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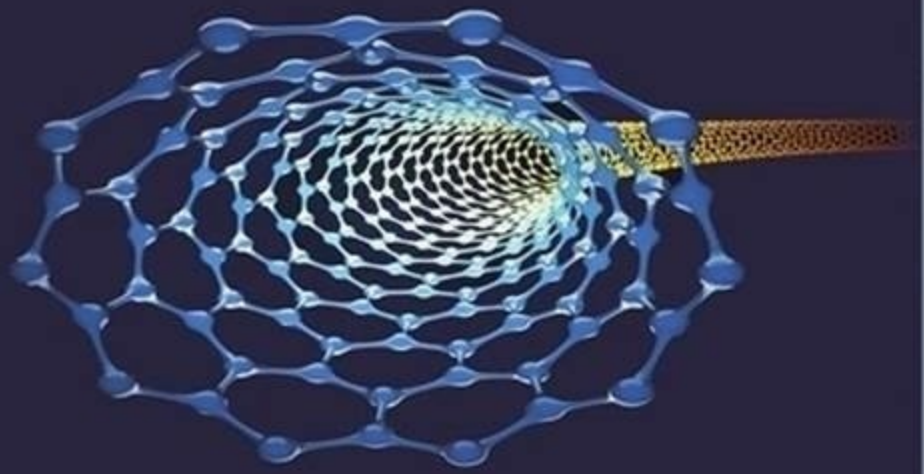

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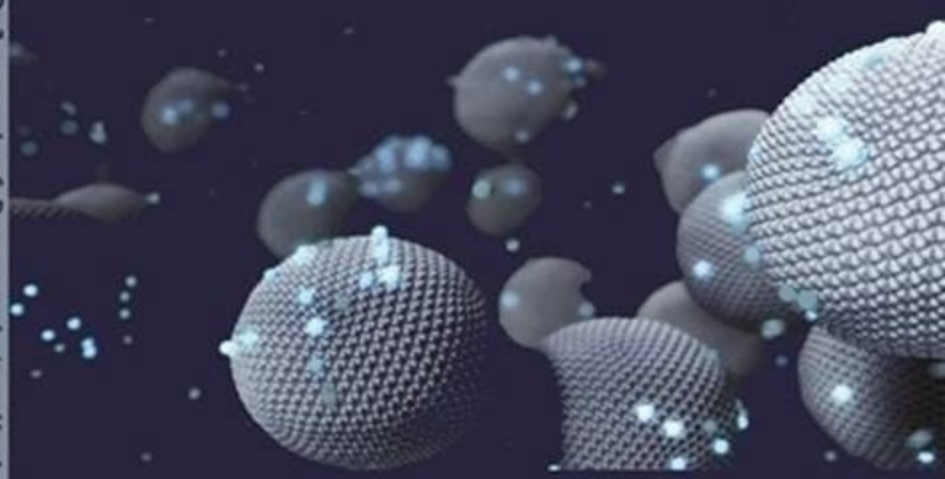

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RESEARCH PAPER

Seroprevalence of Cytomegalovirus Among Voluntary Blood Donors in Erbil Province, North Iraq.

Haween T. Nanakaly [1], Bashdar M. Hussen[2],

[1]Medical Research Centre, Hawler Medical University, Erbil, Kurdistan Region, Iraq
[2]College of Dentistry, Hawler Medical University, Erbil, Kurdistan Region, Iraq

A B S T R A C T:

Background and objectives: Cytomegalovirus (CMV) is one of the most significant agent of infection in immunocompromised patients and transplant recipients. CMV is transmissible through transfusions of blood components. The present study amied to determine the seroprevalence of Cytomegalovirus (CMV) among voluntary blood donors in Erbil province, North of Iraq.

Methods: A prospective study was done on serum samples from 472 HIV, HCV, and HbsAg- seronegative healthy male voluntary blood donors were screened for CMV IgM /IgG using Enzyme-Linked Immunosorbent assay (ELISA).

Results: A total of 472 healthy voluntary blood donors were recruited; 148(31.36%) of the donors were anti-CMV IgG positive. There were no statistically significant differences between CMV seropositivity and age. Further, seven (1.48%) subjects were both anti-CMV IgM and IgG positive.

Conclusions: The moderate seroprevalence of CMV IgG antibodies in the our donor population, underscores the importance of using strategies such as leukoreduction and transfusion with CMV-seronegative blood is recommended for preventing and reducing transmission of CMV with the infected blood or blood products in immunocompromised patients.

KEY WORDS: Cytogemalovirus, blood donors, ELISA.
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ZJPAS (2019), 31(3); 1-6 .

INTRODUCTION:

Cytomegalovirus (CMV), is a double-stranded, enveloped DNA Herpes viruses belongs to the Betaherpesvirinea subfamily of Herpesviridea (Drew et al., 2004; Ljungman et al., 2002). In humans the species is known as Human Herpesvirus 5 (HHV-5) and its seroprevalence varies between 30 to 96.6% in different countries (Crough and Khanna, 2009; Taylor, 2003; Al-Sabri AMA, et al. 2017).

In healthy immunocompetent individuals, primary CMV infection is usually asymptomatic, with seroconversion to an antibody positive status being the only indicator of past infection. After a primary infection the virus persists in a latent state in CD34+ haemopoietic progenitor cell in bone marrow and CD13+, CD14+ peripheral blood monocytes (Crough and Khanna, 2009; Qu and Tran, 2007). Allogeneic stimulation of peripheral blood mononuclear cells by T cells provides an immunologic stimulus that facilitates reactivation of latent HCMV. During latency, an individual can be re-infected with different strains of CMV; the immune response to re-infection needs better clarification (Taylor, 2003). Thus HCMV may be reactivated from latently infected cells after blood transfusion (Döcke et al., 1994; Soderbeg et al., 1997).

Congenital CMV infection in low birth weight (LBW) neonates, immunocompromised

* **Corresponding Author:**
Bashdar M. Hussen
E-mail: Bashdar.hussen@hmu.edu.krd
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patients, solid organ or haemopoietic stem cell recipients is associated with high morbidity and mortality (Drew et al., 2004; (Crough and Khanna, 2009; Qu and Tran, 2007). When the host immune system is compromised, either through infection; for example, by human immunodeficiency virus (HIV), or through iatrogenic means following organ transplantation, the virus is then able to exert its full pathogenic potential (Emery, 2001).

Transmission of the virus can occur horizontally or vertically via direct contact with infectious body fluids (e.g., saliva, urine, breast milk, semen, cervical or vaginal excretions, and tears) or blood, by vertical transmission, through organ transplant, or via blood (Emery, 2001). Transfusion transmitted CMV (TT-CMV) can lead to primary infection in CMV-seronegative recipient or reinfection (superinfection) by a new strain in CMV seropositive recipient who receives blood products from CMV seropositive donor (Sia and Patel, 2000; Diosi and Kazanjian, 2003). There are strategies to prevent the spread of CMV via blood products, such as the transfusion of leukocyte-depleted blood products and screening to select CMV-seronegative blood donors (Ljungman, 2004; Qu and Tran, 2007). In many countries the high prevalence of CMV among blood donors is usually a problem when assembling a CMV-negative blood inventory. Moreover, the cost of maintaining a CMV-negative blood supply can often be quite high (Ljungman, 2004). Thus, the aim of this study was undertaken to determine the seroprevalence of CMV among blood donors at Hawler teaching Hospital and Nanakaly Hospital for Blood diseases and cancer. And inform that might be valuable plan to blood banking for adding CMV test. Besides HCV,HBS and HIV for blood donors.

1. MATERIALS AND METHODS

This study was carried out among blood donors at the Hematology and Virology unite of the Hawler Teaching Hospital and Nanakaly Hospital for Blood Diseases and cancer, a referral blood banks and hemotherapy service in Hawler, Kurdistan region, Iraq for the entire state.

The study was approved by the Ethical and Scientific Protocol Review Committee of Medical Research Centre /Hawler Medical University. A total number of 472 male voluntary blood donors were enrolled for this prospective study. Participants were asked and aided to fill up a structured questionnaires, including demographic information and history of previous exposure to blood transfusion and donation. Inclusion criteria were age 18 - 60 years, weight >50 kg, haemoglobin level >12.5 g/dl, with normal blood pressure, pulse rate and temperature. The exclusion criteria were: individuals younger than 18 years, weight < 50 kg, haemoglobin<12.5 g/dl, any history of jaundice, chronic illness (e.g. hypertension, diabetes, asthma), and blood born infections. The donated blood was routinely screened for HIV 1 and 2, HBsAg, and anti-HCV at Virology Unit in Hawler Teaching Hospital and Nanakaly Hospital for Blood Diseases and cancer. A 5 ml of venous blood samples from cubital vein obtained from voluntary blood donors. Blood samples were centrifuged directly after collection and the serum samples were separated and stored at -20ºC until assays. Sera were tested for IgG and IgM CMV by the enzyme-linked immunosorbent assay (ELISA) test according to the manufacturer's instruction kit (BioCheck, Inc CMV IgG/ ELISA Kit: BC-1089 and CMV IgM /ELISA Kit BC-1091U.S.A.). The cut-off of IgG and IgM was established at 0.5WHO IU/ml (Calibrator 2) by the kit's manufacturer. Samples with a concentration >1.2 and > 1.0 WHO IU/ml were considered positive for CMV IgG and IgM respectively, with samples with concentration below the cutoff as negative results.

2. Statistical Analysis

The Statistical Analysis System using Statistical Package for Social Science (SPSS, version 20.0) was used to complete all data analyses. Seropositivity rates were calculated and compared according to the age group and history of blood transfusion. The differences were evaluated using Pearson Chi-square test. A $p \leq 0.05$ was considered statistically significant.

2. RESULTS AND DISCUSSION

2.1 RESULTS

All 472 subjects were healthy male voluntary blood donors concerning anti-CMV IgG, from the 472 healthy donors screened, 148 (31.36%) were

seropositive and 324(68.64%) were seronegative. Whereas, only 7 (1.48%) were seropositive and 465 (98.52%) were seronegative for anti-CMV IgM (Table 1).

The age among the 472 healthy donors included ranged from 21-57 years with mean age of 32.58± 6.988 years. Table 2 shows the distribution of anti-CMV IgG status according to age group. Age distribution analysis revealed that 68(45.9%), 56(37.8%), 23(15.5%) and 1(0.7%) of blood donors which were of age group 20-29, 30-39, 40-49 and 50-59 year respectively were anti-CMV IgG positive and was no statistically significant difference were found in the CMV IgG seroprevalence between different age group ($p > 0.05$).

Out of 148 healthy donors who were positive for anti-CMV IgG (Table 3), of whom 30(20.3%) had a previous history of blood transfusion and were all positive for anti-CMV IgG (100.0%). Of the 442 healthy donors without a history of blood transfusion, 118(79.7%) were positive for anti-CMV IgG. A highly statistically significant association existed among healthy donors between history of blood transfusion and being anti-CMV IgG positive ($p < 0.01$).

Interestingly, the 30 healthy donors who previously transfused were all anti-CMV-IgM negative (100.0%) (Table 3), whereas 435 (92.16%) of the 442 without a history of blood transfusion were anti-CMV IgM negative. No statistically significant association was observed between anti-CMV-IgM and history of blood transfusion among healthy blood donors.

2.2 DISCUSSION

Several studies have suggested that CMV is an important transfusion-transmitted (TT) pathogen (Qu and Tran, 2007). In contrast to asymptomatic CMV infection in healthy people, TT-CMV can cause diseases with significant risk of morbidity and mortality in immunocompromised individuals (Nichols et al., 2003; Ljungman, 2004).

CMV antibody positive to both IgG and IgM in blood donors harbor CMV in their peripheral blood which potentially infectious. In this study, we found seroprevalence of 31.36% in voluntary blood donors, indicating past exposure to infection; of which 1.48% was CMV IgM positive, representing the percentage of those that had active infection. Active infection could result from reactivation of latent CMV infection or fresh exposure to CMV. Similar seroprevalence rates of anti-CMV IgG was reported by (Eisenfeld et al., 1992; Kraat et al., 1992; Boeke et al., 2008), which was (38%), (34%) and (35.5%) respectively however, they did not find any IgM positive cases. Although it was suggested that anti-CMV IgM positive with anti-CMV IgG+/-donation are more infectious than IgG positive and IgM negative ones but still this remain unproved (Badani, 2001). However, other studies have shown a high worldwide seroprevalence for CMV among blood donors, such as in Nigeria (95.8%), Brazil (96.4%), Turkey (97.2%), Iran (98.5%), and the India (98.6%)(Ojide et al., 2012; Souza et al., 2010; Mutlu et al., 2008; Ziaei et al., 2013; Das et al., 2014, Iraq ((97.4%) Mohammed K. and Abdul-Lateef M. 2014.

On the other hand, other studies reported that CMV IgM in blood donors with positivity of 2.3%, 1.6%, and 0.07% from Souza, Delhi, Pune by (Souza et al., 2010; Souza et al., 1996; Kumar et al., 2008) respectively. These differences in IgM rates could be related to factors such as assay methods, sample size, geographic distribution and socioeconomic status (Arora, et al. 2018).

Although no statistically significant difference between the seropositivity of anti-CMV IgG antibody and age distribution was found. The subjects in the age range 20-29 had the highest anti-CMV IgG antibody (45.95%). This is an indication of past infections among this age range and as this age range represents the most active age group of the study subjects. This is similar to the finding of other investigators (Kumar et al., 2014). Since similar study reported a significant increase seropositivity with the increasing age of blood donors (SurgCdr et al., 2009). The significant association found between histories of blood transfusion among the donors being CMV IgG positive in our approach should be taken into consideration since they could be considered as a risk factor for CMV antibody production as have been reported in many studies (Akinbami et al., 2009). Additionally, a group of researchers pointed that CMV infections in a high risk groups such as HIV/AIDS patients (Badani et al., 20001), renal transplant patients (Choudhury et al., 1996),

and bone marrow transplant patients (Thorat et al., 2004) ; is associated with life threatening CMV disease and CMV induced immunosuppression leading to superinfection by various other pathogens with high mortality((Diosi and Kazanjian , 2003; Drew et al., 2004) . Moreover, it documented that blood transfusion itself leads to immunomodulation with profound negative effects on the immune system that persists for many months (Raghavan and Marik, 2005). All these indicate high risk and need for strategies in prevention of TT-CMV.

As this study demonstrated moderate seroprevalence of CMV in our donors, therefore, we propose that the future strategies for the prevention and /or reduction of TT-CMV in high-risk transfusion recipients in Hawler province. These should include the routine screening of donor blood for CMV antibodies or transfusion of CMV 'free' (i.e. neither positive for CMV IgG nor CMV IgM) or CMV 'safe' as a first step and prestorage leucodepleted blood components. Leucodepleted blood product reduces risk of CMV by reducing the number of latently infected cells of blood components. In addition, it reduces possibility of CMV reactivation in recipients by reducing cytokines release and other immunological trigger from donor leucocytes (Roback, 2002). It is reported risk reduction of TT-CMV to 93.1% and 92.3% in CMV seronegative blood components and leucodepleted blood component respectively as against unscreened and nonleucodepleted blood components (Vamvakas, 2005). Presence of plasma viraemia prior to seroconvertion and failure to achieve adequate removal of leucocytes have been implicated for residual risk of CMV in these blood components which is rarely encountered (Mussa et al., 2005).

3. CONCLUSIONS

Transfusion of CMV seropositive blood can lead to severe TT-CMV with high mortality and morbidity in transfusion recipients. This shows the importance of using strategies such as transfusion of CMV seronegative blood and leuco-depleted blood components for prevention of transfusion transmitted cytomegalovirus infection in high-risk recipients.

