transformational process reengineering, refinements and adjustment of scoping and implementation strategy to achieve the desired service level objectives by the concerned line Ministries/Departments at the Central, State and Local Government levels.

IMPACT OF DIGITAL INDIA:

The digital India programme provides a huge opportunity to use the latest technology to redefine India the paradigms of service industry. A digitally connected India can help in improving economic, social and environmental condition of people living in rural areas by the mean of development of non-agricultural economic activities apart from providing access to education, health and financial services. There are some impacts of digital India which are as follows:-

Economic Impact: - It can play a very significant role in macro economic factors such as GDP growth, employment generation, labor productivity, growth in number of businesses and revenue leakages for the government.

Social Impact: - Social sectors namely, education, healthcare, and banking are unable to reach out to the citizens due to obstructions and limitations such as middleman, illiteracy, poverty, lack of funds and investments. Modern ICT makes it easier for people to obtain access to services and resources even in villages too. The penetration of mobile devices may be highly useful as a complementary channel to public service delivery apart from creation of entirely new services.

Environment Impact: - The major changes in the technology space have not only brought changes to the economic system but are also contributing in to the environment changes. The next generation technologies are helping in lowering the carbon footprint by the way of reducing fuel consumption, waste management, greener management, greener workplaces and thus leading to a greener ecosystem.

CHALLENGES AHEAD:

Infrastructure

India will need massive Data Centres with mirroring i.e. all data will need to be backed up at an alternate site would surely sort out many of the problems. This will require large investment to set up state-of-the-art data centres in various parts of India. These data centres will have to be provided fail proof physical and cyber security cover.

Security

Security is not an act but merely it is a process and a lifestyle. India is a nation where we neither understand security nor practice it, as part of our daily lifestyle. This has left our IT infrastructure vulnerable to security attack either through cyber space or through internal subvert. India is still nowhere in establishing secure and impenetrable networks, as seen in various attacks on critical sites of various government establishments, over the years. Government sites especially are vulnerable. Then there is the real

possibility of internal damage that can result in stealing or damaging of data, at any given point. The biggest challenge to the success of the Digital India project is not on the delivery side but on the security side too. Securing this data for all time is going to be the real challenge that the government has to address before embarking on this ambitious project.

Cyber Laws

When the government stores personal data of the citizens, the government becomes its custodian, which means that the government is responsible for securing the data and also preventing its misuse. The question is how is the government going to prevent litigation in cases of data if misused? For instance, what happens when one individual uses a government official to access another individual's medical records and then uses this information against that individual and so on… Furthermore, India is a country with a VIP culture. What happens in a case of data pertaining to a VIP being accessed, lost or damaged by someone with vested interest? In this scenario, the government becomes a party to the data breach. What happens if the data is damaged or the delay in verification causes the individual a financial loss or a loss of business opportunity? Will the government be liable for the loss? Do we have cyber laws that adequately address such scenarios? These will have to be looked at very closely by the government prior to launching the Digital India programme and with the probable solutions to the above problem.

Training

The Digital India program will have to simultaneously launch a training program to ensure all government officials in order to understand about the available data, its protocols of access and protocols of security, and also the legal complications in case of data breach. Given the large size of the government, it will take time and investment to train and cover all individuals, for successful delivery of the program.

CONCLUSION

Even though India is known as the powerhouse of software, the availability of e-government services to citizens is still comparatively very low . The Digital India programme is just the beginning of a digital revolution, once implemented properly it will open various new avenues for the citizens of our country. It is one of the highly ambitious programme of Indian government. Various MNCs like Microsoft, Google have also agreed to be the partner and help the success of Digital India initiative. If the government can extend the vision to include email, Messaging and cloud services on servers located in India, it will truly usher in a digital revolution in India, besides opening up massive business opportunities for many of us even at our home. This will enable the country to become better in the terms of sustainable development along with transparency. Thus, it can be further said that India can't be a Developed Country until and unless if it is not e-Governed. Therefore making necessary to develop e- governance, cloud storage, elimination of redundancy, thus by facilitating everything online. Thus, Digital India programme is a great opportunity to develop the digital backbone in the country.

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REVIEW ARTICLE

International Journal of Science, Technology and Society
Vol 3, No. 2 (2017) : 36-40

Sustainable management of soil borne pathogens of tomato

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Publication Info

Article history:
Received : 23.11.2017
Accepted : 19.12.2017
DOI: https://doi.org/10.18091/ijsts.v3i02.11409

Key words:
Phytopathogens, Rhizospheric microorganisms, Antibiosis, Mycoparasitism, Competition

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ABSTRACT

Tomato is the second most cultivated vegetable crop in world, which not only used in culinary but also has industrial and medicinal value. Tomato plants are susceptible to various soil borne diseases that severely affect its growth and fruit quality either grown in fields or cultivated in greenhouse under controlled conditions. The use of rhizospheric microorganisms as a biocontrol agent (BCA) against phytopathogens is considered as one of the most promising eco-friendly approach for sustainable management for tomato cultivation. The rhizopsheric microorganisms caters several mechanisms to control or suppress soil borne phytopathogenic fungi, bacteria and nematodes, via direct mechanism of mycoparasitism and/or indirect mechanisms such as antibiosis through production of secondary metabolites or antibiotics; and competition for nutrients and ecological niche. The present work outlines role of rhizospheric microbial isolates in management of phytopathogens affecting quantitative and qualitative production of tomato.

INTRODUCTION

Tomato (*Lycopersicum esculentum* L.) is commercially important and the second most cultivated vegetable crop in the world belonging to the Solanaceae family that encompasses several other important crops *i.e.,* potato, eggplant and pepper (Piquerez *et al*. 2014). Tomato is native species to Mexico, but cultivated and consumed all around the world (Arah *et al*. 2015). It's a warm season crop that requires optimum range of temperature *i.e.,* 21-24^{o}C, however temperature ranging between 10^{o}C to 30^{o}C is suitable for the growth. Tomato plants require well drained soil with preferred pH range 5.5-6.8. Wet conditions increase incidence of diseases and affect fruit ripening.

Commercial and medicinal value of Tomato

It is one of the most versatile, economically important vegetable that not only found space in kitchens but also tops the list of industrial crop because of its outstanding processing qualities (Christopher *et al*. 2010). Tomato has beneficial effects on human health due to presence of antioxidants and essential nutrients such as lycopene, β-carotene, flavonoids, vitamins and essential minerals, and therefore considered as one of the most important "protective foods" (Babu *et al*. 2015; Arah *et al*. 2015). Several studies have demonstrated that consumption of tomato has strong inverse correlation with the risk of certain types of cancer, cardiovascular diseases and age-related macular degeneration (Babu *et al*. 2015; Arah *et al*. 2015).

Factors affecting tomato productivity

Plants are constantly encountered with various biotic stresses that affect crop productivity worldwide. Biotic stresses are the major challenges that affect the tomato productivity worldwide. Tomato plants are susceptible to wide range of pests and pathogens including bacteria, fungi, oomycetes, nematodes, viruses and insects and this is the reason, tomato is considered as an excellent model for research and studying plant-pathogen interaction and helps to establish an effective control against these diseases to enhance productivity worldwide (Piquerez *et al*. 2014; Arie *et al*. 2007; Takahashi *et al*. 2005). Tomato provides a good example of how the use of biocontrol agents can be introduced into practice as an IPM strategy (Minuto *et al*. 2006).

The tomato diseases caused by soil-borne pathogens are widely distributed worldwide that include *Fusarium oxysporum f. sp. lycopersici*, *Verticillium* spp., *Ralstonia solanacearum* causing vascular wilt in tomato, *Pythium aphanidermatum and Rhizoctonia solani causing* Damping off, *Fusarium oxysporum f. sp. radicis-lycopersici* (fusarium crown rot), *Pyrenochaeta lycopersici* (corky root rot), *Colletotrichum coccodes* (Black dot root rot), *Clavibacter*

michiganensis subsp. *michiganensis* (bacterial canker), *Meloidogyne* species (root knot). The soil borne pathogens are considered difficult to control as they have wide range of host and persist in soil for long, by producing resilient surviving structures that remain viable for many years in soil, even in the absence of host plant. In India upto 45% of yield loss has been reported due to incidence of *Fusarium* wilt (Ramyabharathi *et al*. 2012).

Conventional practices for management of pathogens

To enhance the food productivity and to control plant pests and diseases, agrochemicals such as fertilizers and pesticides are being used since green revolution. The input of conventional chemical pesticides for management of plant pests and pathogens has posed safety risk on humans and animals health; has caused environmental pollution and also detrimentally affected the microbial community of ecosystem (Chowdappa *et al*. 2013; Babu *et al*. 2015). The imbalances in the microbial community have created unfavorable conditions for the activity of beneficial organisms (El Hassan *et al*. 2013) that play crucial role in growth and productivity of both plants and soil. To curb the negative consequences of use of chemicals pesticides and produce residue free food, focus of researchers has shifted toward alternative and sustainable approaches to control plant diseases and enhance food production. The alternative methods that makes use of organic amendments to soil involving compost, manures, agricultural waste, etc.; cultural practices including tillage, crop rotation and burning; soil solarization; steam sterilization; use of resistant cultivars and biological methods are considered as powerful tool for management of plant diseases (Mokhtar and El-Mougy, 2014).

The alternative methods like organic amendments to soil, cultural practices, soil solarization and resistant cultivars are most practical and cost efficient strategies, however, these practices do not control plant diseases effectively; their efficiency is limited by the diversity of pathogen host plants, ability to colonize the rhizosphere of non-host plants and persistence in soil for long via resting spore (Jabnoun-Khiareddine *et al*. 2009). Even the efficiency of resistant cultivars is limited by pathogenic variability due to emergence of new pathogenic races and pathotypes (Jimenez-Gasco *et al*. 2004). In search of sustainable, long lasting, cost effective and eco-friendly approach for managing plant diseases, research has been geared toward biocontrol agents that include use of naturally occurring microorganisms which is considered safe and compatible with other organic methods of pest and disease management (Motlagh and Samimi, 2013).

Rhizospheric microorganisms as alternative approach for management soil borne phytopathogens

The microorganisms residing in vicinity of plant roots are termed as Rhizospheric microorganisms. These microbes exert neutral, beneficial as well as detrimental effect on plants. The beneficial rhizospheric microorganisms possess ability to combat with soil borne phytopathogens either by inhibiting or suppressing their growth by deployment of plethora of mechanisms including competition for nutrition and space, mycoparasitism and antibiosis (Patel and Saraf, 2017; Babu *et al*. 2015). Several rhizospheric bacteria and fungi such as species belonging to genera *Bacillus*, *Pseudomonas*, *Trichoderma*, arbuscular mycorrhiza utilizes one or more of these biocontrol mechanisms to control phytopathogens. (Karimi *et al*. 2017; Babu *et al*. 2015; Srivastava *et al*. 2010; Thomma *et al*. 2002). The tomato plants inoculated with pathogens and treated with conidial suspension of *T. hamatum* and its filtrate showed <30 and <40% respectively, colonization of stem vascular tissues by pathogen as compared to 100% in control. Similarly Jabnoun-Khiareddine *et al*. (2009) in their study found that the three species of *Trichoderma* viz. *T. harzianum*, *T. viride* and *T. virens* had antagonistic effect on pathogen *Verticillium dahlia, V. albo-atrum* and *V. tricorpus*, casual agent of vascular wilt in tomato, that resulted in reduce radial growth of mycelia and abundance of resting spores of pathogens, inhibited sclerotinization, degraded melanin and caused profound alteration of mycelia at confrontation zone. Pathogen inoculated tomato plants, treated with *Trichoderma* under green house condition shown reduced discolouration index and increased plant fresh weight by more than 50%, after 90 days of culture.

Mycoparasitism is most significant and effective mechanism which involves lyses of pathogen mycelia by production of cell wall degrading enzymes (CWDE) such as chitinases, β-glucanases and proteases (Troian *et al*. 2014; Benitez *et al*. 2004). Mycoparasitism is an ancestral trait of *Trichoderma*, whereby hyphae of *Trichoderma* parasitize and cause lysis to host hyphae by the action of hydrolytic enzymes alone or in combination with secondary metabolites (El Hasan *et al*. 2013; Gajera *et al*. 2012; Mukherjee *et al*. 2012). Gajera *et al*. (2012) reported that the two *Trichoderma* isolates, *T. koningi* and *T. harzianum* mycoparasitized *Macrophomina phaseolina* and degraded its mycelia by coiling around the hyphae with appressoria and hook-like structures and producing cell wall degrading enzymes, chitinase, β-1, 3 glucanase and total phenol content. Similar findings were reported by Monteiro *et al*. (2010), where *T. harzianum* ALL42 was overgrown and

degraded mycelia of *Rhizoctonia solani* and *M. phaseolina*. The study of interaction between fifteen isolates of *T. harzianum* and the soil-borne plant pathogen, *Rhizoctonia solani* through light microscopy and transmission electron microscopy revealed that *T. harzianum* was efficiently coiled around the pathogens and substantially produced hydrolytic enzymes (Almeida *et al*. 2007).

Competition for nutrients and ecological niche is an indirect mechanism employed by several rhizospheric microorganisms to curtail growth of pathogens. Pathogens are generally excluded by *Trichoderma* and plant growth promoting rhizobacteria (PGPRs) due to depletion of nutrients and physical occupation of site. Competition for nutrients basically concentrates on carbon, nitrogen and essential micronutrients (Schippers *et al*. 1987) such as iron and manganese. Production of siderophores and ability to grow fast and rapidly colonize the substrate allow PGPRs and *Trichoderma* to inhibit growth of pathogens via competition (Btaszczyk *et al*. 2014; Sharma *et al*. 2012; Persello Cartieaux *et al*. 2003). The unavailability of iron to the pathogen, which produce less siderophores with lower binding power (Junaid *et al*., 2013) result in growth inhibition. Siderophore pyoverdin produce by *Pseudomonas* species poses ability to suppress growth of *Pythium* induced post-emergence damping-off of seedlings in many crops (Paulitz and Loper, 1991; Buysens *et al*. 1996). However, the mutant strain *Pseudomonas aeruginosa* 7NSK2 deficient either in siderophore pyoverdin or pyochelin, or both, when compared to wild strains showed that one of these siderophores are essential for antagonism of *Pythium* causing damping-off in tomato (Buysens *et al*. 1996).

Antibiosis is a process that occur when antagonistic microorganism interact with pathogen, causing detrimental effect on later. It is assumed to be an essential mechanism employed by various PGPRs and *Trichoderma* spp. (El-hassan *et al*. 2009; Choudhary and Johri, 2008). The low molecular weight diffusible compounds or antibiotics are produced that inhibit the growth or kill the cells of pathogens. Species of *Bacillus*, *Pseudomonas*, *Trichoderma* have been documented to produces various volatile and non-volatile metabolites that play a major role in antibiosis (Puluputuri *et al*. 2014; Dubey *et al*. 2011; Choudhary and Johri 2008). Qualhato *et al*. (2013) reported the production and secretion of β-1,3-glucanase, N-acetyl-β-D-glucosaminidase (NASase), chitinase, acid phosphatase, acid proteases and alginate lyase by *Trichoderma harzianum*, *T. tomentosum*, *T. asperellum* and *T. ghanense* that was found to be antagonistic against *Fusarium solani, Rhizoctonia solani* and *Sclerotinia sclerotiorum.* The gliotoxin produce by *T. viride* was found to inhibit the growth of *Fusarium* and *Alternaria* spp. (Puluputuri *et al*. 2014). Another metabolite Trichodermin-a potent inhibitor of protein synthesis (Carrasco *et al*. 1973), was isolated from *Trichoderma brevicompactum*, which found to inhibit the activity of pathogens *Rhizoctonia solani*, *Fusarium oxysporum*, *Colletotrichum lindemuthianum*, *C. amphelinum* and *Botrytis cinerea* more than the positive control Carbendazin (Shentu *et al*. 2014). The effect of volatile compounds and culture filtrate of two strains of *Pseudomonas* PS1 and PS2 evaluated under *in-vitro* conditions that showed restriction of colony growth of pathogen *Macrophomina phaseolina* by volatile compounds to 25 and 32%, respectively and 57 and 61%, respectively, by culture filtrate at 20% concentration (Bhatia *et al*. 2003). Iturin A and Surfactin secreted by *Bacillus subtilis* and Zwittermicin A synthesized by *B. cereus* are documented to suppress damping off of Tomato and alfalfa, respectively (Choudhary and Johri 2008).

