

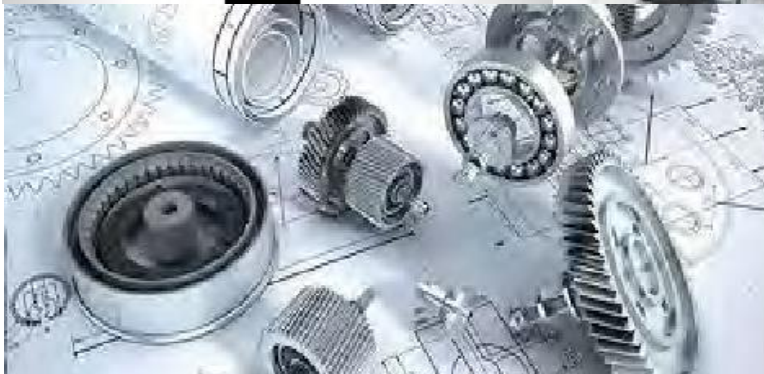


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Physiological Responses of Plant Growth Promoting Rhizobacteria, Biochar and Chemical Fertilizer Under Salinity Stress

Aliya Fazal, Asghari Bano and Farman Ali

Abstract—This research work is conducted to evaluate the physiological responses of phosphate solubilizing rhizobacteria (*Pseudomonas* sp.), biochar and N-fertilizer under salt stress. Biochar and fertilizer are mixed in soil (5:1) while *Pseudomonas* sp. is applied as seed soaking at 10^6 cells/ml prior to seed sowing. Salt stress with 150mM NaCl is applied for three days (3d) at three (3) leaf stage. The obtained results depicted significant effect of *Pseudomonas* sp. on root fresh weight and leaf area both under unstressed and salt stress conditions followed by biochar. Treatment with biochar and *Pseudomonas* sp. resulted in increased root fresh weight, leaf area, chlorophyll fluorescence and decreased osmotic potential by 60% particularly under salt stress. On the contrary, fertilizer treatment is found to be ineffective on seed germination (results not presented here), however increased chlorophyll content by 77%. Under salt stress, fertilizer treatment increased the osmotic potential of leaves. The combined treatments of fertilizer with *Pseudomonas* and biochar significantly increased root fresh weight, chlorophyll content and leaf area under salt stress. It is inferred that combined application of biochar and *Pseudomonas* can augment the effects of N-fertilizer on plants.

Index Terms—Stomatal index, Leaf area, Salt stress, Inoculation, Trichomes, Plant growth promoting rhizobacteria (PGPR).

I. INTRODUCTION

SALINITY is detrimental for several processes occurring in plant like photosynthesis, lipid metabolism and synthesis of protein. The initial response of plant to salinity is decreased in leaf surface area followed by inhibition in the expansion of leaf.

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Plant photosynthesis is badly affected when plant is exposed to salinity [1]. Salinity stress causes significant reduction in water uptake that ultimately results in reduced growth rate due to excessive buildup of salts in the plant causing premature senescence [2]. However, plants have adopted certain biochemical pathways that offer salt tolerance to plants. These pathways are associated with preservation and uptake of water, protection of protoplast, maintaining ion homeostasis, synthesis of osmolytes, specific proteins and certain oxidant scavenging enzymes that protect the plant from detrimental effects of free radicals [1].

Fertilizers both organic and inorganic add fertility to soil [3]. However in tropical conditions, inorganic fertilizers are less competent in weathered and highly degraded soils and there is less accessibility to resource-pool in using inorganic fertilizers [4]. Therefore, organic fertilizers are preferable use as they enhance efficiency of nutrients because of rapid rate of mineralization in the soil [1, 3, 5-20].

Biochar addition seems to be an efficient technique only if it is applied to permanently farmed soils [5]. Enhancement in yield generally occurs when soil is treated with hardwood biochars and chars possessing high N content [21].

Many plant growth promoting rhizobacteria (PGPR) facilitate plant growth indirectly by reducing plant pathogens or directly by facilitating the uptake of nutrients from environment. In addition, PGPR can also affect plant growth by increasing germination rate, root and shoot growth and weight, yield, leaf area, chlorophyll content, K and N uptake, protein content and delayed leaf senescence [22]. In [7], it is stated that combined inoculation of *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Mesorhizobium* increases grain yield and biomass in chickpea. Whereas it increases biomass of maize by 99 % and 96 % respectively, when seeds are inoculated with two

P-solubilizing bacteria (*Serratiamarcescens* EB-67 and *Pseudomonas* spp. CDB-35) [11].

Present investigation is aimed to evaluate the effects of biochar, *Pseudomonas* and N-fertilizer alone and in combination on some physiological traits of maize under control and induced salt stress.

II. MATERIALS AND METHODS

A. Plant material and growing conditions

Maize seeds are surface sterilized by shaking in 95% ethanol for 2 to 3 min followed by shaking in 10% chlorox for 2-3 min. Thereafter, seeds are thoroughly washed with autoclaved distilled water. Pot experiment is conducted and seeds are sown in plastic pots (width 7cm, length 11cm) and grown in growth chamber with 14h photoperiod at 22/26°C. The biochar (derived from Poplar sawdust) is autoclaved prior to mixing in soil with urea (5:1). N-Fertilizer (1.20 g/pot), is put immediately after sowing and then immediately irrigated [8].

B. Inocula preparation

Inoculum is prepared by inoculating LB broth with 24h old fresh culture of *Pseudomonas* sp. (Acc No KF307196) the culture is incubated on a rotary shaker for 48h followed by centrifugation on a centrifuge (Labofuge 400e) for 10 min at 10,000 rpm. The supernatant is discarded while pellet is suspended in distilled water to adjust the optical density 1 at OD 660nm to get bacterial count of 10^6 cells/ml. Sterilized seeds are then soaked in the inocula thus prepared for 2 to 4h. Seeds are sown in plastic pots (6 plants per pot) containing biochar @ 5g/Kg in autoclaved soil and sand mixed in 3:1 ratio.

C. Induction of salt stress

Salt stress is induced after 2 weeks of sowing by adding aqueous solution of NaCl (150mM) for 3d.

D. Osmotic potential

Cell sap (after 17 days of sowing) from the leaves of control and treated plants is extracted to measure osmotic potential with the help of freezing point osmometer using the technique developed by Capell and Doerffling [10].

E. Root fresh weight

Fresh weight of leaf and root is measured using electric balance.

F. Leaf area

After 17 days of sowing, plants are harvested and their leaf area is calculated using equation (1), by taking average height (cm) and width (cm) of three plants from all the treatments [23].

$$\text{Leaf area (cm}^2\text{)} = \text{height} \times \text{width} \quad (1)$$

G. Measurement of stomata and epidermal appendages

Dehydrated leaves are randomly taken from the plant and boiled in lactic acid. The adaxial surface of leaf is peeled and observed in a light microscope at 20x, and the

total number of stomata and other epidermal cells are counted. Stomatal Index (SI) is calculated using equation (2) as [24].

$$SI(\%) = \frac{\text{No. of stomata}}{\text{No. of stomata} + \text{No. of epidermal cells}} \times 100 \quad (2)$$

H. Chlorophyll fluorescence

Chlorophyll fluorescence is measured with a portable Chlorophyll Fluorometer after 20 min of dark adaptation. Chlorophyll fluorescence is expressed as $\frac{F_v}{F_m}$ ratio. It is calculated as:

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \quad (3)$$

Where F_m and F_0 are maximal and minimal fluorescence of dark adopted leaves respectively and F_v is variable fluorescence [25].

I. Chlorophyll content

Chlorophyll content of fully expanded young maize leaves is measured using Soil-Plant Analyses Development (SPAD) instrument [26].

J. Statistical Analysis

Analysis of variance (ANOVA) is performed using factorial randomized complete plot design to assess significant variation. Significant differences between treatment means is determined through (LSD) tests. Least significant difference is measured at $P < 0.05$ and MS Excel software is used to illustrate and compare data on figures [27].

III. RESULTS

A. Osmotic potential

The maximum increase (150%) in the osmotic potential is due to fertilizer treatment which is decreased in combined treatment with *Pseudomonas* sp. under salt stress as shown in Fig. 1. Fertilizer addition to biochar and *Pseudomonas* sp. increased the osmotic potential of leaves over biochar used alone. The combined treatment of *Pseudomonas* sp. with biochar had no significant effect as compared to *Pseudomonas* sp. inoculation alone but the combined treatment of fertilizer with biochar and *Pseudomonas* sp. are 21% and 46% lower over that of salt stress respectively.

B. Fresh weight of roots

All the treatments significantly increased (26%-30%) fresh weight of leaves under both unstressed and salt stressed conditions respectively (results not presented).

The maximum significant increase (28%) in root fresh weight is in plants inoculated with *Pseudomonas* sp. under unstressed condition as shown in Fig. 2. The biochar and fertilizer treatments has similar magnitude of increase and their combined treatment do not differ significantly. *Pseudomonas* sp. has significant (20%) increase over salt stress. Biochar treatment under salt stress is less stimulatory but it's effectively enhanced when combined

application is made with *Pseudomonas* sp. and fertilizer increasing the value by 10% and 12% over control respectively. Combined application of *Pseudomonas* sp. and fertilizer under salt stress also proved effective resulting in 11% significant increase in root weight over control.

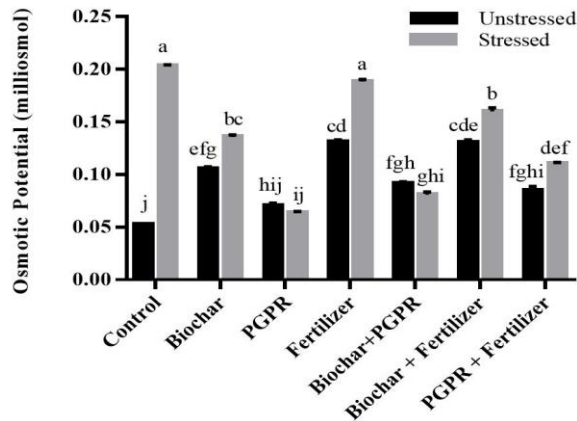


Fig. 1. Effect of Biochar, PGPR (*Pseudomonas* sp.) and N-fertilizer on osmotic potential of maize (*Zea mays* L.) leaves under salt stress. Biochar and fertilizer are mixed in soil (5:1) while *Pseudomonas* sp. is applied as seed soaking prior to seed sowing. Salt stress is induced for 3d after 2 weeks of sowing. The bars containing the same English letters differ non-significantly from each other at $P < 0.05$.

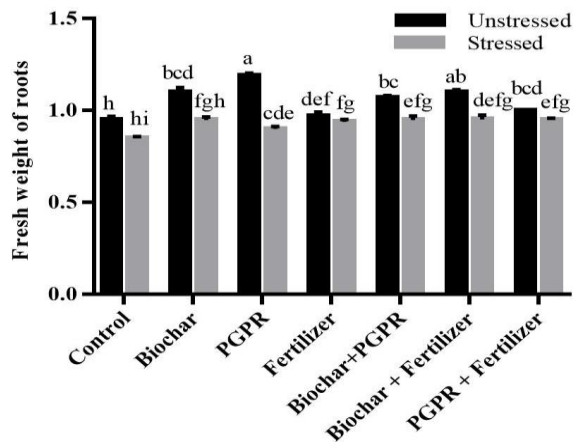


Fig. 2. Effect of Biochar, PGPR (*Pseudomonas* sp.) and N-fertilizer on fresh weight of maize (*Zea mays* L.) roots under salt stress. (Treatments detail shown in Fig. 1).

C. Leaf area (cm^2)

All the treatments under unstressed condition shows significant increase in leaf area over untreated control as shown in Fig. 3. Biochar treatment is less stimulatory but its effectivity is enhanced when combined application is made with fertilizer and *Pseudomonas* sp. and shows significant increase of 76% and 68% in leaf area as compared to control respectively. Similarly, the effect of *Pseudomonas* sp. is enhanced by 25% when combined application is made with fertilizer. Biochar in combination with *Pseudomonas* sp. showed 42% and 53% increases in maize leaf area as compared to *Pseudomonas* sp. and

biochar treatments applied singly. Combined application of biochar with fertilizer shows maximum increase (60%) over salt stress.

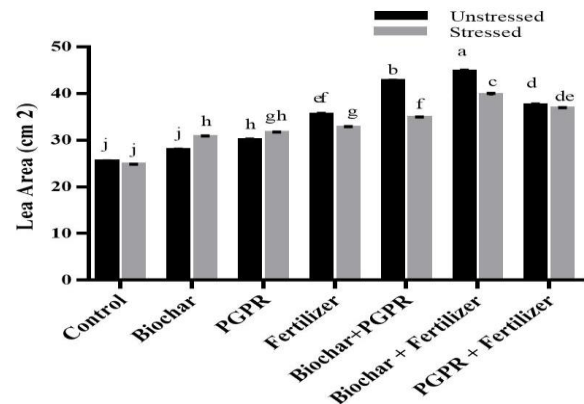


Fig. 3. Effect of Biochar, PGPR (*Pseudomonas* sp.) and N-fertilizer on leaf area of maize (*Zea mays* L.) under salt stress. (Treatments detail shown in Fig. 1).

D. Stomatal index (%)

Significant increases in stomatal index is observed in all the treatments as compared to control as shown in Fig. 4. The maximum increase (60%) is due to *Pseudomonas* sp. Fertilizer treatment showed least (34%) increase in stomatal index over control. The addition of fertilizer to biochar and *Pseudomonas* sp. decreased the stomatal index by 10% and 21% over biochar and *Pseudomonas* sp. under unstressed condition. Under salt stress, the stomatal index is maximum in plants inoculated with *Pseudomonas* sp.

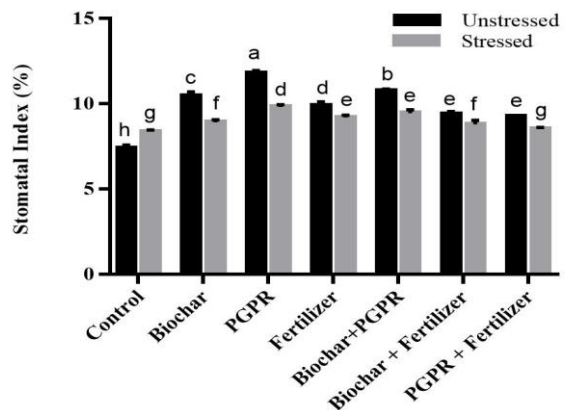


Fig. 4. Effect of Biochar, PGPR (*Pseudomonas* sp.) and N-fertilizer on stomatal index of maize (*Zea mays* L.) leaves under salt stress. (Treatments detail shown in Fig. 1).

E. Chlorophyll fluorescence (F_v/F_m)

All the treatments under unstressed condition display significant increase in chlorophyll fluorescence over uninoculated, untreated control as shown in Fig. 5 Under unstressed condition, fertilizer, biochar and *Pseudomonas* sp. equally stimulates chlorophyll fluorescence of leaves by 11% over control. However biochar in combination

with *Pseudomonas* sp. further augmented its stimulatory effect by 10% as compared to *Pseudomonas* treatment made alone. Under salt stress, fertilizer treatment shows decrease in chlorophyll fluorescence value as compared to control, nevertheless this decrease is compensated by the combined treatments of fertilizer with biochar and *Pseudomonas* which efficiently increased chlorophyll fluorescence.

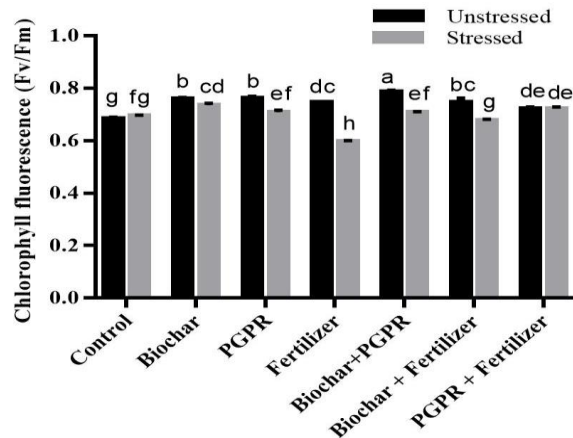


Fig. 5. Effect of Biochar, *Pseudomonas* sp. and N-fertilizer on chlorophyll fluorescence of maize (*Zea mays* L.) Leaves under salt stress. (Treatments detail shown in Fig. 1).

F. Chlorophyll content

Under unstressed condition, the increase in chlorophyll content as shown in Fig. 6 is maximum in the fertilizer treatment made alone and the combined treatment of fertilizer with *Pseudomonas* sp. The *Pseudomonas* sp. and biochar treatment had 13% and 41% increases in chlorophyll content over control. Addition of biochar to *Pseudomonas* sp. inoculated plants assist the *Pseudomonas* sp. to increase the chlorophyll content by 24% higher than that of *Pseudomonas* sp. applied alone under salt stress.

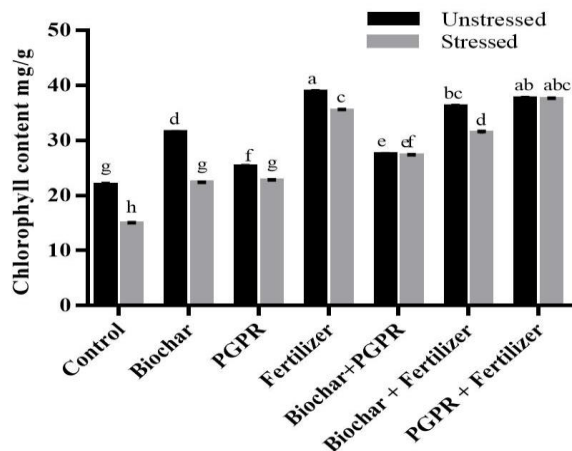


Fig. 6. Effect of Biochar, PGPR (*Pseudomonas* sp.) and N-fertilizer on chlorophyll content of maize (*Zea mays* L.) leaves under salt stress. (Treatments detail shown in Fig. 1).

IV. DISCUSSION

It is evident from the results that both *Pseudomonas* sp. and biochar improves physiological traits of maize plant studied during the present investigation. Salt stress results in increased osmotic potential due to buildup of solutes that gives rise to a secondary stress called osmotic stress thus causing cellular dehydration. Production of compatible solutes and proteins have been reported that function in abiotic stress tolerance [17-18]. It is observed that *Pseudomonas* sp. and also biochar exhibits the highest osmotic tolerance and results in significant increase in proline production [28] that is involved in cellular osmotic adaptation. Hence, the osmotic balance caused due to salinity is encountered by biochar and PGPR but is not addressed by fertilizer treatment. Fertilizer induces increase in leaf area and growth but don't keep pace with osmoregulation as a result, electrolyte leakage is also higher in fertilizer treatment [28]. The osmoregulation appears to be controlled more efficiently by PGPR and biochar applied separately. However, combined treatments of fertilizer with *Pseudomonas* and biochar proved effective in augmenting physiological traits of maize plant. Previous studies have reported that *P. putida* and *B. megaterium* exhibit the highest osmotic tolerance and show increased proline content that is involved in osmotic cellular adaptation thus suggesting that bacteria have developed mechanisms that can alleviate stresses in crop plants [6-21, 29, 30].

In the present research work, the effect of PGPR is more pronounced on root growth and both PGPR and biochar are equally effective to promote shoot growth. In most of the growth parameters, PGPR override the effects of biochar but for leaf area, biochar more effective and fertilizer addition further augments its effect. Spokas et al. [13] reported significant increase in root density and crop productivity following biochar addition while Carlier et al. [14] revealed that inoculation with PGPR produced a substantial increase in plant height and root length in early growth stages of wheat. Dobbelaere et al. [31] studied the physiological responses of the plant roots to inoculation with *Azospirillum* and observed that inoculation leads to an improvement in root development and an increase in the rate of water and mineral uptake. Gholami et al. [32] observed that maize seeds inoculated with *Azospirillum*, *Pseudomonas* and *Azotobacter* strains enhanced seed germination and seedling vigour of maize.

PGPR have also been reported to increase cell division and cell elongation due to production of plant growth promoting hormones [15-37]. The growth promoting property of biochar, *Pseudomonas* sp. and fertilizer is apparent on leaf area of maize under salinity stress which appears to be related with the nutrient content of biochar and fertilizer and growth hormones produced by *Pseudomonas* sp. The combined effect being more stimulatory due to synergism between biochar and *Pseudomonas* sp. can possibly be attributed to the fact that biochar served as a source of nutrients for better proliferation of *Pseudomonas* sp. Busscher et al. [38], Lashari et al. [34] also demonstrated the effects of biochar

on soil properties as well as leaf area index, maize grain yield and observed an increase in overall productivity and performance of plant.

The stomatal density and stomatal index (SI) are indicators of paleomorphologic CO₂ concentration. The application of biochar and *Pseudomonas* sp. inoculation maintained higher stomatal index (SI). This might helped to keep the turgidity higher and facilitate the gaseous exchange efficiently for greater assimilation of leaves but under salt stress the SI is reduced. Varela et al. [4] studied comparative effects of biochar on plant growth parameters and observed higher mean leaf width, leaf length, chlorophyll content, stem size and root size. Increased chlorophyll content can be used as an index of photosynthetic potential as well as an index of plant productivity. Furthermore, trichome has been observed in *Pseudomonas* sp. inoculated plant leaves under unstressed (non-saline) condition [32]. Trichomes are stalked protuberances that contribute to plant resistance against herbivory by physical and chemical deterrents [13]. Moreover, *Pseudomonas* sp. in combination with biochar resulted in 45% (under unstressed) and 13% (under stress) significant increase in stomatal index value over uninoculated control. Vivas et al. [9] demonstrated that PGPR inoculation resulted in an increase in overall plant physiological values including photosynthetic rate, water use efficiency (WUE) and stomatal conductance of lettuce plants.

