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Editorial

The first issue of Current Reports on Science and Technology, a peer reviewed research journal, is a step forward in materialization of the dreams of founding fathers of Khalsa College Amritsar. The most significant achievement of the freedom struggle was the establishment of Khalsa College Amritsar on 5th March, 1892. One of the main objectives of Khalsa College was to spread modern education based on science and technology among the people of the region to keep them abreast with the development of the times. True to its objectives, Khalsa College Amritsar was the first college in the region to offer Honours School in Botany, Chemistry and Physics in second decade of 20th century. Since then courses in science subjects have left everlasting impact.

Dedicated to the centenary celebrations of the introduction of science in Khalsa College Amritsar, the publication of Current Reports on Science and Technology will be a true and rightful homage to the torch bearers who have spread the light of learning and knowledge to the remotest corners of the country.

A lot of efforts have been put in the careful presentation of this issue of Current Reports on Science and Technology. It will be the endeavour of the publishers to maintain the international standards of peer review process in publishing the research articles.

This issue is open for constructive criticism. The suggestions for improvements will be welcome and highly appreciated.

Dr. Taminder Singh
Chief Editor

ਖੋਜਤ ਖੋਜਤ ਤਤੁ ਬੀਚਾਰਿਓ ਦਾਸ ਗੋਵਿੰਦ ਪਰਾਇਣ ॥

(ਅੰਗ ੭੧੪)

Searching and seeking,
I have come to understnad
the essence of reality;
the slave of the Lord of the
Universe is dedicated to Him.

(SGGS Ang 714)

Curr. Rep. Sci. & Tech.1 (1) (2015) 1-5

TL dosimetry of nanocrystalline $Li_2B_4O_7$: Cu irradiated to 120 MeV Ag^{9+} ion beam

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Abstract

Lithium borate ($Li_2B_4O_7$:Cu), a low Z_{eff}, tissue equivalent material was synthesized by using combustion method. The samples were irradiated with silver beam (Ag^{9+}) of different fluences in the wide range ($5x10^{10}$- $1x10^{12}$ ions/cm^2). X- Ray diffraction (XRD) and transmission electron microscopy (TEM) techniques were used to understand the structure and shape of synthesized samples. Further TL characteristics of synthesized samples were studied for observing their potential applications in ion beam dosimetry.

Keywords

$Li_2B_4O_7$:Cu, thermoluminescence, tissue equivalent, ion beam dosimetry.

I. Introduction

$Li_2B_4O_7$ a compound with low effective atomic number (Z_{eff} =7.3) has good tissue equivalence (Z_{eff} = 7.4) but is less sensitive to X-rays [1]. The nanocrystalline $Li_2B_4O_7$:Cu can be used for high dose measurements of gamma radiations [2]. Heavy charged particle beams are also used for radiotherapy because being heavier, trajectories are stiffer, so there is less multiple scattering, sharper field edges can be achieved that is an important consideration for tumors close to critical structures. To the best of our knowledge, thermoluminescent properties of nanocrystalline $Li_2B_4O_7$:Cu irradiated with ion beams have never been reported.

In view of the excellent dosimetric properties of $Li_2B_4O_7$: Cu, we have studied the TL characterstics of nanocrystalline $Li_2B_4O_7$:Cu samples exposed to 120 MeV, Ag^{9+} beam. These results may be helpful in the development of tissue equivalent TL nanocrystalline detectors best suited for wide high range of ion beam exposures.

II. Experimental

$Li_2B_4O_7$:Cu nanophosphors were synthesized by combustion method [2]. Samples were irradiated with 120 MeV (Ag^{9+}) beam using 16 MV Tandem Van de-Graaf type pelletron accelerator facility of the Inter-University Accelerator Center (IUAC), New Delhi. The formation of the compound was confirmed by X-ray diffraction pattern taken at room temperature by using Cu-target (Cu-K_α line, λ=1.54056 Å) on Bruker AXS-D8 diffractometer. The particle size and shape of the concerned phosphors were analysed by using Transmission Electron Microscopy (TEM) Hitachi (H-7500). TL glow curves were recorded using a Harshaw TLD reader (Model 3500) fitted with a 931B photo multiplier (PMT), taking 5 mg of sample each time.

III. Results

The structure of nanocrystalline $Li_2B_4O_7$:Cu sample exposed to 120 MeV Ag^{9+} beam of fluence $5x10^{11}$ ions/cm^{2}was confirmed by studying the X-ray diffraction (XRD) pattern shown in Fig. 1.

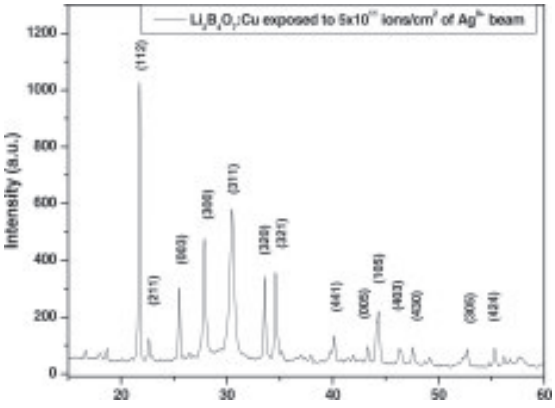

Fig. 1. XRD spectra of $Li_2B_4O_7$: Cu sample exposed to $5x10^{11}$ ions/cm^2 Ag^{9+} beam

The well known Debye-scherrer's relation [3] was used to estimate the particle size for $Li_2B_4O_7$:Cu. The average grain size was calculated to be approximately 20 nm. When the data was fitted with the powder X-ray Data Analysis System, it was revealed that the $Li_2B_4O_7$:Cu compound

exhibits tetragonal structure. Further the transmission electron microscopy (TEM) has been used to determine the shape and size of the particles of the concerned phosphor. The TEM photograph of $Li_2B_4O_7$:Cu exposed to $5x10^{11}$ ions/cm^2 Ag^{9+} beam shown in Fig.2 reveals that the particles are of uniform rod shape with their average diameter approximately 25 nm.

Fig. 2. TEM image of $Li_2B_4O_7$: Cu sampleexposed to $5x10^{11}$ ions/cm^2Ag^{9+} beam

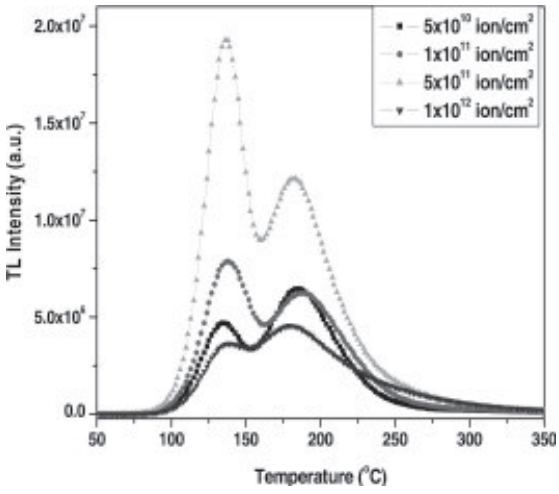

Fig. 3. TL glow curves of $Li_2B_4O_7$:Cu irradiated with 120 MeV Ag^{9+} beam.

The TL properties of synthesized lithium borate dosimeters examined in this study include glow curves and TL response. Fig. 3 shows the TL glow curves of synthesized nanocrystalline $Li_2B_4O_7$:Cu exposed to 120 MeV Ag^{9+} beam of different fluences in the wide range ($5x10^{10}$- $1x10^{12}$ ions/cm^2).

Fig. 3 reveals that for a fluence of $5x10^{10}$ ions/cm^2, the nanophosphor $Li_2B_4O_7$:Cu has two peaks, one at lower temperature of 135 °C and other at higher temperature of 185 °C. The appearance of two peaks in the glow curve of nanophosphor indicates that there are possibly two kinds of trapping sites, one which is shallower leading to the peak at lower temperature and other which is deeper leading to the peak at higher temperature [4]. The peak at 185 °C is the prominent peak. With further increase in dose to $1x10^{11}$ions/cm^2, the lower temperature peak is shifted to 138 °C and higher temperature peak to 190 °C, and the lower temperature peak becomes

dominant. It is also found that with increase in dose from $1x10^{11}$ ions/cm^2 to $5x10^{11}$ ions/cm^2, the TL intensities of both peaks increases. The lower temperature peak is shifted to 137 °C and higher temperature peak to 182 °C. Further when the fluence was increased to $1x10^{12}$ ions/cm^2, the peak intensities of both the peaks decreases drastically. This may be attributed to the fact that on irradiation to high energy radiation, the population of trapping/ luminescent centres (TC/LC) got changed, which reflected on the occurrence of different intensities for the TL glow peaks.

TL response of the samples exposed for higher fluences in the range of $5x10^{10}$ ions/cm^2 to $1x10^{12}$ ions/cm^2 is shown in Fig. 4. It was found that, $Li_2B_4O_7$:Cu nano phosphor irradiated with 120 MeV Ag^{9+} beam exhibits a linear response in the range $5x10^{10}$ ions/cm^2 to $5x10^{11}$ ions/cm^2. The linear behavior over a wide range of fluence can be explained on the basis of track interaction model (TIM) [5,6].

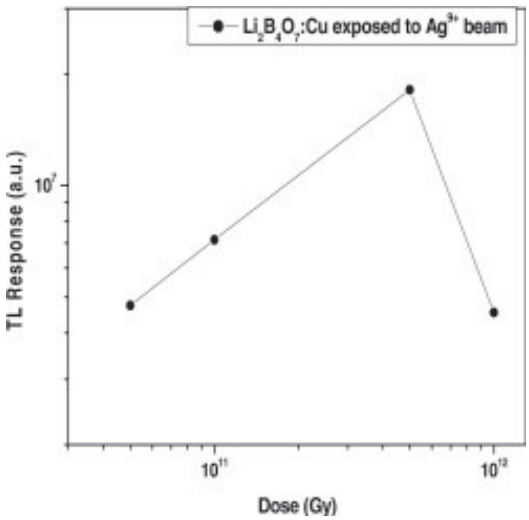

Fig. 4. TL response of of $Li_2B_4O_7$:Cu irradiated with 120 MeV Ag^{9+} beam.

With further increase in fluence to $5x10^{11}$ ions/cm^2, a decrease in the TL intensity has been noticed. The fall in intensity at higher fluences is usually a consequence of competition between radiative and non radiative centre or between different kinds of trapping centres. Therefore the synthesized $Li_2B_4O_7$ nano phosphor exposed to Ag^{9+}beam can be used as TL material within the range $5x10^{10}$ ions/ cm^{2}to $5x10^{11}$ ions/cm^2.

IV. Conclusions

Nanocrystalline $Li_2B_4O_7$:Cu shows a linear response for Ag^{9+} beam in the range $5x10^{10}$ ions/cm$_2$ to $5x10^{11}$ ions/cm^2. So, this material can be useful for their applications in radiation dosimetry of high dose measurements of silver ion (Ag^{9+}) radiations.

Acknowledgments

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Study of performance and emission characteristics of compression ignition diesel engine using biodiesel blends

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Abstract

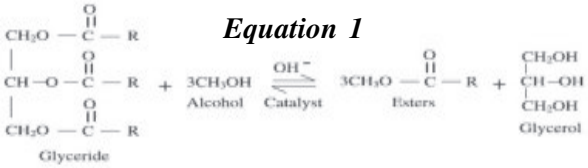
Environmental concerns and energy crisis of the world has led to the search of viable alternatives sources of fuel. Urbanization, industrialization and increasing population has led to steep rise in consumption of fuel all around the world. Biodiesel is a renewable fuel that is produced from vegetable oils and fat tissues. As fossil fuel resources are decreasing daily so biodiesel are attracting increasing attention worldwide. For its production, selection of proper feedstock and reaction parameters is of utmost importance in present scenario. Biodiesel have higher viscosity as compared to other fuels due to which they cannot be directly used for engine operation. Therefore biodiesel is blended with diesel and used as fuel in engine. The aim of present paper is to study the impact of various biodiesel on engine performance. The study reveals that B_{20} biodiesel will be substitute to diesel as an alternative fuel. The results show that BSFC and BTE for B_{20} biodiesel are same as for diesel. Mixed results for NO_x emissions were obtained during the start tests. In addition, HC emissions were higher for the premium blends compared to the corresponding regular blends. CO_2 emissions changes were not significant for the constant-speed tests.

I. Introduction

In today's rising world, the majority of human energy needs are met using petrochemical sources, coal, natural gases, etc. Over the last century,

there has been more than 20 fold increase in the consumption of energy wor ldwide and are likely to be exhausted in near future [1], but these fossil fuels due to their continued use have damaging environmental consequences. Climate change and mitigation of Greenhouse Gases (GHG) emissions are of primary motivation for biodiesel research. Biodiesel is described as fatty acid of methyl or ethyl esters from vegetable oils or animal fats via trans-esterification process through which large molecule of tri-glycerides are transformed into straight chain methyl esters [2, 3]. The process of trans-esterification reduces the viscosity of biodiesel and hence improves combustion characteristics. Biodiesel being a renewable, biodegradable and oxygenated fuel attracts the biodiesel researchers. Biodiesel emits fewer pollutants over the wide range of air–fuel ratio as compared to conventional diesel. Biodiesel emits less GHG and other pollutants due to which it has received wide attention as a replacement for diesel fuel [4, 5]. The present paper attempts to review the work done by the various researchers on the performance and emission characteristics of internal combustion diesel engine using biodiesel and its various blends.

Equation 1



Abbreviations ***Table 1***

B40	40 vol.% Biodiesel in blend with diesel
BTE	Brake Thermal Efficiency
CO	Carbon monoxide
BSFC	Brake Specific Fuel Consumption
CO_2	Carbon dioxide
WCO	Waste Cooking Oil
GHG	Greenhouse gases
UCOME	Used Cooking Oil Methyl Ester
HCs	Hydrocarbon emissions
WFOME	Waste Fried Oil Methyl Ester
SFC	Specific fuel consumption
USEPA	Unites States Environment Protection Agency
NO_x	Nitrogen oxides
DI	Direct Injection
PMs	Particulate matters
ULSD	Ultra low sulphur diesel

II. Performance review

A brief review has been made out form different studies done by researchers regarding the performance of diesel engine blended with biodiesel. Biodiesel as an eco-friendly and renewable fuel substitute for diesel has been getting the attention of researchers/scientists of all over the world. Dwivedi G. et al. investigated that the Brake Specific Fuel Consumption (BSFC) for B_{100} was 14.8% higher than diesel. Also the Brake Thermal Efficiency (BTE) was found higher up to B_{30} in comparison to diesel while BTE of B100 (24%) was almost equals to diesel (24.5%) for Jatropha oil methyl ester (JOME) [3]. Strayer et al. investigated that the Specific Fuel Consumption (SFC) with degummed canola oil and high erucic rapeseed oil as diesel fuel substitutes were higher and concluded that the performance of engine was better with degummed canola oil as compared to crude canola oil [6]. Pryor et al. through his short-term and long-term test concluded that the performance of engine using soybean oil was similar to that of diesel in short term test but could not be carried out in long term test due to power loss and carbon build-up on the injectors [7]. They concluded that the soybean oil can be considered for short-term operation only.

Hamaski et al. studied the performance of a single-cylinder engine at different loads and constant engine speed [8]. They observed that the BTE was similar in all cases when engine is fuelled with the various blends of Waste Cooking Oil (WCO) with diesel fuel having different acid values. Al-Widyan et al studied the performance of direct injection diesel engine at different speed when blended with ethyl ester of waste vegetable oil and diesel fuel in proportions of 75/25, 50/50, 25/75. A higher fuel economy was observed [9]. They concluded that 75/25 blend of the esters of WCO and diesel gives the best performance. Bari et al. tested the performance and durability of a diesel engine fuelled with crude palm oil [10]. They observed heavy carbon deposits in the combustion chamber, wear of piston rings and delivery valve of injection pump with uneven spray formation. Significant improvement in engine performance was observed by Pramanik when diesel engine is fuelled with jatropha oil and diesel fuel when compared to neat vegetable oil. The SFC was reduced due to decrease in the viscosity of the vegetable oil [11]. Acceptable thermal efficiencies were obtained when the

engine was fuelled with blends containing up to 50% of jatropha oil. Canakci and Van Gerpen compared waste oil and soybean oil biodiesel fuels in a 57 kW engine. The tests showed that there was about 2.5% increase in BSFC with 20% blends and 14% from those with pure biodiesel [12]. The study also showed no variations in BTE when using different types of biodiesel blends.

Heavy carbon deposits was observed in a 5.5 kW single cylinder direct injection diesel engine by Ramadhas et al. when engine was fuelled with the blends of rubber seed oil and diesel fuel in proportion of 20/80, 40/60, 60/40, 80/20 [13]. They concluded that the blend up to 80/20 gives acceptable SFC and thermal efficiency. O¨zkan et al. tested WCO biodiesel in a single-cylinder Direct Injection (DI) diesel engine and 25% power loss occurred as compared with diesel. At 1500 rpm the maximum torque of 21.0 Nm was observed for diesel, and a maximum torque of 18.4 Nm was obtained for biodiesel at 2250 rpm. Also, the SFC of diesel was 11.5% lower than that of biodiesel [14]. Murillo et al. studied the performance of a marine outboard three-cylinder naturally aspirated engine fuelled with the blends of WCO and diesel fuel [15]. Results show that at full load, biodiesel resulted in a power loss of 7.14% as compared with diesel fuel. Also there is a reduction in rated power of about 1.502% when using 20% blends and 8% reductions while using pure biodiesel. A higher BSFC was observed by Lapuerta et al. while testing blends of WCO biodiesel with diesel fuel in a DI diesel commercial engine [16]. As the biodiesel concentration in the blend was increased, the BSFC increased while the efficiency of the engine remained unchanged at every tested operation mode.

Rao et al. reported the performance characteristics of Used Cooking Oil Methyl Ester (UCOME) and its blends with diesel fuel in a DI compression ignition engine [17]. The test results shows that BSFC is slightly higher than that of diesel for UCOME and its blends, but BTE for UCOME and its blends was lower as compared to diesel fuel by 2.5%. The BTE of blends of UCOME lies between those of diesel and UCOME at all loads. Reefat et al. tested the performance of microwave-enhanced WCO biodiesel in a Perkins four-cylinder, four-stroke cycle diesel engine [18]. The results shows a slight increase in BSFC of biodiesel blends compared to conventional diesel. Al-Widyan et al. studied the performance of a single-cylinder DI

engine by blending different proportions of the ester with diesel fuel obtained from waste palm oil [9]. Their results shows blend with 100% ester and the 75:25 ester/diesel blends gives lowest BSFC, and highest BTE. Canakci et al. studied the performance of a diesel engine fuelled with waste frying palm oil biodiesel [19]. Results show that the BSFC increases with the increase of biodiesel percentage in the fuel blend whereas brake torques and BTE decreases with the increase in amount of biodiesel in the fuel blend. Engine performance with biodiesel and its blends with diesel fuel depend largely on the factors such as combustion, air turbulence, air-fuel ratio, injector pressure, actual start of combustion which can vary depending upon the quality and origin of biodiesel and engine operating parameters such as speed, load, operating temperature, etc. [20]. Hirkude J. B. and Padalkar Atul S. through their experiment concluded that the BTE decreases with the increase of Waste Fried Oil Methyl Ester (WFOME) in the blend. A BTE of 28.02% for B50 blend was measured which was closer to that of mineral diesel. For B100 blend with diesel fuel BTE was 25.97% at rated output. The SFC increased with increase of WFOME in the blend. B50 gave BSFC of 0.31 kg/kW h while that of diesel was 0.29 kg/kWh [21]. Verma P. and Singh V. M. investigated the performance of diesel engine using cotton seed biodiesel in which a higher BTE was recorded when the blend is preheated with B20, B40 and B60 and was 3.74%, 10.46%, and 3.27% more than that for diesel at full load [22].

III. Emission review

Carbon monoxide (CO), Carbon Dioxide (CO_2), Oxides of Nitrogen (NO_X), Sulphur Oxides (SO_X), Particulate Matters (PMs) and Hydrocarbons (HCs) are the main emission constituents of Biodiesel. A brief review has been made out form different studies done by scientists for these pollutants emitted from the biodiesel [4]. NO_X emissions of biodiesel generally increase because of combustion and some fuel properties but due to high oxygen content CO, CO_2 and PMs Emission reduces as compared to conventional diesel fuel [15]. NO_X emissions can be reduced through the use of water injection, water emulsified biodiesel and injection timing retardation which can further lead to reduction in flame temperature and lastly result to less

production of NO_X [21]. CO concentration depend largely on the air-fuel ratio. Biodiesel is an oxygenated fuel and it contains oxygen atom in its basic form. When its blends are used, it provides more oxygen for the combustion process and lead to the so-called "leaning effect". Due to the leaning effect, CO emission decreases significantly [23-25]. Lapuerta et al. also results that, there is large reduce in PM emissions for the B25 biodiesel blends than that of B50, B75 and B100 biodiesel blends [26].

According to Unites States Environment Protection Agency (USEPA) study on exhaust emission of biodiesel which reported that pure biodiesel can reduce Hydrocarbon (HC) emission by 70%, PMs and CO by 50% when compared with conventional diesel fuel [27]. Dorado et al. tested with the use of methyl ester of used olive oil as fuel in a direct injection diesel engine. According to their report CO, CO_2, NO_X and SO_2 emissions has been decreased by 59%, 8.6%, 32% and 57% respectively and also low smoke emission [28]. Some others literature reviewed that there are no regularity with the increased content of pure biodiesel. For example, Labeckas and Slavinskas found that the B35 blend with 4.075% oxygen produces the maximum NO_X emissions than that of other blends [29]. Narayana Reddy J. and Ramesh A. studied the effect of injection timing, injector-opening pressure, injection rate and air swirl with jatropha oil fuel and concluded that advancing the injection timing and increasing the injector-opening pressure reduces HC and smoke emission level [30].

Lertsathapornsuka et al. experiment on John Deere engine at 1500 r.p.m. speed results that the NO_X emissions were about 12.62% and 1.84% higher for B100 and B50 than conventional diesel [31]. Srivastava et al. with his experiments shows that CO emission decreases by approximately 4-46.5% in a high load condition but increases with the presence of a high percentage of biodiesel in the fuel blend [32]. Murayama et al. through his experiment reported that vegetable oils and methyl ester of rapeseed oil produces less smoke and NO_X [33]. Release of CO and unburned HCs among the exhaust gases represent the loss of chemical energy which is not fully utilized in the Engine [34]. Fontaras G et al. studied that the PM emission at 7 kinds of driving cycles is higher for B50 than that of B100 [35]. Similarly, Aydin and Bayindir has also found the same that higher the content of biodiesel in

the blends, high is the PM emissions and also contributed to the reason of high density and high viscosity which deteriorates the fuel atomisation [36]. Scholl and Sorenson shows that, for soybean ester CO, NO_X and smoke emissions was slightly lower than diesel, whereas there is 50% reduction in HC emission compared to diesel fuel [37]. Further a review literature reveals that the engine operated on biodiesel blends will emit lower gaseous emissions than diesel fuel except NO_X which increase to 2% and 10% with B20 and B100 blends [38]. Barsic and Humke shows that, with the increase in amount of vegetable oil in the blend leads to the increase in amount of carbon deposits on the injector tip as compared to 100% conventional diesel fuel [39].

The NO_x emission has been reported to be slightly elevated by most of researchers. In the past two decades, many researchers and manufacturers have been developed various methods to reduce exhaust emissions including the Low Temperature Combustion (LTC) [40]. Ashraful A.M. et al. through his literature review shows a decrease in PM emissions with increase in biodiesel content in the fuel, whereas NO_X emissions increases by 4.15-14.18% with increase in biodiesel in the fuel blend. On the other hand, it decreases by 4-39% with the presence of low biodiesel in the fuel blend [41].