Conclusion and Future prospects

Tomato is a major crop, cultivated and consumed worldwide. It suffers from yield loses every year due to incidence of soil borne diseases, which also affect quality of produce. The conventional practices adopted to tackle with these constraints have challenged sustainability of agricultural ecosystem and are no more appreciated by environmentalists as well as consumers. These factors have forced to search for alternative approaches that not only shield agriculture sector from constraints but aid in retaining and maintaining its integrity. The end point of search for alternative approach can be attained by appropriate and effective use of beneficial rhizospheric microorganisms that are safe, eco-friendly and cost effective. In-depth understanding of mechanisms deployed by beneficial rhizospheric microorganism assists in harnessing their potential efficiently in management of soil borne diseases of tomato.

Acknowledgement

The authors gratefully acknowledge the financial support to Swati Sachdev from University Grant Commission (UCG), New Delhi, India, as UGC-Senior Research Fellowship.

CONFLICT OF INTEREST

There is no conflict of Interest

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RESEARCH ARTICLE

International Journal of Science, Technology and Society
Vol 3, No. 2 (2017) : 41-53

Nonlinear study of heat transfer in nanofluid saturated horizontal porous medium

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Publication Info

Article history:
Received : 30.11.2017
Accepted : 25.12.2017
DOI: https://doi.org/10.18091/ijsts.v3i02.11403

Key words:
Nanofluid, Porous media, Nanoparticle flux, Ginzburg-Landau Model, homotopy analysis method (HAM).

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ABSTRACT

The present paper deals with weak nonlinear stability analysis of heat transfer in a nanofluid saturated porous layer. We consider a set of new boundary conditions for the nanoparticle fraction, which is physically more realistic. The new boundary condition is based on the assumption that the nanoparticle fraction adjusts itself so that the nanoparticle flux is zero on the boundaries. We use Darcy model that incorporates the effects of Brownian motion and thermophoresis. The governing equations has been reduced to Ginzburg-Landau equation and solved by homotopy analysis method (HAM). The obtained results have been compared with the numerical results obtained by Mathematica NDSolve. The results are valid for the feasible domain with high accuracy. Thermal Nusselt number and Nanoparticle Nusselt number are calculated for different values of parameters. The results have been depicted graphically.

INTRODUCTION

Big impact of small sized nanoparticle on the heat transfer attracts many researchers worldwide to improve the rate of heat transfer of conventional fluid by mixing nanoparticle, which are more efficient carrier for heat transfer. It is reported by Eastman et al. (2001) that the copper nanoparticles increases the thermal conductivity of base fluid ethylene glycol by 40%, and with Multiwalled CNT Choi et al. (2001), the thermal conductivity of the base fluid oil is anomalously increased by 160% by adding only 0.3% and 1% percent volume fraction respectively. The increasingly growth in technology needed high class energy efficient devices and power enhancement which requires rapid heat exchangers for its cooling systems, where the conventional fluids have very limited capacity of heat transfer, therefore we need a relatively new class of fluid which enhances the heat exchange. Nanofluids are colloidal mixture of nanoparticles and a base liquid, its marvelous heat transfer enhancement property is a growing research area in fluid dynamics and engaged many researchers. The work of Choi (1995) introduces the term "nanofluids" during his research in Argonne National Laboratory. Several studies has been performed in this area by taking different, nanoparticles concentration, particle material, base fluid, however, the particle shape and size and effective thermal conductivity is open for discussion. The diverse applications of nanofluids can be found in the areas of engineering, medical, power sectors, nuclear reactors, solar collectors. Experimental and theoretical results on convective heat transfer related to nanofluid are reviewed and documented by Wang and Mujumdar (2007), Daungthongsuk and Wongwises (2007), Trisaksria and Wongwises (2007), Godson et al. (2010), Kakaç and Pramuanjaroenkij (2009), Wen et al. (2009), Eastman et al. (2009), Robert et al. (2013).

Rayleigh-Bénard convection in porous media commonly known as Horton-Rogers-Lapwood convection includes many applications of nanofluid which occur in the porous medium such as electronic cooling system, including food and chemical processes, nuclear reactors, petroleum industry, biomechanics, and geophysical problems. Documented work in this area are well collected and reviewed by Nield and Bejan (2013). Nanofluid finds its application in coolants for advanced nuclear systems, chemical engineering, electronic devices, medical science, storage

devices and in solar collectors. Studies related to nanofluid are mainly focused to thermal conductivity, however a satisfactory explanation for the abnormal enhancement in thermal conductivity and viscosity in nanofluid is yet to be found. The attempt of Buongiorno (2006) is found suitable for stability analysis of nanofluid convection which includes the effect of Brownian diffusion and thermophoresis for non-turbulent flow. Analytical studies have been done by; Nield and Kuznetsov (2009), who studied onset of convection in nanofluid saturated porous media; Kuznetsov and Nield (2010a) investigated thermal instability of nanofluid saturated porous layer using Brinkman model; Kuznetsov and Nield (2010b) performed stability analysis for local thermal non-equilibrium convection in porous media saturated with nanofluid; Nield and Kuznetsov (2011) studied the thermal instability of nanofluid convection in porous media considering the effect of vertical throughflow. Using Buongiorno's mathematical model, Sheremet and Pop (2014) studied natural convection by applying symmetric sinusoidal temperature with respect to the midplane of the square porous cavity. Author's group, Bhadauria and Agarwal (2011a, 2011b, 2011c), Agarwal and Bhadauria (20011, 2014a, 2014b, 2014c) and Agarwal et al. (2011, 2012), studied thermal stability of nanofluid, considering various physical models and boundary conditions. Recently, Nield and Kuznetsov (2014) examined a physically more realistic model for thermal instability by considering a new set of boundary conditions that the normal component of the nanoparticle flux on boundaries is zero. Further, Agarwal (2014) also studied the thermal instability of nanofluid convection in a rotating porous layer considering the new model of Nield and Kuznetsov (2014). Therefore, in this paper, we have made an attempt to study the heat transfer of nanofluid-saturated porous medium with the assumption that there is no nanoparticle flux at the boundaries, which is physically a more realistic condition.

The homotopy analysis method (HAM) is the renowned methods to solve non-linear differential equations, and there is no constrains on parameters involved in the governing equations. This method has been introduced by Liao (1992). This innovative technique has been used by several authors in the field of science and engineering to solve different types of governing differential equations: linear and nonlinear. This method offers highly accurate successive approximations of the solution. Some relevant studies are referred in the following references Liao (2004), Hayat (2004), Domairry (2008).

GOVERNING EQUATION

We consider an infinitely extended horizontal porous layer saturated by nanofluid, confined between the planes $z = 0$ and $z = d$. We choose Cartesian frame of reference with the origin in the lower boundary and the *z*-axis in vertically upward direction. The gravitational force is acting in vertically downward direction. It is assumed that the fluid and solid phases are in local thermal equilibrium. T_h and T_c are the lower and upper plate temperature respectively with the condition that $T_h > T_c$, T_c is taken as reference temperature. Moreover, it is assumed that there is no nanoparticle flux at the boundaries and that the particle fraction value there adjusts accordingly.

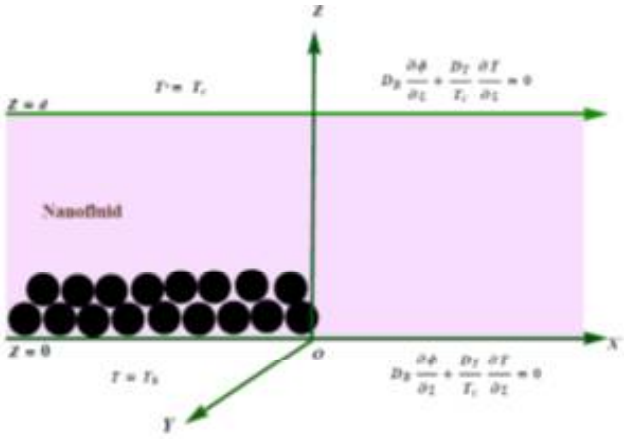

Fig. #: A sketch showing the physical configuration of the problem

Further, the density variation is considered under Boussinesq approximation. Then using the approximated buoyancy term, the governing equations under the above considerations are as follows:

$$\nabla.\mathbf{q}_D = 0, \tag{1}$$

$$-\nabla\mathbf{p} - \frac{\mu}{K}\mathbf{q}_D + \{\phi\rho_p + (1-\phi)\rho\left[1-\beta\left(T-T_c\right)\right]\}g = 0, \tag{2}$$

$$(\rho c)_m \frac{\partial T}{\partial \tau} + (\rho c)_f \mathbf{q}_D.\nabla T = \kappa_m \nabla^2 T + \varepsilon(\rho c)_m \times \left[D_B \nabla\phi.\nabla T + \left(\frac{D_T}{T_c}\right)\nabla T.\nabla T\right] \tag{3}$$

$$\frac{\partial \phi}{\partial \tau} + \frac{1}{\varepsilon}\mathbf{q}_D.\nabla\phi = D_B\nabla^2\phi + \left(\frac{D_T}{T_c}\right)\nabla^2 T, \tag{4}$$

where $\mathbf{q}_D = (u, v, w)$. It is assumed that the boundaries are held at constant temperature and the nanoparticle flux is zero on the boundaries. Thus the boundary conditions are taken as follows

$$w = 0,\ T = T_h,\ D_B \frac{\partial \phi}{\partial z} + \frac{D_T}{T_c}\frac{\partial T}{\partial z} = 0 \text{ at } z = 0, \tag{5}$$

$$w = 0,\ T = T_c,\ D_B \frac{\partial \phi}{\partial z} + \frac{D_T}{T_c}\frac{\partial T}{\partial z} = 0 \text{ at } z = d. \tag{6}$$

We introduce the dimensionless variables by using the following transformations:

$$\left(x^*, y^*, z^*\right) = (x, y, z)/d,\ \left(u^*, v^*, w^*\right) = (u, v, w)d/\alpha_m,$$
$$\tau^* = \tau\frac{\alpha_m}{\sigma d^2},\ \mathbf{p}^* = \mathbf{p}\frac{K}{\mu\alpha_m},\ \phi^* = \frac{\phi - \phi_0}{\phi_0} \text{ and } \mathrm{T}^* = \frac{T - T_c}{T_h - T_c}, \tag{7}$$

where $\alpha_m = \frac{\kappa_m}{(\rho c)_f},\ \sigma = \frac{(\rho c)_m}{(\rho c)_f}$.

The nondimensionlized equations (after dropping the asterisks for simplicity) are:

$$\nabla.\mathbf{q}_D = 0, \tag{8}$$

$$\left(-\nabla\mathbf{p} - \mathbf{q}_D - Rmk\hat{} + RaTk\hat{} - Rn\phi k\hat{}\right) = 0 \tag{9}$$

$$\frac{\partial T}{\partial \tau} + \mathbf{q}_D.\nabla T = \nabla^2 T + \left[\frac{N_B}{Le}\nabla\phi.\nabla T + \frac{N_A N_B}{Le}\nabla T.\nabla T\right], \tag{10}$$

$$\frac{1}{\sigma}\frac{\partial \phi}{\partial \tau} + \frac{1}{\varepsilon}\mathbf{q}_D.\nabla\phi = \frac{1}{Le}\nabla^2\phi + \frac{N_A}{Le}\nabla^2 T, \tag{11}$$

$$w = 0,\ T = 1,\ \frac{\partial \phi}{\partial z} + N_A\frac{\partial T}{\partial z} = 0 \text{ at } z = 0 \tag{12}$$

$$w = 0,\ T = 0,\ \frac{\partial \phi}{\partial z} + N_A\frac{\partial T}{\partial z} = 0 \text{ at } z = 1. \tag{13}$$

The nondimensional parameters, which appeared in the above equations are defined as follows:

$$Le = \frac{\alpha_m}{D_B} \tag{14}$$

is the Lewis number,

$$Ra = \frac{\rho g \beta K d\left(T_h - T_c\right)}{\mu\alpha_m} \tag{15}$$

is the thermal Darcy Rayleigh number,

$$Rm = \frac{\left[\rho_p\phi_0 + \rho\left(1 - \phi_0\right)\right]gKd}{\mu\alpha_m} \tag{16}$$

is the basic density Rayleigh number,

$$Rn = \frac{\left(\rho_p - \rho\right)\phi_0 gKd}{\mu\alpha_m} \tag{17}$$

is the concentration Rayleigh number,

$$N_A = \frac{D_T\left(T_h - T_c\right)}{D_B T_c \phi_0} \tag{18}$$

is the modified diffusivity ratio

$$N_B = \frac{\varepsilon(\rho c)_p}{(\rho c)_f}\phi_0 \tag{19}$$

is the modified particle density increment.

BASIC SOLUTION

The basic state of the nanofluid is assumed to be quiescent thus, temperature field and nanoparticle volume fraction vary in the z-direction only. This gives the solution of the form

$$u = v = w = 0,\ T = T_b(z),\ \phi = \phi_b(z), \tag{20}$$

which satisfy the following equations

$$\frac{d^2 T_b}{dz^2} + \frac{N_B}{Le}\frac{d\phi_b}{dz}\frac{dT_b}{dz} + \frac{N_A N_B}{Le}\left(\frac{dT_b}{dz}\right)^2 = 0 \tag{21}$$

$$\frac{d^2\phi_b}{dz^2} + N_A\frac{d^2 T_b}{dz^2} = 0 \tag{22}$$

Using the boundary conditions (12-13), Eq. (22) may be integrated to give

$$\frac{d\phi_b}{dz} + N_A\frac{dT_b}{dz} = 0. \tag{23}$$

Using the Eq. (23) in the Eq. (21), we get

$$\frac{d^2 T_b}{dz^2} = 0. \tag{24}$$

The solution of the Eq. (24), subject to the boundary conditions (Eq. 12-13), is given by

$$T_b = 1 - z, \tag{25}$$

also the Eq. (22) has been solved subjected to the boundary conditions (12-13) using (25), we get

$$\phi_b = \phi_0 + N_A z. \tag{26}$$

PERTURBATION STATE

Introducing the stream function ψ, eliminating the pressure term and then imposing finite amplitude perturbations on the basic quiescent state as

$$\psi = \Psi,\ T = 1 - z + \Theta \text{ and } \phi = \phi_0 + N_A z + \Phi, \tag{27}$$

we get the following set of equations:

$$\nabla_1^2 \Psi = -Ra\frac{\partial \Theta}{\partial x} + Rn\frac{\partial \Phi}{\partial x}, \tag{28}$$

$$\frac{\partial \Psi}{\partial x} + \left(\frac{\partial}{\partial \tau} - \nabla^2 + \frac{N_A N_B}{Le}\frac{\partial}{\partial z}\right)\Theta + \frac{N_B}{Le}\frac{\partial \Phi}{\partial z} = \frac{\partial(\Psi,\Theta)}{\partial(x,z)} + \left[\frac{N_B}{Le}\nabla\Phi.\nabla\Theta + \frac{N_A N_B}{Le}\nabla\Theta.\nabla\Theta\right], \tag{29}$$

$$-\frac{N_A}{\varepsilon}\frac{\partial \Psi}{\partial x} - \frac{N_A}{Le}\nabla^2\Theta + \left(\frac{1}{\sigma}\frac{\partial}{\partial \tau} - \frac{1}{Le}\nabla^2\right)\Phi = \frac{1}{\varepsilon}\frac{\partial(\Psi,\Phi)}{\partial(x,z)}. \tag{30}$$

Boundary conditions to solve Eqs. (28-30) are

$$\Psi = 0,\ \Theta = 0,\ \frac{\partial \Phi}{\partial z} + N_A\frac{\partial \Theta}{\partial z} = 0 \text{ at } z = 0,1. \tag{31}$$

We now introduce the following asymptotic expansion

$$Ra = Ra_{0,c} + \chi^2 Ra_2 + \chi^4 Ra_4 + ..., \tag{32}$$

$$\Psi = \chi\Psi_1 + \chi^2\Psi_2 + \chi^3\Psi_3 + ..., \tag{33}$$

$$\Theta = \chi\Theta_1 + \chi^2\Theta_2 + \chi^3\Theta_3 + ..., \tag{34}$$

$$\Phi = \chi\Phi_1 + \chi^2\Phi_2 + \chi^3\Phi_3 + ..., \tag{35}$$

where $Ra_{0,c}$ is the critical value of the Rayleigh number at which the onset of convection takes place.