Increasing salinity in soil decreases chlorophyll content which ultimately decreases plant growth causing a shift in many physiological activities like photosynthesis, stomatal conductance and antioxidant activity [16]. However PGPR inoculation helps ameliorating such deleterious effects of salinity stress. An experiment conducted by Fazal and Bano [1] reported that inoculation with *Pseudomonas* sp. enhanced chlorophyll production resulting in significant increase in chlorophyll content over control under unstressed condition. Similar results are observed by Heidari and Golpayegani [23], where PGPR significantly increased the catalase activity and chlorophyll content of leaves under water stress.

The amount of chlorophyll fluorescence (Fv/Fm) indicates thylakoid membrane integrity and the relative efficiency of electron transport from PSII to PSI [19]. Additionally, the flow of electrons through PSII is indicative, under many conditions, of the overall rate of photosynthesis [29]. As the aforementioned results showed increased chlorophyll fluorescence values when combined treatment of biochar and *Pseudomonas* sp. is made, so it could easily be implied that the nutrients present in biochar, and the growth promoting hormones produced by *Pseudomonas* sp. [33] can possibly attribute to increased photosynthetic activity of maize plant.

V. CONCLUSION

In the light of the present results, it is inferred that biochar and *Pseudomonas* sp. are equally effective as fertilizer and can be used to minimize the use of chemical fertilizer. The combined treatment of PGPR and Biochar

may enhance the effect of N-fertilizer on leaf area and root growth.

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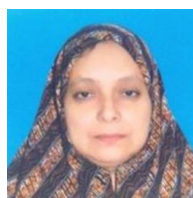
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Synthesis of Sol-Gel Silicas functionalized with Schiff Base Ligands

Shazia Naheed, Ghulam Zakria, Rana Muhammad Talal and Alina Changez

Abstract—Schiff bases containing azomethine linkage in their structure are good ligands and show great metal binding ability. In last few years sol-gel method is found very attractive for synthesis of gels due to its low cost, low operating temperature and ease of synthesis. This work presents the synthesis of Schiff base functionalized gels through sol-gel method and their use in extraction of metal ions from aqueous medium at room temperature and appropriate pH. Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and X-ray diffraction (XRD) analyses of both blank and functionalized gels are presented. The analyses data show successful incorporation of the Schiff base in sol-gel matrix. Metal removal data show high efficiency of Schiff base functionalized gels.

Index Terms—Schiff base functionalized silicas, Sol-gel method, Extraction of metal ions.

I. INTRODUCTION

THE organic compounds consisting of azomethine linkage ($-\text{HC}=\text{N}-$) in their structure are called Schiff bases. Hugo Schiff the pioneer synthesized such compounds by the reaction of aromatic amine with aldehyde or ketone [1]. In a Schiff base nitrogen atom is connected to an aryl or alkyl group but not with hydrogen.

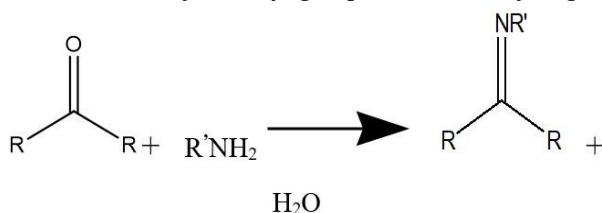


Fig. 1. Formation of Schiff base.

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It is a condensation reaction in which amine, acting as a nucleophile attacks at the electrophilic carbonyl carbon forming Schiff base in which (C=O) of carbonyl group is replaced by (C=N) of imine or Schiff base. A variety of compounds having amino group can be employed for the preparation of Schiff bases as shown in Fig.1 [2-3]. Product (Schiff base) both are very low [4]. pH of the reaction mixture is to be maintained due to high basicity of aliphatic amines. Imines formed from aromatic amines are called Anils. Just like aliphatic amines, aliphatic carbonyls are also not preferred for Schiff bases synthesis as aliphatic carbonyl compounds are less stable [5-7]. Usually aldehyde is the carbonyls of choice due to their better reactivity as compare to ketones. If carbon or nitrogen of (C=N) is attached with aromatic ring, it increases the stability of the Schiff base due to conjugation [8]. Preparation of Schiff base is a reversible reaction. Therefore, the reaction can be used for the protection of amines. It takes place under basic or acidic conditions or simply by refluxing the reactants in a suitable solvent. The equilibrium can be shifted towards forward direction by dehydration with a desiccant such as TiCl_4 [9] or by azeotropic distillation [10-11]. As Schiff bases form crystals they usually show sharp melting points. They are good as ligands and form complexes with metals. Transition metal complexes form a large group in Chemistry [12].

Mechanism of Schiff base formation shows that it is nucleophilic addition reaction due to which an unstable addition product known as carbinolamine is formed. Elimination of water from carbinolamine results in the formation of Schiff base. Its elimination takes place under mild acidic conditions. This step is rate determining step in the synthesis. Therefore the reaction is often done under mild acidic conditions. Harsh acidic conditions can protonate amine diminishing their nucleophilicity and resulting backward reaction. Hydrolysis of Schiff base can be done by water under acidic or basic conditions.

Schiff bases are versatile organic ligands [13-17]. Their biomedical [18] and catalytic [19-21], analytical and other applications are well documented in literature. Previously

Schiff bases have been utilized in estimation [22-23] and separation of metals [24] and estimation of oxo groups [25-26]. They are well known for their biological activities [27]. Azomethine linkage in the structure of Schiff base is thought to be responsible for their bioactivities [28] against cancer [29], bacterial infections [30-31], AIDS [32-33], fungal infections [34-35], viral infections [36, 37], mosquito larvae [3], inflammatory [39] and cancer. Entrapment of bioactive materials in a suitable matrix as support and their transport to the infected parts is an emerging field of research.

Sol-gel process was discovered in 1800. Synthesis of organic-inorganic hybrids through sol-gel process has attained much attention. Metal or semimetal alkoxides being the precursor substance of this process undergo two reactions i.e. Hydrolysis and Condensation, which leads to formation of inorganic network within which the organic moiety get entrapped due to low operating temperature. Occurrence of poly condensation reaction at low temperature is the biggest attraction and advantage of this process [40]. These reactions are shown in Fig. 2.

Industrial development is mainly contributing to environment pollution which is affecting the ecosystem as well as human health [41]. Un-loading of these toxic metals from environment is the urgent need of present era. Some of the previous methods for toxic metal removal are oxidation, precipitation, complexation, electrochemical treatment, filtration, distillation, application of selective membranes and sorption etc [42-44]. Of all, Sorption is superior and more attractive method due to low cost, ecological correctness, higher efficiency and ease of operation. Chelating polymeric materials have been reported for pre-concentration and removal of metals [45-49]. Polymeric resins showed low mechanical and chemical stability, slow kinetics and irreversible adsorption [50]. Unmodified naturally occurring materials [51] and modified naturally occurring materials have also been applied for metal removal.

To overcome the problems of polymeric resins inorganic supports such as silica gel or functionalized silica gel have been applied for metal removal. They have high thermal and mechanical stability, stability to chemicals, negligible swelling, shrinking poisoning and fast sorption kinetics. To avoid some limitations of silica gel modified sol-gel materials have been used as sorbents. Porosity and surface layer composition of sol-gel materials can be tuned for specific application. The sol-gel materials can be physically or chemically functionalized with active chelating ligands for metal removal.

II. EXPERIMENTAL METHODOLOGY

A. General procedure for synthesis of Schiff bases (L1-L4)

10mmol (0.01 M) of 4, 4'-oxydianiline was dissolved in methanol to get a clear solution. 20mmol (0.02 M) aldehyde/ketone was also dissolved in the same solvent. Both the solutions were mixed and refluxed. Progress of reaction was monitored through TLC in appropriate solvent

system from time to time. After reaction completion, mixture was evaporated on rotary evaporator followed by the filtration of the product. Solid obtained was weighted, recrystallized and stored in desiccator.

B. Synthesis procedure for L5

To a stirred solution of Salicylaldehyde (10 mmol, 0.01M) in 15 mL ethanol, 10 mmol (0.01M) of sulfanilamide solution in 15 mL ethanol was added slowly. The reaction was refluxed for 120 minutes. Orange precipitates were formed. The product was filtered, recrystallized with Ethanol and weighed.

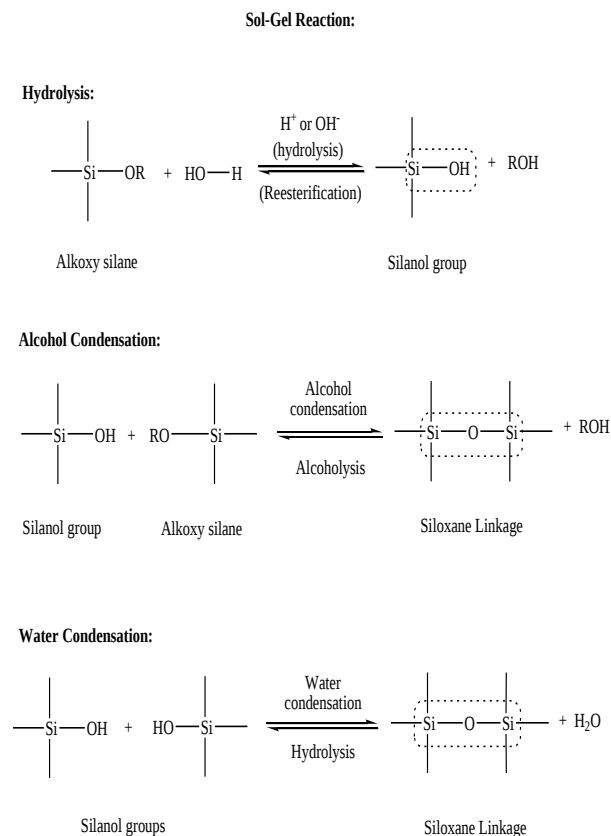


Fig. 2. Sol-gel reaction.

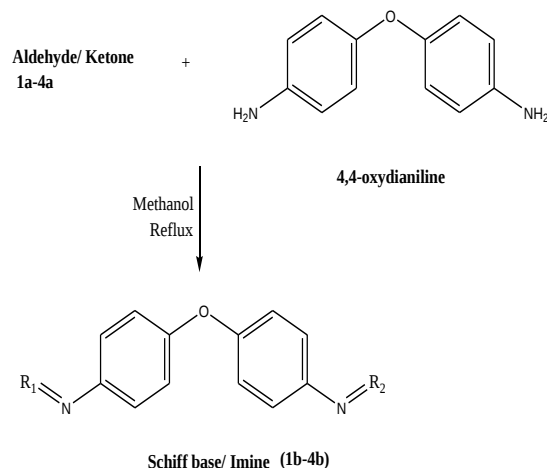


Fig. 3. General reaction for Schiff base (L1-L5).

C. Synthesis of Organically Modified Gel

In a 100 ml container, 20 ml tetraethoxysilane (TEOS), 20 ml distilled water and 40 ml of 0.01M of ligand (L1-L5) solution in ethanol was added, followed by addition of 0.01M ammonium fluoride (NH₄F) solution as catalyst. Shaking of mixture results in immediate gel formation. It allowed the gel to dry for 4-5 days at room temperature.

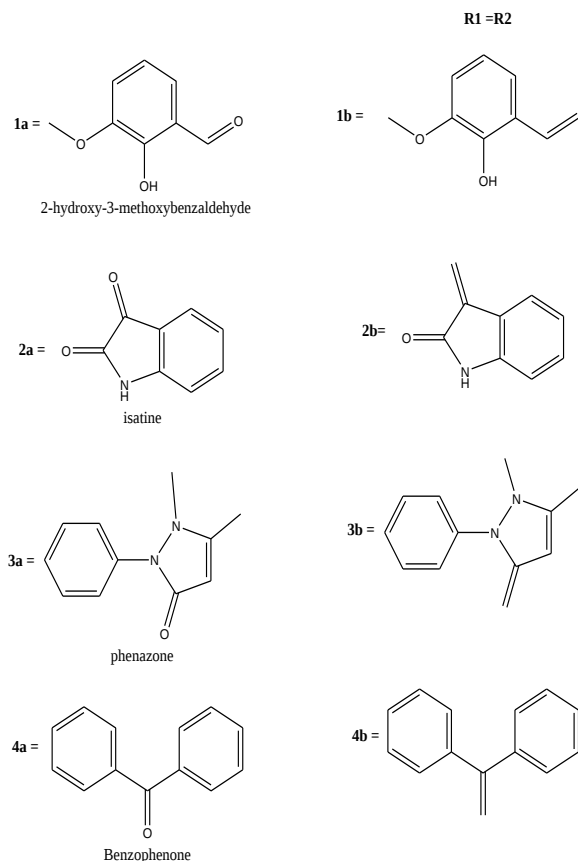


Fig. 4. Aldehydes and Ketones.

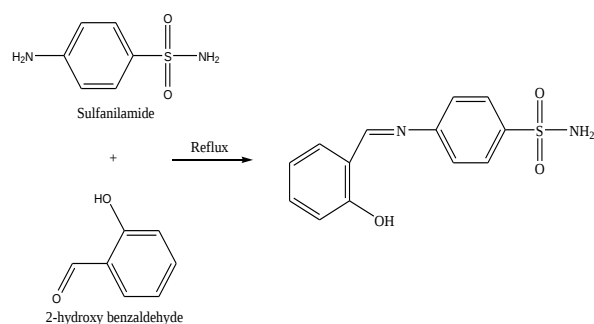


Fig. 5. Synthesis of Schiff base (L5).

Gel got dried and cracks appeared. Later it was placed in an oven at 48°C until weight became constant. After attaining the constant weight gel was crushed into fine powder and soaked in water (washed) in order to remove the undoped reagent. After washing, the gel was again dried (till constant weight by following the same method), crushed, weighed and stored in desiccator. Fig. 3, 4 and 5 shows synthesis of Schiff base.

D. Removal of Zn⁺² from aqueous medium using organically modified gels

10 mg/L Zn(CH₃COO)₂·2H₂O solution was prepared in distilled water. 10 ml of the solution was taken in seven different sample vials and pH was maintained at 1-6 respectively in each vial. 30 mg of functionalized gel was added in each vial and was shaken for 30 minutes at room temperature. The results are reported in Fig. 6.

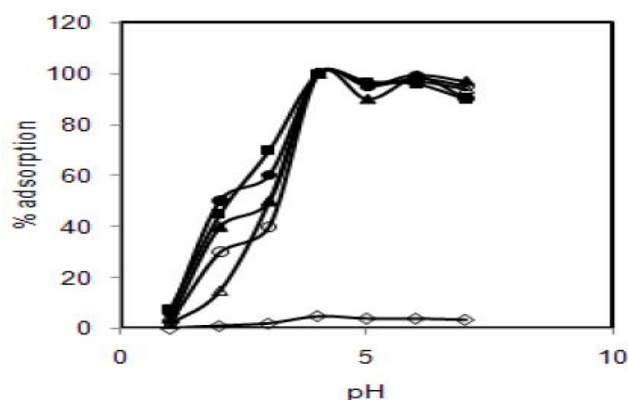


Fig. 6. Removal of metal.

Effect of pH on sorption of Zn (II) by (◇) blank gel, (Δ) L1 Sol Gel, (○) L2 Sol Gel, (▲) L3 Sol Gel, (●) L4 Sol Gel, (■) L5 Sol Gel. Temperature = Room Temperature, Amount of Functionalized gel=30 mg, Shaking time=30 minutes, Zn (II) Concentration=10 mg/L, Volume of suspension=10 mL.

III. RESULTS AND DISCUSSION

A. Schiff base Logands (L1-L5)

Physical properties of the ligands synthesized are reported in Table 1 while the FTIR data of L1-L5 are reported below. Disappearance of C=O stretches of aldehydes and ketones and -NH stretches of primary amines in the FTIR spectrum indicates the Imine formation while physical properties data as shown in Table 1 further confirm the ligand synthesis.

B. 2-((E)-4-(4-((E)-2-hydroxy-3-methoxybenzylideneamino)phenoxy)phenylimino)methyl)-6-methoxyphenol (L1)

Color: Yellow, Yield: 76%, FTIR (ν, cm⁻¹), 1575, 1485 (C=C ring stretch), 3010 (sp² C-H stretch), 3450 (-OH stretch), 1200 (C-O stretch), 1620 (C=N stretch)

C. (Z)-3-(4-(4-((Z)-3-oxoindolin-2-ylideneamino)phenoxy)phenylimino)indolin-2-one (L2)

Color; Deep yellow, Yield; 65%, FTIR (ν, cm⁻¹), 1590, 1459 (C=C ring stretch), 3051 (sp² C-H stretch), 1742 (C=O stretch), 3566 (-NH stretch), 1624 (C=N stretch)

D. (E)-N-4-(4-((E)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazolylideneamino)phenoxy)benzeneamine (L3)

Color, Pale yellow, Yield, 68%, FTIR (ν, cm⁻¹), 1585, 1460 (C=C ring stretch), 3100 (sp² CH stretch), 1200 (C-O stretch), 1624 (C=N stretch)

E. *N*-(diphenylmethylene)-4-(4-(diphenylmethylene-amino) phenoxy) benzamine (L4)

Color, Off-white, Yield, 70%, FTIR (ν , cm^{-1}), 1550, 1478 (C=C ring stretch), 3100 (=CH stretch), 1100 (C-O stretch), 1620 (C=N stretch)

F. *(E)*-4-(2-hydroxybenzylideneamino) benzene sulfonamide (L5)

Color; Yellow, Yield; 78%, FTIR (ν , cm^{-1}) 1540, 1453(C=C ring stretch), 3050 (=CH stretch), 3400(-OH stretch), 1320, 1140 (S=O stretch), 3350, 3400 (-NH stretch), 1620 (C=N stretch).

TABLE I
DATA OF SCHIFF BASE LIGANDS

Code	Molecular Formula	M.Mass (g/mole)	M.P ($^{\circ}\text{C}$)	Colour	Reflux Time (hrs)	% Yield
L1	$\text{C}_{48}\text{H}_{44}\text{N}_4\text{O}_9$	820.88	148-151	yellow	10	76
L2	$\text{C}_{29}\text{H}_{20}\text{N}_4\text{O}_3$	472.49	146-149	deep yellow	5	65
L3	$\text{C}_{40}\text{H}_{28}\text{N}_2\text{O}_3$	584.66	188-192	Pale yellow	3	68
L4	$\text{C}_{34}\text{H}_{32}\text{N}_6\text{O}$	540.66	140-142	off-white	3	70
L5	$\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_3\text{S}$	276.31	210-211	yellow	1.5	78

G. Organically modified gels (L1-SG to L5-SG)

Analytical data of the synthesized gels are reported in Table II. It shows that color of the blank gel was white while the organically modified gels show color similar to Schiff base ligand that indicates the silica gel is doped by the ligand. The FTIR spectra of all modified gels show a very wide band at 1100 and 470cm^{-1} for the siloxane linkage (-Si-O-Si-) and a medium intensity band at 3300- 3400cm^{-1} for -Si-OH linkages. The siloxane band hides/masks the other functional groups of the ligands. Fig. 1 shows the FTIR spectra of the ligand (L5), blank gel (SG) and doped gel (L5-SG) as shown in Table III.

TABLE II
ANALYSIS DATA OF BLANK AND MODIFIED GELS

Product Code	Molecular Formula	Molecular Weight (g/mole)	Melting Point ($^{\circ}\text{C}$)	Colour	% Yield
SG	-	-	Does not melt	White	2.5 g
L1	$\text{C}_{48}\text{H}_{44}\text{N}_4\text{O}_9$	820.88	148-151	Yellow	76 %
L1SG	-	-	-	Yellow	2.9 g
L2	$\text{C}_{29}\text{H}_{20}\text{N}_4\text{O}_3$	472.49	146-149	Deep Yellow	65 %
L2SG	-	-	-	Deep Yellow	2.95 g
L3	$\text{C}_{40}\text{H}_{28}\text{N}_2\text{O}_3$	584.66	188-192	Pale Yellow	68 %
L3SG	-	-	-	Pale Yellow	2.72 g
L4	$\text{C}_{34}\text{H}_{32}\text{N}_6\text{O}$	540.66	140-142	Off-white	70 %
L4SG	-	-	-	Off-white	2.76 g
L5	$\text{C}_{13}\text{H}_{12}\text{N}_2\text{O}_3\text{S}$	276.31	210-211	Yellow	78 %
L5SG	-	-	-	Yellow	2.74 g

Gel modification with organic ligands is also confirmed through Scanning Electron Microscopy analysis (SEM). Data obtained from SEM studies show that porosity is decreased after modification of the xerogel. It shows the filling of the pores of the xerogel after modification with Schiff base. SEM after metal sorption shows the complete filling of pores and surface of the xerogels as indicated in Fig. 7, 8 and 9. XRD Spectra of the xerogels show amorphous nature of the blank and Schiff based gels.

The porosity analysis is reported in Table IV. The interpretation of results was done by getting help from reported literature. The analyses data show successful incorporation of the Schiff base molecules inside the xerogel matrix. Sorption studies further confirm the functionalization of the xerogel. Application of organically modified gel in Zn (II) sorption. The sorption studies were conducted using zinc acetate. Sorption increases with rise in pH as of 1-4, achieves its maximum value at pH 4. Insignificant sorption is examined at pH 1-2. More increase in pH above 4 decreases the sorption to a small degree as shown in Fig. 2. The reduced sorption at low pH value may be due to competition of H_3O^+ ions with metallic ions for complexation with some active positions of Imines.