IV. Biodiesel economy

Biodiesel that is derived from vegetable oils is gaining acceptance and market share as diesel fuel in Europe and the United States. Though biodiesel has relatively high production costs and some of its raw materials used in its production are limited which causes to limit its commercial application. Limiting factors of the biodiesel industry are feedstock prices, biodiesel production costs, crude oil prices, and taxation of energy products.

The economic advantages of biodiesel are that it reduces greenhouse gas emissions, helps to reduce a country's reliance on crude oil imports, and supports agriculture by providing new labour and market opportunities for domestic crops. In addition it enhances lubrication and is widely accepted by vehicle manufacturers [42,43]. Many farmers who raise oilseeds use a biodiesel blend in tractors and equipment as a matter of policy to foster production of biodiesel and raise public awareness. It is sometimes easier to find biodiesel in rural areas than in cities.

V. Economic benefits of biodiesel

Biodiesel is a renewable fuel manufactured from vegetable oils, animal fats, and recycled cooking oils. Biodiesel offers many benefits [44]:

1. It is renewable.
2. It is energy efficient.
3. It displaces petroleum-derived diesel fuel.
4. It can be used in most diesel equipment with no or only minor modifications.
5. It can reduce global warming gas emissions.
6. It can reduce tailpipe emissions, including air toxins.
7. It is non-toxic, biodegradable, and suitable for sensitive environments.
8. It is made from either agricultural or recycled resources.

VI. Present biodiesel policy

The European Union accounted for nearly 89% of all biodiesel production worldwide in 2005. Germany produced 1.9 billion litres, or more than half the world total. Other countries with significant biodiesel markets in 2005 included France, the United States, Italy, and Brazil. All other countries combined accounted for only 11% of world biodiesel consumption in 2005. In Germany biodiesel is also sold at a lower price than fossil diesel fuel. Biodiesel is treated like any other vehicle fuel in the UK. The European Union has set the goal of obtaining 5.75% of transportation fuel needs from biofuels by 2010 in all member states. Many countries have adopted various policy initiatives. Specific legislation to promote and regulate the use of biodiesel is in force in Germany, Italy, France, Austria, and Sweden. New and large single markets for biodiesel are expected to emerge in China, India, and Brazil [45].

VII. Conclusion

The summary of the present literature review is as follows: BSFC has been found to increase in most of the papers while using biodiesel. BSFC increases with increase in percentage content of biodiesel in the blend. This is due to its lower heating value. Some authors found similar results of BTE while others reported decrease of BTE with increase in percentage content

of biodiesel in the fuel. As the percentage of biodiesel in the blend increases the ignition delay of biodiesel decreases which is less as compared conventional diesel. The effective power decrease at full-load conditions but shows no change at partial load conditions. However, biodiesel is still having some drawbacks when compared with petroleum-based diesel fuel which includes worse low temperature properties, greater emissions of some oxygenated HCs, higher SFC, decrease in BTE and higher production cost. A relatively high disparity of results has been found regarding the emissions characteristics of biodiesel blends. Most of the reports recorded slight increases in NO_X when compared to diesel at rated load. Due to high oxygen content, proper combustion of biodiesel takes place; hence CO and unburnt HC emissions are eliminated. It is also reported that there is a sharp reduction in PM and smoke intensity of biodiesel blends. Biodiesel due to its negligible sulphur content score very well as an alternate fuel. This review work shows that in recent years, biodiesel has been in focus as a replacement component of petroleum diesel. Most countries in the world are exploring alternate renewable energy resources which are eco-friendly, in which biodiesel is one of them.

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Impact of surfactant and co-surfactant on the structural, morphological and electrical properties of nanocrytalline copper oxide

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Abstract

CuO assembled in the form of nanoparticle clusters have been prepared via a sol-gel route. The effect of co-surfactant polyethylene glycol (PEG-400) in addition with surfactant cetyltrimethylammonium bromide (CTAB) on the structural, morphological and electrical properties of CuO has been studied. The thermal decompositions, crystallite size, morphology and elemental composition of the product were investigated from the obtained data in thermogravimetric (TGA/DTA), X-ray powder diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) analysis respectively. TGA/DTA analysis indicates a single step decomposition of dried precursor and, suggests suitable calcination temperature of 400^{o}C based upon thermal stabilization of the product. XRD diffractogram of sample shows the formation of prominently (002) oriented monoclinic CuO nanoparticles. SEM and TEM observation reveals that the presence of co-surfactant PEG and surfactant leads to an oriented growth of CuO cluster and mictrostructure finally evolves in nanorods like morphology. The elemental composition as indicated by EDAX data showed that product compose of stochiometric defects created by enrichment of oxygen in samples. The powder based thick films have been investigated to calculate activation energy of charge carriers by two most widely used techniques of two and four probes electrical characterization of samples. The electrical studies of film samples reveals that four probe measurement are quite fit better to the law for thermal activation of charge carriers with high value of correlation coefficients (R^2=0.99). The activation energy of the sample has been found be 0.92 eV. The role played by surfactants CTAB and PEG-400 in affecting the overall properties of CuO has been also discussed.

Keywords

CTAB, PEG, CuO

I. Introduction

The chemical route to synthesize metal oxide nanocrystalline materials with distinct properties over their microcrystalline counterparts have attracted considerable attention owing to their important applications in various fields such as catalyst [1], window material [2], photosensor [3], and gas sensor [4, 5] etc. Nanocrystalline CuO material as a p-type semiconductor has a very wide range of applications in industry and varieties of processing techniques [5-7] have been suggested by various researchers for its synthesis in different forms. The chemical, precipitation and sol–gel methods have drawn considerable attention due to their simple processing route and as no sophisticated instrument are required for large scale synthesis [8, 9]. Moreover, the sol–gel auto combustion synthesis seems to be an interesting and powerful method due to production of single phase ceramics at relatively low temperatures, better compositional homogeneity, huge porosity and purity of the synthesized powder [10-12].

Surfactants (cationic, anionic and non-ionic) can play an important role in oriented growth of nanostructures in different interesting morphologies. They may be used to control the size, shape and agglomeration among the particles. The addition of surfactant reduces surface tension of precursor solution, which facilitates nucleation and allows its easier spreading. Surfactant acts as polymer which have tendency to absorb on the specific crystal planes and hence causes the anisotropic grain growth [14-20]. However the CTAB and PEG have seldom been used simultaneously in sol gel auto combustion process. In this present work the sol gel auto combustion technique has been used for the synthesis of CuO nanoparticles in the presence of both CTAB and PEG-400 as regulators of nucleation and crystal growth.

II. Experimental

All the chemicals (Loba Chemie Mumbai) were of analytical reagent grade used as precursors. Cupric nitrate ($Cu(NO_3)_2 \cdot 3H_2O$) and citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$) were used as starting materials. PEG-400 was

used as co-surfactant in addition to CTAB as surfactant. $Cu(NO_3)_2 \cdot 3H_2O$ and citric acid were dissolved in deionized water by taking metal nitrate to citric acid ratio 1:1 and the pH of the solution was adjusted to 7. In the first step, the solution was continuously stirred using a magnetic stirrer at 80^oC for 4 hours. An aqueous solution of CTAB ($(C_{16}H_{33})N(CH_3)_3Br$) and PEG 400 were prepared by dissolving it in deionized water to give a final concentration of 0.5M in both cases. In the second step the 10 ml of aqueous CTAB and co-surfactant PEG-400 solution were added drop wise to the precursor solution with vigorous stirring. The solution was thermally dehydrated in an oven (80 ± 5^0C), which resulted in a viscous liquid. The viscous liquid was heated on a pre-heated hot plate maintained at temperature around 300^0C. The detailed combustion mechanism has been reported earlier [18-20]. The decomposed gels so formed were calcined at 400^0C for 4 hours with heating rate of 10^0C/min in a muffle furnace (Macro Scientific). This results a complete transformation to fine pure CuO powder without any impurity.

The thermal analysis of dried gel samples with and without the addition of surfactant was carried out using Perkin Elmer (Pyris diamond) Thermal analyzer. The phase identification of samples was performed by taking X-ray diffraction (XRD) pattern using X'Pert Panlytical diffractometer with Cu K_α radiation (λ = 1.5405 Å , 30mA , 40 kV) in 2θ range from 30-80°. The surface topography of copper oxide powder sample was studied by field emission scanning electron micrographs, taken using JEOL JSM-6700F with a beam voltage of 30 kV. TEM images were taken using transmission electron microscope system (HRTEM, model FEI Technai 30) operated at 300 kV. The electrical characterization of film was done by heating with a specially designed heater and temperature was measured using a K-type (chromel–alumel) thermocouple obtained from Omega Engineering Inc. (USA). The four probe measurements were performed on high resistivity measurement set up of Scientific Roorkee (India) make.

III. Results and discussion

Observation from the thermogravimetric analysis shows that surfactant modified nitrate-citrate gel exhibit auto-catalytic combustion behaviour. Fig. 1 shows the TGA/DTG plots of metal-citrate complex which indicates single step thermal decomposition of dried gel samples due to the occurrence of

auto combustion reaction. The plot reveals that CTAB and PEG assisted sample shows a major weight loss at temperature of 240^0C, and large amount of gaseous products were released in this interval. No significant weight loss has been observed beyond 400^0C which indicate the attainment of thermal stabilization of sample. Based upon these observations the optimum temperature of calcination was chosen to be 400^0 C

The XRD diffractogram of decomposed gel and 400^0C calcined CTAB-PEG doped sample illustrated in Fig. 2. The XRD pattern show broad, intense diffraction peaks, characterizing well ordered and nanosized copper oxide phases as indexed by various miller indices. Intense diffraction peaks corresponding to reflection from (002) and (111) atomic planes of CuO phase have been found for the decomposed gel and calcined sample at 2θ value of 35.5^0 and 38.7^0 respectively. These directions are the prominent and require minimum energy for their growth.

Interestingly, no peak corresponding to Cu_2O phase has been noticed in the diffraction pattern of calcined samples thus indicate complete transformation to CuO phase.

The FESEM, TEM and EDAX images of the surfactant assisted CuO samples calcined at 400^0C are shown in Fig. 3. The porous feature of the agglomerates obtained in all the samples may be attributed to the liberation of large amount of gaseous products during combustion reaction. TEM images of CuO samples show the evolution of grains in rod shaped clusters which appear to possess huge porosity and minimum agglomeration. The morphology as indicated by TEM images suggests the formation of rod like micelles in gel which are responsible for final rod like microstructure of product.

The EDAX spectrum reveals elemental composition of Cu and O atoms. The significant oxygen enrichment of about 43% has been observed in sample and suggests that sample is having large number of defects.

PEG is a non-ionic surfactant and able to act as a co-template. Among the common water-soluble polymers, PEG is one of the most flexible polymers in an aqueous medium. There is a close similarity between a polymer-induced micellization and the micellization of the surfactant alone; indeed, PEG, which is located in the outer part of the micelles, tends to strongly induce the micellar growth. In this case, the micelles creep like a

snake through tubes in a porous structure given by the other micelles. At very high concentration the linear growth may lead to branched micelles.

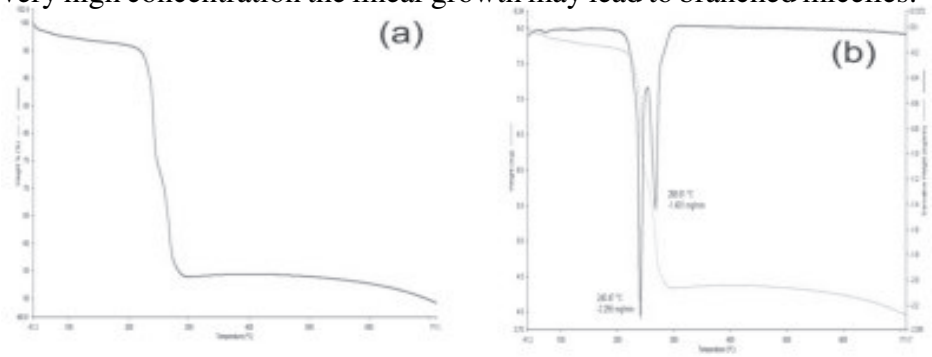

Fig. 1 (a) TGA, (b) DTG curves of the dried gel samples

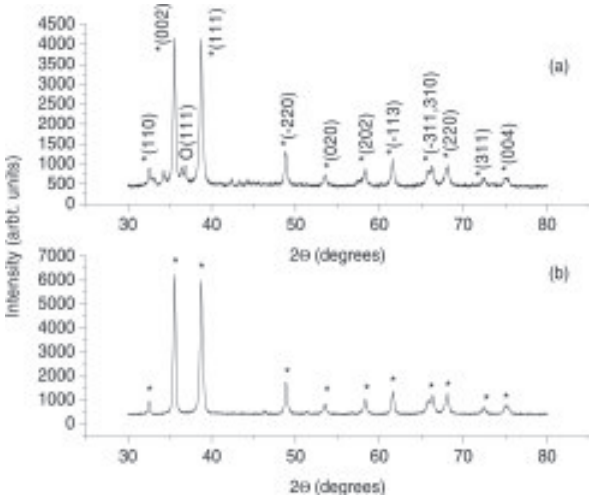

Fig. 2 XRD diffractogram of the (a) decomposed and (b) 400^{o}C calcined samples of PEG-CTAB doped CuO powder

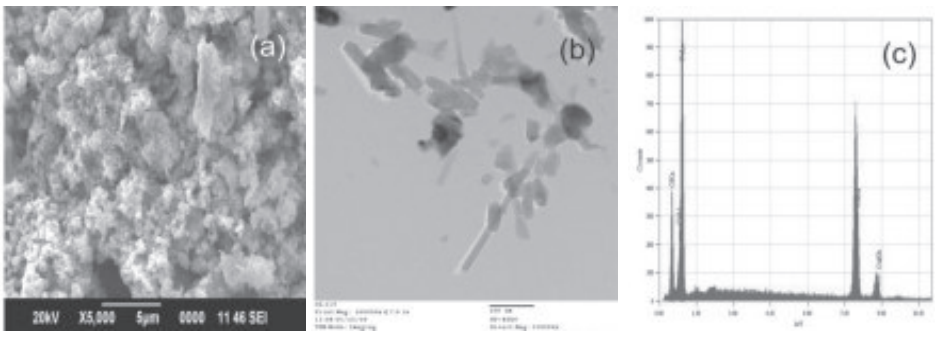

Fig. 3 (a) FESEM, (b) TEM and (c) EDAX spectrum of the PEG-CTAB doped 400^{o}C calcined CuO sample

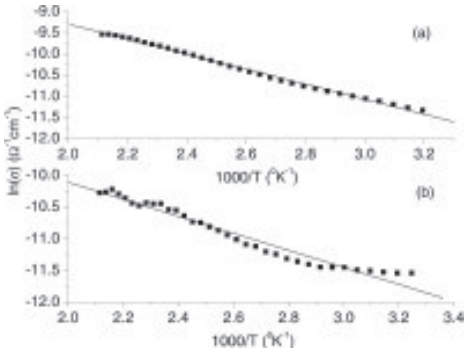

Fig. 4 ln(σ) vs. 1000/T plot for the CPEG-PEG assisted sample.

The electrical resistance of film was measured as function of temperature in the range of 300K-473K. The resistance of film in case of sample has been found to be 10.26 MΩ at room temperature. The decrease in resistance of film with temperature is quite likely as large number of oxygen molecules are chemisorbed at the grain boundary and on surface of film. It may be mentioned that the principal chemisorption species in CuO is O_2^{-1} at room temperature. With the rise in temperature, the rate of chemisorbtion of O_2^{-1} increases on the sample surface, which results in the decrement of surface resistance of films. The dependence of conductivity on temperature can be represented by the Arrhenius equation [21]

$$\sigma = \sigma_0 \exp(-\frac{\Delta E_a}{kT})$$

where E_a is the activation energy that corresponds to energy difference between valence band and conduction band, σ_o is a temperature independent factor, and k is the Boltzman's constant, and T absolute temperature. The increase in conductivity of films with temperature indicate their semiconductive behaviour. Fig. 4 depicts the lnσ vs 1000/T plots for CuO samples by using two probe and four probe measurement techniques. The linear nature of plots is indicated by the high R^2 value of 0.97 and 0.99 for two probe and four probe measurements respectively, implies that the themoionic emission plays a major role in the carrier transport within the experimental temperature range [22]. The activation energies of samples have been obtained from slope of plots and found to be 1.18 eV in case of two probe measurements, whereas it decreases to 0.92 eV corresponds four probe measurements. The high R^2 value in case of four probe measurements of electrical properties of sample suggests that it fits much better experimental data to theoretical law as given by the

arrhenious equation. The high value of activation energy of sample can be understood from the small crystallite size as confirmed by XRD, and TEM analysis. The decrease in crystallite size increases the scattering of carriers at grain boundaries, this results in a decrease in the mobility as suggested by Bouderbala et al. (2009) [23]. The porous nature as induced by the action of surfactant and co-surfactant might also be responsible for the increases in resistance, hence activation energy. Perez-Ramos et al. (2003) [24] have also observed the effect of porosity on the electrical conductivity in case of Fe based samples.

IV. Conclusions

Nanocrystalline CuO powder was synthesized by the addition of surfactant, co-surfactant in sol-gel auto combustion method. Monoclinic CuO crystallites of 29 nm in size entangled in rod shaped clusters possesses minimum agglomeration has been synthesized. The four probe conductivity measurement has been found to be more accurate in determining activation energy of charge carriers. The activation energy has been found to be of 0.92 eV and this high value might be due to small crystallite size as well as porosity induced by surfactant and co-surfactant in CuO microstructure.

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Buccal micronucleus test as a reliable tool to assess genotoxicity

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Abstract

Assessment of DNA damage is an essential part of genetic toxicology because it is an important event in carcinogenesis. The micronucleus assays have emerged as one of the preferred methods of assessing chromosomal damage because they enable both chromosomal loss and chromosomal breakage to be measured reliably. Micronucleus is small chromatin body that appears in cytoplasm by condensation of acrocentric chromosomal fragments or by whole chromosome lagging behind during cell division. Micronucleus assay in buccal exfoliated cells is a minimally invasive method for monitoring genetic damage in human population. This method is now applied to various cell types for population monitoring of genetic damage and screening of chemicals for genotoxic potential. Issues related to sample size and staining based on micronucleus test are also addressed. Micronucleus test can be used as a simple, sensitive and predictive biomarker for the detection of cancer and this paper gives a brief overview of the buccal micronucleus technique.

Keywords

Micronucleus, Buccal, Genotoxicity, Cancer, Protocol

I. Introduction

Genotoxicity is the property of any chemical agent that damages the genetic information within a cell causing mutations which may lead to cancer.

Genotoxicity is often confused with mutagenicity. All mutagens are genotoxic, however, not all genotoxic substances are mutagenic. The alteration can have direct or indirect effects on the DNA including induction of mutations, mistimed event activation and direct DNA damage leading to mutations. The genotoxic substances induce damage to the genetic material in the cells through interactions with the DNA sequence and structure. The transition metal chromium interacts with DNA in its hexavalent oxidation state so as to produce DNA lesions leading to carcinogenesis. The changes can affect either somatic cells of the organism or germ cells which may pass on to future generations. Cells try to prevent the expression of genotoxic mutations by DNA repair mechanisms. To assay the genotoxic effect of different chemicals, researchers are using both in-vivo and in-vitro techniques. The DNA damage can be in the form of single or double strand breaks, loss of excision repair, cross-linking, alkali-labile sites, point mutations and structural and numerical chromosomal aberrations. The compromised integrity of the genetic material has been known to cause cancer. Many sophisticated techniques including Ames test, Comet assay and micronucleus test have been developed to assess the potential of different chemicals causing DNA damage which may lead to cancer. The purpose of genotoxicity testing is to determine if a chemical will damage genetic material or not. Various tests have been developed to assess the genotoxic potential of different chemicals and metals in different animal and plant models including bacteria, yeasts, earthworms, fishes and mammalian cells.

II. Buccal micronucleus

Buccal micronuclei are small chromatin bodies that appear in cytoplasm of Buccal mucosal cells (Fig.1) by condensation of acro-centric chromosomal fragments or by whole chromosome lagging behind during cell division. Thus, it is only biomarker that allows simultaneous evaluation of clastogenic effect in wide range of cells that are easily detected in interphase cell. Micronucleus is name given to small nucleus that forms whenever a chromosome or its fragments is not incorporated into one of daughter nuclei during cell division.

Micronuclei originate from chromosome fragments that lag behind at

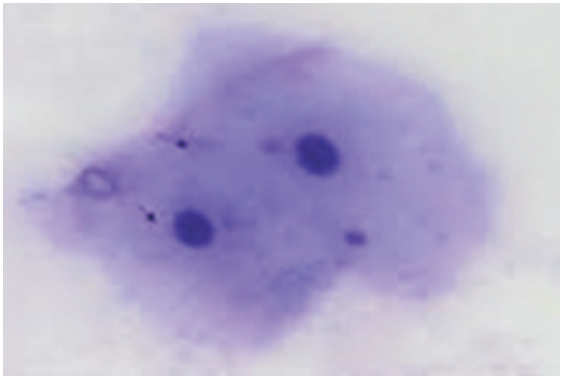

Fig.1 A buccal epithelial cell showing micronuclei

anaphase during nuclear division. This damage may occur due to excessive exposure to chromosomal damaging agent, defects in mitosis and DNA mis-repair. MN can be easily assessed in erythrocytes, lymphocytes and exfoliated epithelial cells to measure the genetic damage. The x chromosome especially tends to lag behind in female lymphocyte anaphase. Two basic phenomena leading to formation of micronucleus in mitotic cells are chromosomal breakage and dysfunctionof mitotic apparatus. Laggards cannot move to poles as they are detached from mitotic spindle and they have bipolar orientation. Beside this some MN may have their origin in fragments derived from broken anaphase bridges formed due to chromosomal rearrangements such as dicentric chromatids, intermingled ring chromosomes or union of sister chromatids.

III. Methodology

Buccal micronuclei are a reliable marker of genetic damage and have been employed by different researchers on different models [1-68]. The steps involved in the making of slides for assessing micronuclei are preparation of smears, fixation in an appropriate fixative, hydrolyses in hydrochloric acid and staining. Then the slides are scored for micronucleated cells.

A. Preparation of slides

Slides for buccal micronucleus test can be prepared according to Singh and Chadha [69]. After moistening the mouth, exfoliated cells are collected from buccal mucosa by swabbing with a wooden spatula. Smears of buccal mucosal cells are prepared on slides. The slides are coded according to the subjects for further identification.

B. Fixation and hydrolysis

Within 3-4 hours of sampling, the air dried slides are fixed in freshly prepared fixative [69] for 15 minutes. Again the slides are to be air

dried. Buccal smear preparation is then hydrolysed in 1 N HCl at 60°C for 8 minutes followed by a rinse in double distilled water.

C. Aceto-orecein staining

After the fixation and hydrolysis procedure, the slides are placed in a coupling jar containing a solution of aceto-orecein stain for 20 minutes at 40°C. After this, the slides are washed with ethanol and subsequently with double distilled water.

D. Counter staining

Counter staining with fast green is carried out by placing washed slides in a coupling jar containing 1% fast green solution for 12 minutes. After rinsing in ethanol and distilled water, the air dried stained slides are mounted in DPX.

E. Scoring of slides

The air dried slides can be scored under a microscope. 2000 to 4000 cells per slide are scored for the presence of micronucleated cells. Photomicrography of normal and cells with MN is done using a digital camera.

F. Recording of observations

The number of micronucleated cells (MNed cells) and number of micronuclei (MNi) are counted from the microscope. Overlapping cells should be avoided. The micronuclei can be observed according to the criteria of Tolbert et al. [70].

a) The diameter of MNi is usually 1/3rd of the mean diameter of the main nucleus.
b) MNi are non-refractile and can be readily distinguished from artifact such as staining particles.
c) MNi are not linked or connected to the main nuclei.
d) MNi may touch but not overlap the main nucleus and the micronuclear boundary should be distinguishable from the nuclear boundary.
e) MNi usually have the same staining intensity as the main nucleus.