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Table (1) Seroprevalence of anti-CMV IgG and IgM antibodies among blood donors

Groups	Anti-CMV IgG (%)	Anti-CMV IgM (%)
Seropositive	148(31.36%)	7(1.48)
Seronegative	324 (68.64)	465 (98.52)
Total	472 (100%)	472 (100%)

Table (2) Age Distribution of Cytomegalovirus (CMV) IgG Seropositivity among blood donors

Age groups-Year	Frequency	CMV IgG positive (%)	Pvalue
20-29	206	68 (45.9)	0.813
30-39	194	56 (37.8)	
40-49	69	23 (15.5)	
50-59	3	1(0.7)	
Total	472	148 (100.0)	

Table (3) Distribution of Cytomegalovirus (CMV) antibody among blood donors with and without history of blood transfusion

History of Blood transfusion	Anti- **CMV IgG positive (%)**	Pvalue	**Anti-CMV IgM positive (%)**	Pvalue
Positive	30 (20.3)	0.0001	0 (0.00)	0.482
Negative	118 (79.7)		7 (100.0)	
Total	148(100.0)		7 (100.0)	

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RESEARCH PAPER

Molecular epidemiology of human Adenovirus among patients with Keratoconjunctivitis

Sazan Q. Maulud

Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq

A B S T R A C T:

Various Human Adenoviruses (HAdV) serotypes are reported to be accompanied with keratoconjunctivitis infections. Seventy-three conjunctival swabs were obtained from (39 male and 34 female) who presented to the ophthalmic unite in the hospital of Erbil province with clinical features of acute keratoconjunctivitis. Immunochromatography (IC) was performed 51(69.86%) samples were positive out of 73 eyes swab. In the present study, nested PCR was employed to amplify the partial hexon gene, and the sequence of the 37 isolates were specified and compared to sequences deposited in NCBI. The results illustrated that all isolates showed 96.7 to 100% nucleotide identity and all sequences were submitted to Genbank-NCBI which documented and received accessions number start from (MK615151 to MK615187) respectively. Phylogenetic analysis classified these isolates into different genotype-related groups A, B, D, E, and F and human adenovirus HAdV-D8 were the most frequent type. The results of nucleotide Blast of HAdV-D8 with the deposited sequence in NCBI were revealed that similar to genotype sequence documented in Germany in (99%).

KEY WORDS: Molecular epidemiology Adenovirus keratoconjunctivitis partial hexon gene
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ZJPAS (2019) , 31(3);7-15 .

INTRODUCTION :

As a highly contagious eye infection with probable global outbreaks, epidemic keratoconjunctivitis (EKC) can cause a severe type of conjunctivitis on the surface of the eye (Wold and Horwitz, 2007). Adenoviruses are regarded as the main cause of viral conjunctivitis, and they contain double-stranded non-enveloped DNA viruses that belong to the family Adenoviridae, genus Mastadenovirus which are highly resistant to environmental influence and can transmit from person to person through infectious secretions and particularly as tear fluids (Aldloch *et al*., 2010). Ocular adenoviral infections are associated with different keratoconjunctivitis (EKC), chronic papillary conjunctivitis, and non-specific follicular conjunctivitis. One of the specifications of EKC is severe bilateral conjunctivitis with involvement of corneal. Period of incubation is 8 to 10 days, and corneal affection is likely to continue for months (O'Brien *et al*., 2009). Sixty-eight types of HAdVs were identified and assigned into 7 groups (A–G) based on immunological distinctiveness, genome sequencing, phylogenetic, biological characteristics and sequence (Walsh *et al*., 2009). Of all conjunctivitis cases, the global prevalence of adenoviral conjunctivitis has been reported to be between 15 and 70% in both sporadic and epidemic forms. Moreover, epidemic keratoconjunctivitis may break out in large scales in schools, hospitals, and military establishments (Weiss *et al*., 1993; Shamsi-Shahrabadi *et al*., 2009).

Adenoviruses have been typed differently in various parts of the world. According to different

* **Corresponding Author:**
Sazan Q. Maulud
E-mail: sazan.maulud@su.edu.krd
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studies, the major causes of adenoviral conjunctivitis are HAdV-4 (in HAdV-E), HAdV-3 (in HAdV-B), and HAdV-8, HAdV-19 and HAdV-37 (in HAdV-D) with three HAdV-D serotypes that are known to cause EKC with severe symptoms including severe discharge, membrane formation, multiple subepithelial corneal infiltrates, and lacrimation (Aoki and Tagawa, 2002; Jin *et al*., 2006; Kaneko *et al.*, 2008; Janani *et al.*, 2012). Different HAdV types are recombined, and there are cases on of co-infection with two or more types of HAdV, as a result of which intermediate types emerge and more virulent strain may be resulted. Moreover, new types like HAdV-53 or changes in tropism as in HAdV-56 may be observed (Kaneko *et al*., 2009; Robinson *et al*., 2011). According to DNA sequence analysis, hexon, penton, and fiber genes were the most frequent variables among various adenovirus serotypes (Madisch *et al*., 2005; Miura-Ochiai *et al*., 2007). Currently, no vaccine exists for the civilians; however, a vaccine against type 4 and 7 are available on the license, which is only provided to U.S. military recruits (Tate *et al*., 2009).

Few studies in Iraq have focused on the molecular epidemiology of adenoviruses, particularly adenoviral keratoconjunctivitis. In this regard, the present study was conducted in order to identify the molecular epidemiology of HAdV infections so as to achieve a clear picture of the prevalent types.

2. MATERIALS AND METHODS

2.1. Patients and clinical specimens

Seventy three conjunctival swabs (39 men, 34 women) were collected from patients with age groups ranging from nine to seventy six year with suspected viral keratoconjunctivitis based on clinical features (with at least one of the findings and complaints below: sudden pinkness or redness, discomfort, pain, tearing, burning, conjunctival hemorrhages, and eyelid swelling) which attending the clinical ophthalmic unite in Rizgary hospital in the period 2016 to 2017. An ophthalmologist examined the patients, and two specimens were retrieved from each patient with two sterile Dacron swabs by ophthalmologists. One of the swabs was employed for IC adenovirus test, and the other one was gathered in 3 ml M5 viral transport medium (Remel Microbiology product, USA), immediately placed in ice and transported to the laboratory. The samples were first vortexed in transport medium for 30 seconds, then the excess fluid was removed from the swabs, and finally the swabs were discarded. The processed specimens were kept at -70°C.

2.2. Immunochromatography test (IC)

The immunochromatography test was carried out based on the manufacturer's instruction (Aden test, SA Scientific^{TM}, USA). Directly after sample collection using sterile swab this test performed in which 4 drops (about 150µl) of the specimen were put into the kit specimen well. Sufficient time was devoted for the specimen to filter through the kit to the specimen and control positions, and the results were read within a period of 15 minutes. Depending on the concentration of antigen, some positive results can be observed in as short as 30 seconds. When two colored lines appeared at specimen and control position, the test was regarded positive, when only one colored line appeared in the C (control) position, the test result was interpreted as negative, and when there was no line, it was considered invalid and the same specimen was retested. Within 30 minutes from application of specimen to the kit, the readings were finalized.

2.3. Molecular detection of HAdV

2.3.1. Human Adenovirus DNA extraction

DNA was extracted from 37 isolates which gave positive IC adenovirus test through QIAGEN DNA extraction kit (Hilden, Germany). According to the protocol of the manufacturers' instructions, the kit reagents were reconstituted before use. The molecular detection of virus was done in Genome center/ Koya University.

2.3.2. Partial hexon gene amplification using nested PCR

Nested PCR was utilized to amplify a partial hexon sequence of HAdV from all of the 37 isolates in two steps (Shimada *et al*., 2004; Miura-Ochiai *et al*., 2007). Using 50pmol of a pair of primers AdTU7 and AdTU4 during the first step, the 1004bp fragment of the hexon gene was amplified (Table 1). With a pair of primers, AdnU-S, and AdnU-A using 10µl of the first step

PCR product, the 956bp DNA fragment was amplified using nested PCR. PCR was conducted for 36 cycles in a PCR Thermal Cycler (Eppendorf, Germany). Each cycle included annealing at 50°C for 1 minute, denaturation at 94°C for 1 minute, and primer extension at 72°C for 2 minutes. Following the last cycle, the extension was continued at 72°C for 7 minutes. The products of the PCR were separated on 1% agarose gels, and a QIAquick gel extraction kit was used to purify them (Qiagen, Germany). PCR amplifications were conducted in 50µl volumes containing 25 µl GoTaq green master mix (Promega, USA), 2 µl of amplicon and 1 µl of each primer needed for the specific reaction and reaction completed using dH_2O. Using a cycle of denaturation at 94ºC for 1 minute, primer extension at 72ºC for 2 minutes, and annealing at 50ºC for 1 minute, PCR was performed in a PCR thermal cycler (fermentas, Germany) for 36 cycles. Following the final cycle, the samples were extended at 72ºC for 7 minutes. This PCR amplification was carried out by utilizing the same protocol as used for the first reaction. Following the nested-PCR amplification, 5 µl of the reaction mixture was subjected to electrophoresis on a 1% agarose gel that contained ethidium bromide.

Table (1): Primer used for partial hexon gene amplification and sequencing

Primer	Sequence	Product size/bp	Reference
AdTU7	5'-GCCACCTTCT TCCCCATGGC-3'	1004	Shimada *et al*., (2004)
AdTU4	5'-GTAGCGTTGC CGGCCGAGAA-3'		Miura-Ochiai *et al*., (2007)
AdnU-S	5'-TTCCCCATGG CGCACAACAC-3'	956	
AdnU-A	5'-GCCTCGATGA CGCCGCGGTG-3'		

2.3.3. Partial hexon gene amplification using nested PCR

An Applied Biosystem 3130 Genetic analyzer was used to determine the nucleotide sequence of the PCR products, and sequencing reaction was done by employing an ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Genome Centre, Koya University). To analyze the nucleotide sequences of the partial hexons, they were compared to those of prototype strains of all HAdV serotypes obtained from GenBank using ClustalX for Windows version 1.81, with the default parameters. MEGA7 version (7.0.26) was used to construct phylogenetic trees. In brief, Kimura's two-parameter method was utilized to compute the evolutionary distances (Kimura, 1980), and the neighbor-joining (N-J) method was employed to construct unrooted phylogenetic trees (Saitou and Nei, 1987). Bootstrap analyses were performed by 1,000 replicates of the data sets (Felsenstein, 1985). The tree is drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The Kimura two-parameter method (Kimura, 1980) with a transition/ transversion ratio of 2.0 was utilized to calculate the similarity of the hexon gene between the HAdV and the prototype strains in each window of 200 nucleotides. The nucleotide sequences of the partial hexon genes (956bp) of all 37 strains of HAdV were determined in Genome center/ Koya University.

3. RESULTS AND DISCUSSION

3.1. Circulating types and associated diseases

Adenoviruses are a main common cause of conjunctivitis all over the world. Recently 68 human adenovirus genotypes were identified and grouped into 7 species A to G. Distinguishing between serotypes associated with simple conjunctivitis and those accompanied with more severe epidemic keratoconjunctivitis was desirable in ocular infections (Sarantis *et al.*, 2004; Walsh *et al*., 2009; Hogan and Crawford, 2018). In 2016, some cases of nosocomial EKC were observed at a Rizgary hospital located in Erbil, the Kurdistan Region of Iraq. The seventy-three samples (39 male, 34 female) with age groups ranging from (9-76) years were tested using IC test in which 51(69.86%) samples (27 male, 24 female) were positive HAdV results and 22 (30.13%) negative to HAdV IC test (See Table 2). This method was developed for rapid diagnosis of human adenovirus and commercially available kit. The frequency of HAdV infections in males and

females was normal and same distribution, as well as age. Genotype and seasonal differences were observed all over the study and the peak season was mostly during the spring months because in the spring season there is a lot of subject travel, pool swimming, and different other activities, as in figure (1).

Table (2): Immunochromatography test (IC) and nested PCR for detection of HAdV in the patient with adenoviral keratocounjuctivitis

IC test		Nested PCR	
Positive (%)	**Negative (%)**	**Positive (%)**	**Negative (%)**
51(69.86%)	**22 (30.13%)**	37(72.54%)	14(27.45%)

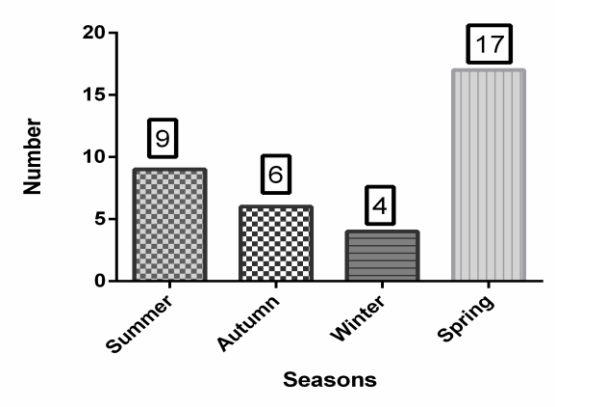

Figure (1): Seasonal genotypes distribution of human Adenovirus in keratoconjunctivitis patients

Furthermore, genotypes of the virus vary based on population and geographical distribution for this the DNA genomes from positive IC test were extracted, and two steps nested PCR was applied. For confirmation and genotyping, a set of primers was employed to amplify a partial hexon gene, which was found to be effective in amplifying all of the HAdVs strains that it selected based on conserved sequences of the hexon gene as in table (1). In this study thirty-seven (72.54%) Adenoviral DNA was detected using nested PCR and 14 (27.45%) were negative, The PCR products of all clinical specimens were 956bp when applied on 1% agarose gel electrophoresis as in figure (2). The targets band excised and purified from agarose gel using DNA purification kit (Fermentas, Germany). Amplification using nested PCR has become more applicable to clinical specimens and popular alternative methods, sensitive, fast, and accurate. Approximately the two used methods gave the same results which indicated high specificity, and these findings are in agreement with those reported by Jin *et al.* (2006), Levent *et al.* (2009), Al-Kasaby *et al*. (2011), and Demian *et al*. (2014).

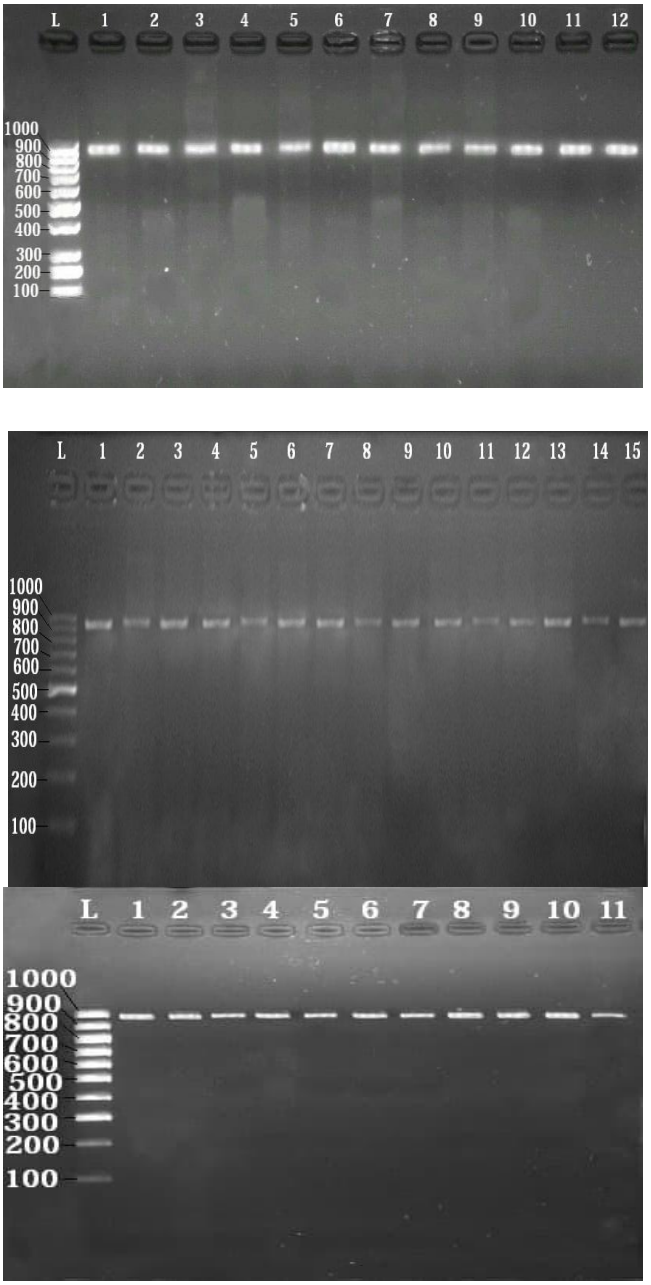

Figure (2): Agarose gel electrophoresis analysis 1% (stained with ethidium bromide) of PCR products showing partial hexon gene amplified from the seventy three Human Adenovirus with specific primer that indicated the expected size 956 bp. Lane L: is 1 kb DNA Ladder; Lanes 1-12,13,15: HAdV Hexon gene product amplified from HAdV genome run on 75V for 45min.