TABLE III
FTIR DATA OF BLANK AND SCHIFF BASE MODIFIED GELS (KBr PELLET, ν cm^{-1})

Name	(C=N)	(Si-OH)	(Si-O-Si)
SG	-	3300-3000	1100, 470
L1	1620	-	-
L1SG	1620	3300-3000	1107, 471
L2	1624	-	-
L2SG	1624	3400-3000	1100, 470
L3	1624	-	-
L3SG	1624	3300-3000	1101, 470
L4	1620	-	-
L4SG	1620	3300-3100	1102, 469
L5	1620	-	-
L5SG	1620	3100-3400	1100, 470

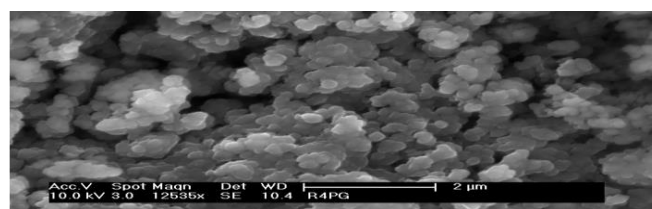


Fig.7. SEM of blank gel (SG).

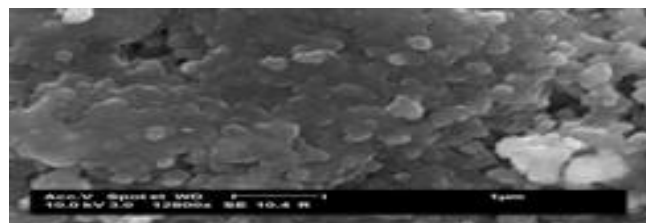


Fig. 8. SEM of organically modified gel (SBSG).

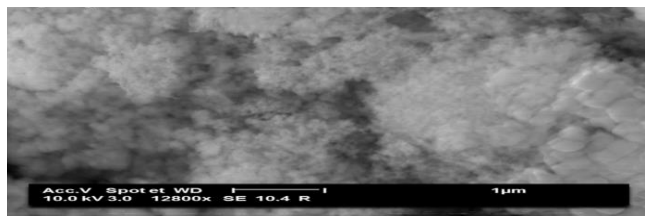


Fig. 9. SEM of modified gel after sorption of metal ions.

TABLE IV
POROSITY DATA OF BLANK AND MODIFIED GELS

Xerogels	Surface Area (m/g) ²	Pore volume (g/cm) ³	Pore diameter (nm)
SG	269	0.73	17.10
L1SG	249	0.53	9.12
L2SG	235	0.54	9.09
L3SG	265	0.60	10.00
L4SG	250	0.56	11.00
L5SG	255	0.49	0.88

functionalized gels. The data shows high efficiency of the functionalized gels as compared with blank gel. The advantage of the work is that it shows high efficiency at room temperature and moderate pH.

IV. CONCLUSION

In the light of above study it is concluded that Schiff bases being good ligands for toxic metals retain metal extraction potential even after doping of silica. Sol-gel method is a good approach for synthesis of organically modified gels. The Schiff base modified gel is more promising for metal extraction than the blank gel. SEM and XRD analysis data show the successful incorporation of the Schiff base ligand into the gel. Present study confirms that Schiff base modified gels are found very promising for extraction of Zinc metal at moderate pH at room temperature as compared to blank gel.

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Development of UAV Octocopter Based on Pesticides Spraying System

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Abstract—The application of pesticides in agriculture has a leading role in increasing the per acre yield of different crops. There are a number of systems through which pesticides can be sprayed on crops. Unmanned aerial vehicles (UAVs) are being progressively used for this purpose due to their improved efficiency and high-speed characteristics. This research work mainly focuses on the construction of an octocopter based on the idea of UAVs for the agriculture sector. The implementation of octocopter for spraying chemicals is prominently increasing to minimize the wastage of pesticides while increasing the production of crops. This research also strives to minimize the spraying time, labor cost and weight of the octocopter for its better utilization and implementation in the field of agriculture. The flight test showed that the octocopter is useful, efficient and beneficial for the intended application.

Index Terms—Octocopter, Spraying drone, UAV, Agricultural pesticides

I. INTRODUCTION

UNMANNED aerial vehicle (UAV) has become less costly due to advancements in the software technology and reduction in the cost of hardware [1]. An unmanned aerial vehicle is basically a human operated vehicle through a remote control. Nowadays, the unmanned aerial vehicles are being used in many fields of daily life such as: agriculture, search, rescue operations [2], traffic surveillance, security, military and police [3], firefighting [4], and during live coverage of different outdoor events by different TV channels. UAVs have the ability to reach such places which are generally impossible for human beings [5].

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Depending on the load to be carried, different, different amendments are made in copters. Nowadays, a multi-rotor vehicle, embedding a remote controlled system, has the ability to carry a load of about two kilograms [6]. UAVs have ability to takeoff vertically, landing, hovering and moving carefully and skillfully.

The octocopter can take off and land vertically, which relates it to the group of the multi-rotor helicopter. In helicopter, fixed-pitched blade is used with rotor, while in multi-rotor vehicle's pitches do not change as in the case of blade rotors. First multirotor dragon flyer was manufactured around 2000 [7]. The star shape configuration of an octocopter is the best to carry the maximum amount of payload [8].

This paper proposes agriculture based application through an octocopter where it is responsible for spraying chemicals on crops fields. In the agriculture sector, UAVs are mostly used for cultivation, production, protection and spraying purposes. The octocopter is generally selected because of its higher stability and maximum payload carrying ability [9]. The spraying of chemicals is controlled through wireless sensors.

The World Health Organization (WHO) estimated that every year more than one million cases of deaths while spraying pesticides are registered. The World Health Organization (WHO) estimated that every year more than one million case of deaths are register with pesticides spraying and it also effect the yield of former and economy of country as well. [9].

The main objective of this research is to reduce spraying time on crops as compared to conventional spraying systems and labor cost. This also increases the efficiency of spraying.

Basically, the idea of an octocopter was presented by Joo et al. [8] but they did not implement it practically. This research is, therefore, focused on the fabrication of a low weight octocopter and its implementation in the field of agriculture. Table I shows the overall literature study related to UAV Octocopter based on Pesticides' Spraying System.

TABLE I
RESEARCH MATRIX

Reference	Researcher	Type	Research Purpose		The weight of Designed Copters
			Design Fabrication		kg
[1]	Girard et.al	Quadcopter	✓	✓	3
[2]	Penga et.al	Helicopter	✓	✓	5
[3]	Pound. et.al	Quadcopter	✓		2
[4]	Mc Kerrow et.al	Quadcopter	✓	✓	4
[5]	Casbeer et.al	Quadcopter	✓		4
[6]	Rayana et.al	Quadcopter	✓	✓	3
[7]	MIT Technology Review	Quadcopter	✓	✓	4
[8]	Joo et.al	Octocopter	✓	-	5
[9]	Gandhi et.al	Quadcopter	✓	✓	4
This Research	Shah et. al	Octocopter	-	✓	3.8

II. WORKING PRINCIPLE AND SYSTEM DESCRIPTION

The Octocopter developed during research work has eight rotor propellers in which four rotates clockwise (CW) and four counterclockwise (CCW). The eight rotors produce different thrust and octocopter hovers and moves according to these motors. Basically, one motor produces a thrust of 1400g and the total thrust produced is, therefore, 11200g. Due to eight rotors plus (+) and star (*), both configurations are used in octocopter. The Octocopters have more lifting capacity and redundancy. They are very popular in carrying heavy payloads and for higher stability. According to safety aspect if copter is high in the sky carrying some expensive camera equipment it is good to have extra protection in case of crash landings.

The system, developed during this research, consists of the mechanical and electrical structures.

A. Mechanical Structure

The mechanical structure consists of frame and propellers. Frame is the basic element for any multirotor because it holds all the components together. If there is vibration or instability in the frame the flight would not be smooth and if the frame is stable and well-designed then it would be easy to move, hover and fly a multirotor.

The frame of an octocopter should be rigid and has the capacity to reduce vibrations produced by brushless motors. In octocopters, the frame is usually large as compared to quadcopter and hexacopter. The frame of an octocopter is star shaped because of its stability

requirements [8]. Its arms are made of Aluminum rods because of its higher strength and lower cost.

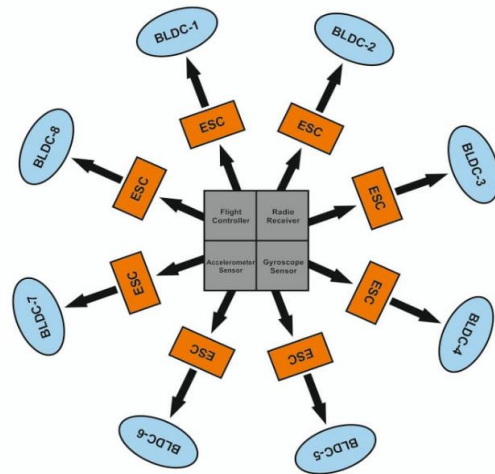


Fig. 1. The physical structure of the system.

The Aluminum is compared with steel and carbon fiber. Steel is lesser in cost but heavier in weight whereas carbon fiber is even more costly but lighter in weight. So, Aluminum is the better option for arms because of lower weight and lesser cost. The length of each rod is 38cm. Total diameter of developed octocopter is 89cm and its weight is 916g excluding electronics. Every arm is equal in length and at an angle of 45 degrees to another adjacent arm [8]. The base and motor mount are made of fiberglass because of higher stiffness and lower weight as compared to carbon fiber which is comparatively more expensive though lighter in weight. The base consists of battery pads, landing gears, controlling pads and legs.

Propellers are the type of fans which convert rotational motion into thrust. Propellers of a multirotor of quadcopter have different diameter and pitch scales; 10 (inch) * 4.7 (inch), 10 (inch) * 4.5 (inch) etc. A multirotor travel according to its pitch. An octocopter has eight propellers, four normal propellers, and four pushing propellers. For counter motor torque, octocopter use four clockwise propellers and four counterclockwise propellers. Normal propellers spin counter-clockwise and pushing propellers spin clockwise. Propellers used in the octocopter, developed here, are 10 (inch) * 4.5 (inch) which is according to the distance between its arms. 10 (inch) is the length of propeller and 4.5 (inch) is its pitch. Propellers are of low weight type made of nylon. Propellers made of nylon are of higher strength as compared to wood and carbon fiber, and also lower in cost. All the propellers used in the octocopter, developed during this research, are of the same diameter and pitch.

B. Electrical Structure

The electrical structure consists of the following components:

Transmitter and receiver having different components like the fly sky, FS-T6 transmitter with receiver, 6 channels, 2.40-2.48Hz range, 12V power DC (1.5A * 8)

and an antenna having length of 26mm. The reason of using specified transmitter is due to its higher range and ease of operation. Fly sky transmitters are lesser in cost and recommended for multirotor vehicles as shown in Fig. 2.

BLDC motor is also called electronically commutated motors as shown in Fig. 3. It is run by a DC source. It provides higher amount of torque as compared to its weight thus having: higher efficiency, lesser noise, longer battery life and higher reliability. The size of BLDC motor is compact as compared to servo and stepper motors. Also, in comparison to stepper and servo motors the BLDC motors have higher RPM, higher efficiency, lesser power consumption and produce lesser amount of vibrations. The BLDC motor used in the octocopter, developed during this research, operates at 10V or 0.4A and at 8000 rpm it produces a thrust of 1400g through propellers having dimensions of 10 (inch) * 4.5 (inch). The weight of BLDC motor used in research work is 63g.



Fig. 2. Transmitter and receiver.



Fig. 3. Brushless motor.

Electronic Speed Controller (ESC) controls the BLDC motors as shown in Fig. 4. Microcontroller gives signal to ESC that converts this signal to BLDC motor. In this octocopter 8 ESCs and 8 BLDC motors are installed. The signal's frequency of all ESCs changes by controlling them independently. The selection of ESC is dependent upon the selected according to motor discharge rate of the motor. It receives a maximum current of 40A and gives a burst current of 50A for 10s to the motors. The weight of the electronic speed controller used is 43g.



Fig. 4. Electronic speed controller.

Accelerometer is used to measure the acceleration as shown in Fig. 5. It senses the gravitational force. The octocopter, developed during this research, uses an accelerometer MPU-6050 that has different features e.g. current required for normal operating conditions is 500μA, whereas other accelerometers require different amounts of current i.e. 1.25Hz take 10μA, 5Hz take 20μA, 20Hz take 60μA, 40Hz take 110μA. In addition to current requirements the other features are signaling and orientation detection, self-testing by a user, integrates 16-bit ADC and sits full-scale range is ±2g, ±4g, ±8g, ±16g. The MPU-6050 works in 6-9 axis, the input voltage is very low 2.3V-3.4V and also have a digital output sensor.

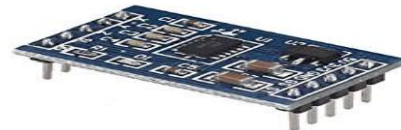


Fig. 5. Accelerometer sensor.

Gyroscope sensor is used to increase the stability of multi-rotors. It controls the angular velocity and angular speed. The gyro-sensor, used in the octocopter, is having a size of 36×36×11.5mm, weight of 8.6g, input voltage of 4.8-6.0V and receiver signal of 1520us (5 channels). It is used for increased stability in every axis.

The battery used here is Lipo (5000mAh-40C) which is lithium based and is therefore mostly preferred as shown in Fig. 6. Its energy to weight ratio is higher as compared to other types. However, before using, it should be charged properly. The battery used here has the capacity of 5000mAh with a discharge rate of 25. The weight of this Lipo battery is 520g. It gives a power output of 88 watts per hour. While operating eight BLDC motors, the 5000mAh battery takes about 15 minutes to completely discharge.



Fig. 6. Lipo battery.

III. SOFTWARE DESCRIPTION

Arduino with open source software is used for controlling the speed and direction of the motors in the developed octocopter as shown in Fig. 7.

Arduino is a microcontroller-based kit which basically is used for communications and controlling/operating many devices.

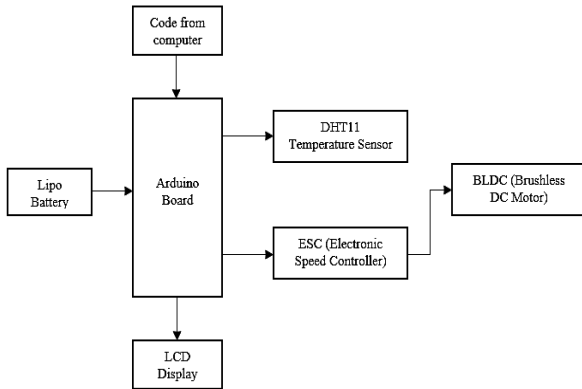


Fig. 7. Block diagram to control the motor speed.

It consists of two memories - program memory and the data memory. The code is stored in the flash program memory, whereas, the data is stored in the data memory. When the program starts it gets information, saved in the program memory, and then compiles it and gives a useful response or re-evaluates it. An LCD display, attached to the Arduino board, displays temperature and variations in speed of DC motor with respect to temperature. Temperature sensor (DHT11) is used to detect the temperature and humidity of the surroundings and display it on the LCD. DHT11 uses the temperature difference to produce a voltage signal which is processed by the Arduino to give a digital output displaying the temperature of the given surrounding. An ESC, connected with the Arduino board, is used to control the speed of the Brushless DC motor.

IV. PESTICIDES SPRAYING MECHANISM

The mechanism, developed here, uses a tank that has a capacity of 3000 ml and connected to a 12V DC pump. The pump is further connected to the battery. Also, a set of mini nozzles (switchable) is also fitted with the end pipes. Different nozzles are used for different crops, so it can be changed as per requirement. When the pump is operated, spray starts. To avoid wastage of pesticides, a balanced pressure is maintained throughout.

V. FABRICATION PROCESS

The main components used in fabrication are brushless direct current motors. The other component used is electronic speed controller (ESC), which generates different signals of high frequency. ESC is controlled to keep the motors running. It increases the power to supply enough current to motors. Aluminum rods of 38 cm are used in the developed octocopter structure. The Aluminum

is selected because of its higher strength and durability. These Aluminum rods are attached to the fiberglass frame. The total width of octocopter is 89 cm, height from the ground is 21cm and its weight is 915g. The next component used is an accelerometer sensor which measures the acceleration and gravity. It also senses the device's orientation. The other component used is the gyroscope sensor which measures the rotational speed around the 3 axes. It also controls the device and keeps the drone safe. Basically, it provides a smooth flight. Finally, a lipo battery is connected that consists of different cells (1-10) 4S1P, commonly suitable for octocopter which supplies the power of 14.8V.

MENG JOO [8] described that star shape configuration of an octocopter is best to carry a maximum amount of payload. Keeping that in view, a star-shaped octocopter is fabricated to carry maximum payload, as shown in Fig. 8. Basic task is to spray on a one-acre field with the help of the octocopter. To evaluate its power and performance three tests are carried out, one ground test and two flight tests. The ground test is to verify the stability of the software system, evaluate the endurance of the power system and the wireless communication range.

VI. TESTING OF DEVELOPED SYSTEM

During the ground test motors are governed to normal RPM and propellers are not installed for safety consideration. After the ground test, two flight tests are conducted to evaluate the power and performance of octocopter. The test of UAV is manually controlled by a skilled pilot. In both flight tests, the target of spraying in one-acre field is achieved easily which showed that the star-shaped design is best in all aspects of increasing payload, performance, and stability. At a minimum and maximum speed, the test results are shown in Table II.

TABLE II
TEST RESULTS

Speed (m/s)	Coverage of sprayer (m)	Coverage Rate (acre/ hour)	
		CR	Average CR
4.71	1.5	6.32	6.235
		6.15	
18.8	1.5	23.25	24.18
		25.11	



Fig. 8. Octocopter fabricated structure.

After the flight tests, it is determined that during a minimum speed of 4.71 m/s the coverage rate is 6.235 acres/hr and at a maximum speed of 18.8 m/s, the 24.18 acres of land is sprayed in 1 hour. After these results, it is cleared that 1 acre of land can be sprayed easily within 2.5 minutes.

VII. CONCLUSION

This research work has introduced the use of UAVs in the field of agriculture for the purpose of spraying pesticides on crops spread over large area of land. Based on this idea the use of UAVs can also be expanded to other aspects of life like: military, firefighting, sports etc. Another point, which can broadly be concluded from this research, is that the use of octocopter can actually save human lives by keeping them away from the harmful effects of chemicals and pesticides. By using UAVs, crops in the fields can also be saved by the beating they normally receive when the same task is completed with the help of heavier machines driven by tractors. Developed system also shows that the octocopter is useful in reducing the spraying time and labor cost when implemented in the field of agriculture.

The flight time of an octocopter is about 10 minutes on lipo battery. For future work, lipo can be replaced by a solar power to increase the timing of octocopter. The spraying of pesticides depends on the number of pesticides to be sprayed. In future, the spraying UAVs can be controlled with the help of Android mobiles. By using universal nozzles powder pesticides can also be sprayed. Carrying of the payload can be increased by changing material of UAVs.

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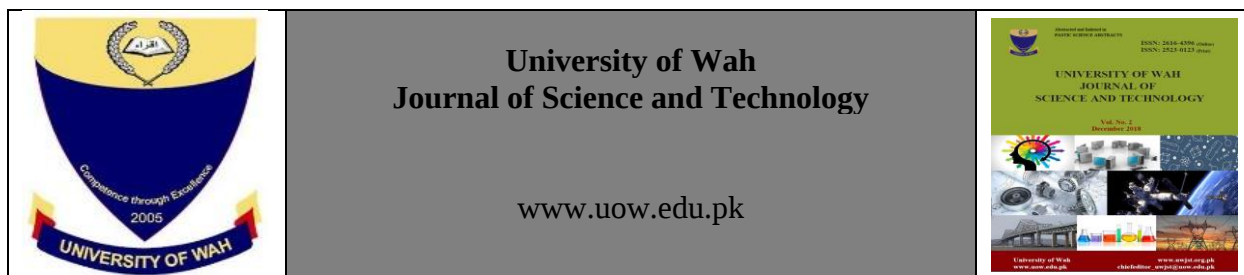
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Determination of Antimicrobial Activity of Mulberry Fruit Extracts against Water-borne Microbial Pathogens Isolated from Different Water Samples

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Abstract—Mulberry (*Morus* sp.) is being used worldwide due to its nutritional values and medicinal properties. In the current study, antimicrobial activities of Ripened and Un-Ripened Black and White mulberry fruit extracts are tested against microbial pathogens isolated from different water samples. Aqueous fruit extracts show significant antimicrobial activity with 35mm zone of inhibition by Ripened White Toot (RWT) against *V. cholera* and 32mm zone of inhibition by Un-Ripened Black Toot (URBT) against *M. luteus*. All mulberry extracts are considerably active against *E. coli* and *B. thuringiensis*. Present findings indicate that mulberry fruit extracts have medicinal potential and will be an excellent choice for the development of alternative antimicrobial drug.

Index Terms—Antimicrobial activity, antibiotics, *Morus nigra* L., *Morus alba* L., bacterial strains.

I. INTRODUCTION

PLANTS have been used as crude drugs and healing agents since pre-historic times [1]. Around 25% of modern medicines are acquired from plants [2]. Almost 65-80% of the world population of developing countries depends on plants for their medicinal needs [3-4]. In flourished countries the preparations of herbal medicines are popular. Recommendation for registration of such medicines exists in developed countries including Germany, France, Italy and the United States [6].