IV. Stains for micronucleus test

Different stains are used to identify the MNi in a cell (Fig.2). The

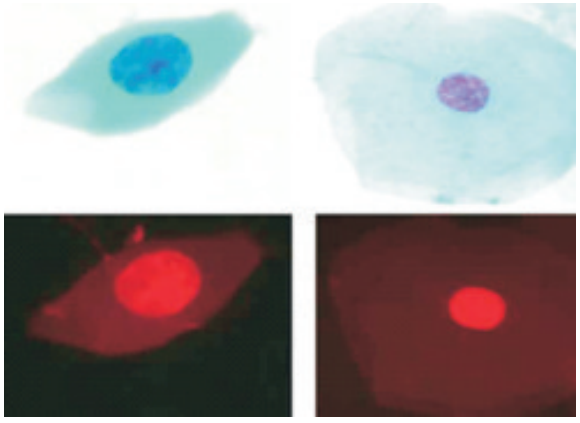

Fig.2 Images of the different cell types stained using Feulgen under light and fluorescence microscope.

most crucial point is that the counting of non-nuclear bodies as MNi should be checked. To prevent this, fluorescent nuclear stains are preferred than general cell stains. Different researchers have used a number of stains for the evaluation of buccal micronucleus. The stains used include feulgen/fast green stain [71, 72], fluorescent propidium iodide stain [73], Wright's stain [74] and fluorescent DAPI stain [75]. Various other stains used are ethydium bromide, aceto-orecein, Giemsa and May-Graunald stain. Large number of earlier studies used DNA unspecific stains like Giemsa which leads to false positive results as they also stain keratin bodies that are formed in epithelial cells as a consequence of cytotoxicity.

V. End points used

There is a criterion for identifying and scoring cell types in the buccal micronucleus assay (BMNA). The scoring criteria for the various distinct cell types and nuclear anomalies in the BMNA are mainly based on those originally described [70]. These criteria are intended for classifying buccal cells into categories that distinguish between normal cells and cells that are considered abnormal on the basis of cytological and nuclear features, which are indicative of DNA damage. A more detailed description of the scoring criteria for the BMNA cell types is given. Normal basal cells have a larger nucleus-to-cytoplasm ratio than the differentiated buccal cells. Basal cells have a uniformly stained nucleus and are smaller in size and more oval in shape when compared to the more angular and flat differentiated buccal cells. No DNA-containing structures apart from the nucleus are observed in these cells. The cytoplasm is typically stained in a darker shade of green as compared to the differentiated cells. Normal differentiated cells have a uniformly stained nucleus which is

oval or round in shape. They are distinguished from basal cells by their larger size and by a smaller nucleus-to cytoplasm ratio. No other DNA-containing structures apart from the nucleus are observed in these cells. These cells are considered to be terminally differentiated.

A. Cells with micronuclei

These cells are characterized by the presence of both a main nucleus and one or more smaller nuclear structures called micronuclei. The micronuclei are round or oval in shape and their diameter should range between 1/3 and 1/16 of the main nucleus. Micronuclei have the same staining intensity and texture as the main nucleus. Most cells with micronuclei will contain only one micronucleus but it is possible to find cells with two or more micronuclei. Cells with multiple micronuclei are rare in healthy subjects but become more common in individuals exposed to radiation or other genotoxic agents. Cells which are pyknotic (with shrunken nuclei) or karyorrhectic are usually not scored for MNi.

B. Cells with nuclear buds

These cells contain nuclei with an apparent sharp constriction at one end of the nucleus (Fig. 3) suggestive of a budding process which might be a sign of elimination of nuclear material by budding. In the original publication by Tolbert et al. [70], the cells with nuclear buds were referred to as 'broken egg' cells. The nuclear bud (NBUD) and the nucleus are usually in very close proximity and appear to be attached to each other. The nuclear bud has the same morphology and staining properties as the nucleus; however, its diameter may range from a half to a quarter of that of the main nucleus. The mechanism leading to nuclear bud formation is not known but it may be related to the elimination of amplified DNA or DNA repair.

C. Binucleated cells

These are the cells which contain two main nuclei instead of one (Fig. 3). The nuclei are usually very close and may touch each other and usually have the same morphology as that observed in normal cells. These cells are probably indicative of failed cytokinesis following the last nuclear division in the basal cell layer.

TABLE 1: End points used in selected population studies using buccal micronucleus assay [76].

End points	N	Percentage
MNi frequency	31	32.29%
MNed frequency	17	17.70%
MNi and MNed frequency	12	12.50%
Binucleated cells	14	14.58%
Broken eggs/nuclearbud	13	13.54%
Pyknotic cell	09	9.37%

D. Buccal cells with condensed chromatin

These cells show a roughly striated nuclear pattern in which the aggregated chromatin is intensely stained. Similar nuclear morphologies have also been shown in other cell types (Fig.3). In these cells it is apparent that chromatin is aggregating in some regions of the nucleus while being lost in other areas. When chromatin aggregation is extensive the nucleus may appear to be fragmenting.

E. Karyorrhectic cells

These cells have nuclei that are characterized by more extensive nuclear chromatin aggregation relative to condensed chromatin cells. They have a dense nuclear pattern indicative of nuclear fragmentation leading to the eventual disintegration of the nucleus. These cells are avoided to be scored for MNi assay.

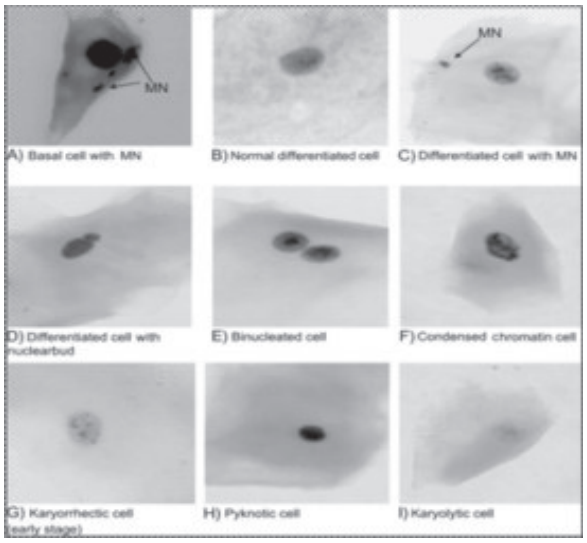

Fig. 3 Showing different deformities in buccal cells.

F. Pyknotic cells

These cells are characterized by a small shrunken nucleus with a high density of nuclear material that is uniformly but intensely stained. The

nuclear diameter is usually one third to two third of a nucleus in normal differentiated cells. It is thought that these cells may be undergoing a unique form of cell death; however, the precise mechanism remains unknown.

G. Karyolytic cells

These are cells in which the nucleus is completely depleted of DNA and is apparent as a ghost-like image that has no Feulgen staining. Therefore, these cells appear to have no nucleus and represent a very late stage in the cell death process.

The most commonly studied endpoints were frequency of MN (32.29% of studies), frequency of micronucleated cells (17.70%), while 12.50% of studies evaluated both the endpoints (Table 1). The other endpoints of the cytome assay (basal cells, binucleated cells, nuclear buds, pyknotic cells, karyolytic cells, karyorrhectic cells and condensed chromatin cells) were much less frequently evaluated.

VI. Sample size

Sample size is an important step of study design and should be large enough to reach statistical significance.Sample size is a critical issue in biomonitoring studies when the frequency of the biomarker is rare. In an another review [76], sample size ranged from 11 to 441 subjects, with an average of 111.03 (S.E., 11.28). In most cases (> 60%), the studied population ranged between 50 and 150 subjects.

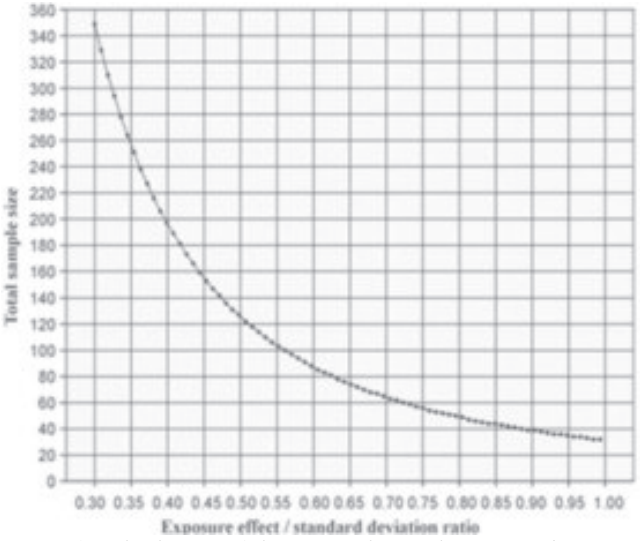

Fig. 4 Calculation of the optimal sample size to observe a statistical significant difference of mean micronuclei frequency between controls and exposed subjects. Total sample size is given by the ratio between the expected exposure effect and the common standard deviation in the two groups. [76]

Table 2 Selected characteristic population studies using buccal MN assay with respect to sample size.

Sample size	N	Percentage
<50	11	17.5
50-99	24	38.1
100-149	16	25.4
≥150	12	19·0

Fig. 4 shows the total sample size necessary to observe a statistically significant difference between the means of controls and exposed expressed as a function of the ratio between the estimated effect of exposure and the common standard deviation in the two groups. Sample size depends on the extent of the expected effect and on the variability of all observations. Since the latter is unknown, it is convenient to express it as a function of the expected effect. If the mean of MNi frequency in buccal exfoliated cells of control subjects is 1 per 1000 cells and the exposure may increase this value by 50%, i.e., the exposure effect is estimated to be 0.50 MNi per 1000 cells, assuming that the ratio between exposure effect and the standard deviation is 0.50, we can conclude that the total number of subjects corresponding to this ratio is a little more than 120, i.e. about 65 exposed and 65 controls are required to obtain a significant result if MNi frequency in buccal cells of exposed subjects is 50% higher than in controls. If the expected difference between the mean of control and exposed is smaller or the variability is larger, the number of subjects has to be increased.

VII. Cell scoring

Recommended number of cells to be scored

The optimal number of cells to be scored for the buccal MN assay represents the point of equilibrium between the sensitivity and stability given by large numbers, and the need to save time and resources. A single best value does not exist and depends on the study constraints. A Monte Carlo method was applied to simulate the distribution of the sample MN means in a hypothetical population of cells. From Fig. 5, it is clear that increasing the number of cells scored results in a smaller confidence interval of the estimates. The width of the confidence interval tends to level off after 4000 cells. Table

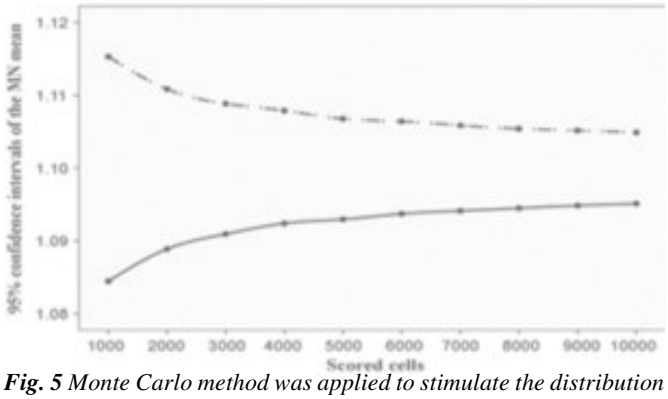

Fig. 5 *Monte Carlo method was applied to stimulate the distribution of the sample MN means in a hypothetical cells population. Ten samples were randomly drawn from a log-normal distribution with mean and standard deviation corresponding to the respective average values observed in the control groups included in the meta-analysis. The size of each sample ranged from 1000 to 10000 (step 1000) which represents a plausible range of cells to be scored.*

3 shows different studies based on number of cells scored.

Indeed between 1000 and 4000 cells scored there was a reduction of about 50% of the width of the confidence interval. It shows that as scoring based on 1000 or 2000 cells is affected by a large variability, raising this number to 4000 cells reduces this factor. Further increase in the number of scored cells does not remarkably increases the efficiency of the test. Thus a minimum of 4000 is a recommended number of cells to score for Buccal micronucleus test. Table 4 shows different studies with respect to variable sample size.

Table 4: Selected characteristic 63 population studies using Buccal MN Assay with respect to number of cell scored.

Sample size	N	Percentage
<50	11	17.5
50-99	24	38.1
100-149	16	25.4
≥150	12	19·0

VIII. Conclusion

Genotoxicity describes the property of chemical agents that damages the genetic information within cell causing mutations. The methods for micronucleus studies with exfoliated cells have been substantially improved in last years by joint efforts of the HUMN consortium. It was shown that MN frequencies of exfoliated cells of buccal mucosa correlate with human cancer risks and are therefore valuable biomarkers for the detection of exposure to genotoxic carcinogens. Conclusively, buccal micronucleus test

may be used as a simple, sensitive and predictive biomarker for the detection of cancer.

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Effect of humic acid derived from vermicompost on the growth of plants: A review

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Abstract

Vermicompost is not only a valuable compost and a biocontrol agent but it is also an effective way of solid waste management.Vermicompost is ideal organic manure for better growth and yield of many plants. It can increase the production of crops and prevent them from harmful pests without polluting the environment. Studies suggested that treatments of humic acids and vermicomposts on plant increased their growth and can be used for a sustainable agriculture and discouraging the use of chemical fertilizers.In the present review, humic acid derived from vermicompost has been described as an excellent soil amendment through a number of studies along with the reasons which make it the best organic fertilizer and more eco-friendly..

I. Introduction

Humic substances are heterogeneous mixtures of naturally occurring molecules which are present in all soils, water and sediments. Humic substances are primarily the microbiological transformation products of lignin and other plant detritus. They cannot be classified as any other chemical class of compounds and are traditionally defined according to their solubility. Humic acids (HA) are insoluble at acidic pH values (pH>2) and soluble at higher pH values, while fulvic acids (FA) are soluble in water at all pH values. From an environmental point of view HA and FA are particularly interesting because in their molecular structures there are both polar and non-polar substituents therefore, they can interact both with water soluble and insoluble compounds [1]. Humic compounds are produced by abiogenic chemical reaction, including condensation, polymerization, oxidations and

reductions, by which relatively low molecular weight compounds such as degradation products of biopolymers (i.e. proteins and carbohydrates) linked to one another [2]. FA is generally more aliphatic and less aromatic than HA and is richer in carboxylic, phenolic and ketonic groups.

II. Effect of humic acids derived from vermicompost on plant growth

It is well established that earthworms have beneficial physical, biological and chemical effects on soils. Many researchers have demonstrated that these effects can increase plant growth and crop yield in both natural and managed ecosystems [3-4]. These beneficial effects have been attributed to improvements in soil properties and structures to greater availability of mineral nutrients to plants, increased microbial populations and biologically active metabolites such as plant growth regulators [5-8].

The earthworms fragment the organic waste substrates, stimulate microbial activity greatly and increase rate of mineralization, rapidly converting the wastes into humus like substances with a finer structure than composts but processing a greater and more diverse microbial activity, commonly referred to as vermicompost [9]. The effects of vermicompost on the growth of a variety of crops including cereals and legumes, vegetables, ornamental and flowering plants have been easy to read in the green-house and to lesser degree in field crops [10]. These investigations have demonstrated consistently that vermicomposted organic wastes have beneficial effects on plant growth. The vermicompost have consistently improved seed germination, enhanced seedling growth and increased plant productivity. When humic acids derived from pig manure and food waste vermicompost were mixed in different ratios to grow tomato and cucumber seedlings, there was a significant increase in the growth and yield of tomato and cucumber**.** List of parameters of various plants enhanced by applying humic acids extracted from vermicompost are given in Table 1.

It was observed that effect of humic acids on plant growth depended upon their applied concentrations, plant species, nature of container medium and source of vermicompost. Authors attributed the increase in growth to hormone like activity of vermicomposts [11]. In another study, Humic acids were extracted from cattle, food and paper-waste vermicomposts substituted

Table 1. List of parameters of various plants enhance by applying humic acids extracted from vermicompost

S.No	Crop/Plant	Parameters enhanced	References
1.	Tomato (*Lycopersicum esculentum* L.) and Cucumber	Plant Height, Leaf area, Root dry weight, Shoot dry weight	Atiyeh et al. 2002
2.	Marigold, Pepper and Strawberry	Plant heights, Leaf areas, Shoot dry weights, Root dry weights, Numbers of fruits	Arancon et al. 2003
3.	Basil (*Ocimumbasilicum* L.)	Wet and dry yield, Essence yield, Chlorophyll content	Befrozfar et al. 2013

Source: Joshi et al. 2014

in soilless growth medium, Metro-Mix 360 (MM360) increased growth and yields of marigold, peppers and strawberry plants. Parameters like plant heights, leaf areas, shoot dry weights, root dry weights increased in all the three crops. A field study was conducted by Befrozfar et al. [12] to evaluate the effect of vermicompost, plant growth promoting bacteria and humic acid on growth and essence of basil. Humic acids were applied as foliar spray and seed treatment. Highest yield, plant height and essence percentage was recorded in combined treatments of humic acids with plant growth promoting bacteria. Authors suggested that treatments of humic acids, plant growth promoting bacteria and vermicomposts can be used for a sustainable agriculture, discouraging the use of chemical fertilizers. Martinez-Balmori [13] also reported that humic acids has the ability to induce lateral root emergence in maize seedlings.

III. Effect of humic substances as related to nitrate uptake and growth regulation in plant systems

A. Nitrate uptake

The relationship between nitrate uptake by barley seedling and different concentrations of humic extracts as well as their LMS fractions were investigated. The reported values show that HE2 was the most effective humic fraction in hastening NO_3-uptake. Also HE5 was able to give an increase of 37.5%(1.98 nM NO_3- mg fresh wt-1 min -1) but only at the

conc. of 750 μGC-1. These results showed that the five humic extracts were active in increasing the rate of nitrate uptake in the order -HE2> HE1> HE5> HE3 = HE4. The effect of soil humus on the stimulation of NO_3^- uptake by plants after 16 h exposure was observed by Albuzio [14]. All of the humic extract showed increased NO_3^- uptake after 16 h incubation although the extent of the stimulation varied with HE concentration. The acid functionally seems to play an important role than aliphaticity since HE3 and HE4, though relatively higher in aliphatic C did not simulate NO3 uptake to the extent of HE2 and HE1 where HE5 aliphaticity as high as HE3 and HE4, but with a higher COOH group content was able to increase uptake rate by 37.5 although only at high concentration (750 μGC-1). The LM5 fractions stimulated much more active NO_3^- uptake by barley seedlings than the original humic extracts again LMS2 showed the highest effect. It is almost doubled the percentage increase but the manner in which they act has still to be elucidated. Low molecular wt. components of humic substances have proved to be biologically active although high molecular components appeared to be similarly active [14-17].

B. Growth regulation

The concentration of the original humic extracts and of their LMS fractions presenting hormone like activity corresponding to 1mg-1 of indo acetic acid (IAA) gibberellic acid (GA) and benzylaminopurine (BAP) were reported. No hormone-like activity was shown by the original humic extracts except for HE2 that reported an IAA-like activity at concentration (12.75 mg x-1) Conversely all LMS fractions showed IAA like activity at concentrations varying from 0.3 (LMS5) to 0.47 mg C1-1 (LMS1). Again, all the LMS material produced GA-like activity at concentrations in the order of 10-1 for LMS3, LMS4 and LMS5 and of 4.1 and 3.7 mg C1-1 for LMS1 and LMS2 resp. Cytokinin like activity was shown only by LMS2 and LMS4 at rather low concentrations.

IV. Effects of vermicomposting on physico-chemical properties of humic acids

Carbon content for the HA fraction (CH_5) apparently increased with

composting or vermicomposting time, which may suggested that a large presence of structural units of humic acids as consequence of the concentration of the more recalcitrant molecules and degradation of easily degradable fraction are present, when longer is the stabilization processes [18-19]. The H/C ratio shows the degree of maturity of the humic substances and can also be considered as a source indicator of organic matter [20]. The H/C values for HA derived from organic amendments were greater than the indicating matter, which is consistent with the technology used to be obtain the stabilized organic amendments studied than that observed for soil HA suggest that compost and vermicompost HA are less condensed or less substituted aromatics rings than soil HA [21].

The C:N ratio is an indicator of the source of humic substances in natural system. The C:N ratio obtained in this study ranged 10 to~20 for the compost and vermicompost. HA analyzed and were relatively high , indicating that vegetable waste were the dominant contributors to these humic substances, specially in HA extracted from sorghum compost in which the raw materials are no more than vegetable waste [21]. The acidic functional groups content obtained for HA extracted from compost and vermicompost are lower than those corresponding to soil HA in agreement with results reported by other authors [22-24].

V. Effect of humic substances on mitochondrial respiration

As mitochondria are the chief of both cellular respiration, and energy conversion to ATP. The effect of humic acids on the processes of respiration and ATP formation (oxidative phosphorylation) were investigated by several authors. Humic molecules play an important role in plant respiratory catalysts in plant respiration by providing an intermediate redox system between dehydrogenase or hydrogen acceptors and oxidases [25-26]. Humic acid are capable of occurring electrons from a wide spectrum of acceptors, linking redox couples which normally are thermodynamically discrete. It is very well possible that humic matter-induced cellular respiration with greater production and availability of ATP which, in turn, will stimulate nutrient upscale and lead to increased synthetic activities.

VI. Effect of compost derived humic acids on vegetable biomass production and microbial growth

A. Vegetable biomass production

Composting is an aerobic biological decomposition of organic soil substrates, with putrescible materials converted to a stabilized end product. This process is in widespread use as a means of treating organic wastes including sewage sludge, animal and agricultural residues and household refuse [27]. Through composting, organic matter undergoes partial mineralization and to a varying degree, transformation into humus-like substances [28]. Thus compost can be used directly in agricultural as an organic amendment to enhance soil fertility. Compost derived humic acids (CDHA) significantly affected the growth of chicory at concentrations >1000 mg/kg. The KCl treatment showed that this productivity increase was apparently not a function of the quantity of potassium introduced into the soil with the CDHA amendments. This suggests that the natural content of K in the soil was not limiting for growth of chicory.

Alterations of membrane permeability by humic compounds may possibly explain the increase of plant productivity through a higher absorption of nutrients [29]. Plant growth in soil, amended with CDHA was compared to that obtained with the addition of the synthetic surface active agent. Pots amended with 1000 and 2000 mg/Kg of Tween 80 only produced larger biomass than untreated pots after 120 days. These results may be evidence that Tween 80 amendments increased the permeability of root hairs to mineral nutrients. In addition, microbial degradation of Tween 80 may have produced organic fragments that also help assist plant growth.

Whenever, Tween 80 was added to the soil in combination with Hoagland's solution. No significant increase of vegetable biomass production was obtained compared with the control (i.e. the soil which had received only the mineral nutritive solution). Although chicory biomass produced in pots treated with Hoagland's solution + Tween 80 appears, in absolute values, greater than vegetative biomass produced by amending soil with only tween 80, the influence of Hoagland's solution on plant productivity seems to prevails over any treatment with the synthetic surfactant. This can be explained by assuming that at low conc. of nutrients

in the soil solution. Tween 80 may influence plant growth by promoting mobility of the mineral elements from soil particles. On the other hand, Tween 80 should lessen its effect on plant trophism when high levels of nutrients are available in the soil solution, as in the case of the Hoagland's mineral medium addition.