We now assume the variation of time only at the slow time scale $t = \chi^2\tau$, and arranging the systems at different order of χ. At the lowest order, we have

$$\begin{pmatrix} -\nabla_1^2 & Ra_{0;c}\frac{\partial}{\partial x} & -Rn\frac{\partial}{\partial x} \\ \frac{\partial}{\partial x} & \left(-\nabla^2 + \frac{N_A N_B}{Le}\frac{\partial}{\partial z}\right) & \frac{N_B}{Le}\frac{\partial}{\partial z}\nabla^2 \\ -\frac{N_A}{\varepsilon}\frac{\partial}{\partial x} & -\frac{N_A}{Le}\nabla^2 & -\frac{1}{Le}\nabla^2 \end{pmatrix}\begin{pmatrix}\Psi_1 \\ \Theta_1 \\ \Phi_1\end{pmatrix} = 0. \tag{36}$$

solution at the lowest order subject to the boundary conditions (31), are assumed to be

$$\Psi_1 = A[t]\psi_F,\ \Theta_1 = B[t]\theta_F,\ \Phi_1 = C[t]\phi_F \tag{37}$$

where

$$\psi_F = Sin(k_c x)Sin(\pi z),\ \theta_F = Cos(k_c x)Sin(\pi z),\ \phi_F = -N_A Cos(k_c x)Sin(\pi z). \tag{38}$$

The critical value of the Rayleigh number and the corresponding wave number for the onset of stationary convection is calculated numerically and the expression for critical Rayleigh number is given by

$$Ra_{0,c} = \frac{\delta^4}{k_c^2} - N_A Rn\left(\frac{Le}{\varepsilon} + 1\right), \tag{39}$$

and the critical wave number is $k_c = \pi$, where $\delta^2 = k_c^2 + \pi^2$.

AMPLITUDE EQUATION

At the second order, we have

$$\begin{pmatrix} -\nabla_1^2 & Ra_{0;c}\frac{\partial}{\partial x} & -Rn\frac{\partial}{\partial x} \\ \frac{\partial}{\partial x} & \left(-\nabla^2 + \frac{N_A N_B}{Le}\frac{\partial}{\partial z}\right) & \frac{N_B}{Le}\frac{\partial}{\partial z}\nabla^2 \\ -\frac{N_A}{\varepsilon}\frac{\partial}{\partial x} & -\frac{N_A}{Le}\nabla^2 & -\frac{1}{Le}\nabla^2 \end{pmatrix}\begin{pmatrix}\Psi_2 \\ \Theta_2 \\ \Phi_2\end{pmatrix} = \begin{pmatrix}R_{21} \\ R_{22} \\ R_{23}\end{pmatrix}. \tag{40}$$

where

$$R_{21} = 0, \tag{41}$$

$$R_{22} = \frac{k_c^2\pi}{2\delta^2}A[t]^2 Sin(2\pi z) - \frac{\pi k_c^2 N_A N_B}{2\delta^4}(k_c^2 Sin^2(k_c x)Sin^2(\pi z) + \pi^2 \mathrm{Cos}^2(k_c x)\mathrm{Cos}^2(\pi z))A[t]^2 \tag{42}$$

$$R_{23} = \frac{\pi k_c^2 N_A}{2\varepsilon\delta^2}\left(\frac{Le}{\varepsilon} + 1\right)A[t]^2 Sin(2\pi z). \tag{43}$$

The second order solution subject to the boundary conditions (31), are assumed to be

$$\Psi_2 = 0,\ \ \Theta_2 = B_S[t]\theta_S,\ \ \Phi_2 = C_S[t]\phi_S \tag{44}$$

where

$$\theta_S = Sin(2\pi z),\ \phi_S = -N_A Sin(2\pi z). \tag{45}$$

The horizontally averaged thermal Nusselt number

and nanoconcentration Nusselt number, Nu and Nu_Φ, for stationary mode of convection (the mode considered in this problem) is given by:

$$Nu[t]=\frac{\left[\frac{k_c}{2\pi}\int_0^{\frac{2\pi}{k_c}}\left(1-z+\Theta_2\right)_z dx\right]_{z=0}}{\left[\frac{k_c}{2\pi}\int_0^{\frac{2\pi}{k_c}}(1-z)_z dx\right]_{z=0}}, \tag{46}$$

$$Nu_\Phi[t]=\frac{\left[\frac{k_c}{2\pi}\int_0^{\frac{2\pi}{k_c}}\left(\phi_0+N_A z+\phi_2\right)_z\ dx\right]_{z=0}}{\left[\frac{k_c}{2\pi}\int_0^{\frac{2\pi}{k_c}}\left(\phi_0+N_A z\right)_z\ dx\right]_{z=0}}, \tag{47}$$

Substituting expressions of Θ_2 and Φ_2 in the above Eqs. (46 and 47) and simplifying, we get

$$Nu[t]=1+\frac{k_c^2}{4\delta^2}(A[t])^2, \tag{48}$$

$$Nu_\Phi[t]=1+\left(\frac{k_c{}^2 Le}{4\delta^2}\left(\frac{1}{\varepsilon}\left(\frac{Le}{\varepsilon}+1\right)+\frac{1}{Le}\right)\right)(A[t])^2. \tag{49}$$

At the third order, we have

$$\begin{pmatrix} -\nabla_1^2 & Ra_{0;c}\frac{\partial}{\partial x} & -Rn\frac{\partial}{\partial x} \\ \frac{\partial}{\partial x} & \left(-\nabla^2+\frac{N_A N_B}{Le}\frac{\partial}{\partial z}\right) & \frac{N_B}{Le}\frac{\partial}{\partial z}\nabla^2 \\ -\frac{N_A}{\varepsilon}\frac{\partial}{\partial x} & -\frac{N_A}{Le}\nabla^2 & -\frac{1}{Le}\nabla^2 \end{pmatrix}\begin{pmatrix}\Psi_3\\ \Theta_3\\ \Phi_3\end{pmatrix}=\begin{pmatrix}R_{31}\\ R_{32}\\ R_{33}\end{pmatrix}. \tag{50}$$

where

$$R_{31}=-Ra_2\frac{\partial\Theta_1}{\partial x}, \tag{51}$$

$$R_{32}=\frac{\partial\Psi_1}{\partial x}\frac{\partial\Theta_2}{\partial z}+\frac{N_B}{Le}\left\{\frac{\partial\Phi_1}{\partial z}\frac{\partial\Theta_2}{\partial z}+\frac{\partial\Phi_2}{\partial z}\frac{\partial\Theta_1}{\partial z}\right\}+2\frac{N_A N_B}{Le}\left\{\frac{\partial\Phi_1}{\partial z}\right\}\frac{\partial\Theta_2}{\partial z}-\frac{\partial\Theta_1}{\partial t}, \tag{52}$$

$$R_{33}=\frac{1}{\varepsilon}\left\{\frac{\partial\Psi_1}{\partial x}\frac{\partial\Phi_2}{\partial z}\right\}-\frac{1}{\sigma}\frac{\partial\Phi_1}{\partial t}. \tag{53}$$

Substituting the value of Ψ_1, Θ_1, Θ_2, Φ_1 and Φ_2 in the above equations to get the expressions of .

Applying the solvability condition for the existence of third order solution, we get the non-autonomous Ginzburg-Landau equation with time periodic coefficients in the form

$$a_1\frac{dA'(t)}{dt}+a_2A[t]+a_3(A[t])^3=0 \tag{54}$$

where .

$$a_1=\frac{1}{\delta^4}\left(Ra_{0,c}+RnN_A\right)+\frac{1}{\sigma\delta^4}LeRnN_A\left(\frac{Le}{\varepsilon}+1\right),$$

$$a_2=-\frac{1}{\delta^2}Ra_2$$

$$a_3=\frac{k_c{}^2}{8\delta^4}\left(Ra_{0,c}+RnN_A\right)+\left(\frac{k_c{}^2N_ALe}{8\delta^4\varepsilon}\right)LeRn\left(\frac{1}{\varepsilon}\left(\frac{Le}{\varepsilon}+1\right)+\frac{1}{Le}\right).$$

The Ginzburg-Landau equation given by Eq (54) is a Bernoulli equation and to obtained its solution HAM method has been employed, subject to the initial condition $A[0]=a_0$, where a_0 is the chosen initial amplitude of convection. In our calculations, we may assume $Ra_2=Ra_{0,c}$ to keep the parameters to the minimum.

METHOD OF SOLUTION

Let us assume the following non-linear differential equation in the form of

$$N\left[A(t)\right]=0 \tag{55}$$

where N is a non-linear operator, t is an independent

variable and *A(t)* is the solution of equation. We define the function, $\varphi(t,p)$ as follows:

$$lim_{p\to 0}\varphi(t,p) = A_0(t) \tag{56}$$

where, p:[0,1] and is the initial guess which satisfies initial or boundary conditions and

$$lim_{p\to 1}\varphi(t,p) = A(t) \tag{57}$$

And by using the generalized homotopy method, Liao's so-called zero-order deformation Eq.(55) is

$$(1-p)L\left[\varphi(t,p) - A_0(t)\right] = phN\left[\varphi(t,p)\right] \tag{58}$$

where *h* is the auxiliary parameter which helps us to increase the convergence results, *L* is the linear operator. It should be noted that there is a great freedom to choose the auxiliary parameter *h*, the initial guess $A_0(t)$ and the auxiliary linear operator *L*. This freedom plays an important role in establishing the keystone of validity and flexibility of HAM as shown in this paper. Thus, when *p* increases from 0 to 1, the solution $\varphi(t,p)$ changes between the initial guess $A_0(t)$ and the solution *A(t)*. The Taylor series expansion of with respect to *p* is

$$\varphi(t,p) = A_0(t) + \sum_{m=i}^{+\infty} A_m(t)p^m \tag{59}$$

and

$$A_0^{[m]}(t) = \frac{\partial^m \varphi(t,p)}{\partial p^m}|_{p=0} \tag{60}$$

where $A_0^{[m]}(t)$ for briefly is called the m[th]-order of deformation derivation which reads

$$A_m(t) = \frac{A_0^{[m]}(t)}{m!} = \frac{1}{m!}\frac{\partial^m \varphi(t,p)}{\partial p^m}|_{p=0}. \tag{61}$$

Indeed, in HPM we solve the non-linear differential equation by separating any Taylor expansion term. Now, we define the vector of

$$\vec{A}_m = \{\vec{A}_1, \vec{A}_2, \vec{A}_3, ..., \vec{A}_n\}. \tag{62}$$

According to the definition in Eq. (61), the governing equation and corresponding initial conditions of $A_m(t)$ can be deduced from zero-order deformation Eq. (55). Differentiating Eq. (55) m times with respect to the embedding parameter *p* and setting *p=0* and finally dividing by m!,we will have the so called *m[th]*-order deformation equation in the form:

$$L\left[A_m(t) - \chi_m A_{m-1}(t)\right] = hR\left(A_{m-1}\right), \tag{63}$$

where $R\left(A_{m-1}\right) = \frac{1}{(m-1)!}\frac{\partial^{m-1} N[\varphi(t,p)]}{\partial p^{m-1}}|_{p=0}$

and

$$\chi_m = \begin{cases} 0 & m \le 1 \\ 1 & m > 1 \end{cases}$$

So by applying inverse linear operator to both sides of the linear equation, Eq.(55), we can easily solve the equation and compute the generation constant by applying the boundary condition.

APPLICATION

$$\frac{dA'(t)}{dt} = Q_1 A(t) - Q_2 A(t)^3 \tag{64}$$

Where $Q_1 = -\frac{a_2}{a_1}$ and $Q_2 = \frac{a_3}{a_1}$ according to nature of the GL equation, the initial solution may be taken in the form:

$$A_0(t) = c_1 + (a_0 - c_1)e^{\gamma t}, \tag{65}$$

where $c_1 = \sqrt{\frac{Q_1}{Q_2}}$ and γ is as yet unspecified. The determination of γ can and will be dealt with at the time of seeking a series solution of the GL equation with a time-periodic coefficient. Quite obviously $A_0(t)$ has been so chosen that it satisfies the conditions $A_0(\infty) = c_1$ and $A_0(0) = a_0$. The choice of the form of $A_0(t)$ is most important in obtaining a convergent series solution by the HAM. Now, we are introducing the two notations to obtain the series solution of the GL equation by HAM.

$$L[A(t)] = \frac{dA'(t)}{dt} + \gamma A(t) \tag{66}$$

$$N[A(t)] = \frac{dA'(t)}{dt} - Q_1 A(t) + Q_2 A(t)^3. \tag{67}$$

The required equation for $\varphi(t,p)$ can be constructed using L[A(t)] and N[A(t)] , and we also remind ourselves at this point that $\varphi(t,p)$ varies from $A_0(t)$ to $A_{NL}(t)$ as *p* varies

from 0 to 1. The required equation is as follows:

$$(1-p)L[\varphi(t,p)-A_0(t)]=phN[\varphi(t,p)], \qquad (68)$$

where h is a convergence-control parameter. Eq. (68) and $\varphi(t,0)=a_0$ are called the zeroth-order deformation equations. Now, in order to obtain the *p*-derivatives of $\varphi(t,p)$ we differentiate m-times the zeroth-order deformation equations with respect to *p*. To make use of the notation $A_m(t)$ defined in Eq. (61), we set *p=0* in the resulting equations, and also divide by *m!*. The above procedure results in the following infinite system of linear equations:

$$L[A_m(t)-\chi_m A_{m-1}(t)]=hR_m\left(\tilde{A}_m(t)\right) \qquad (69)$$

subject to the initial condition

$$A_m(0)=0,(m=1,2,3....) \qquad (70)$$

$$\tilde{A}_m(t)=\left(A_0(t),A_1(t),A_2(t),A_3(t),...,A_{m-1}(t)\right), \quad m\geq 0 \qquad (71)$$

and

$$R\left[\tilde{A}_{m-1}(t)\right]=\frac{dA'_{m-1}(t)}{dt}-Q_1A_{m-1}(t)+Q_2\sum_{k=0}^{m-1}A_{m-1-k}(t)\times \sum_{j=0}^{k}A_{k-j}(t)A_j(t), \qquad (m=1,2,3....) \qquad (72)$$

After some simplification, we get recurrence relation

$$A_m(t)=\chi_m A_{m-1}(t)+he^{-\gamma t}\int_0^t e^{\gamma\tau}R\left[\tilde{A}_{m-1}(\tau)\right]d\tau \qquad (73)$$

In fact, the solution of Eq.(73) may be obtained with the aid of Mathematica. Some iterative solutions are as follows.

$$A_1(t)=\frac{1}{2\gamma}e^{-3\gamma t}h\Big\{-(a_0-c_1)^3Q_2-6(a_0-c_1)^2c_1e^{\gamma t}Q_2+2c_1e^{3\gamma t}\left(-Q_1+c_1^2Q_2\right)+e^{2\gamma t}\left(2c_1Q_1+a_0^3Q_2+3c_1\left(a_0^2-3a_0c_1+c_1^2\right)Q_2-2(a_0-c_1)\gamma\left(\gamma+Q_1-3c\right)_1^2Q_2\right)t\Big\} \qquad (74)$$

Other iterative solutions are too long to be mentioned here, therefore, we demonstrate our result graphically.

To determine an appropriate *h*, we define a residual error in the form:

$$E_R(h)=\frac{1}{t_0}\int_0^{t_0}\left[\frac{dA'(t)}{dt}-Q_1A(t)+Q_2A(t)^3\right]^2dt \qquad (75)$$

where t_0 is time domain in which we want to capture the error. We have to choose the value of *h* in such a way that error is going to minimum. Here, it is noticed that *h* is a helpful parameter that influences the rate of convergence of the HAM solution but the convergent solution is independent of the choice of *h* as proved by Liao (2009).

m^{th}-order HAM solution can be written in the following form

$$A_{NL}^m(t)=\sum_{i=0}^{m}A_m(t),m=1,2,3..... \qquad (76)$$

RESULTS AND DISCUSSION

We perform a weak nonlinear analysis of heat transfer for nanofluid in a closely packed anisotropic porous media by considering the Darcy model. The effects of Lewis number, modified thermophoresis to Brownian-motion diffusivity ratio, concentration Rayleigh number porosity and specific heat ratio on the heat transport have been studied. Using power series expansion in terms of perturbation parameter, which is assumed to be small, the problem has been studied using the Ginzburg-Landau amplitude equation. Such an assumption will help us in obtaining the amplitude equation of convection in a rather simple and elegant manner and is much easier to obtain than in the case of the Lorenz model.