Medicinal plants contain synergistic combinations of active compounds which can be used against common pathogens with low or no side effects. In Pakistan more than 500 species of flowering plants are used in traditional medicine [7-9]. The numbers of plant species which have been used worldwide for medicinal purposes are approximately more than 50,000 [10]. In the past years, worldwide the mass production and growth of chemically synthesized drugs have revolutionized health care. However, in developing countries large portions of the people still depends upon herbal medicines and traditional practitioners for their health concerns. Ninety percent of population in Africa and up to 70% in India relies on traditional medicine. In China, traditional medicine accounts for around 40% of all health care delivered and more than 90% of general hospitals have units for traditional medicine [11].

Due to the hot and humid environment in the region, our local environment remains on the great risk of water-borne diseases. The diseases associated with the contamination of water are believed to be transmitted through water borne pathogens. The widespread water borne infectious diseases caused by protozoan, bacteria and viruses includes diarrheal diseases, Amoebiasis, Microsporidiosis, Giardiasis, Cyclosporiasis, Cryptosporidiosis, *E. coli* Infection, Cholera, Typhoid fever, Leptospirosis, Dysentery, Salmonellosis, Legionellosis, *M. marinum* infection, Campylobacteriosis, Botulism, Vibrio Illness, Otitis Externa, SARS, Hepatitis A, *Poliomyelitis* (Polio) and *Polyomavirus* infection [12-15].

Many efforts have been made for the development of new antibiotics against resistance pathogens [16-17]. Bacteria resistant to antibiotics are actively increasing in aquatic environment. In general, physicians are using wide range of antibiotics for treatment against infections. The noticeable reason is that most of the bacterial strains are

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now become resistant to one or more of antibiotics and some may be resistant to all available antibiotics [18].

Mulberry plant (commonly called Toot in local language) is one of conventional herbs which are used in medicine due to its chemical composition and pharmacological function. *Morus alba* L. (white mulberry) is native to Eastern and Central China, Japan, and India. It has a long history of medicinal use in Chinese herbal medicine. According to Chinese pharmacopoeia the leaves, stems, barks, roots and fruits of *M. alba* L. were active ingredients in medicinal preparations [19].

Morus nigra L. (black mulberry) is native to Western Asia. It is widely spread through-out all regions from the tropics to the sub-arctic and from sea level to altitudes as high as 4000 m [20]. The fruits of black mulberry are famous for their flavor and nutritional qualities particularly due to carotene [21]. In different parts of the world, this is used as vegetable and is cultivated for fruit production in European countries. The leaves of mulberry are used as infusion in Asian countries (most common in Japan and Korea). Leaves of Mulberry are also used as powder, tea and juice in Japan [22].

In recent years, several studies have been carried out for the treatment of bacterial infections through alternative drugs. The current study is the assessment of antibacterial properties of *Morus alba* L. and *Morus nigra* L. against pathogenic bacterial strains isolated from different water samples. There is a great potential in this area of research because it will contribute to promote the commercial use of these plants as antimicrobial agent.

II. MATERIALS AND METHODS

A. Collection of Water Samples

Water samples are collected in sterilized falcon tubes (50 ml) from different areas of Punjab which includes Attock City, Kala Chitta Pahar, Gulistan Colony, Officer's Colony Wah Cantt and Taxila.

B. Determination of Different Properties of Water Samples

Water qualities like type of water, pH, optical density and bacterial count are determined. For the growth of bacterial culture, nutrient agar media (Oxoid) is used. One ml of each collected water sample is spread with the help of sterilized glass spreader. Same process is followed for each sample and incubated the plates overnight at 37 °C using Electro-thermal Incubator (DNP5092, SERICO). Bacterial count (CFU/ml) is determined by means of the surface viable counting Technique [23].

C. Screening of Bacterial Colonies

Streak plate method is used to isolate and purify bacterial cultures obtained from tested water samples. The streaking method is repeated until pure isolated colonies are obtained.

The Gram's staining and standard biochemical tests including coagulase, urease, catalase, oxidase, citrate utilization, sugar fermentation, indole and motility test are



Fig.1. Ripened fruit of *M. nigra* L. (RBT).

performed for the phenotypic identification of isolated bacterial strains [24-29].

D. Antibiotic Sensitivity by Disc Diffusion Method

Sensitivity and resistance of identified bacteria is checked against standard antibiotics such as Ciprofloxacin 5 µg (CIP5), Gentamicin 10 µg (CN10), Kanamycin 30 µg (K30), Chloramphenicol 10 µg (C10), Sulphamethoxazole-trimethoprim 25 µg (SXT20), Cephalexin 30 µg (CL30), Cefotaxime 30 µg (CTX30), Ofloxacin 5 µg (OFX5), Ampicillin 10 µg (AMP10), Tetracyclin 30 µg (TE30) and Ceftazidime 30 µg (CAZ30) by disc diffusion method.

E. Preparation of Plant Extracts

The fresh fruits of *Morus* sp. are collected from the University Campus. Fresh fruits are washed with distilled water. 20 g of fresh fruit material is grinded with the help of mortar and pestle. 200 ml distilled water is added in the grinded fruits and incubated in the shaking incubator at 100 rpm for 8 hours. The aqueous extracts are then filtered through Watman No. 1 (Schleicher and Schuell 125 mm Cat No. 1001 125) filter paper. The different types of Mulberry fruit extracts i.e. Ripened Black Toot (RBT) as shown in Fig. 1, Un Ripened Black Toot (URBT), Ripened White Toot (RWT) as shown in Fig. 2 and Un Ripened White Toot (URWT) are prepared and passed through bacterial filter for sterilization.

F. Antimicrobial Activity of Plant Extracts by well Diffusion Method

To determine the antimicrobial activity the well diffusion assay for RBT, URBT, RWT, and URWT is performed on Muller Hinton agar plates. 40 µl of each fruit extract was poured in respective well under sterile conditions. Plates are incubated at 37 °C for 24 hours. Zones of inhibition for each bacterial sample are determined.

In addition, the antimicrobial properties of mulberry extracts are further evaluated by determining the growth curves of bacterial isolates in presence and absence of the extracts. For this purpose optical density of bacterial cultures is measured at 600 nm by using spectrophotometer at different time intervals ranges between 2 - 26 hours.

III. RESULTS AND DISCUSSION

All the water samples are soft water having pH ranges 7.0 – 8.5. Different properties of these water samples are summarized in Table I.

TABLE I
SUMMARY OF DIFFERENT PROPERTIES OF WATER SAMPLES

Sampling Site	pH	*OD at 600nm	Density g/ml	Bacterial count **CFU/ml
Officer's colony	8.53	0.050	2.27	32
Taxila	7.50	0.045	2.31	960
Gulistan	8.25	0.049	2.32	800
Kala Pahar	8.36	0.050	2.31	228
Attock	8.28	0.046	2.32	52
Chitta Pahar	7.18	0.049	2.37	1200

*OD: Optical Density; **CFU: Colony Forming Units

Sample analysis shows the presence of wide range of bacteria. Total 65 bacterial colonies are isolated and purified for further investigations. Bacterial identification results confirm the presence of *Clostridium* sp., *Listeria monocytogene*, *Legionella pneumophila*, *Micrococcus luteus*, *Proteus mirabilis*, *Serratia* sp., *Aeromonas hydrophila*, *E. coli*, *Corneibacterium* sp., *Salmonella enteric*, *Bacillus subtilis*, *Moraxella catarrhalis*, *Bacillus megaterium*, *Bacillus thuringiensis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Actinomyces* sp., *Vibrio cholera*, *Bacillus anthracis*, *Salmonella typhi* and *Streptococcus pneumonia*.

Antimicrobial susceptibility of isolated bacterial strains against commercially available sterilized antibiotic discs (CIP5, CN10, K30, C10, SXT20, CL30, CTX30, OFX5, AMP10, TE30 and CAZ30) is determined by disc diffusion method. The zone of inhibition is interpreted according to CLSI [30]. The *Clostridium* sp. and *M. luteus* show resistance against SXT20, CAZ30, CTX30 and AMP10. The *L. monocytogene* is resistant against SXT20 and OFX5. The *L. pneumophila* is resistant against C10, SXT20, CTX30 and AMP10. *Proteus mirabilis* show resistance against SXT20 and AMP10. The *Serratia* sp. shows resistance against SXT20. The *A. hydrophila* is resistant against C10, SXT20 and AMP10. *E. coli* is resistant against CAZ30. The *Corneibacterium* sp is resistant against C10, SXT20 and CAZ30. The *B. subtilis* shows resistance against SXT20. The *M. catarrhalis* and *S. pneumonia* are resistant against C10 and SXT20. The *B. megaterium* and *B. thuringiensis* are resistant against C10, SXT20 and AMP10. The *S. aureus* and *Actinomyces* sp were resistant against SXT20 and CAZ30. The *B. anthracis* shows resistance against AMP10 while *S. typhi* shows resistance against TE30 and CTX30 respectively as shown in Table II.

In the present study RWT and URBT extracts shows maximum antimicrobial activities with 35 mm zone of inhibition against *V. cholera* and 32 mm against *M. luteus*, respectively. All fruits extract of mulberry (URBT, RBT, URWT, and RWT) show antimicrobial activity against *M. luteus*, *E. coli*, *B. subtilis*, *B. anthracis* and *B. thuringiensis*. In case of *L. pneumophila*, *P. mirabilis*, *Serratia* sp., *A.*

hydrophila, *M. catarrhalis*, *B. megaterium*, *S. pyogenes* and *S. typhi* these extracts show no antibacterial activities.

Current work show that *E. coli* is sensitive to all standard antibiotics except CAZ30. All mulberry extracts are also effective against *E. coli* and show zone of inhibition in RBT (20 mm), URBT (22 mm), RWT (19 mm) and URWT (17 mm). Antibacterial activity of mulberry extracts is even better in case of *B. thuringiensis* RBT (13 mm), URBT (23 mm), RWT (10 mm) and URWT (23 mm). The *B. thuringiensis* show resistance against standard antibiotics C10, SXT20 and AMP10 while it exhibits significant zone of inhibition against all mulberry extracts as shown in Table II and Fig. 3. URBT (32 mm) and RWT (35 mm) show greater zone of inhibition than all standard antibiotics used in this study except K30 (35 mm), OFX5 (32 mm) and TE30 (32 mm).

Present findings reveal that antimicrobial effects of mulberry fruit extracts vary in different bacterial species. Further investigations on phytochemical constitutions present in mulberry fruit extracts can determine and compare their antimicrobial properties. Antibacterial activity present in fresh fruit juice of mulberry against the Gram-positive and Gram-negative bacteria has been also observed in some previous studies. Maximum zones of inhibitions against *B. subtilis* (18.46 mm) and the minimum zone of inhibition against *E. coli* (9.98 mm) are reported in [24]. Among the Gram- positive species, *Bacillus* species show highest zones of inhibition while for Gram- negative bacteria, had higher inhibition than *S. typhimurium* or *E. coli* [31]. Current Results reveal that *E. coli* shows maximum zones of inhibition against all fruits extracts of mulberry i.e., RBT (20 mm), URBT (22 mm), RWT (19 mm), and URWT (17 mm) as shown in Fig. 3.

Growth patterns of *M. catarrhalis*, *B. megatrium*, *S. pyogenes*, *S. typhi*, *P. mirabilis*, *V. cholera*, *E.coli*, *M. luteus* and *B. thuringiensis* are studied. The growth curves show two types of patterns: mulberry fruit extracts significantly inhibited the bacterial growth of some bacteria as compared to the control while the same extract enhances the growth of other bacteria as compared to control. The difference of response in different types of bacteria might be related with species specific effect of these extracts. It is found that *M. catarrhalis* growth was inhibited in the presence of RBT mulberry extracts as compared to control as shown in Fig. 4. In case of *B. megatrium* maximum growth inhibition is observed in

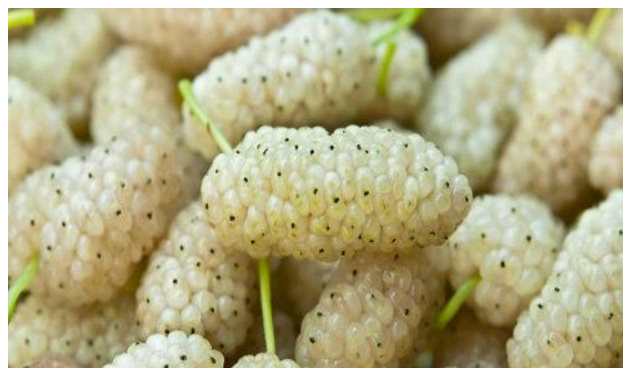


Fig.2. Ripened fruit of *M. alba* L. (RWT).

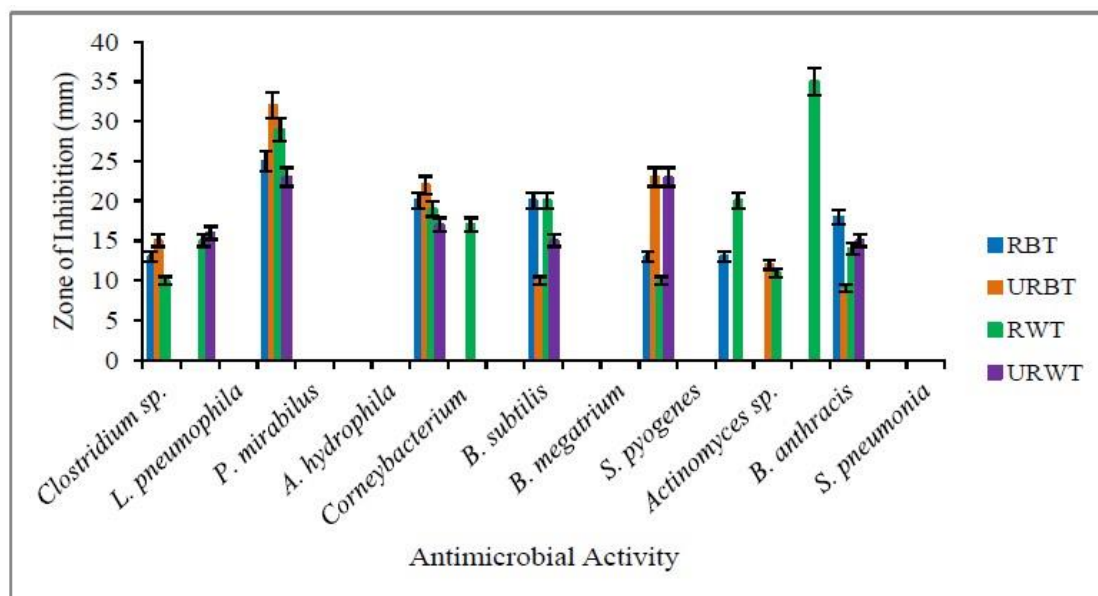
URWT while growth inhibition was minimum in case of RWT as compared to control as shown in Fig. 5. *S. pyogenes* show enhanced growth in presence of mulberry fruits extracts as compared to the control. Maximum growth is observed in URBT as depicted in Fig. 6. Growth of *S. typhi* is inhibited by almost all fruit extracts of mulberry as compared the control while RWT shows

significant growth inhibition as shown in Fig. 7. In case of *P. mirabilis* maximum growth inhibition is observed in RBT as depicted in Fig. 8 whereas in *V. cholera*, it is observed in RWT and is shown in Fig. 9. *E. coli* in the presence of URBT show maximum inhibition of growth as shown in Fig. 10. *M. luteus* also exhibits growth inhibition in all fruit extracts of mulberry but maximum inhibition in

TABLE II
SUMMARY OF ANTIBIOTIC SENSITIVITY OF BACTERIAL ISOLATES OF DIFFERENT
WATER SAMPLES WITH THEIR MEAN ZONE OF INHIBITION

Bacterial Species	Zone of inhibition (mm) Against Antibiotics										
	CIP 5	CN 10	K 30	TE 30	C 10	SXT 25	CAZ 30	CL 30	CTX 30	OFX 5	AMP 10
<i>Clostridium sp.</i>	27	15	16	28	15	0	0	16	0	17	0
<i>L. monocytogens</i>	26	27	16	17	13	0	19	22	21	0	13
<i>L. pneumophila</i>	20	10	20	16	0	0	0	22	10	19	0
<i>M. luteus</i>	25	14	15	25	16	0	0	16	0	17	0
<i>P. mirabilis</i>	18	14	16	20	15	0	20	19	10	24	0
<i>Serratia sp.</i>	18	10	16	10	20	0	25	23	9	23	13
<i>A. hydrophila</i>	20	10	24	15	0	0	22	19	10	22	0
<i>E. coli</i>	25	13	16	14	16	24	0	25	12	27	29
<i>Cornebacterium</i>	24	17	20	18	0	0	0	24	14	21	9
<i>S. enteric</i>	30	15	16	26	20	23	20	23	9	23	13
<i>B. subtilis</i>	26	10	23	15	15	0	20	26	17	26	25
<i>M. catarrhalis</i>	16	15	17	19	0	0	25	30	26	17	33
<i>B. megatrium</i>	20	10	24	13	0	0	21	19	10	22	0
<i>B. thuringiensis</i>	21	10	22	14	0	0	24	21	11	26	0
<i>S. pyogenes</i>	20	10	15	20	11	12	12	22	15	18	20
<i>S. aureus</i>	25	14	21	16	15	0	0	19	12	20	10
<i>Actinomyces sp.</i>	12	12	15	10	11	0	0	20	15	20	10
<i>V. cholera</i>	30	25	35	32	25	15	20	25	32	35	15
<i>B. anthracis</i>	11	15	18	20	23	16	15	25	30	20	0
<i>S. typhi</i>	22	16	13	0	10	9	14	16	0	19	23
<i>S. pneumonia</i>	20	15	10	10	0	0	20	25	18	24	17

Abbreviations: CIP5: Ciprofloxacin 5 µg, CN10: Gentamicin 10 µg, K30: Kanamycin 30 µg, TE30: Tetracyclin 30 µg, C10: Chloramphenicol 10 µg, SXT20: Sulphamethoxazole-trimethoprim 25 µg, CAZ30: Ceftazidime 30 µg, CL30: Cephalixin 30 µg, CTX30: Cefotaxime 30 µg, OFX5: Ofloxacin 5 µg and AMP10: Ampicillin 10 µg.



Abbreviations: Ripened black toot (RBT), Un-ripened Black Toot (URBT), Ripened White Toot (RWT) and Un-Ripened White Toot (URWT)

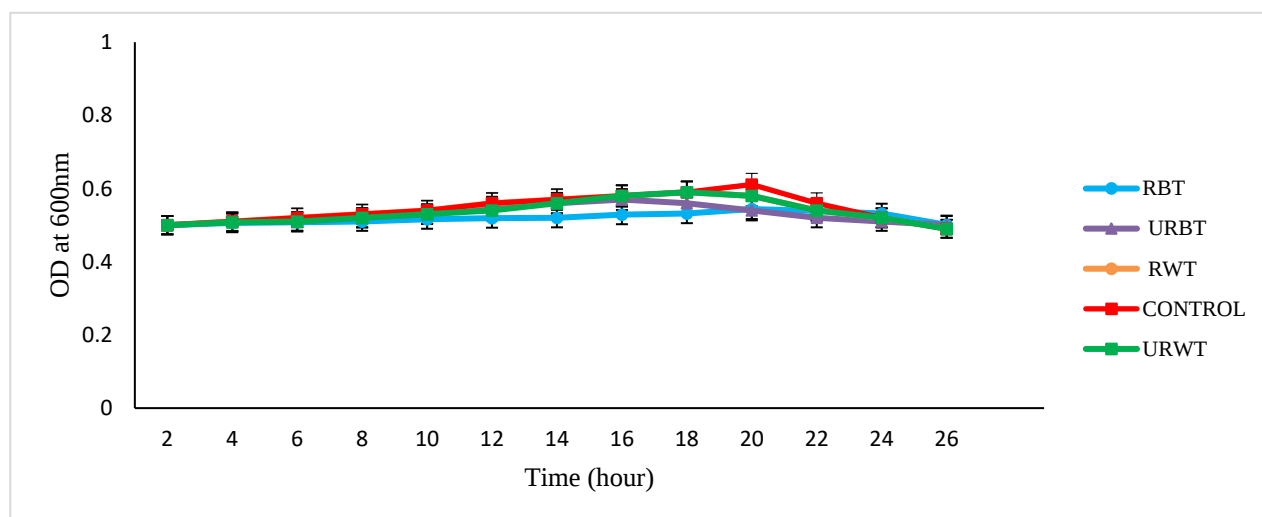
Fig.3. Antimicrobial activities of mulberry fruit extracts against isolated bacterial strains.

growth is found in presence of RBT as depicted in Fig. 11. *B. thuringiensis* growth is inhibited when grown with URBT as compared to the control as shown in Fig. 12.

The mulberry extracts are known to have many active ingredients which inhibit bacterial growth. The phytochemistry, pharmacologically active constituents and nutritional profile of *M. alba* revealed its importance. It is studied in [32] that mulberry extracts are rich in phytochemicals and have antimicrobial properties against harmful pathogens. Kuwanon G (purified from methanolic extract of *M. alba*) showed antimicrobial activity against dental caries associated *Streptococcus mutans* [32]. Mulberries also have antimicrobial chemicals such as kuwanon C, mulberrofurin G and albanol B [33]. It is also reported in [34-35] that phyto-constituents isolated from the aqueous and ethanolic extract of *M. alba* have antibacterial and antifungal activities against oral pathogens such as *Streptococcus mutan*. These findings along with present work indicate that Mulberries extracts have antimicrobial properties against various bacterial species.