3.2. Phylogenetic analysis and sequencing of partial hexon region

An ABI BigDye Terminator v3.1 Cycle Sequencing Kit (Genome Centre, Koya

University) was used to sequence and type 37 purified partial hexon region genes successfully. After the DNA sequences were analyzed, the BLAST search engine National Center for Biotechnology Information (NCBI) was used. Through a BLAST search of all partial hexon gene (956bp), it was showed that the highest homology was found with the hexon proteins sequences of human adenovirus deposited in NCBI and it was found that samples belonged to different groups of HAdV-A, -B, -D, -E, and -F (Figure 3) and all 37 sequences were submitted and received Genbank-NCBI accessions number start with (MK615173 to MK615187) respectively. Moreover, the sequences obtained in this study and HAdV reference sequences retrieved from GenBank(http://www.ncbi.nlm.nih.gov/Genbank) were utilized to construct a phylogenetic tree. Based on the phylogenetic analysis, there was similarity between most of the isolates and the selected reference strains; however, in some cases a degree of divergence from the reference strains was observed but the analysis gave acceptable bootstrap values. Furthermore, Serotypes belonging to the same species made cluster together in which serotypes 8, 9, 19, and 37 were encompassed the species D. Genotype 7, 11, 16, and 50 encompassed together within the species B. Also serotype 40 and 41within the species F. Genotypes 18 and 4 within species A and E respectively as in figure (3). Results showed that 19 (51.3%) isolates were HAdV-D, 9 (24.3%) isolates were HAdV-B, 4 (10.8%) were HAdV-F, 3 (8.1%) were HAdV-E and 2 (5.4%) were HAdV-A with different genotypes (Table 3).

The constructed phylogenetic trees demonstrated that the amplicon of each serotype possessed a readily identifiable sequence and belonged to different human adenovirus groups A, B, D, E, and F with a different genotype beside that it showed that HAdV-8 was most prevalent causing adenoviral keratoconjunctivitis and it showed serotypes are quite distinct and the bootstrap values are overall quite high particularly at the nodes between species; however, they were less good for some nodes within species. This finding agrees with other studies reported in Turkey and India (Erdin *et al*., 2015; Akhil *et al*., 2016). It should also be noted that group B was second predominates genotypes. According to the results of most previous studies, EKC is typically resulted from one of the three HAdV-D serotypes -8, -19, and -37, and these strains lead to frequent nosocomial outbreaks. These viruses originally were isolated from patients that had EKC in 1951, 1955, and 1976. So far, several variants of these viruses have been reported as the causes of keratoconjuctivitis (Tabery, 1995; Tanaka-Yokogui *et al*., 2001; Ariga *et al*., 2005; Zhou *et al*., 2012). Many subjects and travellers from various regions around the world and have moved to Erbil, and they may bring viruses such as adenovirus with them, and this may have contributed to the spread of epidemics of adenoviral keratoconjunctivitis. Beside that the virus may obtain pathogenesis through some unknown mechanisms, leading to large nosocomial EKC infections because mutations of the hexon gene might change the antigenicity of the adenovirus, these changes of antigenicity may allow the virus to escape from acquired immunity and may result in an outbreak of EKC. Another reason simultaneous infection of two or more HAdV types results in recombination, and a large number of these intermediate strains are accompanied with different diseases of varying intensity, which has been well documented between 1973 and 1992 and typed by De Jong *et al*. (1999) and Janani *et al*. (2012).

Genotypes of the virus are diverse and differ according to population and geographical distribution. The HAdV-D8 was most predominant genotypes were compared with the other nucleotide sequences of HAdV-D8 which isolated and sequenced from other countries and deposited in NCBI using Blast engine. Results were showed that 33 samples from Germany have 99% identity rate to detected HAdV-D8 of study, 20 samples from Denmark in 97.2%, 18 samples from the USA in 93.2% identity (Table 4).

Table (3): Adenovirus serotypes and frequency associated with keratoconjunctivitis

HAdV subgroup	Serotype	Frequency of serotype (percentage)
A	18	2 (5.4%)
B	7, 11, 16, 50	9 (24.3%)
D	8, 9, 19, 37	19 (51.3%)
E	4	3 (8.1%)
F	40, 41	4 (10.8%)

Table (4): HAdV-D8 identity with the other sequences isolated from others country.

No. of Sample	Country	Identity %
33	Germany	99%
20	Denmark	97.2%
18	USA	93.2%
14	Tunisia	89.1%
8	Japan	89%

Few epidemiological and limited data exist on ocular adenoviral infections and genotype distribution in Iraq and to the best of the author's knowledge, this study has been the first report on the molecular epidemiology of HAdV infections from Iraq but several outbreaks of keratoconjunctivitis caused by adenovirus have been reported within other countries supported our study in Saudi Arabia, Egypt, Iran, and Turkey (Casas *et al*., 2005; Tabbara *et al*., 2010; Ayoub *et al*., 2013; Sohrabi *et al*., 2014; Lee *et al*., 2015; Akçay *et al*., 2017).

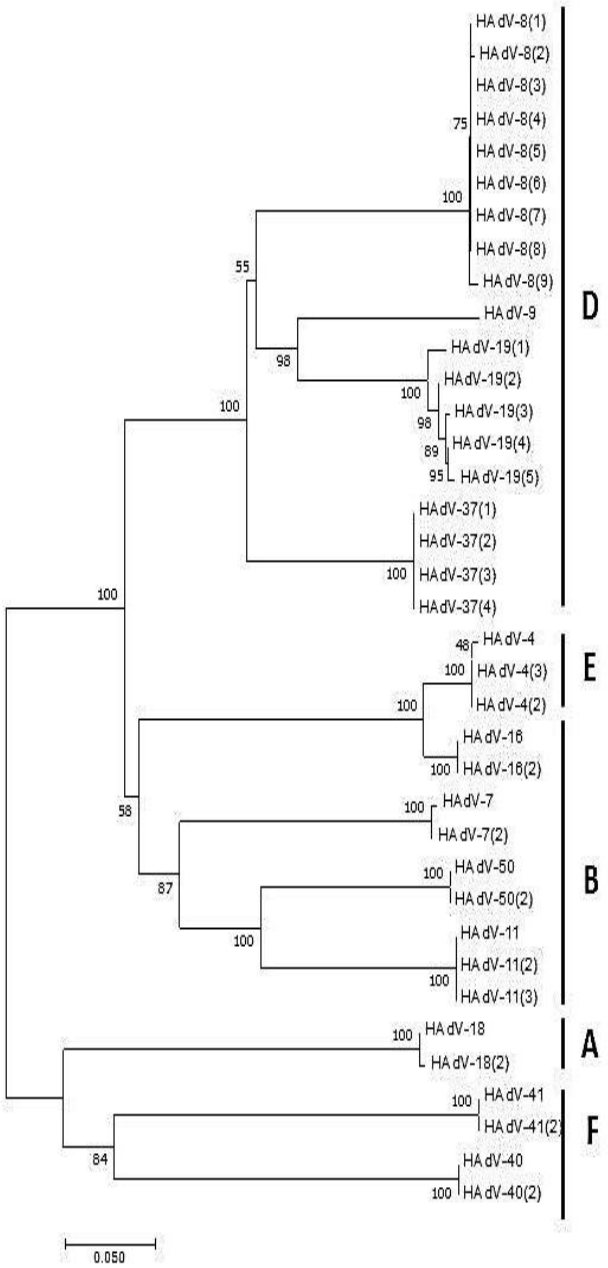

Figure (2): The phylogenetic analysis included 37 nucleotide sequences of HAdV inferred using the Neighbor-Joining method [1]. The percentage of the replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is indicated next to the branches [2]. The evolutionary distances were computed using the Kimura 2-parameter method [3] and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7 version (7.0.26).

4. CONCLUSIONS

In the present study the circulating HAdV strains and their epidemiology in Erbil, the Kurdistan region of Iraq between 2016 and 2017 were documented that causes keratoconjunctivitis

using direct sequencing techniques is an accurate, efficient, and rapid means of diagnosing and the most common cause of adenoviral keratoconjunctivitis was human adenovirus types 8 these data will be useful in predicting future outbreaks of adenovirus infection. However, due to the limited sample size, the seasonal peak of adenovirus infection was in spring. It is recommended that a well-designed epidemiological study to be conducted in order to obtain generalizable findings on the relationships among the serotypes, the sequences of their hexon gene, and prevalent serotypes and their associated keratoconjunctivitis diseases in Erbil province. Epidemiological surveillance of HAdV serotypes will improve understanding of the global infection of HAdV.

Conflict of Interest

There is no conflict of interest.

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RESEARCH PAPER

Evaluation of *femA* gene and different primers for *mecA* gene for detection of methicillin-resistant *Staphylococcus aureus* (MRSA)

Bushra Amin[1], Nada Nasir[2]

[1]Department of Biology, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq
[2]Qalla College for Gifted Students

A B S T R A C T:

The clinical specimens (wound and burn swab, abscess and pus, urine and blood culture) were collected from 122 patients admitted to different hospitals in Erbil city/Iraq. Depending on cultural, morphological and biochemical characteristics 38 isolates were identified as *S. aureus.* Antibiotic sensitivity testing was done for all isolates by using 8 antibiotics. Vancomycin was the most effective antibiotics against *Staphylococcus aureus* while only (31.57%) isolates were resistant to it. While Penicillin and Methicillin were the least effects when (94.87%) and (86.84%) isolates were resistant to them respectively. Through phenotypic detection for Methicillin-resistant *S. aureus* (MRSA) among the 38 isolated *S. aureus*, 28 (73.68%) isolates were detected as MRSA and 10 (26.31%) isolates were detected as Methicillin-sensitive *S. aureus* (MSSA). Among *S. aureus* isolates, only 16 (42.10%) gave a positive result for *mec*A gene detection by PCR when used *mec*A 1st set primers, while 18 (47.36%) positive result for *mec*A 2nd set primers. Otherwise, all 38 (100%) *S. aureus* isolates showed a positive result for *fem*A gene by PCR method.

KEY WORDS: **:** Antibiotics, mecA and femA, MRSA, and S. aureus
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1.INTRODUCTION :

Staphylococcus aureus is gram-positive bacteria which is one of the most common bacterial pathogens in humans (Guillemot et al., 2004). It has been identified as an important cause of disease globally also it becomes a dangerous pathogen for both community-acquired as well as hospital-associated infections (Humphreys et al., 2009; Patel et al., 2010).

* **Corresponding Author:**
Bushra Amin
E-mail: bushra.amin@su.edu.krd or dr_bushrah@yahoo.com
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S. aureus resistance ability increased over the time due to the extensive abuse of common antibiotics. It has reached to dangerous proportions of methicillin resistance (Voss and Doebbeling, 1995). MRSA are *S. aureus* that has acquired one of the Staphylococcal Cassette Chromosomes encoding methicillin resistance (SCCmec) which is a comparatively stable piece of DNA incorporated into the chromosome (International Working Group on the Classification of Staphylococcal Chromosome Elements, 2009).

*fem*A gene is a chromosomally encoded universal gene in Staphylococci, which is involved in the formation of the peptidoglycan pentaglycine interpeptide linkages (Berger-Bachi et al., 1989). Besides, it has a role in the expression of methicillin resistance, the mechanism of which is not clearly understood. *femA* gene can be used as a

marker for the differentiation of *S. aureus* from the coagulase-negative Staphylococci (Abimanyu et al., 2012; Lina et al., 1999).

Various PCR methods have been revealed to detect the *mecA* gene (Murakami et al., 1991; Tokue et al., 1992). The main purposes of this study were to determine the occurrence of MRSA among *S. aureus* isolates also detect the presence of *mec*A gene and *fem*A gene among MRSA isolates. As well as compare two primers for detecting the *mec*A gene in MRSA.

2. MATERIALS AND METHODS

2.1 Specimens collection

From February to July 2014 one hundred and twenty-two specimens were collected from patients who admitted to West Emergency Hospital Management Center and Rizgary hospital in Erbil city. The clinical specimens were included wound swab (26), burn swab (15), abscess and pus (26), urine (45) and blood culture (10). The specimens were cultured on blood agar and mannitol salt agar. Microscopical characteristics (gram stains), cultural characteristic (colonial morphology), and biochemical tests coagulase, catalase, urease, citrate, nitrate reduction and voges proskaue, were identified for all isolates.

2.2 Antibiotics Sensitivity testing

The effect of different antibiotics on the isolated bacteria was studied by using disc diffusion method (known as Kirby- Bauer method). Antibiotics that used for this study were: Penicillin-G (P) (10µg), Methicillin (ME) (5µg), Erythromycin (E) (15µg), Cephalothin (CEP) (30µg), Gentamicin (GM) (10µg), Oxacillin (OX) (1µg), Cefoxitin (FOX) (30µg), Vancomycin (VA) (30µg) (Green and Sambrook, 2012).

2.3 Phenotypic detection method for MRSA

Oxacillin (1µg) disc diffusion method is used for detection of MRSA according to the guidelines recommended by Clinical and Laboratory Standards Institute (Green and Sambrook, 2012), due to the presence of a unique Penicillin-binding protein (PBP2a or PBP2`) in the cell wall of MRSA .

2.4 Genomic DNA extraction

The DNA purification kit was used for extraction of genomic DNA of the isolates according to the instructions recommended by the manufacturer Thermo, EU. It is designed for rapid isolation of Genomic DNA from a variety of sample source.

2.5 PCR reaction

PCR amplification was done for genomic DNA extraction using *fem*A gene primer and two sets of *mec*A gene primers as in table (1)

Table 1: PCR stages, temperature and time for each stage.

Stages	Temperature	Time/ min
Initial denaturation	94℃	4:40
Cycles (35x) a- Denaturation b- Annealing c- Extension	 95℃ 56℃ 72℃	 0:30 0:30 0:30
Final Extension	72℃	5:00
Hold	4 ℃	∞

The PCR reactions were performed in a volume of 25µl as shown in table (2).

Table 2: Reagents and PCR reaction mixture volume that used in this study.

Reagents	PCR reaction mixture volume
Master Mix	12.5 µl
Forward Primer	1 µl
Reverse Primer	1 µl
Template DNA	3 µl
Nuclease-Free Water	7.5 µl
Total Volume	25 µl

Primers

The primers used in this study are listed in table (3).