From the expression of *Rn*, it is observed that *Rn* is defined as a typical nanofluid fraction instead of the difference of two fractions so that, *Rn* cannot be negative, the modified diffusion ratio is positive. Also, it is not necessary to take large values of *Le* as mentioned by Nield and Bejan (2009). As there are no two opposing agencies which affect the instability, therefore, the oscillatory instability is not possible. One can observe that the solution of first order, second order, third order and the Ginzburg - Landau equation is independent of modified particle-density increment , therefore, heat transfer is unaffected by modified particle-density increment N_B, this happens due to orthogonality of the solution of the trial functions.

If one wants to quantify heat and mass transfer, which linear stability analysis is unable to do, the problem needs to perform a nonlinear analysis, thus the need for nonlinear stability analysis is justified.

It is difficult to control the nanoparticle fraction at the boundaries, therefore, we consider new set of boundary conditions by assuming that the normal component of the nanoparticle flux on boundaries is zero, such an assumption can be taken as more realistic and suits the real world problem. It is important to study the effect of nanoparticle concentration and modified diffusion ratio in nanofluid convection in porous media. The objective of this article is to consider nanoparticle concentration and modified diffusion ratio for either enhancing or inhibiting convective heat transport as is required by the real application.

For the critical wave number, the Eq. (39) takes the form

$$Ra_{0,c} + N_A Rn\left(\frac{Le}{\varepsilon}+1\right) = 4\pi^2. \qquad (77)$$

The Eq. (77) can be taken as a useful upper bound for the value of critical Rayleigh number in case of stationary convection.

Figs. (1-10) show the effect of parameters on the heat transport, initially the value of Nusselt number is one which shows that the heat transfer is by conduction alone, and as time increases, the value of Nusselt number increases showing that heat transport is being effected by convection. Furthermore, after reaching a fixed value, there is no change in magnitude of Nusselt number with respect to time, showing the saturation state for heat transfer. Figs. (1-5) shows the effect of parameters on the heat transport for the base fluid, while Figs. (6-10) shows the effect of parameters on the heat transport for the nanoparticles. We keep our parameters to the fixed value of Rn=1.0, ε=0.6, $N_B = 1$, $N_A = 1$ and Le=10, except for the varying one.

From Fig. (1), we observe that for increasing values of concentration Rayleigh number, heat transfer decreases, Fig. (2) shows the effect of porosity that for increasing values of porosity, heat transfer increases; moreover, we take the value of porosity near to one as some manmade artificial porous matrix has porosity nearly one. From Fig. (3), we observe that the effect of specific heat ratio is very frail and appeared only for short time. Fig. (4) shows the effect of modified thermophoresis to Brownian-motion diffusivity ratio, it is observed that for its increasing values heat transfer decreases. The effect of Lewis number is shown in Fig. (5), and it is found that for increasing value of Lewis number, heat transfer decreases.

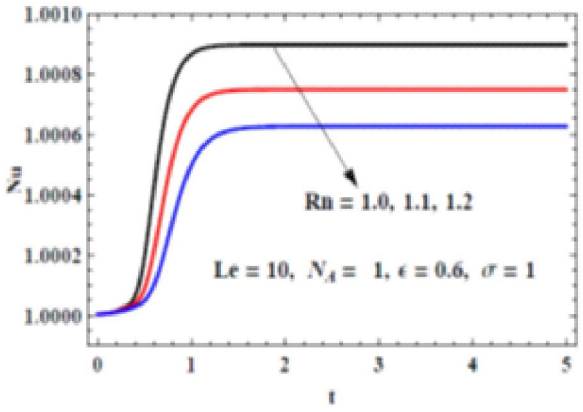

Fig. 1: Variation of Nusselt number with time for the different values of Rn

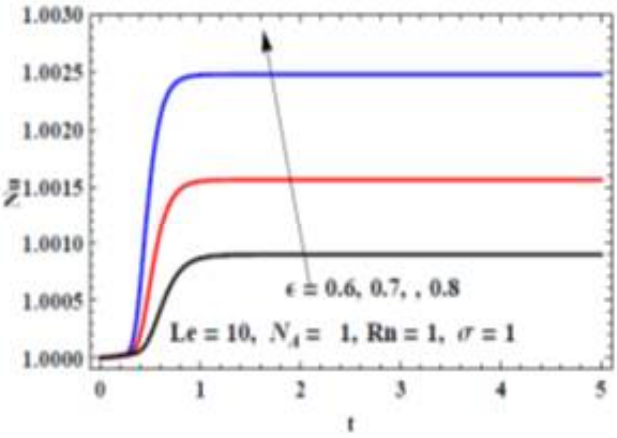

Fig. 2: Variation of Nusselt number with time for the different values of ε

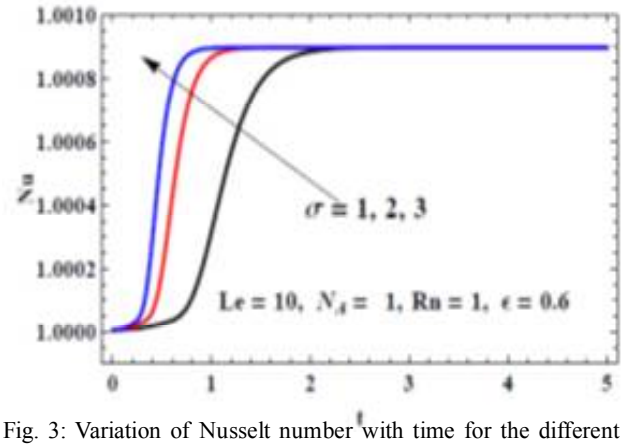

Fig. 3: Variation of Nusselt number with time for the different values of σ

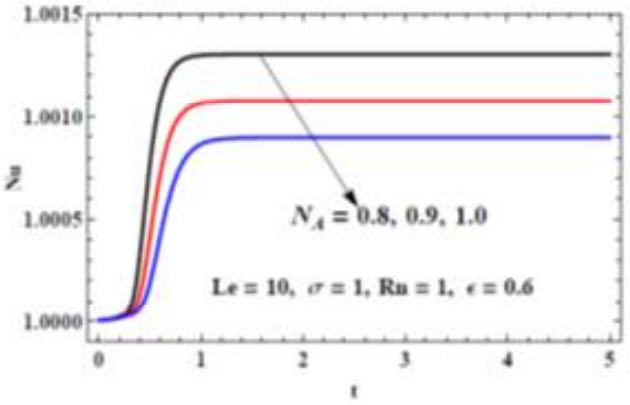

Fig. 4: Variation of Nusselt number with time for the different values of N_A

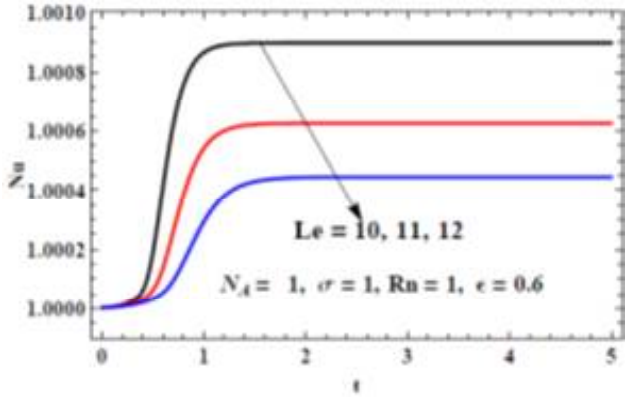

Fig. 5: Variation of Nusselt number with time for the different values of Le

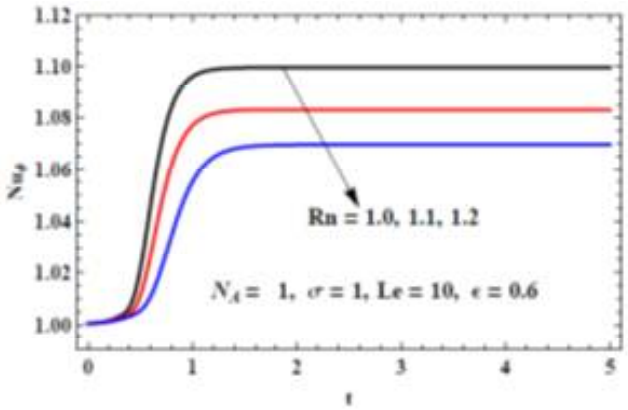

Fig. 6: Variation of nanoparticle concentration Nusselt number with time for the different values of Rn

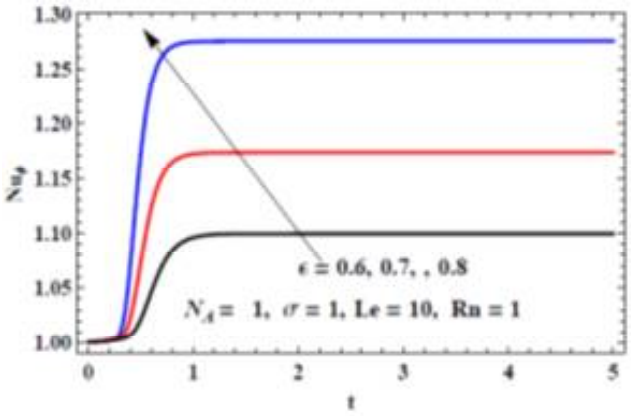

Fig. 7: Variation of nanoparticle concentration Nusselt number with time for the different values of ε

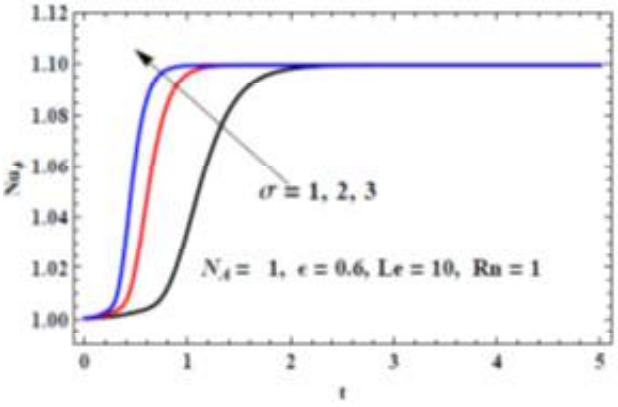

Fig. 8: Variation of nanoparticle concentration Nusselt number with time for the different values of σ

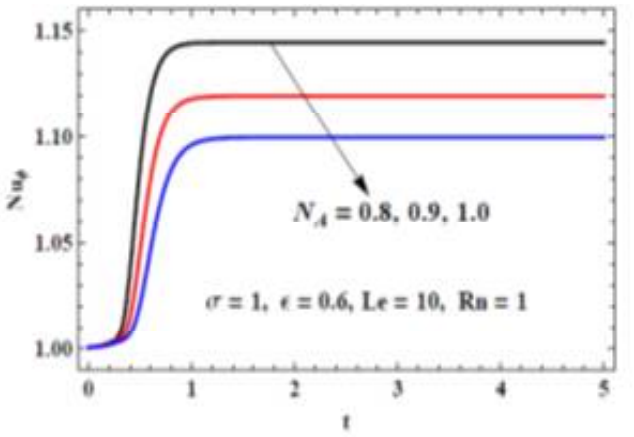

Fig. 9: Variation of nanoparticle concentration Nusselt number with time for the different values of N_A

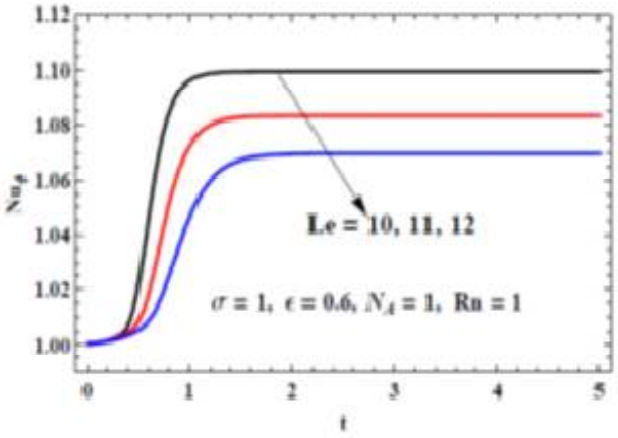

Fig.10: Variation of nanoparticle concentration Nusselt number with time for the different values of Le

From Fig. (6), it is observed that for increasing values of concentration Rayleigh number, heat transfer decreases. Fig. (7) reveals the effect of porosity that for increasing values of porosity, heat transfer increases. From the Fig. (8), we find that the effect of specific heat ratio is very frail and appeared only for short time. From Fig. (9), it is clear that the effect of modified thermophoresis to Brownian-motion diffusivity ratio decreases heat transfer for its increasing values. Fig. (10) shows the effect of Lewis number that increasing value of Lewis number decreases heat transfer.

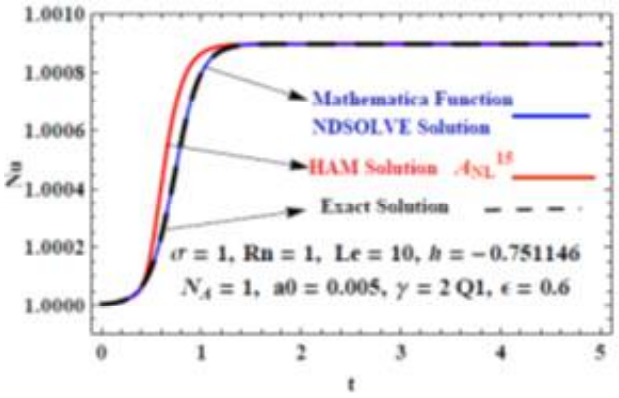

Fig. 11: Comparison between various solutions for Nu verses t

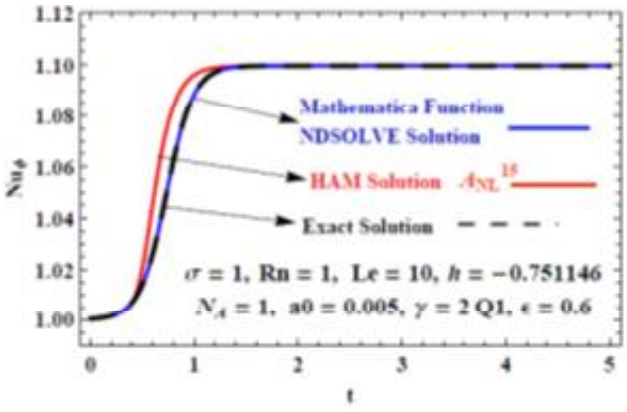

Fig. 12 : Comparison between various solutions for Nu_ϕ verses t

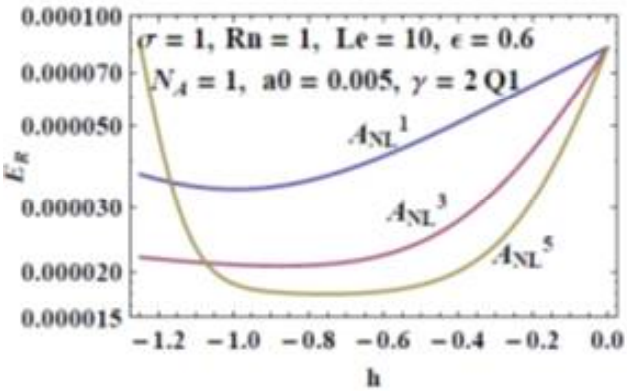

Fig. 13 : Curve of residual error E_R versus convergence controlling parameter h

Following results have been found from Figs. (1, 2, 4, 5) for the heat transport

1. $[Nu]_{Rn=1.2} < [Nu]_{Rn=1.1} < [Nu]_{Rn=1.2}$
2. $[Nu]_{\varepsilon=0.6} < [Nu]_{\varepsilon=0.7} < [Nu]_{\varepsilon=0.8}$
3. $[Nu]_{N_A=1.0} < [Nu]_{N_A=0.9} < [Nu]_{N_A=0.8}$
4. $[Nu]_{Le=12} < [Nu]_{Le=11} < [Nu]_{Le=10}$

Further, from Fig. (3), we find that the effect of σ on Nu[t] is felt only for short times.