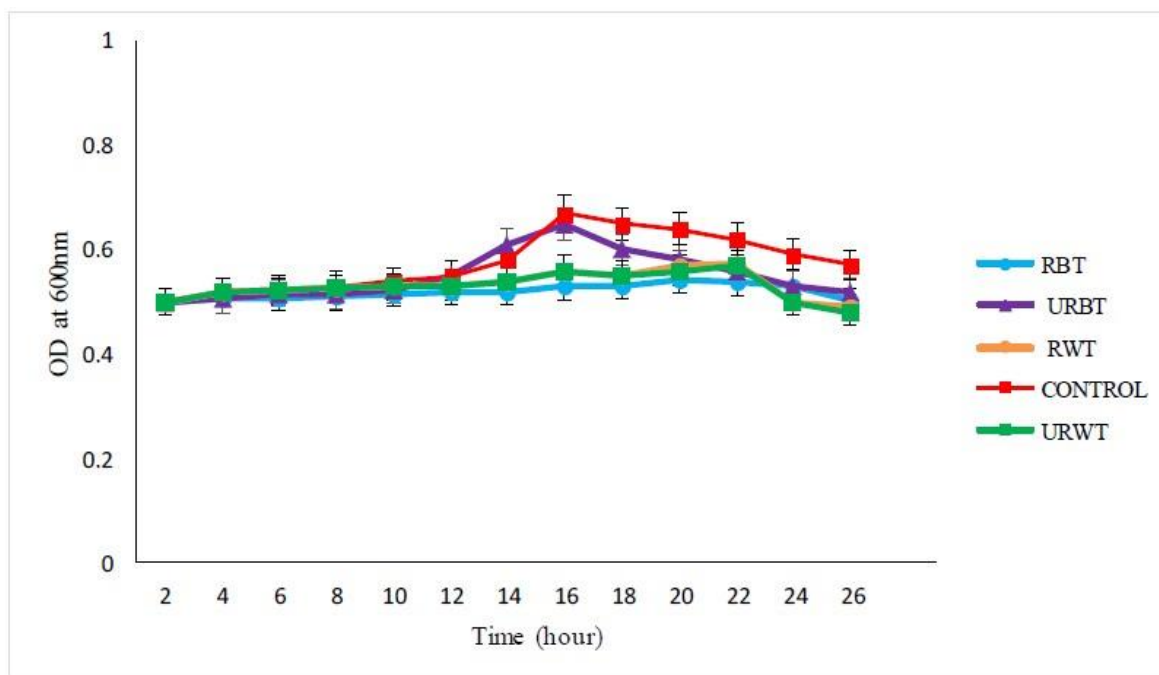
IV. CONCLUSION

The search of bioactive compounds from plants is considered to be the important paramount to control diseases caused by water-borne pathogens. Presented results indicate that mulberry fruit extracts are effective against *M. Luteus*, *E. coli* and *B. thuringiensis*. Previous studies reported that mulberry fruit extracts contain high phenolic contents, amino acids, vitamins, flavonoids, steroids, tri terpenes and other trace elements [36-37]. In the light of current findings it is concluded that mulberry fruits extracts have a great potential for future discovery of potent antimicrobial agents of plant origin. There is a great potential in this area of research because it would contribute to promote the commercial use of these plants.



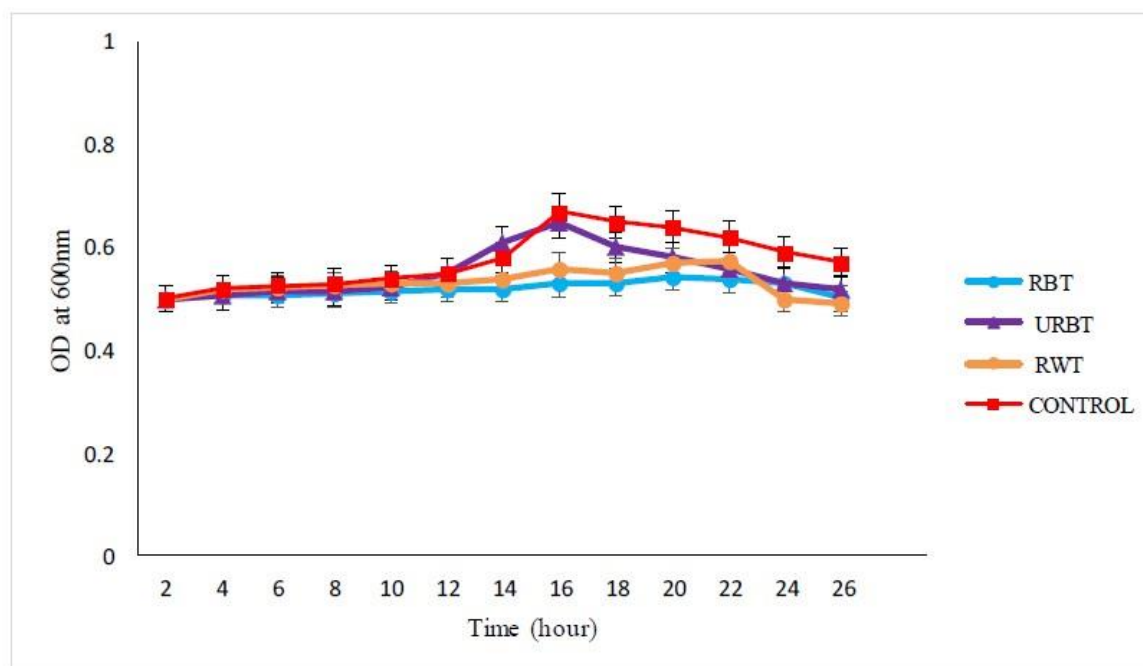
Abbreviations: Ripened black toot (RBT), Un-ripened Black Toot (URBT), Ripened White Toot (RWT), Un-Ripened White Toot (URWT), Optical Density (OD)

Fig. 4. Growth pattern of *M. catarrhalis* against mulberry fruit extracts.



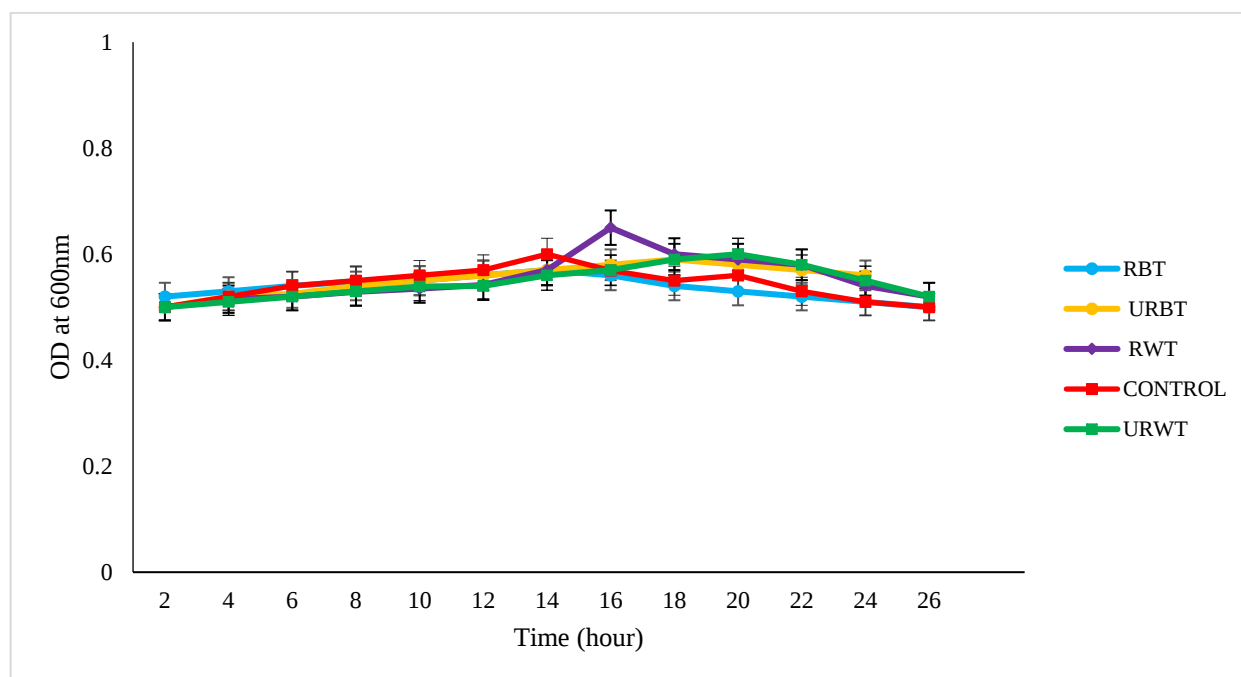
Abbreviations: Ripened black toot (RBT), Un-ripened Black Toot (URBT), Ripened White Toot (RWT), Un-Ripened White Toot (URWT), Optical Density (OD)

Fig. 5. Growth pattern of *B. megatrium* against mulberry fruit extracts.



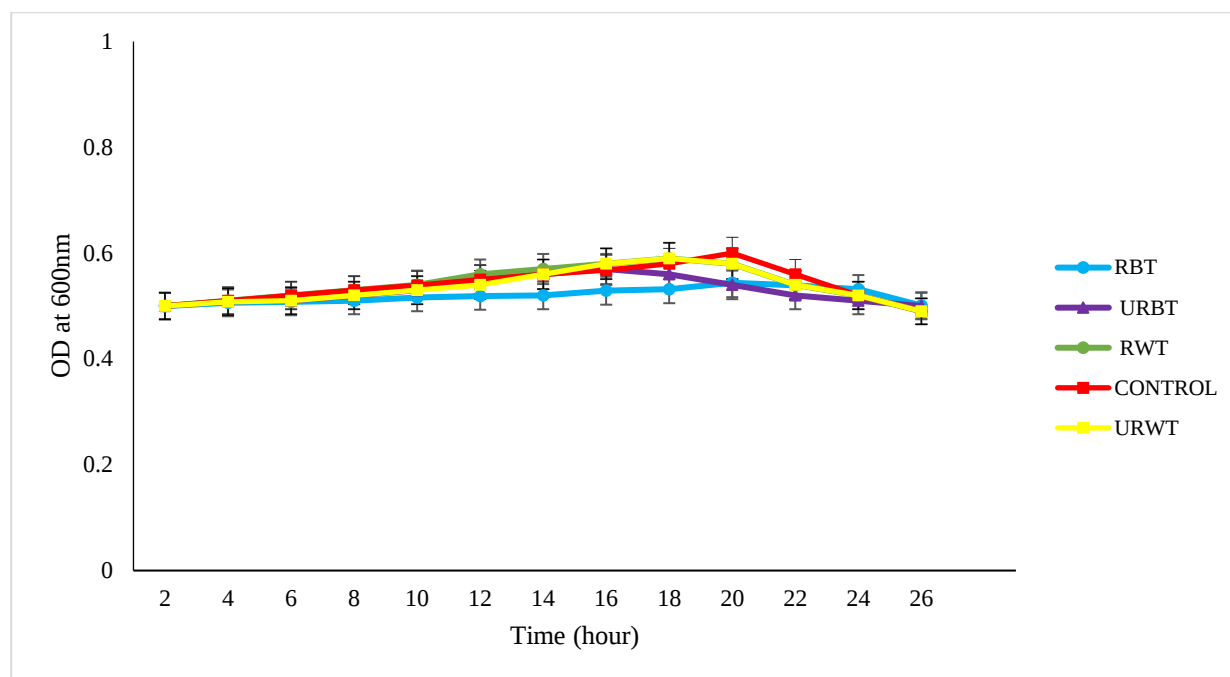
Abbreviations: Ripened black toot (RBT), Un-ripened Black Toot (URBT), Ripened White Toot (RWT), Optical Density (OD)

Fig. 6. Growth pattern of isolated *S. pyogenes* against mulberry fruit extracts.



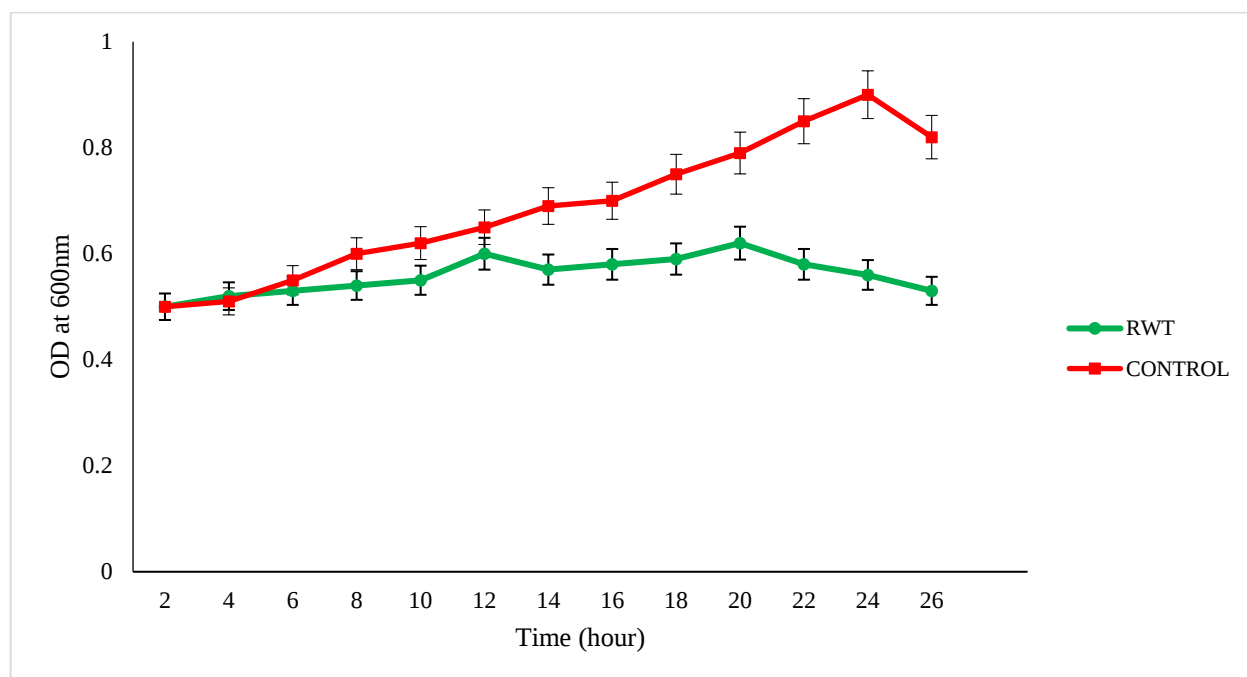
Abbreviations: Ripened black toot (RBT), Un-ripened Black Toot (URBT), Ripened White Toot (RWT), Un-Ripened White Toot (URWT), Optical Density (OD)

Fig. 7. Growth pattern of isolated *S. typhi* against mulberry fruit extracts.



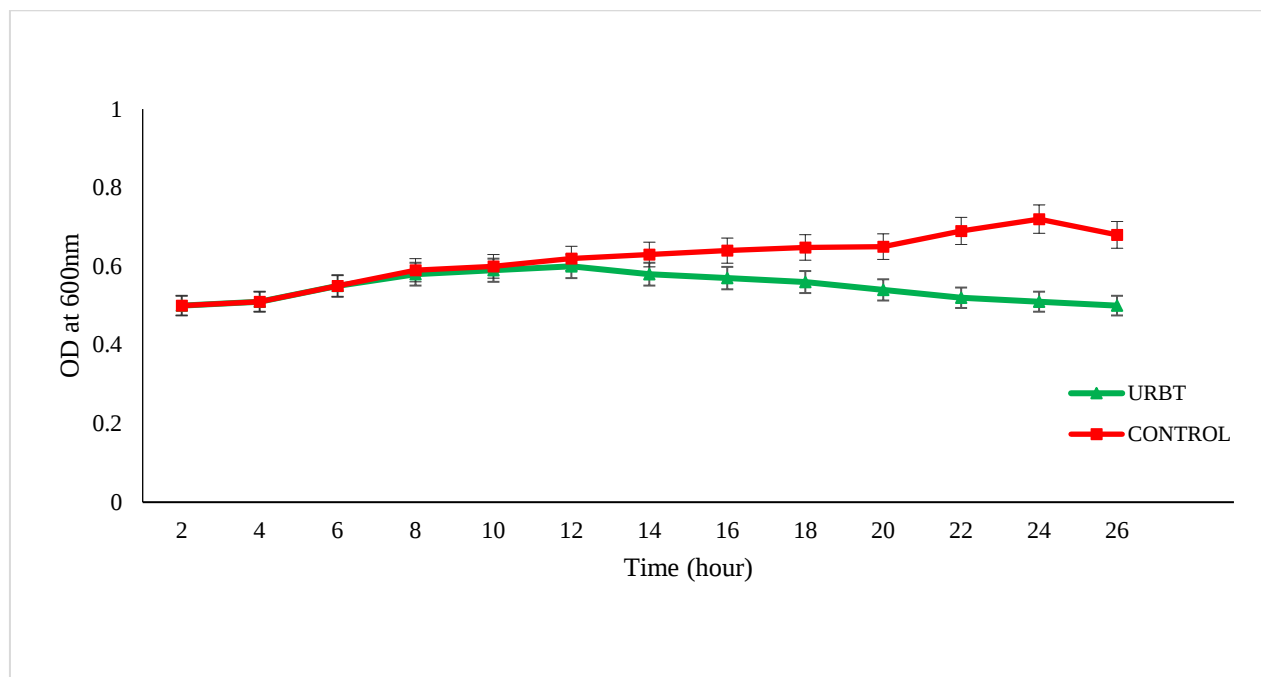
Abbreviations: Ripened black toot (RBT), Un-ripened Black Toot (URBT), Ripened White Toot (RWT), Un-Ripened White Toot (URWT), Optical Density (OD)

Fig. 8. Growth pattern of isolated *P. mirabilis* against mulberry fruit extracts.



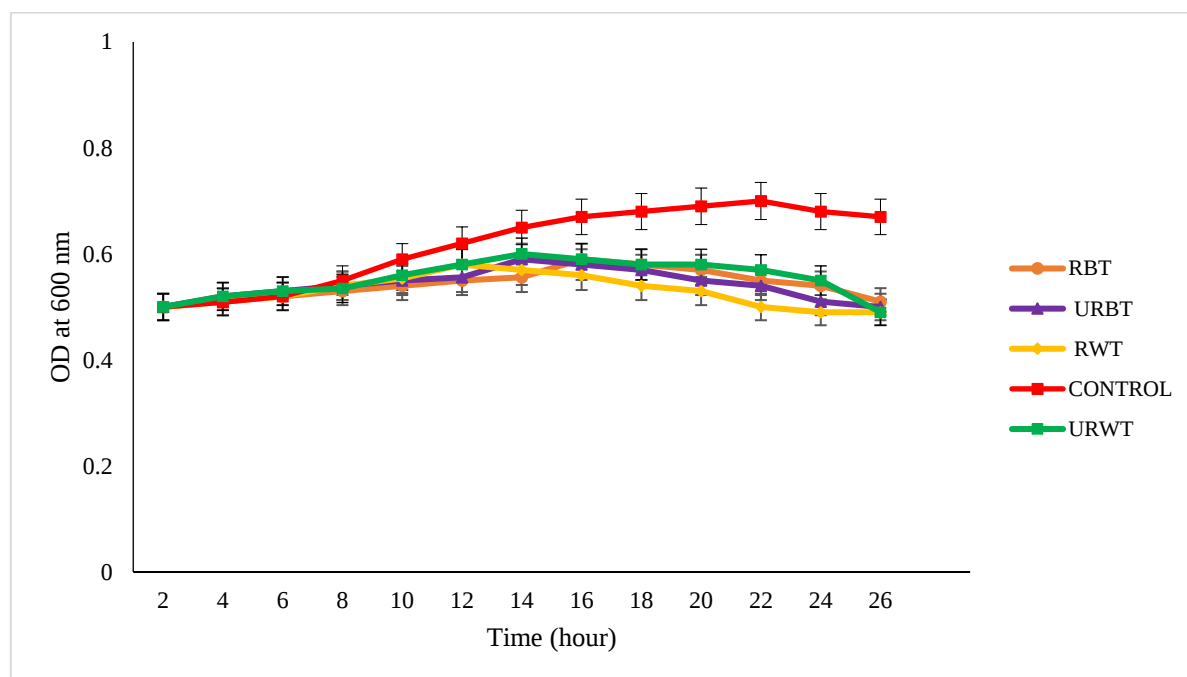
Abbreviations: Ripened White Toot (RWT), Optical Density (OD)

Fig. 9. Growth pattern of *V. cholera* against mulberry fruit extracts.