B. Effect on soil microbial populations

The various groups of the soil microbial community reacted differently to the amendments of potassium humates. Total aerobic bacteria were stimulated by CDHA given at higher rates (4000-8000 mg/Kg) from the earliest sampling. During the next 80 days, bacterial growth in the soil progressively increased upto 2000 mg/Kg of humates. After 120 days, differences in bacterial counts became negligible. Pots treated with KCL did not show any significant increase in bacterial counts in comparison with the soil previously wetted with Hoagland solution significantly affected total aerobic bacteria at 1000 mg/Kg and 2000 mg/Kg upto 60 days but not thereafter. The increase in bacterial biomass from humates may be related to the availability of carbonaceous substrates for heterotrophic growth. Further more the combination of Hoagland's solution and humates would improve bacterial degradation and utilization of humified substances by rendering their C/N ratio more favourable for microbial growth. Positive effects on the growth of total aerobic bacteria were also observed in pots amended with Tween 80 alone or in combination with Hoagland's solution. However, bacterial counts only increased with Tween 80 at 1000 mg/Kg or greater, and the increase lasted for 90 and 60 days for Tween 80 and Tween 80 + Hoagland's solution resp. Again these results suggest the involvement of membrane mechanisms enhancing a higher permeability to nutrients [27].

Although humates may contribute to microbial growth by rendering available small carbonaceous fragments, surprisingly microbes with possible degrading capabilities towards humic acids did not clearly benefit from the addition of CDHA to the soil. However, neutralization of humic acids proceeds rather slowly and takes longer than the experimental period used here. In respect of soil actinomycetes and cellulolytic microorganisms, the present study confirms previous observations [27], [30] that humic acids

exert only a weak stimulative effect of these groups of micro-organisms. Even CDHA added to the soil in combination with Hoagland's solution produced a similar response. On the other hand, Tween 80 alone or in combination with Hoagland's solution did not affect either actinomycetes or cellulolytic microbes. None of the treatments or treatment combinations had any significant effects on counts of filamentous fungi.

Humic acids stimulate growth of autotrophic nitrifying bacteria, especially the nitrite oxidizing ones, at the higher conc. (1000-8000 mg/Kg) of CDHA supplied to the soil. This beneficial effect was already detectable after 7 days from the start of treatment. The addition of Hoagland's solution amended with humates gave analogus results. Also Tween 80 alone or in combination with Hoagland's solution visibly increased the growth of nitrifying bacteria. Autotrophic nitrifiers probably take advantage of the presence of humic substances at either the physiological or biochemical levels [31], [32]. Similar responses to humic products and surfactants such as Tween 80 suggests that CDHA affected the nutrifiers through improved cell permeability with biological membranes being one of the prime targets of such surface active agents.

VII. Conclusions

It is evident that vermicompost can improve physical, chemical and biological properties of soil and is an excellent organic fertilizer. Presence of microflora and phenolic compounds in vermicompost help to effectively control a number of plant pathogens and pests. Humic acids are useful in increasing the plant growth, rate of respiration, vegetable biomass production and microbial growth within a plant. Farmers must be educated and made aware for the use of organic amendments especially in developing nations like India. Cost effectiveness of vermicompost should also be reflected in future studies. Net economics of applied vermicompost and inorganic fertilizers must be compared to know whether such organic amendments are cost-effective for farmers or not and how these amendments can be made more cost effective in future so that the farmers of both developed and developing world can take its advantage without hesitation.

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Predisposing factors responsible for onset of human myiasis

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Abstract

Myiasis, the infestation of tissues of live humans and vertebrate animals with dipterous larvae (maggots) is a commonly observed throughout the tropical regions of the world. It is commonly seen in the domestic and wild animals but occurs rarely in man also. The tissue invasion in man by maggots is generally a well recognized complication of neglected wounds. The condition is generally associated with traumatic injury, erosive or ulcerative lesions, carcinoma or lymphoedema. However man is affected accidently in rural and slum areas where unhygienic conditions are prevalent and domestic animals are in close vicinity. Infestations with maggots cause severe pain and mental agony among humans besides hammering economic loss significantly among domestic animals.Human myiasis is an outcome of increased fly's population due to ever increasing garbage dumps and poor hygienic conditions due to human activities. Flies flourish on litter that man produces wherever he lives, and while man has been domesticating the pig, the goat, the horse and the dog, he has also been unwillingly domesticating the flies.

Keywords

Myiasis, Human, Maggots, Parasitosis

I. Introduction

Myiasis is the infestation of live human and other vertebrate animals with dipterous larvae which for certain periods, feed on host's dead or living tissue, body fluids or ingested food [1]. Myiasis has a widespread incidence among domestic animals all over the world especially in tropical

countries like India which fulfil all the favourable conditions for the abundant growth of flies. In man it is frequent in rural and slum areas where unhygienic conditions are prevalent. Old and sick people, mentally retarded patients, drug addicts and people of low socio economic status are especially prone to attack. Flies are attracted to open wounds and natural body openings like eye, ear, nose anus and vagina to lay their eggs. Oviposition is encouraged by foul smelling purulent discharge from the diseased tissues which is followed by development of larvae. On hatching, the larvae invade the broken skin and burrow in dermal layers or pre-existing wounds resulting in enlargement of wounds. Myiasis causes lesions and makes the animal deficient of blood, debilitated and in severe cases results into death, if vital organs like brain and lungs are invaded. Similarly in untreated wounds of humans the destructive activity of maggots may lead to severe problems like deafness if internal ear is invaded, blindness in case of eye infestation and even death. The fully grown third instar larvae fall off in the soil in order to pupate. After about 7-10 days the adult flies emerge out of the pupae in tropical environment and 21 to 24 days in subtropical conditions.

Human myiasis may be asymptomatic or may result in more or less severe problems and even death when larvae invade the body cavities or the areas that forbid their direct visual examination. Many cases of myiasis, however, usually do not reach the attention of medical personnel because of the tendency of the mature larvae to migrate out of the lesion which subsequently heals. The immediate response of the traditional physicians would be to extract the maggots from the wound and throw them away. To the utter surprise of the present author, many physicians even didn't know that maggots were the developmental stages of flies. Cases of human traumatic myiasis have been reported in 42 cases with pre existing wounds from USA [2], scratch wound of scalp [3], a mentally retarded patient[4], schizophrenic patients [5], a patient with necrotic hip wound [6] and a wounded soldier [7]. Other basis for the onset of myiasis are necrotic lesions due to adenocarcinoma [8], squamous cell carcinoma [9, 10], basal cell carcinoma [11,12] and filarial lymphaedemea [13].

II. Flies capable of causing myiasis

There are many species of flies that infest human or animal tissue in larval stages. Some require a mammalian host for larval development; other may deposit their eggs on open wounds as an alternate to decaying animal or vegetable matter. Three major categories of flies i.e. Blowflies (Family: Calliphoridae), Flesh flies (Family: Sarcophagidae) and Bot flies (Family: Oestridae) are commonly responsible for causing myiasis, although the common housefly (*Musca domestica*) is also known to cause this problem.

The fly larvae that complete or at least for a certain period continue their normal development on or in the vertebrate body are classified into two categories: the obligatory parasites and facultative parasites. Obligatory parasites are those which develop exclusively in or on the living vertebrates. To this group belong the maggots which live in nasopharyngeal cavities of various groups of mammals, for example maggots of bot flies or in dermal and sub dermal tissues as do maggots of warble flies. Other larvae, however develop in alimentary canal, for example equid and rhino bots.

The other groups of fly larvae, the facultative parasites, comprise those maggots which are normally free living and develop in decaying organic matter such as carcasses, decomposing vegetables, faeces, sewage etc. Occasionally such a maggot may gain access to a live animal and act as a parasite for certain period of life. Examples include maggots of blowfly and flesh flies which cause myiasis in various domestic animals as well as humans.

In addition, there is another group of myiasis causing flies that cause accidental myiasis when their eggs or larvae are ingested with food and pass through alimentary canal passively, whether dead or alive. The condition is termed as pseudomyiasis. Infestation probably comes through food, drinking water, from soiled hands, direct deposition in the mouth, or other means. Larvae may be passed with faeces, sometimes in enormous number, or may be expelled in vomitus. However in various experimental studies the larvae could not survive in normal digestive system. If they happen to survive, that might be due to some abnormality in the gastrointestinal system or the condition under which the patient have been receiving acid suppressive therapy for long periods.

III. Types of human myiasis

Depending upon tissue involved myaisis is classified into various categories such as:

A. Cutaneous myiasis

The maggots infest the dermal and subdermal tissues. The dipterous larvae cause burrows or boils in the dermal layers or invade pre existing wounds and enlarge them or form wounds after having actively gained access to the tissue. They burrow into the skin or even some migrate under the skin resulting into swelling and itching.

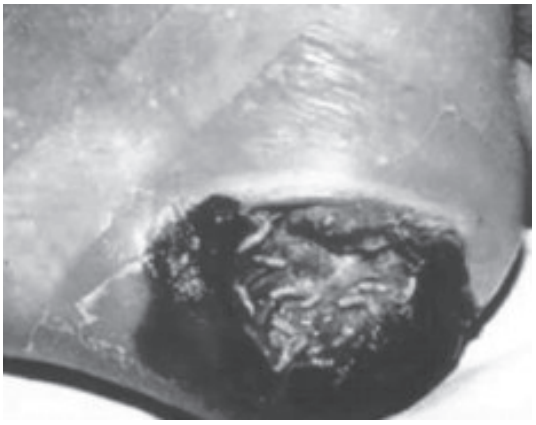

Fig. 1 Cutaneous Myiasis of a diabetic foot

B. Oral myiasis

Infestation of oral cavity by dipterous larvae is termed as oral Myiasis. Neglected wounds of patients suffering from oral cancer have been reported to be the one of the predisposing factors of Myiasis. In certain parts of India and Southeast Asia, the practice of chewing cured tobacco with beetle nut has been associated with oral cancers. More than 200 million persons are thought to be engaged in this practice worldwide. Oral Myiasis in patients of oral cancer has been reported from India [14] and Hong Kong [15].

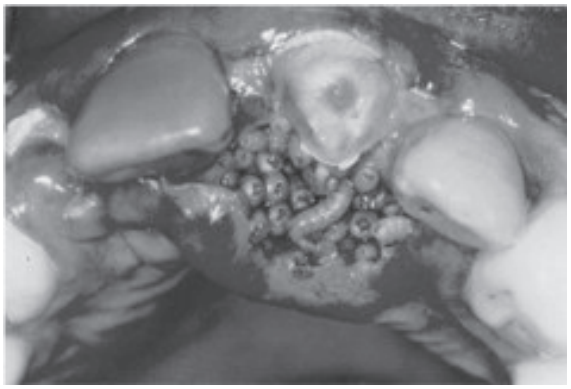

Fig. 2 Oral Myiasis in a patient of oral cancer

C. Nasopharyngeal myiasis

It involves the infestation of nasal fossae, frontal sinuses, and pharyngeal cavities by highly adapted fly maggots, which are obligatory parasites. The head cavities, including the outer ear may also be invaded by maggots which cause traumatic myiasis and creates more or less extensive open wounds.Invasion of nasal cavities may result in direct extension to paranasal sinuses, adjacent bony structures and meninges. It has been observed that the human myiasis by far the majority of deaths had occurred from the involvement of nasal cavities.

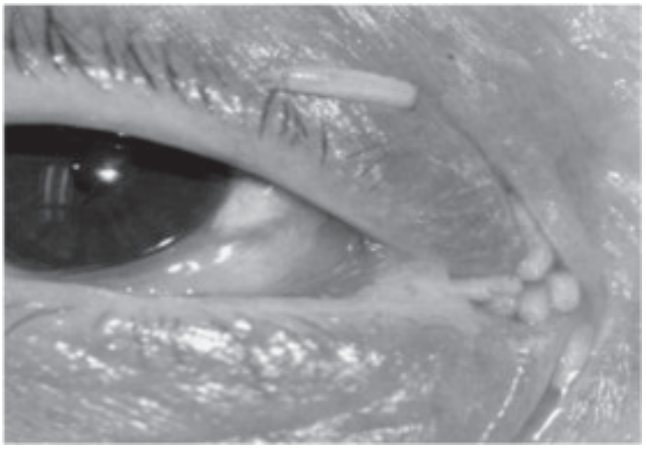

Fig. 3 Opthalmomyiasis

D. Opthalmo myiasis

In case of eye ball and surrounding tissue are more or less clinically involved, the condition is called opthalmomyiasis. It may result in the destruction of eye ball and blindness.

E. Aural myiasis

Maggots infesting the accessory sinus of the nose may easily invade the ear either externally or through the eustachian tube. The symptoms of auricural myiasis include pain and discomfort accompanied by deafness and a ringing in the ear if the maggots are in the external auditory meatus and a bloody purulent discharge if they have entered the middle ear.

F. Intestinal myiasis

This type of myiasis is sometimes also called 'enteric myiasis'. Several groups of mammals like cow and buffalo harbor dipterous larvae as obligatory parasites. For example the larvae of two species of flies-

Hypoderma lineatum and *Hyoderma bovis*, commonly called 'cattle grubs' affect domestic cattle in the Northern Hemisphere. The larvae may be attached to the wall of, or in alimentary tract, from pharynx down to the rectum and anus. Men has no obligatory dipterous parasites, but facultative are sometimes found causing 'rectal myiasis'.

G. Urogenital myiasis

Infestation of the urogenital system is termed as urogential myiasis. The larvae are excreted with urine or are found in vagina. If traumatic lesions are present, the larvae are usually involved in the dermal myiasis .The attractant of the fly is sometime the purulent discharge or a preexisting sore or diseased condition, such as carcinoma. The flies may be attracted and stimulated to lay eggs by the presence of leucorrhoeal, gonorrheal, menstrual, spermatic and purulent discharge of the female and male genital organs.

H. Nosocomial myiasis

When the myiasis occurs in the patient after hospitalization, the condition is termed as nosocomial myiasis. It usually occurs when the patients mental or physical functions are completely or partially impaired and he /she cannot brush the flies away. Similarly, infants cannot defend themselves against the approaching flies. Other factors contributing to nosocomial infestation include hypoesthesia or disturbed consciousness, preventing the patient's sensation of fly contact. Beside, paralysis or immobility may prevent a patient from fending off a fly even if detected.

IV. Discussion

Fly larvae usually infest man when the conditions favour enormous multiplication of the flies such as in foul smelling wounds and body orifices which also act as suitable oviposition sites. Oviposition is encouraged by discharges from diseased tissues. It has also been remarked that debilitated persons such as newborns and patients who are mentally disturbed or in comatose condition are vulnerable to fall victims to myiasis[4, 16, 17]. In vast majority of cases of human myiasis, local factors such as traumatic lesions, inflammatory and malignant diseases play an important predisposing

role [17]. A rare case of aural myiasis in a patient suffering from diabetes, hypochondriasis and depression [18] has been reported. Diabetic wound is another important factor for onset of myiasis. Besides disturbing glucose metabolism, diabetes may affect some other systems of our body such as loss of sensation of certain body parts. The disorder is commonly known as Diabetic neuropathy. The symptoms include numbness, paresthesia, severe hyperesthesia and pain. Loss of sensation in extremities generally makes the patients unaware about the presence of maggots in their wounds. Cases of Myiasis have been reported in the patients of diabetic foot from India [19]. It is emphasized that particular attention should be given to hospitalized patients with wounds and consciousness problems and windows of the hospital should be protected the help of screens.

V. Conclusions

There is urgent need to curb the menace of myiasis among man and domestic animals. It can be achieved mainly by educating the people about the actual cause and main factors responsible for occurrence of myiasis. As the old saying goes "prevention is better than cure" the disease can be prevented by controlling fly population, maintaining good personal hygiene. Special care needs to be taken in medically compromised dependent patients as they are unable to maintain their hygiene. The maintenance of neat and clean surroundings, reduction of odours of decomposition, control of fly populations, use of screens and cleaning and covering of wounds are the most effective means of prevention of myiasis.

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Salinity stress induced changes in growth and yield contributing parameters in *Vigna mungo* (L.) hepper genotypes

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Abstract

The present investigation was undertaken to study the effect of salt stress (30mM NaCl and 45mM NaCl) on growth and yield contributing traits in two mashbean genotypes i.e KUG 363 and KUG 529. A check variety, UL 338 of mashbean was also grown along with these genotypes. The plants were grown in plastic pots and kept under a rain-out shelter. NaCl was applied in split dose: 50% at the time of sowing and remaining 50% at 15 days after sowing (DAS). Data was recorded at 35 (Vegetative stage), 50 (flowering stage) and 65 (pod setting stage) DAS. Salt stress caused marked reduction in plant dry biomass, yield and yield contributing parameters of studied genotypes at various stages of development in comparison to control. The decrease in plant dry biomass also led to reduction in relative growth rate (RGR) and crop growth rate (CGR). However, genotype KUG 529 showed tolerance where as KUG 363 was sensitive to salt stress.

Key words

Mashbean, Salinity, Tolerant genotype, Yield

I. Introduction

Salinity is one of the world's most serious environmental problem as elevated levels of NaCl are naturally present in agricultural fields [1]. Salinity reduces soil fertility and drastically affect the growth and survival of crop plants. Mashbean or urdbean is the third most important pulse crop in India mostly grown under rainfed conditions. Although India is the largest producer

of mashbean, yet its average production is quite low. Its cultivation in India is about 3.26 million hectares with an annual production of 1.74 million tonnes and productivity of 534 Kg/ha [2].

Crop performance is adversely affected by salinity as a result of nutritional disorders derived from effect on nutrient availability, competitive uptake, transport or partitioning within the plant [3]. Plants grown in saline soils face three main problems; high salt concentration in the soil solution, high concentrations of potentially toxic ions and nutrient imbalance as a result of depressed uptake, impaired internal distribution and transport of minerals [4]. Salinity reduces uptake of water, causing reduction in growth rate. Growth becomes stunted as result of reduced rate of cell expansion and subsequent cessation of expansion. High concentrations of salt in soil cause enormous reduction in yield for wide variety of crops [5].

Strategies for alleviation of salt stress involve developing salt tolerant cultivars. Keeping in view, the present study was conducted to screen two mashbean genotypes to salt stress by studying yield and yield related traits.

II. Materials and methods

The present investigation was carried out to study the effect of salt stress on growth and yield contributing parameters in two mashbean genotypes i.e. KUG 363 and KUG 529 along with check variety UL 338. These seeds were procured from the Department of Plant Breeding and Genetics, PAU, Ludhiana. The experiments were carried out in an open-air facility equipped with a rainout shelter. Plants were grown under saline and non-saline conditions in 27 cm diameter plastic pots containing 8 kg of soil supplied with fertilizers as per recommended package of practices for mashbean. The saline treatment at 30mM NaCl and 45 mM NaCl was applied in split dose: at the time of sowing and 15 days after sowing (DAS) in a sufficient volume to wet the soil to field capacity. Non-saline treated controls were irrigated with tap water. In all the treatments, six seeds were sown in each pot and later thinned to 3 plants per pot at 20 DAS. The experiment was conducted with twelve replications. Three genotypes were subjected to control, 30mM NaCl and 45 mM NaCl treatments. Data on following parameters were recorded at 35 DAS (vegetative stage), 50 DAS (flowering stage) and 65 DAS (pod setting stage).

A. Total plant dry biomass

Samples were collected during different developmental stages. The plants were dried at 80±2 ºC for a period of 48 hours till constant weight was obtained and the dry weight of plants was recorded and expressed in g.

B. Crop growth rate (CGR)

The dry matter accumulation rate per unit area is referred to as crop growth rate (CGR), normally expressed as grams per square meter land area per day (gland area^{-1} day^{-1}). It was calculated with the help of formula given by [6]:

CGR = (ΔDM/ ΔH) where,

ΔDM = Change in Dry matter (g/m^2),

ΔH = change in temperature

C. Relative growth rate (RGR)

RGR is defined as increase in plant dry matter per unit plant material per unit time. It is expressed as g-1 day-1 and was calculated using the following formula given by [7]:

$$RGR = \frac{1}{W} \times \frac{dW}{dt}$$ where,

W = weight of tissue (g)

dW = change in weight

dt = Change in time

D. Yield contributing parameters

1) Number of pods plant-^{1}:Total numbers of pods of nine plants were counted at harvest and average was recorded.
2) Number of seeds pod-^{1}: Number of seeds in ten randomly selected pods of nine plants was counted and average was recorded.
3) 100 seed weight (g) and Seed yield plant-^{1}(g):
   Weight of 100 seeds of each line was recorded and expressed in grams (g). Seeds from all the plants of a line were harvested and weighed in grams (g) respectively.

III. Results and discussion

The present investigation is aimed to screen two mashbean genotypes (KUG 363 and KUG 529) by studying the effect of salt stress (45 mM NaCl and 30mM NaCl) on total plant dry biomass, crop growth rate, relative growth rate and yield contributing parameters along with a check variety UL 338.

A. Plant biomass

Higher salt concentration (45mM NaCl) caused more decrease in dry biomass plant-1. It is assumed that reducing cell division and plant growth metabolism induced by accumulation of Na+ cause changes in ion balances and the imbalance of mineral nutrients result in a reduction or an inhibition of plant growth [8]. Decrease in dry weight under the influence of salt was also reported in sugar beet [9] and maize [10] plants.

Since salt stress decreased the dry biomass of plants in both genotypes including check variety UL 338. In our studies, At 35 DAS genotype (KUG 529) showed 46.2% reduction and sensitive one showed 55.9% decline as compared to control under 45mM NaCl stress (Table 1). Check variety UL 338 showed 53.2% decrease in plant dry biomass as compared to KUG 363

Table 1: Effect of NaCl on Plant Dry Biomass (G) in Mashbean Genotypes at Different Stages of Development

Treatments	Genotypes		
	UL 338	KUG 529	KUG 363
35 DAS			
Control	2.82±0.052	2.94±0.064	2.02±0.047
NaCl 30	1.64±0.040	1.77±0.069	1.02±0.046
NaCl 45	1.55±0.023	1.58±0.058	0.89±0.074
CD (p=0.05) Genotypes = 0.94, Treatments = 0.13			
50 DAS			
Control	7.83±0.075	8.09±0.156	6.19±0.116
NaCl 30	4.13±0.052	4.34±0.081	2.99±0.056
NaCl 45	3.66±0.046	3.49±0.069	2.59±0.073
CD (p=0.05) Genotypes 0.27, Treatments = 0.29			
65 DAS			
Control	16.35±0.091	16.89±0.121	12.92±0.074
NaCl 30	8.12±0.087	8.51±0.110	5.61±0.075
NaCl 45	7.09±0.080	6.59±0.078	4.69±0.81
CD (p=0.05) Genotypes 0.15, Treatments = 0.27			

Concentrations were used in mM

(sensitive) which showed 58.1% at flowering stage (50 DAS). Dry biomass showed significant decrease in the salt stressed mashbean genotype as compared to control. Genotype KUG 529 maintained highest dry biomass under the influence of all treatments and at all the stages. This genotype showed higher dry biomass even than check variety UL 338 followed by KUG 363. It was observed that the dry yield of the plants decreased significantly with exposure to 45mM NaCl salinity.

B. Relative growth rate (RGR)

The decrease in dry weight of plants under salt stress decreased the relative growth rate (RGR). The RGR was calculated between 35 to 50 DAS (flowering stage) and 50 to 65 DAS (pod setting stage). A reduction in RGR was recorded between both the stages.

Table 2 Effect of NaCl treatments on relative growth rate in mashbean genotypes at different stages of development.

Treatments	Genotypes		
	UL 338	KUG 529	KUG 363
50 DAS			
Control	0.1184±0.0016	0.1168±0.0004	0.1376±0.0086
NaCl 30	0.1012±0.0020	0.0968±0.0034	0.1288±0.0125
NaCl 45	0.0908±0.0004	0.0806±0.0025	0.1273±0.0223
CD (p=0.05) Genotypes = 0.091, Treatments = 0.17			
65 DAS			
Control	0.0725±0.0006	0.0725±0.0017	0.0725±0.0034
NaCl 30	0.0644±0.0003	0.0641±0.0041	0.0584±0.0007
NaCl 45	0.0625±0.0002	0.0592±0.0040	0.0541±0.0055
CD (p=0.05) Genotypes = 0.003, Treatments = 0.005			

Concentrations were used in mM.