Following results have been found from Figs. (6, 7, 9, 10) for nanoparticle transport

1. $[Nu_\Phi]_{Rn=1.2} < [Nu_\Phi]_{Rn=1.1} < [Nu_\Phi]_{Rn=1.0}$
2. $[Nu_\Phi]_{\varepsilon=0.6} < [Nu_\Phi]_{\varepsilon=0.7} < [Nu_\Phi]_{\varepsilon=0.8}$
3. $[Nu_\Phi]_{N_A=1.0} < [Nu_\Phi]_{N_A=0.9} < [Nu_\Phi]_{N_A=0.8}$
4. $[Nu_\Phi]_{Le=12} < [Nu_\Phi]_{Le=11} < [Nu_\Phi]_{Le=10}$

Further, from Fig. (8), it is found that the effect of σ on $Nu_{\Phi}[\tau]$ is felt only at short times.

Comparison of exact and Mathematica solutions with the fifteen-order HAM solution is depicted in Fig (11) and Fig (12). Fig (11) exhibits the plot of *Nu* verses *t*, on the other hand Fig (12) shows the plot of Nu_{ϕ} verses t. It is evident that the convergent solution (for $h=-0.751146$, $\gamma = 2Q_1$) of the fifteen-order HAM solution for a fixed values of parameters is very close to the exact solution.

When , increasing the order results in increasing the residual error, thus the series is divergent. Besides, choosing any value of h in the region $-1.25 < h < -1$ results in divergent series. However, choosing any value of in the region $-0.80 < h < 0$ results in convergent series. Obviously, there exists such a region $h_B \le h < 0$ where h_B is a constant, that choosing any value of *h* in this region results in convergent HAM series. It is unnecessary to determine the exact value h_B of the boundary. For example, from Fig (13), it is obvious that the HAM series converges by choosing any value of h in the region $-0.80 < h < 0$. Besides, as proved by Liao (2009), all of these convergent HAM series converge to the same result for given physical parameters, although the convergence rate depends upon the chosen value of *h*.

Variation of stream lines, isotherms, isoconcentrations at different instant of time are shown graphically in Figs. (14-16). From Fig (14), it is clear that the magnitudes of stream lines increases as time increases. Fig. (15) shows the variation of isotherms at different instant of time, it is found that initially the isotherms are flat and parallel showing the heat transport is only by conduction alone, and as time increases isotherms starts oscillating showing convective regime is in place. Fig (16) shows that initially the isoconcentrations are flat and parallel showing that the transfer of nanoparticle concentration is by conduction only, and as time increases isoconcentrations starts oscillation and form contours which shows that the convective regime has become stronger. Moreover, it is clear from the Fig. (14-16) that after certain instant there is no changes in stream lines, isotherms, isoconcentrations.

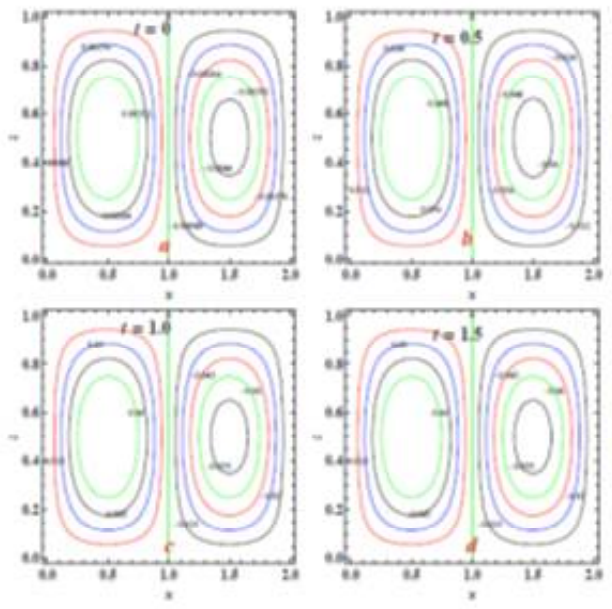

Fig -14: Streamlines at (a) *t* = 0.0, (b) *t* = 0.5, (c) *t* = 1.0, (d) *t* = 1.5

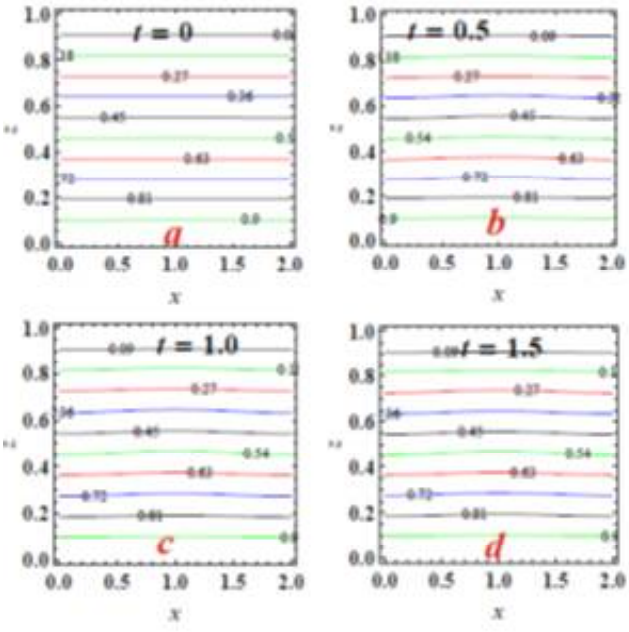

Fig -15: Isotherms at (a) *t* = 0.0, (b) *t* = 0.5, (c) *t* = 1.0, (d) *t* = 1.5

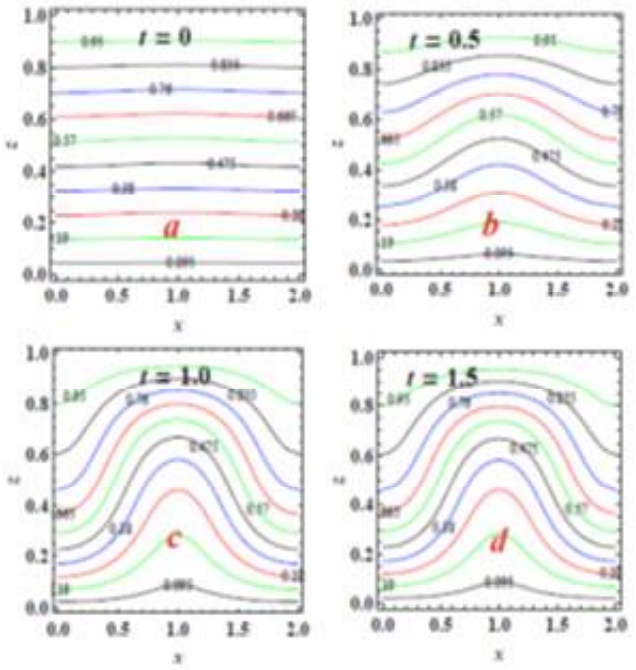

Fig -16: Isohalines at (a) t = 0.0, (b) t = 0.5, (c) t = 1.0, (d) t = 1.5

CONCLUSIONS

We investigated weak nonlinear effect using the Ginzburg-Landau equation for nanofluid convection in an infinite horizontal porous layer which is heated from below. Effects of concentration Rayleigh number, modified thermophoresis to Brownian-motion diffusivity ratio, Lewis number, porosity, specific heat ratio on the heat and nanomass transfer have been studied. Following conclusions have been made from our analyses, for the increasing values of parameters:

1. Concentration Rayleigh number parameter *Rn* : heat and nanomass transfer decrease.
2. Lewis number *Le* : heat transfer decreases, while mass transfer increases.
3. Porosity ε : heat and nanomass transfer decrease.
4. Specific heat ratio σ : Effect is felt only for short time.
5. Modified thermophoresis to Brownian-motion diffusivity ratio N_A : heat and nanomass transfer decrease.

ACKNOWLEDGMENT

Author Alok Srivastava gratefully acknowledges the financial assistance from N.B.H.M. (D.A.E) as a Post Doctoral fellowship and the necessary facility provided by Department of Mathematics, Institute of Science, Banaras Hindu University, Varanasi. Author Vineet Kumar also gratefully acknowledges the financial assistance from UGC, New Delhi as a junior research fellowship (Award Letter â" F 17-1/2006 (SA-1)).

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RESEARCH ARTICLE

International Journal of Science, Technology and Society
Vol 3, No. 2 (2017) : 54-61

Application of *Rhizobium-Pseudomonas* consortia for enhanced production of mungbean in sustainable manner

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Publication Info

Article history:
Received : 11.09.2017
Accepted : 12.12.2017
DOI: https://doi.org/10.18091/ijsts.v3i02.11406

Key words:
Rhizobia, *Pseudomonas*, PGPR, Co-inoculation, mungbean

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ABSTRACT

In this study plant growth promoting rhizobacteria (PGPR) were isolated from root nodules of *Vigna radiata* and rhizospheric soil of *Abrus precatorius* respectively. On the basis of morphological, biochemical and physiological characterization, isolate R5 (from root nodules of *V. radiata*) was identified as *Rhizobium* and P21 (from rhizosphere of *A. precatorius*) as *Pseudomonas*. Isolates R5 and P21 showed positive results for phosphate solubilization, zinc solubilization, nitrogen fixation, production of indole acetic acid (IAA), siderophore and exopolysaccharides (EPS). Isolates (R5 and P21) were applied as coinoculant on mungbean (*V. radiata*) to check their impact on its growth in laboratory conditions and pot trials. Seed germination rate and growth analysis of plants were observed by measuring growth parameters (root length, shoot length, leaves count, root nodule count, fresh weight, dry weight and chlorophyll content). It was observed that R5 and P21 significantly enhanced the plant growth in comparison to control (without treatment), but consortial inoculation was more effective in comparison to mono-inoculation of R5 and P21.

INTRODUCTION

Leguminosae is economically and ecologically very important family of Kingdom Plantae (Harborne, 1994). Legumes are the second most important food crops for world agriculture (ILDIS, 2006). Mungbean is a significant pulse crop with high nutritive value and used as a world's major source of food (Yadav *et al.,* 2014). India is the largest producer and consumer of mungbean and accounts for about 65% of the world acreage and 54% of the world production of this crop (Lambrides *et al*., 2007; Sehrawat *et al.*, 2014). Although mungbean is required on large scale but due to some agro-ecological conditions, the nodulation of mungbean is very poor, which causes lower yields (Ahmed *et al.,* 2006). The excessive uses of chemical fertilizers are used for higher yield, but this approach has several negative implications on our environment (Arora *et al*., 2012; Hosseini *et al.,* 2014). The application of PGPR as biofertilizers causes a beneficial and cost effective strategy in growth enhancement (Mayak *et al.,* 2004; Mishra and Arora, 2016; Arora *et al*., 2017).

PGPR impart a great agronomic importance and influence plant development directly by producing metabolites such as plant growth regulators (hormones and other metabolites), siderophore production, phosphate solubilisation and symbiotic nitrogen (N_2) fixation and indirectly through modification to the activity of other plant-microbe interactions or by inducing changes in the microbial population balance, for instance, exerting biological control against plant pathogens (Tewari and Arora, 2013; Ahemad and Kibret, 2014; Vejan *et al*., 2016; Mishra *et al.,* 2017). The root nodulating bacteria (rhizobia) are well known for their symbiotic association with legumes mainly for biological nitrogen fixation (BNF) (Sessitsch *et al.,* 2002) and used for sustainable crop production (Laranjo *et al.,* 2014; Gopalakrishnan *et al.,* 2015; Arora *et al.,* 2017). One of the most promising groups of bacteria amongst PGPR are *Pseudomonas,* which also have various beneficial plant growth promoting traits (Moeinzadeh *et al.,* 2010; Mayz *et al.,* 2013; Tewari and Arora 2016; Mishra *et al*., 2017). Currently applications of various PGPR as co-inoculant are growing as a very beneficial trend in sustainable agriculture for higher crop yield.

Co-inoculation of PGPR especially *Pseudomonas* with *Rhizobium* is visualized as an important practice in the development of sustainable agriculture (Singh *et al.,* 2013; Arora *et al*., 2014). Co-inoculation improves plant growth by affecting some physiological functions such as by reduction in ethylene level (Shaharoona *et al.,* 2006), direct

stimulation of rhizobial growth/survival in the soil, enlargement of the root system by hormone production for enhanced nutrient uptake and increase in the number of potential colonization sites by rhizobia (Gull *et al.,* 2004; Barea *et al.,* 2005). This type of co-inoculation is reported to enhance nodulation and N fixation, plant biomass and grain yield in various leguminous crops such as alfalfa (Knight *et al.,* 1988), soybean (Dashti *et al.,* 1997), pea (Kumar *et al.,* 2001), chickpea (Verma *et al.,* 2010), white clover (Arora *et al*., 2008), galega (Egamberdieva *et al*., 2010), lentil (Caamano *et al.,* 2018) and common bean (Korir *et al*., 2017). The seed inoculation with the appropriate PGPR are recommended for higher crop yield since long time, but recently the co-inoculation of N_2 fixing rhizobia and PGPR with diverse characters is emerging as a popular approach rather than inoculating a single organism (Khanna *et al.,* 2011). The aim of this study was to check the plant growth promoting activities of bacterial isolates (*Rhizobium* and *Pseudomonas*) and their co-application as consortia to enhance the growth of mungbean.

MATERIALS AND METHODS

1) Sample collection and isolation of bacteria:

The intact root system bearing nodules of *V. radiata* plant were uprooted carefully and after cutting off the shoot portion plants were aseptically placed in plastic/ polypropylene bags, and immediately brought to laboratory for isolation of rhizobia. Rhizospheric soil of *A. precatorius* plant was collected and used for isolation of PGPR. Sites for sample collection was within the campus of Babasaheb Bhimrao Ambedkar University, Lucknow (26.8467° N, 80.9462° E). Rhizobial isolate R5 was isolated and grown on yeast extract mannitol agar (YEMA) media (Hi Media, Mumbai) (Vincent, 1970). Isolate was also preserved on YEMA slants and in 25% glycerol stock solution at -80 ºC. Isolate P21 from rhizospheric soil was isolated and grown on King's B agar media (King *et al.,* 1954) (Hi Media, Mumbai). Colonies were purified and preserved on King's B agar slants and in 25% glycerol stock solution at -80 ºC.

2) Characterizations of isolates

The bacterial isolates (R5 and P21) were characterized by various morphological, biochemical and physiological tests according to Bergey's Manual of Systemic Bacteriology (Garrity *et al*., 2005). Both the isolates were checked for nodulation on mungbean by tube assay.

3) Plant growth promoting characteristics of isolates:

Plant growth promoting potential of isolates was determined by various tests:

a) Phosphate solubilisation

This assay was done on plates containing Pikovskaya's agar media by spot inoculation method (Pikovskaya, 1948). After inoculation plates were incubated at 28 ˚C for 48 hrs and observed for halo formation around the bacterial colonies.

b) Zinc (Zn) solubilzation

For this, Zn solubilising basal agar medium containing 0.1% insoluble zinc sources such as zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc phosphate ($Zn_3(PO4)_2$) was prepared and spot inoculated with bacterial culture (Fasim *et al.,* 2002). After inoculation plates were incubated at 28±1ºC for 3 days and observed for the formation of clear zone around the colonies.

c) Siderophore production

The detection of siderophore production was done on chrome-azurol sulfonate (CAS) agar media (Schywn and Neilands, 1987). In this assay CAS agar media plate was spot inoculated with culture, incubated at 28 ºC for 4-5 days and observed for the formation of orange coloured zone around the colonies which indicated the positive result for siderophore production. Siderophore production of isolates was also estimated according to the modified microplate method given by Arora and Verma (2017).

d) IAA production

IAA production by bacterial culture was determined by the process of Brick *et al.,* (1991). In this assay tryptophan (0.1%) supplemented broth was prepared and inoculated with bacterial culture followed by incubation at 28±2ºC for 5 days. After incubation culture were centrifuged and 1 ml of culture supernatant was mixed with 4 ml of Salkowski's reagent (50 ml 35% perchloric acid + 1 ml 0.5 M ferric chloride solution) and 2 drops of ortho-phosphoric acid and incubated for 20 minutes at room temperature. After incubation development of pink colour showed the positive result.

e) EPS production

EPS production was monitored by chilled ethanol precipitation method (Hong *et al.,* 2002). YEM broth media was prepared, inoculated with a loop full of culture and incubated for 4-5 days. After incubation broth was

centrifuged at 8000 rpm at 4°C for 10 minutes. Once the cells get separated the culture supernatant was used for extraction of EPS. EPS was precipitated when supernatant was mixed with double volume of chilled 96% ethanol (2:1).

f) Nitrogen fixing activity

Glucose nitrogen free minimal medium (GNFMM) with BTB (0.0025%) as colour indicator was used for this assay (Nakbanpote *et al.,* 2013). GNFMM plate was inoculated with bacterial culture, incubated for 3-7 days at 30ºC and observed the colour change from green to blue around the colonies which indicated the nitrogen fixing ability of isolates.

