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Research Papers

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BIO-BASED NANOCOMPOSITE FILMS FOR FOOD PACKAGING - A REVIEW

Haritha Devanand
Assistant Professor, Department of Food Science and Technology, Satavahana University, Karimnagar

ABSTRACT

Concerns on environmental waste problems caused by non-biodegradable petrochemical-based plastic packaging materials as well as the consumer's demand for high quality food products has caused an increasing interest in developing biodegradable packaging materials using annually renewable natural biopolymers such as polysaccharides and proteins. So Biofilms have arised, Several edible materials have had their film forming properties studied, to produce edible films to be used in food packaging. But these biofilms have poor mechanical and physical characteristics so this is replaced by bio-nanocomposite edible films, which have better properties similar to petroleum-based polymers. The nanocomposites are having the improved mechanical properties. Both the water uptake and the diffusion coefficient of water were found to decrease. The nanocomposites are prepared by three major pathways- In situ polymerization, In solution intercalation, In melt intercalation. Starch-Based Nanocomposites, Cellulose-Based Nanocomposites, Chitosan-Based Nanocomposites, Protein-Based Nanocomposites are prepared by any of these three major path ways. The food packaging application of these films found better results as the active packaging for enhanced food quality, safety and innovative packaging.

This review concludes that by replacing the biofilms with the bio nanocomposite films there is an improvement in the mechanical and physical characteristics of the films similar to the petroleum based polymer films. Therefore these petroleum-based films can be replaced to some extent and can reduce the packaging related pollution problems.

Keywords: active packaging, bio-films, bionanocomposite, food packaging, nanocomposite, Natural biopolymer,

INTRODUCTION

At the turn of the 20th century, most non-fuel industrial products like inks, dyes, paints, medicines, chemicals, clothing, synthetic fibres and also plastics were made from biologically derived resources. However, 70 years later petroleum-derived chemicals to a major extent replaced these.

Now, at the turn of the 21st century recent developments are raising the prospects that naturally derived resources again will be a major contributor to the production of industrial products. Currently, scientists and engineers successfully perform developments and technologies that will bring down costs and optimize the performance of biobased products. At the same time, environmental concerns are intensifying the interest in agricultural and forestry resources as alternative feed stocks. A sustained growth of this industry depends on the development of new markets and cost and performance competitive biobased products. A potential new market for these materials is food packaging, a highly competitive area with great demands for performance and costs.

Food and beverage packaging is responsible for about 70% of the packaging market in the united states, and more than half the worldwide market. Most of this large volume of food packaging material is meant to be discarded without recycling, because of recycling costs and difficulties in polymer separation.

To come across the recycling and other pollution problems, several biodegradable food packaging materials are used for reducing the usage of non biodegradable material. Edible materials have had their film forming properties studied, to produce edible films for food packaging, not to completely replace synthetic plastics, but rather to improve their efficiency, thus reducing the amount of synthetic polymers required for each application.

So Biofilms have arised but they are of poor mechanical and physical properties. And now these Biofilms are replaced by bio-nanocomposite edible films, which have better properties similar to petroleum-based polymers.

The use of edible materials serve a number of important functions, such as extending the food shelf life, enhancing food quality, biodegradability. Azeredo. (2009)

Cooksey.(2005) studied on establishing methods for coating low-density polyethylene film or barrier films with methyl cellulose as a carrier for nisin and found that they significantly reduced the Listeria monocytogenes in solutions and vacuum packed hot dogs. And also studied the use of chitosan in films to inhibit the Listeria monocytogenes. These were found effective.

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BIO-BASED MATERIALS

In general, biobased polymers may be divided into three main categories based on their origin and production:

Category 1: polymeric materials

Polymers are extracted from marine and agricultural products. Examples are polysaccharides such as cellulose, starch and chitin, and proteins such as casein, whey, collagen and soy.

All these polymers are by nature hydrophilic causing processing and performance problems especially in relation to packaging of moist products. On the other hand, these polymers make materials with excellent gas barriers.

Category 2: polymeric materials

Polymers produced by classical chemical synthesis using renewable biobased monomers. A good example is polylactic acid, a biopolyester polymerized from lactic acid monomers. The monomers themselves may be produced via fermentation of carbohydrate feedstock.

To date, polylactic acid (PLA) is the polymer showing the highest potential for a commercial major-scale production of renewable packaging materials. The PLA materials have a good water vapour barrier and have also relatively low gas transmittance.

The feedstock can be agricultural resources, e.g. corn or wheat, or alternatively agricultural waste products, such as whey or green juice.

Category 3: polymeric materials

Polymers produced by microorganisms or genetically modified bacteria. To date, this group of biobased polymers consists mainly of the polyhydroxyalkonoates , but developments with, for example, bacterial cellulose are in progress.

Poly (hydroxyalkanoate)s (PHAs) are a family consisting of renewable, biologically degradable, biocompatible, optically active polyesters. They are produced by many bacterial species in the form of intracellular particles, functioning as an energy and carbon reserve material.

The presence of functional groups in the side chains of the polymer makes it possible to modify the polymer chemically, increasing the number of potential food-packaging applications of PHAs. Presently, the production of PHAs is not cost-effective for the production of packaging materials.

Bacterial strains of *Acetobacter xylinum* and *A. pasteurianus* can produce an almost pure form of cellulose (homo- β -1,4-glucan). Its chemical and physical structure is identical to the cellulose formed in plants. It also has an enormous potential within the food-packaging industry, but is so far largely unexploited.

Masaya *et* al.(2008)Transparent polymers were reinforced by bacterial cellulose BC nanofibers, which are 10_50 nm ribbon-shaped fibers. They exhibited high luminous transmittance at a fiber content as high as 60 wt %, and low sensitivity to a variety of refractive indices of matrix resins. Due to the nanofiber size effect, high transparency was obtained The optical transparency was also surprisingly insensitive to temperature increases up to 80 °C. As such, BC nanofibers appear to be viable candidates for optically transparent reinforcement.

Table-1: Category 1 polymers directly extracted from biomass

Polysaccharides	Proteins	Lipids
Starch	***Animal source***	Bees wax
Potato	Casein	Cadellila wax
Maize	Whey	Triglycerides
Wheat	Collagen/gelatine	Acetylated monoglycerides
Rice	Fish myofibrillar protein	Fatty acids
Derivatives	Keratin	Fatty alcohols
	Egg white	Sucrose fatty acid esters
		Resins such as shellac and terpene resin.

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Cellulose Cotton Wood Other derivatives	***Plant source*** Zein Soya Gluten cotton seed protein Protein from sorghum - kafrin Rice bran Peanuts Pea	
Gums Guar Locust bean Alginates Carragenan Pectins Derivatives		
Chitosan /Chitin		

(Webert 2002)

Category 2: polylactic acid (PLA), other polyester.

Category 3: poly(hydroxyalkanoate)s (PHAs), bacterial cellulose. (Webert 2002)

NATURAL BIOPOLYMER-BASED FILMS

These are the materials often formulated with natural biopolymers, such as polysaccharides, proteins, and natural gums, capable of forming a cohesive and continuous matrix.

Film is regarded as a stand-alone thin layer of materials composed of a polymer matrix providing structural integrity. Generally, films are prepared from polymers for matrix formation and other additives. The formulation of films needs the use of at least one component capable of forming a structural matrix with a sufficient cohesiveness. Only high molecular weight polymers, owing to their sufficient cohesive strength and capacity for coalescence, can produce such film structure.

Natural biopolymer films can be prepared with various hydrocolloids (polysaccharides and proteins), lipids, and their composites as polysaccharide films, protein films, lipid films, composite films.

Abayomi *et* a/.(2008) prepared Rice bran protein-based edible films and reported that the puncture strength (PS) of RBP films increased up to pH 8.0 and then decreased.

Wang *et* al.(2007) assessed the film-forming abilities of six types of proteins, as well as six types of polysaccharides at various concentrations (proteins: 0-16%; polysaccharides: 0-4%) and heating temperatures (60-80 C). Biopolymer films evaluated included: sodium caseinate (SC), whey protein isolate (WPI), gelatine (G); caboxymethyl cellulose (CMC), sodium alginate (SA) and potato starch (PS). Screening trials showed that optimal film-forming conditions were achieved and good tensile strength, flexibility, tear strength, puncture resistance, respectively.

The commercial use of edible films has been limited because:

- In some instances relatively poor mechanical properties
- Many are relatively hygroscopic nature.
- The present low level of production and high cost.

Therefore to develop biodegradable plastics with properties comparable to petroleum-based plastics an attractive option is the biobased nanocomposite materials.

NANOCOMPOSITES

Nanocomposites refer to multiphase materials where at least one of the constituent phase has one dimension less than 100nm.

A VARIETY OF NANOFILLERS INCLUDE

- Solid layered clays
- Synthetic polymer nanofibers

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- Polysaccharide nanocrystals
- Carbon tubes.

IMPORTANCE OF BIO-NANOCOMPOSITE FILMS

When polymers are combined with nanofillers, resulting in bio-nanocomposites exhibit significant improvements in

- Mechanical properties
- Dimensional stability
- And solvent or gas resistance
- Low density
- Transparency
- Good flow
- Better surface properties
- Recyclability.

Up to now only the layered silicate attracted the attention of packaging industry. And polysaccharides are among the most promising sources for the production of nanoparticles.

Zhao *et al.* (2008).

A) POLYSACCHARIDE NANOCRYSTALS

Stable aqueous suspensions of polysaccharide nanocrystals can be prepared by acid hydrolysis of the biomass. The suspended colloidal particles include whiskers (elongated rod-like nanoparticles), nanocrystals and monocrystals.

a) Cellulose and chitin colloidal aqueous suspension

- The biomass is generally first submitted to a bleaching treatment with NaOH in order to purify the cellulose or chitin by removing other constituents.
- The bleached material is then disintegrated in water, and the resulting suspension is submitted to a hydrolysis treatment with acid. The amorphous regions of cellulose or chitin act as structural defects and are responsible for the transverse cleavage of the microfibrils into short monocrystals under acid hydrolysis.
- The resulting suspension is subsequently diluted with water and washed by successive centrifugations. Dialysis against distilled water is then performed to remove free acid in the dispersion.
- Complete dispersion of the whiskers is obtained by a sonication step.
- The dispersions are stored in the refrigerator after filtration to remove residual aggregates with the addition of several drops of chloroform. Then these solutions are used for reinforcing the polymers to form bio-nanocomposite film.

Azeredo *et* al. (2009) developed cellulose reinforced mango puree edible films and found that the addition of CNF was effective in improving the water vapor barrier strength, tensile strength, effective young's modulus of the films.

Leitner *et* al.(2007) prepared sugar beet cellulose nanofibril sheets and found higher strength and stiffness than the non-homogenised cellulose sheets.

Alemdar and sain. (2008) isolated and characterized cellulose nanofibers from agricultural residues wheat straw and soy hulls and found that their degradation temperature reached beyond 290 degrees c. These can be used for reinforcing the polymers.

Wang *et* al.(2009) studied on preparation and properties of nanocomposite films composed of cellulose nanocrystals and polyvinyl alcohol. They found that rod like cellulose nanocrystals were approximately 20nm in diameter and 200nm in length, the nanocomposite films were uniform and stable, showed an increase in thermal stability and tensile strength with an increase of the filler content.

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Daniell and Kritiina (2007) studied on Biodegradable nanocomposites based on 5 wt% cellulose nanowhiskers (CNW) and polylactic acid (PLA). The results from mechanical testing showed a maximum modulus for the composite with 5 wt% surfactant and as the surfactant content increased, the CNW dispersion improved and the tensile strength and elongation at break was improved compared to its unreinforced counterpart.

B) Starch Nanocrystals Aqueous Suspension

Aqueous suspensions of starch nanocrystals can be prepared according to the "lintnerization" procedure.

The kinetics of lintnerization shows two main steps.

During the initial step, the hydrolysis kinetics is fast and corresponds to the hydrolysis of amorphous domains.

In the second step, the hydrolysis kinetics is slow and corresponds to the hydrolysis of crystalline domains.

With a starch concentration of 14.69 wt%, the preparation of aqueous suspensions of starch nanocrystals was achieved after 5 days of hydrolysis with 3.16 molIL H2SO4 at 40 °C and 100 rpm, with a yield of 15.7 wt%. Waxy maize starch nanocrystals consist of platelet-like particles about 5-7 nm thick, 20-40 nm long, and 15-30 nm wide.

The use of sulfuric acid for preparing polysaccharide nanocrystals leads to more stable aqueous suspensions than the use of hydrochloric acid. Then these nanocrystal solutions are used for reinforcing the polymers to form bio-nanocomposite film.

IMPROVEMENTS

- The nanocomposites are having the improved mechanical properties.
- Due to hydrogen bonding the theinial stabilization of the composite is upto 500k, the temperature of degradation.
- Both the water uptake and the diffusion coefficient of water were found to decrease.
- Natural rubber reinforced with waxy maize starch nanocrystals showed reduced oxygen diffusion, permeability and water vapour transmission rate.
- The sorbitol plasticized pullulan films reinforced with starch nanocrystals showed enhanced barrier properties at higher filler contents
- The water vapour transmission rate of cotton nanocrystals reinforced CMC films was found to decrease slightly in heat treated nanocomposites.

Dufresne.(2008).

B. Clay nanoscale fillers

These are the nanofillers which include

- Montmorillomite
- Hectrite
- Saponite
- Pristine layered silicates.

These are combined with polymeric materials to form nanocomposites.

Among all these the Montmorillonite (MMT) is of particular interest and has been studied widely.

MMT

MMT is a clay mineral consisting of stacked silicate sheets with a high aspect ratio (length to thickness ratio) and a plate-like morphology. Chemically, MMT consists of two fused silicate tetrahedral sheets sandwiching an edge-shared octahedral sheet of either magnesium or aluminum hydroxide.

These clays usually contain hydrated sodium or potassium ions and in this state these silicates are miscible only with hydrophilic polymers such as poly(ethylene oxide)(PEO), poly(vinyl alcohol) (PVOH), and natural biopolymers such as starches and proteins.

In the interlayer region of MMT there exists Na+ and Ca2+,which can be replaced by the alkylammonium and alkylphosphonium ions, rendering the clay into an organophilic nature. Which plays an important role for producing the nanocomposite.

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When these organoclays are mixed with a polymer, three types of composites are commonly obtained:

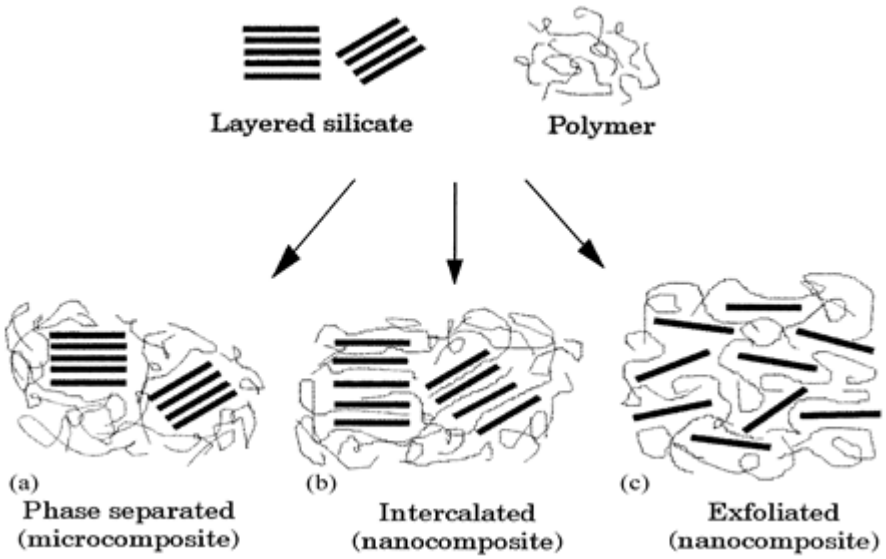
- Tactoid,
- Intercalated,
- and Exfoliated structures.

TACTOID NANOCOMPOSITE
Complete clay particles are dispersed within the clay matrix and the layers do not separate.

INTERCALATED NANOCOMPOSITE
Often a single polymer chain will be driven between the clay silicate layers, but the system still remains quite well ordered in a stacked type of arrangement.

EXFOLIATED NANOCOMPOSITE
The silicate layers are completely delaminated from each other and are well-dispersed. The exfoliated nanocomposite, which has been shown to exhibit the most significant improvements in physical properties.

Figure-1



SYNTHESIS OF NANOCOMPOSITES
There are three major pathways for the formation of nanocomposites.

In situ polymerization
It involves the combination of clay and monomer, followed by the polymerization of the monomer, which ideally locks the exfoliated clay particles.

In solution intercalation
The clay is first swollen in a solvent and the polymer (intercalant) is dissolved in the solvent. Both solutions are then combined, and the polymer chains intercalate and displace the solvent within the interlayer of the clay.

In melt intercalation
The clay and polymer are added together above the melting temperature of the polymer. They may be held at this temperature for a period of time, put under shear, or other conditions to encourage the intercalation and the exfoliation of the clay.

NATURAL POLYMER-BASED NANOCOMPOSITES (WITH CLAY NANOFILLERS)
Starch-Based Nanocomposites
Starch is one of the natural biopolymers most widely used to develop environmentally-friendly packaging materials to substitute for petrochemical-based non-biodegradable plastic materials.

Native starch is not a true thermoplastic but it can be converted into a plastic-like material called "thermoplastic starch". In the presence of plasticizers at high temperature (90-180ºC) and under shear, starch readily melts and flows, allowing for its use as an injection, extrusion or blow molding material, similar to most conventional synthetic thermoplastic polymers. However, the pure thermoplastic starch still has the same

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limitations as native starch: it is mostly water sensitive and has poor mechanical properties. In order to improve the properties, including the resistance to water and mechanical properties of starch plastics, reinforcement of starch with nano-scale minerals has been considered without interfering in the biodegradability of the composites.

Cyras *et al.* (2008) prepared on Glycerol-plasticized potato starch/clay nanocomposites films to study the effect of the nanoclay in the properties of starch. They found an improvement in the thermal resistance of starch. The water absorbed by the nanocomposites was reduced and improvement in the Young's modulus up to 500% for the nanocomposite containing 5 wt% of clay.

Cellulose-Based Nanocomposites

Cellulose is the most abundant naturally occurring biopolymer. It is composed of unbranched, linear chains of D-glucose molecules, linked to one another by 1,4-β-D glucosidic bonds. Hubbe.(2008).

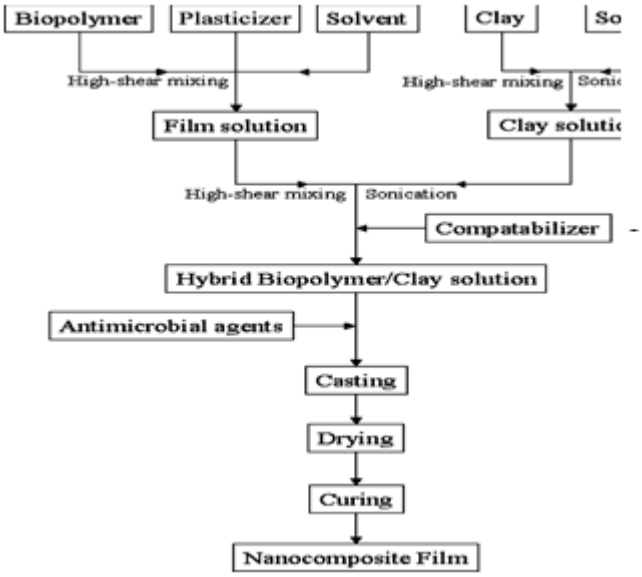
Leitner *et a/.* (2007) prepared polyvinyl alcohol and phenol-formaldehyde films reinforced with cellulose nanofibers and obtained the best mechanical, tensile, modulus of elasticity performance of the films.

Chitosan-Based Nanocomposites

Chitosan is a partially deacetylated derivative of chitin. It is the second most abundant natural biopolymer next to cellulose. Structurally, chitosan is composed of glucosamine and Nacetylglucosamine units linked by the β-1-4 glucosidic bond.

Rhim, Perry.(2007)

Figure-2



Preparation flow chart for nanocomposite film

Protein-Based Nanocomposites

α-Amino acids are the basic structural units of proteins. These are highly complex polymers made of 20 different amino acids.

In proteins, four levels of protein structure exist: primary, secondary, tertiary, and quaternary. Due to these complexity in their composition and structure they possess multiple function properties, such as solubility, gelation, elasticity, emulsification, and cohesion-adhesion.

Zhao et al.(2008), Rhim, Perry et al.(2007)Nayak *et* a/.(2008) prepared polycaprolactone (PCL)/soy protein isolate (SPI) blend with Organoclay and found great enhancement of tensile, dynamic mechanical properties and strong shear-thinning behavior, percolated network of clay particles in melted state.

APPLICATIONS IN THE FOOD PACKAGING

The use of proper packaging materials and methods to minimize food losses and provide safe and wholesome food products have always been the focus of food packaging. In addition, consumer trends for better quality, fresh-like, and convenient food products have intensified during the last decades. Therefore, a variety of active packaging technologies have been developed to provide better quality, wholesome, and safe foods and also to limit package related environmental pollution and disposal problems.

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These bio-nanocornposites can exhibit many advantages. Followings are some examples:

- Biodegradable;
- Enhanced organoleptic characteristics of food, such as appearance, odor, and flavor; Reduced packaging volume, weight, and waste
- Extended shelf life and improved quality of usually non-packaged items.
- Individual packaging of small particulate foods, such as nuts and raisins;
- Function as carriers for antimicrobial and antioxidant agents
- Controlled release of active ingredients
- Annually renewable resources.

Because of these advantages they are useful for packaging different food products for different purposes.

Active packaging

It is a type of packaging that changes the condition of the packaging to extend shelf-life or improve the safety or sensory properties while maintaining the quality of the food. Examples are antimicrobial packaging, antioxidant packaging.

As one of the innovative active packaging methods, antimicrobial packaging, applying antimicrobial compounds in combination with food packaging materials, has been receiving considerable attention as a potential application for a variety of foods including meat, fish, poultry, bread, cheese, fruits and vegetables. The potential application of these films with antimicrobial activities would allow surface contact with food that could help control the growth of pathogenic and spoilage microorganisms.

Dutta *et al* (2009) studied on the perspectives for chitosan based antimicrobial films in food applications and found that they exhibited high antimicrobial activity against a wide variety of pathogenic and spoilage microorganisms.

Antioxidant packaging will protect the packaged food like cut apples from undergoing oxidation and avoid browning of the foods.

Disposable tableware

The use of compostable tableware opens up for new perspectives for, for example, fast-food restaurants. If all packagings and tableware for the consumption of burger meals were produced using compostable packagings, it would be possible to dispose all waste from fast-food restaurants by composting. Several companies are aiming at developing a suitable biobased and compostable burger clamshell.

Chilled or frozen products

PHA and PLA materials have relatively low gas and water vapour barriers, which makes them interesting for a long range of food applications. Especially, dairy products have been suggested as a very likely application for PLA.

Weber et al.(2002); Zhao et al.(2008); Nayak et al.(2008).

CONCLUSION

The usage of large volumes of packaging materials in food and beverage industries should ultimately discarded causing environmental pollution due to reduced recycling problems. So to avoid this, edible materials which have the film forming properties are used to produce biofilms but has poor application. This resulted in bio-nanocomposite films. They have improved properties which are similar to the petroleum-based packaging materials and so have better application in food packaging. For the production of bio-nanocomposite films they use the agricultural waste, byproducts of food industry, and are biodegradable which ultimately reduces the pollution problems.

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RESPIRATORY RESPONSES OF *GARRA MULLYA* (SYKES) UNDER BIFENTHRIN AND CARBOSULFAN INTOXICATION IN SUB LETHAL TENURES

Surekha Bansode[1] and Ajendra Patil[2]
Assistant Professor[1] and Associate Professor[2], Department of Zoology, A. C. S College, Navapur, Nandurbar

ABSTRACT
Aquatic Pollution Due To Wide Array Of Xenobiotic Compounds Causes Serious Threats To Plants And Animals Living In The Water. Pesticides Discharged In The Aquatic Bodies Though Get Diluted But Forms Sublethal Concentration And May Affect Functioning Of Organisms. The Prominent Manifestation Of Pesticidal Toxicity Is Either Altered Respiration Or Over Stimulation. Respiratory Responses Of Fresh Water Fish Garra Mullya (Sykes) Were Studied Under Sublethal Tenure Of Bifenthrin And Carbosulfan. Acclimatized Fishes Were Exposed To Pesticides And The Rate Of Oxygen Consumption Was Recorded After 7, 14 And 21 Days. Present Study Revealed Initial Increase Followed By Decrease In The Rate Of Oxygen Consumption.