At both the stages higher concentration of salt caused more decreased in RGR. Under 30 mM NaCl stress, (Table 2) Check variety showed 11% decrease and KUG 363 showed 19.44% decrease with respective to control at pod setting stage (65DAS). However, the reduction was less in KUG 529 genotype than KUG 363. Higher concentration of salt caused more decrease in RGR. Salinity stress reduced the RGR and the reduction was in a concentration dependent manner.

C. Crop growth rate (CGR)

Decrease in dry biomass of salt stressed plants resulted in decline of CGR also. Crop growth rate also showed a decreasing trend in both the genotypes under salt stress. All the genotypes showed a decrease in CGR recorded between 35 to 50 DAS and 50 to 65 DAS. A decrease in CGR of mashbean genotypes growing under saline conditions has earlier been observed by [11].

Table 3 Effect of NaCl treatments on crop growth rate in mashbean genotypes at different stages of development

Treatments	Genotypes		
	UL 338	**KUG 529**	**KUG 363**
Control	17.58±0.081	18.07±0.324	14.63±0.571
NaCl 30	8.74±0.041	9.02±0.041	6.91±0.357
NaCl 45	7.40±0.081	6.70±0.041	5.96±0.519
CD (p=0.05) Genotypes = 0.85, Treatments = 0.92			
65 DAS			
Control	29.89±0.057	30.88±0.122	23.61±0.669
NaCl 30	14.00±0.122	14.63±0.669	9.19±0.067
NaCl 45	12.04±0.117	10.88±0.517	7.37±0.541

Genotype KUG 363 showed more decrease as compared to KUG 529 at both the concentrations of NaCl. The decrease in crop growth rate was due to lesser dry matter in plants under the influence of salt. At 65 DAS, 32.2% increase occurred in CGR of KUG 529 as compared to KUG 363 with exposure to 45mM NaCl salinity (Table 3).

D. Yield contributing parameters

The reduction at higher salinity level in most of the yield attributing characters may be because of adverse affect on growth and dry matter accumulation. A significant decrease in yield contributing parameters was observed in salt stressed mashbean plants and the decrease was in a concentration dependent manner. 45mM salinity level caused about 33.7% decrease in number of pods in KUG 529 and about 45.3% in KUG 363. Similarly, the number of seeds per plant decreased by about 17.4% in KUG 529 and about 20.7% in KUG 363 in comparison to their respective controls and check variety showed 19.6% decrease in same parameter under the influence of 45mM NaCl stress (Table 4,5,6& 7). 100-seed weight and seed yield per plant also showed a similar trend. 100-seed weight increased upto 4.6% in check variety UL 338 as compared to KUG 363.

Table 4: Effect of NaCl treatments on number of pods plant-1 in mashbean genotypes

Treatments	Genotypes			
	UL 338	KUG 529	KUG 363	KUG 310
Control	39.2±1.85	44.5±0.87	27.8±0/74	29.2±0.74
NaCl 30	30.5±1.73	30.3±0.95	17.6±0.70	18.4±1.48
NaCl 45	29.8±1.67	29.5±1.28	15.2±0.84	16.5±1.42

CD (p=0.05) Genotypes = 2.15, Treatments = 0.98

KUG 529 genotype showed tolerance to NaCl stress and the reduction in 100 seed weight and total seed weight were minimum as compared to KUG 363 mashbean genotype. Similar results were obtained in mungbean genotypes [12], [13] and in chickpea [14]. Salinity induced 62.9% decrease in seed yield per plant in KUG 363 genotype, where as 54.2% decrease showed in KUG 529. Reduction of yield and its components under salt stress condition may be attributed to reduced photosynthetic rate due to low production and expansion of foliage.

Table 5: Effect of Nacl treatments on seeds pod-1 in mashbean genotypes

Treatments	Genotypes		
	UL 338	**KUG 529a**	**KUG 363**
Control	6.1±0.29	6.3±0.18	5.3±0.08
NaCl 30	5.3±0.12	5.4±0.12	4.7±0.10
NaCl 45	4.9±0.13	5.2±0.13	4.2±0.08

CD (p=0.05) Genotypes = 0.25, Treatments = 0.31;

Table 6: Effect of NaCl treatments on 100-seed weight of mashbean genotypes

Treatments	Genotypes		
	UL 338	KUG 529	KUG 363
Control	4.28±0.17	4.44±0.15	4.04±0.13
NaCl 30	3.51±0.18	3.95±0.09	3.45±0.10
NaCl 45	3.42±0.16	3.83±0.12	3.2d6±0.20
CD(p=0.05) Genotypes = 0.29, Treatments = 0.31			

Also, under salt stress, the efficiency of plants to fill the developing seeds might get reduced leading to lesser number of seeds per pod and lesser dry matter accumulation of individual seed. NaCl disrupts the translocation of assimilates [15] and decreases the activity of various enzymes. All these factors may have contributed to poor seed yield in NaCl treated plants.

Table 7. Effect of NaCl treatments on seed yield plant-1 of mashbean genotypes

Treatments	Genotypes		
	UL 338	KUG 529	KUG 363
Control	10.36±1.39	12.81±0.17	5.89±0.26
NaCl 30	5.69±0.50	6.46±0.21	2.87±0.26
NaCl 45	4.99±0.38	5.86±0.23	2.18±0.20
CD(p=0.05) Genotypes = 0.29, Treatments = 0.31			

IV. Conclusions

Salt stress caused marked reduction in plant dry biomass, yield and yield contributing parameters of studied genotypes at various stages of development. In the present study, it was concluded that genotype KUG 529 performed even better than the check variety UL 338 under saline conditions, so it can be recommended to be grown under saline conditions and used by the breeders for producing salt tolerant mashbean varieties.

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Effects of water stress on nodule biomass, leghaemoglobin content and yield in chickpea genotypes

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Abstract

The present study was conducted to screen twenty chickpea genotypes to water stress tolerance. The crop was sown in the field area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab, India in split plot design with three replications. Plot included three irrigation treatments i.e. Control (C) - by irrigating as and when required, WS_{VFP}- sown with one pre-sowing irrigaton, WS_F- with-holded irrigation at flowering stage. Effect of water deficit conditions on nodule biomass and leghaemoglobin content in chickpea was observed at flowering stage. Yield was recorded at harvest. Significant reduction in nodule biomass and leghaemoglobin content was recorded under stress treatments in comparison to control. This decline showed positive and highly significant correlation with yield which suffered marked reduction under drought conditions. Among studied genotypes, RSG963 and GL28151 were observed as tolerant while GL22044 as sensitive to water stress.

Keywords

Water stress, Chickpea, Genotypes, Treatments

I. Introduction

Pulse crop chickpea (*Cicer arietinum* L.) is the fourth largest grain legume crop in the world, with a total production of 13.10 million tons from an area of 13.54 million ha and a productivity of 0.97tha^{-1} [1]. Major producing countries

include India, Pakistan and Iran. Seeds contain 12 to 30% proteins and are rich in essential amino acids, carbohydrates, minerals and vitamins A and C [2]. During the life cycle, crop is constantly challenged by a wide range of biotic and abiotic stresses, among which water deficit is the single stress that causes the most severe yield losses and thus threatens world food security. Drought conditions limit production of legumes by affecting nodule functioning and causing alterations in nodule metabolic activity. Legume nodules fix nitrogen gas from the atmosphere and convert it into ammonia, which is then assimilated into amino acids (the building blocks of proteins), nucleotides (the building blocks of DNA and RNA as well as the important energy molecule ATP), and other cellular constituents such as vitamins, flavones and hormones. Under water deficit conditions, alterations in nodule structure, weight and metabolic activity may be attributed to less available water which hindered the transport of nitrogen products away from nodules [3].

Legume nodules harbor an iron containing protein called leghaemoglobin, closely related to animal myoglobin, to facilitate the conversion of nitrogen gas to ammonia. Leghaemoglobin content suffer decline under water stress conditions due to restriction of carbohydrate transport from leaves to nodules. During water deficit stress, many changes occur in nodules, like decrease in leghaemoglobin content, decrease in nodule membrane integrity and loss of nitrogen fixation activity regardless of physiological and biochemical mechanisms of N_2 fixation inhibition by water deficit stress [4]. Moisture stresses experienced by crop at different growth stages cause yield losses ranging between 30% to 100% [5]. Severe decline in yield traits of crop plants due to water deficiency may be probably by disrupting leaf gas exchange properties which not only limited the size of the source and sink tissues but impair the phloem loading, assimilate translocation and dry matter partioning [6].

Keeping in view, present study was conducted to screen twenty chickpea genotypes by studying nodule biomass, leghemoglobin content at flowering stage and yield at harvest. Water stress was imposed in chickpea by withholding irrigation at different sampling stages i.e. C- By irrigating as and when required, WS_{VFP}- sown with one pre-sowing irrigaton, WS_F- with-holded irrigation at flowering stage.

II. Materials and methods

The present work was carried out in the laboratories and field area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Seeds of chickpea (*Cicer arietinum* L.) genotypes were procured from Pulses section, Department of Plant Breeding and Genetics, PAU. Sowing of seeds was undertaken in the field during rabi season. All the required field management practices were followed according to specifications given in the package of practices for rabi crops, Punjab Agricultural University, Ludhiana. The crop was grown in three replications in split plot design. The main plots included following irrigation treatments: C - Without stress (control) given irrigation as and when required, WS_{VFP} - Sown with one pre-sowing irrigation, WS_F - stressed by withholding irrigation at flower initiation. Dry weight of nodules and leghaemoglobin were measured at flowering stage and yield was recorded at harvest.

A. Dry weight of nodules plant

The nodules of roots at flowering stage were washed and then dried in the oven and weighed. The dry weight of nodules was expressed in g plant-^{1}.

B. Leghaemoglobin content of nodules

The leghaemoglobin content was determined by Drabkins solution in nodules at flowering stage and expressed in mg g^{-1} fresh weight nodules. It was estimated by [7] method.

Fresh tissue of nodules (0.5 g) was extracted by crushing in a 10 ml round bottom centrifuge tube with 3ml drabkin's solution. The resulting mixture was centrifuged (15 min, 5000 rpm) so that large particles of nodule tissue settle down. The supernatant was transferred to 10 ml volumetric flask. The nodule tissue was extracted twice more and supernatant combined with the first one in the flask. The total volume was made to 10 ml with drabkin's solution, and centrifuged at 2000 rpm for 30 minutes. The absorbance of the clear supernatant was read on UV2600 spectrophotometer at 540 nm using drabkin's solution as a solvent blank. The amount of leghaemoglobin was expressed in mg g^{-1} fresh weight nodules.

C. Grain yield

All the plants from each plot were sun dried for 2-3 days and the yield obtained was expressed in kg/ha.

III. Results and discussion

A. Dry weight of nodules (At flowering stage)

Dry weight of root nodules decreased under stress over controlled environment. Reduction under treatment WS_{VFP} was comparatively higher than WS_F treated plants. Under control conditions (Table 1), dry weight was found maximum in RSG963 (0.73g). Detrimental effects were more obvious in GL22044 showing least dry weight (0.44g) followed closely by GL21107 (0.43g). Percentage reduction as shown in Fig. 1 was least in GL28151 under treatment WS_{VFP} (11.47%) and WS_F (6.56%) whereas tremendous difference in GL22044 under WS_{VFP} (61.36%) and WS_F (40.91%) was recorded.

Similar results were reported in common bean [8], *Vigna unguiculata* (L) Walp. [9] and *Vigna radiate* L. [10]. Drought altered nodule structure and weight and caused premature senescence of nodules in legumes [11]. Low soil moisture during the early stages of the chickpea growth decreased nodule formation [12]. The reduction in nodule mass may be due to decreased number of nodules which is attributed to the effect of drought on the process of nodulation and the activity of nitrogenase enzyme as reported [13]. The higher sensitivity of chickpea nodule development as compared to other plant parts suggests that water deficit specifically affected nodule development.

B. Leghaemoglobin content (At flowering stage)

Leghaemoglobin content reduced under stressed conditions in comparison to control, reduction was comparatively higher under treatment WSVFP than WSF (Table 1). At 90 DAS, under control, maximum leghaemoglobin content was observed in GL28137 (6.88 mg g^{-1}) followed by RSG963 (6.52 mg g^{-1}), while least value was observed in GL21107 (4.31 mg g^{-1}). Under WS_{VFP} treatment, steep reduction depicted in Fig. 2 was observed in GL22044 (61.31%), followed by GNG1861 (45.54%) and minimal changes were witnessed in GL28137 (7.85%), followed by RSG963 (9.97%). Similar trend was followed under treatment WS_F- where least alterations appeared in RSG963 under WS_{VFP} (10.12%) and WS_F- (6.44%) stress treatments and pronounced percent reduction recorded in GL22044 (45.80%) followed by GNG1861 (35.95%).

The leghaemoglobin content declined in dehydrated nodules under severe

drought in common bean [14]. The reduction of nodule leghaemoglobin content can be attributed to early nodule degeneration related probably to the production of O_2 [15]. Similar reports were given in chickpea [16].

C. Yield (At harvest)

Water stress treatment on chickpea grain yield was significant. Under control conditions (Table 2), maximum yield was observed in RSG963 (3401.67 kg/ha) whereas minimum yield was noticed in RVSSG4 (2529.44 kg/ha) followed by GNG1861 (2709.44 kg/ha). GL28151 under stress treatment WS_{VFP} (2677.78 kg/ha), and RSG963 under stress treatments WS_F (3111.11 kg/ha) showed maximum yield. Under stress treatment WS_{VFP}, shown in Fig. 3, least variations were observed in GL28151 (13.04%) while eminent percentage reduction was observed in GL22044 (55.91%) showing their tolerant behavior towards moisture stress. Under stress treatment WS_F, percentage reduction varied between GL28151 (7.40%) to marked difference in GL22044 (37.70%).

Results depicting yield reduction under water deficit in chickpea were reported earlier [17]. Rate of yield reduction due to water deficit depends on the genotypes, crop growth stage, severity and duration of water stress [18]. In present study, highest and lowest average grain yield (kg/ha) has been reported in no drought stress treatment and severe drought stress treatment respectively in chickpea genotypes, tolerant genotypes performing significantly better than susceptible. It appears that water deficit in chickpea generative stage prevents yield potential attainment through flower and pods shedding [19].

D. Phenotypic correlation of yield with nodule biomass and leghaemoglobin content:

In the present study, phenotypic correlation of yield with nodule biomass and leghaemoglobin content was found to be highly significant showing magnitude of 0.75 and 0.66 respectively. Dry weight of nodules also showed positive and highly significant correlation (0.8369) with leghaemoglobin content of nodules. Positive correlation among the studied traits and their interdependence under control and stress conditions as well, shows that these parameters can be used as indicators for screening of drought tolerant cultivars.

Table 1: Dry weight of nodules (g) and leghaemoglobin content (mg/g) of various chickpea genotypes in response to stress imposed at flowering stage.

Genotypes	Dry weight of nodules (g)			Leghaemogloin content of nodules (mg/g)		
	C	WS_{VFP}	WS_F	C C	WS_{VFP}	WS_F
GL21107	0.45	0.21	0.33	4 31	2.53	3.11
GL22044	0.44	0.17	0.26	5.48	2.12	2.97
GL26054	0.56	0.36	0.4	6.17	4.35	5.12
GL26074	0.59	0.38	0.41	6.24	5.12	5.45
GL28137	0.72	0.53	0.59	6.88	6.34	6.4
GL28151	0.61	0.54	0.57	5.48	4.91	5.12
GL28186	0.52	0.41	0.45	6.12	5.45	5.5
GNG1594	0.54	0.43	0.46	5.87	4.33	4.68
GNG1861	0.63	0.35	0.39	5.73	3.12	3.67
DCP 92-3	0.48	0.28	0.31	4.33	2.54	3.12
GG1362	0.56	0.42	0.37	6.12	4.79	5.22
RSG811	0.58	0.4	0.42	5.73	4.32	4.74
RVSSG4	0.63	0.38	0.41	6.13	4.12	4.66
RSG963	0.73	0.6	0.66	6.52	5.87	6.1
RSG957	0.51	0.38	0.41	6.37	4.83	5.12
BGM547	0.58	0.41	0.47	5.82	4.66	5.11
PDG3	0.52	0.42	0.46	5.77	5.1	5.34
PDG4	0.51	0.35	0.37	4.32	3.21	3.76
PBG1	0.49	0.35	0.38	5.12	4.22	4.38
GPF2	0.52	0.38	0.4	5.76	4.77	4.92
LSD (5%)	**G = 0.01; T = 0.03; G x T =0.05**			**G = 0.01; T =0.03; G xT =0.05**		

LSD (0.05) - Least significant difference at 5% level of probability;
G-Genotype; T-treatment,
C-Control; WS_{VFP} - grown with one presowing irrigation;
WS_F- stressed at flower initiation stage

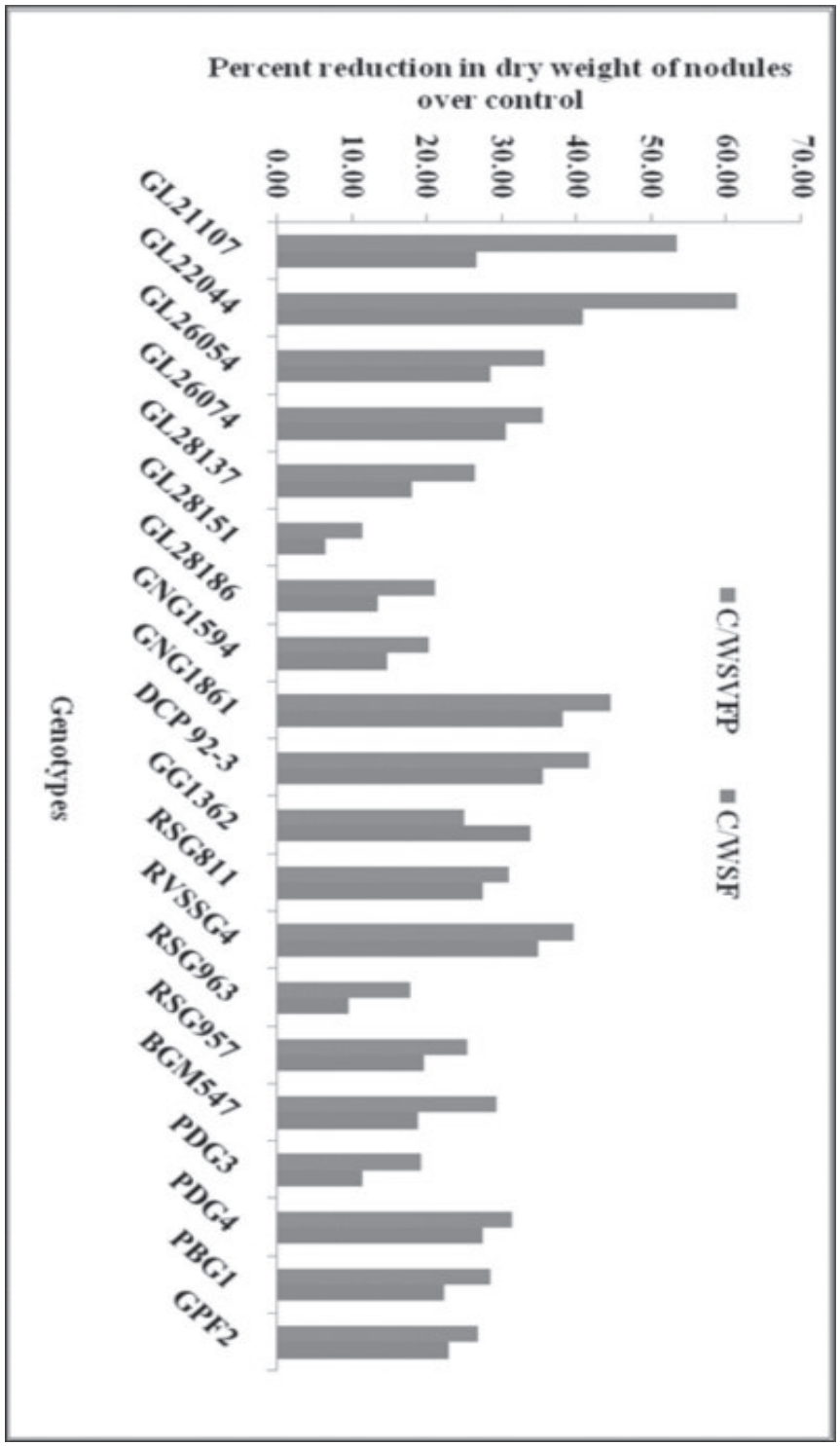

Fig 1. Percentage Reduction in dry weight of nodules under water stress conditions over control; C/WSVFP- Percent reduction in treatment WSVFP in comparison to control; C/WSF-Percent reduction in treatment WSF in comparison to control.

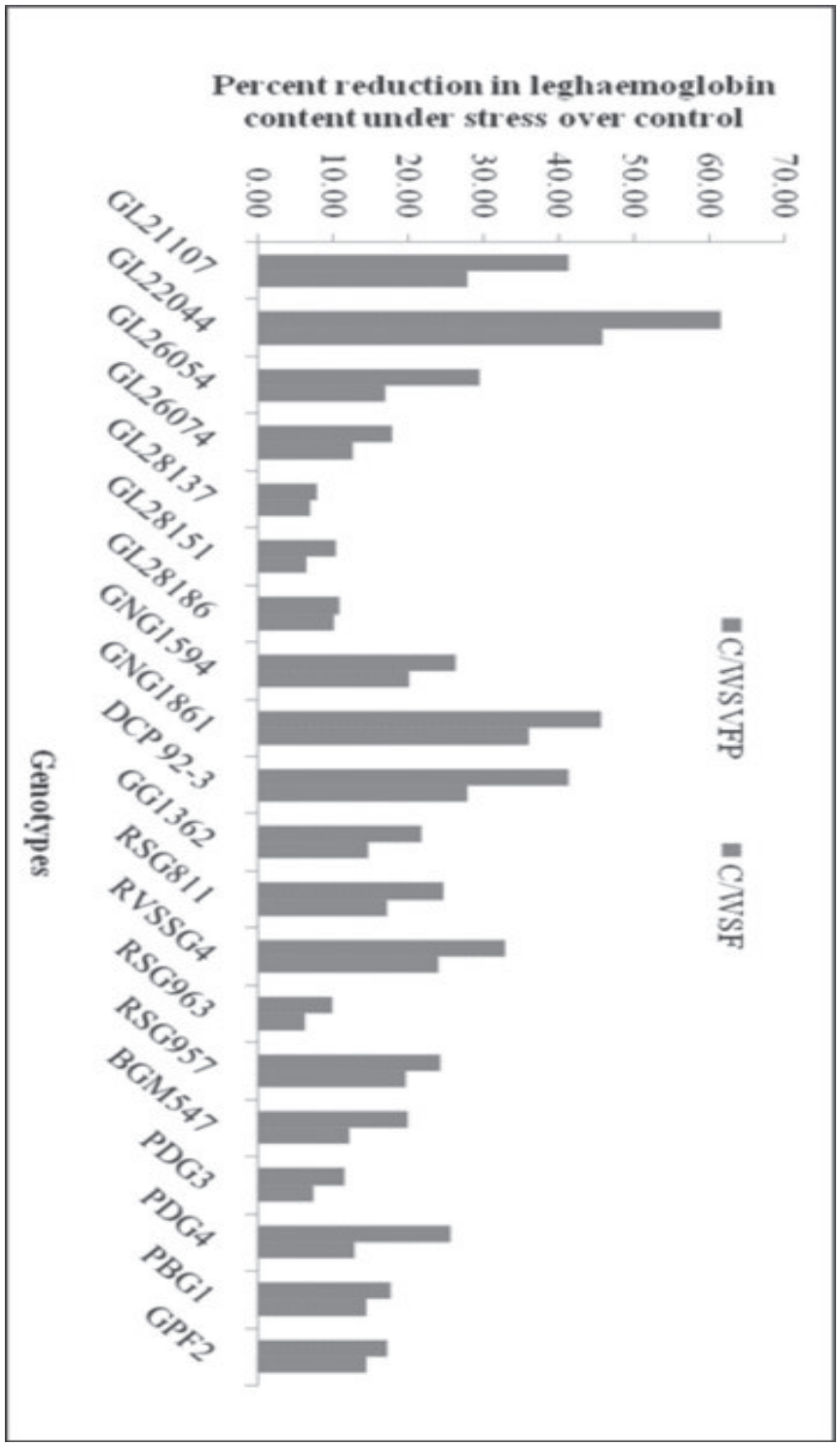

Fig 2.Percentage reduction in leghaemoglobin content of nodules under water stress conditions over control; C/WSVFP- Percent reduction in treatment WSVFP in comparison to control; C/WSF-Percent reduction in treatment WSF in comparison to control.