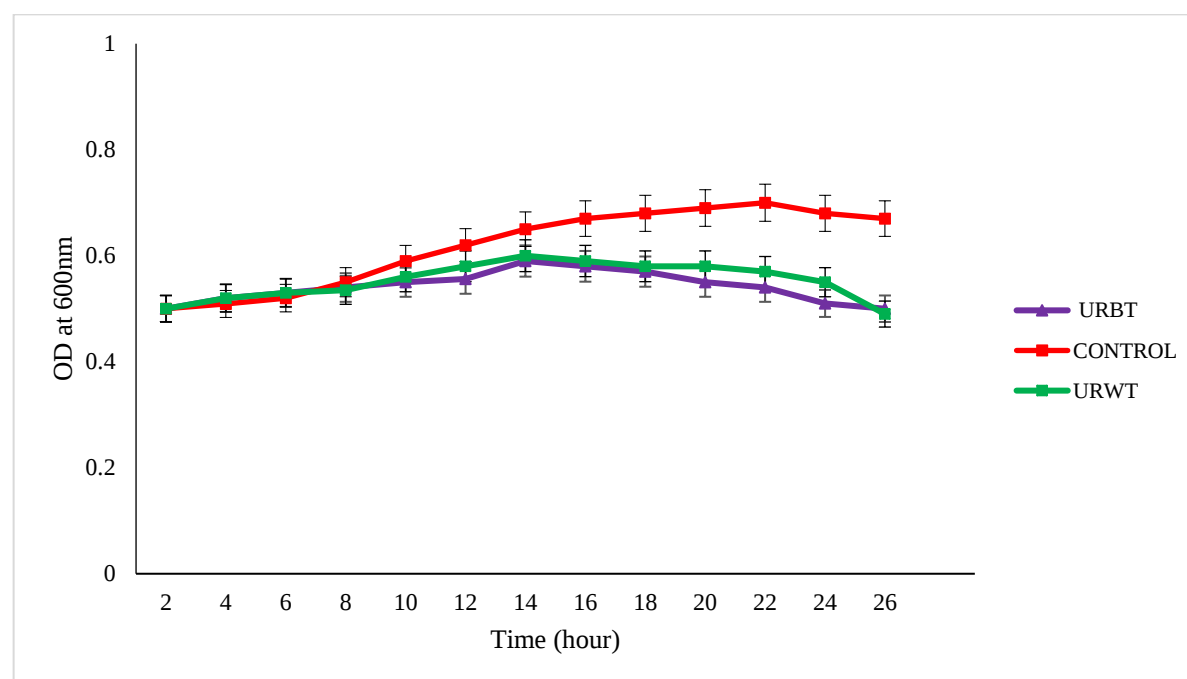
Abbreviations: Un-ripened Black Toot (URBT), Optical Density (OD)

Fig. 10. Growth pattern of *E. coli* against mulberry fruit extracts.



Abbreviations: Ripened black toot (RBT), Un-ripened Black Toot (URBT), Ripened White Toot (RWT), Un-Ripened White Toot (URWT), Optical Density (OD)

Fig. 11. Growth pattern of *M. luteus* against mulberry fruit extracts.



Abbreviations: Un-ripened Black Toot (URBT), Un-Ripened White Toot (URWT), Optical Density (OD)

Fig. 12. Growth pattern of *B. thuringiensis* against mulberry fruit extracts.

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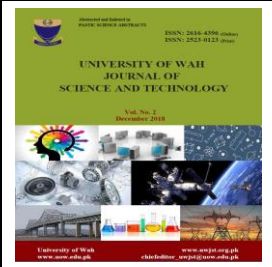


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Top Quark Mass Measurement at TEVATRON and LHC Energies

Fakhra Ghafoor, Nameeqa Firdous, Fatima Ali and Wajeeha Khalid

Abstract—This research work provides a review of a well heaviest known quark i.e. Top Quark (a fundamental particle ever observed). In hadron collisions, it produces in particle-antiparticle combination, which provides an important probe of the strong interactions i.e. one of the four fundamental interactions. Top quark was discovered in 1995 at Fermi lab, with a mass that is about as heavy as an entire atom of gold, still remains a topic of intense research interest. The top quark mass is one of the key parameter of SM of Particle Physics. Its exact value effects directly to the key predictions of the SM including the production rates of top at the Large Hadron Collider (LHC). This study presents an overview of the top quark mass measurement at TEVATRON to LHC including the precise measurement of the top given by CMS (Compact Muon Solenoid) experiment at LHC.

Index Terms—Standard Model (SM), Hadronization, Jet Scale Factor (JSF), Parton Distribution Function (PDF), Luminosity, Transfer Function, Top Quark, Compact Muon Solenoid (CMS).

I. INTRODUCTION

IN Standard Model, Top Quark is known to be heaviest fundamental particle so far. It possesses unique features. In addition, its short lifetime makes it worth studying. Observed lifetime is $\sim 10^{-25}$ sec which is much shorter than the hadronization time. Top quark is also termed as bare quark as it does not exist in bound states due to its short lifetime and decays via W boson and a b quark in very short span.

During Top Quark Run I, proton-antiproton ($p\bar{p}$) collision had been placed at 1.8 TeV. In 1995, CDF and D0

experiments discovered the top quark with 67pb^{-1} and 50pb^{-1} of integrated luminosity, respectively [1-4].

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In 2001, Run II started with 1.96 TeV, $p\bar{p}$ collisions with provision of approximately 10.5fb^{-1} of integrated luminosity. It also leads us towards Beyond Standard Model (BSM). In Past, different simulation techniques have been developed for mass measurement of top quark.

Whilst, Large Hadron Collider (LHC) at CERN is also known as top factory where proton proton (pp) collisions at center of mass energy (7 TeV) has delivered already more than 3fb^{-1} of data to ATLAS and CMS (Two general purpose detectors). Most dominant processes of top quarks production are $\bar{q}q \rightarrow t\bar{t}$ and $gg \rightarrow t\bar{t}$ [5-6].

At TEVATRON, quark-antiquark annihilation is dominant with 85% of production cross section measurement. Whereas gg fusion shows less dominant process with 15% production cross section measurement. While at LHC energies, gg fusion is dominant with 80% of the production at ($\sqrt{s} = 7\text{TeV}$). Due to the high energy, the production rate of $t\bar{t}$ at LHC is much larger than at the TEVATRON [7, 8]. The large datasets at all main experiments like ATLAS and CMS enable us to measure top quark cross section production with high precision.

II. LITERATURE SURVEY

A. Production of Top Quarks

There exist many channels by which top quarks can be produced. Figs. 1 and 2 show one of the most occurring channels of hadron colliders via strong interaction in which top anti-top is produced in pair [9, 10].

Alternative production via electroweak interaction involving a W_{tb} vertex. Top quarks produced in this way are often referred to as single top quark as shown in Figs. 3 and 4, which means only one top is produced. Additionally, single top quark events have a much larger background due to their lower jet multiplicity. These two

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factors make the search for single top quark far more difficult than that of top quarks produced by the strong interaction.

Three different mechanisms have been predicted by electroweak theory for the production measurements of single top quarks are t – channel, s – channel, $|V_{ts}|$ and $|V_{td}|$ vertex that has less probability to get top quark as a result.

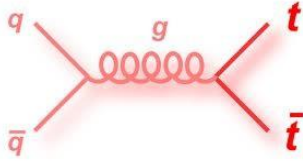


Fig. 1. Top Pair Production via quark-antiquark.

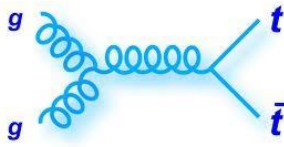


Fig. 2. Top Pair Production Via gg Fusion.

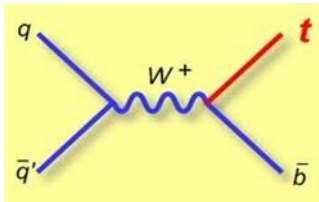


Fig. 3. (S-Channel Mode): single top quark.

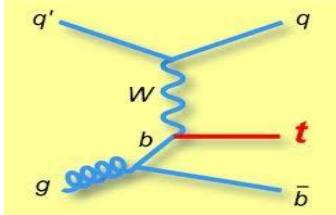


Fig. 4. (T-Channel Mode): single top quark.

B. Decay modes of Top Quark

The top disintegrates into W-boson and b-quark with nearly 100% branching fraction. There are two most familiar decay modes for $t\bar{t}$ production. One of the most important $t\bar{t} \rightarrow$ dilepton decay channel that is used in previous mass analysis at LEP as shown in Fig. 5. During this interaction, both W bosons decay via lepton decay i.e. $t\bar{t} \rightarrow W^-bW^+\bar{b} \rightarrow \bar{\nu}_l \bar{\nu}_l l^+ l^+ b\bar{b}$. Only factors that differentiate it from previous analysis are more weights assigned to more precisely measured events or that corresponds to $t\bar{t}$ signal (2). Final decay products (leptons and jets) and missing transverse momentum are hard enough to handle in identification of precise mass measurement. Also, because of leading order Matrix Element (ME) involvement to calculate the event weights.

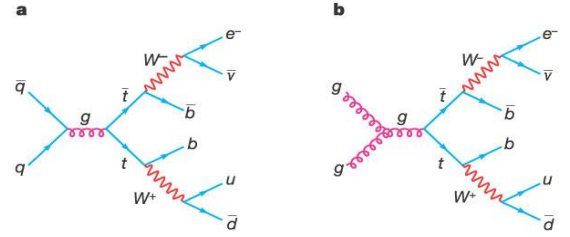


Fig. 5. Decay channels of top quark $q\bar{q}$ annihilation and gg fusion.

Alternating way for the production of top pair includes (1 lepton, one missing energy and four jets) $t\bar{t} \rightarrow \bar{\nu}_l q \bar{q} b \bar{b}$ used in ATLAS and CMS mostly.

III. RESULTS AND DISCUSSION

A. Top-quark mass measurement at CMS Detector

CMS is a general-purpose detector. Over the last years, the CMS collaboration has measured top-quark mass with high precision. All possible final products of the $t\bar{t}$ pairs are used to measure their production rate at both 7TeV and 8TeV center of mass energies. Many analyses have been carried for its mass measurement, which is explained as follows:

Top Pair channels:

- Di Lepton Channel
- SSemi Lepton Channel
- Fully Hadronic Channel

Single Top Channel:

- s-Channel
- t-Channe
- tW Associated Channel

In the Mass measurement of CMS,

$$t\bar{t} \rightarrow \text{lepton} + \text{jets} \quad (1)$$

$$t\bar{t} \rightarrow \text{alljets} \quad (2)$$

Where ideogram method is used in (1) and (2) channels and further kinematic fit is done on following decay modes:

$$t\bar{t} \rightarrow l \bar{\nu}_l q \bar{q} b \bar{b} \quad (3)$$

$$t\bar{t} \rightarrow q \bar{q} b \bar{q} b \quad (4)$$

For these ideograms, different likelihood functions are employed that depends on m_{top} and Jet Scale Factor (JSF) [11-12].

In $t\bar{t} \rightarrow$ dilepton channel, m_{top} is obtained from Matrix Element (ME) method, by reconstructing event kinematics [3].

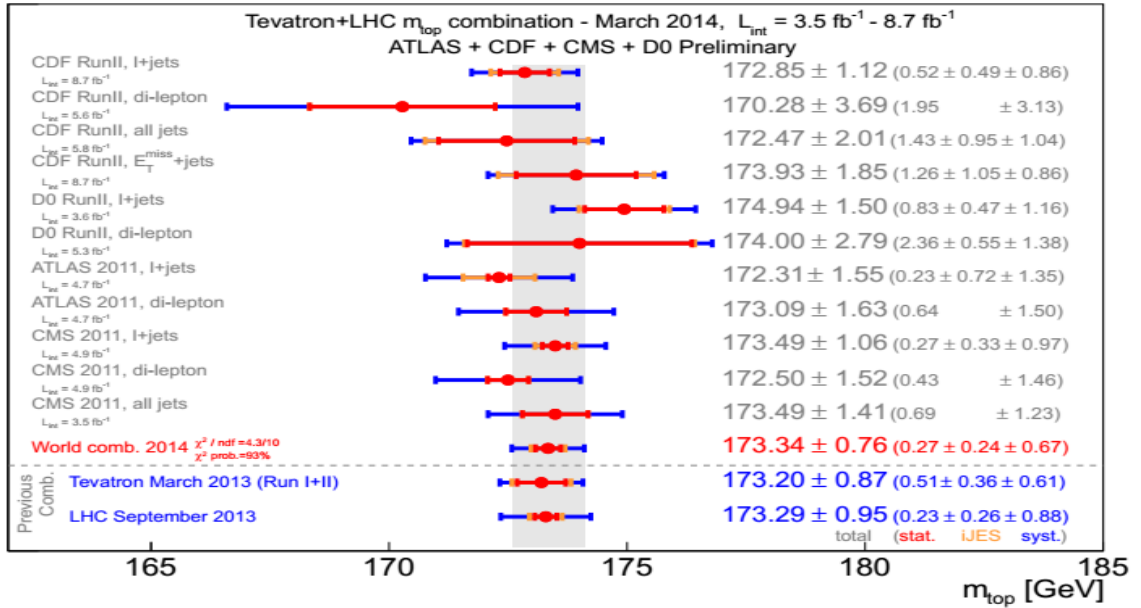


Fig. 6. Comparison of the m_t combination result with the individual m_t determinations as per $t\bar{t}$ decay channel [19].

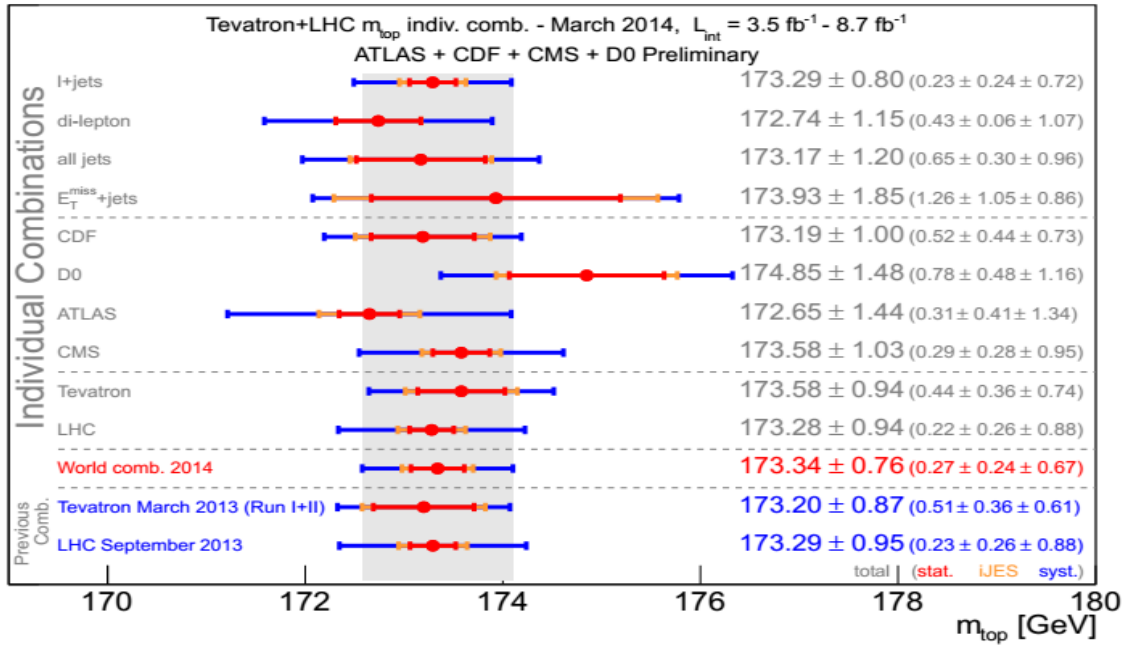


Fig. 7. Input measurements and result of their combination compared with the TEVATRON and LHC combined m_t values [19].

Di-lepton Channel which provides highly precise measurement with 2.3 fb^{-1} of data. ($<4.5\%$ uncertainty) for top quark (two W's decay into leptons) at 7TeV and constrained by jet energy scale (JES) [11].

Similarly, mass and cross section measurement for single tops depends on the CKM matrix element V_{tb} . In the t-channel, high transverse momenta top quark is produced with 4.7 fb^{-1} cross section (i.e. 9% uncertainty attained) by CMS at 7 TeV. Most recent searches include 4.8% uncertainty for V_{tb} with highly precised measurement at 8 TeV from the tW-channel. Current work is done at 95% confidence level. By applying the likelihood fit technique, CMS measures $m_{\text{top}} = 173.3 \pm 0.76 \text{ GeV}$.

B. Top-quark mass measurement at TEVATRON

TEVATRON is a hadron collider with $p\bar{p}$ collision runs at 1.96 TeV with 7.5 fb^{-1} . Most of the top produce in pair at TEVATRON collider detected by CDF and DØ Collaborations. These measurements in the equations (channels) (1) and (2) with 8.7 fb^{-1} data set have been studied [13, 14]. Similarly to reconstruct mass of Top quark, equations (1) and (2) uses data of 5.6 fb^{-1} and 5.8 fb^{-1} respectively [15, 16].

In all analyses, template method with event reconstruction uses kinematic fit so that to extract the top mass with very high precision [17, 18].

Template method picks those set of variables x_i sensitive to m_{top} which maximises the likelihood consistent with observation, whereas in matrix element method, Parton Distribution Function (PDF) is calculated on event-by-event basis by using following equation.

$$P_i(\vec{x}_1) = \frac{1}{N} \int TF(\vec{x}_1 | \vec{y}_1) d\sigma(\vec{y}_1, m_i) \quad (5)$$

TF in equation (5) is transfer function that is used by parton level quantities to map them accordingly. The reconstructed m_{top} after likelihood fit calculated by DØ collaboration at 1.96 TeV with 3.6fb^{-1} luminosity is $173.34 \pm 0.76 \text{ GeV}$ [19].

IV. CONCLUSION

The précised value of m_{top} will allow experimentalists for verification of further mathematical predictions that will provide the strong connections between the Higgs top and their intermediate particle of electro weak force i.e. W boson. Theorists are currently working to explore its more precise value that will change the predictions regarding Higgs field and its ultimate effect on exploration of universe [18]. It will also provide the framework for searching beyond standard model that will lead for our better understanding of the universe.

In future, BSM constraints derived from top physics measurements will continue to improve until 2035. Top quark studies at future facilities have the potential to deliver the transformation that this field needs to have higher energy hadron colliders so that Yukawa coupling goes to one with 0% control to systematics.

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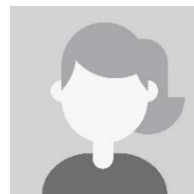
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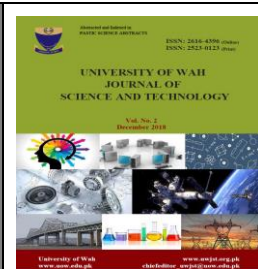


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Bioremediation of Environmental Waste: A Review

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Abstract—Nearly 1.6 billion tons of waste produced annually poses several environmental and health threats and, therefore, efficient waste disposal and management programs require urgent attention. Biodegradation and bioremediation are environmental friendly methods with reference to reduced production of toxic residues and release of trapped nutrients in the process of mineralization. The so-called ‘waste substances’ have high calorific values and huge amount of energy that is often not utilized and is discarded. This waste matter can be utilized for energy, fuel and electricity production with the help of micro-organisms. Currently, ‘Waste to Energy (WtE) Program’ is being practiced by many countries for the production of clean fuels and electricity utilizing a number of methods e.g. fermentation, anaerobic digestion, landfill gas, bio-sparging, bio augmentation, microbial fuel cells, bioventing, dendro liquid energy, etc., with the latter being most efficient of all as it is based on zero waste technology and has high efficiency. Many bacterial, fungal and other species have been reported that efficiently degrade the waste matter. In addition, genetically modified organisms are being produced for the degradation of specific kinds of wastes e.g. polymers, hydrocarbons, plastics, industrial wastes, etc. Efficient system of waste collection, recycling and reuse along with active public participation are essential for any successful waste management program. This review aims to discuss few biological methods for waste management

Index Terms—Biodegradation, Bioremediation, Microbial fuel cells, Dendro liquid energy, WtE program

I. INTRODUCTION

WASTE are the substances discarded as useless and are the byproducts of human activities that can be of solid, liquid and gas in nature. Annually, nearly 1.6 billion metric ton waste is produced globally and its management cost is expected to rise to US\$50 billion by 2025 [1]. Solid

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wastes are most hazardous as they are source of many problems e.g. health-associated risks, ground water contamination, accumulation of particulate matter, soil and air contamination and difficulty in their management and disposal, especially in developing countries [2]. Because of ever-increasing pollution, health-associated risks and economic losses on the behalf of governments and public, waste management has gained worldwide attention. Waste management refers to collection, separation, handling and disposal of waste matter in an eco-friendly way [3]. Biodegradation refers to the reduced complexity of substrate (chemical compounds mostly), mediated biologically by the action of living microorganisms [4]. The process is termed as mineralization when microbes completely reduce the substrate in the form of smaller compounds [5]. Now-a-days, waste matter is regarded as a useful material that can be re-utilized for a lot of purposes including incineration, compost formation, fertilizers etc.

II. IMPACTS OF INAPPROPRIATE WASTE DISPOSAL

The major effect of inappropriate disposal of waste matter is soil pollution, surface and ground water contamination. Such waste disposal sites serve as a breeding place for several pathogens, vectors, pests and germs. Emission of toxic gases, air pollution by waste combustion, leachates, release of methane by decomposition of waste matter, etc. are most commonly encountered precarious effects. Landfills located near populated areas pose additional health risks [6].

Inappropriate dumping of wastes affects the number and kind of micro-organisms present in that area as well as their enzymatic activities and physiochemical properties thereby disturbing soil-environment homeostasis [7]. Several types of wastes and their sources are enlisted in Table I. Clogging of drains caused by municipal solid waste generates pools of stagnant water that provide brooding sites for insects and other pathogens transmitting several diseases such as cholera, dengue etc. and causes floods in rainy season. MSW-contaminated water used for drinking, irrigation, baths, etc. increases the risk of exposure to pathogens and contaminants. According to The U.S. Public Health

Service, twenty two (22) diseases of human beings are related with inappropriate waste management including cancer, low birth weight, chemical poisoning caused by inhalation of toxic chemicals, neurological disorders, vomiting, nausea, congenital malformations, Hg toxicity, high algal populations, etc. Accumulation of heavy metals is increasing day by day in the food chains [8].

Exposure to waste matter increases the chances of cancer in liver, lung, soft tissues and testis [9]. Asthma, skin irritation, allergies, gastrointestinal diseases, are also found in people inhabiting areas near waste disposal sites [10].

III. WASTE MANAGEMENT STRATEGIES

In order to devise efficient waste management strategies, accurate assessment of quantity of different categories of waste production is the foremost necessity.