Keywords: Bifenthrin, Carbosulfan, Garra Mullya, Oxygen Uptake, Sublethal Dose.

INTRODUCTION

Aquatic pollution due to various chemicals including pesticides is the most critical and burning environmental issue of the centuries.(Magar and Shaikh,2012) Most of the pesticides are species specific but when they enter into aquatic environment may disturb the life of non target species. Except some anaerobic organisms life without oxygen in impossible and hence respiration is said to be the vital process of any organism that provides oxygen for oxidation of food material to release energy. This energy is then utilized for various life activities and normal development of an individual. Oxygen consumption is a very sensitive physiological process. The activity of animal can be measured in terms of O2 uptake. Respiratory activity is the first physiological response given by the fishes when exposed to various toxic substances present in the aquatic bodies. Aquatic organisms respire through gills which are in continuous contact with water. Toxic pesticides present in the aquatic environment may bring about the changes in the gill architecture that finally affect rate of oxygen consumption. An abnormal opercular movement is an indicator of respiratory stress but more direct method to measure pesticide stress on any aquatic organism is the estimation of oxygen consumption. Any change in respiratory activity, including altered rate in opercular movement indicates physiological stress in pollutant exposed animals (Anderson, 1971; Sharp *et.al,* 1979). All aquatic organisms including fishes shows stress responses which are dependent on concentration of pesticides as well as the duration of pesticidal contact. More the exposure more will be the physiological stress. Lethal concentration of pesticides causes death of organisms but pesticides in sublethal concentrations though not causing death, tend to accumulate in the tissues of aquatic organisms and brings severe lesions in them affecting normal functioning of the system.

According to Klein (1959) and Jones (1973) oxygen consumption is an important physiological parameter to assess the toxic stress in animals. It is the valuable indicator of expenditure of energy and the metabolic processes in general. In the present study an attempt has been made to find out the effect of sub lethal concentrations of Bifenthrin and Carbosulfan on the rate of oxygen consumption of fresh water fish *Garra* mullya (Sykes).

MATERIALS AND METHODS

The fresh water fishes *G. mullya* (Sykes) were collected from Bhaware Dam, Tal-Navapur, and acclimatized to the laboratory condition for 7-8 days. Healthy fishes measuring 9-10 cm in length and 5-7gms in weight were considered for experimentation. The acclimatized fishes *G. mullya* were exposed to sub lethal (LC50/10 values of 96 hours) concentrations of pesticides i. e. Bifenthrin (0.1253 ppm) and carbosulfan (0.6980 ppm). After an interval of 7 days up to 21 days the oxygen consumption by control and treated groups was determined by standard Winkler's method.

Only one fish was introduced into each respiratory chamber. Once the fish was completely settled in the respiratory chamber, the initial sample was collected. The next sample was collected from the respiratory chamber at an interval of 7 days up to 21 days. One chamber without fish was maintained throughout the experiment to record initial amount of oxygen. Simultaneously control was also maintained to find out the rate of oxygen consumption in *G. mullya* when exposed to sub lethal concentrations of pesticides.

OBSERVATION AND RESULTS

In the present study significant alteration in the rate of oxygen consumption was noted. Increased opercular movements, clogging of the gill surface with mucous, surfacing phenomenon and gulping of air in the early

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exposure period was the noticeable behavioural changes which were more in Bifenthrin exposed fishes than Carbsulfan. The comparative data on the rate of oxygen consumption of control and experimental fish, calculated per gram body weight per hour after chronic treatment of Bifenthrin and Carbosulfan for *G. mullya* was given in the Table. The results of the control and experimental values are graphically represented in Fig by taking time on X axis and the amount of oxygen consumed per gram body weight on Y axis.

Table: The rate of oxygen consumption of *Garra mullya* (Sykes) after sublethal exposure of Bifenthrin and Carbosulfan.

Sr.No	Treatment	Average oxygen consumption ± S.D. ml/gm/hr/lit en		
		7 days	14 days	21 days
1	Control	0.44 ± 0.024	0.44 ± 0.017	0.38 ± 0.021
2	Bifenthrin	0.21 ± 0.027** 51.63 %	0.36 ± 0.030* 18.67 %	0.29 ± 0.011** 22.09 %
3	Carbosulfan	0.28 ± 0.028** 35.03 %	0.37 ± 0.041^{NS} 15.30 %	0.20 ± 0.05* 46.01 %

1. Each value is a mean of three observations ± S.D

2. (+) or (-) indicate present variations over control.

3. Values are significant at N. S. = Not Significant

* = P < 0.05

** = P < 0.01

*** = P < 0.001

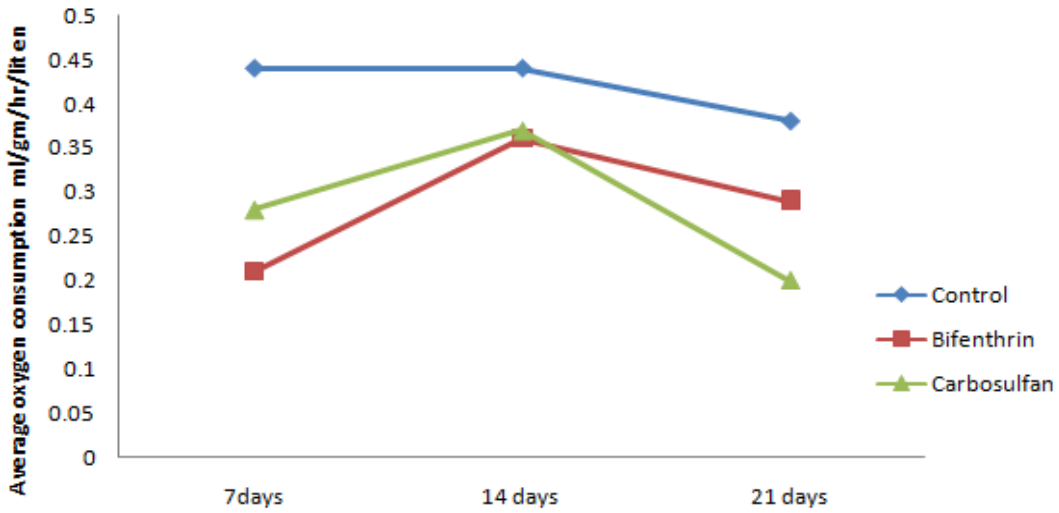

Fig: The rate of oxygen consumption of *Garra mullya* (Sykes) after sublethal exposure of

Bifenthrin and Carbosulfan.

Initial increase after 7th day of exposure followed by decrease in the rate of oxygen consumption after 14th day was observed in Bifenthrin and Carbosulfan exposed fishes which was 51.63%, (P < 0.001), 18.67% (P < 0.05) and 22.09% (P < 0.01) in Bifenthrin and 35.03% (P < 0.01), 15.30% (NS) and 46.01% (P < 0.05) in Carbosulfan exposed *G. mullya* on 7th, 14th and 21st day.

DISCUSSION

Most fishes are gill breathers and transports oxygen from surrounding water to the tissue level. Pesticidal discharge into aquatic bodies changes chemical properties of water that reflects abnormal ventilatory activity of the gills which are in intimate contact with surrounding environment. Alteration in the architecture of gill lowers its diffusion capacity that results in decreased oxygen consumption. Luther Das *et.al* (2000) reported increased rate of oxygen consumption during initial period in fresh water fish *Labeo rohita* exposed to cypermethrin. Similar observations were also reported by Nagaraju, And Venkata Rathnamma (2013), Rajmannar and Manohar (1992) and Sambasiva Rao et al (1981).

Present study shows agreement with these investigators. *G. mullya* showed steady decrease in the rate of oxygen consumption in the control group but initial elevation of oxygen uptake was reported in Bifenthrin and

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Carbosulfan exposed fishes. Gradual decrease in the rate of oxygen uptake can be attributed to the starvation of fishes as starved fishes shows reduced metabolic rates. Stress imposed by the pesticides in the aquatic environment make the animal active to combat the stress and this may be the reason of initial increase in the uptake of oxygen to incur an increased energy requirement. It can be explained that the sudden rise in the oxygen uptake during early period of exposure up to 7^{th} day might be due to the adjustment efforts of the fish *G. mullya* to the toxic stimulus of pesticides. The decrease in the rate of oxygen consumption at the later period can said to be a protective measure to ensure low intake of the toxic substance. According to Jhingran (1983) reduction in oxygen uptake after subsequent exposure of pesticides might be due to failure of fishes to attempt boosting oxidative exposure period. Tilak et al; (2005a) correlated reduction in oxygen uptake with the extent of damage of gill epithelium.

CONCLUSION

From the observations and analysis of the data of the present work it can be evidenced that pesticides Bifenthrin and Carbosulfan have shown profound effect on respiratory activity in fresh water fish *G. mullya*. Pesticide induced stress causes increased metabolic activity to combat its harmful effect thus increases oxygen uptake similarly clogging of gill surface to avoid the direct contact of pesticides as a defence may be responsible for the decreased rate of respiration. Estimation of respiratory rate in aquatic organisms can be used as a potential tool for assessment of aquatic pollution.

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UNRAVELING DIVERSITY IN SHAPES OF SOMATIC EMBRYOS OF *HARDWICKIA BINATA*: A UNIQUE CASE STUDY OF HIGH FREQUENCY SECONDARY SOMATIC EMBRYOGENESIS

Bihani Priyanka[1] Jain Monica[2] Shrivastav Pankaj[3]
[1,3]Forest Research and Extension Circle, Indore
[2]Department of Life Sciences, Maharaja Ranjit Singh College of Professional Sciences, Indore

ABSTRACT
Somatic embryogenesis in Hardwickia binata was obtained by culturing cotyledons from immature seeds in MS medium supplemented with low concentrations of 2,4-D. Secondary embryogenesis was then initiated when explants were subsequently cultured on MS medium fortified with Abscisic acid (ABA) plant growth hormones. The embryogenic lines remained productive for eleven cycles and repetitive induction of somatic embryos (SE) was obtained which resulted in inexhaustible production of embryos. Including globular, torpedo, heart, and cotyledonary shapes many other novel shapes obtained during secondary embryogenesis under the influence of Abscisic acid are presented here for the first time in tissue culture studies.

Keywords: H. binata, woody plant, somatic embryogenesis, conservation, in vitro propagation

INTRODUCTION
Somatic embryogenesis is a well-known phenomenon in tissue culture and is the most desirable pathway for *in vitro* plant regeneration. Since 1958 (Steward 1958a,b) numerous studies have been performed in diverse plant species with variations in growth regulators, concentrations and combinations, genotypes, culture conditions, embryonic stages and altered requirement for different stages like somatic embryo induction, maturation and development etc. Somatic embryogenesis plays a critical role in having its practical application in *in vitro* regeneration of woody plants, especially for those tree species which are problematic to propagate by conventional methods (Isah 2016). However, various recalcitrant forest tree species of economic value are still difficult to establish *in vitro* mainly due to reduced or lack of morphogenetic ability, increased level of contamination and insubstantial rooting of the regenerated shoots (Bonga 2010). Under tissue culture approach the plant spp. belonging to the family Leguminoseae is in general considered to be recalcitrant to *in vitro* regeneration. *Hardwickia binata* Roxb. (family Fabaceae) commonly known as Anjan, is one such economically useful tree species found in Eastern Asia which has now become vulnerable, owing to heavy biotic pressure as the wood is used as timber. The wood of the tree is among hardest and most dense of trees growing in India. The bark yields a fiber which is used for making ropes. The leaves are used as fodder and green manure. Oleo-resin extracted from the heart wood is used in manufacture of varnishes. The natural propagation of this tree sp. greatly suffers due to poor seed setting and low seed germination percentage (Mandora et al. 2014). Very few *in vitro* regeneration studies have been reported for propagation of this economically useful timber yielding species where, *in vitro* propagation from callus cultures by Das et al. (1995), from mesocotyls, shoot tips and axillary buds by Anuradha et al. (2000) somatic embryogenesis from semi mature zygotic embryos by Chand and Singh (2001) and Das P. (2011) and recently *in vitro* propagation from axillary bud of the seedling nodal segments was perfected by Mandora et al (2014). However, complete plants from somatic embryos could not be obtained and still raise possibilities for a successful protocol to be worked out. Under these efforts, we subjected *Hardwickia binata* to tissue culture technique for effective somatic embryogenesis from immature cotyledons leading to development of avenues for mass production and development of elite genotype.

MATERIALS AND METHODS
Explant collection and Surface Sterilization
Fresh and immature pods of *H. binata* were collected from trees growing in the forest of Rajpura valley, located 20 km from Indore (M.P.), India. The pods were surface sterilized by washing under running tap water for 20 min followed by washing with T-20 sol. (1ml/100ml) for 10 min and subsequently washed with distilled water. The pods were then subjected to savlon antiseptic solution treatment (0.6ml/100ml) for 15 min and rinsed by distilled water for 15 min Further sterilization was done under Laminar Air Flow, where pods were disinfected with 0.1% freshly prepared aqueous mercuric chloride ($HgCl_2$) for 10 min followed by washing with sterile distilled water. Finally the pods were treated with 70% ethanol for 60 s and again washed 3 times with sterile distilled water to remove all the traces of the disinfectants.

Culture conditions
The seeds were taken out and seed coat was removed carefully. After removing seed coat small segments of cotyledons were aseptically cut and cultured into test tubes containing MS medium having 3% sucrose and

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0.6% agar supplemented with various concentrations of 2,4-D (0.25- 2.0 $mg.l^{-1}$). The pH of the medium was adjusted to 6.8 before sterilization. The medium was steam sterilized at 121°C under 1.06 kg/cm^2 pressure for 15 min Based on preliminary studies, the cultures were maintained in dark for two weeks and then transferred to light conditions. In light condition, the cultures were provided with 16/8 h. of photoperiod and were maintained at 35±2°C. The cultures were transferred to fresh medium after an interval of every four weeks. Once somatic embryogenesis was achieved, the embryos were transferred to MS medium supplemented with ABA (0.25 - 2.0 $mg.l^{-1}$) for three weeks and then the mature embryos were transferred to MS medium fortified with BAP (0.5-2.0 $mg.l^{-1}$) and IAA (0.25-1.0 $mg.l^{-1}$) (Table 1).

Table-1: Growth regulators used during various stages of somatic embryogenesis

Basal Media	SE Induction	SE Maturation	SE Germination	
	2,4-D ($mg.l^{-1}$)	ABA ($mg.l^{-1}$)	BAP ($mg.l^{-1}$)	IAA ($mg.l^{-1}$)
MS	0.25	0.25	0.5	0.25
	0.5	0.5		0.5
	1.0	1.0		1.0
	2.0	2.0	1.0	0.25
	-	-		0.5
	-	-		1.0
	-	-	1.5	0.25
	-	-		0.5
	-	-		1.0
	-	-	2.0	0.25
	-	-		0.5
	-	-		1.0

RESULTS AND DISCUSSION

During the first week of culture the cotyledons showed expansion with swollen cut ends and after another week the whole surface got swollen and fully expanded. When transferred to light, the cotyledons exhibited small globular protuberances pale whitish in color, directly from the surface (fig.1a) after two weeks of culture period. The primary somatic embryos further grew and started acquiring heart and torpedo shapes (fig. 1b). These embryos grew further (fig. 1c) and acquired cotyledonary shape within two weeks growth period. Best embryogenic response was obtained in cultures growing in MS medium supplemented with 2,4-D (0.5 $mg.l^{-1}$) where from each explant 30-35 embryos were obtained. Use of 2,4-D to initiate somatic embryogenesis has been reported in various plant species; like Norway spruce (Hakman et al. 1985), Loblolly pine (Becwar et al. 1990), *Alnus glutinosa* (Corredoira et al. 2013) and on elite trees (Peeris and Senarath 2015). Even the use of immature and mature zygotic embryos has been reported in various plant species by many researchers (Yuan Guan et al., 2016)

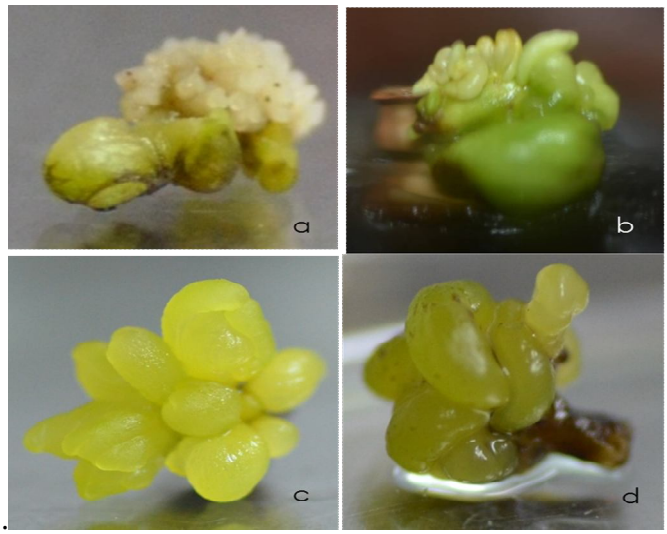

Fig. 1a: Globular somatic embryo induction; b: Heart and torpedo shaped somatic embryos; c: Somatic embryos acquiring growth; d: Cotyledonary shaped somatic embryos.

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SECONDARY SOMATIC EMBRYOGENESIS

The mature embryos were transferred to ABA (0.25 - 3.0 mg.l^{-1}) supplemented medium for maturation, but the embryos proliferated further and the mature embryos started forming new embryos from their surface (Fig. 2a). This phenomenon where new somatic embryos are formed from the existing ones is very well known as secondary somatic embryogenesis (Raemakers et al. 1995). The secondary somatic embryogenesis was highly repetitive which started along the axes (fig. 2a), and gradually covered whole surface (fig. 2b). During this period, while few embryos grew in size, the others kept on giving rise to new embryos further (fig. 2c). Secondary somatic embryo induction was so vigorous that huge number of new embryos was obtained from all the primary embryos. These new embryos again acquired torpedo and cotyledonary shapes during their maturation, but after that they turned black and went under dormant phase of 10-12 days.

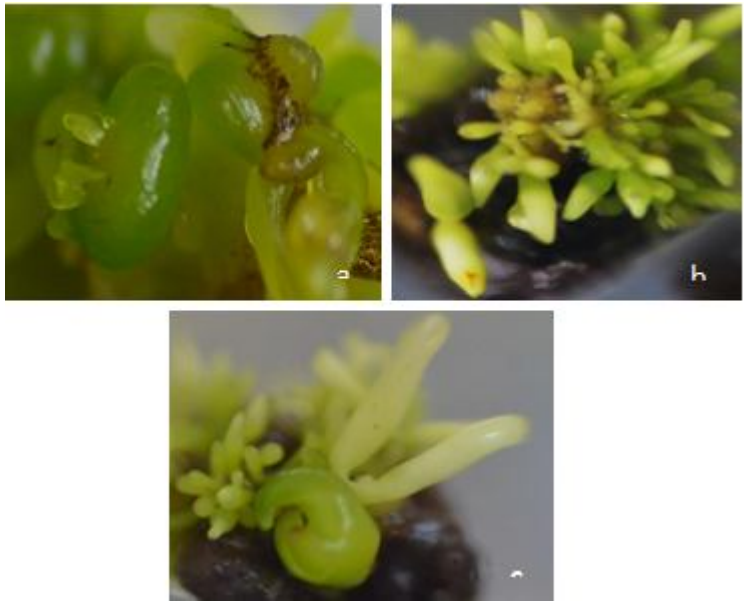

Fig. 2a: Induction of secondary somatic embryos; 2b: Whole surface covered by secondary somatic embryos; 2c: Maturation of secondary somatic embryos.

Soon, this dormant phase was broken and new embryos again emerged from all over the surface of the old embryos. They multiplied and acquired maturity (fig. 3a-b). During this phase the embryos accumulated of pigmentation, green (fig. 3c) and red (fig. 3d). Very soon pigmentation was lost, the embryos re-entered dormancy and initiated the cycle all over again. Similar process continued for eleven more cycles and innumerable embryos multiplied exhibiting immense embryogenic proliferation potential. The concentration of abscicic acid played a very crucial role. On raising the concentration from 0.25 to 0.5mg.l^{-1}, the embryos enlarged in size and new interesting shapes mostly like florets and curled whorls (fig 4a-d) were observed.

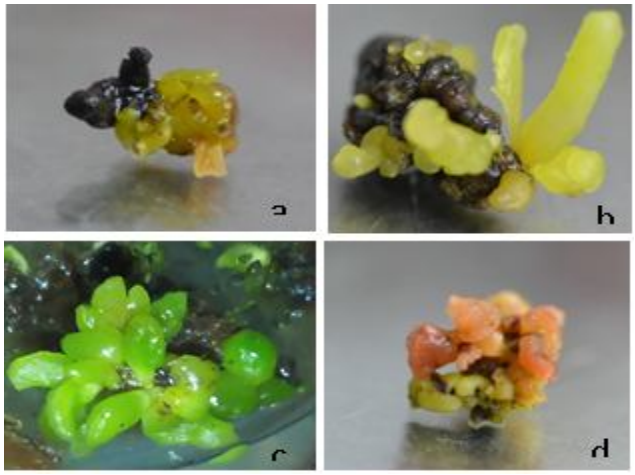

Fig-3a-b: Multiplication and maturation of secondary somatic embryos; 3c-d: Embryos accumulating green and red pigmentation.

Fig. 4a-d: Secondary somatic embryos exhibiting curled whorls and florets under the influence of raised ABA concentration (0.5mg.l^{-1}).

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Further increment in the concentration of ABA (1.0 mg.l^{-1}) led to more compact and fused morphology (fig. 5a-b). The accessory embryos did not show distinct identity instead aberrant shape morphology was observed. Advancement in concentration of hormone, further led to enlarged size and compact cotyledon shaped structures (fig.5c-d) were observed. All these embryos however continued proliferating new embryos; which seemed to be a never ending process. In woody plants however, secondary somatic embryogenesis is reported to maintain the embryogenic competence of cultures for many years and thus embryos prove to be a useful research material (Martinelli et al. 2001; Martinez et al. 2015). The stimulus triggered by the growth regulator was so intense that the somatic embryo proliferation was unstoppable even after complete removal of ABA from the medium. ABA is found to stimulate embryo development to the precotyledonary stage on media (Becwar et al. 1990) and is considered to trigger cell fate transition under stress conditions (Karami and Saidi (2010); Ikeuchi et al. 2015).

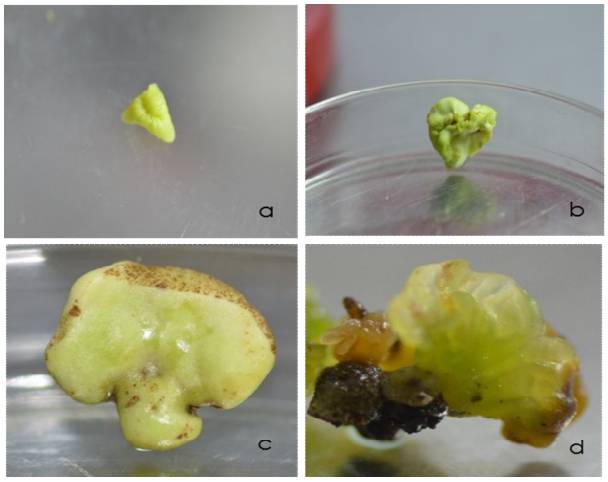

Fig-5a-b: Embryos exhibiting compact and fused morphology; c-d: Embryos exhibiting expanded cotyledon shapes.