Table 2: Yield (kg/ha) of chickpea genotypes in response to water stress recorded at harvest (120 DAS) days after souring.

Genotypes	C	WS_{VFP}	WS_{F}
GL21107	3146.33	1775.95	2148.17
GL22044	3218.33	1418.89	2005.00
GL26054	3220.56	2185.19	2503.89
GL26074	2765.00	2114.72	2450.00
GL28137	3283.33	2004.45	2568.33
GL28151	3079.44	2677.78	2851.67
GL28186	2773.89	1923.89	2289.50
GNG1594	3142.78	2289.50	2626.11
GNG1861	2709.44	1296.28	2011.11
DCP-392	2781.67	1751.67	2290.56
GG1362	2973.89	1923.89	2338.89
RSG811	3112.78	2177.78	2368.33
RVSSG4	2529.44	1668.55	1944.45
RSG963	3401.67	2666.66	3111.11
RSG957	2973.89	1831.67	2288.89
BGM547	2851.67	1698.34	2289.55
PDG3	3122.22	2057.22	2646.89
PDG4	2918.33	2211.11	2535.83
PBG1	2909.44	1781.11	2213.56
GPF2	2979.06	1844.45	2224.11
LSD (5%)	**G=30.65**	**T=79.65**	**GxT=137.11**

LSD (0.05) - Least significant difference at 5% level of probability; G-Genotype; T-treatment,

C-Control; WS_{VFP} - grown with one presowing irrigation; WS_F- stressed at flower initiation stage

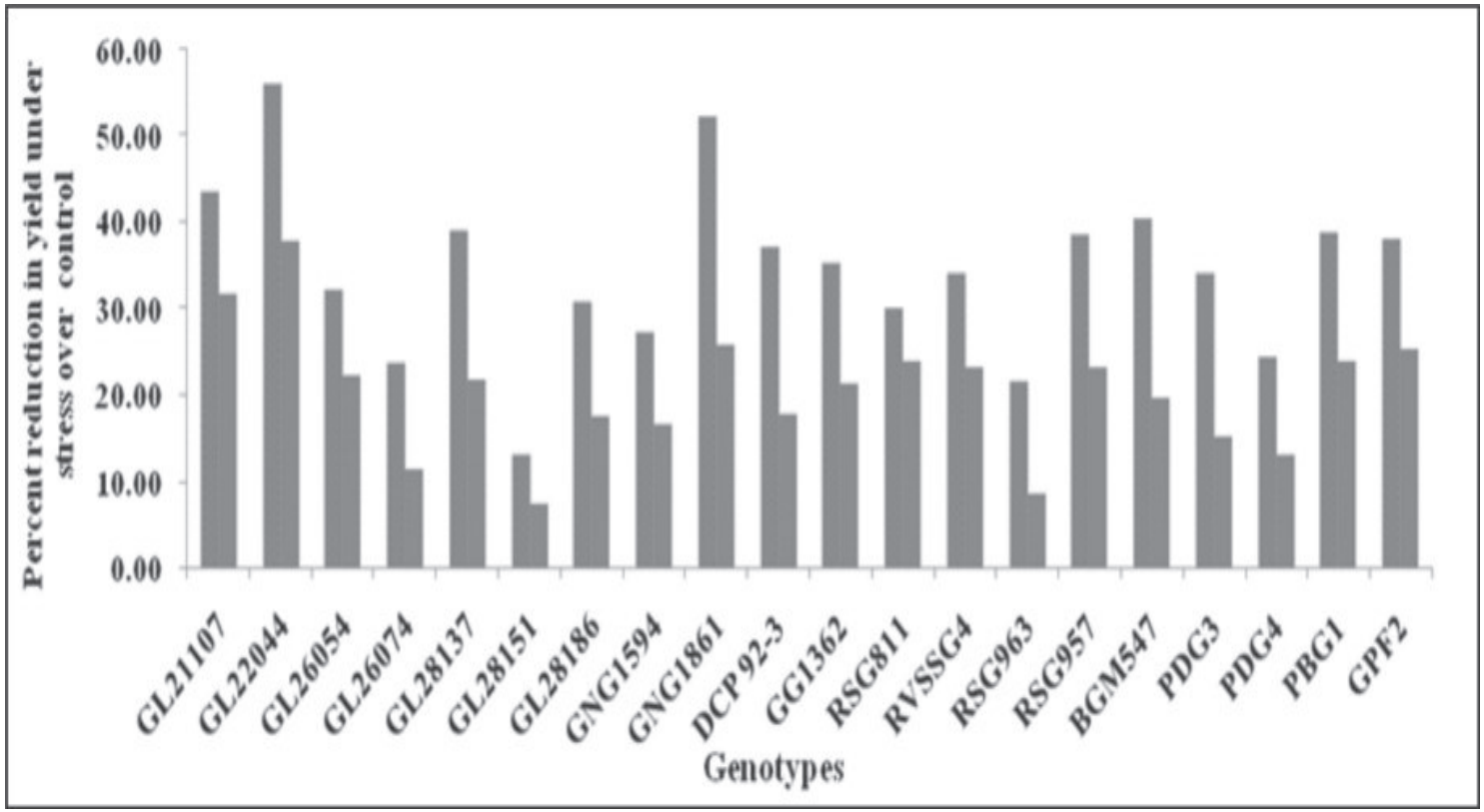

Fig 3.Percentage reduction in yield under water stress conditions over control; C/WSVFP- Percent reduction in treatment WSVFP in comparison to control; C/WSF-Percent reduction in treatment WSF in comparison to control.

IV. Conclusions

Water deficit conditions adversely affected the nodule biomass and leghaemoglobin content due to premature senescence and degeneration of nodules. Yield also reduced significantly as a result of impaired translocation of assimilates and dry matter partitioning. In the studied traits, genotypic variations were apparent, tolerant genotypes exhibited lesser alterations than sensitive ones, attributable to their greater resistance to water stress conditions, because of better adaptation and genetic constitution. Based on this research, genotypes GL22044, GNG1861 were observed to show sensitivity whereas RSG963, GL28151 remained tolerant during stress conditions in the field.

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The susceptibility of bacterial biofilms to eugenol

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Abstract

Bacteria can grow as single cells as well as biofilms. Bacteria growing in biofilm are more dangerous. Many antibiotics are available in market but many bacterial species are increasingly becoming resistant to many antibiotics. So an alternative approach is being followed to kill many infection causing bacteria. The aim of this study was to evaluate the efficiency of a monotrepene, Eugenol against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. All these bacteria were grown as biofilms in laboratory conditions. Staphylococcus aureus showed greater capability to grow as biofilm. After checking the biofilm capabilities of these gram positive and gram negative bacteria, individual minimum inhibitory concentrations of Eugenol were calculated against both planktonic cells as well as against their biofilms. The MIC of Eugenol against E.coli biofilm was twice that against planktonic cells whereas the MIC was same against Pseudomonas aeruginosa biofilm and planktonic cells. Staphylococcus aureus biofilm was four times more resistant than planktonic cells. Eugenol was more effective against Staphylococcus aureus than Pseudomonas aureus and E.coli.

Key words

Biofilms, Eugenol

I. Introduction

Biofilms are well organized communities of microorganisms living together, protected by extracellular matrix. Their growth reaches several millimeters in thickness and they become very resistant and difficult to remove [1]. Many human chronic infections are caused by biofilms that grow on

medical implants or devices [2]. Biofilms can withstand several antimicrobial treatments and evade host defenses [3]. Treatment of biofilms with highest effective antibiotics kills most of cells but few cells resist the antibiotics and hence when the treatment is stopped, they regrow and form biofilms again at different place [4]. There are many theories explaining the resistance of biofilms to antibiotics [5]. There are four factors which are considered as main reasons for resistance Inability of the antibiotics to penetrate the biofilm [6]. Nutrient limitation across the matrix which leads to decrease in growth rate of cells [7]. The enzymes which some cells produces in order to protect themselves [8]. Multidrug efflux pumps when get activated by some cells [8]. Monoterpenes have antimicrobial effect and can easily penetrate cellular membranes. Eugenol is a monocyclic monoterpene and it is widely used. Monoterpene save less side effects on mammalian cells and biodegrade. So all these benefits encourages the use of monoterpene as antimicrobial agent.

The primary mechanism of action of Eugenol is through the disruption of the bactericidal membrane, thereby potentially increasing nonspecific permeability of the antibiotic. Other secondary effects at sub lethal concentration cannot be discounted and can be expected as a consequence of the interactions with the bacterial membrane. The ability of Eugenol to sensitize the bacterial cells towards a heterogeneous group of antibiotics which underlines the nonspecific and general nature of its activity.

Research in recent past explains a lot about antibiotic resistance of biofilms and it is clear now that the resistance is multifactorial [9]. Keeping in view, the present study was conducted to evaluate monoterpene, Eugenol for eradication of several biofilm producing bacteria.

II. Materials and methods

A. Bacterial strains and reagents

Staphylococcus aureus NCTC 4163, *Escherichia coli* NCTC 8196 and *Pseudomonas aeruginosa* ACTC 9027 were used. These were maintained on nutrient agar(Acumedia 7145A) slopes at 4 degree celsius in duplicates and were sub cultured monthly. The Eugenol used throughout the experiments was purchased from SIGMA - ALDRICH (Lot 17H0239). This monoterpene was stored at room temperature for the duration of the

experiments. Nutrient broth(Oxoid CM0001) was used for dilutions and growing medium for bacteria.

B. Determination of biofilm formation capabilities

Biofilm formation capability of the Gram-negative bacteria and Gram-positive bacteria were determined by inoculating 10ml of Nutrient Broth (NB) with each of the isolates and then incubating overnight at 37 degree Celsius. Broth cultures were diluted 1:100 with nutrient broth and 200μL volumes were placed in the wells of a clear 96-well tissue culture microtiter plate. Negative controls were put in the 12th column from A to H rows 36-40 replicates were used. The microtiter plate was then sealed with Para film and incubated at 37 degree celsius for 2 days. After this time the A595 was read using the Bioreader plate reader. The well contents were then tipped out, the wells were rinsed twice with sterile distilled water and the plate air dried for 45 minutes. 200μL of 1% crystal violet was added to each well using a multichannel pipette and was left to stain for 20min at room temperature. After this time the crystal violet was removed from each well and the wells were washed with sterile distilled water five times. Purple rings were then visible around some of the wells. 200ul of 95%ethanol was then added to the wells in order to destain them and after 5 minutes A490 was read using the micro plate reader [10].

C. Minimum inhibitory concentration (MIC) - broth micro dilution method

1) MIC of antimicrobials against planktonic test organisms*:* The MIC was determined for Eugenol used throughout this study on test organisms. Eugenol was diluted in nutrient broth (containing 0.15 %(w/v) technical agar) to a final concentration of 6 % (v/v). Clear 96-well flat bottom microtitre plates were used and the dilution medium was nutrient broth. Ten two-fold dilution of Eugenol were prepared in nutrient broth (100 μL volumes), then 100μL of bacterial suspension added to each well to give a final volume of 200μL. Depending on the experiment, the concentration of bacteria was 10^6 cells/ml. Column 11 was the positive control containing 100μL of nutrient broth and 100μL of cells and Column 12 was the negative control containing only 200μL nutrient broth. Eight replicates were performed in each experiment. The plates were read in a Bio-Rad

plate reader at 595 nm before incubation at 37ºC for 24 hours. The results were clearly determined visually after incubation and findings were confirmed by measuring absorbance at 595 nm.

2) MIC of antimicrobials against test organisms as biofilms: Biofilms were established for 72 hours, procedure was similar as used in determination of biofilm capacity of test microorganisms. After 72 hours, reading was taken at 600nm to see amount of planktonic growth. Planktonic cells were then discarded and wells were washed with 0.85 % saline water. Similar concentrations of Eugenol were used as in planktonic cells. Ten two-fold dilutions of Eugenol were prepared in nutrient broth (200μL volumes) and were added in each well. Reading was again taken at 600 nm. Then plates were incubated for 24 hours. Again reading was taken at 600 nm. Absence of microbial growth at least 7 of 8 from tests was considered as the MIC level.

III. Results and discussion

A. Comparison of biofilm producing properties

The following table shows the biofilm formation capability of the three organism

Table 1: Comparison of Biofilm Producing Properties

Microorganisms	Planktonic (O.D at 600)	Biofilms(O.D at 480)	Ratio of planktonic growth/biofilms
E.coli	0.247	0.195	1.26
Staphylococcus aureus	0.305	0.295	1.03
Pseudomonas aeruginosa	0.370	0.100	3.7

Of both Gram positive and Gram negative bacteria which were used, Gram positive *Staphylococcus* aureus showed highest capability to produce biofilms followed by the Gram negative bacteria *Escherichia* coli and Pseudomonas aeruginosa.

B. Minimum Inhibitory concentrations of eugenol against planktonic cells and Biofilms of three microorganisms used:

The following are the graphs showing MICs of Eugenol against planktonic cells of three microorganism and against their biofilms. The lowest point after which there is decline in the O.D (O.D represents cell growth) is the MIC.

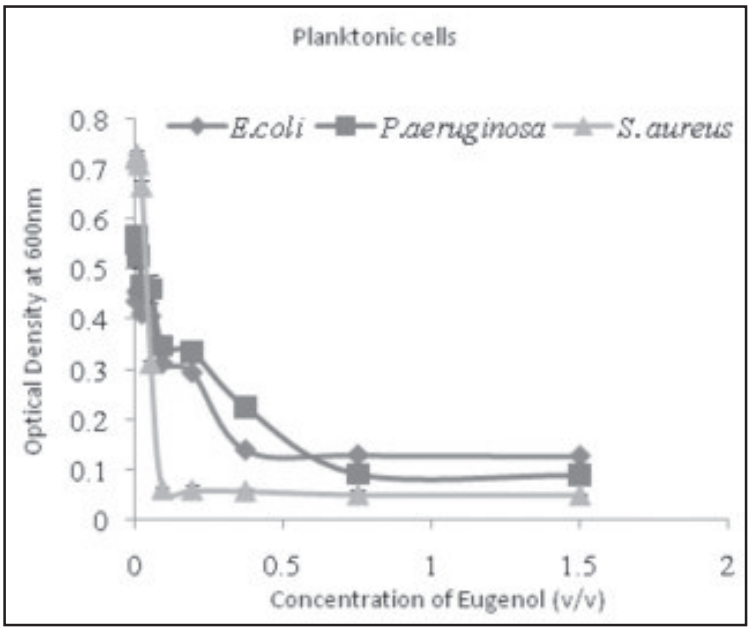

Fig 1: The MIC of Eugenol against the planktonic cells of three microorganisms

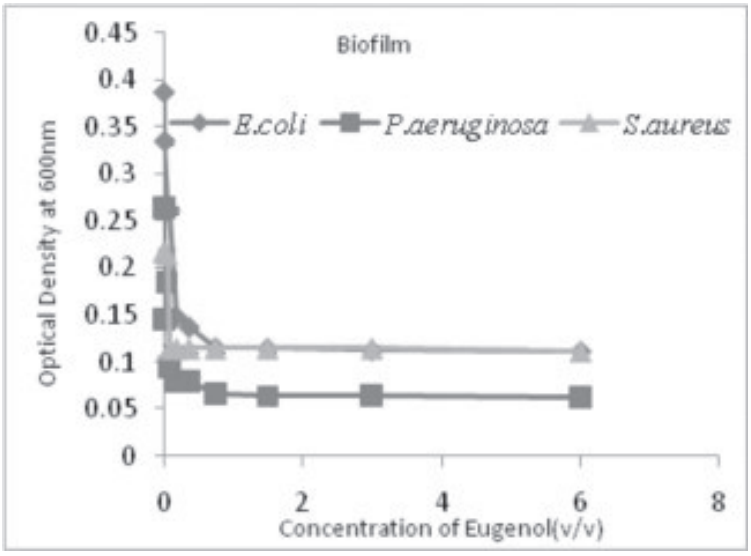

Fig 2: The MIC of Eugenol against the biofilms of three microorganisms

Table 2: The summary table of mics of eugenol against planktonic cells and biofilms of three microorganisms

	MIC of Eugenol(%v/v)	
Microorganisms	Planktonic Cells	Biofilms
E.coli	0.37	0.75
Pseudomonas aeruginosa	0.75	0.75
Staphylococcus aureus	0.09	0.37

The MIC of Eugenol is equal or higher for biofilms of three microorganism used than for their planktonic cells. The MIC of Eugenol against E.coli biofilm was twice that against planktonic cells whereas the MIC was same against *Pseudomonasaeruginosa* biofilm and planktonic cells. Staphylococcus aureus biofilm was four times more resistant than planktonic cells. Bacterial cells are much more protected from antimicrobial agents when embedded in biofilm cells. This protection is due to number of reasons. E.g. reduced diffusion of biocides due to the exopolymeric matrix, physiological changes in the cells. Against planktonic cell as well as biofilms, Eugenol was more effective against *Staphylococcus* aureus than *Pseudomonas* aureus and E.coli. The main difference is between the structure of the cell walls of gram negative and gram positive bacteria [12]. 90-95 % of the cell wall of gram positive bacteria consists of peptidoglycan, to which other molecules, such as teichoic acid and proteins are linked. The structure of the gram positive bacteria cell wall allows hydrophobic molecules to easily penetrate the cells and act on both the cell wall and within the cytoplasm [12]. Phenolic compounds, which are also present in the essential oil generally show antimicrobial activity against gram positive bacteria. At lower concentration essential oil interfere with enzymes involved in the production of energy. The cell wall of Gram negative bacteria is more complex. It has a peptidoglycan layer that is 2-3 nm thick, which is thinner than in the cell wall of Gram positive bacteria. An outer membrane lies outside of their peptidoglycan. It is composed of double layer of phospholipids that is linked to inner membrane by lipopolysaccharides[12]. The peptidoglycan layer is covered by an outer membrane that contains various proteins as well as lipopolysaccharide. LPS consist of lipid A, the core polysaccharide and Q

side chain which provides "quid" that allow gram negative bacteria to be more resistant to essential oil and other natural extracts[12].

IV. Conclusions

Out of gram positive and gram negative bacteria, *Staphylococcus aureus* have more capability to produce biofilms followed by *Escherichia coli* and *Pseudomonas aeruginosa*. MIC of Eugenol against biofilms were higher than planktonic cells.

Acknowledgment

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Heuristic approach for cloud computing with parallel computing environment

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Abstract

Cloud computing is an emerging trend in the field of Computer Science. It is a booming area. It is a service focused for the high quality, high reliability and low cost information services for the task scheduling of different tasks in the parallel environment. The cloud is helpful for anyone to access any information anytime from anywhere. The cloud services scheduling to any customer provides in such a way that takes place minimum amount of time with minimum cost. Task here should be scheduled in such a way that it minimizes the cost and time in parallel computing environment. Here a meta-heuristic approach having genetic algorithm technique with task scheduling in parallel computing environment be presented which not only minimize the execution cost, it also minimize the execution time for a particular cloud. By merging the scheduling algorithms and genetic algorithms, a new technique naming genetic algorithm with task scheduling (GATS) for the cloud computing (CC) environment be designed and implemented for the fulfilment of the different services in the cloud computing environment. It reduced the computational complexity of any processor used in the parallel cloud computing environment. With the experimental results by using the MATLAB optimization genetic tool, the proposed algorithm exhibits a good performance for such environment.

Keywords

Genetic Algorithm (GA), Task Scheduling (TA), Cloud Computing (CC), Makespan, Heuristic Techniques

I. Introduction

Cloud computing is the one of the upcoming latest new computing

paradigm where a simple format can be executed for the data and the applications on the internet [1]. The advantage and usability of the cloud computing attracted the number of business organization and educational institutes in today's life. Task management plays a vital role in the processing and management of the different tasks and scheduling of jobs in the cloud environment. Scheduling in the cloud means selection of best suitable resources for the task execution. A tasks scheduler in the cloud computing environment give the best performance and satisfy its all cloud users and improve the job scheduling criteria's by providing a specific quality control mechanisms. In cloud computing, a cloud user commit to an agreement called service level agreement (SLA) [2] with a cloud provider. Any best cloud system provides the three main features for the performance in the parallel computing environment for a cloud. These are cloud consumer, cloud service provider and cloud services. Cloud consumes all the cloud services provided by the cloud service provider. A cloud system can be developed with the help of a several virtual machines which are interconnected. So cloud system be constructed by a service provider and it control the parallel environment. It also set a number of tasks scheduled to these machines and minimizes the execution time by processing different tasks from the different users in different types of environment in a cloud. Therefore how to allocate tasks to virtual machines efficiently is an important and challenging issue in the cloud computing [3]. Actually a cloud is type of parallel and distributed system and is a collection of interconnected and virtualized computers that are dynamically provisioned. Such type of system presented as one or more unified computing resources based on service level agreements established through negotiation between service providers and consumers [4]. The existing models are suitable for cloud computing. Old techniques focus on the importance of the system performances. However there are lots of tasks scheduling models given by the various researchers for tasks scheduling and will be discussed in the related work.

Number of models emphasis on the minimization of completion time of group of jobs [5]. Here the proposed technique is the GATS (Genetic Algorithm with Task Scheduling) for the cloud computing environment. The aim of this technique is to improvement of some specific metric like

computing speed and storage availability. Another powerful aim of such type of techniques is user's satisfaction and provider's profit. Actually with the help of Genetic Algorithm, task scheduling procedure can be used for minimization of make span as well as the reduction of cost in the cloud computing environment. This paper is subdivided into five parts. Section II is the detail study of the related work in the field of task scheduling, genetic algorithm and cloud computing. Section III describes the GATS technique for the CC environment. Section IV elaborates the experimental work with its performance. At last but not least, the section V is the conclusion with the future work.

II. Related work

In 2007, Independent task scheduling in cloud computing by improved genetic algorithm proposed by the Pradeep Kumar et. al. and firstly done showing the proper use of VMs and cloudlets as parameters using the cloud sim tool. Further in 2008, a new evolutionary approach heuristic algorithm to schedule bag-of-tasks (tasks with short execution time and no dependencies) in a cloud is presented by B. Hays so that the number of virtual machines to execute all the tasks. The purpose is to minimize the cost and execution time. In 2009, a study of genetic algorithm based task scheduling for cloud computing was done under the parameter throughput, simulator time, average VM utilization, average response time, average processing cost and number of task using the GA based scheduling optimization tool by Sung Ho Jung et. al. Also in 2009, two level scheduler was proposed by the Sudha et. al. It uses user centric meta scheduler for selection of resources and system centric VM scheduler for dispatching jobs in cloud computing environment based on Qos. A new scheduler who makes the scheduling decision by evaluating the entire group of tasks in a job queues by Yujia et. al. . A genetic algorithm is designed as the optimization method for a new scheduler that shows a minimum makespan and maximum balanced load across all evaluates the entire group of tasks in a job queue on the basis of prediction of execution time of tasks assigned to certain processors and makes the scheduling by Sandeep Tayal. In 2010, a better optimal scheduling policy based on linear programming, to outsource deadline constraint

workloads in a hybrid cloud scenario is proposed [10]. In 2011, a DRR (Deadline, Reliability, Resource-aware) scheduling algorithm, which schedules the tasks such that all the jobs can be completed before the deadline, ensuring the Realiability and minimization of resources by Laiping Zhao, Yizhi Ren & Kouichi Sakurai.