A. Waste to Energy Program

A rapidly developing waste management approach is waste to energy transformation strategy in which waste matter is utilized to produce energy and is being rapidly adopted by many countries around the globe. In 2013, the global market of waste to energy was evaluated at about \$25.32 billion with a profound increase in successive years [11]. India, Thailand and Philippines have started this program for the generation of electricity [12].

Direct combustion of waste substances generate heat that is used to drive a turbine, with 15-27% efficiency. Gasification of solid waste involves the generation of 'Syngas' i.e. combustible synthetic gas that can either be

production is captured and then utilized for industrial, domestic or commercial purposes [13].

B. Biological Management of Waste Matter

Using living organisms for the management, recycling or decomposition of waste substances is an efficient method often combined with other chemical or physical procedures. This method can be used for several materials but is most suited for management of domestic, municipal and agricultural wastes. Bioremediation can be carried out by in-situ and ex-situ means. In in situ remediation, nutrients and oxygen are supplied in the form of a solution to contaminated soil that stimulate biodegradation by naturally found bacteria. This method is generally used for soil and ground water. Intrinsic bioremediation and engineered in situ bioremediation are two types of in situ remediation. In ex situ remediation, soil excavation and ground water pumping activities facilitate biodegradation by microbes. This technique can involve solid and slurry phase systems, sub-categorized into land farming, composting, biopiles - hybrids of land farming and composting; and bioreactors, bioventing i.e. indigenous bacteria are stimulated by using wells to supply nutrients and oxygen to contaminated soil; bio-sparging i.e. rate of biological degradation is enhanced by injecting air under pressure below ground water table in order to increase oxygen concentrations; bioaugmentation-importing microbes to the site of contamination in order to enhance degradation; respectively [14]. Some of the bioremediation strategies are enlisted in Table II.

Anaerobic digestion involves the degradation of wastes

TABLE I
DIFFERENT TYPES OF WASTES AND THEIR SOURCES [13]

Source/Type		Composition
Municipal Solid Waste	Residential	Food waste, paper, cardboards, plastics, textiles, leather, yard wastes, wood, glass, metal, ashes, special wastes (e.g. bulky items, consumer electronics, white goods, batteries, oils, tyres), household hazardous wastes, e-wastes.
	Industrial	Housekeeping wastes, packaging, food wastes, wood, steel, concrete, bricks, ashes, hazardous wastes.
	Commercial and Institutional	Paper, cardboards, plastics, wood, food wastes, glass, metals, special wastes, hazardous wastes, e-wastes.
	Construction and Demolition	Wood, concrete, steel, soil, bricks, tiles, glass, plastics, insulation, hazardous wastes.
Process Waste	Municipal Services	Street sweepings, landscape and tree trimmings, sludge, wastes from recreational areas.
Medical Waste		Scrap materials, off-specification products, slag, tailings, top soil, waste rock, process water and chemicals.
Agricultural Waste		Infectious wastes (bandages, gloves, cultures, swabs, blood and bodily fluids), hazardous wastes (sharps, instruments, chemicals), radioactive wastes, pharmaceutical wastes.
		Spoiled food wastes, rice husks, cotton stalks, coconut shells, pesticides, animal excreta, soiled water, silage effluent, plastic, scrap machinery, veterinary medicines.

used to drive a steam turbine for the generation of electricity or direct generation in gas turbines or engines after further refining. This Syngas can be converted further into methane for domestic utilization and can be used as transport fuel e.g. diesel, ethanol, hydrogen, biodiesel, jet fuel, etc. after being upgraded to bio-methane and various others substances [13].

Many waste to energy plants use combined heat and power units to increase the efficiency of energy production up to 40%. Heat generated during the process of electricity

in the absence of oxygen resulting in the release of 'biogas' which is rich in methane and can be used as fuel for electricity production and contains many nutrients that are employed in fertilizers as shown in Fig.1. The duration of this procedure varies from 15 to 30 days. Bio-fertilizer can efficiently replace conventional chemical fertilizers after they have been made pathogen free. Waste water treatment is an example of this type of bioremediation [13]. The fertilizer or compost is beneficial with reference to a number of crops. Tea waste and tea bark composts were

experimentally proven to enhance the yield in 'Great Lakes 118' lettuce [16].

Fermentation involves anaerobic conversion of organic wastes to produce acids or alcohols, leaving behind a residue rich in a number of useful nutrients. Production of bioethanol using various yeast strain and substrates is an excellent example of fermentation. This process can provide an annual production of about 2-3 lac tons of bioethanol which is regarded as clean fuel [13].

Landfill gas (LFG) emitted from landfills as a result of decomposition of organic wastes, is mainly composed of 50% of CO₂ and CH₄ each. It is an eco-friendly method as methane has 25% greater global warming capacity as compared to CO₂. LFG is used mainly in thermal kilns, boilers, infrared heaters, sludge dryers, forgers, electricity generations, leachate evaporations, blacksmithing forges, etc. [13].

Microbial Fuel Cell (MFC) are systems that involve the

electrode materials, inadequate manufacturing capabilities for producing reactor cathodes etc. [18].

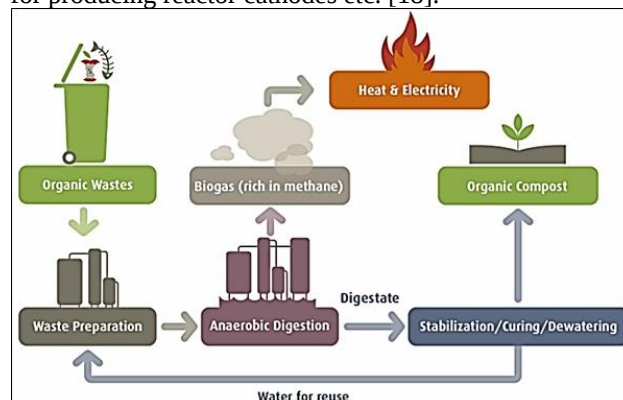


Fig. 1. Anaerobic digestion of waste matter.

Recently, a German innovation named Dendro Liquid

TABLE II
BIOREMEDIATION STRATEGIES [15]

Technology	Examples	Benefits	Limitations	Factors to Consider
In situ	In situ bioremediation Bio-sparging Bioventing Bio augmentation	Most cost efficient Non-invasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties	Bio degradative abilities of microorganisms Presence of metals and other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Land farming Composting Bio piles	Cost Efficient Low Cost Can be done on site	Space requirements Extended treatment time control abiotic loss Mass transfer issues Bioavailability limitations	See Above
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhance mass transfer Effective use of inoculants and surfactants	Soil requires excavation Relatively high cost capital Relatively high operating cost	See above Bio augmentation Toxicity of amendments Toxicity concentrations of contaminants

production of electricity from bacteria or enzymes from the oxidation of organic wastes. In MFC chambers, proton exchange membranes separate the anode and cathode chambers; the former chamber is maintained under anaerobic conditions and latter submerged in aerobic solutions or is exposed to air as shown in Fig.2. External circuit regulates the flow of electrons from anode to cathode [17]. MFC systems find applications in many areas ranging from power sensors in devices used to monitor corrosion and those that measure pressure levels in deep sea gas and oil pipelines; electricity generation on small scale; waste water treatment; production of bio-hydrogen; bioremediation and biosensors for monitoring of various processes and analysis of many pollutants. This technique is not yet being used commercially because of expensive

Energy (DLE) involves bio-processing of mixed wastes e.g. plastics, wood logs etc. to produce clean fuels e.g. CO, H₂, etc.; needed for electricity production. The system is based on 'zero waste technology' and is 4 times more efficient as compared to anaerobic digestion involving no combustion, effluents, emissions discharges or nuisance [19].

Insects driven Bioremediation is gaining popularity now days. Many insets like black soldier fly, *Hermetia illucens* L, can actively degrade the waste matter for example, can reduce kitchen waste, poultry feed, fruits and vegetables, pig liver, rendered fish and pig manure, that can be used in animal feed [20]. In addition, bioremediation of organic wastes can be promoted by using earthworms due to their physical, chemical, and biological activities. Earthworms are reported to improve the aeration, fertility

and nutritional status of the soil. These factors are often thought to be limiting the process of biodegradation. Earthworms decreases the binding of organic chemicals and soil; release any formerly bound soil contaminants followed by subsequent degradation; disperse and promote other organic contaminant-degrading microbes [21].

C. Biodegradation of Polymers and Xenobiotic

With the advancements of science and technology, nearly 140 million of polymers are produced every year [22]. These polymers are increasingly polluting surface and ground water sources and are hindrance to waste treatment plants. Many such synthetic chemicals are resistant to physical and chemical degradation, and hence they are hard to dispose off. Therefore, there has been an increasing trend towards the production of biodegradable polymers and plastics [23].

Xenobiotic are synthetics, often halogenated, difficult to degrade by microbial species. Saturated, long chain and straight alkanes are more prone to aerobic degradation as compared to unsaturated aliphatic hydrocarbons. Most common mechanism for the degradation is the oxidation of terminal methyl group into a carboxylic acid through an alcohol intermediate, and eventually complete mineralization through β -oxidation [24]. Aromatic compounds are mostly degraded under anaerobic, iron-reducing, nitrate-reducing, methanogenic and sulfate-reducing conditions. Oxidation of aromatic compounds produces molecular intermediates that enter metabolic pathways e.g. β -oxidation and Krebs Cycle [25].

D. Biodegradation of Industrial Effluents

Industrial effluents are the most important source of toxic waste accumulation in the environment. Several species of cyanobacteria have been reported to show efficient potential in the bioremediation of terrestrial and aquatic habitats, waste water treatment, biodegradation of toxic components in industrial effluents, etc. Species mainly cyanothecae, Oscillatoria, Nodularia and Synechococcus have high biosorption and biodegradation capability [26]. For the bioremediation of pulp and paper mill effluents, *Alteromonas* species can be efficiently utilized because of their alkali tolerant and osmophilic characteristics [27]. For the biodegradation of textile effluents, several *Bacillus* species e.g. *Bacillus cereus*, *B. subtilis*, *B. mycoides*, *Micrococcus* sp. and *Pseudomonas* sp. are considered to be best microbial organisms [28].

Moderate heavy metal concentrations in domestic and industrial effluents can be remediated by the action of *Bacillus licheniformes* and *Pseudomonas putida* [29]. 95% BOD and COD can be reduced by naturally found consortia of microbes including *Bacillus*, *Arthrobacter*, *Micrococcus* and *Pseudomonas* in industrial effluents from steel mills [30]. Effluents from sugar mills can be remediated biologically by the action of *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter aeruginosa*, with *Staphylococcus aureus* having maximum degradation potential [31]. In the biodegradation of municipal sewage water, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Acinetobacter*, *Entero-*

TABLE III
VARIOUS SPECIES OF MICRO-ORGANISM THAT
UTILIZE DIFFERENT HEAVY METALS

Micro-organisms	Elements
<i>Bacillus</i> spp., <i>Pseudomonas aeruginosa</i>	Cu, Zn
<i>Zoogloea</i> spp.	U, Cu, Ni
<i>Citrobacter</i> spp.	Co, Ni, Cd
<i>Chlorella</i>	Cd, U, Pb, Au, Cu, Ni
<i>Aspergillus niger</i>	Hg, Zn, Cd, Ag, Th
<i>Pleurotus ostreatus</i>	U, Cd, Cu, Zn
<i>Rhizopus arrhizus</i>	Ag, Hg, P, Cd, Pb, Ca
<i>Stereum hirsutum</i>	Cd, Co, Cu, Ni
<i>Phormidium valderium</i>	Cd, Pb
<i>Ganoderma applanatus</i>	Cu, Hg, Pb
<i>Volvariella</i>	Zn, Pb, Cu

bacteriaceae and *Zoogloea* sp. are the dominant bacterial species as shown in Table III [32].

E. Biodegradation of Waste Papers

Because of the cellulose-digestion abilities of bacteria, they can be efficiently used in the degradation of waste papers. Using bacterial species namely *E. coli*, *Pseudomonas fluorescens* and *Bacillus subtilis* isolated from soil, 1 gram paper waste was incubated at 35°C for 90 days resulting in a zone of clearance of about 13.3mm diameter with *Pseudomonas fluorescens* most actively hydrolyzing the paper [33]. Microbial and fungal consortia including *Bacillus cereus*, *A. niger* etc. can actively degrade waste papers [34]. Macroalgae from the genus *Oedogonium* has the potential to lower the concentration of a number of heavy metals e.g. Cd, Ni, Al, Zn, Cu, As, Cr, etc. in the ash water from coal-fired power plants [35].

F. Biodegradation of Plastic

Annually, about 300 million metric tons of plastic is produced, 50% of which is discarded within one (1) year

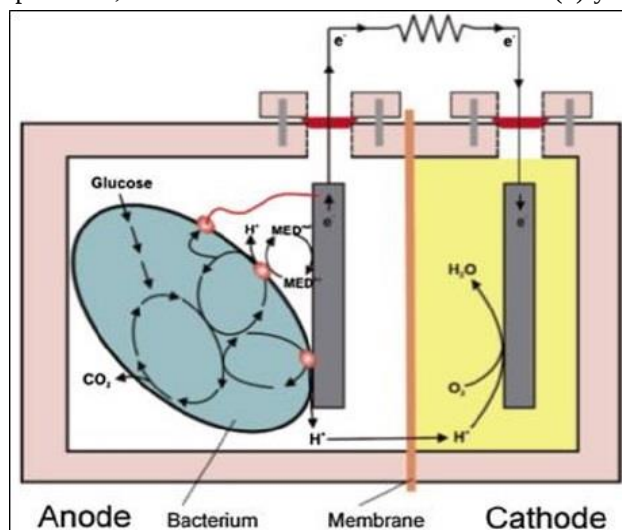


Fig. 2. Components of a two-chambered MFC system [17].

of purchase. Plastics usually have high molecular weight and have high demand in various fields. They pose several health and environmental hazards because of their non-degradable nature. Therefore, there has been an increased concern about the proper management of plastic wastes. The calorific value of plastics is high therefore they can be used as an alternative fuel with lesser CO₂ emissions. Plastic wastes can be managed by recycling, incineration, reuse, landfills and utilization in the construction of roads and can be co-processed in cement kilns [36]. Fig. 3 explains the microbial action of biodegradation.

G. Waste Management in Pakistan

Waste management strategies are currently inefficient in Pakistan as 51-69% of waste remains uncollected [38]. Solid waste management (SWM) approaches include open

Waste generated in Pakistan can be broadly categorized as biodegradable, e.g. food and animal wastes, plant-based wastes etc.; non-biodegradable, e.g. rubber, plastic, metals, textile waste, stones; and recyclable material e.g. paper, rags, cardboards etc. [37].

In Islamabad, open dumping of waste material has resulted in deteriorated quality of water, reduced abundance and diversity of vegetation along with significant alterations in the quality and properties of soil, e.g. elevated pH levels; increased concentration of heavy metals, mainly lead, chromium, nickel, copper and zinc; EC regime and TDS [41]. Rate of waste generation and collection in five (5) major cities of Pakistan is shown in Fig. 4.

The adaptability of above-mentioned waste management techniques depends upon the efficiency of waste collection

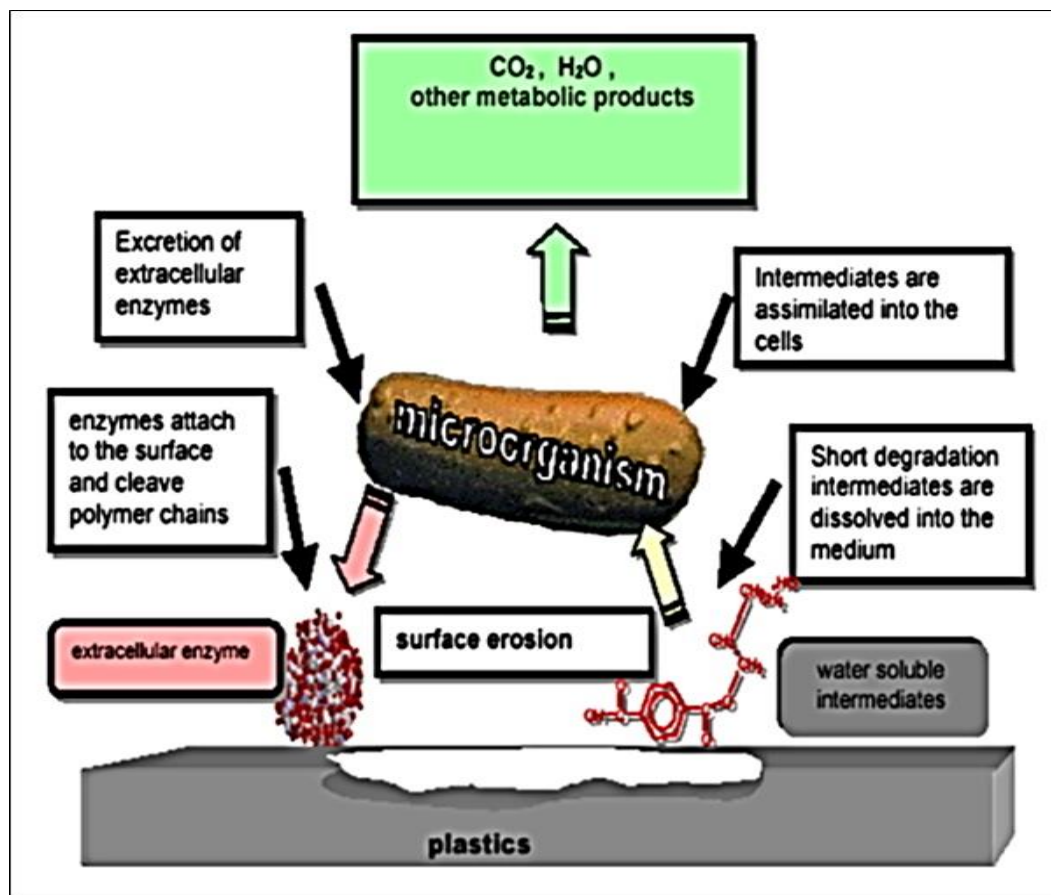


Fig. 3. Mechanism of plastic biodegradation under aerobic conditions [37].

dumping of waste matter and landfills, posing serious environmental and health threats. Burning of solid wastes releases a number of highly toxic gases posing further environmental risks and causing ground water contamination. According to a study conducted in Peshawar, Pakistan, 43% of respondents were willing to pay for the betterment of solid waste management techniques and the remaining 57% were content with existing SWM strategies [39]. Waste generation in Pakistan ranges from 1.896-4.29Kg per house per day [40].

system and the infrastructure available for extraction of energy from so-called waste matter. Waste to energy programs can be immensely helpful in eco-friendly production of energy and clean fuel for domestic and commercial usage. These programs can also be helpful in lessening the burden on already-diminishing water resources of Pakistan for electricity production.

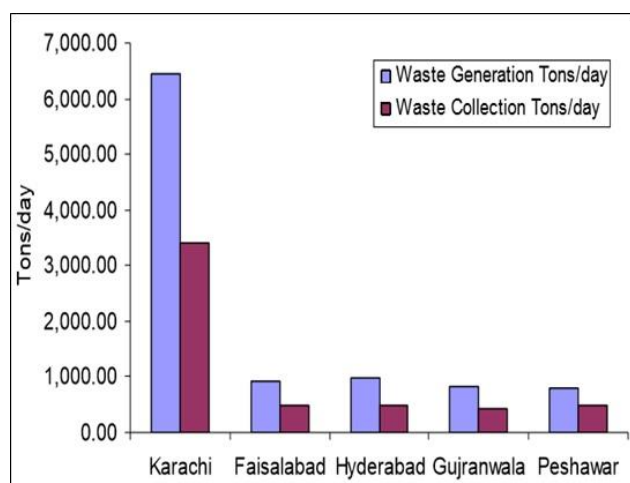


Fig. 4. Rate of waste generation and collection in five (5) major cities of Pakistan.

IV. IMITATIONS OF BIOREMEDIATION AND WASTE MANAGEMENT

Although bioremediation is an environmental friendly process, it has certain limiting factors as well. In case of in situ remediation, several additives are added for the enhancement of degradation activities of microbes. These additives can be disruptive or damaging for many other microbes inhabiting the same environment [15]. The removal of these organisms from that particular environment becomes difficult with the passage of time even if genetically modified organisms are used. Bioremediation is labor intensive, costly and time consuming in order to reach a prescribed level of remediation. In addition, neither all compounds are biodegradable nor can be degraded quickly and completely as shown in Table IV. Nature of contaminants and waste can vary in accordance with their physical state e.g. solid, liquid, gas etc.

Several compounds formed as a result of bioremediation and degradation are more toxic or persistent than their parent compound. These toxins can inhibit the growth of microbes and can even kill them. In addition, biodegradation requires specific types of microbes for particular contaminants or toxins; optimum environmental conditions for the degradation to take place; and optimum levels of contaminants or nutrients; etc. Large scale bioremediation operations are difficult to carry out as compared to laboratory or pilot scale projects [42].

The foremost limiting factor for efficient waste matter management is lack of awareness about the gravity of this matter among general public. In addition, lack of adequate legislations, infrastructure and institutional deficiencies, limited financial and human resources, inadequate short and long-term planning, etc. exacerbate the problem leading to waste accumulation [6].