GERMINATION OF SOMATIC EMBRYOS

The somatic embryos were transferred to germination medium containing BAP (0.5-2.0 mg.l^{-1}) and IAA (0.25-1.0 mg.l^{-1}). After one week of growth period, the embryos gained pigmentation and exhibited radicular end with hypocotyl formation. The shooty end however continued exhibiting fused morphology under the influence of elevated concentrations of BAP and IAA, leading to merged cotyledonary structures (fig.6a), anisocotyledonary (fig.6b), multi cotelydonary (fig.6c), funnel shaped (fig.6d), trumpet shaped (fig.6e), cone head shaped (fig.6f), bulged head (fig.6g), fan head (fig.6h), bean head (fig. 6i) etc. Few embryos under the influence of lower concentrations of BAP (1.0 mg.l^{-1}) and IAA (0.2 mg.l^{-1}) converted to plantlets exhibiting root shoot morphology (fig. 7a-b); however efficient conversion to whole plantlets needs further research. Vahdati et al. (2008) found low efficiency of embryo maturation, germination and conversion to plantlets, a major problem which affects efficacious completion of somatic embryogenesis.

Fig.6. Morphology acquired by somatic embryos during germination under the influence of BAP and IAA. Merged cotyledonary shape (fig.6a), anisocotyledonary (fig.6b), multi cotelydonary (fig.6c), funnel shaped (fig.6d), trumpet shaped (fig.6e), cone head shaped (fig.6f), bulged head (fig.6g), fan head (fig.6h), bean head (fig. 6i)

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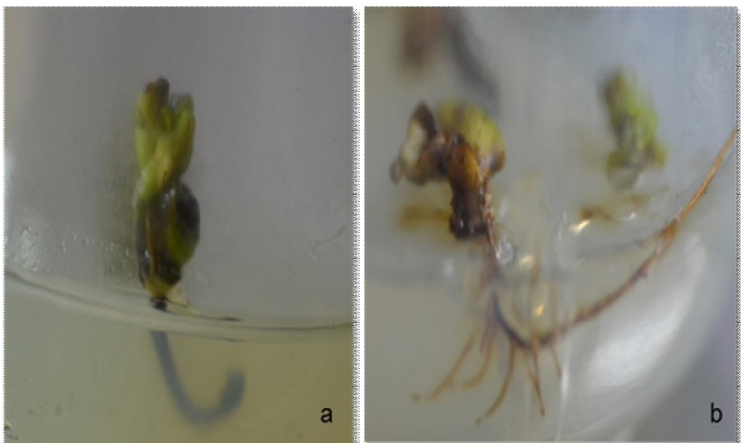

Fig-7: Conversion of embryos into plantlets exhibiting bipolar morphology

CONCLUSION

The somatic embryos emerge out as bipolar propagules from the undifferentiated mass of cells having potential to give rise to new plantlets. Our study unravels various shapes of somatic embryos displaying the morphological and developmental disparity with the natural zygotic embryos. A high frequency rapid somatic embryogenesis obtained in our study provides a way to new research on developmental biology of plants. The protocol set forth, raises great promises synthetic seed production of such woody plant species of commercial value.

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EVALUATION OF TWO VARIETIES OF CHICKPEA GROWN UNDER THERMAL POWER PLANT WASTEWATER AND COAL FLY ASH APPLICATION

Irfan Ahmad[1] and Akil A Khan[2]
[1]Department of Botany, ABPG College Ranapar, Gorakhpur
[2]Department of Botany, Gandhi Faiz-E-Aam College, Shahjahanpur

ABSTRACT
A field experiment was conducted to investigate the effect of thermal power plant wastewater (TPWW), ground water (GW) and coal fly ash (FA) on the growth and seed yield of chickpea at Department of Botany, Aligarh Muslim University, Aligarh. Two chickpea varieties (BG-256 and Avarodhi) were evaluated against TPWW, GW and four fly ash-soil amendments (0, 10, 20 and 40%). Uniform dose of NPK fertilizers were applied and the seeds were sown. The results revealed that all growth and yield traits of chickpea were significant at ($P<0.05$) and positively influenced by TPWW and fly ash soil amendments; and chickpea varieties also responded differently. TPWW proved better as compared to GW, while fly ash (10%) showed better response in comparison to control i.e. FA_0 whereas, FA_{20} and FA_{40} proved deleterious for both the varieties of chickpea. However, least performance was resulted by the crop at control. In case of varieties, BG-256 showed its superiority over Avarodhi. It was concluded that the growth and seed yield of chickpea varieties improved with the application of TPWW and FA (10%); while chickpea variety BG-256 showed its superiority over Avrodhi for all the growth and yield traits. Hence, variety BG-256 may preferably be cultivated and along with TPWW, FA (10%) may be applied for maximizing the chickpea yields.

Keywords: Chickpea, thermal power plant wastewater, fly ash, NPK, yield.

INTRODUCTION
Grain legumes are a major source of protein in human and animal nutrition and play a key role in crop rotations in most parts of the world. Chickpea (C. arietinum L.) is the third most widely grown grain legume in the world after bean and soybean. The agronomical significance of chickpea depends on its high protein content (approx. 19.3– 25.4%) for the human and animal diet, being utilized increasingly more as an elective protein source. Moreover, Growth is generally a function of environmental factors (such as temperature and solar radiation) and mineral nutrition, along with genotype and production practices (Alam and Haider, 2006). Growth analysis is one way to verify the crops ecological adaptation to new environments, the competition between species, crops management effects and the identification of the productive capacity of different genotypes. The elements of dry matter distribution to different plant organs, their yielding and efficiency might be described by utilizing various indices of growth analysis (Zajac et al., 2005; Kibe et al., 2006). Growth investigation is as yet the most straightforward and exact strategy to assess the commitment of various physiological procedures in plant development. It provides a considerable insight into the functioning of a plant as depends on genotype or environment. The motivation behind growth analysis is the assurance of the expansion in dry matter alluded to a reasonable reason for photosynthetically active tissue, leaf area and measure of leaf protein (Ali et al., 2004; Gupta and Gupta, 2005; Alam and Haider, 2006; Yasari and Patwardhan, 2006).

Fly ash is produced by burning coal in thermal power plant and it poses a serious environmental hazard. Disposal of the huge amount of ash produced by burning of coal for energy purpose in different industry is a major concern today (Gautam et al. 2012). The disposal of fly ash by conventional methods leads to degradation of arable land and contamination of ground water therefore, development of proper technologies for disposal of this solid waste in an eco-friendly manner becomes essential to derive maximum benefit from its heterogeneous nature, since it is a store house of readily available plant macro and micronutrients (Gupta et al 2002). In combination with organic manure, microbial inoculants or fertilizers, fly ash can be used to design a soil benefaction strategy, which can help in improving soil properties and enriching its nutrient status. The presence of almost all essential plant nutrients in ionic form and the ameliorating effect on the physical, chemical and microbial nature of soil makes fly ash an important input for biomass production, especially on various degraded soils and waste land (Gupta et al 2002). Lower amendment levels of fly ash caused enhancements of both growth and yield while adverse effects at higher levels were observed for several crops including maize, soybean barley, cabbage, apple, alfalfa, beet (Kumar et al, 2002; Marten, 1971).

Water is visibly so much that its value is accounted low as it is believed that the water resources are inexhaustible or at least more than sufficient for all our needs. However, the habitable land areas have only limited fresh water resources, and only about 0.5% is present either as ground water or as surface water in lakes, rivers, ponds and dams etc. (Cunningham and Saigo, 1995). Contrarily, enormous amount of waste water is

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generated every year from urban population and industrialization and may cause environmental threat worldwide including India. Therefore, planned collection, treatment and disposal of the waste water are an important component in the protection of public health, surface soil and fresh water. The treatment of waste water is given low priority in India due to the financial constraints. Thus, alternative option appears to be reuse of waste water in agriculture for profitable crop production. This option may lessen the problem of water pollution and also serve as fertilizing components. The waste water contains nutrients of fertilizing value (Soumare et al., 2003) that enhances growth and yield of crop plants (Shah et al., 2004; Gupta et al., 2005; Javid et al., 2006). Therefore, keeping in mind the beneficial effects of TPWW and FA, two varieties (BG-256 and Avarodhi) were evaluated to find a suitable method for waste management and to reduce the use of inorganic fertilizers. Both the varieties were also tested to understand the varietal differences on the basis of growth and yield parameters.

MATERIALS AND METHODS

To achieve the aim, five pot experiment was performed in the net house of Environmental Plant Physiology, Department of Botany, Aligarh Muslim University, Aligarh. TPPW was collected from the outlet of the leachate reservoir of Harduaganj Thermal Power Plant, Kasimpur, located 13km away from Aligarh city, whereas tap water was the source of GW. Fly ash was also collected from the fly ash pond of the same thermal power plant. Each pot received 500ml water on alternate days for the duration of about 125 days starting from 10^{th} day after sowing (DAS) i.e. after seedling emergence. Four different concentrations of fly ash as 0, 10, 20 and 40% were thoroughly mixed with soil making the total of soil/fly ash weight up to 7kg pot^{-1}. The control consists of only soil without fly ash. Uniform starter basal dose of nitrogen (20kg ha^{-1}), phosphorus (20kg ha^{-1}) and potassium (20kg ha^{-1}) was also applied before sowing. The sources of NPK were urea, single super phosphate (SSP) and muriate of potash (MoP) respectively.

Seeds of BG-256 and viable *Rhizobium* culture (*Rhizobium sp*.) specific for chickpea were procured from Indian Agricultural Research Institute (IARI), New Delhi. Seeds of locally grown variety of chickpea (Avarodhi) were obtained from Agricultural Directorate, Aligarh. Healthy seeds were surface sterilized with absolute alcohol and dried in shade before applying the inoculum (Rao 1982). Before irrigation the water samples were collected and analyzed for physico-chemical characteristics (Table 1) adopting the procedures outlined in the standard methods (APHA 1998). Similarly, soil/fly ash samples were collected before the start of the experiment and analysed (Table 2) for standard physico-chemical properties (Jakson 1973; Ganguly 1951; Walkley and Black 1934; Dickman and Bray 1940; Chopra and Kanwar 1982; Richards 1954; Ghosh et al 1988).

For investigating the comparative effect of TPPW, GW and fly ash under inoculated conditions, observations were carried out at 60 days after sowing (DAS). For the study of the root, the plants were uprooted carefully and washed gently to clear all the adhering particles. For assessing dry weight, three plants form each treatment were dried, after taking their fresh weight, in hot air oven at 80ºC for two days and weighed. The area of leaves was measured using leaf area meter (*LA 211, Systronics, India*). For nodule number, whole plant was uprooted with the precaution that the roots or the nodules may not be damaged. Samples were washed gently to wipe away all the adhering foreign particles and the number was carefully counted.

NRA and chlorophyll were estimated (Jaworski 1971; Mac-kinney 1941). Healthy leaves were for the estimation of N, P and K contents (Lindner 1944; Fiske and Row 1925). Potassium was estimated with the help of flame photometer. At harvest, yield attributes including seed yield per plant, biomass, seed protein (Lowry et al 1951) and harvest index were measured.

The data for the growth and yield of each experiment were analysed statistically taking into consideration the variables (Panse and Sukhatme 1985). The 'F' test was applied to assess the significance of data at 5% level of probability ($p \leq 0.05$). The error due to replication was also determined.

RESULTS AND DISCUSSION

The results (Table 3) clearly indicate that TPWW proved better in enhancing all the parameters studied of both the varieties (BG-256 and Avarodhi) when compared with GW. While growth and yield parameters get benefitted by the lowest concentration of FA i.e. 10%, however higher concentrations of FA (20%, 40%) proved deleterious for chickpea. Regarding the performance of both the varieties of chickpea, BG-256 gave better results as compared to locally grown Avarodhi suggesting sowing of BG-256 for better production. Nitrogen is the single most important element limiting plant growth and is invariably required in large quantities deserves special consideration in this regard. As vegetative growth includes the formation of new leaves, stems and roots, the involvement of N through protein metabolism controls the growth (Table 3). On application to soil, most of the non-organic forms of N remain readily available for uptake, during vegetative plant growth. In comparison,

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only about 5-75% of the organic forms is commonly mineralized and that too in about one year after application (Sommers and Giordano, 1984). This lends support to the above observation of the suitability of wastewater as a good source of this nutrient. Another aspect that requires consideration here is the fact that both NH_4^+–N and NO_3^-–N were present in wastewater, the former being about five times more than the latter (Table 2). It is noteworthy that applied NH_4^+–N is toxic for some higher plants, including bean and pea (Maynard and Barker, 1969). However, in the presence of NO_3^-–N, it has been reported to benefit sunflower (Weisman, 1964) and wheat (Cox and Reisenaver, 1973). The observed nutritional superiority of wastewater (containing both NH_4^+–N and NO_3^-–N) for growth of chickpea in this study is thus not exceptional. Similarly, the presence of additional P in wastewater might have primarily influenced root growth (Tables 2&3). It is known that for the effective use of P, various factors operate together, such as rooting pattern, length of crop growth, soil characteristics including pH as well as dose and source of P, in addition to the presence of water. Since wastewater was one source of irrigation and was comparatively richer than the other source (groundwater) by about 58% in all experiments (Table 2) the observation of improved performance of the crop under wastewater is understandable. It is all the more noteworthy because application of phosphate fertilizers was its limitation as P fertilizer applied to the soil are very rapidly changed to less soluble forms and, therefore, become less and less available with time (Russel, 1950). Admittedly in short season crops, like some vegetables, growth responses to applied P may persist upto harvest. However, long season crops, like corn and chickpea, show only early growth responses and comparatively much lesser effect at seed formation and maturity. Frequent wastewater application until this late stage, therefore, enhanced P availability to the crop and ultimately lead to higher seed productivity (Table 3) in chickpea.

It is well known that N is fully utilized for crop production only when K is adequate (Mengel and Kirkby, 1982). The presence of K in almost double the amount in wastewater than in groundwater (Table 2). Therefore, benefited the treated crop not only due to its own physiological role (Wolf *et al*., 1976) but also by enhancing the effect of N. While it increased the chlorophyll content of alfalfa leaves and also the CO_2 exchange rate on plant^{-1} basis (Collin and Duke, 1981), it is not surprising that this nutrient (along with Mg) improved the chlorophyll content in the present study also (Table 3).

The presence of higher NPK contents in leaves (Table 3) grown under wastewater further confirm these observations. This ultimately led to increase seed yield (Table 3). In addition to N, P and K, presence of S also improves growth and N fixation (Walker and Adams, 1958). Therefore, in our study S as well as Ca and Cl present in wastewater (Table 2) might have contributed further towards enhanced growth and led to the promotion of the crop's yield. It may be pointed out that yield potential is the yield of a crop grown in an environment to which it is adapted and is provided with sufficient nutrients and water, in addition to other stresses being effectively controlled. Thus, considerable yield increases are possible by improving one or more physiological or morphological traits of crop, which in turn are dependent upon the availability of essential nutrients (Evans and Fischer, 1999). Obviously, all these were provided by the wastewater.

Nodule number and nodule dry weight was increased under wastewater. As pointed out earlier, with the increased amount of nutrients in the medium roots had a better chance to exploit them. This could not only result in increased root proliferation but also nodulation. Franco (1977) has cited several authors who obtained increased nodulation and N_2 fixation in legumes by utilizing optimum amounts of N in the medium. Similarly, frequent supply of additional P and K in the wastewater also play an important role in enhancing nodulation. In this connection the review by Andrew (1977) gives support to this report of the effect of P contention. Moreover, the importance of K for tropical legumes, specially in N_2 fixation by increasing either nodulation or nodule productivity (Duke *et al*., 1980) further strengthens our assertion. Add to it the role of Ca (Table 2) in symbiotic N_2 fixation (Lowther and Loneragan, 1968; Freire, 1977) and the picture becomes brighter.

Increase in NRA was observed (Tables 3). The presence of nitrate-nitrogen in the irrigation water (TPPW) as recorded in Table 2 could be mainly responsible for it. NR is a substrate-dependent enzyme (Afridi and Hewitt, 1964; Campbell, 1999). After absorption by roots the N was translocated to leaves (Table 3), which is a major site for its reduction. NRA seems to be indirectly affected by the presence of P in wastewater. P is involved in phosphorylation and diversion simple sugars towards respiration as a result of which oxidation of photosynthates produces more reducing power subsequently for nitrate-mediated NO_3^- reduction. In comparison to N and P, K is proved to be an activator of many enzymes including NRA (Suelter, 1970).

Similar to above observations, effect of wastewater was noted to be significant (Table 3). Increase in seed protein content was due to the presence of N, P and K in wastewater (Table 2). The data reveals that protein content of seeds of the plants grown with wastewater was at par with the seed protein content of those grown under GW. This was also observed earlier at Aligarh by Aziz *et al*. (1999) while working with petrochemical

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refinery wastewater. The reason may be traced to the "dilution factor". Because of increased seed yield, apparently due to enhanced send production in wastewater treated plants. Thus, the tendency to cross the level of significance was nullified by the dilution effect. However, it may be inferred that wastewater has neither deleterious nor beneficial effect on seed quality. This may still be considered as a plus point for wastewater irrigation of chickpea.

All the growth and yield parameters were found to be increased due to 10% fly ash application, whereas higher levels of FA (20% and 40%) proved deleterious for chickpea (Table 3). It has been supported that fly ash can increase the soil fertility by improving its texture (Chang *et al*., 1989) and water holding capacity (Sharma *et al*., 1990), thereby affecting the plant growth indirectly. Its most important direct role is to correct the nutrient balance in the medium (Hill and Lamp, 1980) as some of the naturally existing essential nutrients enrich it (Klein *et al*., 1975; Koakinen *et al*., 1975). It is known to be source of B (Wallace and Wallace, 1986), Ca (Martens and Beahm, 1976), Cu (Wallace *et al*., 1980), K (Martens *et al*., 1970), Mg (Hill and Lamp, 1980), Mo (Cary *et al*., 1983), S (Elseewi *et al*., 1978) and Zn (Schnappinger *et al*., 1975). Expectedly, it was due to the presence of these essential element in our fly ash samples (Table 1) that supplemented those supplied by the soil and wastewater. However, the benefit of fly ash proved only of limited nature as noted above. The decrease in yield was probably due to increased levels of sulphate, chloride, carbonate and bicarbonates (Table 1). Some toxic compounds i.e. dibenzofuran and dibenzo-p-dioxine mixture (Helder *et al*., 1982; Sawyer *et al*., 1983) and elements like Ni, As, Cd, Cr, Pb, Se, Zn, Cu (Wadge and Hutton, 1987) were reported to occur in fly ash might have also contributed towards the lesser yield under higher fly ash concentrations. Detrimental effects of higher levels of fly ash on plants have also been reported earlier due to either the phytotoxicity of B (Adriano *et al*., 1978) or a shift in the chemical equilibrium of the soil (Singh and Yunus, 2000).

Nodulation, like growth and yield, was increased on adding fly ash albeit upto a limited level (10%). More than 10% amendment decreased it due to variation in pH. At higher levels, toxic amounts of soluble salts released from fly ash seem to affect roots and rhizosphere adversely. It may also be added that high doses of fly ash added to the soil decrease the microbial activity due to change in soil salinity or concentrations of potentially toxic elements (Singh and Yunus, 2000). This could not only delay nodulation but also cause a decrease in their number as noted by Martensson and Witter (1990). NRA and leaf N, P and K were also decreased by higher doses of fly ash (Table 3). Although fly ash contained an extremely small amount of nitrogen, an increase in NRA by its application was observed in the present study. The presence of Mo (Cary *et al*., 1983) in fly ash and sufficient quality of available nitrogen in the soil (Table 1) might have accelerated the rate of NR activity. Considering the increase in seed protein content due to the application of fly ash (Table 3) the pressure of additional P and K in it may be responsible for it. This has also been reported by Bhaisare *et al*. (2000), Khan *et al*. (1996), MiLovsky (1992), Sriramachandrasekharan (2001). Similarly, due to phytotoxicity of some heavy metals and conversion of some trace elements like Mo and B into some inorganic complexes availability of nutrients including NPK was adversely affected under high levels of fly ash (Bilteanu *et al*., 1973). The ameliorative effect of nutrients present in the applied wastewater and fly ash, together with the N and P applied as fertilizers, was pronounced when interaction was considered. On defining interaction, Russell (1973) states that if two factors are limiting or nearly limiting growth, adding only one of them will have little effect, while adding both together will have a very considerable effect. In the context of crop plants, two such factors show a positive interaction if the response of the crop to both together is larger than the sum of responses to each separately. . It may be emphasized that wastewater and fly ash supplemented these nutrients thereby proving economically efficacious on the one hand and environmentally acceptable on the other. Among the significant interactions, low concentration of fly ash plus wastewater i.e. TPPW × FA_{10} proved beneficial due to the positive nutritional role played by the constituents of the wastewater products generated from the same source (Thermal Power Plant). As mentioned earlier fly ash was deficient in N which was amply compensated by the application of wastewater having sufficient nitrogen in the form of NH_4^+ and NO_3^- in the presence of low doses of N and P fertilizers. Crop species differ in their morphological and physiological characteristics as well as yielding ability in response to their surroundings. Genetic variability is supposed to be largely responsible for such observations (Frageria *et al*., 1991) although environmental factors do play a role. In agricultural crops, therefore, genetic potential must be of sufficient magnitude and flexibility so that they may be grown over a wide range of agroclimatic conditions. This accounts for differences in crop productivity and simultaneously allows a particular crop or cultivar to adapt itself to a particular environmental conditions (Lafever, 1981; Heinrich *et al*., 1983; Bruckner and Frohberg, 1987). Under fly ash treatments, BG-256 performed better than Avarodhi as growth of the former was enhanced by fly ash in general and FA_{10} in particular. The present investigation thus showed that due to the superior inherent genetic potential of BG-256, it proved more efficient in comparison to Avarodhi. However, it must be admitted that the amount and kind of nutrient applied for better

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growth and yield in particular crop, and even its species or cultivars, is important as the magnitude of differences various between the species well adopted to the same climate, same soil and in some cases even the same management. The superior performance of BG-256 was due to increased nodulation and dry weight as well as better developed root system (Table 3) in comparison to Avarodhi. It also showed enhanced leaf area resulting into higher matter accumulation (Table 3). The results showed that BG-256 significantly differed from Avarodhi in leaf NRA, chlorophyll content, leaf NPK contents, seed yield and seed protein contents (Table 3) under wastewater and fly ash. The better performance of BG-256 in this regard could be traced back to enhanced shoot dry weight and root dry weight – the most important criteria to access vegetative growth. The differences in N, P and K status of the two cultivars reflected their differential efficiency to absorb and accumulate these nutrients (Table 3). These findings thus provide a positive conclusion with regard to the objectives of the present study. Therefore, for the cultivation of chickpea, basal application of 10 kg fly ash ha^{-1} may be recommended under TPPW irrigation. Among the available varieties, BG-256 may be preferred for cultivation in this region (Western Uttar Pradesh, India). Finally, TPPW and fly ash, which is by all means waste product of Thermal Power Plant, may be profitably utilized for agriculture purpose.

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Table-1: Chemical characteristics of soil and fly ash before sowing. All determinations in mg l^{-1} in 1: 5 (soil-water extract) or as specified.

Soil		Fly ash	
Determinations		Determinations	
Texture	Sandy loam	CEC (meq $100g^{-1}$ fly ash)	9.20
CEC (meq $100g^{-1}$ soil)	2.78	pH	8.70
pH	7.6	Organic carbon (%)	1.42
Organic carbon (%)	0.382	EC (µ mhos cm^{-1})	990.00
EC (µ mhos cm^{-1})	226.00	NO^{-}_{3}–N (g kg^{-1} fly ash)	0.02
NO^{-}_{3}–N (g kg^{-1} soil)	0.217	Phosphorus (g kg^{-1} fly ash)	2.13
Phosphorus (g kg^{-1} soil)	0.109	Potassium	13.00
Potassium	16.00	Calcium	21.24
Calcium	32.37	Magnesium	16.37
Magnesium	18.66	Sodium	13.29
Sodium	11.01	Carbonate	12.37
Carbonate	19.65	Bicarbonate	51.86
Bicarbonate	62.17	Sulphate	26.13
Sulphate	16.34	Chloride	19.71
Chloride	22.43		

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Table-2: Chemical characteristics of ground water (GW) and thermal power plant wastewater (TPPW). All determinations in mg l^{-1} or as specified.