Table 3: Primer sequences, product size and references

Primers	Primer Sequence (5'-3')	Product Size (bp)	Reference
***femA* – F** ***femA* – R**	AAAAAAGCACATAACAAGCG GATAAAGAAGAAACCAGCAG	**132**	(Aziz et al., 2014)
MR1 ***mecA* – F** ***mecA* – R**	TGCTATCCACCCTCAAACAGG AACGTTGTAACCACCCCAAGA	**286**	(Aziz et al., 2014)
MR2 ***mecA* – F** ***mecA* – R**	AAAATCGATGGTAAAGGTTGGC AGTTCTGCATACCGGATTTGCA	**533**	(Kaya et al., 2009)

2.6 Agarose gel electrophoresis of PCR products

For running PCR products, (1gm) Agarose powder was weighed and added to 100 ml of (1x) TBE buffer and boil. After cooling, the gel cast and after solidification the PCR product were loaded into the wells in the gel, 5ml/well. DNA ladder (5 µl) added into one well in the gel to determine the size of the amplified products. The gels were run at 45V/15 minutes. Then at 85V/45 minutes the gel stained with safe stain, visualized by UV-trans illuminator and photographed (Sambrook and Russell, 2001).

3.RESULTS AND DISCUSSION

This study was conducted on 38 *S. aureus* isolates to detect *femA* and *mecA* gene of MRSA by PCR. The distribution of *S. aureus* according to the type of specimen showed that UTI accounted for 45 (36.88%) while blood sample 10 (8.19%) the least one. *S. aureus* is recognized as one of the most important bacterial pathogen that seriously contribute to the problem of nosocomial and community acquired infections. Transmission of MRSA occurs primarily from colonized or infected patients or staff to other patients or vice versa. The genus, *Staphylococcus* comprises about 34 different species and methicillin-resistance was reported in most of the species, which included the most pathogenic species S. aureus and other commensal species. Early and accurate diagnosis of MRSA is crucial in effective management and control of spread of MRSA infections. PCR-based assays are considered as the gold standard for the detection of MRSA, due to the heterogeneous resistance by various phenotypic detection methods displayed by many clinical isolates. Genotypic methods are more accurate in detecting methicillin resistant Staphylococci as compared to conventional susceptibility methods (18). Among the resistant pathogens, MRSA is of great concern because of its particular importance in causing various clinical conditions. Therefore, the risk of acquiring *S. aureus* infection is increased in the wards in the presence of other hospitalized 'shedders' who may be possibly infected with the antibiotic resistant strains (17). Specimen distribution of MRSA showed that wound swab accounted for the highest 14 (36.82%) isolates (Table 4). It has a wide distribution and carriage rate among the hospital personnel as well as the patients (Chambers, 2003).

Table 4: Distribution of *S. aureus* isolates according to sample type.

Specimens	No. (% of clinical specimens	MRSA (%)	MSSA
UTI	45 (36.88%)	2 (5.26%)	43
Wound swabs	26 (21.31%)	14 (36.82%)	12
Abscess swabs	26 (21.31%)	12 (31.57%)	14
Burn skin swab	15 (12.29%)	8 (21.05%)	7
Blood sample	10 (8.19%)	2 (5.26%)	8
Total	122	38	84

Biochemical tests were used for the identification of the isolates includes coagulase, catalase, urease, citrate, nitrate reduction and voges proskaue. The result shows positive for all these tests. An antibiotic sensitivity test was done to detect the antibiotic-resistant properties of each isolate. Eight different antibiotics used, including penicillin-G (P), (ME), erythromycin (E), cephalothin (CEP), gentamicin (GM), oxacillin (OX), cefoxitin (FOX), vancomycin (VA). The most effective antibiotic was VA (31.57%) this result was in agreement with (Alalem, 2008; Mohmood, 2013) and differ with (Kampf et al.,

1998) who reported that *S. aureus* 100% sensitive to VA. The high levels of resistance to VA among isolates of the current study due to the fact that VA is recently widely used in our area that make these bacteria develop resistance against them. As well as resistance to P was (94.87%) this revealed with (Mohmood, 2013; Tel et al., 2012). The frequency of resistant isolates to each antibiotic was shown in (Figure 1).

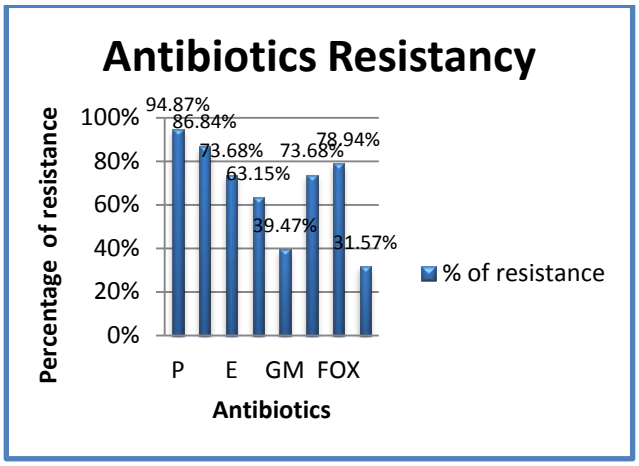

Figure (1): antimicrobial resistant frequency in the studied population

The Oxacillin disc diffusion test is the important and standard method for phenotypic MRSA detection. The screening by disc diffusion method was done for all 38 *S. aureus* isolates by using the antibiotic disc as a screening agent which was Oxacillin 1. The 28 (73.68%) isolates were MRSA and the rest (26.3%) were methicillin sensitive (MSSA). The result agrees with that reported by (Kobayashi et al., 1994; Mohmood, 2013; Tel et al., 2012), but higher from those results reported by (Alalem, 2008) who mentioned the present result of the OX resistance only 56% and (Aziz et al., 2014) who founded 26.54%.

Detection of *mecA* and *femA* genes together by PCR was considered to be a more reliable indicator to identify MRSA by differentiating it from *mecA*- positive CNS than single detection of *mecA* (Kobayashi et al., 1994). The *mecA* and *femA* genes were carried out on 38 MRSA isolates. The PCR result shows that all *S. aureus* isolates given a positive result for a *fem*A gene, which represented in figure (2). The result agreed with those reported by (Aziz et al., 2014; Kareem, 2013; Kobayashi et al., 1994; Singh et al., 2014). The significance of the *femA* gene in the mechanism of methicillin resistance was supported by the demonstration that *S. aureus* strain with *femA* gene inactivated lost the methicillin resistance trait, but with the transduction of *femA* gene restored the resistance.

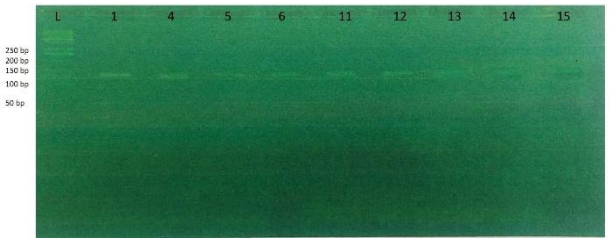

Figure (2): Agarose gel electrophoresis (1% agarose) of amplified PCR production of *femA* gene (chromosomal gene); Lane 1: 50 bp DNA Ladder; Lane 1-15: Amplified PCR products of *femA* gene.

Only 16 (42.10%) isolates from the total 38 isolates showed *mec*A gene positive for *mec*A 1st primer as present in figure (3).

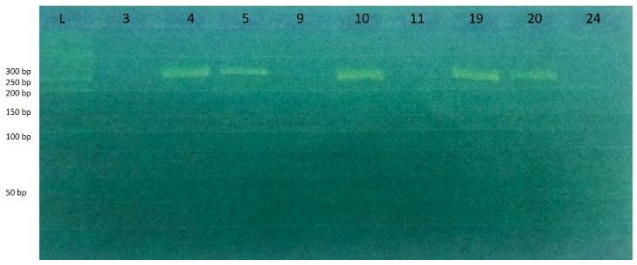

Figure (3): Agarose gel electrophoresis (1% agarose) of amplified PCR production of *mecA* gene (chromosomal gene) using *mecA* first one primer; Lane 1: 50 bp DNA Ladder; Lane 4, 5, 10, 19, 20: Amplified PCR products of *mecA* gene.

The result was positive for *mec*A 2nd primer for 18 (47.36%) isolates as shown in (figure 4)

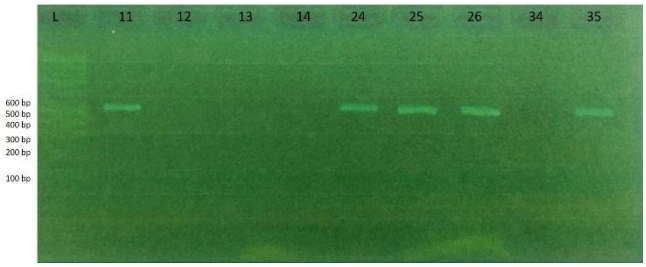

Figure (4): Agarose gel electrophoresis (1% agarose) of amplified PCR production of *mecA* gene (chromosomal gene) using *mecA* second primer; Lane 1: 100 bp DNA Ladder; Lane 11, 24, 25, 26, 35: Amplified PCR products of *mecA* gene.

Different results in the PCR products for detection *mecA* gene in *S. aureus* isolates when used different primers and this result agrees with that reported by (Siripornmongcolchai et al., 2002). The virulence genes *mecA* and *femA* were examined because they are believed to be major contributors to methicillin resistance.

The accuracy of oxacillin disk diffusion test for detection of MRSA is not as much as the accuracy of PCR assay. The presence of mecA gene not correlated with the methicillin phenotypic resistance in all MRSA isolates. These results indicated that the methicillin resistance mechanism in the *mecA*-positive isolates was due to the production of PBP2a by *mecA* gene (Fluit et al., 2001).Expression of *mecA* is either constitutive or inducible by some beta-lactam antibiotics, but not by methicillin or oxacillin, or heterogynous, with only a few cells in a population expressing the gene (Berger, 1994). In spite of the general agreement that PCR assay is the gold standard for detection of different genes, however molecular assays for the detection of resistance have a number of limitation. New resistance mechanisms may be missed, and in some cases the number of different genes makes generating an assay too costly to complete with phenotypic assays.(Fluit et al., 2001)

From a clinical perspective, it is important to differentiate isolates that have *mecA* positive resistance, which is the classic type of oxacillin resistance, from the frequency encountered isolates that have one of the other types of more subtle or borderline resistance due to hyper production of beta-lactams (chambers *et al* ,1997).

1. CONCLUSIONS

It can be concluded that there is a highly prevalent of MRSA (36.82%) among wound infections of *S. aureus* isolates. Most of the *S. aureus* isolates were multi-resistant to antimicrobial agents. The most effective antibiotic against *S. aureus* is Vancomycin (31.57%). Further researches should be implemented on bacterial resistance to Vancomycin to take a resolve measure to curb the spread of it.

Conflict of Interest

There is no conflict of interest.

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RESEARCH PAPER

The Phenetic Study of Distributed Species of Valerianaceae Batsch Family in Kurdistan Region-Iraq

Serwan T. S. Al-dabbagh[1]* and Jawhar F. Saeed[2]

[1]Department of Field Crops, College of Agriculture, Salahaddin University-Erbil, Kurdistan Region, Iraq
[2]Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq

A B S T R A C T:

The present study was conducted in Kurdistan region of Iraq, the field trips from all geographical districts of the region have been done during the years (2016-2018). The species of Valerianaceae family have been collected from the Jabal Sinjar (MJS), Amadiya (MAM), Rowanduz (MRO), Sulaimaniya (MSU), Erbil (FAR), Kirkuk (FKI), Upper Jazeera (FUJ), and Persian Foothills (FPF) districts within the studied area. The various characters of morphological, anatomical, palynological and cytological studies have been analyzed. The achieved data were processed by MVSP windows program, and the different dendrograms are constructed for each cluster attributes to determine the various relationships among the studied species.

KEY WORDS: Iraq, Kurdistan Region, Phenetic study, Valerianaceae.
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1.INTRODUCTION:

The term of phenetic was initiated by Cain and Harrison (1960) to express a correlation of overall similarities and relationships among taxa (Sneath and Sokal, 1973), depending on all available features without any weighting (Stuessy, 1990). Numerical analysis of taxonomy is recently best known to a phenetic (Mondal, 2009), also called taxometrics Rogers (1963) and Mayer (1966), or multivariate morphometrics (Blackith and Reyment, 1971).

(Sneath and Sokal, 1973) interpreted that the sensible crucial unit in a large majority part of examples is the individual organs. The individuals are the fundamental units applied, would throw light on resemblances among intraspecific variants, but would not be likely to offer much scope for comparisons at the sub-generic, generic, and higher levels. The comparisons of numerous individuals would lead to similarity matrices of excessive size and therefore be an individual (or an average) standing for a race, a species, a genus or a higher ranked taxon. (Sokal, 1966; 1986) believes the numerical taxonomic relations of three kinds: the phenetic, cladistic (close cladistic, close phenetic similarity) and chronistic or temporal.

The phenetic taxonomy is arrangement the operational taxonomic units (OTUs) in a stable and convenient classification (Sokal, 1966). There are units such as individuals, genera, and family which calculate the similarity. Taxonomic characters are attributes of a member of a taxon by which it differs from a member of different taxa. In selecting taxonomic characters, it is wise to

* **Corresponding Author:**
Serwan Taha S. Al-dabbagh
E-mail: serwan.saleh@su.edu.krd
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avoid characters that do not reflect the inherent nature of the OTU or are the logical correction to other units or are invariant. In addition, a priori weighting of each taxonomic characteristic must be equal (Pedersen *et al*., 2001).

The Valerianaceae family comprises 10 genera and 370 species (Lawrence, 1951). and consists of 10 genera about 300 species, mostly annual or perennial herbs, the leaves are opposite, estipulate, inflorescence is bracteates, composed of cyme units. The calyx of 5 sepals or reduced to teeth, and/or forming an accrescent pappus in some taxa, the corolla is gamopetalous with 5 [3,4] imbricate lobes and saccate or spurred. Stamens are 1- 4, whorled, gynoecium is 3 carpelled, syncarpous, ovary inferior with one fertile and two sterile locules. Fruit is an achene, sometimes cypsela. Seeds have an oily endosperm (Simpson, 2006). The final number of the genera and species of the family are counted by (Takhtajan, 2009) were: *Belonanthus*:5 species; *Centranthus*: 9 species; *Nardostachys*: 1-2 species; *Patrinia*: 15 species; *Phyllactis*: 25 species; *Plectristis*: 5 species; *Stangea*: 7 species; *Valeriana*: 250 species; *Valerianella*: 50 species. In APG III (2009) system, the Caprifoliaceae expanded to include Diervillaceae, Dipsacaceae, Linnaeaceae, Morinaceae, and Valerianaceae.

In Iraq the lists of Valerianaceae genera and species included as: (Guest, 1933) cited the presence of 7 species of *Valerianella*, (Blakelock, 1948) only 2 species of *Valeriana*, with 7 species of *Valerianella*, (Zohary, 1946) pointed out the presence of 2 species belonging to the genus *Valeriana* and 10 species of *Valerianella*. While (Rechinger, 1964) listed 13 species belonging to the *Valerianella*, (Al-Rawi, 1964) mentioned the presence of 1 species of *Centranthus*, and 2 species of *Valeriana* and 15 species of *Valerianella*, (Rechinger, 1969) pointed out the presence of 1 species of *Centranthus*; 2 species of *Valeriana* and 15 species of *Valerianella* most of them distributed in Iraqi Kurdistan.

The list of (Ridda and Daood, 1982) included 1 species of *Centranthus*, 2 species of *Valeriana* and 15 species of *Valerianella*, finally, (Ghazanfer and Edmondson, 2013) elucidated 1 species belonging to *Centranthus*, 2 species belonging to *Valeriana* and 15 species belonging to *Valerianella*.

The current study attempts to reconsider the comprehensive similarity and dissimilarity of Valerianaceae members and investigate the relationships and diagnostic ability of morphological, anatomical, palynological and cytological evidence to separate the studied taxa.