III. Genetic algorithm for cloud computing

Execution of tasks is the major problem occurs in cluster, grid and cloud computing environment. From the observations on the basis of the previous study, the existing scheduling strategies in cloud are based on the approaches developed in the related area such as distributed system and grid computing [12]. There are many algorithm like Min-Min, Max-Min, Suffrage, Shortest cloudlet to Fastest processor (SCFP), Longest Cloudlet to Fastest processor (LCFP) which are the generic algorithm to minimize the execution speed of different tasks in the task scheduling environment and maximum optimize such type of system. Also there are some meta-heuristic algorithm like Genetic Algorithm (GA), particle swarm optimization (PSO), Ant-colony optimization, A Bee Colony (ABC) optimization and simulated Annealing (SA) algorithms and techniques are used for the minimization of the make span and proper utilization of tasks in the tasks scheduling.

There are so many techniques having different way of representation in the form of algorithmic approach are given by various researchers for cloud, but none of the above discussed existing algorithms and techniques in the related area. Introduction parts of the GATS for CC have considered for the two major events. These are

i). Computational complexity

ii). Computing cost

These two above said parameters can be early handled by the Genetic Algorithm approach for the different tasks in the tasks scheduling mechanisms for the well formed operations and informative approaches for the cloud computing. So here a GATS algorithm be proposed for the proper utilization of computational complexity and computing cost for the cloud computing system.

IV. GA operators

Before applying any GA operation, there is need of computation of fitness function.

A. Fitness function

The fitness value of each chromosome is generated by the fitness function. This fitness value evaluates and shows the suitability of a chromosome set in solving the tasks scheduling problems. This is based on execution time of tasks. But, task scheduling problems in Cloud computing are different from general task scheduling problems because computing services in Cloud computing are offered through a SLA between cloud users and providers [6]. The minimization of execution time is a goal of task scheduling in Cloud computing. Another major point here is cost minimization. For computation and best results following GA operations can be done.

i) Selection operator

Selection operation is the basic design of fitness function, so how to design the fitness function will directly effect the performance of genetic algorithm [7]. To select the superior and eliminate the inferior, GA uses the selection operator. According to their fitness value individual are selected. Once fitness values have been evaluated for all chromosomes, it is very east to select good chromosomes through rotating roulette wheel strategy. This operator generate next generation by selecting best chromosomes from parents and offspring.

ii) Crossover

The crossover operation is a simple mechanism to swap one part of a chromosome for that of another chromosome. In this paper, the two-point crossover [8] is used for transferring genetic material of parent to children.

iii) Mutation

The mutation operation is to expand the search space by changing one part of a chromosome. In the beginning of the genetic algorithm, the quality of generated chromosomes is not particularly good [9]. As time goes by, the quality of chromosomes becomes more improved. There is the potential for improvement in quality by the mutation operation in

the early part of the genetic algorithm. But, it is hard to improve the quality of chromosomes by the mutation operation after the quality attains a certain standard. In this case, the mutation operation increases execution time of the genetic algorithm.

B. Evaluation

Evaluation is based on the execution time and execution cost. Those schedules will be selected for next generation whose makespan (execution time of all cloudlets) and execution cost is less than the defined genetic algorithm.

C. Termination

Genetic Algorithm gets terminated after user specified number of generations.

Above all the operations occurs and gives best results according to the following algorithm:

V. Genetic algorithm (GA) for cloud computing

a) Generate an initial population of individuals with output schedules of algorithms Longest Cloudlet to Fastest Processor (LCFP), Smallest Cloudlet to Fastest Processor (SCFP) and 8 Random Schedules.

b) Evaluate the fitness of all individuals, while termination condition not met do
   i. Select filter individuals for reproduction with minimum execution time.
   ii. Crossover between individuals by two-point crossover.
   iii. Mutate individuals by simple swap operator.
   iv. Evaluate the fitness of the modified individuals having relevant fitness.
   v. Generate a new population

c). Stop the above operations while it gives a best set of chromosome and generation showed be decision making. Here, Cloudlets refer to user jobs in Cloud Computing.

VI. Experimental work with performance

The experiment was done using the designed Simulator by doing coding

with Java programming and also the optimization tool of the Genetic algorithm in the MATLAB 13.1 version. A scheduling algorithm as discussed above was experimented and it was found that it leads to better resource utilization, less average Make-span and better system throughput. Make-span refers to the completion time of all cloudlets in the list [10] [11]. To formulate the problem here let be considered cloudlets (C1, C2,C3…..Cn) run on processors (P1, P2, P3…..Pn). Main objective here is to minimize Make-span. The speed of processors is expressed in MIPS (Million instructions per second) and length of job can be expressed as number of instructions to be executed. Each processor is assigned varying processing power and respective cost in Indian rupees. Here algorithm is tested by varying the number of cloudlets and randomly varying the length of cloudlets. Experimental results show that under heavy loads our proposed algorithm that is modified Genetic Algorithm exhibits a very good performance. Below given Fig.1 shows that how the variations occurs between number of processors in a particular set and number of cloud set.

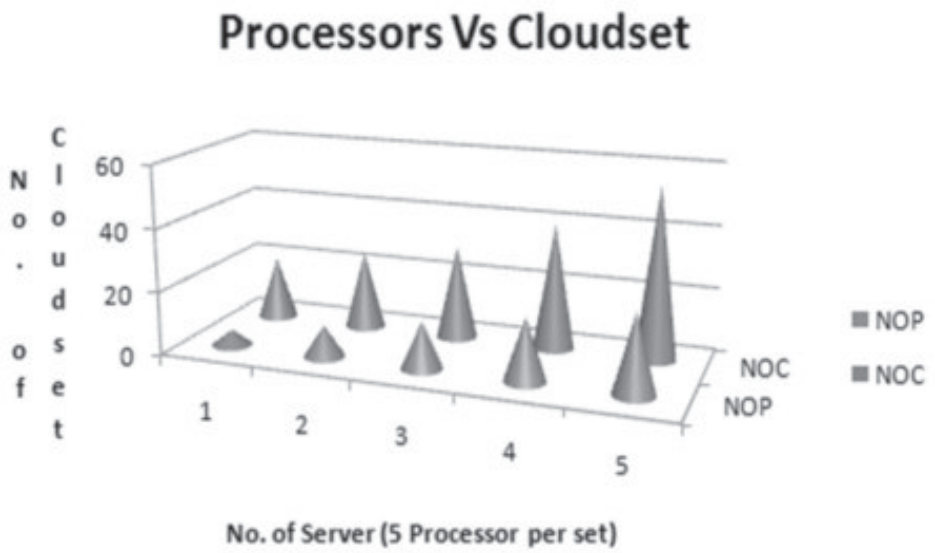

Fig. 1: Relationship between Processors and the Cloudset

Below shown Fig.2 shows the number of iterations occurs on the particular set of processor so that a better enhanced result of a particular cloudset can be obtained.

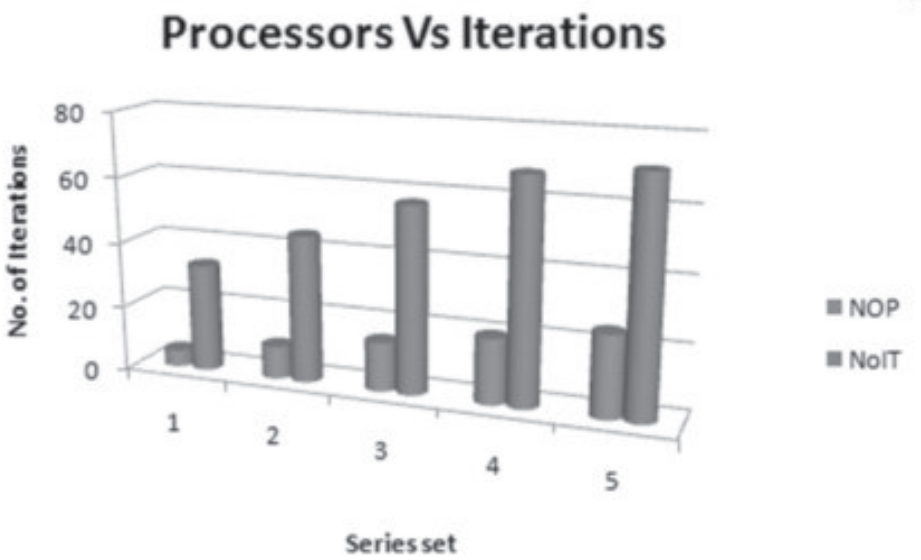

Fig. 2: Occurrence of iterations on a particular set of Processors

Below is the computed table for the cloud set with Genetic Algorithm as below:

Table 1: GA Parameters

Parameter with Genetic Algorithm	Quantity
Number of Cloudlets	5-40
Number of Processors	5-35
Number of Iterations	10-80
Cost occurs	1-5

Below showing Fig.3 will gives the results of processor capacity with cost computation and it gives the results that as the capacity of processor increases the cost per unit also increases.

The figures above shows the makespan refers to execution time calculated in seconds of all cloudlets in each set of algorithms. Experimental resulting values show that our proposed algorithm takes less execution time as compared to existing algorithm which is based on the random generation of schedules. As shown in Fig.3, it shows the comparison of execution cost of these algorithms. Resulting values show that performance of proposed algorithm is better than the existing algorithm and keep on increasing with increase in workloads.

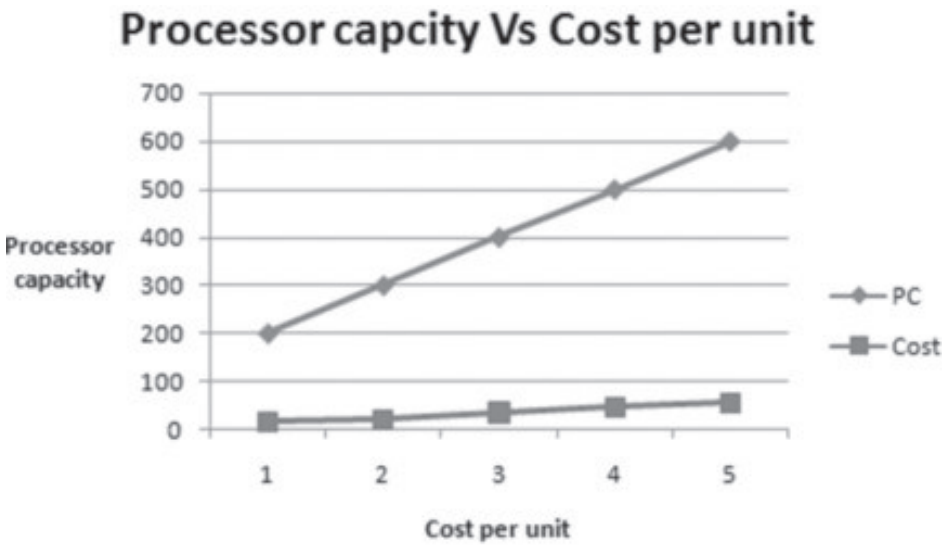

Fig. 3: Processor capacity (in Mips unit) and the cost per unit with set of processor

VII. Conclusions

In this paper, it presents scheduling problems in Cloud computing and proposed a task scheduling technique for optimization of execution speed of tasks and processing cost in parallel environment. In the proposed task scheduling model, the task scheduler calls the GA scheduling function to make task schedules based on information of tasks and virtual machines. The GA scheduling function creates a population, a set of task schedules, and evaluates the population by using the fitness function considering user satisfaction and virtual machine availability. The function iterates reproducing populations to output the best task schedule. The restart operation is also applied to the GA scheduling function for the improvement in quality of task schedules. For performance evaluation, an experimental setup was designed and simulates the proposed task scheduling model and conducted diverse experiments. Empirical results prove that the proposed task scheduling model outperforms existing task scheduling models.

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Catalytic oxidation of benzyl alcohol to benzaldehyde

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Abstract

The present review summarizes novel methods of benzyl alcohol oxidation to benzaldehyde. These approaches are not only facile but they provide an ecofriendly oxidation route employing transition metal catalysis, electrolysis and mixed metal oxides.These methodologies are a excellent alternative to conventional inorganic oxidants like $KMnO_4$ or $K_2Cr_2O_7$.

Keywords

Benzaldehyde, benzyl alcohol, transition metal catalysis, hydroperoxides, electrolysis.

I. Introduction

The selective oxidation of aliphatic and aromatic alcohols to corresponding carbonyl compounds is an important transformation which provides valuable synthetic intermediates having extensive applications in different areas such as pharmaceuticals, dyes, perfumery and agro-chemical industries [1]. In the recent years, a wide variety of oxidizing agents and the catalysts have been developed for selective oxidation of benzyl alcohol to benzaldehyde [2]. Consequently, traditional inorganic oxidants such as $KMnO_4$ or $K_2Cr_2O_7$ have been replaced by new, more selective and efficient reagents which provide an environmentally benign green oxidation technology to high yield products [3]. The aim of this review is to provide an overview on the recent developments for selective oxidation of benzyl alcohol to benzaldehyde. The literature survey throws light on a lot of reports but the aim of the present review is to selectively choose those methodologies

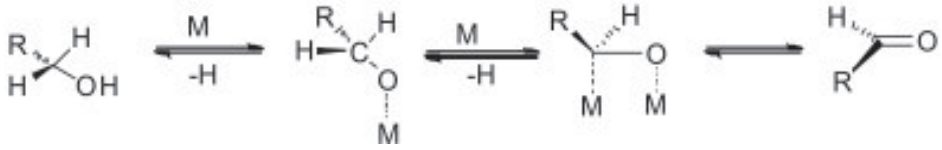
for conversion of benzyl alcohol to benzaldehyde which are facile, environmentally benign and employ ecofriendly catalytic approaches.

II. Catalytic oxidation of benzyl alcohol to benzeldehyde

A. Oxygen

The main focus of the recent research on oxidation methods for benzyl alcohol is to use environmentally friendly oxidants such as oxygen in place of stochiometric amounts of toxic heavy metal oxidants such as dichromates and permanganates. The use of molecular oxygen as oxidant under heterogeneous catalysis is an ecofriendly route for dehydrogenation of alcohols in the gaseous as well as in the liquid phase conditions. These oxidations have been reported with a variety of transition metals and metal salts of V [4], Co [5], Cu [6], Mo [7], Ru [8], Rh [9], Pd [10] as well as Mn and Zn [11] catalysts.

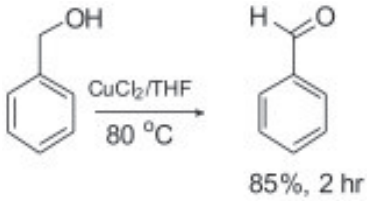
The most accepted mechanism of dehydrogenation of the alcohols suggests that the reaction involves two elementary steps (Scheme 1). Firstly the O–H bond of the alcohol breaks upon adsorption on the active sites resulting in an alkoxide species and hydrogen atom.



Scheme 1. *Mechanism for oxidation of alcohols over metal catalyst surface.*

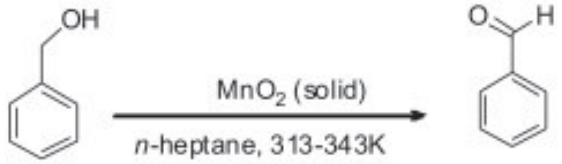
In the second rate-determining step, the weakest C-H bond adjacent to oxygen undergoes cleavage to form the carbonyl compound. The dehydrogenation is generally accelerated by bases. The role of oxygen (or similarly adsorbed OH species in alkaline medium) is to oxidize the co-product hydrogen and thus shift the equilibrium towards the carbonyl compound.

Lokhande et. al. have reported environment friendly oxidation of benzyl alcohol using $CuCl_2$ in THF at 80^{o}C [12]. Both primary and secondary alcohols can be oxidized using the same catalyst (Scheme 2). It was further observed that the reaction time was shorter for oxidation of benzylic alcohols bearing electron-donating substituents and longer for those bearing electron-withdrawing substituents which might be attributed to co-ordination of the phenolic OH group with cupric chloride.



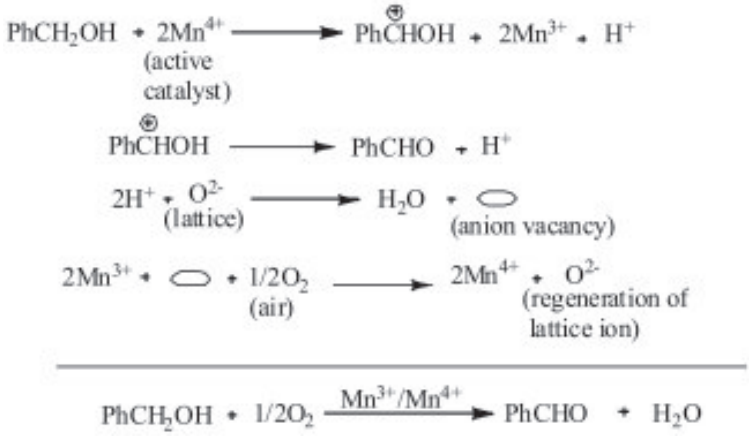
Scheme 2. *Conversion of benzyl alcohol to benzaldehyde using $CuCl_2$/THF.*

Mohammad et. al. reported liquid phase oxidation of benzyl alcohol by molecular oxygen using solid manganese oxide as a catalyst in *n*-heptane as the solvent [13]. Catalyst so developed was highly chemoselective and brought about complete conversion of benzyl alcohol to benzaldehyde and it can be recovered and re-used. *n*-Heptane was found to be an excellent solvent for the oxidation reaction as catalyst did not leach into the solution (Scheme 3).



Scheme 3*. MnO_2 catalyzed oxidation of benzyl alcohol.*

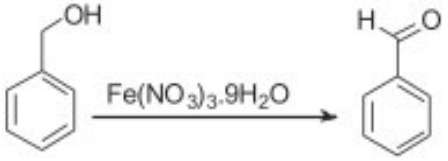
Fernandes et. al. have reported aerial oxidation of benzyl alcohol in liquid phase employing pure and Al^{3+}doped manganese oxides, mainly of the type OMS-2 (Octahedral molecular sieves). It was further observed that Al^{3+} doped OMS-2 has significantly higher activity (~ 58%) as compared to pure OMS-2 (~ 43 %) [14]. Characterization of catalyst was further done by XRD, IR, H^+ ion exchangeability, thermal analysis, BET surface area and temperature



Scheme 4. *Catalytic oxidation of benzyl alcohol using Al^{3+}doped MnO2.*

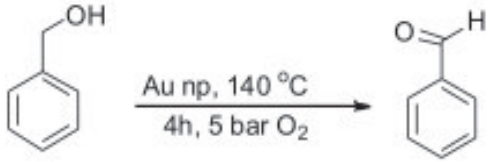
programmed desorption (TPD) of ammonia. Catalytic activity depends on the ease of availability of lattice oxygen and presence of Mn^{4+}/Mn^{3+} redox couple.

Hulshof and co-workers have optimized reaction conditions for the oxidation of benzyl alcohol with iron (III) nitrate nonahydrate under conventional and microwave heating conditions (Scheme 5) [15]. Based on the results obtained, it was observed that conventional method was recommended as the method of choice for a scalable process as microwave heating led to somewhat uncontrolled temperatures.



Scheme 5. *Oxidation of benzyl alcohol with iron(III) nitrate nonahydrate.*

Bagabas et. al. have developed a new method for the oxidation of benzyl alcohol to benzaldehyde by using gold nanoparticles deposited over different oxidic supports (MgO, CaO, ZrO_2, TiO_2 and Al_2O_3) by wet impregnation method [16]. Among all tested catalysts, TiO_2 supported Au nanoparticles exhibited the best catalytic activity with a benzyl alcohol conversion of 81% and benzaldehyde selectivity of 95 % at 140 °C and 5 bar O_2 in 4 h (Scheme 6).



Scheme 6. *Gold nanoparticle catalyzed oxidation of benzyl alcohol.*

Benzaldehyde can also be prepared by an environmentally friendly solvent-free liquid phase oxidation of benzyl alcohol with molecular oxygen, using nano-gold supported U_3O_8, particularly prepared by the homogeneous deposition precipitation method. The Au/U_3O_8 was reported as highly promising, easily separable and reusable catalyst for the solvent-free selective oxidation process. Higher conversion yield and better selectivity was achieved by increasing the concentration and downsizing the gold particles. This method has the advantage over other metal oxides supported catalysts such as Au/MgO, Au/Al_2O_3 and Au/ZrO_2 [17].

B. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide has gained considerable interest in the recent years as an oxidant for organic reactions [18]. Its distinct advantage over other oxidizing agents is that it produces water as the only by-product. Various research groups have reported both homogenous and heterogeneous catalysis of alcohols employing H_2O_2. Liquid phase alcohol oxidations proceed *via* a dehydrogenation mechanism on surface of metal catalyst. The alcohol is dehydrogenated to aldehyde through the formation of alkoxide as illustrated below:

$$RCH_2OH(sol) \rightarrow RCH_2OH\ (ads) \rightarrow RCHO(ads) + 2H(ads)$$

Alcohols and aldehydes can be oxidized by Fenton's reagent which is a system of Fe^{2+} and hydrogen peroxide [19]. The reaction proceeds *via* a free radical reaction mechanism as illustrated by following equations (Scheme 7).

Chain initiation: $OH{-}OH + Fe^{2+} \longrightarrow Fe^{3+} + \dot{O}H + OH^{\ominus}$

$$RCH_2OH + \dot{O}H \longrightarrow R\dot{C}HOH + H\text{-}OH \text{ (reversible)}$$

$$R\text{-}\dot{C}HOH + OH\text{-}OH \longrightarrow \dot{O}H + RCHO + H_2O$$

Chain termination at low alcohol concentration:

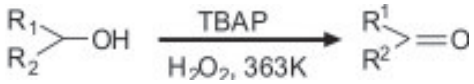
$$Fe^{2+} + \dot{O}H \longrightarrow Fe^{3+} + OH^-$$

Chain termination at high alcohol concentration:

$$2R\text{-}\dot{C}HOH \longrightarrow R\text{-}CHO + R\text{-}CH_2OH \text{ (disproportionation)}$$

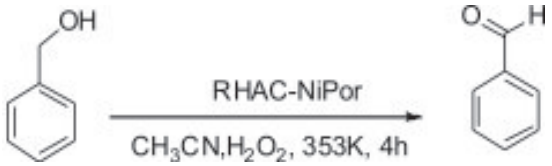
Scheme 7. Oxidation of alcohols to aldehydes by Fenton's reagent.

Lu et. al. [20] have developed a very facile and practical oxidation of alcohols to ketones with H_2O_2 in the presence of TBAP (triethyl {2,4,6-trimethyl-3,5-bis}[(triethylazaniumy) methyl]azanium (tribromide) based phosphotungstate complex) under mild conditions that afforded products in high yields and shorter time (Scheme 8).