Determinations	Sampling			
	I		II	
	GW	WW	GW	WW
Ph	7.6	7.7	7.8	8.3
EC (μ mhos cm^{-1})	750	930	720	930
TS	934	1281	917	1314
TDS	519	622	504	630
TSS	423	674	431	687
BOD	17.28	70.34	16.16	68.29
COD	40.24	129.37	38.31	120.17
Mg	18.83	26.24	16.58	25.19
Ca	26.21	42.31	25.39	43.17
K	8.34	17.29	8.09	18.36
Na	16.30	47.20	16.11	48.34
HCO_3^-	62	90	60	86
CO_3	18	37	19	35
Cl^-	72.48	104.18	68.37	100.24
PO_4	0.73	1.15	0.65	1.04
NO_3–N	0.80	1.04	0.78	1.10
NH_3–N	2.58	5.27	2.41	5.22
SO_4	49	75	51	70

Table-3: Effect of ground water (GW) and thermal power plant wastewater (TPPW) on growth and yield parameters of chickpea (*Cicer arietinum* L.) grown with different levels of fly ash

N.B: Subscript values denote the amount of fly ash (FA) in kg ha^{-1}. A uniform basal dose of nitrogen, phosphorus and potassium at the rate of 20 kg ha^{-1} each was applied at sowing.

	Shoot length $plant^{-1}$ (cm	Shoot dry weight $plant^{-1}$ (g)	Leaf area $plant^{-1}$ (cm^2)	Root length $plant^{-1}$ (cm)	Root dry weight $plant^{-1}$ (g)	Nodule number $plant^{-1}$	Nodule dry weight $plant^{-1}$ (g)	Leaf nitrate reductase activity (μ mol g^{-1} fresh weight h^{-1})	Total chlorophyll content (mg g^{-1} fresh weight)	Leaf nitrogen content (%)	Leaf phosphorus content (%)	Leaf potassium content (%)	Seed yield (g $plant^{-1}$)	Biomass (g $plant^{-1}$)	Seed protein content (%)	Harvest index (%)
Avarodhi																
GW FA_0	34.81	4.34	596.72	10.91	0.188	19.08	0.033	346.93	1.070	1.303	0.149	1.906	2.41	6.84	19.79	35.21
$WWFA_0$	40.70	5.14	728.19	12.75	0.263	24.12	0.042	395.69	1.159	1.522	0.166	2.115	2.99	7.87	19.98	37.99
GW FA_{10}	39.82	5.59	896.49	13.76	0.282	24.38	0.047	423.71	1.258	1.487	0.176	2.236	3.07	8.34	21.26	36.82
$WWFA_{10}$	46.13	6.66	1100.60	16.33	0.395	31.38	0.060	497.74	1.377	1.768	0.198	2.496	3.88	9.77	21.42	39.72
GW FA_{20}	36.01	4.68	660.19	11.53	0.219	20.34	0.037	368.35	1.130	1.352	0.154	1.973	2.56	7.17	20.45	35.69
$WWFA_{20}$	42.11	5.56	811.62	13.40	0.306	26.08	0.047	402.68	1.227	1.579	0.172	2.211	3.19	8.28	20.62	38.51
GW FA_{40}	35.01	4.40	598.23	10.99	0.191	19.15	0.035	347.24	1.093	1.314	0.151	1.917	2.46	6.98	19.84	35.23
$WWFA_{40}$	40.97	5.17	729.42	12.85	0.265	24.18	0.044	395.84	1.168	1.530	0.168	2.121	3.07	8.07	20.05	38.03
BG-256																
GW FA_0	49.88	6.81	805.46	16.11	0.288	31.04	0.051	433.29	1.197	1.337	0.165	2.039	4.57	10.78	21.89	42.38
$WWFA_0$	58.40	7.81	1002.04	19.56	0.440	39.92	0.064	487.11	1.300	1.606	0.191	2.229	5.70	12.47	22.14	45.72
GW FA_{10}	57.62	8.86	1262.68	21.76	0.431	40.37	0.075	544.60	1.446	1.549	0.202	2.437	5.94	13.34	24.04	44.54
$WWFA_{10}$	67.34	10.34	1562.39	26.78	0.667	52.40	0.094	624.96	1.578	1.855	0.234	2.687	7.53	15.67	24.38	48.05
GW FA_{20}	51.57	7.30	923.60	18.00	0.336	33.74	0.059	480.08	1.287	1.402	0.174	2.154	5.03	11.70	23.18	42.99
$WWFA_{20}$	61.46	8.80	1157.10	22.21	0.502	43.69	0.074	526.39	1.399	1.688	0.204	2.350	6.29	13.56	23.51	46.37
GW FA_{40}	49.98	6.92	808.38	16.37	0.289	31.13	0.053	435.01	1.204	1.342	0.167	2.043	4.59	10.82	22.09	42.39
$WWFA_{40}$	58.68	7.94	1005.30	19.69	0.442	39.99	0.066	489.12	1.315	1.613	0.193	2.234	5.73	12.53	22.26	45.74

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DESIGN AND COMPARATIVE ANALYSIS OF WELLBORE CENTRALIZERS FOR CASING PIPE APPLICATIONS

Shubham Suri[1], Aman Gupta[2], Dr Rahul Malhotra[3]
Student[1], Guide[2] and Director/Principal[3], Swami Devi Dayal Institute of Engineering & Technology, Kurukshetra University Kurukshetra, Golpura, Barwala, Haryana

ABSTRACT
A centralizer is a mechanical device protected around the casing at different locations to keep the casing from touching the wellbore walls. Its outcome of covering centralization, a continuous annular go-ahead everywhere the casing allow cement to completely seal the casing to the borehole wall. It results in less friction between centralizer and hole pipe as the bows of welded type centralizer are curved by the dies and bows are not straightly in contact with whole pipe so there is no touching between bows and hole pipe. High resistance power is attained in the Welded semi rigid type centralizers by curving the bows which improves bow strength to hold the weight of hole pipe or outer pipe. The survey of Different Models is done in order to examine the Centralizer, Stop Collar and Float Equipment. As they are used in oil Field Industry so the motive of Testing of Centralizer is to analyze the Starting, Running and Restoring Force of Centralizer and Float Equipment for Ductility and high Pressure and High Temperature Test. For upgrading the Design of Centralizer, Stop Collar and Float Equipment, the Resulting Data from these Tests are intended to review the Quality and base.

Keywords: centralizer, design, bows, wellbore, casing

1. INTRODUCTION

A centralizer is a mechanical device protected around the casing at different locations to keep the casing from touching the wellbore walls. Its outcome of covering centralization, a continuous annular go-ahead everywhere the casing allow cement to completely seal the casing to the borehole wall. Casing centralization device assures the worth of a cementing work by dodging mud channeling and poor zonal separation. Centralizers can also influence in the movement of the casing and the avoidance of difference spike. Its method is extensive. It is expectable that 10 million centralizers are bulk-produced and used every year globally. Centralizer producers possibly desire to increase the demand for centralizers. To ensure keep casing from contacting the bore wall Casing centralizer is used. For following reasons Tool centralization is needed.

a) To prevent tool from hanging up on obstructions or blockage on wellbore wall.

b) To pass fluid efficiently and prevent excessive standoff.

There are four various kinds of centralizers.

a) Bow spring design

b) Semi rigid design

c) Rigid blade design

d) Mold on design

The separation of casing from the wall of the hole is called Stand-off or the level to which pipe is centered is known as Stand-off. If the casing is exactly centered than the stand-off is 100% and the 0% stand-off means the pipe is in contact with wellbore. The stand-off should be more than 67% throughout the casing string with respect to the API standards. Stand-off = C/ (A-B). For learning the deflection of casing we must study the force balance for pipe section. Two types of forces on casing:

a) Axial tension force at the ending, pushing casing upwards.

b) Gravitational force on the pipe body, pulling casing downwards.

The direction (upward or downward) of the net side force depends on the weight and tension of casing.

To set the position of the centralizer at an efficient place, three methods are used.

a) Specify spacing- 40 feet is the standard spacing between the two centralizers (1 centralizer per joint). The standoff at the central point is forever lower than on centralizer because of bow spring centralizer used here. The standoff at central point between centralizers is summation of casing sag among the centralizer and at bow spring compression at centralizers.

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b) Specify standoff- Stand-off is called the parting of casing from the wall of hole. The stand-off will be setup on the mid span between centralizer. The Specify standoff mode ensures minimum standoff will be setup. The "specify standoff" mode ensures minimum standoff of casing between centralizer. According to API the minimum 67% standoff will be required as per requirement.

c) Optimum spacing- Both approaches must have specifying standoff 70% with 20ft incremental space requirement to get the optimum position of the centralizer on pipe.

2. RESEARCH METHODOLOGY

Introduction of 5S

5S is the name of place of work involvement method that uses an inventory of five Japanese words: seiri, seiton, seiso, seiketsu, and shitsuke. 5S engage community through the exercise of 'Standards' and 'Discipline'. It is not just about maintenance, but focused to maintain the values & guideline to administer the group - all achieved by continuation & viewing respect for the office every day.

The 5 Steps are as follows:

a) Sort: reform & split the one which is desirable & not needed in the area.

b) Straighten: place items that are desirable so that they are complete & simple to use. Undoubtedly recognize locations for all items so that anybody can locate them & return them once the job is finished.

c) Shine: Clean the place of work & tools on a usual base in order to preserve values & spot the defects.

d) Standardize: re-examine the first three of the 5S on a common root and verify the state of the workplace using normal actions.

e) Sustain: maintain to the rules to preserve the standards & go on to improve every day.

Introduction of Seven QC Tools

The Seven Basic Tools of Quality is a description given to a predetermined set of graphical technique recognized as being most supportive in resolving issues allied to quality. They are called basic because they are appropriate for community with minor proper training in figures and because they can be used to resolve the vast mass of quality-related issues.

1. Cause-and-effect diagram (also known as the "fishbone" or Ishikawa diagram)
2. Check sheet
3. Control chart
4. Histogram
5. Pareto chart
6. Scatter diagram
7. Stratification (alternately, flow chart or run chart)

The Seven necessary Tools rest in distinction to other highly developed numerical methods such as survey sampling, acceptance sampling, statistical hypothesis testing, design of experiments, multivariate analysis, and various methods developed in the field of operations research.

Introduction of Kaizen

Kaizen is the practice of continuous improvement. Kaizen was originally introduced to the West by Masaaki Imai in his book Kaizen: The Key to Japan's Competitive Success in 1986. Today Kaizen is recognized worldwide as an important pillar of an organization's long-term competitive strategy. Kaizen is Continuous Improvement that is based on certain guiding principles:

1. Good processes bring good results
2. Go see for yourself to grasp the current situation
3. Speak with data, manage by facts
4. Take action to contain and correct root causes of problems
5. Work as a team
6. Kaizen is everybody's business
7. And Much more

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3. PROPOSED WORK

Welded bow type centralizer are made up of cast iron and available in various size and made by different types of materials. These centralizers have high resistance power and long lasting. In the welded type centralizer bows are made under high temperature conditions with correct grade electrodes. In this type bows are not directly in contact with hole pipe so no contact between bows and hole pipe. So this results the less friction between centralizer and hole pipe. The bows are of high quality alloy steel and change into required shape by use of dies and then heat treatment will be done under temp/time cycles for required spring characteristics. These centralizers are used where stress points are few.

Table-1: Parameters of Welded Type Centralizer

Size	6" X 24"
Weight	5.400 Kg
Height	24 inches
Thickness	5 mm

Fig-1: Welded type Centralizer

Welded semi rigid bow spring centralizer

The semi rigid bow spring centralizer are good robust design, reliable and are of high performance. These are made up of cast iron and available in various size. The highly treated bows are welded on end collar of centralizer. The bows are first bent as per requirement with the help of dies then we do heat treatment of bows at temp/time cycle under requirements. The small area that bent are in contact with hole pipe which increases friction between centralizer and hole pipe. These type of centralizers are used where stress points are large or where stress is too high.

Fig-2: Welded semi rigid type centralizer

Table-2: Parameters of Welded semi rigid type centralizer

Size	6" X 24"
Weight	4.500 Kg
Height	24 inches
Thickness	5 mm

4. Results and Analysis Starting Force Test

The starting force indicates the detailed force required to put the inner pipe into the outer pipe (after make up for the weightiness of the inner pipe and additions).

a) First step to test starting force of centralizer mount the centralizer in fully assembled condition. We must ensure that centralizer assembled in same manner as used in actual service.

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b) The test should be performed at an angle of 5 degree of vertical position.

c) The contacting area of centralizer to the outer pipe must lubricate with petroleum based grease so that centralizer must move easily.

d) Apply the load by universal testing machine (UTM) on the inner pipe to insert centralizer into outer pipe.

e) Then take the readings at various load/time conditions. Check the maximum force that is required to fully insert the centralizer inside the outer pipe.

f) Check whether the centralizer was pulled or pushed into the outer pipe

g) Check the holding device used to conduct the starting force test.

Running Force

The running force signifies the extreme force needed to move the inner pipe inside the outer pipe when the force reading has become fixed (after reimbursing for the heaviness of the inner pipe and additions

a) Install First step to test starting force of centralizer mount the centralizer in fully assembled condition. We must ensure that centralizer assembled in same manner as used in actual service.

b) The final outcome of this test is not compulsory to note the maximum value. This test should be performed and final outcome will be recorded.

c) This test can be done when the starting force test going on or do this test separately.

d) Take different readings of force at different period of time when centralizer is moving inside the outer pipe until the inner pipe is fully placed.

e) Write down the maximum force as the running force after reparation as in 1.

Restoring Force

The restoring test can be used to test the bows strength that how much force absorbed by the bows of centralizer.

a) The restoring test can be done by setting up the pipe at an angle of 5 degree horizontal.

b) The force applied to the bows up to 12 times to check strength ability of bows.

c) Put the load by universal testing machine to the outer pipe so that load will be transported to the inner pipe vertically over the point of contact of the centralizer with the outer pipe

d) Put on load and read load-deflection analyses at least possible of 1, 6 mm (1/16 in) rises until three times (□5 %) the smallest restoring force has been attained. The travel distance to obtain 67 % standoff shall be discover out for each test position.

e) Repeat the process, testing the centralizer until each spring and each set of springs has been tested in situations 1 and 2 as shown in Figure.

f) Determine the total load at each deflection by recompensing for the mass of the move pipe and attachments.

g) Make the final load-deflection curve using the calculation average of the force readings at corresponding deflections. Restoring force shall be find out from this curve at 67 % standoff ratio.

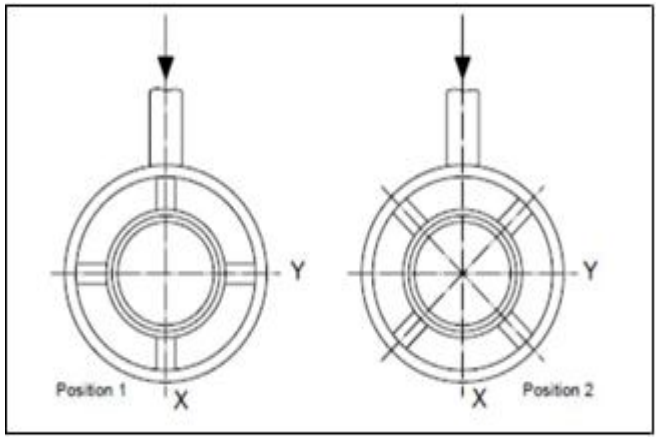

Fig-3: Example of Casing Centralizer for Restoring Force Equipment

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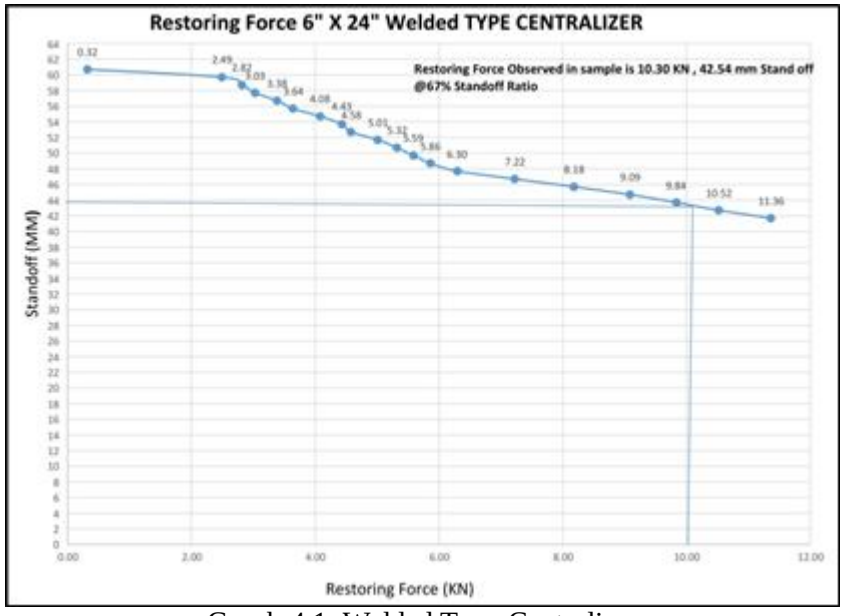
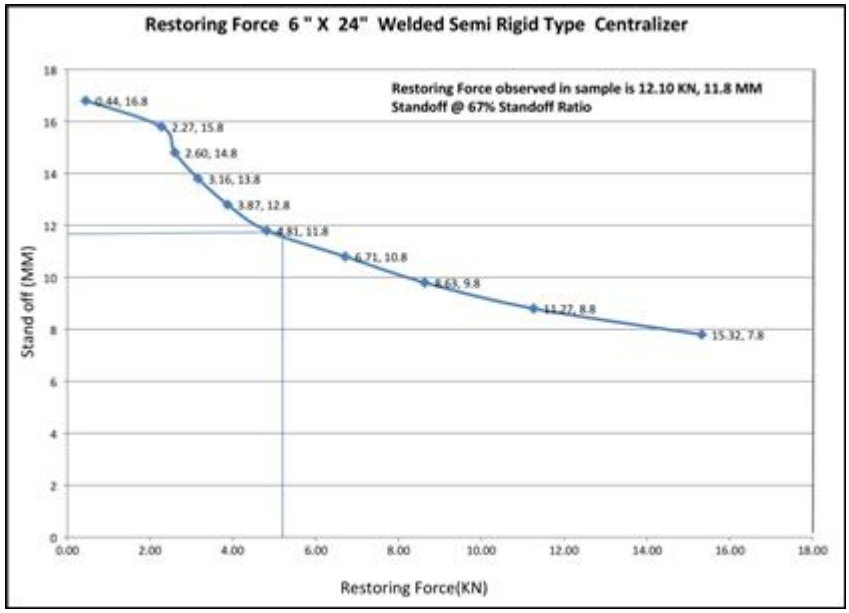


Graph-4.1: Welded Type Centralizer



Graph-4.2: Welded Semi Rigid Type Centralizer

Compression Test of Sledge Chakra

Compressive strength is the ability of a material or assembly to endure loads inclining to decline size, as opposite to tensile strength, which persists loads tending to extend. In other words, compressive strength repels compression (being pushed together), whereas tensile strength struggles tension (being pulled apart). In the study of strength of materials, tensile strength, compressive strength, and shear strength can be analyzed independently. Compressive strength can be measured by plot graph between applied forces compared to deformation on a universal testing machine.

Table-3: Results of Compression Strength

TEST	RESULT
Load At Peak	140.740KN
Elongation at Peak	10.130MM
Compression Strength	15.667N/MM^2

5. CONCLUSION AND FUTURE SCOPE

A Welded semi rigid bow spring centralizer for wellbore applications is proposed. This type of centralizer is flexible and can operate in various wellbore applications. Introducing bending of bows improve bow strength to hold the weight of outer pipe or hole pipe. A Welded semi rigid bow spring centralizer provides larger Starting

force, running force. These types of centralizers also have more absorption energy than welded type centralizer. The nut bolt attached to both sides of centralizer which removes the usage of stop collar. The main highlights of this thesis are the welded semi rigid bow spring centralizer of proposed structure with given dimensions promise the large restoring force and larger starting and running force than welded type centralizer. Future scope of these centralizers are functions as centralization and mud removal. It is one of the most important factors in obtaining a good cement job. Effective centralization assists in mud removal and helps ensure an even cement coat around the casing. Certain running procedures, such as pipe reciprocation and rotation, improve the mud displacement process. Centralizers for horizontal wells have to fulfill two requirements: They should have a high restoring capability and a low moving force, and they should allow pipe rotation and reciprocation. Conventional bow-type centralizers have been used effectively in some horizontal wells. But as the horizontal section length increases, special centralizers, such as low-moving-force, bow-type centralizers and rigid centralizers, may be essential. The welded semi rigid type these centralizer with stop collar is designed for future wellbore applications with API Specifications.

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PARAMETRIC STUDY OF PERFORMANCE AND EMISSION CHARACTERISTICS FOR 4S SI ENGINE USING CURCUMA LONGA L LEAVES BASED BIOFUEL

Manish S Deshmukh[1] and Dheeraj S Deshmukh[2]
Associate Professor[1], ME Department, AISSMS College of Engineering, Pune, Maharashtra
Associate Professor[2], ME Department, G H R College of Engineering, Nagpur, Maharashtra

ABSTRACT
This paper describes an experimental study concerning the feasibility of using bio-oil namely turmeric leaves-based oil obtained from the resin of turmeric plants. The emission and performance characteristics of a 4-stroke spark ignited engine were studied by using gasoline and turmeric leaves oil. The performance and emission parameters of both the fuels were evaluated and compared. The performance parameters investigated were torque, brake mean effective pressure (BMEP), brake power, specific fuel consumption (SFC), and thermal efficiency. Carbon monoxide (CO), carbon dioxide (CO2), hydrocarbons (HC) and oxides of nitrogen (NOX) exhaust emissions levels are also presented. The results showed that torque and BMEP were slightly lower when the turmeric leaves bio-fuel was used as fuel as compare to gasoline on all engine speeds.

Keywords: Curcuma longa leaves oil, biofuel, 4S SI Engine, emission and performance analysis.

I. INTRODUCTION
The world energy demand has for the last two decades, witnessed uncertainties in two dimensions. Firstly, the price of conventional fossil fuel is too high and has added burden on the economy of the importing nations. Secondly, combustion of fossil fuels is the main culprit in increasing the global carbon dioxide (CO_2) level, a consequence of global warming. The scarcity and depletion of conventional sources are also cases of concern and have prompted research world-wide into alternative energy sources for internal combustion engines. Biofuels appear to be a potential alternative "greener" energy substitute for fossil fuels[1].

Petroleum resources are finite and therefore search for alternative is continuing all over the world. Development of bio-fuels as an alternative and renewable source of energy for transportation has become critical in the national effort towards maximum self-reliance- the corner stone of our energy security strategy.

The production of the biofuels from the various food sources results in many problems such as, increasing food prizes and worldwide food crisis. These problems forced the researchers to look for the new sources of alternative fuels and these new alternatives are known as Second generations biofuels. This project work is all about to give a better second generation biofuel.

Turmeric (*Curcuma longa*) (Family: *Zingiberaceae*) is used as condiment, dye, drug and cosmetic in addition to its use in religious ceremonies. The rhizomes of turmeric are used in many ways but the leaves of the turmeric are having no use so far. However, in this work we are proposing to extract the oil from the turmeric leaves and used the oil as fuel for the petrol engine[2].

II. COMPOSITION OF TURMERIC LEAVES OIL
The turmeric leaves oil is extracted from the agricultural waste of the turmeric corp. by using the leaves we are producing a volatile oil by the hydro-distillation process. Leaf samples of *C. longa* and *C. aromatic* on hydro distillation yielded 1.32 and 1.00% essential oil respectively, containing *a*-phellandrene (38.24%), C8- aldehyde (20.58%), 1,8-cineole (8.64%), *a*-pinene (2.88%) and *b*-pinene (2.36%) in *C. longa*, and 1,8-cineole (28.01%), linalool (7.67%), *a*-pinene (4.74%), *b*- pinene (3.70%) and C8-aldehyde (2.62%) in *C. aromatica*, as confirmed by GLC analysis. The major compounds of *C. longa* leaf oil samples were *a*- phellandrene and C8-aldehyde. Such predominance of C8-aldehyde is novel. In *C. aromatica*, the major compounds are 1, 8-cineole (28.01%) and linalool (7.67%). The aroma of turmeric is due to its volatile oil, while the phenolic compounds and its analogues account for its bright yellow colour. Due to its lower commercial importance, the chemistry of turmeric oil has not received much attention earlier. Kelkar and Sanjeev Rao (1933) reported that steam distilled volatile oil is predominantly a mixture of sesquiterpene ketones and alcohols. Malingre (1975) reported p-cymene, b-sesquiphellandrene, turmerone, arturmerone and sesquiterpene alcohols from C. longa. The turmeric oil mainly consist of the phenols which are some kinds of alcohols[3].