2. MATERIALS AND METHODS

In the current study, the numerical methods have been applied for investigating the morphological, anatomical, palynological and cytological relationships among the studied taxa of Valerianaceae family. The individuals used as OTUs, both quantitative and qualitative characters were used. Cluster analysis by UPGMA (Unweighted Pair-Group Method using arithmetic Averages)

Cluster analysis was achieved by MVSP (Multi Variate Statistical Package) for Windows, version 3.22. The characters states were taken from the different features for the purposes of analyzing each of the variantsas follows:

1- 32 key characteristics were taken for numerical analyzing for 3 genera of the family.
2- The taken morphological characters for numerical analyzing were 64 characteristics for all studied taxa.
3- The taken anatomical characters for numerical analyzing were 66 characteristics for all family's taxa.
4- The taken chromosomal characters for numerical analyzing were 6 characteristics for all family's taxa.
5- The taken palynological characters for numerical analyzing were 9 characteristics for 14 taxa of the family.

The similarity and dissimilarity have been collected by comparing each OUT with all other OTUs, all the data from the individual sets were consolidated to carry out the whole of 64 characters states analysis, include 15 species within 3 genera which belonging to Valerianaceae.

For all the data sets, similarity coefficients *S*(*j*)*k* was calculated to measure the similarity among OUT *j* and *k*, using GGSc (Gower General Similarity coefficient) that elucidated by (Pedersen *et al*., 2001).

$$S(j)k = \frac{\sum_{i=1}^{n} Wijk.sijk}{\sum_{i=1}^{n} Wijk}$$

I = the *i*th character € [1,. . . n]

j-k = the two OTUs under comparison
Wijk = 1 for valid comparisons, 0 for invalid

$$Sijk = 1 - \frac{x(i)j - x(i)k}{x(i)max - x(i)min}$$

Subsequently, the similarity coefficient was calculated by Gower that was used to construct dendrograms using UPGMA.

3. RESULTS:

3.1. The general relationship within genera:

The dendrogram of Valerianaceae genera and the similarity coefficient shows that the higher similarity at GGSc=0.625 is between the two genera *Centranthus* and *Valeriana*, the reasons of convergence refer to the affinity or similarity in their height, perennial duration, mountain habitat, the presens or absens of the trichomes, number of fruit locules, variation in inflorescence types, and chromosome numbers. The genus *Valerianella* meets with the two genera *Centranthus* and *Valeriana* at the lower level GGSc=0.188, the divergence of the genus from them is caused by the dissimilarity at the previous mentioned attributes. Figure (1) and table (1).

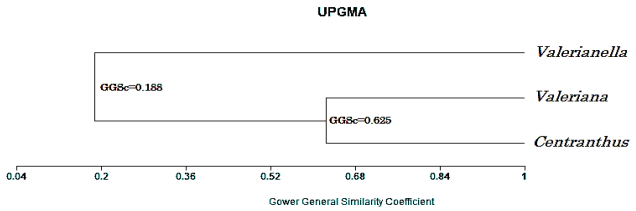

Figure (1): Dendrogram of the similarity degrees among Valerianaceae genera.

Table (1): Similarity matrix coefficient for Valerianaceae genera.

CLUSTER ANALYSIS
Imported data- Key Data: Analyzing 32 variables (characters) x 3 cases (genera)
Gower General Similarity Coefficient

Similarity matrix			
	Centranthus	*Valeriana*	*Valerianella*
Centranthus	1.000		
Valeriana	0.625	1.000	
Valerianella	0.156	0.219	1.000
	Centranthus	*Valeriana*	*Valerianella*

3.2. Morphology:

The similarity coefficient for morphology data sets was cleared in table (2), and the dendrogram formed by cluster analysis of morphology showed in figure (2). The absolute similarity is marked by 1.000. At the upper level GGSc=0.938, *Valeriana alliariifolia* and the *Valeriana sisymbriifolia* are similar. At the level GGSc=0.813 the *Centranthus longiflorus* is single in group *Va. alliariifolia*, *Va. sisymbriifolia* and *C. longiflorus*. The level which they are meted is depended on an average of values of the unweighted arithmetic. The relationship of the species of former both genera to the *Valerianella* species is correlated in the very low level at GGSc=0.434.

Table (2): Similarity

matrix coefficient of morphological characters for Valerianaceae taxa.

CLUSTER ANALYSIS UPGMA

Imported data – Morphological Data: analyzing 64 variables (characters) x 15 cases (species)

Gower General Similarity Coefficient Similarity matrix

	C. longiflorus	*Va. alliariifolia*	*Va. sisymbriifolia*	*V. pumila*	*V. carinata*	*V. muricata*	*V. coronata*	*V. kotschyi*	*V. vesicaria*	*V. dufresnia*	*V. szovitsiana*	*V. tuberculata*	*V. oxyrrhyncha*	*V. dactylophylla*	*V. discoidea*
C. longiflorus	1.000														
Va. alliariifolia	0.844	1.000													
Va. sisymbriifolia	0.781	0.938	1.000												
V. pumila	0.406	0.406	0.406	1.000											
V. carinata	0.484	0.484	0.484	0.828	1.000										
V. muricata	0.469	0.469	0.438	0.781	0.766	1.000									
V. coronata	0.391	0.422	0.391	0.672	0.625	0.703	1.000								
V. kotschyi	0.406	0.406	0.375	0.750	0.609	0.719	0.922	1.000							
V. vesicaria	0.391	0.391	0.391	0.703	0.656	0.734	0.938	0.891	1.000						
V. dufresnia	0.359	0.359	0.359	0.828	0.750	0.703	0.781	0.859	0.813	1.000					
V. szovitsiana	0.469	0.531	0.500	0.688	0.797	0.781	0.609	0.625	0.609	0.734	1.000				
V. tuberculata	0.469	0.469	0.438	0.625	0.672	0.719	0.734	0.750	0.734	0.703	0.781	1.000			
V. oxyrrhyncha	0.422	0.422	0.391	0.703	0.750	0.734	0.688	0.734	0.656	0.781	0.859	0.859	1.000		
V. dactylophylla	0.422	0.484	0.453	0.672	0.625	0.703	0.875	0.859	0.813	0.719	0.672	0.797	0.719	1.000	
V. discoidea	0.453	0.516	0.484	0.641	0.625	0.734	0.844	0.797	0.844	0.719	0.703	0.734	0.594	0.781	1.000
	C. longiflorus	*Va. alliariifolia*	*Va. sisymbriifolia*	*V. pumila*	*V. carinata*	*V. muricata*	*V. coronata*	*V. kotschyi*	*V. vesicaria*	*V. dufresnia*	*V. szovitsiana*	*V. tuberculata*	*V. oxyrrhyncha*	*V. dactylophylla*	*V. discoidea*

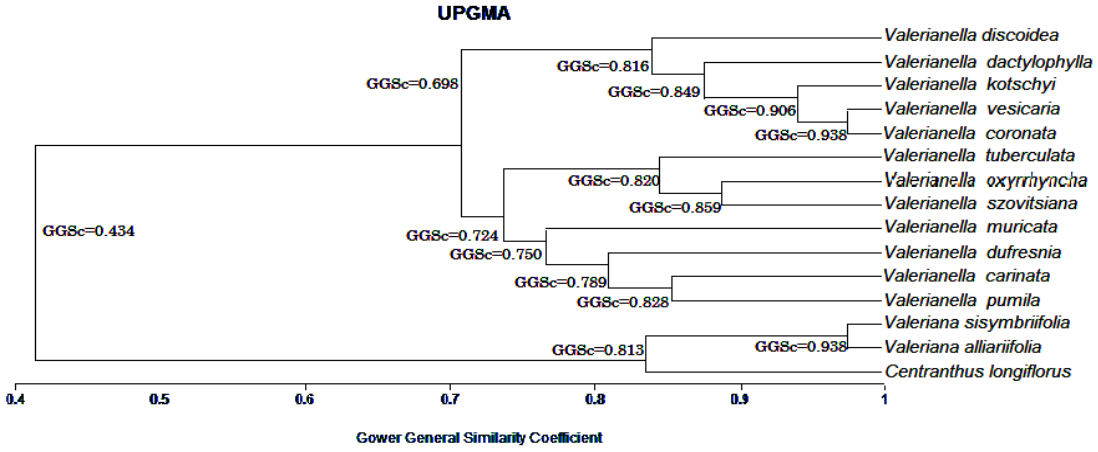

Figure (2): Dendrogram of morphological characters, the similarity degrees among studied taxa of Valerianaceae.

Among the *Valerianella* species, *Valerianella coronata* and *Valerianella vesicaria* are similar at the level GGSc=0.938, this pair meets *Valerianella kotschyi*, *Valerianella dactylophylla*, and *Valerianella discoidea* at levels GGSc= 0.906, 0.849 and 0.816 respectively. *Valerianella szovitsiana* and *Valerianella oxyrrhyncha* are similar at level GGSc=0.859, and *Valerianella carinata* and *Valerianella pumila* similar at level GGSc=0.828 and GGSc=0.724, and both of them are also included in group. *Valerianella tuberculata*, *Valerianella dufresnia*, and *Valerianella muricata*, are more similar than *V. coronata* and *V. vesicaria* which contacted at the level GGSc=0.698.

3.3. Palynology:

The similarity coefficient for pollen morphology data sets was cleared in table (3), and the dendrogram formed by cluster analysis of pollen morphology showed in figure (3). The absolute similarity is marked by 1.000. According to the resultant dendrogram indicating to a close affinity among the OTUs as *V. oxyrrhyncha*, *V. tuberculata* and *V. szovitsiana* which are similar at the absolute level 1.000, and the OTUs *V. dufresnia*, *V. dactylophylla* and *V. discoidea* which are similar at the absolute level 1.000. These two groups are similar to each other at the level GGSc=0.778. The *V. carinata*, *Va. sisymbriifolia*, and *V. muricata* are similar at the level GGSc=0.778, and the *Va. alliariifolia* and *V.*

pumila are similar at the level GGSc=0.778, this confusion between the species of the two genera suggests the indistinctness of pollen grain in family's taxa delimitation. *V. coronata*, *V. kotschyi,* and *V. vesicaria* are similar at the level GGSc=0.833, connects the former groups at the low level of GGSc=0.566, and all groups converge at the lowest similarity level GGSc=0.471, with a less similar cluster of *V. carinata*, *Va. sisymbriifolia,* and *V. muricata*.

Table (3): Similarity matrix coefficient of pollen morphology characters for Valerianaceae taxa.

CLUSTER ANALYSIS UPGMA Data in random input order
Imported data - Palynological data: Analyzing 9 variables (characters) x 14 cases (species)
Gower General Similarity Coefficient Similarity matrix

	Va. alliariifolia	*Va. sisymbriifolia*	*V. pumila*	*V. carinata*	*V. muricata*	*V. coronata*	*V. kotschyi*	*V. vesicaria*	*V. dufresnia*	*V. szovitsiana*	*V. tuberculata*	*V. oxyrrhyncha*	*V. dactylophylla*	*V. discoidea*
Va. alliariifolia	1.000													
Va. sisymbriifolia		0.556	1.000											
V. pumila	0.778	0.556	1.000											
V. carinata	0.333	0.778	0.333	1.000										
V. muricata	0.333	0.778	0.333	0.778	1.000									
V. coronata	0.667	0.444	0.444	0.444	0.444	1.000								
V. kotschyi	0.556	0.333	0.333	0.556	0.333	0.889	1.000							
V. vesicaria	0.778	0.556	0.556	0.333	0.333	0.889	0.778	1.000						
V. dufresnia	0.556	0.556	0.333	0.778	0.556	0.667	0.778	0.556	1.000					
V. szovitsiana	0.778	0.333	0.556	0.556	0.333	0.667	0.778	0.556	0.778	1.000				
V. tuberculata	0.778	0.333	0.556	0.556	0.333	0.667	0.778	0.556	0.778	1.000	1.000			
V. oxyrrhyncha	0.778	0.333	0.556	0.556	0.333	0.667	0.778	0.556	0.778	1.000	1.000	1.000		
V. dactylophylla	0.556	0.556	0.333	0.778	0.556	0.667	0.778	0.556	1.000	0.778	0.778	0.778	1.000	
V. discoidea	0.556	0.556	0.333	0.778	0.556	0.667	0.778	0.556	1.000	0.778	0.778	0.778	1.000	1.000
	Va. alliariifolia	*Va. sisymbriifolia*	*V. pumila*	*V. carinata*	*V. muricata*	*V. coronata*	*V. kotschyi*	*V. vesicaria*	*V. dufresnia*	*V. szovitsiana*	*V. tuberculata*	*V. oxyrrhyncha*	*V. dactylophylla*	*V. discoidea*

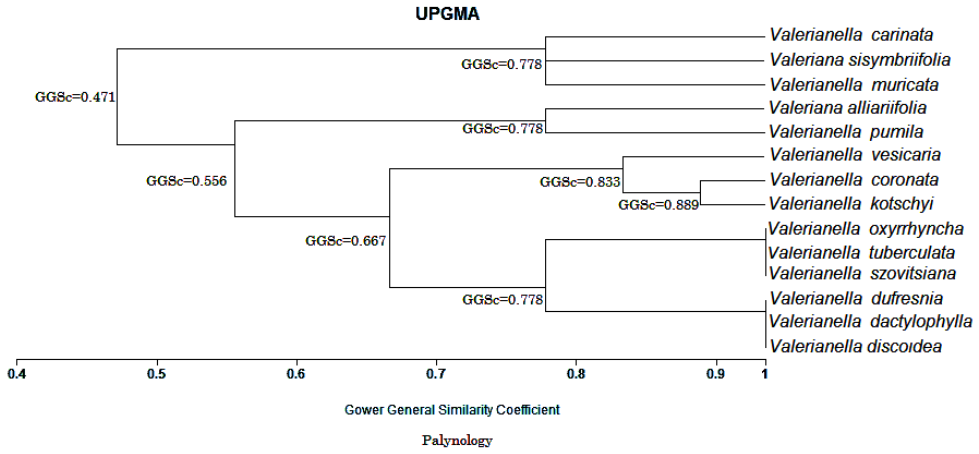

Figure (3): Dendrogram of pollen characters, the similarity degrees among studied taxa of Valerianaceae.

3.4. Anatomy:

The similarity coefficient for pollen morphology data sets was cleared in table (4), and marked by 1.000. The anatomical analysis dendrogram showed that the two species *V. muricata* and *V. pumila* have high similarity at level GGSc=0.848, whereas the species *V. dactylophylla* is more similar with *V. oxyrrhyncha* at level GGSc=0.808 than *V. muricata* and *V. pumila* which they made a group with *V.* the dendrogram formed by cluster analysis of pollen morphology showed in figure (4). The absolute similarity is

tuberculata and *V. carinata* at different levels. The other OTUs group of *V. vesicaria*, *V. kotschyi*, *V. coronata*, *V. dufresnia*, and *V. discoidea* have similarity at different levels. At level GGSc=0.716 there are two groups: *Va. alliariifolia* with *C. longiflorus*, and *Va. sisymbriifolia* with former groups. Finally, *V.*

szovitsiana has single clade sister at lower similarity level GGSc=0.683, and suggesting the less resemblance with all other family's taxa.

Table (4): Similarity matrix coefficient of anatomical characters for Valerianaceae taxa.