4) Compatibility test between isolates

Before co-inoculation compatibility between both the isolates was checked by cross streak assay on nutrient agar media plate. In this assay both isolates R5 and P21 were streaked together in form of a single line on nutrient agar plates, incubated for 48 hrs at room temperature and clear zone (if any) along the streaked line was observed at the interaction of the streaks (Anandaraj and Delapierre, 2010).

5) Application of isolates on mungbean

The isolates (R5 and P21) were applied on mungbean as test crop and observed for their impact on plant growth both *in vitro* and *in vivo* conditions. Before bacterial inoculation seeds of mungbean were surface sterilized with 70% ethanol (2 min) and 2% sodium hypochlorite (10 min) followed by 7-8 times washings with sterilized distilled water. Surface sterilized seeds were inoculated with R5 and P21 as following treatments: i) uninoculated seeds as control (T1), ii) treatment with R5 (T2), iii) treatment with P21 (T2) and iv) treatment with both R5and P21 (T4). For bacterial treatments broth of R5 (48 hrs old culture) and P21 (24 hrs old culture) were taken and suspended in 1.0% sterilized carboxymethylcellulose (CMC) solution (Weller and Cook, 1983). For consortial inoculation bacterial culture were equally mixed (1:1) and added to the seeds (Vidhyasekaran and Muthamilan, 1999). Seeds were kept for overnight and air dried before sowing. All the treatments were applied in three replicates. Treated seeds were allowed to germinate on plain water agar media (0.8 %) in glass tubes under laboratory conditions. In each tube 3 seeds were placed. After 2-3 days germination of seeds were observed and recorded. Treated seeds were also planted (5 in each pot) in medium sized plastic pots (24x12x12cm) filled with sterilized soils. The physical properties of experimental soil were also examined (Chapman and Pratt, 1961). The soil characteristics were: pH-8.2, electrical conductivity-1.5ds/m, water holding capacity-55%, organic carbon-0.34%, total nitrogen-973.45Kg/H, total phosphate-11.23Kg/H, soluble potassium-210.34Kg/H and organic matter-0.61%. In laboratory conditions plants growth were observed after 15 days, while in pot trial after 30 days of sowing (DAS). Plants were carefully removed and various growth parameters were recorded such as root length, shoot length, leaves count, root nodule count, fresh weight and dry weight. Chlorophyll content was also analyzed according to Arnon, (1949) by using fresh leaf tissue.

8) Statistical analysis of growth parameters

All the data of plant growth parameters were analysed statistically by analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5% level to compare difference between treatment means (Gomez and Gomez 1984). Statistical analysis was done by software statistical package for the social science (SPSS) (2016) for windows.

RESULTS AND DISCUSSION:

1) Isolation and characterizations of isolates

In this study bacterial isolates R5 and P21 were used for coinoculation study. R5 was isolated from root nodules of *V. radiata* plant which formed white mucilaginous colonies on YEMA plate; while P21 from rhizospheric soil of *A. precatorius* formed green fluorescent colonies on King's B medium. R5 formed root nodules on its host *V. radiata.* On the basis of morphological, biochemical and physiological characterizations R5 was identified as *Rhizobium* (Holt *et al.,* 1994) and P21 as *Pseudomonas* (Garrity *et al.,* 2005). The results of biochemical and physiological nature of isolates are mentioned in Tables 1 & 2. Isolate P21 showed fluorescence on King's B medium, was Gram-negative, motile, rod shaped and positive for oxidase and catalase (Garrity *et al.,* 2005). R5 showed distinguishing characters of rhizobia, (Tables 1 and 2) (Deshwal and Chaubey, 2014).

2) Plant Growth Promoting characters of isolates

Both isolates R5 and P21 were observed for various plant growth promoting characters (Table 3). Both isolates were able to solubilize phosphate and zinc showing clear zones around the colonies on Pikovaskya agar plate and zinc supplemented media plates. Results indicated that P21 was more potent for phosphate solubilization while R5 for zinc solubilization. R5 and P21 were able to produce IAA and EPS but R5 was better than P21 for both the attributes. R5 and P21 were able to produce siderophore and P21 was more efficient (siderophore producer) in comparison to R5. Nitrogen fixing ability of isolates was also checked and only R5 was able to fix nitrogen. Rhizobia are very well known for

Table 1. Morphological and biochemical characterization of bacterial isolates

Characteristic	R5	P21
Gram staining	-	-
Shape	Rod	Rod
Colour	White	Greenish yellow
Fluorescent green pigment	-	+
Motility	+	+
Citrate	+	-
Urease	-	-
Indole	-	-
MR	+	+
VP	-	-
Catalase	++	+
Amylase	+	-
Protease	-	-
Lipase	-	-
Cellulase	-	++

(-) = negative for the test, (+) = positive for the test (++) = more positive for the test

MR = Methyl red, VP = Voges Proskauer

their outstanding property of BNF (Lupwayi *et al.,* 2004). *Rhizobium* and *Pseudomonas* are well known PGPR and show various plant growth promoting activities such as production of phytohormones, phosphate solubilizing activity and siderophore production (Deshwal *et al.,* 2011; Tewari and Arora 2014; Gopalakrishnan *et al*., 2015) and are used in bioformulations.

Table 2. Carbon and nitrogen source utilization pattern of the isolates

Carbon sources	Isolates		Nitrogen sources	Isolates	
	P21	R5		P21	R5
Mannitol	+	+	Yeast extract	+	+
Glucose	+	+	KNO_3	+	+
Dextrose	+	+	$NaNO_3$	+	+
Lactose	+	+	NH_4Cl	+	+
Galactose	+	+	NH_4F	-	-
Sucrose	+	+	NH_4So_4	+	+
Maltose	+	+	Glycine	-	-

(-) = negative growth, **(+)** = positive growth

Fig. 1. Effect of bacterial isolates and their consortia on growth of mungbean

3) Compatibility test between isolates:

It was observed that isolates R5 and P21 were compatible with each other and neither of the isolates inhibited each other's growth. Earlier studies have suggested that PGPR strains can be antagonistic or synergistic to each other and it is important to check the compatibility before using them as consortia (Anandaraj and Delapierre, 2010; Singh *et al.*, 2013; Santiago *et al.*, 2017). The compatibility of strains is very crucial for formulating bioinoculants with more than one strain.

4) Application of isolates on mungbean:

R5 and P21 were applied on mungbean alone and in combination. Although R5 (*Rhizobium*) and P21 (*Pseudomonas*) both caused the significant increase in plant growth parameters but co-inoculant (R5 and P21) gave best results (Figure 1). Co-inoculation resulted in significant

Table 3. Plant growth promoting characterization of bacterial isolates

S. No.	PGP activity	R5	P21
1.	Phosphate solubilisation	+	+++
2.	IAA production	+++	+
3.	Siderophore production	+	++
4.	Zinc solubilisation	++	+
5.	EPS production	++	+
6.	Nitrogenase assay	++	-

(+) Positive, (++) Good positive, (+++) Excellent positive, (-) Negative

Table 4. Results of *in vitro* application of isolates on mungbean

Treatments	Growth Parameters							
	Seed Germination (%)	Root length (cm)	Shoot length (cm)	Root Nodule count	Fresh Weight (gm)	Dry weight (gm)	Leaves count	Chlorophyll content (mg/L)
Control (un-inoculated)	81.33±1.15^{a}	3.16±0.05^{a}	8.90±0.01^{a}	-	0.96±0.01^{a}	0.29±0.01^{a}	3.33±1.15^{a}	42.33±0.57^{a}
R5 (*Rhizobium*)	93.33±1.15^{b}	4.52±0.01^{b}	10.85±0.01^{b}	1.66±1.12^{a}	1.25±0.01^{b}	0.45±0.01^{b}	6.66±1.15^{b}	45.00±1.00^{b}
P21 (*Pseudomonas*)	94.66±1.15^{b}	5.45±0.01^{c}	12.95±0.01^{c}	-	1.29±0.01^{c}	0.49±0.01^{c}	7.33±1.15^{b}	47.00±1.00^{c}
R5 + P21 (*Rhizobium*+ *Pseudomonas*)	98.66±1.15^{c}	6.61±0.01^{d}	14.82±0.01^{d}	3.33±1.15^{b}	1.98±0.01^{d}	0.65±0.01^{d}	8.66±1.15^{b}	50.66±0.57^{d}

increase in root length, shoot length, leaves count, root nodule count, fresh weight, dry weight and chlorophyll content of mungbean plants in lab (Table 4) as well as pot conditions (Table 5). It was observed that nodulation was also enhanced by about 30% on co-inoculation in comparison to R5 inoculation alone. The cooperative interaction between rhizobia and other PGPB are found to be very relevant in improving the nodulation as well as nitrogen fixation in legumes (Barea *et al.,* 2005; Figueiredo *et al.,* 2008; Rajendran *et al.,* 2012; El-Nahrawy and Omara 2017). Besides nodulation, co-inoculation between compatible strains can increase plant growth parameters such as root length, shoot length, fresh weight, dry weight, chlorophyll content due to the increased plant nutrition and photosynthesis (Stajkoviæ *et al.,* 2011; Hosseini *et al.,* 2014; Korir *et al.,* 2017). It was observed that root and shoot length of R5 and P21 treated plants were higher with increment of 38% and 20% and 44 and 28% respectively. In comparison to control there was 66 and 46% increase in root and shoot length when both the isolates were applied in combination. Fresh and dry weight of co-inoculated plants was highest (102 and 205% more in comparison to

Table 5. Results of *in vivo* application of isolates on mungbean:

Treatments	Growth Parameters							
	Seed Germination (%)	Root length (cm)	Shoot length (cm)	Root Nodule count	Fresh Weight (gm)	Dry weight (gm)	Leaves count	Chlorophyll content (mg/L)
Control (uninoculated)	73.70±1.15^{a}	5.01±0.01^{a}	17.26±0.01^{a}	-	3.19±0.01^{a}	1.13±0.01^{a}	7.00±0.57^{a}	42.33±0.57^{a}
R5 (*Rhizobium*)	80.76±0.57^{b}	6.93±0.01^{b}	20.73±0.01^{b}	3.33±0.57^{a}	4.63±0.01^{b}	2.44±0.01^{b}	10.66±0.57^{b}	45.66±0.57^{b}
P21 *(Pseudomonas*)	90.30±1.15^{c}	7.23±0.01^{b}	22.16±0.01^{b}	-	5.14±0.01^{c}	2.54±0.01^{c}	13.33±0.57^{c}	46.66±0.57^{b}
R5 + P21 (*Rhizobium*+ *Pseudomonas*)	99.80±0.57^{d}	8.33±0.01^{c}	25.23±0.01^{c}	4.33±0.57^{b}	6.46±0.01^{d}	3.45±0.01^{d}	17.33±1.00^{d}	50.01±1.00^{c}

Data are represented by the mean of three replicates ± standard deviation; Data followed by the same letters are not significantly different by DMRT at 0.05 levels

control plants. Although individual inoculation of R5 and P21 also increased plant growth parameters significantly less than co-inoculation. Growth enhancement by co-inoculation of rhizobia with other PGPR has been reported as an important method to improve the nitrogen availability in sustainable agricultural practices for other crops (Cambolat *et al.,* 2006; Mishra *et al.,* 2017; El-Nahrawy and Omara 2017).

CONCLUSION

On the basis of this study it is concluded that co-inoculation of *Rhizobium* R5 and *Pseudomonas* P21 showed an increase in the growth and nodulation of *V. radiata.* Application of consortia of both the isolates showed significant increase in germination percentage and biomass over the uninoculated seeds and individual inoculation. Thus co-inoculation of such compatible strains can be used for higher yields of an important pulse crop and lead to sustainable agriculture by replacing chemical fertilizers.

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RESEARCH ARTICLE

International Journal of Science, Technology and Society
Vol 3, No. 2 (2017) : 52-67

Enhancement in growth promotion and production of wheat (*Triticum aestivum* L.) by application of a native strain of *Trichoderma virens* (T2) in pot condition

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Publication Info

Article history:
Received : 17.11.2017
Accepted : 26.12.2017
DOI: https://doi.org/10.18091/ijsts.v3i02.11405

Key words:
Trichoderma virens, sustainable agriculture, wheat,

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ABSTRACT

An endemic strain of *Trichoderma virens* was isolated from wheat field of village Nagram Lucknow, Uttar Pradesh, India. This strain showed positive test for production of indole 3-acetic acid, phosphate solubilizing activities and siderophore production. The strain was identified on the basis of morphological and molecular characterizations by 18S rRNA partial gene sequencing as *Trichoderma virens*. Fifteen seeds were primed with conidia of *Trichoderma* at the rate of $2x10^8$ conidia/ml of *Trichoderma* and grown. The root length, shoot length, number of tillers, fresh weight and dry weight were increased at 120 days, over control in which no additional nutrient source was applied. The plants applied with *Trichoderma virens* produced more significant biomass. The fungus enhanced length of spikelet and grain weight per plants by 35% and 69%, respectively. The data indicated that the endemic strain of this fungus can be used as plant growth promoting microbe for cultivation of wheat in this region in addition to its well described role as effective for enhancing wheat production.

INTRODUCTION

Wheat is the most important staple food crop used to support humanity as provides more calories in the diet. Wheat is second most important cereal crop in India, the second producer of wheat in the world. Due to the deficiency of nitrogen and phosphorus in soil, farmers often over load urea and di-ammonium phosphate (DAP) or nitrogen, phosphorus and potassium (NPK) in agricultural fields during wheat cultivation in northern part of India . The use of chemical fertilizers and synthetic herbicides and pesticides has significantly influenced the environment by increasing pollution and destruction (Molla et al., 2012). To improve crop production, fertilizers and synthetic pesticides have been used without concern for environmental problems and soil health (Elkoca et al., 2010). Besides, the soluble chemical fertilizers enhance plant production and yield very significantly but require heavy irrigation as a support measure. It is already reported that the excessive use of chemical fertilizers are unsustainable for ecosystem and also, uneconomical (Singh et al., 2006; 2008a; 2010). Few of these problems can be solved by the use of microorganism, as these are natural, useful and ecological product (Mirzakhani et al., 2009).

Although, reactive nitrogen (nitrate, nitrite and ammonia) specie have been reported in leaves and other parts of plant including wheat (Dahiya el al. 2004; Sanchez-Bragado, et al. 2017). But, only 30-50 % of applied nutrients are obtained by the plant and rest is lost either through runoff or leeching in the surface or ground water (Zhou et al., 2014). Moreover, a significant amount is emitted as NOx gases and in building up the level of these gases which contribute significantly in greenhouse gas (GHG) effects. The nutrients taken by the plants are also not assimilated completely. To cope up these problems the rhizospheric microbes are used as significant assimilator of nutrients to the plants (Hoyos-Carvajal et al., 2009). Many plant growth promoting microbes have been reported to be used as biofertilizer in wheat and other crops (Sharma et al., 2012). *Trichoderma* sp. has been reported to be a very potential soil microbe which can easily be isolated and applied in the agriculture field for bio control of plant disease (Buensanteai et al., 2010). *Trichoderma* sp. has been reported as to be potential promoter of plant growth and development (Gravel et al., 2007; Shaban and El-Bramawy, 2011).

Trichoderma sp. has been reported to be a very potential soil microbe which can easily be isolated, proliferated and applied in the agricultural fields for bio-control of various diseases (Jabnoun-Khiaredinne et al. 2009; Foroutan, 2013; Zhang et al., 2014), degradation of organic matter (López-Mondéjar et al., 2011; Molla et al., 2012) and

growth promotion of plants (Akter et al., 2013; Kowalska et al., 2014; Verma et al., 2014). No report, however, is available in our data base which shows its effect on growth and productivity of wheat. We have isolated and characterized an endemic *Trichoderma* from wheat rhizosphere of the local soil, which was identified as a strain of *Trichoderma virens*. In this study, we have demonstrated its plant growth promoting activities and its effect on growth promotion.