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III. CHEMICAL AND PHYSICAL PROPERTIES OF FUEL

PROPERTIES	GASOLINE	TURMARIC LEAVES OIL	ETHANOL	METHANOL
Typical formula	$C_{6.97}H_{14}$	$C_{21}H_{20}O_6$	C_2H_5OH	CH_3OH
Molecular weight	106.22	368.3799	46..07	32.04
Density (kg/m3)	750	925 at 20° c	785	792
Research octane number	95.8	Not determined	107	106
Motor octane number	85	Not determined	89.7	88.6
Kinematic viscosity (mm2/s)	0.494 at 40°c	0.782 at 25° c	1.221 40°c	0.596 40°c
Heating value (kJ/kg)	42,600	43671.13	26,700	19,850
Flash point (°C)	95-100	102		
Fire Point (°c)				
Pour point (C)	<=-30	<=-20	<=-25	<=-20

Table-1: Comparison of properties of Turmeric leaves oil with Gasoline and other alternative

The chemical characteristics play a very important role in case of fuel all the properties are explained in the above section along with their importance in combustion. In this section we will compare the properties of the turmeric leaves oil with the gasoline and other alternate oils. The table shows the various properties of all the turmeric leaves oil, gasoline and other alcohol-based fuel.

The turmeric leaves oil has a higher Calorific value as compare to the gasoline and alcohol-based oils. The density of the turmeric oil is slightly higher but it can be compensated as it reduces as the temperature increases and also it can be reduced by adding some additives. The turmeric leaves oil has equivalent properties with the gasoline and also it is having superior quality as compare to the other alternative oils and hence this fuel will be better alternative for Gasoline[4].

IV. EXPERIMENTAL SET-UP

The figure shows the details of the experimental test rig. These test rigs is used for the evaluation of the performance and emission characteristics of turmeric oil and are compared with the gasoline. The emission coming out from the engine is analyzed with using AVL 4 gas analyzer.

Experiments were carried out on a single cylinder 150cc engine. The engine is four-stroke, single cylinder, spark ignition and naturally aspirated. Bore and stroke are 76mm and 60mm respectively the engine works at a compression ratio of 8:1. Maximum power is 14bhp at 5500 RPM and maximum torque 1.42 kg-m at 6000 RPM. Intake valve opening at 10^o BTDC and closing at 49^o ABDC and exhaust valve opening and closing takes place at 55^o BBDC to 12^o ATDC respectively.

Engine type	single cylinder, 2 Valve, SOHC
Bore X stroke (mm)	76mm X 60mm
Displacement volume (cc)	150cc
Compression ratio	8:1
Maximum torque (kg-m/rpm)	1.42 kg-m 6000 RPM
Maximum power (kW/rpm)	14bhp at 5500 RPM
Maximum speed (rpm)	7000 RPM
Cooling system	naturally aspirated

Table-2: Engine Specifications

The test engine was coupled to an eddy-current dynamometer for measuring engine speed & load.

Mass of air intake is measured by air box method with U-tube water manometer and exhaust gas mass flow rate measured by exhaust gas calorimeter.

The two separate fuel tanks were used for gasoline and turmeric leaf oil. Two separate fuel-metering systems were provided to meter both primary fuel and pilot fuel. Fuel consumption of an engine was measured manually by a graduated burette.

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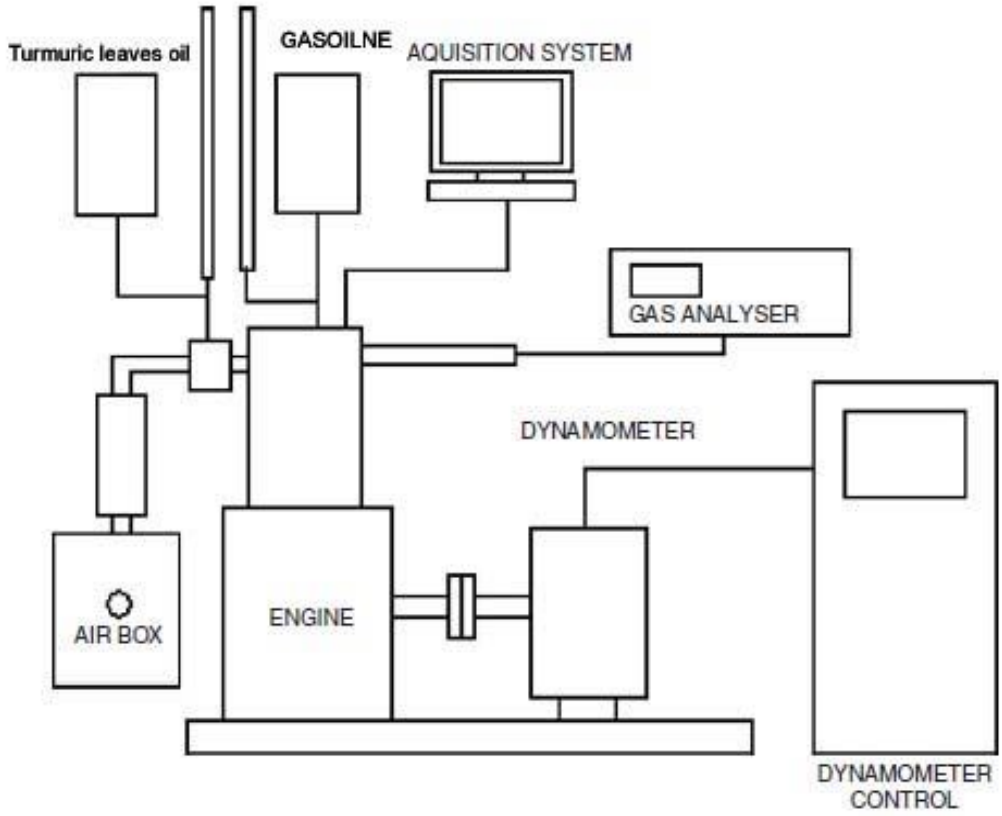
An orifice meter attached with an anti pulsating drum measures air consumption of an engine with the help of U tube manometer.

Data were collected simultaneously from sensors and sent to data acquisition system. Also, data from engine torque and exhaust gases were recorded which included, the concentration of NOx, unburned Hydrocarbon (UBHC), Co_2, Co and O_2 in exhaust emission.



V. EXPERIMENTAL METHOD

1. The whole test was conducted for the standard engine pressure and intake timing.
2. There are two separate fuel tanks are used for gasoline and Turmeric leaves oil.
3. There were two separate fuel-metering systems provided with test rig to measure primary and pilot fuel consumption, respectively.
4. The first test was conducted using unleaded gasoline emission, for determining fuel consumption and performance.
5. First 25% load was applied; consequently, speed of the engine decreases.
6. Then the second fuel i.e. turmeric leaves oil is used and fuel consumption and performance are determined.
7. Before taking the results, the engine is kept running for some time on the turmeric leaves oil so as to remove any presence of gasoline in the fuel systems.
8. Fuel consumption and cylinder pressure were measured at each load.
9. Emission is measured on all loads by using AVL 4 gas analyzer.
10. 50%, 75% and 100% loads were applied one by one and steps 8 and 9 were repeated for each load and each fuel.

VI. EXPERIMENTAL RESULTS

A. Engine power

When the turmeric leaves oil used the engine power slightly decreased for all engine speeds. The loss of power can be attributed to the low calorific value, which is slightly lower as compare to gasoline. the density & volumetric efficiencies is more and are also responsible for engine power. The brake engine power for gasoline and turmeric leaf oil at engine speed of 2200 RPM is 2.00 kw & 1.938 kw respectively.

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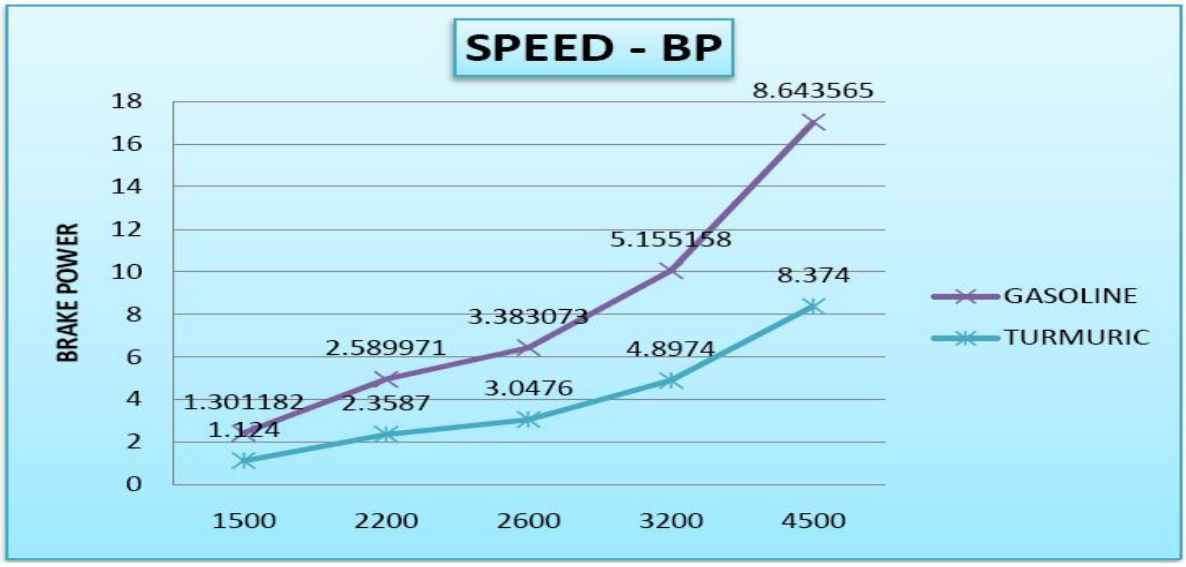
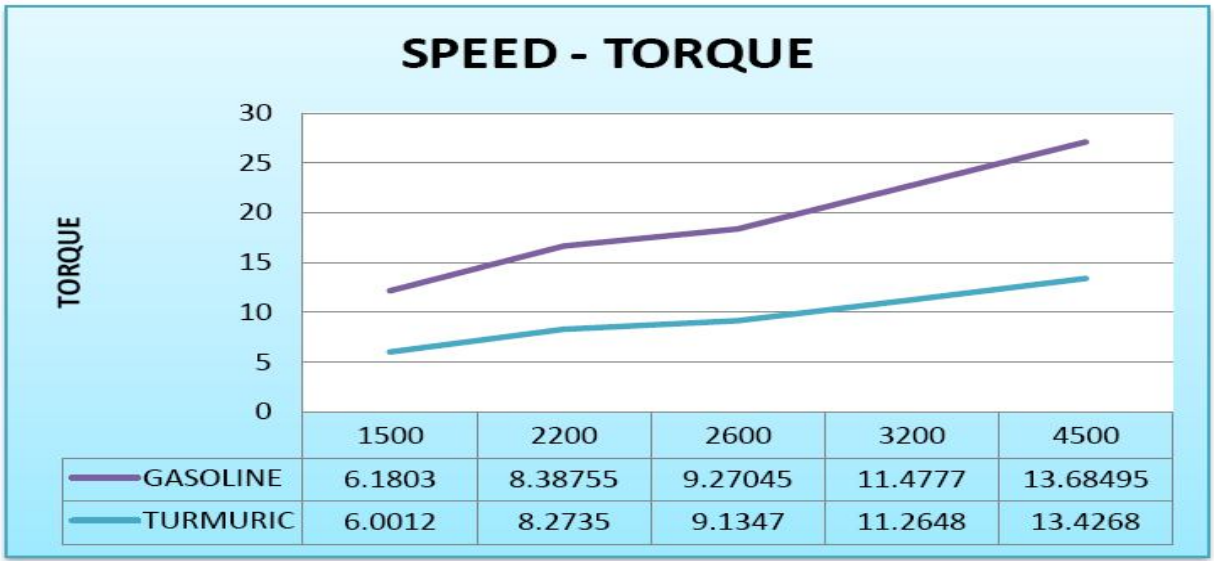
Figure-1: Experimental Set-Up



B. Torque output

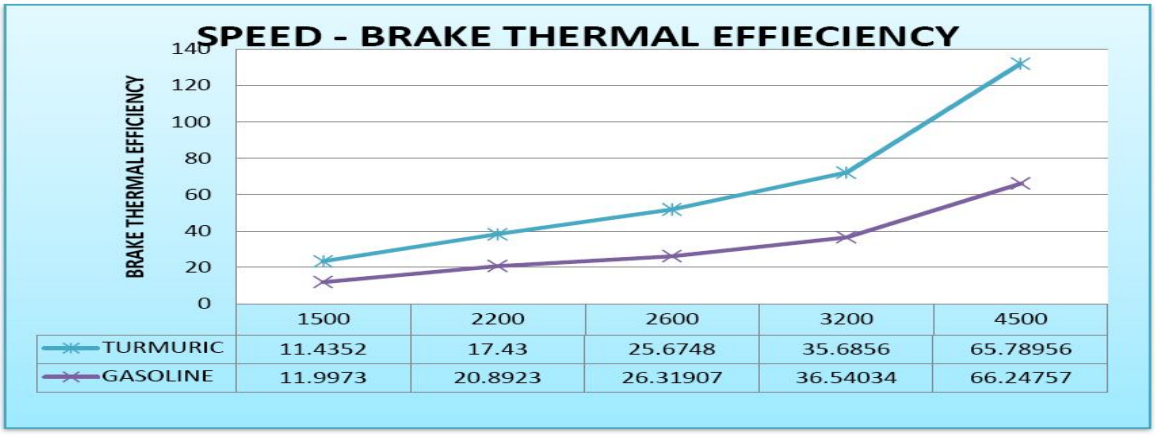
The torque output of engine with turmeric leaf oil is 8.39 N-m turmeric leaves oil produces slightly rich mixture that produces more power the rich mixture allows a more advanced timings that results in high combustion pressure and thus higher torque.



	1500	2200	2600	3200	4500
GASOLINE	6.1803	8.38755	9.27045	11.4777	13.68495
TURMURIC	6.0012	8.2735	9.1347	11.2648	13.4268

C. Brake thermal efficiency

A brake thermal efficiency of engine with, turmeric leaves oil is 14.92% which is slightly less as compare to gasoline which is 17.1.14% as the engine increase the brake thermal efficiency increases and it is maximum at 4500rpm for both gasoline & turmeric oil.



	1500	2200	2600	3200	4500
TURMURIC	11.4352	17.43	25.6748	35.6856	65.78956
GASOLINE	11.9973	20.8923	26.31907	36.54034	66.24757

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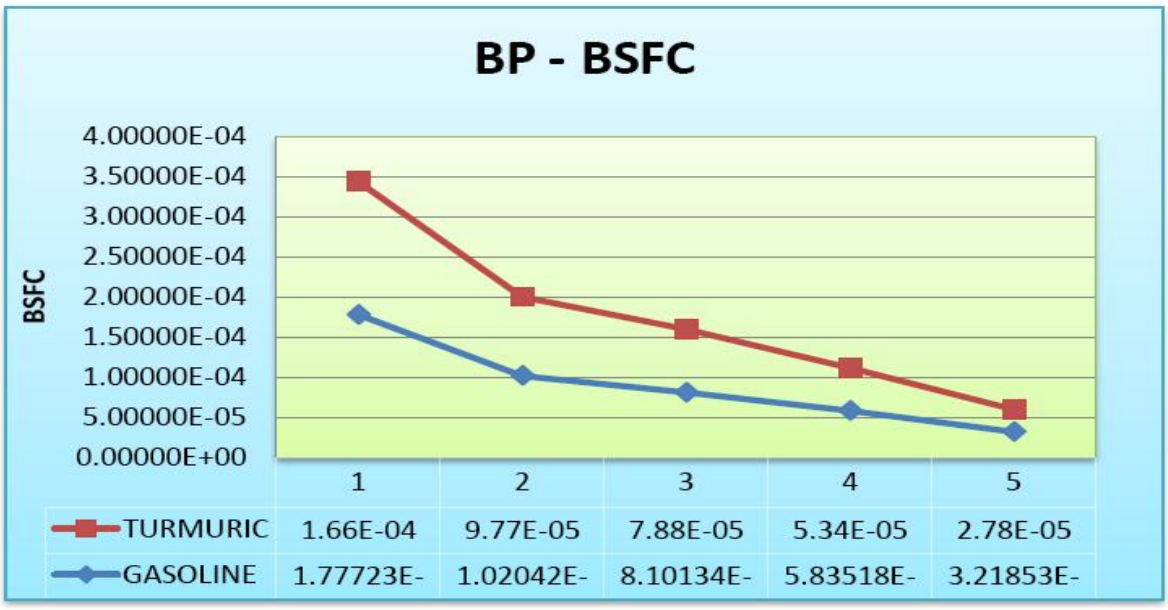
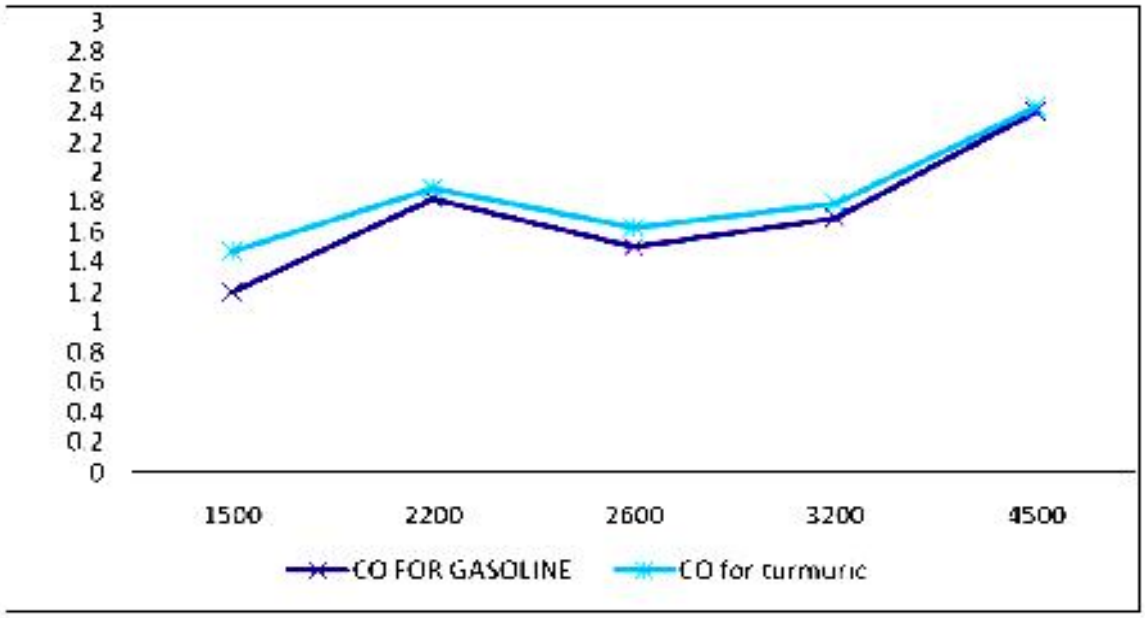
D. Brake specific fuel consumption (BSFC)

The BSFC for lower engine speed is more, then it start decreasing and, it is minimum at engine speed of 3500rpm, and then its starts increasing the BSFC for the turmeric leaf oil is 0.5517kg/kw-hr and for petrol it is, 0.4757.1 kg/kw-hr. This is due to the increased in brake thermal efficiency.



Engine emission studies

CO emission



The concentrations of CO emission for different engine speeds are evaluated by AVL gas analyzer. The CO concentration in the exhaust gas emission at 3000 rpm for gasoline fuel was 4.69 (%V), while the CO concentration of turmeric leaves oil at 3000 rpm was 4.05 (%V). The CO concentrations at 3000 rpm using turmeric leaves oil was decreased by 13.7%, in comparison to gasoline. The significant reason of this reduction is that the oxygen content in the blended fuels increases the oxygen-to-fuel ratio in the fuel-rich regions. The most significant parameter affecting CO concentration is the relative air–fuel ratio (k).

E. CO_2 emission

CO2 emission depends on relative air–fuel ratio and CO emission concentration. The CO2 concentration in the exhaust gas emission at 3000 rpm for gasoline fuel was 12.4 (%V), while the CO2 concentration of turmeric leaves oil at 3000 rpm was 12.9. The CO2 concentrations at 3000 rpm using turmeric leaves oil was increased by 3.87 in comparison to gasoline.

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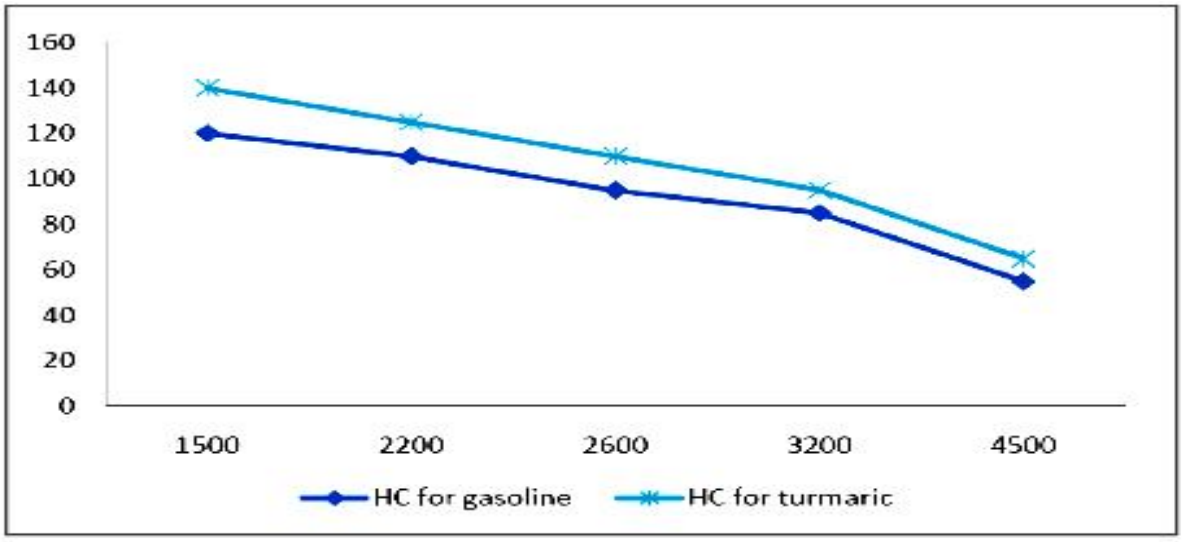
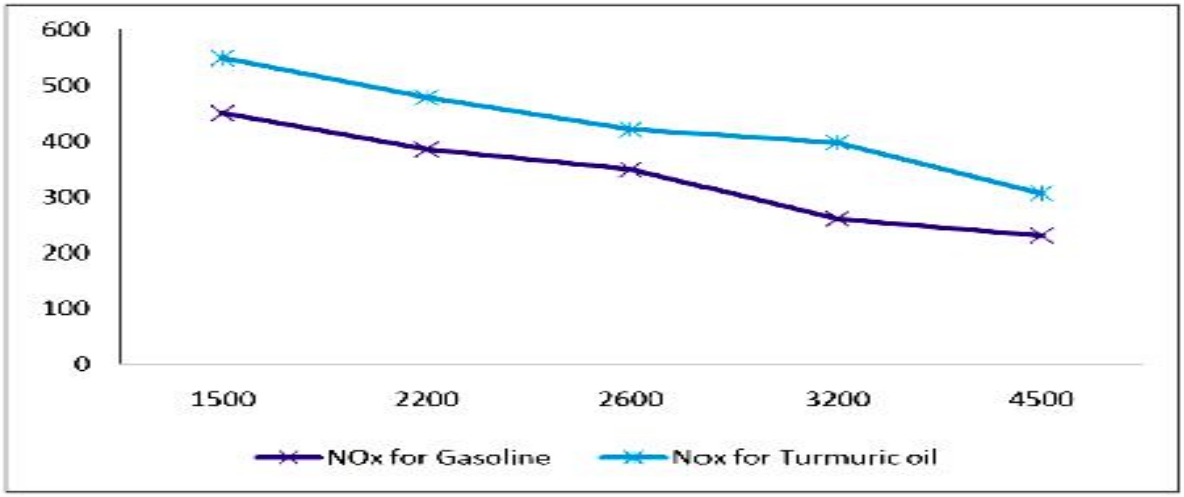
F. HC emission



The HC concentration in the exhaust gas emission at 3000 rpm for gasoline fuel was 183 ppm, while the HC concentration of turmeric leaves oil at 3000 rpm was 152. The HC concentration at 3000 rpm using turmeric leaves oil was decreased by 16.94% at 3000 rpm, in comparison to gasoline. This result indicates that turmeric leaves oil can significantly reduce HC emissions. The concentration of HC emission decreases with the increase of the relative air–fuel ratio, the reason for the decrease of HC concentration is similar to that of CO concentration described above.