CLUSTER ANALYSIS UPGMA

Imported data:- Anatomical data: Analyzing 66 variables x 15 cases (species)

Gower General Similarity Coefficient Similarity matrix

	C. longiflorus	*Va. alliariifolia*	*Va. sisymbriifolia*	*V. pumila*	*V. carinata*	*V. muricata*	*V. coronata*	*V. kotschyi*	*V. vesicaria*	*V. dufresnia*	*V. szovitsiana*	*V. tuberculata*	*V. oxyrrhyncha*	*V. dactylophylla*	*V. discoidea*
C. longiflorus	1.000														
Va. alliariifolia	0.818	1.000													
Va. sisymbriifolia		0.742	0.803	1.000											
V. pumila	0.727	0.727	0.773	1.000											
V. carinata	0.742	0.803	0.788	0.773	1.000										
V. muricata	0.697	0.697	0.773	0.848	0.803	1.000									
V. coronata	0.652	0.652	0.697	0.712	0.788	0.773	1.000								
V. kotschyi	0.652	0.652	0.667	0.773	0.788	0.803	0.758	1.000							
V. vesicaria	0.712	0.773	0.697	0.773	0.788	0.773	0.758	0.818	1.000						
V. dufresnia	0.636	0.697	0.742	0.727	0.773	0.788	0.773	0.773	0.712	1.000					
V. szovitsiana	0.576	0.606	0.712	0.667	0.712	0.667	0.682	0.682	0.682	0.667	1.000				
V. tuberculata	0.742	0.773	0.788	0.773	0.818	0.803	0.727	0.758	0.758	0.773	0.712	1.000			
V. oxyrrhyncha	0.667	0.697	0.773	0.818	0.803	0.818	0.773	0.773	0.773	0.758	0.758	0.773	1.000		
V. dactylophylla	0.727	0.727	0.742	0.818	0.803	0.818	0.803	0.742	0.773	0.727	0.727	0.803	0.788	1.000	
V. discoidea	0.652	0.682	0.697	0.742	0.788	0.773	0.788	0.818	0.788	0.803	0.712	0.727	0.773	0.803	1.000
	C. longiflorus	*Va. alliariifolia*	*Va. sisymbriifolia*	*V. pumila*	*V. carinata*	*V. muricata*	*V. coronata*	*V. kotschyi*	*V. vesicaria*	*V. dufresnia*	*V. szovitsiana*	*V. tuberculata*	*V. oxyrrhyncha*	*V. dactylophylla*	*V. discoidea*

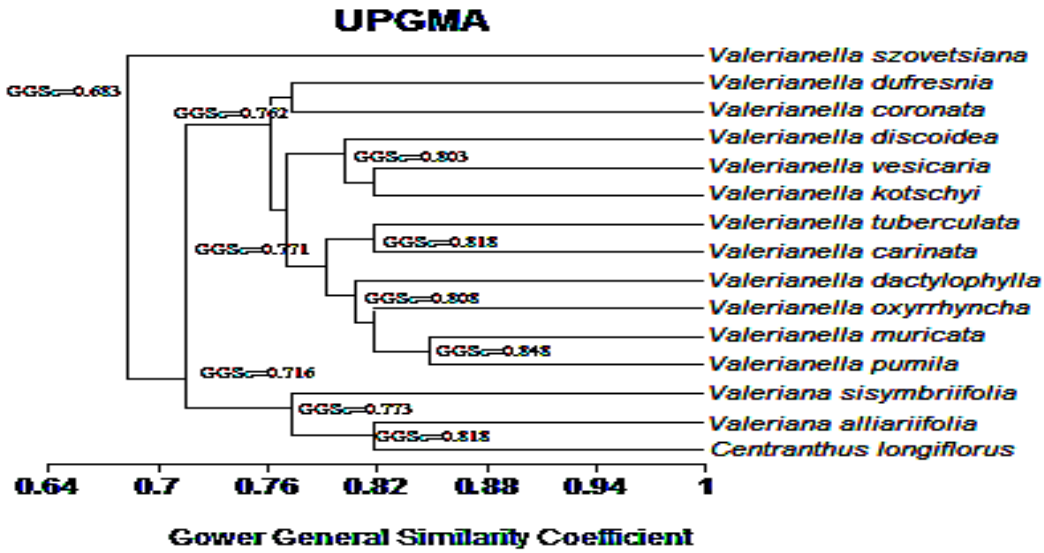

Figure (4): Dendrogram of anatomical characters, the similarity degrees among studied taxa of Valerianaceae.

3.5. Cytology:

The similarity coefficient for cytologydata sets was cleared in table (5), and the dendrogram formed by cluster analysis of cytology showed in figure (5). The absolute similarity is marked by 1.000. The dendrogram constructed from cytological investigation, revealed that the cluster of *V. discoidea*, *V. vesicaria*, *V. coronata*, and *V. pumila*, and cluster of *V. dactylophylla*, *V. dufresnia*, *V. muricata*, *Va. sisymbriifolia* and *Va. alliariifolia* were similar at absolute level, and they have a low similar level at GGSc=0.625. These two groups are more similar than the cluster of *C. longiflorus* and *V. szovitsiana* (GGSc=1.000), and *V. oxyrrhyncha* (GGSc=0.883). The former groups contact with the latter group at the lowest level of resembling GGSc=0.417.

Table (5): Similarity matrix coefficient of cytological characters for Valerianaceae taxa.

CLUSTER ANALYSIS UPGMA

Important data- Cytological Data: Analyzing 6 variables (caryotypes) x 15 cases (species)

Gower General Similarity Coefficient Similarity matrix

	C. longiflorus	*Va. alliariifolia*	*Va. sisymbriifolia*	*V. pumila*	*V. carinata*	*V. muricata*	*V. coronata*	*V. kotschyi*	*V. vesicaria*	*V. dufresnia*	*V. szovitsiana*	*V. tuberculata*	*V. oxyrrhyncha*	*V. dactylophylla*	*V. discoidea*
C. longiflorus	1.000														
Va. alliariifolia	0.500	1.000													
Va. sisymbriifolia	0.500	1.000	1.000												
V. pumila	0.167	0.667	0.667	1.000											
V. carinata	0.333	0.833	0.833	0.500	1.000										
V. muricata	0.500	1.000	1.000	0.667	0.833	1.000									
V. coronata	0.167	0.667	0.667	1.000	0.500	0.667	1.000								
V. kotschyi	0.167	0.667	0.667	0.833	0.667	0.667	1.000								
V. vesicaria	0.167	0.667	0.667	1.000	0.500	0.667	1.000	0.667	1.000						
V. dufresnia	0.500	1.000	1.000	0.667	0.833	1.000	0.667	0.667	0.667	1.000					
V. szovitsiana	1.000	0.500	0.500	0.167	0.333	0.500	0.167	0.167	0.167	0.500	1.000				
V. tuberculata	0.667	0.833	0.833	0.500	0.667	0.833	0.500	0.500	0.500	0.833	0.667	1.000			
V. oxyrhyncha	0.833	0.667	0.667	0.333	0.500	0.667	0.333	0.333	0.333	0.667	0.833	0.833	1.000		
V. dactylophylla	0.500	1.000	1.000	0.667	0.833	1.000	0.667	0.667	0.667	1.000	0.500	0.833	0.667	1.000	
V. discoidea	0.167	0.667	0.667	1.000	0.500	0.667	1.000	0.667	1.000	0.667	0.167	0.500	0.333	0.667	1.000
	C. longiflorus	*Va. alliariifolia*	*Va. sisymbriifolia*	*V. pumila*	*V. carinata*	*V. muricata*	*V. coronata*	*V. kotschyi*	*V. vesicaria*	*V. dufresnia*	*V. szovitsiana*	*V. tuberculata*	*V. oxyrrhyncha*	*V. dactylophylla*	*V. discoidea*

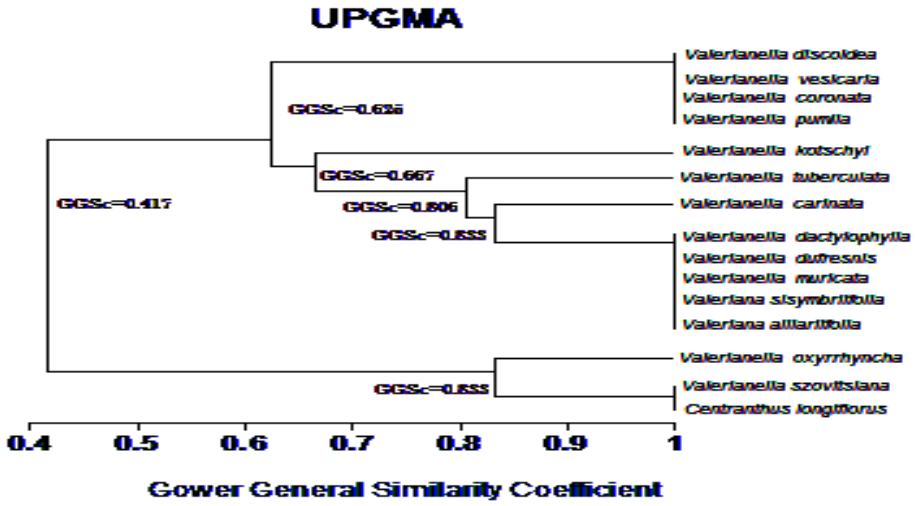

Figure (5): Dendrogram of cytological characters, the similarity degrees among studied taxa of Valerianaceae.

4. DISCUSSION:

In the present study, the cluster analyzing based on the epitome of different morphological, palynological, anatomical and cytological characters. As mentioned by (Sneath and Sokal, 1973) and (Stuessy, 1990).

The computer cluster analyzing for the main characters of the family's genera showed the distinct differences between *Valerianella* with *Valeriana* and *Centranthus* which they more similar in their characters than the *Valerianella*.

The dendrogram constructed from morphological characters, manifested morphologically the great similarity between the *Valeriana* species than the *C. longiflorus*, as well as this group has a single sister clade connected with the cluster of *Valerianella* species at the lower level. It seems these distances among them comes from the dissimilarity in duration, dense and types of indumenta, branching pattern, calyx modification, inflorescence type, number of stamens and ovary locules, fruit shapes, and habitat of growth, as (Aras *et al*., 2005) revealed that the existence of environmental and geographical groups with higher resemblances. The convergences among *Valerianella* species due to the similarity from habit, leaf type, indumenta types, branching and inflorescence pattern, and the divergences caused by dissimilarity from calyx tube shape and lobes number, and all fruits characters such as fruit shape, seed shape, and type of trichomes.

Dendrogram formed from pollen morphology showed some confusion among the family members. The species were divided into four similarity groups, three of them are similar at the GGSc=0.778 level, the convergence is a false synapomorphy caused by parallelism by the similarity in pollen size and polarity view. But the fourth group has the actual similarity at GGSc=0.889 and 0.833 level, the convergence due to the similarity in pollen shape, size and polarity.

Dendrogram constructed from anatomical characters showed the regular relationship among all taxa, the similarity and dissimilarity mostly caused by the internal characters. The divergence of *V. szovitsiana* due to the dissimilarity in epidermal tissue, stomata types and index, type of trichomes, ordinary cells, stem outline, leaf margin, and midrib outline. The species was a single taxon out of groups, meets other OTUs at a lower level of similarity GGSc=0.716. The other OTUs were divided into two groups; the first group has interpenetrated relationships among all other *Valerianella* species, where they are in different similarity levels, and the second group indicates the close relationship between both *Valeriana* species and *C. longiflorus*. These convergences due to the similarity of epidermal cells, absent or present the trichomes, the cross section outlines of stems, leaves, and fruits and occurrence of inulin similarities.

Dendrogram constructed from cytological karyotypic characters suggests the close relationship among all taxa at a high level of similarity. The convergences due to the equivalents of chromosome numbers for OTUs *V. discoidea*, *V. vesicaria*, *V. coronata* and *V. pumila*, and the occurrences of *V. dactylophylla*, *V. dufresnia*, *V. muricata*, *Va. sisymbriifolia* and *Va. alliariifolia*, with *V. szovitsiana* and *C. longiflorus*, each group is similar at high level GGSc=1.000 in their groups. The clusters divergences of groups are caused by polyploidy appearance of the chromosomes.

5. CONCLUSIONS:

The current work demonstrated a fully supported relationship among studied genera and species of Valerianaceae family, and it has shown approximately a fair relationship between the species whether with their morphology and internal characters or even the environment they were distributed.

Conflict of Interest (1)

There is no conflict of interest.

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RESEARCHPAPER

Spatial Distribution of Heavy Metals in Surface Soil Horizons Surrounding Erbil Steel Company (ESC) Areas

Ismaeel T. Ahmed and *Muna M. Abd-Alhameed
Department of Soil and Water, College of Agriculture, Salahaddin University-Erbil, Kurdistan Region, Iraq

A B S T R A C T:

This study was carried out during September 2015 to investigate the impact of Erbil Steel Company (ESC) on soil pollution of the surrounding area. Soil samples were collected from 40 sites around Erbil Steel Company at different distances and eight directions (N, NE, E, ES, S, SW, W and NW) on five different circled belts named (50, 100, 500, 1000 and 2000 m) far from the source of the pollution in the study area and 1 sample as control sample. The samples were analyzed for some physico-chemical properties, as well as for the five heavy metals Iron (Fe), Chromium (Cr), Lead (Pb), Zinc (Zn) Copper (Cu) and Nickel (Ni) by using a portable x-ray fluorescence (XRF). As a results of the distance that effected on the heavy metals concentration are: Fe had the highest concentration value and ranged from 4752.00 to 15873.69 $mg.kg^{-1}$ while Cr had the lowest concentration value and ranged from 4.00 to 74.00 $mg\ kg^{-1}$ and Pb, Zn, Cu and Ni concentrations ranged from (56.00 to 378.00, 1.27 to 220.40, 8.00 to 85.20 and 1.43 to 10.17 $mg.kg^{-1}$) respectively. An interpolation property was applied to prepare the special map of natural distribution of heavy metals of the soils in the studied area using Arc GIS 10.1 programs.

KEYWORDS : Soil pollution ,Erbil steel company, Heavy metals, Interpolation map.
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INTRODUCTION :

Soil pollution is one of the most serious problems in the world with long term consequences on human life. In recent years, with the rapid development of industry, various dangerous pollutants such as heavy metals (Cu, Cr, Fe, Mn, Ni, Pb and Zn) have been released due to production, smelting and tailings and got deposited in soils around industrial areas causing serious pollution and soil quality degradation. Iron and steel manufacturing produces important quantities of solid wastes containing heavy metals.Heavy metals and some trace elements are biologically toxic and can affect and threaten the health of human being owing to their accumulation and persistence in the compartments of the food chain. Hence, it is very important to investigate and monitor soil contamination for economic sustainable development and people's health (Ene and Bosneaga, 2010).

Several analytical techniques have been extensively employed for environmental pollution monitoring, such as: X-Ray Fluorescence Analysis (XRF), Atomic Absorption Spectrometry (AAS) and Inductively Coupled Plasma Spectrometry-Atomic Emission Spectroscopy (ICP-AES) (Popescu*et al*., 2009). XRF is one of the preferred methods for the assessment of the elemental composition of soils and sediments, because XRF has the advantage of being a rapid and inexpensive method with a simple sample preparation. Quantitative and qualitative analyses by XRF without chemical digestion and a great number of elements can be determined

***Corresponding Author:**
Ismaeel T. Ahmed
E-mail: ismaeel.ahmed@su.edu.krd
Haval_haji90@yahoo.com
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simultaneously in a short time (Ene*et al.*, 2009).The main aims of the present study was to produce the point interpolation theme maps of the heavy metals concentration of the selected area.

2. MATERIALS AND METHODS

2.1. Descriptions of the study area of (ESC)

The current study was the 1st investigation that carried out at different locations surrounding Erbil Steel Company (ESC) which is located at about 15 km west outskirts of Erbil city (Latitude.36˚ 08' 18"N, Longitude.43˚ 47' 43"E), in order to assess the effect of steel industry refuses on soil pollution Fig (1).

Fig.1. Erbil Steel Company (ESC)

2.2. Erbil Steel Company (ESC)

Its establishment commencing in 2006 in Erbil Kurdistan region, ESC has started integrated steel production in December 2006, taking into account its production and employment capacity. Erbil steel company currently is the most prominent heavy industry investment in the region and produces as an average of about 700 ton/day collected scrap irons from different sources and locations in the main raw material of the steel bar manufacturing. All incoming raw materials are melted in induction furnace at 1700˚C in the furnace most of the generated or melted matter are heavy metals, including cadmium, chromium, lead and nickel. ESC has also a fume extraction system and this causing pollution (Anonymous, 2010).