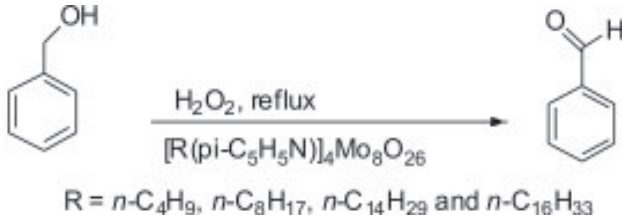
Scheme 8. *Oxidation of alcohols with hydrogen peroxide catalyzed by TBAP.*

Adam and Ting [21] have synthesized highly stable silica-supported metalloporphyrin complex, in which [tetrakis (*o*-chlorophenyl) porphyrinato] Ni(II) was successfully immobilised onto the functionalised silica support prepared from (Rice Husk Ash) RHA. RHAC-NiPor can be used further as an efficient catalyst for the liquid phase oxidation of benzyl alcohol with the environment friendly H_2O_2 as a sole oxidant (Scheme 9).



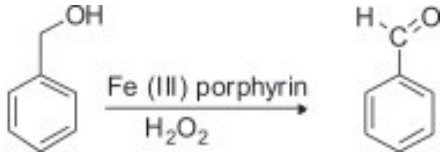
Scheme 9. *Oxidation of benzyl alcohol to benzaldehyde over RHAC-NiPor.*

Guo Ming-Lin et al found that selective oxidation of benzyl alcohol with aqueous hydrogen peroxide as oxidant was more efficient when tetra-alkylpyridinium octamolybdate catalysts were employed (Scheme 10) [22].



Scheme 10. *Oxidation of benzyl alcohol with aqueous H_2O_2 and tetra-alkylpyridinium octamolybdate.*

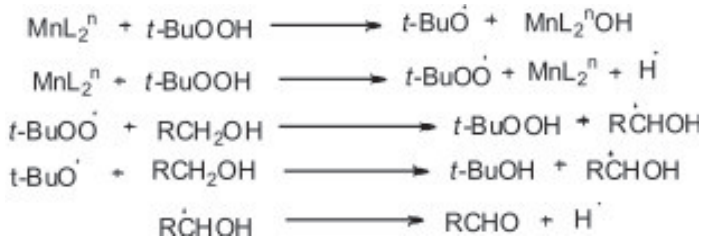
Mild and efficient oxidation of alcohols with hydrogen peroxide catalyzed by iron (III) tetrakis (*p*-sulfonatophenylporphyrinato)acetate, supported on polyvinylpyridine and Amberlite [23] IRA-400 at room temperature was also reported (Scheme 11). The catalysts used in this study showed high activity, not only in the oxidation of benzyl and linear alcohols but also in the oxidation of secondary alcohols at room temperature. The catalysts can be reused several times without significant loss of their activity.



Scheme 11. *Oxidation of benzyl alcohol with hydrogen peroxide and supported Fe (III) porphyrins.*

C. *tert*-butylhydroperoxide (*t*-BuOOH)

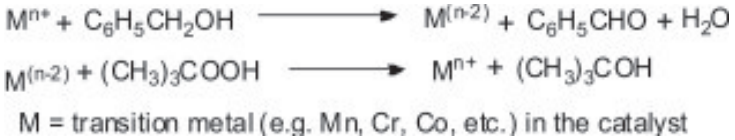
tert-Butyl hydroperoxide (*t*-BuOOH) is an organic peroxide widely used in a variety of oxidation processes. Mahdavi et. al. evaluated the catalytic activity of the supported Mn(II)bipy complexes, $[Mn(bipy)_2]$ 2^+/HMS in the oxidation of benzyl alcohol in the liquid phase using tert-butylhydroperoxide (TBHP) as an oxidant. They also studied the effects of loading Mn^{2+} and of various solvents on the conversion and selectivity of benzyl alcohol oxidation at different temperatures using $[Mn(bipy)_2]^{2+}$/HMS and excess TBHP [24].The report revealed that metal-catalysed oxidation involving alkyl peroxides may proceed either through a homolytic or heterolytic mechanism. Transition metal salts of Co, Mn, Fe, Cu or the metal oxides are normally involved in homolytic cleavage. The following oxidation pathway was suggested for the conversion of benzyl alcohol to benzaldehyde (Scheme 12).



where RCH_2-OH = benzyl alcohol, R = benzaldehyde.

Scheme 12. Oxidation of benzyl alcohol to benzaldehyde with alkyl peroxides.

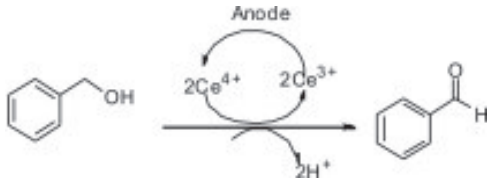
Chaudhary and group [25] investigated the liquid phase oxidation of benzyl alcohol under solvent free conditions using a number of synthetic hydrotalcite materials (Layered double hydroxides) LDHs containing one or two different transition elements (Mn-Al, Co-Al, Ni-Al, Zn-Al, Mg-Fe, Mn-Fe, Co-Fe, Ni-Fe, Mn-Cr, Co-Cr, Ni-Cr, Cu-Cr, and Zn-Cr) and tert-butyl hydroperoxide (TBHP) as an oxidizing agent (Scheme 13). The LDHs containing transition elements Mn, Cu and Co showed very good catalytic activity in the oxidation. Oxidation of benzyl alcohol by TBHP over the transition metal containing LDH and/or HD catalysts is expected to involve a redox mechanism.



Scheme 13. *Oxidation of benzyl alcohol using transition metal LDH.*

D. Electrolysis method

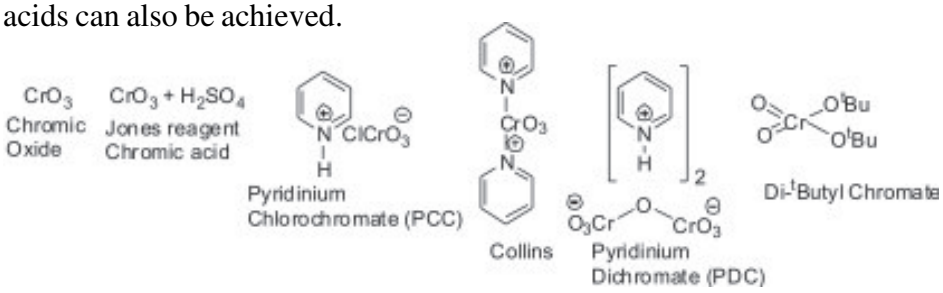
Xavier et.al. have developed a novel, non-conventional method for the preparation of benzaldehyde using bi-phase electrolysis [26]. The electrolysis was carried out in an undivided cell using graphite electrodes in the presence of ceric ammonium sulphate (CAS) redox couple (Scheme 14).



Scheme 14. Proposed electrochemical oxidation mechanism of benzyl alcohol.

E. Chromium oxidants

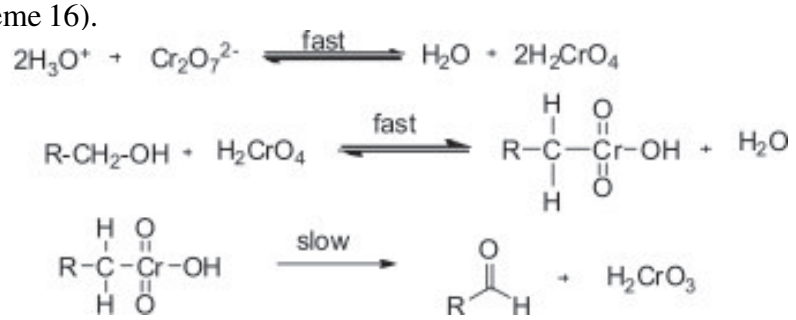
A variety of chromium (VI) oxides derived from CrO_3 are among the most popular reagents for oxidation of primary and secondary alcohols to aldehydes and ketones respectively (Scheme 15). The properties of the reagent can be altered by Lewis base complexation. Most commonly used reagents are pyridinium chlorochromate, pyridinium dichromate and chromic oxide-pyridine (Collins reagent). The oxidation of primary alcohols can usually be stopped at the aldehyde stage, although oxidation to carboxylic acids can also be achieved.



Scheme 15. *Reagents based on CrO_3.*

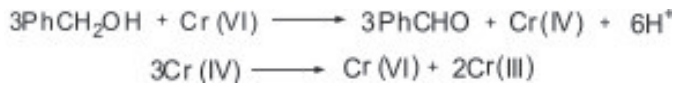
Westheimer first proposed the mechanism for the oxidation of alcohols by dichromate ion in 1949 [27]. In the first step the dichromate ion is protonated to form chromic acid in a rapidly established equilibrium. The chromic acid then undergoes a rapid, reversible reaction with the alcohol to form a chromate ester, which then decomposes in the rate-determining step to form H_2CrO_3 and the aldehyde or ketone. There are subsequent steps in which H_2CrO_3 and various other chromium species react with the resulting

carbonyl compound until all of the chromium is in the +3 oxidation state (Scheme 16).



Scheme 16. *Mechanism for the oxidation of alcohols by dichromate ion.*

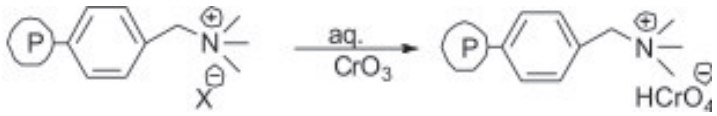
Bijudas and co-workers [28] have recently reported a rapid, efficient and selective method for the oxidation of benzyl alcohol to benzaldehyde using acidic potassium and dichromate in various quaternary ammonium salts as phase transfer catalysts under various organic solvents by dichromate (Scheme 17). The phase transfer catalysts (PTC) used were tetrabutylphosphonium bromide (TPBP), tetrabutylammonium bromide (TBAB) and tetrabutyl-ammonium hydrogen sulphate (TBAHS).



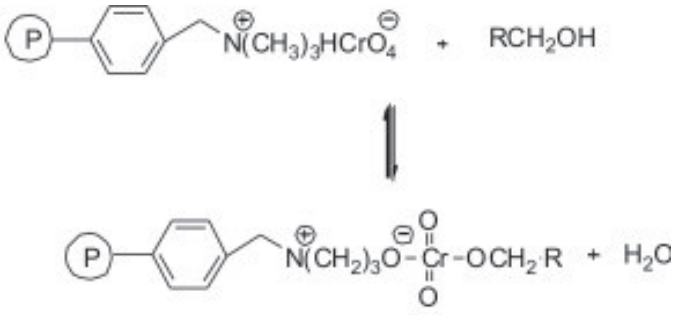
Scheme 17. Phase transfer catalyzed oxidation of benzyl alcohols.

Benzyl alcohol and substituted benzyl alcohols on oxidation under heterogeneous condition gave corresponding aldehydes as the product with very high yield (above 90%).

Vilas Y observed that the reagent supported on anion exchange resin was found to be more efficient in the oxidation reaction of alcohol [29]. In this report, oxidation of benzyl alcohol with chromic acid supported on anion exchange resin like Tulsion-T-52 A [Cl-] in 1,4-dioxane has been studied. The supported oxidizing agent was prepared by reported method [30].

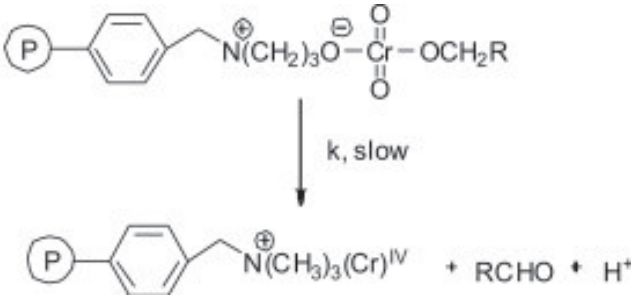


The proposed mechanism for this oxidation suggests that the polymer supported reagent first reacts with a molecule of benzyl alcohol to form a chromate ester (A).

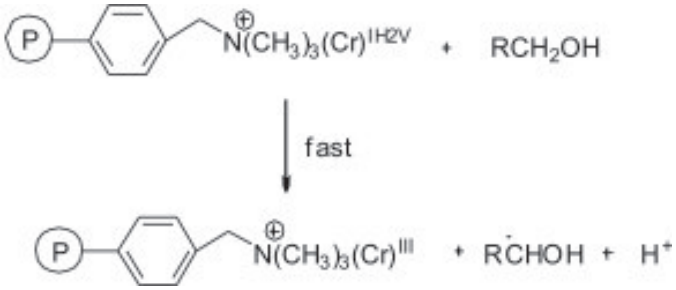


(A)

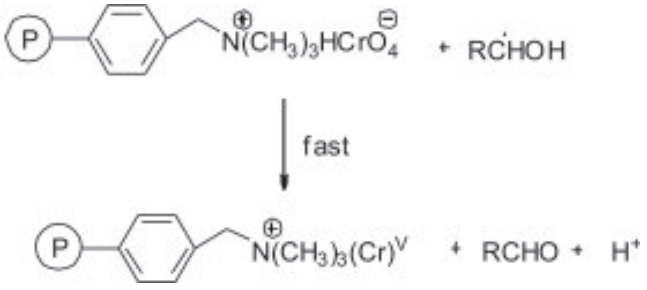
The ester (A) formed then decomposes into aldehyde and the intermediate chromium (IV) in the second and slow step.



The intermediate chromium (IV) further reacts with another benzyl alcohol molecule to produce a free radical species.



Subsequently the free radical reacts with another oxidant site in the polymeric reagent in a fast step leading to the formation of chromium (V).



The intermediate chromium (V) in the last step reacts with benzyl alcohol to produce benzaldehyde.

F. Metal oxides and mixed metal oxides

Heterogeneous catalysis using metal oxides, supported metal oxides and mixed metal oxides has several advantages over the homogenous catalysis due to the ease in the separation of product and reuse of catalyst. A wide variety of metal oxide catalysts such as basic oxides ZnO, CuO, MgO and acidic oxides such as chromium containing copper have been used for selective oxidation of benzyl alcohol to benzaldehyde. It was found that when copper-based catalysts were used for dehydrogenation of alcohols, alkaline additives such as BaO, MgO increased the yield and selectivity of copper oxide catalyzed reactions [31].

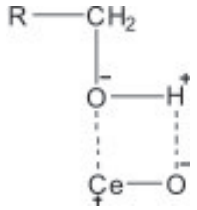
Pozan et. al. reported catalytic dehydrogenation of tetradecanol over a series of CuO/BaO catalysts in liquid phase and under inert atmosphere conditions [32]. El-Molla et. al. reported the catalytic conversion of alcohol over CuO/MgO treated with K_2O at 150-400 ºC using a flow technique [33]. The activity depended on the reaction temperature, textural properties, the nature and the concentration of the dopant used.

The use of metal oxides nano-particles supported over a material has additional benefits. Sadiq et. al. investigated the performance of iron oxide nanoparticles supported on activated carbon for the liquid phase oxidation of alcohol to aldehyde under various reaction conditions [34]. The catalyst showed maximum selectivity for aldehyde at lower temperature and lower partial pressure of oxygen.

In many cases, the use of alumina supported and zirconia supported metal oxides of precious metals like Pt and Pd have shown high activity for oxidation of alcohol at temperature 373 K [35]. Similarly Pd nanoparticles supported on magnesia or entrapped in aluminum hydroxide were found to be efficient catalysts for the aerobic oxidation of alcohols [36].

Idriss studied the reactions of ethanol on the surfaces of platinum, palladium, rhodium and gold supported on relatively inexpensive oxide such as alumina, silica, titania or ceria (of size 10-20 nm) and the bimetallic compounds: Pt-Rh, Rh-Au, Rh-Pd and Pt-Pd. The initial acid-base

interactions of dipole-dipole type between polar molecule such as ethanol with the CeO_2 surface have been proposed for the reaction [37]. As alcohol molecules approach the catalytic surface, hydrogen atom of the alcohol interacts with one surface O^{2-} of the catalyst and Ce^{4+} site interacts with the p orbitals of oxygen of adsorbed ethanol molecule leading to the formation of ethoxide and surface hydroxyl species. This is observed in the reaction over most oxide surfaces and some metals. This may be followed by the transfer of one H from the methylene group to the catalyst resulting in the formation of aldehyde (Scheme 18).



Scheme 18. Interaction of an alcohol with the surface of CeO_2 catalyst.

Cobalt oxide, a non-precious metal oxide, has been used as oxidation catalysts in several chemical processes. Ilyas and Saeed reported oxidation of benzyl alcohol in liquid phase with cobalt oxide catalyst and found that the oxidation in the liquid phase resulted in an easy separation of the catalyst by simple filtration [38]. The reaction was found to be 100% selective for benzaldehyde formation until 65% of benzyl alcohol was used up. They also investigated the effects of various parameters like reaction time, temperature, catalyst loading and partial pressure of oxygen on the reaction performance.

Recently there has been a considerable interest in the use of dual site catalysts possessing two active sites for the selective cleavage of functional groups that cannot be manipulated easily or selectively with single site systems. Williams et. al. reported alcohol dehydrogenation with a dual site Ruthenium, Boron Catalyst and proposed a mechanism which most likely involves reactivity only at the ruthenium center [39].

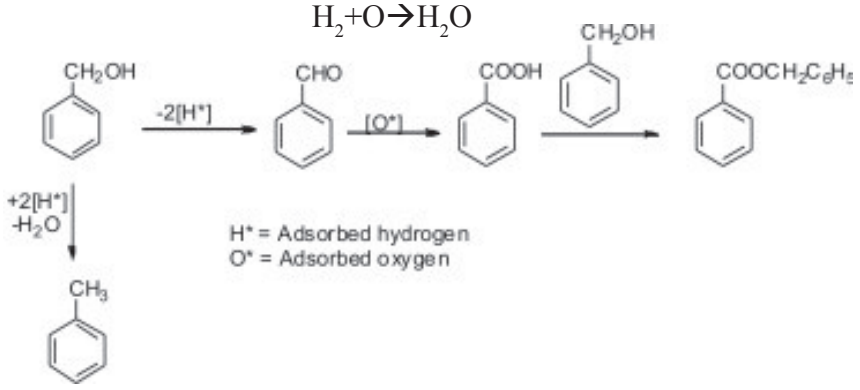
The use of mixed metal oxide CoO-CeO_2 and tert-butyl hydroperoxide (TBHP) for liquid phase oxidation of alcohols in n-hexane as a solvent was reported by Deshpande and Jayaram [40].

Vishwanathan et. al. carried out the selective oxidation and

dehydrogenation of benzyl alcohol on $ABB'O_3$(A=Ba, B=Pb, Ce, Ti and B'=Bi, Cu, Sb) type perovskite oxides in the absence and in the presence of oxygen. In the absence of oxygen or at its low partial pressures partial reduction of the catalyst was observed [41]. It was found that copper-containing perovskites were highly reducible while $BaTiO_3$ and $BaCeO_3$ were the least reducible. The selective oxidation of benzyl alcohol on all the perovskite oxides in the absence of oxygen provided benzaldehyde and toluene as the main product while for the same reaction in the presence of oxygen, small amounts of benzoic acid and benzylbenzoate are also obtained. (Scheme 19). In the absence of oxygen, hydrogen and water were also observed in the product stream suggesting the involvement of dehydrogenation step in the reaction process.

$$C_6H_5CH_2OH \rightarrow C_6H_5CHO + H_2$$

$$H_2+O \rightarrow H_2O$$

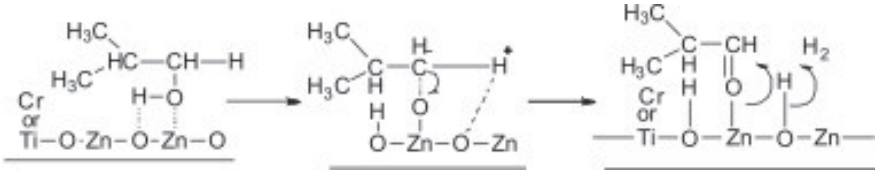


Scheme 19 Dehydrogenation of benzyl alcohol on ABB'O3-type perovskite oxides.

Benzoic acid and benzylbenzoate were formed only when the reaction is carried out in the presence of oxygen. This showed that adsorbed oxygen was responsible for the formation of benzoic acid and benzyl benzoate. Benzyl benzoate may be formed by the condensation of benzoic acid with benzyl alcohol.

Saad et. al. studied the dehydrogenation of isobutanol over zinc oxide catalyst and the role of adding TiO_2 and Cr_2O_3 on the performance of zinc oxide catalyst towards the isobutanol conversion reaction. It was proposed that acid base interactions between ($Zn^{2+}O^{2-}$) catalytic sites with strongly basic oxygen play an important role leading to hydrogen abstraction by the

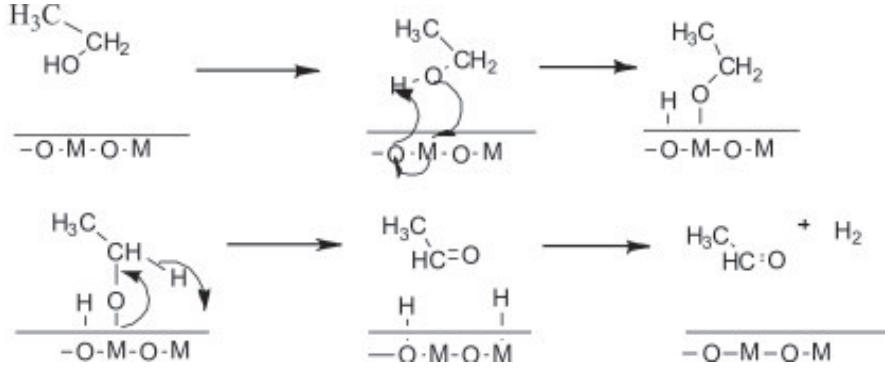
catalyst surface and formation of an alkoxy intermediate. Chemisorption of alcohol molecules on $Zn^{2+}O^{2-}$ site pairs of the catalyst results in the cleavage of the H bond to form a surface alkoxide intermediate bound to a Zn^{2+} acid center. This is followed by abstraction of α-hydrogen in the alkoxide group by a neighbouring basic site in order to form adsorbed aldehyde. Dehydrogenation of isobutanol to isobutyraldehyde on the catalysts surface takes place by the following reactions (Scheme 20) [42].



Scheme 20 *Mechanism of dehydrogenation of alcohol over zinc oxide catalyst.*

The catalysts having Ti or Cr surface enrichment are believed to provide additional Lewis acid centers which may stabilize the alkoxy intermediates; hence Ti-O-Zn and Cr-O-Zn species may be particularly effective as abundant sites for dehydrogenation. Moreover, benefit from the presence of these species is suggested to be enhancement of H_2 dissociation and O-H or C-H activation during the dehydrogenation of isobutanol.

Mahdavian proposed a mechansim for dehydrogenation of ethanol to acetaldehyde on SnO_2 (110). According to the proposed mechanism, after adsorption on the catalyst surface, the O-H bond of the alcohol dissociates hydrolytically to yield an ethoxide and a proton as shown in Scheme 21. The ethoxide formed in turn gets converted into acetladehyde by transferring the second H to the catalyst. The interaction of alcohol with the catalyst surface leads to the formation of aldehyde [43].



Scheme 21. Mechanism involving conversion of ethanol to acetaldehyde on SnO2.

III. Conclusions

Benzaldehyde is an important organic compound which finds applications in pharmaceuticals, dyes, perfumery and agro-chemical industries. In this review, different methods reported for the selective oxidation of benzyl alcohol to benzaldehyde have been explored. Literature survey has revealed a large number of new catalysts and reaction conditions that were employed for this oxidation in recent years. The oxidation has been reported under both homogenous and heterogeneous in gas/liquid phase reaction conditions. At many places researchers have used clean oxidants such as oxygen and hydrogen peroxide in place of stoichiometric toxic heavy metal oxidants such as dichromate and permanganate. Use of a number of catalysts such as transition metals, their salts, oxides as such or supported on an inert support has further widened the available options for this selective oxidation. Even noble metals such as gold supported over some reagents or catalysts in their nano form have been employed. The study of mechanism for this reaction has revealed how hydrogens from OH and methylene (CH_2) groups get transferred to the catalyst system during dehydrogenation.

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