G. NOx emission



Considering the NOx emission, it is found that the NOx concentration is higher when turmeric leaves oil is used. The NOx concentration in the exhaust gas emission at 3000 rpm for gasoline fuel was 876 ppm, while the NOx concentration of turmeric leaves oil at 3000 rpm was 1002. The NOx concentrations at 3000 rpm using turmeric leaves oil was increased by 12.57% in comparison to gasoline. When the combustion process is closer to stoichiometric, flame temperature increases, therefore, the NOx emission is increased, particularly by the increase of thermal NO.

VII. CONCLUSION

In this project we look for the development of the new source of the Alternative fuel. We used the agricultural waste of the turmeric crop and we evaluated the fuel on the single cylinder spark ignited engine and compared it with the gasoline.

When the turmeric leaves oil used the engine power slightly decreased for all engine speeds. The loss of power can be attributed to the low calorific value, which is slightly lower as compare to gasoline. A brake thermal efficiency of engine with, turmeric leaves oil is 14.92% which is slightly less as compare to gasoline which is 17.1.14%. The BSFC for lower engine speed is more, then it start decreasing and, it is minimum at engine speed of 3500rpm, and then its starts increasing the BSFC for the turmeric leaf oil is 0.5517kg/kw-hr and for petrol it is, 0.4757.1 kg/kw-hr. This is due to the increased in brake thermal efficiency.

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The CO concentrations at 3000 rpm using turmeric leaves oil was decreased by 13.7%, in comparison to gasoline. The CO2 concentrations at 3000 rpm using turmeric leaves oil was increased by 3.87 in comparison to gasoline. The HC concentration at 3000 rpm using turmeric leaves oil was decreased by 16.94% at 3000 rpm, in comparison to gasoline. The NOx concentrations at 3000 rpm using turmeric leaves oil was increased by 12.57% in comparison to gasoline.

we can concluded that the turmeric leaves oil can be a good alternative fuel for the spark ignited engines. We have found this fuel eco-friendly and economical as compare to the other alternative fuels such as ethanol, methanol etc. we found that the performance of the engine is reduces slightly when turmeric leaves oil is used as fuel. The turmeric leaves oil gives a lower emission which is under the Emission norms.

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STUDIES ON "*O*-NITROBENZALDEHYDE DERIVATIVE OF 1, 2-DIPHENYLETHANE-1, 2-DIENE HYDRAZONE OXIME

Madhuree K. Jagare[1*], Raj R. Badekar[2], S. W. Kulkarni[3] and R. S. Lokhande[4]
[1,4]School of Basic Sciences Jaipur National University, Jaipur
[2]RIVA Industries, Ambernath MIDC, Thane, Maharashtra
[3]Department of Chemistry, K. M. Agrwal College, Kalyan

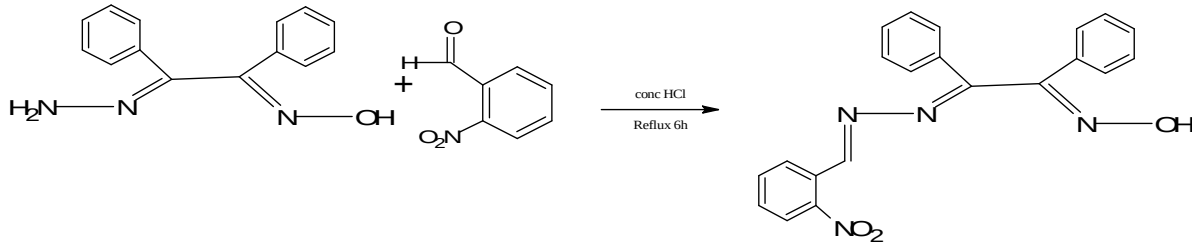
ABSTRACT
Reaction between hot ethanolic solution of α-benzilmonoximehydrazide (HBMOH) and o-nitrobenzaldehyde (o-NBA) yields Benzilmonoximehydrazide-o-nitrobenzaldehydes (HBMHONB). Synthesized compound were characterized on the basis of various physico-chemical and spectral techniques such as UV-Visible, PMR and IR spectra.

INTRODUCTION
Schiff's base is the organic compound in which aldehyde or ketone like compounds in that, the carbonyl group is replaced by an imine or azomethine group[1]. Schiff bases and their metal complexes are used in various biological systems as a catalyst as well as in the manufacturing of polymers and dyes also.[2-6]

α-Benzilmonoximehydrazone is one of the example of Schiff base derived compound and its various metals complexes are studied recently.[3-8] In view of this we wish to report *o*-nitrobenzaldehyde derivative of 1, 2-diphenylethane,1-2-diene hydrazone oxime. The structure of synthesize compound elucidated by various physico-chemical and spectral studies. The IUPAC name of the title compound is 2-(2-nitrobenzylidene)hydrazinylidene-1,2-diphenylethanimine for sake of convenience able as HBMHNB.

MATERIAL AND METHODS
All chemical used were of analytical reagent grade. Distilled water obtained from a glass distillation unit. UV-visible spectra of the compound were recorded on JASCO V-650 spectrophotometer, methanol and 0.1N NaOH was used as a solvent to record UV- spectrum of the compound. FT(IR)spectra KBr discs were recorded on Perkin-Elmer spectrum 100 model. PMR spectra were recorded on Brucker AV300 NMR spectrometer using TMS as internal standard.

PREPARATION OF COMPOUND
α-Benzilmonoxime was prepared by reported by method[4-8]. The title compound was prepared by hot ethanolic solution of 3.107gm.(0.013 mol) of α-benzilmonoximehydrazide (HBMOH) and 2ml conc HCl was added in 100ml three necked RBF(Round Bottom Flask), stirred 15min, then added 2.012gm(0.0135mol) of *o*-nitrobenzaldehyde (*o*-NBA) under stirring. Final mixture was reflux 6 hours and after refluxed, the reaction mixture was cool, filters the precipitated washed with hot water.



RESULT AND DISCUSSION
Characterization of the prepared compound is done by using analytical data obtained from UV-VISIBLE, FT(IR), ^{1}H NMR spectroscopy and elemental analysis etc. The molecular weight of proposed compounds is 372gmol^{-1} determined by Rast method[9]; they are melts at 210-215^{0}C. They are yellowish brown crystalline solids, soluble in common organic solvents such as, methanol, chloroform, acetone, DMF, DMSO, dioxane, dilute alkali etc. partially soluble in ethanol. Structural studies of the synthesized compounds of FTIR, PMR, UV-VISIBLE spectroscopy and elemental analysis etc. the prepared compounds are monobasic in nature by compounds-KOH titration curve.

ELEMENTAL ANALYSIS

%	C	H	N	O
Theoretical	67.7	4.3	15.0	12.8
Observed	66.3	4.1	14.7	12.4

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SPECTRAL MEASUREMENTS

A) UV- Visible spectrum (In methanol Solvents)

The electronic spectrum of HBMHONB in methanol for the UV region shows two high intensity bands at 264nm and 227nm respectively. These bands are due to π→π* transition possible for oximino and Azomethine group in the synthesized molecules.

Table-1: UV-Visible spectra of the *o*-nitrobenzaldehyde derivatives α-Benzilmonoximehydrazide

λ nm	Abs.	ε	Transition	Assignment
264	1.505	20476	π→π*	Oximino group > C=N-O
227	0.445	6054	π→π*	Azomethine group > C=N-N

B) FT(IR) Spectrum

In FT(IR) spectrum of HBMHONB compound, absence of band between 3300 - 3350cm^{-1} due to the –NH_2 vibration reported[8] at 3387cm^{-1} in α-Benzilmonoximehydrazide, indicates a successful replacement of the amino group by the hydrazonyl group during Schiff base formation. The spectrum of HBMHONB shows peak at 3235.97cm^{-1}, assigned as the hydroxyl group of the oxime. The bands at 1616.06 and 1571.70 are due to (C=NN) and (C=No) i.e. Azomethine and oximino respectively.

Table-2: FT(IR) spectra of the *o-nitro*-benzaldehyde derivatives α-Benzilmonoximehydrazide in cm^{-1}

Bands	Assignments
3235.97	-OH (Oximino)
3118.33	-Ar (C=C)
2949.59	-Ar (C-H)
1616.06	>C=NN (Azomethine)
1571.70	>C=NO (Oximino)
1365/1332	-NO2 (Two bands)

C) PMR- Spectrum

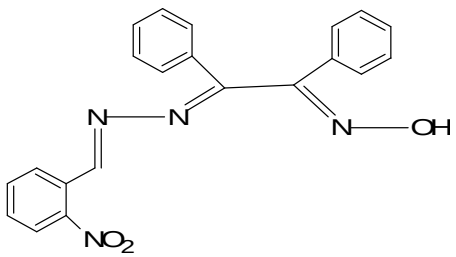
^{1}H NMR spectra of the synthesized compound was recorded in d_6 DMSO solvent and important bands summarized in **Table-3**. The pmr spectrum of HBMH*o*NB reveals a broad singlet at δ10.17 due to –OH of oximino group. A multiple observed around δ8.15-8.43 due to phenyl rings in the compound. Another singlet observed at δ2.51 assigned as –CH= group of the title compound.

Table-3: PMR data of *o*-substituted nitrobenzaldehyde derivatives α-benzilmonoximehydrazide in ppm

δ (PPM)	Assignments
10.17(*s*)	-OH (oximino proton)
8.15-8.43 (*m*)	Phenyl rings
2.508(*s*)	-CH=(methane group)

CONCLUSION

The synthesized compound is insoluble in water but it is soluble in dilute alkali and common organic solvents. The synthesized compound structure elucidated by spectral studies such as UV-Visible, pmr, FT(IR) spectra and this structure assigned as;



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SYNTHESIS AND CHARACTERIZATION OF NEWLY SYNTHESIZED COMPOUND OF (2*E*)-2-[(2*E*)-(2-BROMOBENZYLIDENE) HYDRAZINYLIDENE]-1, 2-DIPHENYLETHANIMINE

Premkumar Yadav[1], Raj Badekar[2] and Rama Lokhande[3]
[1,3]School of Basic Sciences, Jaipur National University, Jaipur
[2]Riva Industries (p) Ltd, Ambernath MIDC, Dist, Thane-Maharashtra

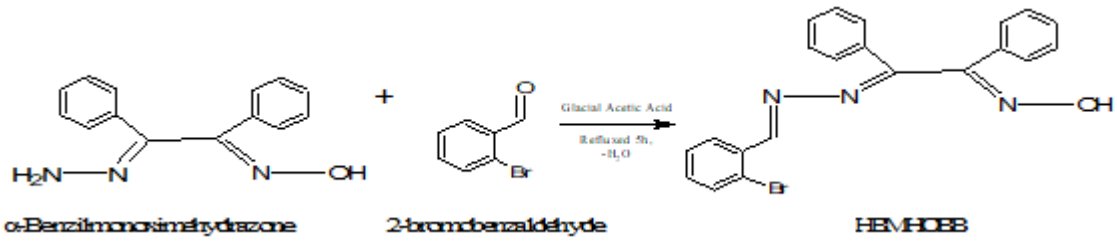
ABSTRACT
The '(2E)-2-[(2E)-(2-bromobenzylidene) hydrazinylidene]-1,2-diphenylethanimine' compound, were prepared by condensation between α-Benzilmonoximehydrazone and o-bromobenzaldehyde in the presence of Acetic acid and methanol. Synthesized compounds structure elucidated by elemental analysis, IR, UV spectra, PMR spectra. The bromobenzaldehyde derived compound have high melting point, indicate that strong bonding between all the atoms. Proposed compound insoluble in water, but soluble in common organic solvents.

Keywords: α-Benzilmonoxime, Schiff base, Oximino group, Bromobenzaldehyde

INTRODUCTION
A large number of Schiff bases and their various metal complexes have been studied for their important properties. Many Schiff bases reported their catalytic activity[1], photochemical properties[2] and from the complexes towards some toxic metals[3-7]. Schiff bases and their various metal complexes were plays n important role in the development of coordination chemistry. They are providing important and essential ways for chemical and biological activity of compounds. The high affinity for the complexation of the Schiff bases towards the transition metal ions utilized in preparation of their solid complexes. Schiff base with donors (N, O etc.) have structural similarities with natural biological systems, imports in elucidating the mechanism of transformation, recemination reaction in biological systems physiological and pharmacological activities associated with them[6-7], Schiff bases derived α-benzilmonoxime and their metal complexes reported earlier[8-13]. A-Benzilmonoxime derivatives are known to better coordinating agents. In view of this, we wish to report Spectral studies of '(2*E*)-2-[(2*E*)-(2-bromobenzylidene) hydrazinylidene]-1, 2-diphenylethanimine' and title compound abbreviated as HBMHOBB. The prepared compound was characterized by IR, UV spectra, PMR, elemental analysis.

EXPERIMENTAL
All reagents and chemicals used AR grade. All solvents were purified by before using. Melting point determined in an Electrothermal 9200. ^{1}H-NMR spectrum in CDCl3 was recorded on Brucker AV300 NMR spectrometers using TMS as internal standard. The FT-IR spectrum was recorded in the range 400–4000 cm^{-1} by KBr pellet using a 'Perkin- Elmer spectrum 100' model FT-IR spectrophotometer. The UV–Vis spectrum in methanol was recorded with a JASCO V-650 Spectrophotometer.

PREPARATION OF HBMHOBB
α-Benzilmonoximehydrazone was prepared by reported method[14]. The title ligand was prepared by mixing of methanolic solution of the α-Benzilmonoximehydrazone (0.10mol) and 2-bromobenzaldehyde (0.15mol), added 2-3 drops of glacial acetic acid. The resulting mixture was refluxed 5h using water condenser. After complete refluxation the solution was cooled at room temperature, solid separated, dried at 110°C in oven.



RESULTS AND DISCUSSIONS
A novel compound '(2*E*)-2-[(2*E*)-(2-bromobenzylidene) hydrazinylidene]-1,2-diphenylethanimine (HBMHOBB) has been synthesized and reported first time. The first H in the abbreviation of the compound assigned to the presence of one ionizable proton. Characterization of the HBMHOBB is done by using analytical data obtained from FTIR, PMR, UV-VISIBLE spectroscopy, elemental analysis etc. Physical data of the ligand (**Table-1**) corresponds to the molecular formula $C_{21}H_{16}N_3OBr$, molecular weight is 406g/mole. The molecular weight determined by Rast method[15], which is in agreement with the molecular weight calculated

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from molecular formula. The HBMHOBB is obtained in a crystalline form, which decompose at 205^0C. Synthesized compound found yellow crystalline solid, soluble in chloroform, acetone, DMF, DMSO, 1,4-Dioxane, dilute alkali etc. and is partially soluble in methanol and ethanol. Since HBMHOBB is soluble in dilute alkali solution indicating the acidic nature. The HBMHOBB has ionizable proton, yet our studies reveal that it is monobasic in nature.

Table-1: Analytical and physical data for HBMHOBB compound

Compounds	Color	% Yield	MP in ^{0}C	% of the expected (observed)				
				C	H	N	O	Br
HBMHOBB	Yellow	76.98	205	62.08 (61.92)	3.97 (3.88)	10.34 (10.02)	3.94 (3.85)	19.67 (19.00)

UV-Visible Spectra

The UV- spectra of the prepared compounds in the ultra-violet region show high intensity band at around 339nm (ε = 12723dm^{3}mol^{-1}cm^{-1}), 226nm (ε = 20905dm^{3}mol^{-1}cm^{-1}). These bands are due to the $\pi \rightarrow \pi^*$ (allowed) transitions of azomethine and oximino environment in the synthesized molecule. In many isonitrosoketones[16-17], a bands occurs at similar positions and intensity, are reported as the ($\pi \rightarrow \pi*$) transitions in the present compound.

Table-1: UV-Visible spectra of the *o* -substituted bromo-benzaldehyde derivatives α-Benzilmonoximehydrazone

Compounds	λ (nm)	ε (dm^{3}mol^{-1}cm^{-1})
HBMHOBB	339	12723
	249	20905

PMR

PMR spectrum of the synthesized compound was recorded in $CDCl_3$ solvent and important bands summarized in **Table-2**. A significant feature of the PMR spectrum of HBMOBB is the absence of any singlet band between δ7.5-8.0 ppm, due to the Amino group, reported at δ7.9 ppm in α-benzilmonoximehydrazone[14], indicating a successful replacement of the Amino group by the methine group during condensation process. This observation also support by new singlet band observed at δ2.5 ppm ascribed to the methine group in the prepared compound. The singlet at observed at δ10.17ppm (*s, 1H)* region indicated oximino proton in the prepared compound and since it is expected to be rather acidic and therefore the weakest shielded proton in the molecules. The multiplets in the region δ 8.15 - 8.43ppm were ascribed to the aromatic ring protons in synthesized compound.

Table-2: ^{1}H NMR data of *o* -substituted bromo-benzaldehyde derivatives α-Benzilmonoximehydrazone in ppm

Compounds	-OH	-CH=	Phenyl rings
HBMHOBB	10.17	2.5	8.15-8.43

FTIR Spectra

A significant feature of the FT(IR) spectra of the *o* -substituted bromo-benzaldehyde derivatives α-Benzilmonoximehydrazone is the absence of band between 3300 - 3400cm^{-1} due to the $-NH_2$ vibration reported[14] at 3387cm^{-1} in α-Benzilmonoximehydrazone indicating a successful replacement of the amino group by the methine group during condensation reaction. The spectrum shows a band at 3209cm^{-1} due to the presence of –OH of the oximino in the synthesized compound and another band observed at 3082cm^{-1} in the FT(IR) spectra of the synthesized compound is ascribed to aromatic C-H stretching vibrations and the aliphatic C-H group band is merged into aromatic C-H stretching which are observed in synthesized compound. Rest of the bands observed in title compounds are almost at the same frequencies in comparisons with bands of benzilmonoximehydrazone[14].

Table-3: FT(IR) spectra of the *o* -substituted bromo-benzaldehyde derivatives α-Benzilmonoximehydrazone in cm^{-1}

Compounds	-OH	ν(C=NO)	ν(C=NN)	ν(N-O)	ν(N-N)	Ar(C=C)	Ar(C-H)	ν(Ph-Br)
HBMHOBB	3209	1569	1625	1018	1095	3107	3082	740

CONCLUSION

The title compound is soluble in most of the common organic solvents and they have high melting point, indicating strongly bonded all functional groups and other molecules. The synthesized compound is monobasic

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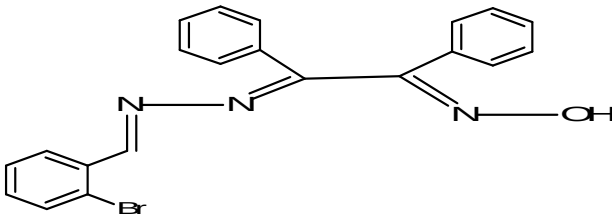
in nature concluded on the basis of the compounds-KOH titration curve method. On the basis of the spectroscopic methods tentatively assigned the structures of the proposed compound as follow;



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THE SURFACE AREA, ELEMENTAL AND PHOTO LUMINESCENCE STUDIES OF $SR_2P_2O_7$ NANO PHOSPHOR

P. D. Solanki, Dr. B. V. Jogiya and Dr. M. J. Joshi
Research Scholar, Department of Physics, Saurashtra University, Rajkot

ABSTRACT
Strontium pyrophosphate finds application as phosphor material. The nano-particles of strontium pyrophosphate were synthesized by the surfactant assisted technique.The Brunauer–Emmett–Teller (BET) measurements were performed for the surface area and the particle size. The majority of particles were found to be in the range of 10 nm to 40 nm.An elemental analysis was carried out by employing EDAX which confirmed the elemental composition. Photoluminescence (PL) study of nano $Sr_2P_2O_7$ phosphor was conducted using the excitation of 244 nm wavelength; the PL emission showed two peaks at 420 nm and 439 nm with good intensity in blue colour range. This indicated down conversion and possible application as bio imaging material and lighting material.

Keywords: BET measurement, EDAX, Photoluminescence, down conversion.

INTRODUCTION
Strontium pyrophosphate has been suggested as adhesive in luminescent screens [19]. Photoluminescence and thermally stimulated luminescence has been reported in europium doped strontium pyrophosphate by Natarajan et al [9].Divalent europium doped strontium pyrophosphate phosphor giving emission at 420 nmis considered to be a potential candidate for use in lamps for photo-therapy for treating infants jaundice. Strontium pyrophosphate activated by europium has been used in the fluorescent lamps in photocopying system, which enables higher production rates [9, 19]. There are several patents obtained based on strontium pyrophosphate phosphors [16] and europium activated strontium -magnesium pyrophosphate phosphors [4]. $Sr_2P_2O_7$Doped with Sm^{3+}, Ce^{3+}, Mn^{2+}, has been reported great potential for high□resolution display devices[13, 8].Altogether, the thermo- luminescence properties of strontium pyrophosphate doped with metals [5] as well as luminescent properties of strontium–zinc pyrophosphate co-activated by silver[2] have been reported. The down conversion materials $Sr_2P_2O_7$:Eu^{2+},Y^{3+} and $SrCaP_2O_7$: Eu^{2+}exhibits long lasting bright blue phosphorescence which find its application in blue-emitting solid-state lighting useful in bio-imaging and phototherapy [11,6].The structural, FTIR and UV –NIR spectroscopy, dielectric, thermal and TEM studies have been reported earlier by the present authors [14,15]. As the nano-particles possess high surface to volume ratio and that affects the nature of the various properties, the present communication further characterizes strontium pyrophosphate nano particles by BET surface areameasurements, EDAX for compositional confirmation and Photoluminescencespectroscopy for different phosphor applications.

MATERIALS AND METHOD
Synthesis of strontium pyrophosphate ($Sr_2P_2O_7$) nano-particles was carried outby using surfactant assisted technique [17].Equal amount of 0.25 M aqueous solution of freshly prepared $Na_4P_2O_7.10H_2O$ and 0.5 M aqueous solution of $SrCl_2.6H_2O$ were used. $Na_4P_2O_7$ solution was added in a drop-wise manner into the mixture of 100ml $SrCl_2.6H_2O$ and 20ml triton-X 100 surfactant with constant stirring at room temperature.The resulting precipitates were quickly filtered by Whatman filter paper; washed with de- ionized water and air dried.

EXPERIMENTAL
The specific surface area of $Sr_2P_2O_7$ is measured by Brunauer–Emmett–Teller(BET) measurement with use of volumetric adsorption equipment (Micromeritics, ASAP 2010, USA) at 77 K. The presence of the elements Sr, P, O was identified from the EDAX spectra obtained on equipment (ESEM EDAX XL-30, Philips, Netherlands). Photoluminescence emission spectrum was recorded at room temperature, using spectro-fluorometer, (Horiba Jobin, Japan) with monochromatorof 330nm and 550 nm PMT detectors.