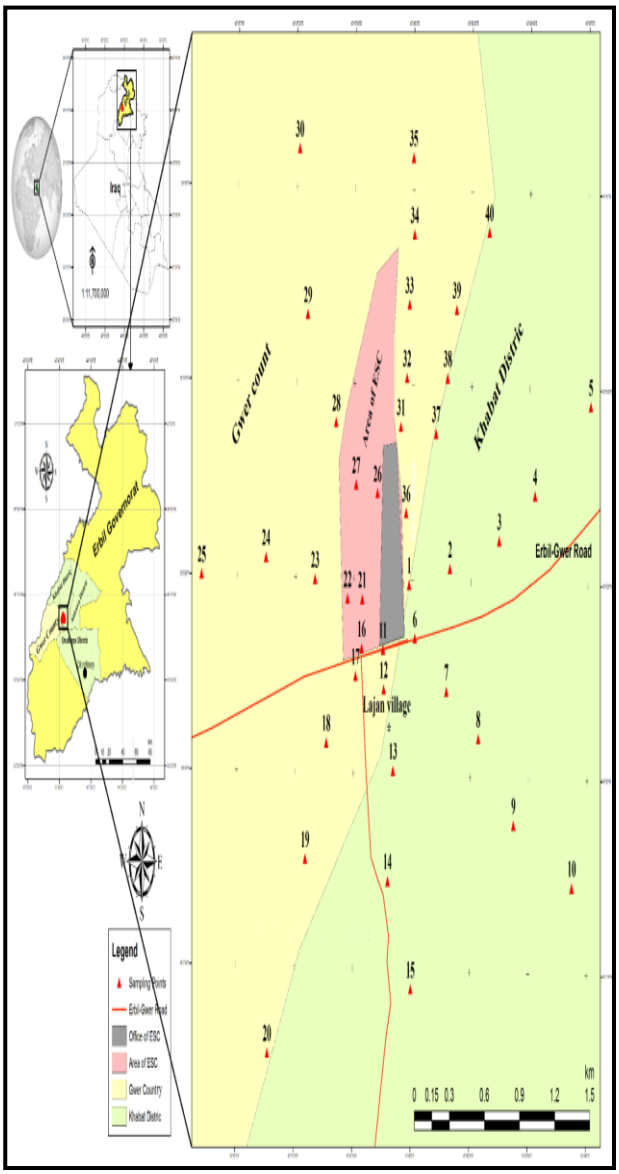

Fig. 2. Location map of the Study area of Erbil Steel Company

2.3. Soil Sampling around the (ESC)

Soil samples were collected in September 2015 from 41 sites of the surface layer using an auger. The soil samples were taken from sites at eight directions (N, NE, E, ES, S, SW, W and NW) on five different circled belts named (50, 100, 500, 1000 and 2000 m) far from the source of the pollution (Ruffel*et al*., 2011). Soil sample were mixed thoroughly and then passed through a 2 mm sieve. Quantitative amount of soil samples were taken around the ESC in order to measure total heavy metal concentration in soil samples (mg kg^{-1}) after drying, sieving by 2 mm and powdering of the material. Soil samples were analyzed by XRF method and heavy metals were measuring by portable (X-MET7500) XRF analyzer (X-Ray Fluorescence) (Sitko*et al*., 2004).The selected sites for soil sampling around ESC are shown in Fig (2).

2.4. The point interpolation maps

Soil pollution by heavy metals is commonly assessed by interpolating concentrations of heavy metals (Fe, Cu, Cr, Ni, Zn and Pb) sampled at location points so that each heavy metal represented in a separate map Fig. (3), using Arc GIS 10.1 kriging methods. (Delava and Safari, 2016).

Interpolation refers to the process of estimating the unknown data values for specific locations using the known data values for other points. In many instances we may wish to model a feature as a continuous field (i.e. a 'surface'), yet we only have data values for a finite number of points. It therefore becomes necessary to interpolate (i.e. estimate) the values for the intervening points. For example, we may have measurements of the depth of a particular geological stratum from a number of bore holes, but if we want to model the stratum in 3-dimensions then we need to estimate its depth for places where we do not have borehole information (Burrough and McDonnell, 1998). Interpolation is a process of using points with known values to estimate values at other points forming the surface. Also interpolation is used to convert the data from point observations to the continuous fields so that the spatial patterns sampled by these measurements can be compared with the spatial patterns of other spatial entities. Spatial interpolation is thus a means of converting point data into surface data. For example while mapping precipitation if there is no weather reporting station within the grid cell, an estimate is based on nearby weather stations. (Setiyoko and Kumar, 2012).

2.5 Statistical analysis

The statistical analysis were conducted using statistical package for social science program version (Stat graphics-plus-professional-version 16.0).Software program and the person correlation coefficient. The correlation was produced at a 95% confidence level (2-tailed) (Hartman, 2000) were used for analysis the data. The following analysis variances were compared by the Least Significant Difference (LSD) at ($P \leq 0.05$) level among treatments, including three replications (Depth) with forty treatments (Distance).

3. RESULTS AND DISCUSSION

3.1. Soil map on the basis of interpolation heavy metals.

Mapping the spatial distribution of contaminants in soils is the basis of pollution evaluation and risk control. Interpolation methods are extensively applied in the mapping processes to estimate the heavy metal concentrations at sampled sites. Interpolation accuracy is related to the precise definition of the polluted area and its boundaries. Consequently, this directly affects the accuracy of pollution assessment (Xie*et al*., 2011). Figure 3 shows the spatial distribution patterns of Cr, Cu, Fe, Ni, Pb and Zn concentrations respectively. The results indicated that the point interpolation method helps to make soil chemical andphysical properties and heavy metals concentration maps and their distribution patterns for selected study area. Table (1) declared the results in the present study of the heavy metals in the soil around of Erbil steel company were higher than the other studied sites or in comparing with control sample this agree with (Khudhur*et al*. 2016).

Table 1. Concentration of heavy metals of studied soils at different distance from ESC

Samples Direction	Locations No.	Distance from the factory (m)	Heavy metals (mg kg^{-1})					
			Cr	Cu	Fe	Ni	Pb	Zn
E	1	50	ND	ND	5732	8.60	56	22.80
	2	100	ND	ND	5032	6.45	ND	3.80
	3	500	ND	ND	5510	4.30	ND	4.43
	4	1000	ND	ND	5540	7.17	ND	3.17
	5	2000	ND	ND	5366	8.93	ND	2.53
	LSD .05		-	-	789.1	6.72	-	15.07
SE	6	50	6	14.0	6325	4.30	103	37.75
	7	100	17	ND	5840	8.60	ND	5.90
	8	500	ND	ND	5766	5.73	ND	ND
	9	1000	ND	ND	5256	5.73	ND	1.27
	10	2000	ND	ND	5842	5.73	ND	0.63
	LSD .05		-	-	348.0	8.80	-	13.12
S	11	50	5.0	16.0	6700	1.43	90	37.60
	12	100	ND	ND	4952	5.73	ND	12.63
	13	500	4.0	ND	5910	7.17	ND	3.80
	14	1000	ND	ND	6008	5.73	ND	3.80
	15	2000	ND	ND	5068	7.87	ND	3.17
	LSD .05		-	-	1322.2	6.50	-	13.73
SW	16	50	6.0	44.8	1075	ND	288.0	115.90
	17	100	6.2	13.0	6435	2.87	56.0	39.90
	18	500	ND	ND	5596	5.73	ND	3.80
	19	1000	4.7	ND	5386	5.73	ND	1.90
	20	2000	ND	ND	5298	7.17	ND	1.90
	LSD .05		-	-	1676.	9.47	-	63.77
W	21	50	14	24.0	1194	ND	252.0	155.8
	22	100	ND	54.0	1134	9.00	378.0	180.5
	23	500	ND	ND	5728	9.80	ND	7.60
	24	1000	8.0	ND	5262	10.2	ND	3.80
	25	2000	ND	ND	5500	5.73	ND	2.85
	LSD .05		-	-	5535.	4.33	-	102.96
NW	26	50	ND	58.8	1122	0.00	207	153.90
	27	100	ND	85.2	1587	2.87	234	220.40
	28	500	ND	ND	5692	5.73	ND	4.75
	29	1000	ND	ND	6299	5.73	ND	1.90
	30	2000	ND	ND	5184	0.00	ND	ND
	LSD .05		-	-	1013.98	7.00	-	126.77
N	31	50	74.	ND	6084	9.90	ND	3.17
	32	100	ND	ND	5276	8.67	ND	3.80
	33	500	4.0	ND	4752	5.73	ND	5.07
	34	1000	ND	8.00	5178	1.50	ND	1.27
	35	2000	ND	ND	5432	4.30	ND	1.27
	LSD .05		-	-	710.9	5.92	-	4.28
NE	36	50	9.0	ND	5672	2.87	ND	5.70
	37	100	5.0	ND	5244	8.60	ND	1.90
	38	500	4.0	ND	5960	5.73	ND	1.90
	39	1000	ND	ND	5676	5.73	ND	1.27
	40	2000	4.7	ND	6121	5.73	ND	1.27
	LSD .05		-	-	827.7	7.28	-	3.79
Control		4750	ND	ND	5263	ND	ND	ND

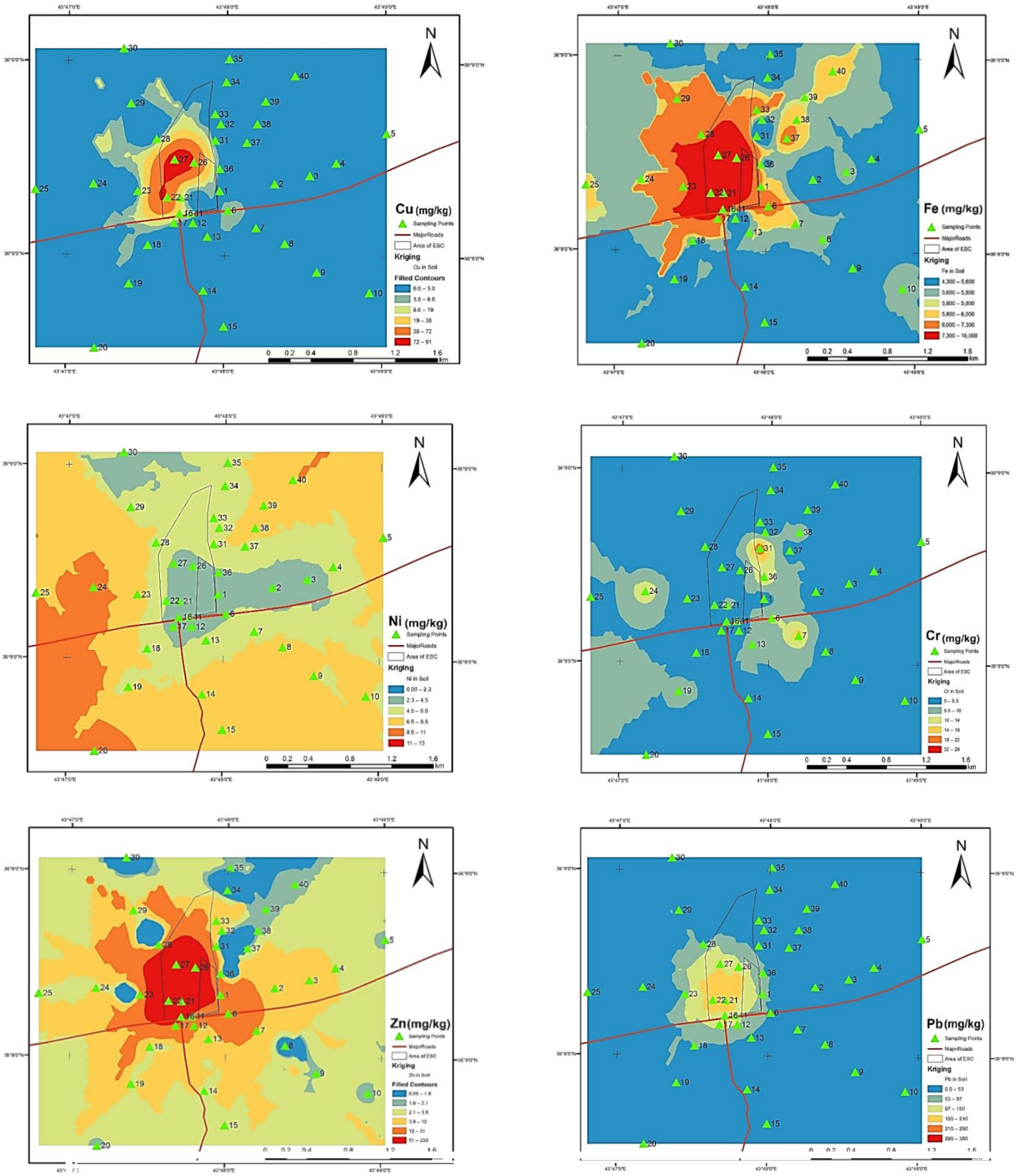

Fig. 3. Patterns of (Cu, Ni, Zn, Fe, Cr and Pb) contents for the selected area derived by point interpolation

In general, the high accumulation of (Cr, Cu, Fe, Ni, Pb and Zn) in the center of the regions and decreasing these metals concentrations by moving away from the center of study area. Obviously the results of metal occurrence indicated that while Fe was the most dominant metal and highest concentration this result agree with (Khudhur*et al.,* 2018),This may be due to the locations were used as a storage of scraps most of wastes were scrap cars which manufactured from steels or iron this result agree with (Lak, 2007) observed that the Iron has earlier been reported to the most abundant mineral in Erbil plain soils. while the lowest value of iron may be due to this location was away from the source of pollution (steel scrap site) and this agree with (Simon and Fadoju, 2016) obtained that the concentration of iron decreased with increasing horizontal distances from the scrap metal dump. There was a negative correlation between iron and nickel so the results of interpolation showed that the distribution of Ni was unlike iron which was lower in the center and being higher by faring away from the center of study area. While the leachate passes into soil, polluted materials are adsorbed on the soil. The high amount of chromium in location (31) may be due to this location lies near from the ESC and chromium originates in the environment mainly from anthropogenic sources industry emissions and combustion processes. The dumping of industrial waste materials significantly increase chromium concentration in soil Further, Cr found in particles of the atmosphere which released from the smoke of factories, while the low amount of Cr in locations (33 and 38) may be due to these locations lies away from factory and Cr in the soil were higher within the factory than outside of the factory (Salami *et al.,* 2014). This high value may be due to this location is located inside the ESC, this company smelting scraps of steel and these scraps melting containing amount of copper. This is agree with (Janke*et al.,* 2000) detected that Cu is introduced into steel melts by the smelting of scrap which originates from steel grades containing an increased amount of copper. For example, structural steels can contain up to 0.5% Cu. As well as this high value of copper may be due to the dust released from steel factory which contained amount of heavy metals like Cu, Pb and others and this agree with (Skorbiłowicz and Samborska, 2014) studied that the emissions of dust from factories containing heavy metals, especially Cu and Pb, into the atmosphere from steel works, while the lowest mean value of copper (8 mg. kg^{-1}) was obtained from location (34) in the N direction which lies (1000 m) away from the source of pollution and this low value may be due to this location far away from the source of pollution The lowest and highest value of Pb both lies 100 m away from source of pollution but the highest value located at W direction while the lowest value located at SE direction and this means that this metal is deposited into soil at various distances depending on wind velocity, metal concentration in soil can vary greatly according to the strength and direction of wind, type of soil, composition and soil pH (Michael *et al.,* 2015). The high value of Zn may be due to result from dust or fume of steel company, this location is inside of ESC and this site exposed to dust of company more than another site and this is agree with (Xu*et al.,* 2003) obtained that high amount of zinc is a result of inhaling large amounts of zinc as dust or as fumes associated with metal fume fever. As well as the causes of increasing Zn is a result of steel industry and this is agree with (Elekes, 2014).