MATERIALS AND METHODS

Isolation, purification and molecular characterization of *Trichoderma* sp.

Soil samples were collected from rhizospheric region of wheat Nagram, Lucknow, Uttar Pradesh, India. Potato dextrose agar (PDA) and rose bengal agar (RBA) medium were used for isolation of fungi. All the microbiological media was purchased from Hi Media Lab. Pvt. Ltd. Mumbai, India. The samples collected were diluted to the extent ranging between 10^{-3}–10^{-5}. Diluted sample (0.1ml) was spread on PDA/RBA medium plates at 27±1°C for 5-8 days (Aneja, 2003). Colonies appeared were purified by repeated and checked daily. To confirm *Trichoderma* identity and morphology, phase contrast trinocular microscope (Olympus CX41) was used. The molecular characterization was done on the basis of partial gene sequencing (18S rRNA) by Akar Biotech Private Limited, Lucknow, Uttar Pradesh, India.

Indole 3-acetic acid (IAA) production:

The fungal cultures were grown on minimal broth with 0.1% tryptophan for 3-5 days (Brick et al., 1991). After growth broth was centrifuged and culture supernatant was used for IAA estimation. 1 ml supernatant of each broth was mixed with 4 ml of Salkowski reagent and incubated for 30 minute. The formation of pink color indicates IAA production.

Phosphate solubilization:

The cultures were screened for their in-vitro phosphate solubilizing potential in NBRIP medium NBRIP-BPB liquid medium consist of $(NH4)_2SO_4$(0.1 g/l), Ca3(PO4)2 (5.0 g/l), $MgSO_4.H_2O$ (0.25 g/l), $MgCl2.6H_2O$ (5.0 g/l), KCl (0.2 g/l), BPB (0.025 g/l), glucose (10 g/l) (Nautiyal, 1999 and Onyia et al., 2013) inoculated with a 1% (v/v) inoculums pre-culture grown in the same medium. The phosphate solubilization activity of isolates was deter-mined by growing the isolates in NBRIP medium containing a pH indicator (bromophenol blue) for 12 days taking observation at 4 days intervals at 29°C. At the end of the incubation period, spectro-photometric readings were recorded at OD_{600} nm finally we can compare with control.

Ammonia production:

Ammonia production was tested in peptone water broth media (Cappuccino and Sherman, 1992). Fungal isolates were tested for ammonia production in freshly cultured peptone water were inoculated into 10 ml of peptone water after incubation of 48-72h at 28 ± 2°c Nessler's reagent (0.5ml) was added development of yellow to brown color would be considered as a positive test for ammonia production spectrophotometric data were recorded at 412nm

Siderophore production:

Isolates were assessed for Siderophore production on Chrome Azurol S agar medium determined by the method of Schwyn and Neilands (1987). Pure fungal culture grown in LB (Luria broth) was inoculated into plates containing chrome azurol S (CAS) agar plate. Incubated at 30 ° C and observed daily for orange color formation each colony for up to 3-4 days.

Pot experiments

An earthen pot experiment was set up, using soil from research field station BBAU fields for sowing the wheat crop. 12 inch diameter size of earthen pots was filled with 8kg of soil. Treatments with completely randomized design were replicated with three times. The performance of the inoculants was compared with single inoculation *Trichoderma virens*. Inocula primed wheat seeds were sown at 3cm depth with equal spacing. Irrigation was given thrice at tillering pre and post flowering stages during the life cycle of the. Control pots without any inoculation were also maintained. The wheat plants were uprooted at interval of 40,80and 120 days analysed growth and yield parameters root length, shoot length, fresh weight, and dry weight of grain per plant and length of spikelet.

RESULTS

Isolation and characterization of *Trichoderma virens* strain.

The soil samples were obtained from rhizospheric region of wheat from peri urban area of Lucknow, Uttar Pradesh, India and the fungus was isolated by serial dilution method at 10^{-5}-10^{-6} on potato dextrose agar(PDA) plate and purified on the same solid media. The colony color was green and the microscopic study its resemblance with *Trichoderma* species (Fig1 a, b & c).

Fig. 1(a). *Trichoderma virens*

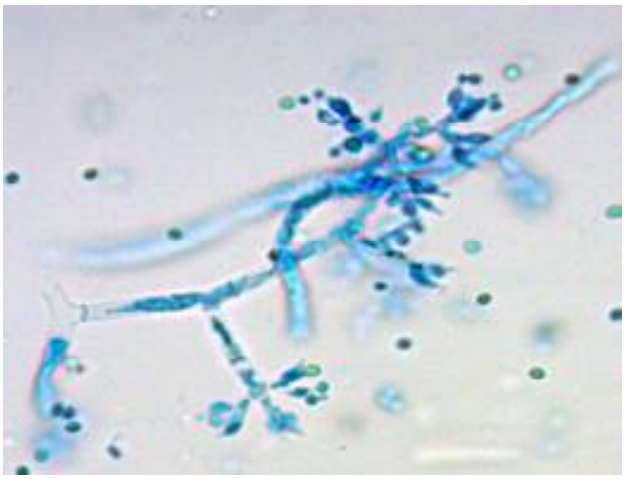

Fig. 1 (b). Microscopic image of *trichoderma virens*

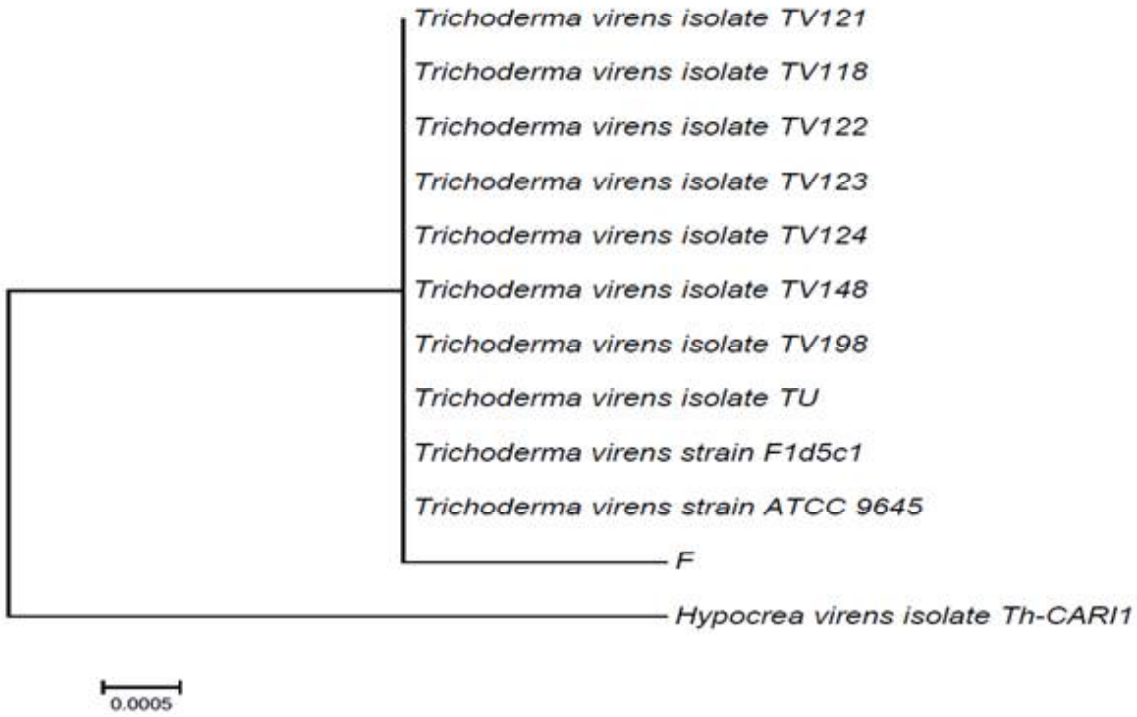

Fig. 1 (c). Neighbor-joining tree based on analysis of partial 18S rRNA nucleotide sequences of *Trichoderma virens* evolutionary analyses was conducted in MEGA6

Table 1. Plant growth promoting activities

Strains	Indole 3-acetic acid (IAA)	Phosphate solubilizing fungi (PSF)	Ammonia	Siderophore
Trichoderma virens	+	+	+	+

The fungus showed the production of indole 3-acetic acid, ammonia, solubilization of phosphate and siderophore, the data is presented in table-1. On partial gene sequencing (18S rRNA) by Akar Biotech Private Limited, the fungus was identified as a strain of *Trichoderma virens.* To observe the effect of fungus on growth of wheat under pot cultivation, fifteen uniform and healthy seeds coated with *Trichoderma virens* ($2x10^8$conidia/ml) were selected and

Table 2. Growth enhancement

Parameters	Days after Sowing	Control	*Trichoderma virens*
Root length (cm.)	40	04.33±0.12	06.03±0.12
	80	06.77±0.19	09.37±0.21
	120	09.33±0.12	14.13±0.17
Shoot length (cm.)	40	20.43±0.12	27.83±0.05
	80	35.57±0.05	53.53±0.12
	120	45.43±0.17	71.37±0.12
Fresh wt. (g)	40	03.28±0.05	06.43±0.12
	80	05.70±0.08	10.27±0.09
	120	04.40±0.08	09.03±0.05
Dry wt. (g)	40	00.18±0.00	00.36±0.01
	80	00.70±0.01	01.51±0.02
	120	01.54±0.02	02.80±0.08
No of Leaves	40	04.33±0.47	07.00±0.00
	80	06.33±0.47	11.33±0.47
	120	04.00±0.00	07.67±0.00
Root hairs	40	04.67±0.47	09.67±0.47
	80	06.67±0.47	11.67±0.47
	120	08.67±0.47	13.33±0.47

Table 3. Variation of weight of grain/plant and length of spikelet of wheat treated with *Trichoderma virens*

Parameter	Control	*Trichoderma virens*
Weight of grain/plant (g)	0.78±0.02	1.32±0.01
Length of spikelet's (cm)	6.70±0.16	9.10±0.14

sowed in earthen pots. The root length, shoot length, number of leaf, number of tiller, number of root hairs, fresh weigh and dry weight were studied on 40, 80 and 120 days of sowing.

On completion of 120 days, the length of spikelet and weight of grain per plant were also measured as presented in Table 3. The data in table 2 showed that there were about 36, 50 & 57% increase in shoot length of the plant after 40, 80 and 120 days of sowing, respectively in comparison to the control. Two tillers were produced in the plant after applying *Trichoderma* as compared to control. The fresh weight increased by about two fold than control. The plants after *Trichoderma* treatment produced about double number of leaves and root over the control plants. The length of spikelet was more (35%) and the weight of grains was also increased by 69% per plant, over control.

DISCUSSION

The dwarf variety is more prone towards the soluble chemical fertilizers and requires enhanced irrigation for increasing the productivity many folds. However, the use of these chemical fertilizers is no more considered as ecologically and economically sustainable due to their high cost and toxicity to the living beings. These are disturbing the native soil microbial profiles and also causing long time fertility reduction (Murali et al., 2012). In addition, these losses cause serious threat to zooplankton and organisms present in the water and air (Ansari et al., 2012; Xue et al., 2014; Tomar et al., 2015). Fungus like *Trichoderma* sp. has the potential to

Fig. 2. Comparison of vegetative growth in pot experiment

provide green nutrients in agricultural fields. However, for different marketing aspects, their stability during mass production and storage beyond lab, and its application in the agricultural fields is not satisfactory. As a result, farmers are not accepting these alternative bio-fertilizers to enhance the productivity by eco-friendly and cost effective ways.

The survival of plant growth promoting microbes (PGPMs) in different agro climatic conditions is not consistent and special measures are realized to be taken for their affectivity. Recently, the consortium approaches have been considered as an effective measure to promote the sustainable agriculture, by different researchers (Yadav et al., 2013; Sharma et al., 2012). The compatible organisms present in consortium with more diverse communities with different species showed more stability to the biofertilizers (Ashutosh Awashthi, Alok Kalra and R.P Singh, unpublished result). The study showed that the isolated *Trichoderma virens* performed growth promotion in pots, which subsequently enhanced the productivity of wheat PBW 343. The mechanism of growth promotion was studied in-vitro, which was found to be associated with production of indole 3-acetic acid as well as solubilization of phosphate and siderophore. Further studies are in process to confirm the response of this endemic species of *Trichoderma* for enhancing the growth and productivity of wheat in field conditions, alone or in consortium with other plant growth promoting rhizobacteria.

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RESEARCH ARTICLE

International Journal of Science, Technology and Society
Vol 3, No. 2 (2017) : 68-74

Combating Global warming through carbon sequestration using carbonic anhydrase enzyme and photo thermal techniques

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Publication Info

Article history:
Received : 11.12.2017
Accepted : 27.12.2017
DOI: https://doi.org/10.18091/ijsts.v3i02.11410

Key words:
Global warming, carbon sequestration, carbonic anhydrase enzyme, photo thermal techniques

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ABSTRACT

Radiative Forcing (RAF), due to ever increasing release of green house gases (GHG), is resulting rise in global temperature. The level of CO2 in the atmosphere has gone up to about 384 ppm due to annual release of over 36 billion ton CO2. This is contributing 50% of total RAF and there by environmental heating. Methane and N2O being 25 and 285 times higher in RAF than CO2 on molecular basis correspondingly contribute to 18% and 5% of total RAF. Other GHG e.g. CO, CFC. SO2, lead particles etc. contribute 35% of total RAF. Hence carbon deactivation vis-a-vis carbon sequestration in to useful material through biotechnological ways using eco-friendly physic-biological system e.g. enzyme and photo-thermal techniques to sustain eco-friendly environment is only option.

Carbonic Anhydrase (CA) is richly present in *Pisum sativum* L. and non-saccharide super sweet plants (NSSP) like *Abrus precatorius* L. and *Glyrrhiza glabera* L. besides several bacterial cells e. g. *Methano-bacterium, Thermoautotrophicum, Porphyridium purpureum, E. coli etc*. It catalyses reversible conversion of CO_2 in to bicarbonate ($CO_2+H_2O \longleftrightarrow HCO_2+H$) with turnover rate (Kcat) ranging between 10^4 to 10^6 S^{-1}. Because of efficient CO_2 conversion, CA has been used in carbon fixation and industrial carbon sequestration to produce useful materials like bricks. cement, building materials, lime stone, green-fuel, formic acid, methanol, urea, 3 'phosphate glycerate etc. using bio-reactor. Zincacted industrial effluents, being heavy metal pollutants are also utilised in the bioreactor system since zinc constitute the protein of CA.

Few eco-friendly photo-thermal techniques developed with combination of eco-friendly components for the deactivation of carbon dioxide, carbon mono oxide and methane and thereby carbon sequestration are "photo thermal cell energy", "Sun shine to petrol" and synthetic fuel production by "Freeing and activating carbon and hydrogen" have become feasible . Immobilization of methanotrophs/methane/mono/oxygenase through bioreactor system diminishes industrial and wet land released methane, heavy metal pollutants and petroleum hydro carbon in much faster way on large scale as compared to free living methanotrophs bacteria. This finally reduces heavily GHG pressure. Environmental/Industrial methane liquidification using "Methane Liquefier" to liquid methane for its use as Rocket fuel is important way of GHG diminution, weakening RAF factors and mitigating global warming.' Further researches are in progress in these directions.

INTRODUCTION

Fast burgeoning human population and over exploitation of natural resources are deteriorating continuously and impacting negatively the environment and the earth's biodiversity. The anthropogenic activities are raising toxic chemicals, pesticides, heavy metals e.g. Mercury, lead, cobalt etc. and green house gases (GHG) e.g. CO_2, CO, methane, SO_2, nitrous oxide,nitrogen oxide, chlorofluro carbon etc. in the edaphic, atmospheric and aquatic environment. All these in turn, are polluting natural environment and causing global warming and there by

climate change. Consequently, the natural ecosystems are becoming unsustainable for supporting the life of all living organisms including human being.