V. CONCLUSION

Bioremediation provides an efficient way to tackle different categories and types of wastes with the help of

TABLE IV
FACTORS LIMITING BIOREMEDIATION [43]

Types of Limitations	Effects
Microbial	Growth until critical biomass is reached
	Mutation and horizontal gene transfer
	Enzyme induction
	Enrichment of the capable microbial populations
	Production of toxic metabolites
Environmental	Depletion of preferential substrates
	Lack of nutrients
	Inhibitory environmental conditions
Substrate	Too low concentrations of contaminants
	Chemical structure of contaminants
	Toxicity of contaminants
	Solubility of contaminants
	Oxidation/reduction potential
Biological aerobic vs anaerobic process	Availability of electron acceptors
	Microbial population present in site
Growth rate vs co-metabolism	Type of contaminants
	Concentration
	Alternate Carbon source present
Physico-chemical bioavailability of pollutants	Equilibrium sorption
	Irreversible sorption
	Incorporation into humic matters
Mass transfer limitations	Oxygen diffusion and solubility
	Diffusion of nutrients
	Solubility/miscibility in/with water

microbes. Not only can bioremediation discard waste materials, but it can also be used for the removal of unwanted substances from soil, water, air and raw materials from industrial wastes. Recovery of recyclable and reusable materials is crucial in a world that is quickly running out of resources. Inappropriate waste management practices could result in the loss of huge amount of energy that could be recovered from these wasted materials. Waste to energy program is beneficial in this regard for the generation of electricity and fuels producing less toxic gases and environmental hazards. Increasing the efficiency of these processes can lead to huge economic and social benefit in terms of reduced health risks and incidence of diseases; reduced costs of fuel and electricity; cleaner environments; reduced public and government cost being spent on waste management; and creating new chances for employments.

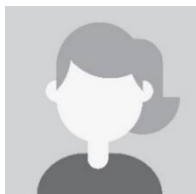
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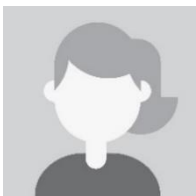
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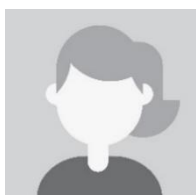
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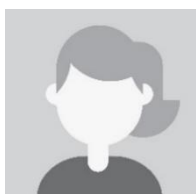
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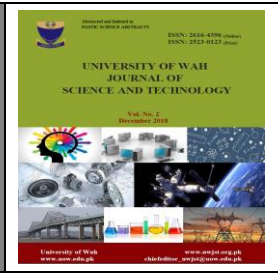


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A Study of Different Factors in Single and Multiple Server Queuing Models in Banking System

Sadaf Malik, Naheeda Akhtar, Samra Shoukat and Sajida Javed

Abstract—Queuing theory is discipline of operational research in mathematics. It is mostly dealt with demonstration and analysis of system that deliver service to random demands. In this research work, the queuing numeral, service windows numerals and the maximum service figures are checked out by the help of queuing theory. Queuing models signifies the formation of physical systems. It specifies the server number and their arrangement in order to give services to their clients, and statistical or probabilistic demand nature, by stipulating inconsistency in process of arrival and service. Main concerns of different counters such as “cash deposits counter”, “cheque deposits counter” and “cash draw counter” are studied in the field of bank such as arrival and departure of customers and service time provided to them. Distinctive queues and quantities of servers engaged with the procedures are additionally observed by utilizing proper probability distributions. The arrival procedure figured by exponential distributions and administration process is measured by Poisson Distributions. Single server and various server queuing models are utilized in order to dissect lining parameters and execution measures of the framework. WinQsb programming is utilized to perform queuing investigation and queuing simulation in order to process these parameters and execution measures. Moreover, qualities of connection between various execution measures are computed utilizing SPSS (Statistical Package for Social Sciences) 16.

Index Terms—Waiting lines, Single Server, Multiple Server, Queuing Model

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I. INTRODUCTION

IN 1930's, Operations Research was used as a subject of science and main subdivision of applied mathematics. It is used to apply adequate analytical methods for assessment creating. Operations Research application can help in making better decisions and solve complicated decision issues. In this research work, an associate optimized model is projected to enhance the bank queuing system supported by queuing theory. This technique will optimize the number of server and improve the service potency that might effectively can cut down service prices and customer's waiting time. One of the extraordinary classes of lining framework that experienced in daily lives, is business benefit framework, in which customers get hold of supplier from business organizations. Many gatherings involve individual-to-individual service at a tough and quick pace, alongside a hair salon, cafeteria, petroleum pump and bank. Numerous business banks have done extraordinary endeavor to blast the bearer proficiency and buyer fulfillment however the most extreme of them are managing a difficult issue of prepared line of clients. In money related organization (i.e. banks), the waiting line of clients appears to be because of low productivity of the lining machine. It emulates the missing of the endeavor reasoning of client driven, and low amenity cost of the device.

Numerous analytical mechanisms used in Islamabad region includes, network analysis, game theory, mathematical logic, queuing theory and simulation etc. But most generally used analytical strategy in numerous field of life is Queuing precept. It is nearly a hundred years old theory. Telephone waiting times for a profitable service in two unlike experiments and consumer thoughts of waiting times considering a psychophysical power function is described in [1]. The negative impact of perceived waiting time on wait evaluations is increased by the pecuniary expenses of waiting. The waiting times are packed in numerous ways like: with music, queuing data, and knowledge regarding expected waiting time. A supplementary variable technique was introduced by David

R. Cox [2]. The basic work of many of the analysis methods of queuing theory is positioned by A.K. Erlang, known as the father of queuing theory for the time period of 1909-1929. He firstly introduced the Poisson process to congestion theory, for the procedure of developing the balance state equations to illustrate the notion of statistical equilibrium mathematically. Allen Flick and Ming Liao obtained the queue time taken by multiple-server queuing system considering arrival and repair rates once these rates are high [3].

Basically there are two types of queuing models; deterministic and probabilistic. In deterministic queuing model, probability distributions is not associated with the arrival of customers and service time, while in a case of probabilistic model, the arrival of events/entities and service time are associated with the probability distribution. For the analysis of non Markovian queues, David G. Kendall introduced the method of Markov chain. Queuing models contradict according to probability distribution of service time, service discipline and consumer arrival rate [4]. Subject to the number of channels, a system of waiting line can be a single server queuing model or multiple servers queuing model. A non-equilibrium queuing system with a finite interval was described by F. Pollaczek [5].

The single server queuing system consists of only one server with multiple or only a single queue. Server provide services to the entities/ customers, which arrive at a system in systematic way in order to be served. M/M/1 queuing model is the basic queuing model where arrival of a customers is according to a Poisson distribution and the service time is exponentially distributed. Arrival rate of a customer is denoted by λ , and a server service rate is denoted by μ , and mean service rate is denoted by $1/\mu$. FCFS (first come first served) is a basic discipline of serving a single server queuing model. Modeling and analysis of a discrete-time two-phase queuing system is discussed in [6].

Multiple server queuing models consist of two or more than two servers. In a system where servers are identical, if customer arrives and finds that at least one server is free, than a customer can connect to this server to avail service. In a multiple queuing system, single queue and multiple queues are formed. In a case of multiple identical servers and multiples queues jokey behavior exist in a queue, where a customer can be switched to another queue from their queue to get immediate service. The choice of a server does not affect the service, if all servers are identical. FCFS is a basic discipline of a multiple server queuing models. Mansour Tabari et al studied the queuing theory to acknowledge the optimum variety of essential human assets in an instructive establishment distributed in Iran [7]. The queue analysis is performed for various numbers of employee's members.

The main objectives of the current research work are to analyze the waiting lines of banking system by developing the queuing models and measured the performance and utilization factor of the system by analytical methods and queuing simulation technique.

This research paper is organized in six (6) sections. Section II, explains materials and methods. Data collection and analysis for proposed work is done in section III and IV. Section V comprises of interpretation of results. Finally, Section VI concludes the whole research work.

II. MATERIAL AND METHODS

Data for the study is collected for two days per week for a bank located in Islamabad. Three counters in the Bank are utilized for data collection, namely 'Cash Deposit Counter', 'Cash Draw Counter' and Cheque Deposit Counter. The subject is limited to customers and service employees of the bank for the reception counter. Information is gathered by means of 'direct observation', 'questionnaires' and 'interviews', while fulfilling the underlined constraints for building a queuing model.

In current research work, two strategies are used. Queuing analysis and Queuing device simulation. Result of Queuing models and Queuing simulation are then compared. Comparison between the only server and multiple server queuing models are additionally created, in step with the steady state probabilities that tends to be estimated through it.

The subsequent assumptions are satisfied by means of records for queuing models.

- Customer's arrival follows a Poisson Probability Distribution.
- Inter-arrival time of consumers are independent and becomes exponentially distributed.
- Service time of the customer is exponentially distributed.
- Purchasers are served by method of any server on first-come first-served basis.
- No customer can depart the queue without obtaining service.
- Queue is incalculable.
- Rate of serving become not obsessed with the queue length. Serving charge remained slight regardless of queue duration.

A. Terminologies and Notations

The following terms and notations are utilized in the model formulation and computation:

- P_n = probability of exactly 'n' customers in the system.
- N = number of consumers in the system.
- L_s = estimated number of customers in the system.
- L_q = estimated number of customers in the queue.
- W_s = consumer's waiting time in the system.
- W_q = consumer's waiting time in the queue.
- A_n = average arrival rate of new customers in systems.
- μ_n = average service rate for overall systems when n consumers are in systems.

B. Queuing Model for Cash Deposit Counter

The primary queuing model determined during cash deposit counter is multiple server multiple queue model and is measured as M/M/s, wherein m represent the

Poisson probability distribution of arrival and departure and s (positive integer) denote wide variety of servers. The average time period of a cash deposit counter became approximately 25-30 minutes for every day for a queuing model of cash deposit counter as shown in Fig. 2. This model is analyzed by queuing analysis and Queuing simulation.

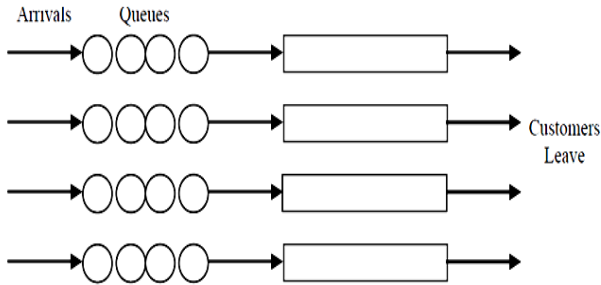


Fig. 1. Queuing Model with multiple queues and multiple parallel servers for cash deposit counter.

C. Queuing Model for Cash Draw Counter

The primary queuing model determined during this counter is multiple server multiple queue model and is measured as $M/M/s$, wherein M/M represent the Poisson probability distribution of arrival and departure and s (positive integer) denote wide variety of servers. The average time duration of data collection is distinct for every day however it's roughly 20- 25 min for each day for a queuing model for cash draw counter as shown in Fig. 2. This model is analyzed by queuing analysis and queuing simulation.

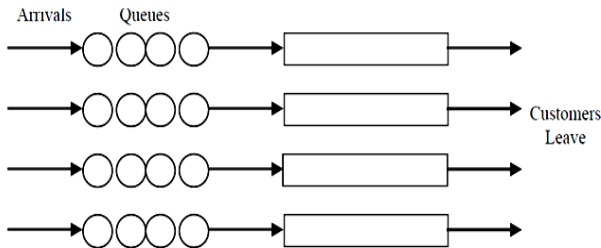


Fig. 2. Queuing model with multiple queues and multiple parallel servers for cash draw counter.

D. Queuing Model for Cheque Deposit Analysis

The primary queuing model with central queuing procedure determined in this counter is single-queue with single-servers, and symbolized as $M/M/1$, where M/M represents the Poisson chance distribution of arrival and departure and 1 characterize numeral of server that is 1. The time length of cheque deposit counter is 40-45 minutes approximately. The Queuing Model with single queue and single servers is analyzed by queuing analysis as shown in Fig. 3.

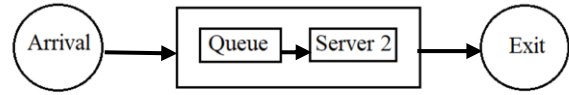


Fig. 3. Queuing Model with single queue and single servers.

E. Parameters of Queuing Models

Parameter of queuing models are,

n = sum of a patrons.

s = figure of parallel channels.

λ = Arrival amount

$$= \frac{1}{\text{average no of customer in a system at one hour}}$$

μ = Serving amount

$$= \frac{1}{\text{average no of customer served per hour per server}}$$

s_μ = Rate of serving for $s > 1$ in a system

ρ = Utilization factor

Where,

$$\rho = \frac{\lambda}{s_\mu} \quad (1)$$

ρ in above equation determines service competency being utilized on the common incoming customers.

F. Performance Measures/ Steady State Probabilities

Performance measures are calculated to estimate projected queue time-span of waiting consumers in a queue that is L_q and is calculated by equation (2) given below.

$$L_q = \left[\frac{1}{(s-1)!} \left(\frac{\lambda}{\mu} \right)^s \frac{\mu\lambda}{(\mu s - \lambda)^2} \right] \quad (2)$$

Projected waiting time of the customers in the queue denoted by W_q and can be written as follows.

$$W_q = \frac{L_q}{\lambda} \quad (3)$$

Projected queue time-span of waiting customers in the system is L_s and can be calculated by using following equation.

$$L_s = L_q + \left(\frac{\lambda}{\mu} \right) \quad (4)$$

Projected waiting time of the consumers in the system which is W_s and can be measured as in equation (5) below.

$$W_s = \frac{L_s}{\lambda} \quad (5)$$

III. DATA COLLECTION

In this research work, occurrence of events such as, a customer's arrival, a beginning of a service in a counter and end of service in counters. Two days data is collected from one of the banks of Islamabad. The methods engaged during data collection are "direct observation", "personal interrogated" and feedback form "administering" by the investigator. Service time is recorded through stop watch. Number of consumers are different for 3 counters in both days. After collection of data, it is tabulated in a spread sheets and then analysis is carried out.

Two techniques, Queuing analysis and Queuing system simulation are used in the current research work. Result of Queuing models and Queuing simulation are compared. Comparison between the single server and multiple server queuing models is also made, according to the steady state probabilities that are estimated through it. Simulation is used to create a model. Arrival rate and service time are random number generated for each entity to fit distribution, which is determined by study time of original system. Simulation and queuing model both have their own advantages. Queuing models are simpler and easy to apply on data and provide more standard results. Once the user create a model and then validates and verifies it, then one can easily analyze the response and adjust the system.

When the model M/M/C is with multiple queues, it means multiple servers with multiple queues and the number of customers are infinite, so the solution is difficult to obtain. So when μ is difficult to obtain through analytical method a Monte Carlo simulation is used. It is a discrete event simulation and provides a very reliable answer as in this case when simulation is run for more than thousand times. In banks "cash deposit" and "cash draw" counters composed multiple server with multiple queues. Queuing simulation is used in such situation to measure the performance of an individual as well as overall system. Simulation is performed using computer based software WinQsb.

IV. ANALYSIS OF DATA

WinQsb software is used to predict the evaluation of queuing analysis and queuing simulation of single server and multiple servers queuing model at bank using arrival rate (λ), service rate (μ), and number of server. Queuing simulation is performed for cash deposit and cash draw counter.

V. INTERPRETATION

Tables I and II shows the results of queuing analysis and queuing simulation. The result of queuing analysis shows that for M/M/2 model, the waiting time of customer in cash deposit is 4.5 minutes, for cash draw the waiting time is 5 minute and waiting time of cheque deposit counter is 2.5 minutes. As in cash draw and cash deposit counter jokey behavior exists so queuing simulation is also performed for these counter because queuing simulation clearly evaluate the performance of multiple servers multiple queues. In cash deposit counter, in queue 2 each customer has to wait for 27.9 minute and in queue 1 each customer has to wait

TABLE I
PARAMETERS AND OVERALL PERFORMANCE MEASURES OF
QUEUING MODEL USING QUEUING ANALYSIS FOR 1ST DAY

Performance Measures	Cash Deposit Counter		Cash Draw Counter		Cheque Deposit Counter
	m/m/2	m/m/1	m/m/2	m/m/1	m/m/1
Arrival rate(λ) per hour	96.91	96.91	72	72	49.7
Service rate (μ) per hour	54.5	54.5	41.09	41.09	67.9
Utilization factor (ρ)	88%	88%	87%	87%	73%
Average number of customer in the system(L_s)	8.48	7	7.66	7.2	2.7
Average number of customer in the queue(L_q)	6.7	6.12	5.9	6.3	1.9
Average time customers spends in the system (W_s)	0.08	0.1653	0.1065	0.2	0.0549
Average time customers spends in the queue (W_q)	0.0692	0.1492	0.0821	0.1756	0.040

for 20.8 minute. In cash draw counter, in queue 2 each customer has to wait for 32 minute and in queue 1 each customer has to wait for 28 minute.

Tables III and IV shows the results that for M/M/2 model, the waiting time of customer in cash deposit is 2.12 minute, for cash draw the waiting time is 9.09 minute and waiting time of cheque deposit counter is 2.25 minute. Queuing simulation clearly evaluate the performance of cash draw and cash deposit counter as these counter have multiple servers multiple queues behavior. In cash deposit counter, in queue 2 each customer has to wait for 27.67 minute and in queue 1 each customer has to wait for 20 minute. In cash draw counter, in queue 2 each customer has to wait for 30 minute and in queue 1 each customer has to wait for 23.3 minute.

A. Correlation between L_q , W_q and ρ

Results of queuing analysis show that the value of utilization factor varies as the waiting time of customers and average number of customers increases or decreases. To analyze this behavior, a spear's man correlation coefficient by using SPSS is used.

Results of Table V clearly shows that positive correlation exist between factors of strong correlation between ρ and L_q as compared to the correlation which

occurs between ρ and W_q . It is proved that when queue that when queue length increase ρ also increase rapidly, by increasing the number of servers, ρ inevitably reduces.

TABLE II
PARAMETERS AND OVERALL PERFORMANCE MEASURES OF
QUEUEING MODEL
THE USAGE OF QUEUEING SIMULATION FOR 1st DAY

Performance Measures	Cash Deposit Counter			Cash Draw Counter		
	*S 1	*S 2	S 1 + S 2	S 1	S 2	S 1 + S 2
Mean inter-arrival time (min)	0.66	0.66	0.66	0.76	0.76	0.76
Mean Serving time (min)	1.1	1.1	1.1	1.46	1.46	1.46
Server utilization	99%	99%	99%	99.28%	99.28%	99.28%
Average number of customers in the queue (L_q)	32.7	44.1	76.9	33.6	45.6	79
Average time customers spends in the queue (W_q)	20.8	27.9	24.4	24.8	32	28

*S 1 = Server 1, *S 2 = Server 2

VI. CONCLUSION

The queueing distinctiveness at the bank of Islamabad is analyzed using queueing evaluation and queueing simulation in three different counters. Result of evaluation show that waiting time, average queue length of customers as well as utilization of servers could be reduced when the multiple servers queueing model is used as compared to the single server queueing model. Strength of correlation between three different performance measures are calculated and it is observed that the strong correlation exist between ρ and L_q . It is therefore needed to use optimal number of servers to reduce the queue length. This research work will help banks to increase their quality of services (QOS), by forestalling, in a case when there are more customers in a system. Hence, presented work will not be only helpful for under study departments but also for all those fields of life having waiting line phenomena such as bank, post offices, railway ticketing, airlines, sales checkout etc.

This research work will not only help in analyzing the existing system but it can also be employed for the improvement of the system. The system can be used by the banks to calculate the number of waiting customers in a queue and the number of going away customers in each

TABLE III
PARAMETERS AND OVERALL PERFORMANCE MEASURES OF
QUEUEING MODEL
THE USAGE OF QUEUEING ANALYSIS FOR 2nd DAY.

Performance Measures	Cash Deposit Counter		Cash Draw Counter		Cheque Deposit Counter
	m/m/2	m/m/1	m/m/2	m/m/1	m/m/1
Arrival rate(λ) per hour	79.41	79.41	75.8	75.8	50
Service rate (μ) per hour	49.58	49.58	41.3	41.3	69.2
Utilization factor (ρ)	79%	79%	91%	91%	72%
Average number of customer in the system (L_s)	4.39	3.98	11.6	11.13	2.6
Average number of customer in the queue (L_q)	2.79	3.18	9.79	10.22	1.8
Average time customers spends in the system (W_s)	0.0556	0.1007	0.1543	0.2941	0.0521
Average time customers spends in the queue (W_q)	0.0354	0.0422	0.1516	0.2699	0.0376

day. The system can also be used for computing the the number of incoming and out going customers in each day so that banks can set a good goal regarding how many servers will be required in main branch of a bank to server customers. Presented research work can also be utilized for the new installations of ATM machine.

TABLE IV
PARAMETERS AND OVERALL PERFORMANCE MEASURES OF
QUEUEING MODEL
THE USAGE OF QUEUEING SIMULATION FOR 2nd DAY

Performance measures	Cash deposit counter			Cash draw counter		
	*S 1	*S 2	S 1 + S 2	S 1	S 2	S 1 + S 2
Mean inter-arrival time (min)	0.76	0.76	0.76	0.8	0.8	0.8
Mean Serving time (min)	1.28	1.28	1.28	1.4	1.45	1.45
Server utilization %	99.22	99.22	99.22	98.6	98.6	98.6
Average number of customers in the queue (L _q)	27.7	39.3	67.1	29	41.8	71.8
Average time customers spends in the queue (W _q)	20	27.67	23.8	23	30.8	27.01

*S 1 = Server 1, *S 2 = Server 2

TABLE V
STRENGTH OF ASSOCIATION BETWEEN ρ , L_q AND W_q

Type of Correlation	Utilization Factor (ρ)	No. of customers waiting in a queue (L _q)	Customer waiting time in a queue (W _q)
Pearson Correlation	1	.926	.899
Sig. (2-tailed)	0.000	.000	.000
Pearson Correlation	.926	1	.982
Sig. (2-tailed)	.000	.000	.000
Pearson Correlation	.899	.982	1
Sig. (2-tailed)	.000	.000	.000

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