RESULTS AND DISCUSSIONS
Brunauer–Emmett–Teller (BET)
Qingbo Liu et al [12]employed the BET analysis to rare earth pyrophosphates to study the catalysis in the production of acrolein from vapour phase dehydration of glycerol. The specific surface area of a powdered sample or nano-particle sample is determined by physical adsorption of a gas on surface of a solid and by calculating the amount of adsorbate gas corresponding to a monomolecular layer on the surface.This physical adsorption results from relatively weak forces, i.e., van der Waal's forces, between the adsorbate gas molecules

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and the adsorbent surface area of the test powder.For the specific surface area measurement of $Sr_2P_2O_7$ nano-particle, the Brunauer–Emmett–Teller (BET) study was conducted.The sample was degassed under vacuum at 373 K for the data collection. The N_2 adsorption–desorption isotherms of $Sr_2P_2O_7$ nanostructures are shown in fig-1,where Volume Adsorbed versus Relative Pressure (P/Po) plots are drawn for adsorption and desorption. The specific surface area of $Sr_2P_2O_7$ was determined by a multipoint BET method using the adsorption data in the relative pressure P/Po range of 0.06– 0.20 as shown in fig-2, where the plot of 1/[VA*(Po/P-1)]versus Relative pressure(P/P_O) is drawn.The BET surface area of $Sr_2P_2O_7$ nanostructures was found 1.9537m^2/g from N_2 adsorption–isotherms. The pore-size distribution was determined by using the Barrett–Joyner–Halenda (BJH) method. This method is applied to nitrogen desorption data measured at 77 K on porous material. It employs modified Kelvin equation to relate the amount of adsorbate removed from the pores of the materials to the size of pores. The BJH adsorption and desorption curves are shown in fig.3 in terms of plots of Pore Volume versus Pore Diameter. The desorptionisotherm was used to calculate the pore- size distribution. The average pore diameter of the sample is found in range of 2.0 – 9.5815 nm, which is attributed to the nano-particles assembled. This data corresponds to our earlier results of the average crystallite size calculated from the powder XRD patterns using Scherrer's formulation and Williamson and Hall formulation [13], which was found to be 33.57nm and 32.80nm, respectively. It is important for nano-particle samples to determine the average surface and average crystallite size because many physical properties are governed by large surface to volume ratio of nano-particles.

Energy Dispersive X-RAY Analysis (EDAX)

An elemental analysis was carried out for strontium pyrophosphate ($Sr_2P_2O_7.6H_2O$) by employing the EDAX,fig-4 shows the EDAX spectra of $Sr_2P_2O_7.6H_2O$ in which the presence of Sr, P and O are clearly identified from the spectral data. The atomic percentage and weight percentage of Sr, P, O elements in the sample is found to be 11.75 %, 10.43 %, 77.82 % and 39.64 % 47.92 %, 12.44 %, respectively. The theoretical values of atomic percentage and weight percentage are 11.76%, 11.76%, 76.47% and39.36%, 46.72% , 13.91% respectively, for Sr, P and O, which corresponds to the EDAX results. This suggests the highly pure compound.

PHOTOLUMINESCENCE STUDY (PL)

Most of the strontium pyrophosphate PL spectra are studied for rare earth or lanthanide ion doped systems [7, 3]. However, very few reports are available on the PL study on un-doped pyrophosphates [10]. The PL measurements were performed under an excitation power 20 mW using the UV-244 nm line of a continuous wave Ar^+ Laser frequency doubling unitat room temperature. When the sample was kept at continuous excitation of 244 nm, the emission peaks appeared at 313 nm, 420 nm and 439 nm.Fig. 5 shows the emission spectrum for different wavelengths. The intensity of the emission peak at 313 nm is as low as four times as compared to 420 nm and 439 nm peaks. A strong and narrow emission peaks are observed in the wavelength range 420 nm - 445 nm reveals the emission in the blue region. The PL spectra can be attributed to the oxygen vacancies in the absence of dopant rare earth ions. The possible role of the oxygen vacancies in the visible PL observed is considered[1]. The UV luminescence is expected to be accompanied with Sr-O charge transfer and is related to radiation decay of self-trapped excitons, where the electron component is located at Sr^{+4} and the hole is at O^{2-} ion. The visible luminescence character can be explained on the basis of the structure of $Sr_2P_2O_7$. In$Sr_2P_2O_7$each Sr^{+2}cation is coordinated by nine O^{2-} anions belonging to five different phosphate groups.These Sr^{+2} cation sites can be divided in to two different types. The higher energy component can be due to radiation decay of excitations localized at a defective Sr octahedronSrO_5^{6-} plus oxygen vacancy. The low energy component may be due to the recombination processes with the participations of F centres formed on the basis of oxygen vacancies of two types; i.e.; one is the vacancy of 'bridge' oxygen and other is the "non-bridge" positions. Further the law energy component can be due to recombination processes with the participation of F centres $6H_2O$ phosphor shows good photoluminescence in the blue region, may be useful in lighting applications.

CONCLUSION

The surface area of $Sr_2P_2O_7$ nanostructures was found 1.9537m^2/g by multipoint BET method.The average pore diameter of the sample determined by BJH method was found in range of 2.0–9.5815 nm. An elemental analysis carried out using EDAX confirmed the presence of Sr, P and O and the formation of the compound. The PL excitation spectra of the un-doped $Sr_2P_2O_7.6H_2O$ phosphor exhibited strong and narrow emission peaks in the wavelength range 420 nm - 445 nm revealed the emission in the blue region indicating the usefulness in lighting applications. It was suggested that the oxygen vacancies of two different locations in the structure of the compound playing important role in the low energy photoluminescence.

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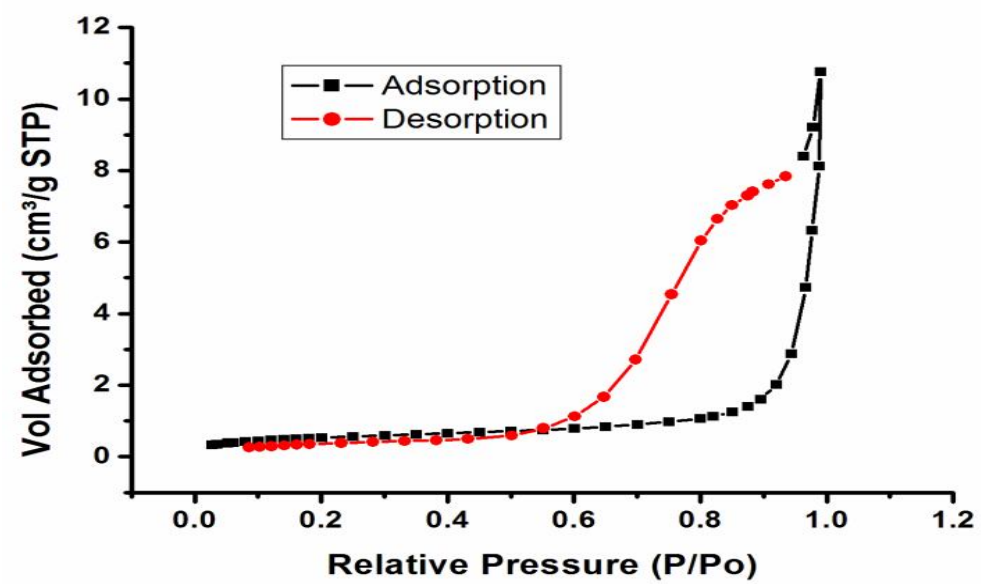

Figure-2: BET adsorption and desorption curves of Volume Adsorbed versus Relative Pressure (P/Po).

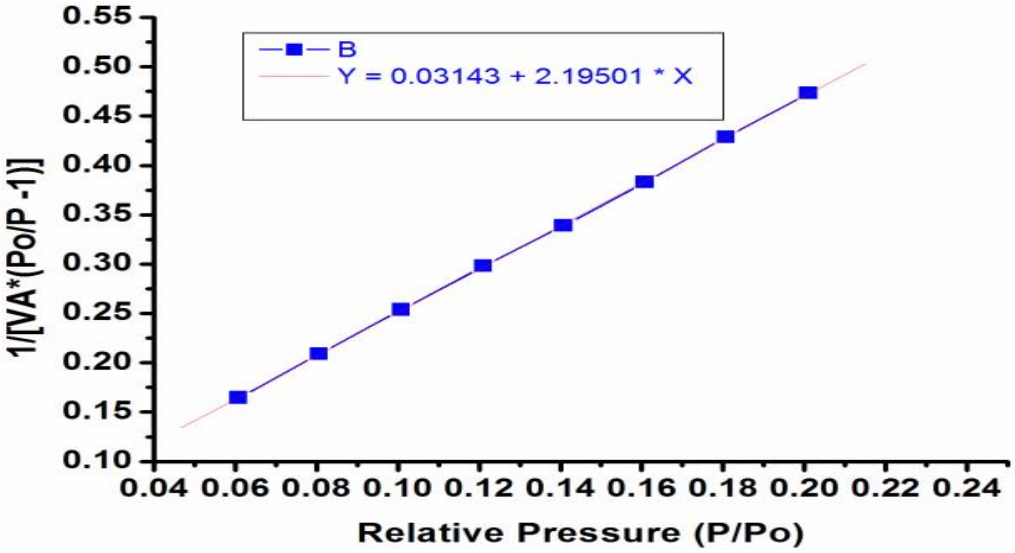

Figure-3: Plot of 1/[VA*(Po/P-1)] versus Relative Pressure(P/P_o)

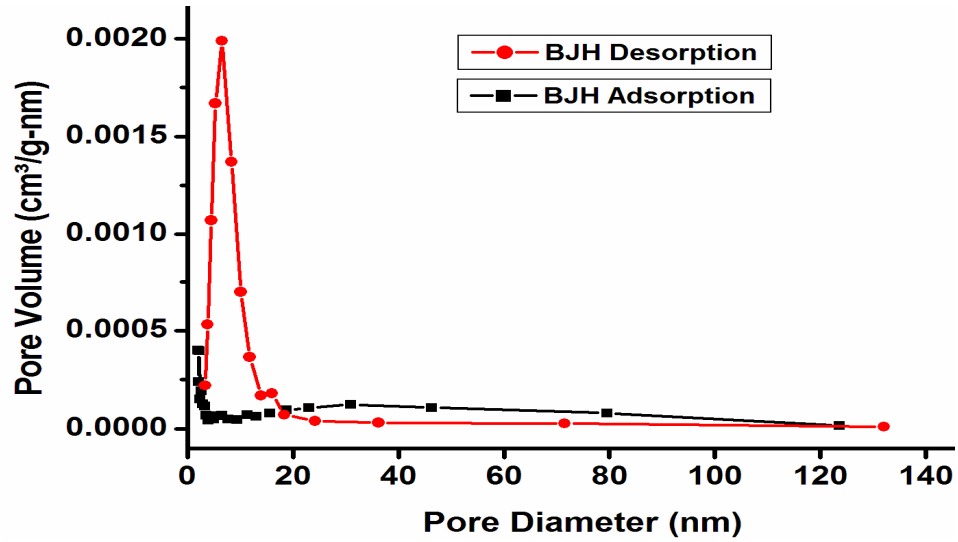

Figure-4: Plots of Pore Volume versus Pore Diameter for BJH adsorption and desorption curves.

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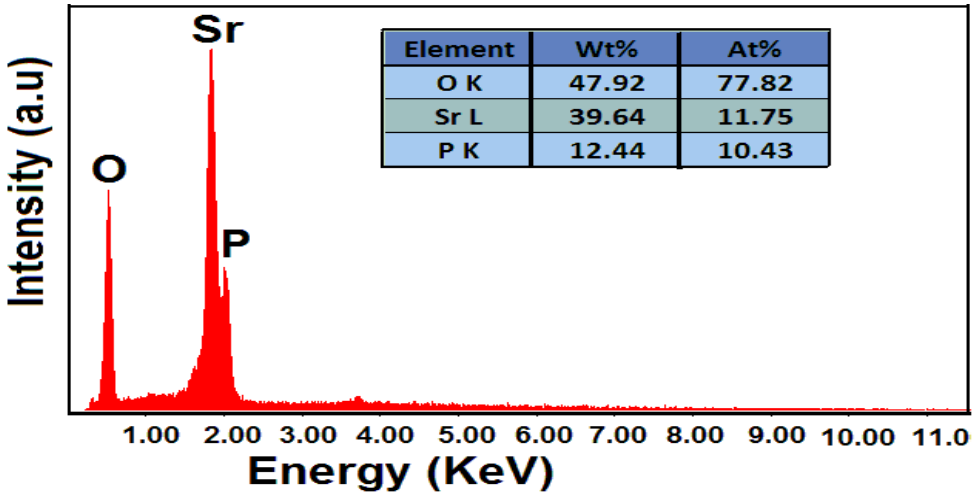

Element	Wt%	At%
O K	47.92	77.82
Sr L	39.64	11.75
P K	12.44	10.43

Figure-5: EDAX spectrum

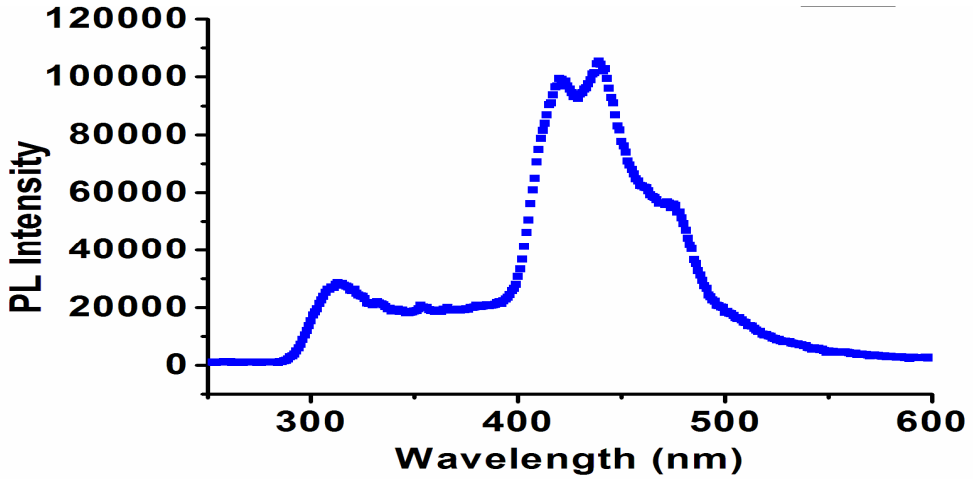

Figure-6: Photoluminescence emission spectrum of PL intensity versus wavelength for un-doped strontium pyrophosphate.

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INTERACTIVE EFFECT OF THERMAL POWERPLANT WASTEWATER, COAL FLY ASH AND DIFFERENT NITROGEN LEVELS ON GROWTH AND YIELD ATTRIBUTES OF CHICKPEA (CICER ARIETINUM L. cv. BG-256)

Irfan Ahmad[1] and Akil A Khan[2]
[1]Department of Botany, ABPG College Ranapar, Gorakhpur
[2]Department of Botany, Gandhi Faiz-E-Aam College, Shahjahanpur

ABSTRACT
The use of thermal power plant wastewater (TPPW) and coal fly ash in agriculture for irrigation need specific studies to evaluate their effect on different soils, crops and agro-climatic conditions. This study was therefore conducted to observe the suitability of wastewater for irrigation, and Cicer arietinum L.cv. BG-256 was used as a test crop. The experiment was conducted in the winter season of 2000–2001 to evaluate its effect together with the basal application of four doses of nitrogen (N_0, N_{10}, N_{20}, N_{30} kg ha^{-1}). Fly ash (@ 10%) selected from previous study, conducted in the year 1999 was amended with soil to make the final weight 7 Kg ha^{-1}. Wastewater irrigation resulted in the increased growth and yield of the crop. Lower fertilizer dose of nitrogen @ 10 Kg ha^{-1} together with wastewater irrigation and fly ash amended soil (FA_{10}) proved optimum, resulting in greater leaf area, plant fresh weight, dry matter (DM) and leaf NPK content, number of pods per plant, 100 seed weight and protein content relative to control which is found to be at par with higher N doses (N_{20} and N_{30}). Thus fertilizer rates could be lowered without reducing yields when using wastewater for irrigation and fly ash (FA_{10}) as an amendment to the soil.

Keywords: Thermal power plant wastewater, fly ash, yield, chickpea, nitrogen.

I. INTRODUCTION
In most parts of the developing world, fresh water supply is becoming increasingly limited due to over consumption by the fast growing population of these countries. More than 60% of the valuable water used each year is diverted for irrigating crops. For Asia, which has two third of the world's irrigated land, the figure is still higher (85%) due to unscientific irrigation. The colossal wastage of our scarce freshwater resources can be reduced by various ways, important being the reuse of wastewater in agriculture which is gaining importance nowadays because of its value as a potential irrigation source and a nutrient supplier. In addition to the manorial ingredients, it effectively augments the supply of water, the most important requirement of cultivated crops. Wastewater not only offers an alternative water irrigation source, but also the opportunity to recycle plant nutrients (1). Its application might ensure the transfer of fertilizing elements, such as nitrogen (N), phosphorous (P), potassium (K+), organic matter, and meso and micro-nutrients, into agricultural soil and has been reported to increase crop yield (2, 3, 4, 5, 6, 7, 8 & 9). Hence, wastewater nutrients can contribute to crop growth (10). Wastewater rich in organic materials and plant nutrients is finding agricultural use as a cheap way of disposal (11). Application of wastewater e. g. thermal power plant wastewater (TPPW) to cropland is an attractive option for disposal because it can improve physical properties and nutrient contents of soils (12). Thus, its use would help in water conservation recycling nutrients (NPK) in wastewater, reducing direct fertilizer inputs and minimizing pollution loads to receiving water bodies (13, 14 & 15).

Similarly, Disposal of high amount of fly-ash from thermal power plants absorbs huge amount of water, energy and land area by ash ponds. In order to meet the growing energy demand, various environmental, economic and social problems associated with the disposal of fly-ash would continue to increase. Every year thermal power plants in India produce more than 100 million tonnes of fly ash, which is expected to reach 175 millions in the near future (16). Disposal of this huge quantity of fly ash is posing a great problem due to its limited utilization in the manufacturing of bricks, cements, ceiling and other civil construction activities. This would further bring changes in land-use patterns and contribute to land, water and atmospheric degradation, if proper management options for handling ash are not undertaken (17, 18 & 19). Therefore, fly-ash management would remain a great concern of the century. Fly-ash has great potentiality in agriculture due to its efficacy in modification of soil health and crop performance. The high concentration of elements (K, Na, Zn, Ca, Mg and Fe) in fly-ash increases the yield of many agricultural crops. But compared to other sectors, the use of fly-ash in agriculture is limited.

While, the most important role of N in the plant is its presence in the structure of protein and nucleic acids which are the most important building and information substances of every cell. In addition, N is also found in chlorophyll that enables the plant to transfer energy from sunlight by photosynthesis. Thus, the supplies of N to the plant will influence the amount of protein, amino acids, protoplasm and chlorophyll formed. Consequently,

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it influences cell size, leaf area and photosynthetic activity (20, 21, 22, 23 & 24). Therefore, adequate supply of N is necessary to achieve high yield potential in crops. In general, N deficiency causes a reduction in growth rate, general chlorosis, often accompanied by early senescence of older leaves, and reduced yield (23 & 25).

Pulses, being an integral part of vegetarian diet in the Indian sub-continent, are a known rich source of protein. However, it must be admitted that the area under their cultivation has not increased in proportion to population explosion. Consequently the per capita availability of pulses has progressively declined from 60.7g day^{-1} in 1951 to nearly 36g in 2000 against the FAO/WHO recommendation of 80g (26). Chickpea (*Cicer arietinum* L.), an important pulse crop grown throughout the country, accounts for more than a third of the area under pulses and about 40% of their production in India, the average annual area and production being about 7-8 million hectares and about 4-5 million tonnes respectively (27).

Keeping in mind the importance of nitrogen (N), disposal problem of thermal power plant wastewater and fly ash, that can be used as nutrients for betterment of plant, and to minimize the use of chemical fertilizer, an experiment was conducted in the year 2000 at Department of Botany, Aligarh Muslim University, Aligarh on chickpea.

II. MATERIALS AND METHODS

An experiment was conducted on chickpea cultivar BG-256, to strengthen the findings of earlier experiment with inorganic fertilizer doses. Here, the comparative effect of TPPW and GW was studied. On the basis of observations made earlier, the best concentration of fly ash i.e. 10% was selected and added to the soil, making the final weight of fly ash amended soil up to 7kg ha^{-1}. Different doses of nitrogen i.e. 0, 10, 20 and 30kg ha^{-1} were supplemented in order to work out the optimum dose for cultivar BG-256. A uniform basal dose of phosphorus and potassium at the rate of 20kg ha^{-1} each was also applied before sowing. Healthy seeds of more or less uniform size were surface sterilized and then inoculated (28). Seeds were procured from Indian Agricultural Research Institute (IARI), New Delhi and viable *Rhizobium* culture (*Rhizobium sp*.) specific for chickpea was also obtained from IARI, New Delhi. Before irrigation the water samples were collected and analysed for physico-chemical characteristics adopting the procedures outlined in the standard methods (29). The soil/fly ash samples were collected before the start of the experiment. These samples were also analysed for standard physico-chemical properties according to some workers (30, 31, 32, 33, 34, 35 & 36). For investigating the comparative effect of TPPW, GW and fly ash under inoculated conditions, observations were carried out at vegetative, flowering, fruiting and at harvest stages. For the study of the root, the plants were uprooted carefully and washed gently to clear all the adhering particles. For assessing dry weight, three plants form each treatment were dried, after taking their fresh weight, in hot air oven at 80ºC for two days and weighed. The area of leaves was measured using leaf area meter (*LA 211, Systronics, India*). For nodule number, whole plant was uprooted with the precaution that the roots or the nodules may not be damaged. Samples were washed gently to wipe away all the adhering foreign particles and the number was carefully counted.

NRA and chlorophyll were estimated (37 & 38). Healthy leaves were collected at different samplings stages for the estimation of N, P and K contents (39 & 40). Potassium was estimated with the help of flame photometer. Ten millilitres of aliquot was taken and K was read using the filter for potassium. A blank was also run side by side with each set of determinations. The readings were compared with a calibration curve plotted against known dilutions of standard potassium chloride solution. At harvest, yield attributes including seeds per pod, pods per plant, 100-seed weight, and seed yield per plant were noted and protein content (41) in the seeds was measured. The data for the growth and yield of each experiment were analysed statistically taking into consideration the variables (42). The 'F' test was applied to assess the significance of data at 5% level of probability ($p \leq 0.05$). The error due to replication was also determined.

Table 1. Chemical characteristics of soil and fly ash before sowing. All determinations in mg l^{-1} in 1: 5 (soil-water extract) or as specified.

Soil		Fly ash	
Determinations		Determinations	
Texture	Sandy loam	CEC (meq $100g^{-1}$ fly ash)	7.34
CEC (meq $100g^{-1}$ soil)	2.88	pH	8.90
pH	8.30	Organic carbon (%)	2.19
Organic carbon (%)	0.789	EC (µ mhos cm^{-1})	1037.00
EC (µ mhos cm^{-1})	281.00	NO^{-}_{3}–N (g kg^{-1} fly ash)	–
NO^{-}_{3}–N (g kg^{-1} soil)	0.243	Phosphorus (g kg^{-1} fly ash)	2.22
Phosphorus (g kg^{-1} soil)	0.120	Potassium	11.00

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Potassium	21.00	Calcium	19.03
Calcium	30.29	Magnesium	16.59
Magnesium	18.24	Sodium	14.27
Sodium	13.18	Carbonate	13.26
Carbonate	18.36	Bicarbonate	64.37
Bicarbonate	81.64	Sulphate	26.25
Sulphate	18.28	Chloride	19.11
Chloride	28.13		

Table-2: Chemical characteristics of ground water (GW) and thermal power plant wastewater (TPPW). All determinations in mg l^{-1} or as specified.