With the advancement of knowledge and technology, the infusion of ingenious idea have become an impetus for providing pragmatically results. In fact, to mitigate global warming due to CO_2, the technologies for carbon sequestration on one hand and splitting of carbon dioxide into harmless carbon and oxygen on the other hand are immerging. The former is oriented to biological/ biotechnological approach (water et. al. 2011) while the later is based on biophysical and thermodynamic principles (Miller 2017).

There are three major pathways for CO_2 utilization: conversion of CO_2 into fuels, utilisation of CO_2 as a feedstock for synthesis of value added chemicals and direct use of CO_2 as a solvent or as working fluid. Some of the conversion routes of CO_2 in to chemical feed stocks and various other intermediates are presented in succeeding pages.

Production of methanol and formic acid from CO_2 has been widely targeted. These are formed by hydrogenation of CO_2 using wide range of catalysts. For the synthesis of methanol three equivalents of hydrogen per molecule of CO_2 are required, out of which two are incorporated into the product while the third is consumed in the production of water. Formic acid is another valuable product, as it can store hydrogen in it. It is a more manageable liquid form requires only a single equivalent of hydrogen for the synthesis without any by-product formation and therefore, proved as highly efficient system (Styring et al., 2011).

I. Carbonic anhydrase (CA) mediated carbon sequestration, related success and limitations.

CA is very potential in catalysing reversible reaction of CO_2 into bicarbonate (carbonic acid) and H+ and bicarbonate (carbonic acid) into CO_2 and H_2O

$$CO_2 + H_2O \longleftrightarrow HCO_3 + H^+$$

The turnover rate of CA is 10^4–10^6 per second. This activity is highest among the photosynthetic enzymes activities of plants. The entire reaction takes place in natural conditions and with fixed / stationary condition of enzyme on bioreactor. Green plants and biota using CA enzyme through photosynthetic energy and many non green organisms using chemosynthetic energy / processes especially under marine conditions, perform reversible conversion of CO_2 into bicarbonate. Zinc constitutes the protein of this enzyme and stimulates its activity (Dwivedi and Randhawa 1974). Cobalt, Cadmium, Fe++ and CO_2 stimulates CA activity on one hand and on other hand, lead, mercury, more than 50m MNO_3 and 100 m MSO_4 inhibit CA activity.

Faridi and Satyanarayana (2015) depicted five ways through which CA regulates CS by depleting environmental CO_2.

1. Flue gas depletion and bioreactor mediated sequestration
2. Biological resource mediated sequestration
3. CA immobilization mode of sequestration.
4. Mineral carbonate sequestration,
5. Miscellaneous valuable products sequestration.

1. Flue gas depletion and bioreactor mediated sequestration

Flue gas (industrial smoke) contain 10%-20% CO_2 and sufficient amount of N-oxide, SO_2,besides the particle of Pb, Hg, Co, copper iron etc and thereby pollute environment. The economic and greener way of capture of CO_2 is through carbonic anhydrase enzyme which catalyses rapid inter conversion of CO_2 and H_2O to HCO_3+H. This is least energy requiring carbon sequestration process for CO_2 released from anthropogenic sources.

$$CO_2 + H_2O \xleftrightarrow{CA} HCO_3 + H^+$$

The use of CA in capturing CO_2 makes large scale carbon capture and sequestration, (CCS) using bioreactor. The technical design involves immobilization of CA on solid matrix in bioreactor. At bottom of reactor there is entry of flue gas. Water is sprinkled with pump from top of bioreactor to maintain aqueous condition. As flue gas bubbles through the reactor it gets dissolved into water. The dissolved CO_2 get converted into bicarbonate ion by action of immobilised CA. Bhatacharya et al. (2003) reported that by grafting CA on silica coated porous steel and horizontal inflow and outflow of CO_2 carrying gas at 60°C combined with continuous sprinkling of water increases efficiency of CO_2 dissolution and carbon sequestration.

NASA (National Aeronautics and Space Administration) designed a bioreactor for capturing CO_2 from ambient atmosphere of confined inhabited cabin which has CO_2 concentration of 0.1% or less. They employed thin

aqueous film carrying dissolved CA (Crown *et al.* 2003) in a reactor. The diffusion through membrane has better efficiency for low CO_2 diffusion in ratio of 1400 to 1 by comparisons of N_2 and 866 to 1 by comparison with O_2. The carbon sequestration through this type of bioreactor was found to be more suitable for natural environments where CO_2 is rising above 400 ppm.

2. Biological resources mediated sequestration.

Fixed type of CA in bioreactor do not work well when a flue gas besides high CO_2 concentration has concentrated impurity of sulphur, Nitrogen oxide, organic amines, metal ions etc.. This render CA in active. Also, flue gas has high temperature range from 50°C to 125°C which can denature CA enzyme. Further, for efficient CO_2 sequestration alkaline media is required. Hence under aforesaid odd conditions, the fixed CA does not work properly. The natural resource CA is the only option for carbon sequestration on large scale.

Natural microbes/bacteria for example in *Bacillus subtilis* CA efficiently fix CO_2 over wide range of pH 7-11 (Ramanan et al. 2009). This enzyme was found to be inhibited by $Pb2^+$ and $Hg2^+$ and stimulated by CO_2, $Cu2^+$ and SO_4- which are present in flue gases (Ramanan et al. 2009.Few examples of natural bio-organisms are presented below (Table 1.)

3. CA immobilization mode of Carbon sequestration (CS)

Immobilization of CA on solid or any suitable matrices enhance CA activity, favours reuse of enzyme, avoid desaturation, improves storage stability with retention of 50% of its initial activity after 30 days (Sharma and Bhattacharya 2010) and increase the precipitation of $CaCO_3$ as compared to free enzymes system (Wanjari *et al.* 2011). Immobilization also enhances temperature stability upto 67.5°C and 74.0°C in *Micrococcus lylae and M. luteus* respectively (Bhattacharya et al. 2013). Hence this system become more suitable in bioreactors or similar artificial systems for carbon sequestration by C.A.

Table 1. Carbonic anhydrase (CA) enzyme in natural biological organisms, its CO_2 fixation, inhibitory and stimulatory factors and products formed after carbon sequestration (CS).

CA in natural Biological organism	Inhibitory Factors	CA stimulatory factor	Product formed	Reference
αCA : Myceliophthora thernophilla; Dunaliella sp. (DsP-αCA opt) (Cloned CA)	-	Thermostable (85oC)	Calcite	Kanth *et al.* (2012)
αCA: E. coli (Cloned CA) (Ssp CA) *Sulfurihydrogenibium yellow stonens*	Oxide of S and N. Inhibition const. 0.58-0.68 mM	Thermostable (110°C)	Any industrial carbonated product	Capasso *et al.* (2012)
Abrus precatorius Pisum sativum PSCA (2cystein -1 Hist.-1 H_2O:Zn)		Zn	HCO3	Smith and Ferry (1993)
Bacillus subtilis CA *Bacillus* spp.	Pb2+ &Hg2+	pH 7-10 Co, Cu, Fe2+, SO4	Carbonic acid	Ramannan *et al.* (2009)
Pseudomonas fragi, Micrococus luteus, M. lylae (MLCA)	More than 35°C-45°C & >50 mM NO3 and >100 MSO4	Zn, Cd, 60, Fe2+	HCO3	Sharma & Bhattacharya (2010)
γCA : from wild microbes *E. coli (cloned CA)* *Azospirillum brasilense*	-	High CO2 stationery phase	Protein & CaCO3	Kaur *et al.* (2010)
β CA-cynobacteria *coleofasciculus chthonoplaste*	Selenate, selenocyanide Holide, heavy metals	-	HCO3	Vello *et al.* (2014)

Few examples of CA immobilization matrices are mentioned below : -

1. Chitosan beads
2. Chitosan activated alumina carbon composite beads
3. Alignale beads
4. Sylylated chitoson beads
5. Core shell CA chitosan nano particle (SEN-CA)
6. Mesoporous aluminosilicate
7. Octa (Aminophenyl) silse squioxane –Fe_3O_4/SiO2 nanoparticles
8. Electro spun polystyrene / poly (styrere-co-maleic an hydride) nanoubers (CLEA)
9. g Amino – propylriethoxysilane coated ion particles
10. Polyurethane foam

4. Mineral carbonate sequestration

Presently in the process of carbon capture and sequestration (CCS), the process of carbon capture, utilization and storage (CCUS) has been given practical importance with a view of products formed (i.e. stored) after CS on industrial and bioreactors levels.

Thus mineralization based sequestration of CO_2 is a major area of CCUS. In nature or on earth large amount of lime stone formation has been resulted by the process of atmospheric Co_2 utilization. Mineral rocks such as wollastonite ($Ca.SiO_2$), serpentine ($Mg_3 Si_2O_5 (OH)_4$) and olivine ($Mg_2 SiO_4$), weather slowly by action of wind and rain and release free mineral ions which react with CO_2 and water and form silica and carbonates (Huijgen *et al.* 2007). Formation of few mineral carbonates are depicted below (Farrel 2011)

$$HCO_3 + OH \rightarrow CO_3 + H_2O$$

$$CO_3 + Ca \rightarrow CaCO_3 \text{ (Calcite)}$$

$$CO_3 + Mg \rightarrow MgCO_3 \text{ (Magnetite)}$$

$$CO_3 + Ca + Mg \rightarrow CaMg(CO_3)_2 \text{ — (Dolomite)}$$

$$CO_3 + Fe^{2+} \rightarrow FeCO_3 \text{ (Siderite)}$$

Buffered in alkaline range pH is essential for aforesaid mineral carbonate formation.

This process takes place in nature and is slow. Mineral carbonate containing the sequestered CO_2 can not only be permanently stored in silica mines but also used to produce several industrial bio products such as chemicals, cement, white paints, calcium supplements, lime stone aggregate etc. for making building.

$CaCO_3$ CA based CO_2 mineralization can also be used to produce glycerol carbonate, ethylene and poly propylene carbonate. Also polyurathane in their polymetric forms can be produced which are used in plastic for food and beverage container, flexible housing and foam insulation (Nguyen and Demiral 2011).

All these natural carbon reduction is carried by the CA present in microbes, snails, eggs, algae, diatoms and various cyanobacteria, eucaryotic microalgae, *Bacillus, Pseudomonas,* Vibrio and sulphate reducing bacteria (Pomar and Hallock, 2008, Barabesi et al. 2007).

6. Miscellaneous valuable products Sequestration

From flue gas, the CO_2 depletion result the formation of bicarbonate which is further utilized in the formation of many valuable products like 3-phosphoglycerate. This is feasible by attaching a subunit with bioreactor through solar panel. (Bhattacharya et al. 2004).

CA based CO_2 conversion after HCO_3 formation can also produce methanol when combined with mixture of formate dehydrogenase, aldehyde dehydrogenase and alcohol dehydrogenase (Amao and Watanabe 2009). Different strains of microalgae can be utilized to produce biofuel from CA captured carbon / HCO_3. The algal biomass can produce lipid, fertilizers and hydrocarbon with nutrients supply (Gonzalez-Fernandez and Ballesteros (2012./ Production of formic acid and methanol from the CA formed carbonate has been achieved by hydration of CO_2 using wide range of catalysts (Styring et al. 2011).

II. Photo thermal mediated carbon sequestration, related success and Limitation.

Photo thermal approaches for carbon sequestration are new but require thermal energy for acceleration of process. Hence, sometimes carbon sequestration remain behind that of the quantum of CO_2 released during energy production through coal ignition. Solar energy supplemented Photo thermal sequestration is therefore,the only alternative for success.

Solar radiation is essential for human existence but GHG impede the reflection of solar radiation back to solar system. This process heats up earth ground and earth atmosphere. N_2O and CH_4 raise temperature of atmosphere 4.2 and 10.5 times higher respectively than CO_2 (Table 2). Impact of photo- thermal energy (Sun+coal) on carbon sequestration and problems creeping in controlling global warming and related success are discussed here :

i) Radiative Forcing ((RAF) and Environmental Warning

Earth gets light and temperature from sun. Various GHG on earth viz. CO_2, CO, CH_4, NO_2, N_2O, SO_2, chloroflurocarbon etc. impede/check the reflection of sunlight especially heat providing ray (Red & Far-red) back to solar system. Consequently the earth surface and its environmental-sphere become warm. Thus the process of impeding the reflection of solar radiation from earth surface back to solar system and thereby warming of earth environment is called radiative forcing.

Carbon dioxide is the one of the largest amount of gas on the earth. Its concentration during preindustrial age ranged from 250-280 ppm but now it has attained the concentration 380-410 ppm due to annual release of over 3 billion ton of CO_2 in the atmospheres a result of unbalanced anthropological activities. Methane (CH4) and Nitrous oxide (N_2O) are important gases which were earlier heard as a little dangerous have attained the level of threat in augmenting environmental warming. The release of CH_4 in different parts of world has been recorded as: 0.09-1.23/m2/yr in India (Panneer *eta* 2014) to 32.41-4.6 39 g/m2/hr in China (Song *et al.* 2009) and that of N_2O from 0.0014 mg/m2/hr in Pacific north west (Erickson and Perakish) to 250-500 mg/m^2/yr in Denmark (Audet *et al.* 2014). Because of release of huge volume of CO_2 it contribute to 50% environmental warming (vis-a-vis) but based on molecular basis RAF value of N_2O and CH4 are heigher and are very dangerous green house gases in raising environmental warming (Table 2).

Considering RAF 1 for CO_2 the RAF 25 and 298 have been recorded for CH_4 and N20 respectively (Dijkstra et al. 2012). Hence special attention need to be given to tackle the problem of rising CH_4 &N_2O Level in the atmosphere.

ii) Kinds of Photo-thermal approaches for carbon sequestration.

Till today three kinds of photo thermal approaches have appeared in literature

1. Split harmful CO_2 into harmless carbon and oxygen
2. Use of CO_2 as input to generate useful energy rich products
3. Conversion of methane into useful fuel.

Miller (2017) from Sandia National Laboratories, California, USA, attempted to split CO_2 into harmless carbon and oxygen but it required more energy. If energy from Coal to be supplied to drive the splitting, chances of more CO_2 release was visualised than that of the amount CO_2 sequestration. Hence solar energy trapping technique to be strengthen to accumulate higher energy and thereby splitting of CO_2.

Use of CO_2 into useful materials directly or by breaking methane in to CO_2 and thereby carbon sequestration using different quantum of energy is illustrated in various reactions as shown below. Carbon mono-oxide and H+ form basic building blocks that find use in producing synthetic fuel. This was called as "Sunshine to Petrol" production technology at Sandia National Laboratories. Concentrated very high sun generated ecofriendly energy is required to drive these reactions and achieve the products.

Conversion of methane into liquid rocket fuel has attained significant success in USA and it is being patented. Sandia National Laboratories of Livermore, Albuquerque, California, New Mexico (USA) have succeeded in this direction while working on biofuel production and national security aspects with a team work of J.E. Miller, J. Henderson and Devon Pa to mitigate harmful GHG.

Table 2. Radiative forcing (RAF) and thereby contribution of different green house gases (GHG) in raising global warming.

	GHG	RAF values (compared to CO2)	Total RAF (%)*	Temperature rise (°C) compared to CO2
i)	CO2	1 (supposed)	50%	1.0
ii)	Methane	25	18%	4.2
iii)	Nitrous oxide	298	5%	10.8
iv)	Others (CO, CFC,SO2, Pb, NO2 etc.	-	3.5%	2.5

* Calculation Based on total quantum of annual release

Deactivation of green house gases

i. $CH_4+2O_2 \xrightarrow{\text{Methanotrops}} CO2+2H2O\ (\Delta H222\ Cal/mol.)$

ii. $CO_2+H_2O \longrightarrow HCHO+O_2\ (\Delta H112000\ cal/mol)$ (Formaldehyde)

iii. $CO_2+H_2O \longrightarrow CO+1/2\ O_2+H_2O$ (Photo thermal I energy-J.E. Miller 2017)

$CO+O_2+H+H$

Sunshine to Petrol ⟶ synthetic fuel

iv. Liquid methane for Rocket fuel: Production of liquid methane/conversion of methane into liquid methane and thereby its use for rocket fuel.

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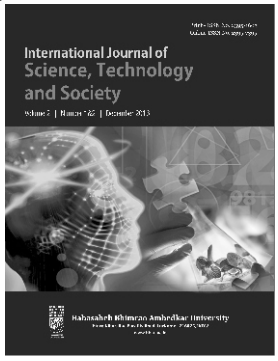

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