Determinations	Sampling			
	I		II	
	GW	WW	GW	WW
Ph	7.3	7.9	7.5	8.0
EC (µ mhos cm^{-1})	710	880	700	840
TS	902	1298	947	1288
TDS	520	637	528	621
TSS	404	658	431	694
BOD	16.17	68.10	17.35	69.24
COD	38.24	124.18	37.19	129.24
Mg	17.84	26.36	18.18	28.22
Ca	26.17	41.84	24.18	38.36
K	7.52	16.67	8.24	14.39
Na	17.13	44.29	15.38	41.37
HCO_3^-	67	93	69	92
CO_3	21	38	22	37
Cl^-	69.13	111.17	65.84	105.67
PO_4	0.70	1.22	0.74	1.34
NO_3–N	0.74	1.19	0.76	1.13
NH_3–N	2.19	5.31	2.10	5.12
SO_4	46	65	47	64

III. RESULTS AND DISCUSSION

In this factorial randomized pot experiment, the comparative effect of two irrigation water sources and three basal levels of nitrogen, supplemented with phosphorus and potassium at the rate of 20kg ha^{-1} each applied uniformly before sowing, was studied on chickpea (*Cicer arietinum* L.) cv. BG-256. The growth characteristics and physiological parameters were recorded at three stages. Yield attributes including seed yield and seed protein content were recorded at harvest.

TPPW proved efficacious for all the growth parameters studied, while GW gave significantly lowest value at vegetative, flowering and fruiting stages respectively. Among various nitrogen treatments (N_0, N_{10}, N_{20} and N_{30}), N_{10} proved optimum for all growth parameters studied, being at par with N_{20}; followed by higher dose of nitrogen i.e. N_{30} which gave at par values at all the sampling stages. Nitrogen treatment of 10kg ha^{-1} proved optimum when interacted with TPPW as well as GW. TPPW-nitrogen combination gave better results than GW-nitrogen combinations, whereas lowest values were recorded by GW×N_0. The wastewater was enriched with considerable amount of nutrients which are considered essential for maintaining soil fertility and enhancing plant growth and productivity. Among them, nitrogen (N) is the most important element limiting plant growth. It is invariably required in large quantities and in wastewater it was present in both ionic forms (Table 2) and thus deserves special consideration. As vegetative growth includes formation of new leaves, stem and roots, the involvement of N through protein metabolism controls them. This was also clearly evident from the enhanced growth under the wastewater irrigation (Fig. 1, 2 and 3). Suitability of ammonium (NH_{4+}–N and NO_{3-}–N) ions for the growth and development of plants depends upon many factors (43). However, normally the highest growth rate and plant yield (Fig. 7b) are obtained by a combined supply of both; therefore, in the present study, the enhancement in growth could be due to cumulative effect of ammonium as well as nitrate ions together (44). It is noteworthy that applied ammonium nitrogen (NH_{4+}–N) is toxic for some higher plants (45). However, in the presence of nitrate-nitrogen (NO_{3-}–N), it has been reported to benefit wheat (Triticum

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aestivum) (46) and chickpea (47). Thus the observed nutritional superiority of wastewater for growth of chickpea was not exceptional and possibly explains better performance of crop growth under wastewater irrigation (Fig. 1, 2 and 3). A substantial increase in dry matter of test plants was also observed (Fig. 1, 3 and 4) because of the increased leaf area and expansion (Fig. 2) which might have influenced the light absorption within plant causing stimulation of PN, thereby optimizing the CO_2 assimilation and photosynthetic production (48). The increase in leaf area brought about by the N supply causing expansion of individual leaves has also been reported by Taylor et al. (49) and Gastal and Lemaire (50). The possible reason for this may be through its effect on cell division and cell expansion (51). Another essential nutrient, P when it is supplied in limited amounts to sugar beet (Beta vulgaris); it has much greater impact on growth than on photosynthesis (52 & 53). During the present study better growth of plants was observed receiving wastewater having phosphorus (Table 2) in addition to other nutrients, and it was also comparatively richer than ground water. The observation of improved performance of the crop receiving wastewater was therefore understandable. But, a regular supply of the enriched wastewater up to harvest ensured availability of P and thus improved the growth and which ultimately led to higher seed productivity. Next to N and P, K is the third most important macronutrient required in the largest amounts by the plant. It is known to play a significant role in stomatal opening and closing (54) and under light conditions the guard cells produce abundant adenosine triphosphate (ATP) in photosynthetic phosphorylation, thus supporting active K+ uptake with sufficient energy (55), and the resulting high-turgor pressure thus causes the opening of the stomata. The diffusion of carbon dioxide (CO_2) into the stomata is followed by its transport into the chloroplasts where it is reduced by ribulose-1, 5-biphosphate carboxylase/oxygenase (RuBPCO). It is this supply of CO_2 which catalyzes reversible dehydration of bicarbonate (HCO_{-3}) to CO_2 in close proximity to the CO_2-fixing enzyme (48). It is also well known that N is fully utilized for crop production only when K^+ is adequate (56) and the presence of K^+ in wastewater was nearly double the amount present in groundwater (Table 2). Therefore, the crop under study was benefitted not only due its own physiological role (57) but also by enhancing the effect of N. This was also strengthened by the presence of higher N, P and K contents in the leaves of the plants receiving the wastewater (Fig. 6). In addition to these three major macronutrients explained above, the presence of other essential nutrients like sulphur (S) could have also played a vital role in plant metabolism (58). It may be pointed out that the application of N in the form of urea is ineffective unless S is applied simultaneously, and its deficiency reduces the leaf area (59) besides decreasing the chlorophyll contents (60). Moreover, in S-deficient plants not only does the protein content decrease but also the S content in proteins, indicating that proteins with lower proportions of methionine and cysteine but higher proportions of other amino acids such as arginine and aspartate are synthesized (48). This decrease in the S-rich proteins is not confined to wheat grains but can also be found in other cereals and legumes (61), and the lower S content of the proteins influences the nutritional quality considerably (62). In the present study the total protein was significantly enhanced in the wastewater-fed plants (Fig. 7c). Similarly, the presence of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions (Table 2) could have further added benefits, as Ca^{2+}, being an essential component of the cell wall, is involved in cell division (63) while Mg^{2+} is a central atom of chlorophyll and is required for structural integrity of the chloroplast (64) on which the rate of photosynthesis is directly dependent. It may be pointed out that the chlorophyll content was enhanced in plants grown under wastewater (Fig. 5 and Table 2) indicating the possible involvement of Mg^{2+} in addition to other nutrients. The observed enhanced growth ultimately led to increase in 100-seed weight (Fig. 7a). Ensured supply and availability of the above mentioned nutrients might have played a cumulative role in enhancing the metabolic activities and finally the seed yield and protein (Fig. 7b&c).

Fly ash (FA_{10}) when applied with nitrogen (N_{10}) gave better results being at par with $FA_{10}N_{20}$ and $FA_{10}N_{30}$ as compared to $FA_{10}N_0$ for all growth, physiological and yield parameters. FA amendment increases the porosity and water-holding capacity, due to the fine-textured nature of fly ash, which helps in improving the physical health of the soil for supplying all essential nutrients in significant quantities for plant growth. The addition of fly ash into soil increased the organic carbon content which helps in binding soil particles in aggregates and improving the water-holding capacity of soil. Such improvement in agronomic properties of soil by constituents of fly ash has also been reported elsewhere as well at Aligarh (65, 66, 67 & 68).

Plant growth is the expression of interplay between meristematic activities and metabolic processes leading to an increase in biomass 64). In addition to the role of N in cell division and expansion (69), it is also essential for a number of biologically important molecules. Therefore, the requirement of N (and the other essential nutrients) during the vegetative growth of a plant is determined primarily by the rate of CO_2 assimilation and if it is high, the required nutrients must be correspondingly at optimum levels in order to convert the photosynthates efficiently into other metabolites.

Thus, growth and yield parameters were noted to be significantly affected by N application (N_{10} proving

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optimum) as a result of the cumulative enhancement of growth and yield parameters, including seed yield (Figs. 1-7). This dose was also found to be optimum for leaf area, NRA and chlorophyll content (Figs. 2&5) which finally led to more pods and the heavier seeds (Fig. 7b). By contrast application of excess N (N_{30}) resulted in decreased grain yield and proved deleterious. Toxicity due to N, when applied as urea is known to appear at two stages of plant growth. The first at seedling stage may be due to accumulation of NH_4^+ (after hydrolysis of urea) which becomes toxic at pH 8 and above. The second is due to accumulation of NO_2 under certain conditions damages young plants (70). Contrary to above findings, higher optimum doses up to 30 kg N ha^{-1} were reported for chickpea by Sharma *et al*. (71) and Krishna *et al*. (72). It was not surprising that in our study comparatively lower dose (N_{10}) proved effective as the applied wastewater had sufficient N in the form of NH_4^+ and NO_3^- ions. In case of legumes due to rhizobial activities, host plants grow well in soil even with low N doses and no benefit from this association may occur if high levels of fertilizer N are given (73). This was in conformity with the present observations. Since N_{10} and N_{20} were at par in their effect therefore it may be concluded that N_{20} led to luxury consumption, thereby proving wasteful, while N_{30} affected adversely thereby proved toxic when wastewater was the source of irrigation, which proved economically as well as environmentally viable.

When nodulation was considered similar observations were made. The beneficial effect of lower dose (N_{10}) was noted to increased root formation (Fig. 3). This provided more surface area for bacterial infection. However, application of N beyond a certain level is known to delay and even suppress nodulation (74, 75, 76, 77 & 78). On the other hand, the crop grown without nitrogen (N_0) expectedly gave significant lowest values as some starter dose of N is always needed even by the leguminous plants to grow normally. Nitrate reductase levels have been shown to fluctuate in response to changes in environmental conditions, including availability of N (79 & 80). Enzymes are sensitive to nutrient levels as is indicated in the present study where NRA was found to decrease with comparatively higher N dose (Fig. 5a). Similar observation has been made in trifoliate leaves in *Phaseolus lunatus* at different canopy positions by Wallace (81) and Andrews *et al*. (82). The induction of NRA requires very low concentration of nitrate suggesting that nitrate is actually sensed more as a hormone than as a nutrient (83). Nitrogen also increased the leaf chlorophyll and NPK contents as it increased the availability of substrate for protein synthesis allowing the development of more and larger chloroplasts with extensive thylakoid system and larger stomal volume (84). The increase in leaf NPK (Fig. 6) was due to the synergistic interplay of the three nutrients, which are known to accelerate root proliferation, thus, extracting more nutrients present in the root zone leading to development of larger canopies (Fig. 2) and greater dry matter accumulation (Figs. 1&2). Similar positive interactions between N and P were also noted by Russell (85) and between N and K by Murphy (86). N as an essential macronutrient has the distinction of being absorbed both as cation as well as an anion. This puts N in a unique relationship of both an anion-cation as well as cation-cation interaction.

Expectedly the application of N enhanced seed protein contents (Fig. 7c) as it chief constituents of proteins. Its adequate supply can increase the amino acid levels through the conversion of organic acids produced from carbohydrates during respiration. As pointed out by Pretty (87), some quality factors in a few grasses were related to the effective utilization of N and the conversion of N-compounds into true proteins. Improvement in seed protein content was also boosted due to the addition of K, applied uniformity as the starter dose alongwith N, as K influences the level of some non-protein N components and positive role in converting these proteins. The N effect on seed protein was also dependent upon the type of crop, its cultivars and other environmental factors including water. Smika and Greb (88) observed the relationship of soil NO_3^-–N and soil water for the protein in wheat. The former was positively correlated with grain protein where opposite relationship was noted due to available soil water. In their opinion adequate soil moisture in addition to N was the important factors for this parameter. Since, the present work was carried but in pots and water was given regularly, therefore, possible protein in the present study was increased.

Finally it was concluded that Plants irrigated with TPPW performed better when supplemented with low fertilizer N level, N_{10}, thus proving the utility of wastewater in saving some amount of costly nitrogenous fertilizers which simultaneously solving the problem of its disposal partially. N_{30} proved deleterious, while N_{20} showed luxury consumption when given with wastewater. Nodulation and seed protein content were also increased by the application of N_{10}, while N_{30} decreased nodulation.

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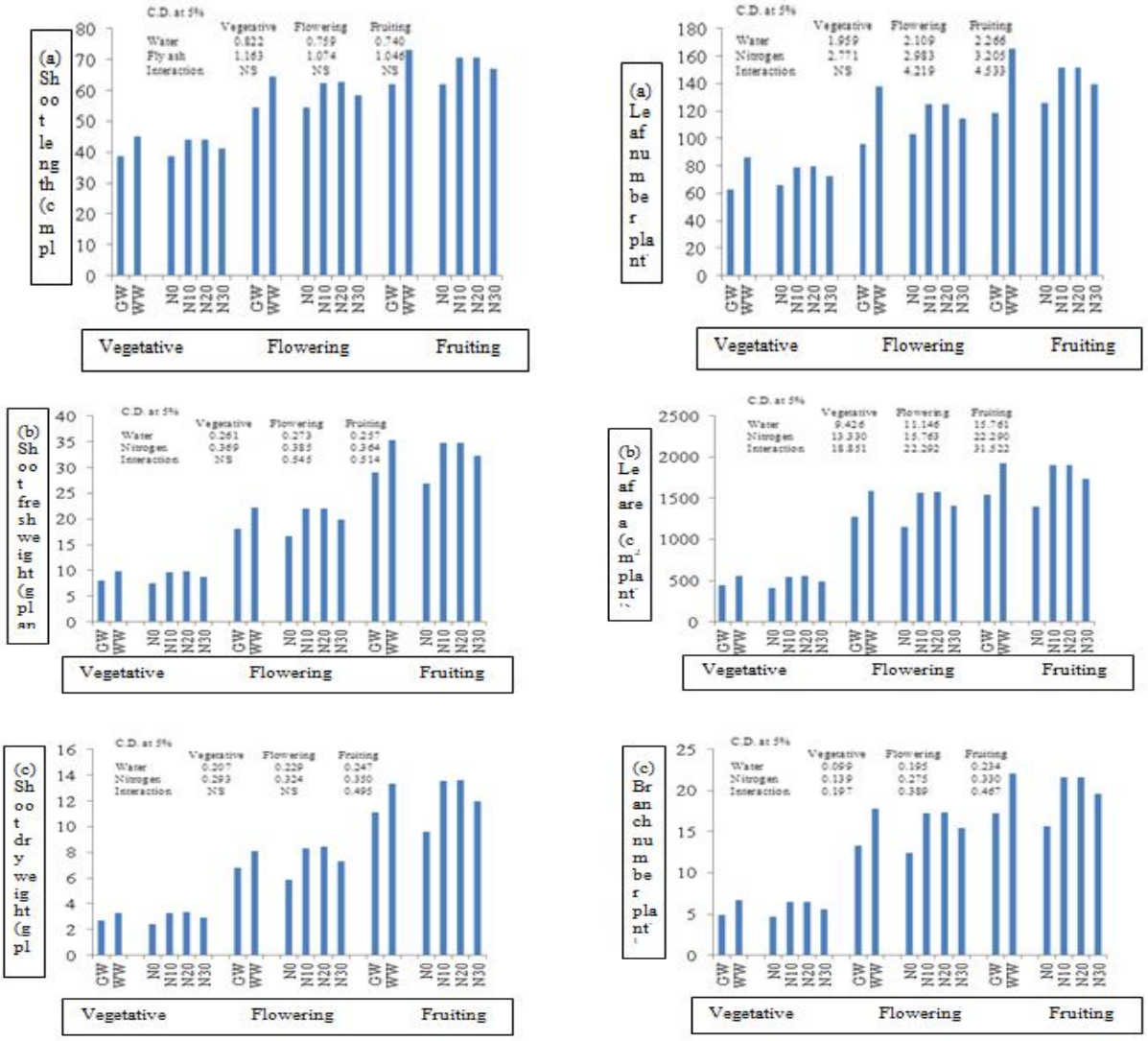
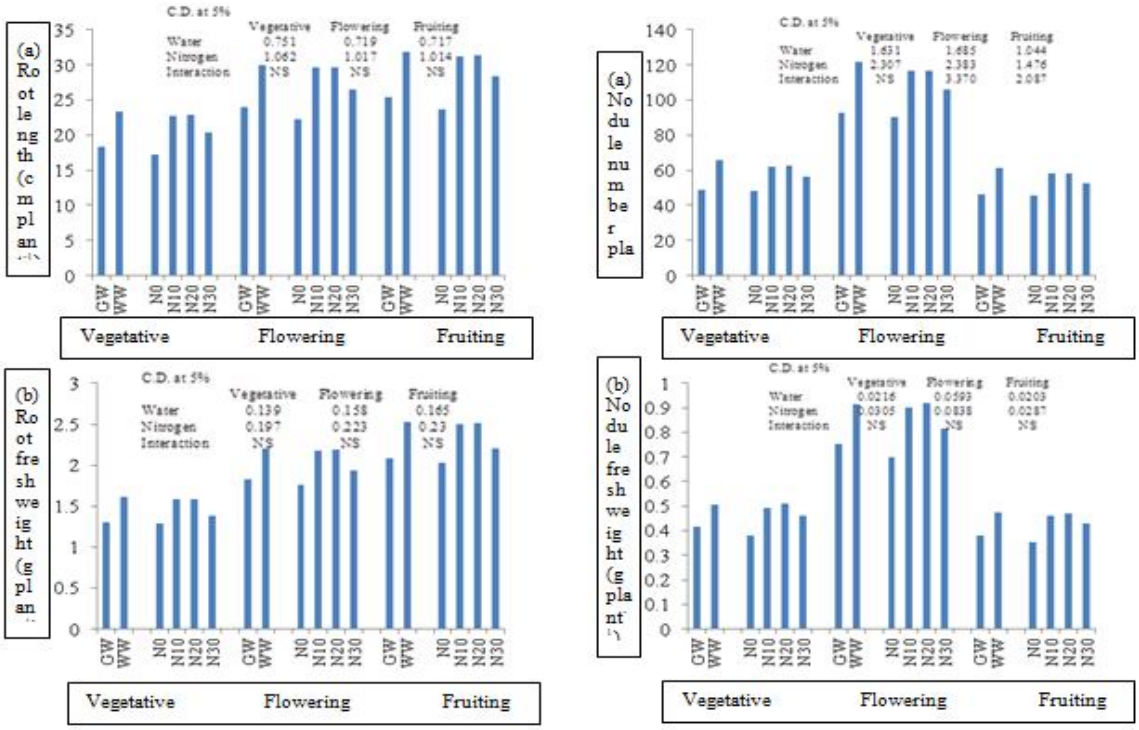


Fig-1: Effect of wastewater and nitrogen on chickpea cv. BG-256

Fig. 2. Effect of wastewater and nitrogen on chickpea cv. BG-256



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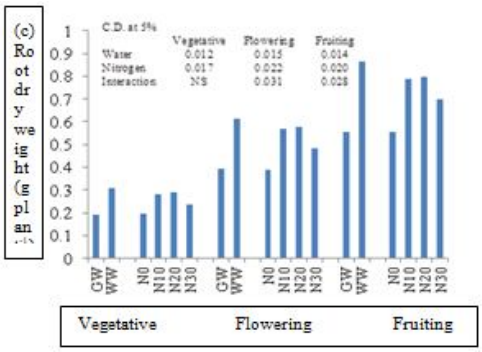
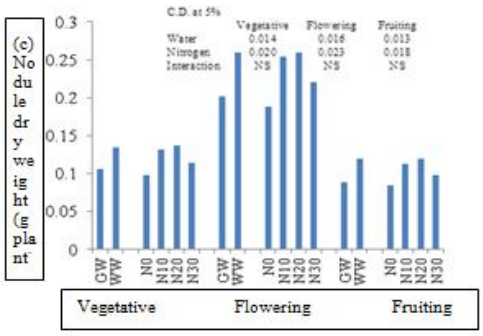


Fig-3: Effect of wastewater and nitrogen on chickpea cv. BG-256



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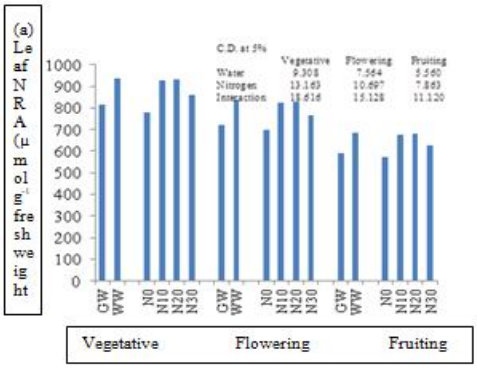


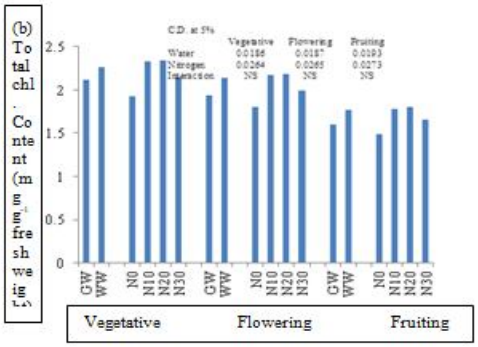
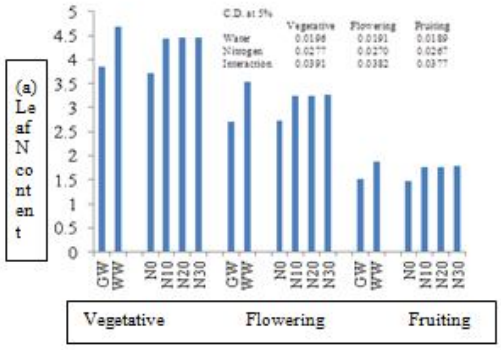
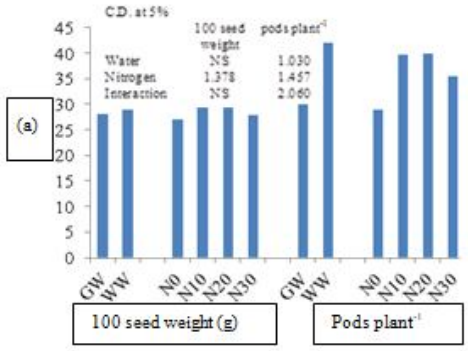
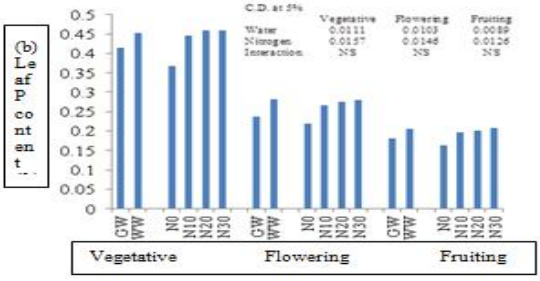
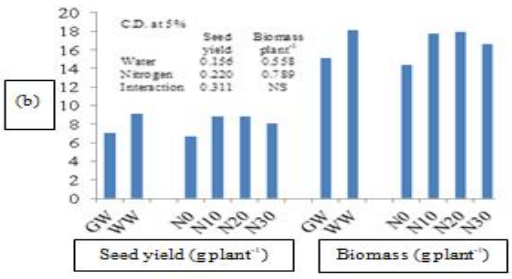


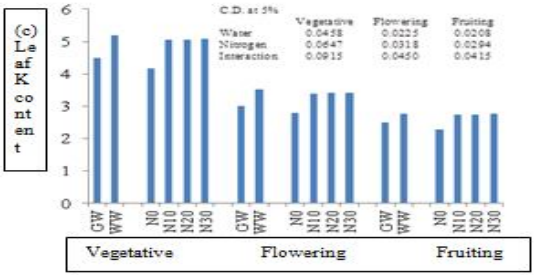
Fig. 5. Effect of wastewater and nitrogen on chickpea cv. BG-256











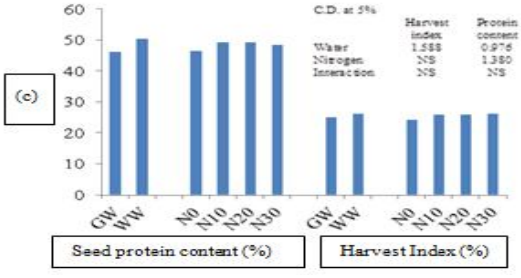


Fig. 6. Effect of wastewater and nitrogen on chickpea cv. BG-256

Fig. 7. Effect of wastewater and nitrogen on chickpea cv. BG-256

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