4. CONCLUSIONS

According to the results obtained from this study the ESC refuses effects on soil properties such as increasing the heavy metals concentration at surrounding area of the factory. The velocity and direction of wind affected upon the concentration and transferring of heavy metals that present at the direction of the wind. The distance and depth effects on the distribution of heavy metals in the study area of ESC and some of the heavy metals were increased with depth and distance while some of them were decreased.

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RESEARCH PAPER

Genetic Diversity Among horse Lines in Erbil Region Using RAPD Markers

Hurea S. Abdulrazaq [1], Chiman H. Saeed [2], Nazhad H. Qader [3]

[1] Department of Animal Resource ,College of Agriculture, Salahaddin University-Erbil / Kurdistan Region, Iraq
[2] Medical Research Center, Hawler Medical University, Erbil / Kurdistan Region, Iraq
[3] Department of Animal Resource ,College of Agriculture, Salahaddin University-Erbil / Kurdistan Region, Iraq

A B S T R A C T:

Genetic diversity is the basis for present day diversified living systems and future genetic improvement needs. Within the framework of breed conservation, genetic characterization is important in guarding breeds and is a prerequisite for managing genetic resources. The objective of this study was to use the RAPD technique to evaluate genetic diversity and relatedness within and among four horse line (white, Gray, Brown, Black). To our knowledge there is currently no information about RAPD genetic markers that detect genetic polymorphism in Erbil/Iraqi horse breeds. Information from this work provides basic genetic knowledge that is critical for conservation and breeding programs. Random amplification of polymorphic DNA (RAPD-PCR) was done by using 10 primers from GenScript USA company. A total of (6) Primers out of the (10) Primers gave results to find a complementary DNA Genomic sites, (OPQ-05, OPQ-06, OPQ-08, OPQ-09, OPQ-10, OPQ-12). These primers amplified on average 7 to 53 bands of sizes varying from 100bp to 1500bp. A total of 150 diagnostic bands were scored within RAPD profiles amplified by these 6 primers. Among 150 scorable bands 28 (18.67%) were recognized as polymorphic. UPGMA dendrogram based on Nei's genetic distance grouped the investigated horse line genotypes into two clusters. The first cluster includes(white, Brown, Black) whereas the second cluster include Gray which appeared to be most distant from the other lines. In conclusion, these results indicated the effectiveness of RAPD in detecting polymorphism between horse lines and their applicability in lines studies and establishing genetic relationships among the horse lines.

KEY WORDS: Genetic diversity, Genetic improvement, Polymorphism, RAPD markers, Horse.
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1. INTRODUCTION :

Horses have participated in civilization in close association with man as well as in both historical and contemporary societies. They have played major agricultural, economic and cultural roles. Horses are members of a family. In Erbil / Iraq, there are four lines of horses (white, gray, brown, black). Different horse has been developed to perform different functions.

The horses are used for racing, and others are used as high-stepping horses. Many people began to use horses in various activities such as horse sports. The development of molecular techniques has provided new possibilities for selection and genetic improvement of livestock. Research into eukaryotic genomes is influenced by the discovery of polymerase chain reaction and has contributed to the development and application of various DNA markers (Marle-Koster and Nel, 2003). The most important part of the evolutionary genetics toolkit is the molecular markers derived from the PCR reaction of genetic DNA (Holsinger et al., 2002). Meeting current production needs in different environments requires the genetic

* **Corresponding Author:**
Hurea Saber Abdulrazaq
E-mail:hurea.abdulrazaq@su.edu.krd or hurea.saber@gmail.com
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diversity of farm animals to allow for sustainable genetic improvement and to facilitate rapid adaptation to changing breeding targets (Notter, 1999). Genetic markers can measure genetic diversity. These have been used to estimate the genetic diversity of species (Zhang et al., 2006). Genetic patterns to identify genetic diversity that combines the attributes of interest and can be used for the introduction of important agricultural traits (Rehman et al., 2006). Using observable morphological characteristics that require a lot of time and effort and to solve the problem of maintaining pure strains, the use of molecular markers in maintaining the horse strain is more appropriate and less time consuming. To identify and estimate genetic distances, many researchers used multiple-randomized DNA (Williams et al., 1990; Welsh and McClelland, 1990) to study genetic diversity within the lines (Apostolidis et al., 2001; El-Soudy et al. , 2005; Eroglu and Arica, 2009) and gene mapping in farm animals (genetic link maps now available for horses, Shiue et al., 1999). The RAPD technology has provided a fast and efficient screen-based polymorphism sequence of DNA in a very large number of sites. The main advantage is that no advance information about the DNA sequence is required. A wide range of potential primers that can be used gives a great technique to diagnose power. Reproductive RAPD ranges can be found by careful selection of primers, improved PCR conditions for target species RAPD markers have been successfully used to estimate the genetic correlation between different groups of cattle, sheep, goats, buffalo and chickens (Mahfouz et al., 2008; Hassan et al., 2007; 2006; Abdul Rahman and Hafez, 2007; Okumos and Kaya, 2005, respectively). RAPD has the potential to detect polymorphisms among horse populations and their applicability in population studies and the establishment of genetic relationships between groups of horses (Bailey and Lear, 1994; Shiue et al., 1999; Apostolidis, 2001 and Egito et al., 2007). The aim of this study was to use RAPD technique to assess genetic diversity and interrelationships within and between four lines of horses (white, gray, brown, black). To our knowledge, there is currently no information on the genetic markers RAPD that reveal the genetic polymorphism in Erbil / Iraqi horse breeds. Information from this work provides basic genetic knowledge that is critical to conservation and education programs.

2. MATERIALS AND METHODS

2.1. samples collection

Blood samples were collected about 3 ml of 40 horse belonging to four different lines (white, gray, brown, black). Blood samples were collected in tubes containing anticoagulant (EDTA), stored at -20 ° C until DNA extraction.

2.2. Genomic DNA extraction

Extract DNA from 300 µl of blood Using Kit Promega USA (Beutler et al., 1990). All laboratory work was carried out at Erbil Medical Research Center / Hawler Medical University.The purity of the DNA samples ranged from 1.5 to 1.9 after the DNA quality isolated by the Nano Drop® spectrometer. The samples were then diluted to 30 ng / µl for use of RAPD PCR In the Salahaddin Research Center in Erbil / University of Salahaddin Erbil.

2.3. RAPD- PCR analysis

The amplification of RAPD was done using 10 primers (Table 1) from GenScript USA. A total of (6) Primers out of (10) Primers gave results to find Genomic DNA complementary sites, (OPQ-05, OPQ-06, OPQ-08, OPQ-09, OPQ-10, OPQ-12). The final volume was 25 µl and had 30 ng of DNA polymerase chain reaction (PCR), and 10 µM of each primer. Calculation temperatures for the Tm parameter were calculated based on GC sequence composition. The PCR program included an introductory step for attenuation at 94 ° C for 5 min followed by 40 cycles with 94 ° C for 1 min for DNA transcription, as illustrated with each primer, extension at 72 ° C for 1 min and final extension at 72 ° C 5 minutes. PCR products were tested with electrophoresis on 2% agarose gel in 1X TBE buffer (Promega, USA) stained with ethidium bromide. The pattern was amplified by ultraviolet light and photographed.

2.4. statistical analysis

Data recording and statistical analysis RAPD patterns were recorded because of (1) or absence (0). The similarity index between each group was

calculated using the formula: similarity = 2nxy / nx + ny. And use, genetic distance = 1- (2nxy / nx + ny). The polymorphism of each primer was calculated on the basis of the following formula: - polymorphism = (Np / Nt) × 100, NP = # polymorphic forms of random primer Nt = total number of sample primer domains (Bowditch et al., 1993).

3. RESULTS AND DISCUSSION

The discovery of polymerase chain reaction has contributed to research in eukaryotic genomes and in the development and application of various DNA markers (Marle-Koster and Nel, 2003). Molecular markers derived from the polymerase chain reaction (PCR) of DNA are an important part of the evolutionary genetics toolkit (Holsinger et al., 2002). By detecting genetic variation, genetic markers have provided useful information in areas such as population structure, gene flow, evolution, and relationship and relationship analysis (Feral, 2002). In this study, RAPD technique was used and random primers were tested to amplify the genetic DNA of these lines. Six of them were selected for further analysis, based on the existence of replicated RAPD profiles and distinct in the horse line (Table 1).

These primers are inflated on average from 7 to 53 groups of sizes ranging from 100bp to 1500bp. This observed range of products is assumed to be due to limitations in the ability to dissolve agarose particles in low molecular weights as well as inefficient prolongation interaction under the PCR conditions described in the higher molecular weights (Bowditch et al., 1993). A total of 150 diagnostic ranges were recorded within RAPD profiles amplified by these six prefixes. The total number of ranges, the multiform range, the percentage of the polymorphism and the size of the random prefixes are shown in Table (2).

Of the 150 bands, 28 (18.67%) were recognized as polymorphisms. In another study, (Egito et al. 2007) reported that 13 of 146 primers produced 44 polymorphic bands among different breeds of horses (Pantaneiro, Arabian and Thoroughbred). (Bailey and Lear 1994), who study the original Arabian breeds, found an average of 3.6 polymorphic band / primer groups in the RAPD. (Martinez .1996), who studies three Brazilian breeds (Lavradeiro, Crioulo and Campolina), found 2.9 bands / primer, using 29 markers. (Apostolidis et al. 2001) found 10.2 bands / primer and 51 polyphonic bands in Greek horses (Thessalian, Skyros Pony, Pinia, Cretan and Andravida). (Egito et al. 2007) Using RAPD-PCR markers in Study of genetic variability between Pantaneiro, Arabian and Thoroughbred horses they found 3.38 bands / primer. Thus, the percentage of primer capable of detecting polymorphisms among the assessed strains depends on the genetic background of the breeds and the genetic distance between the genome strains and their complexity (Ahlawat et al., 2004). The present study also revealed that the OPQ-8 primer produced the maximum number of bands (53), while the minimum number of bands (7) was recorded in the OPQ-12 primer in all strains (Table 2)**.**

It has been suggested that the OPQ-8 primer sequence may occur frequently in all breeds and record the largest number of polymorphic bands while the OPQ-12 Primer has been found to have the least polymorphism between the lines. (Sharma et al. 2001) found the RAPD technique that detects sufficient polymorphism within and between strains. Current and previous studies (Wei et al., 1994; Bailey and Lear, 1994; Smith et al., 1996; Egito et al., 2007) suggest that RAPD analysis requires a large number of random primers to detect polymorphism, The amplification of arbitrary primers depends on the presence or absence of corresponding primer binding sites in the genome. Thus, relatively large numbers of random primers are needed to detect enough polymorphisms to be used for gene analysis. Figures 1, 2, 3, and 4 show amplification pattern of genomic DNA of different breeds with various random declaimer primers .

The RAPD profile generated from these prefixes was used to estimate genetic diversity and interrelationships within and among horse lines based on the band frequency. The number of bands amplified in each primer was variable between the three horse breeds (Table 3). The

maximum number of bands were found in the gray line (44) followed by black (41).

UPGMA dendrogram is based on the Nei's genetic distance grouped the investigated horse line gene investigation structures into two clusters. The first clusters (white, brown, black) includes and the second clusters include (gray) that appears to be farther than the other lines. The dendrogram, based on the genetic distance, was constructed to show the phylogenetic relationships between the horses' lines (Fig. 4). Gray seems to be far from the other lines while the lines (white, brown, black) are closely related to the highest genetic similarity. The close identity of the white, brown and black lines suggests that all the original breeds are relatively similar to the same evolutionary tree line.

4. CONCLUSIONS

The present work was designed to determine the effectiveness of RAPD markers in detecting the polymorphism and estimating the genetic relationship between horse lines. (Gray) seems to be far from the other lines while the lines (white, brown, black) are closely related to the highest genetic similarity.

Table 1. Sequence, operon codes and GC content of random primers used to study variation in horse lines.

Primer Name	Sequence 5' to 3'	%GC content
OPQ-01	GGGACGATGG	70%
OPQ-12	TCTCCGCAAC	60%
OPQ-15	GGACGCTTCA	60%
OPQ-05	GGGTAACGCC	70%
OPQ-06	CAATCGCCGT	60%
OPQ-8	CAGCACCCAC	70%
OPQ-9	CCCCGATGGT	70%
OPQ-10	ACGGACGTCA	60%
OPQ-12	AGTGCGCTGA	60%
OPQ-11	TGTGCCCGAA	60%

Table (2) Total number of bands, polymorphic band, % of Polymorphism and their Size ranges from the random primers.

Primer number	Total number of bands	polymorphic band	% of Polymorphism	Size (bp) Min.	Max.
OPQ-05	15	1	6.67	500	1000
OPQ-06	25	12	48.00	100	1400
OPQ-8	53	5	9.43	220	1200
OPQ-9	15	7	46.67	100	800
OPQ-10	35	2	5.71	200	1500
OPQ-12	7	1	14.29	400	500
	150	28	18.67		

Table (3) Number Of Band Per Primer In Different Horse Line

Primer number	White	Gray	Brown	Black
OPQ-05	4	3	4	4
OPQ-06	6	5	2	12
OPQ-8	15	15	10	13
OPQ-9	1	11	1	2
OPQ-10	10	8	8	9
OPQ-12	2	2	2	1
Total	38	44	27	41

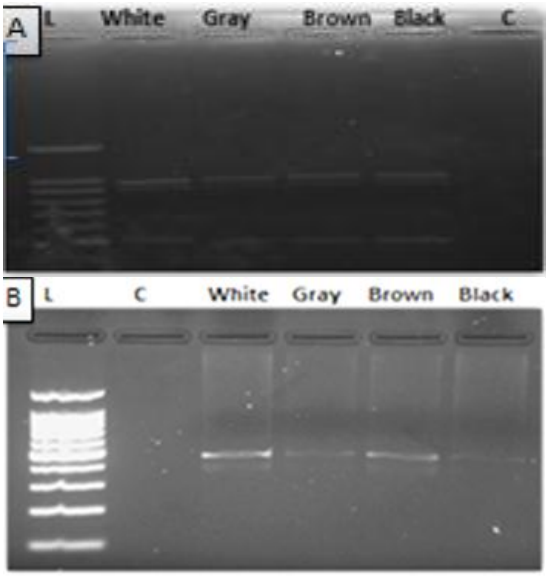

Fig (1). A. Gel electrophoresis for (OPQ-05) RAPD primer products. B. (OPQ-012) primer, for four different lines (white, Gray, Brown and Black) samples, C-Control, L - Molecular marker(100 bp plus ladder).

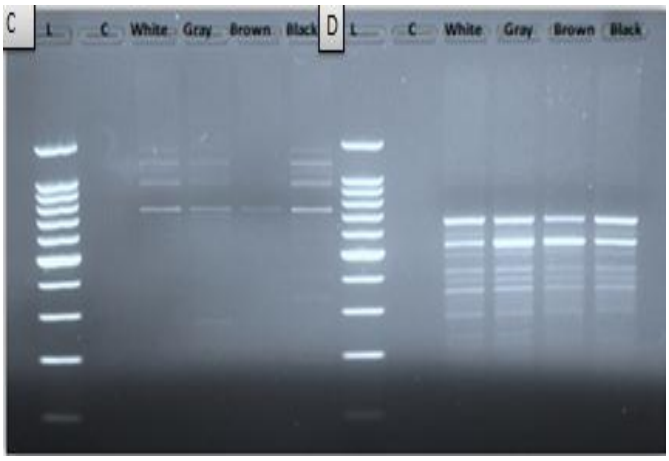

Fig (2) C. Gel electrophoresis for (OPQ-06) RAPD primer products. D.(OPQ-08) primer, four different line(white, Gray, Brown and Black) samples, C-Control, L - Molecular marker (100 bp plus ladder).

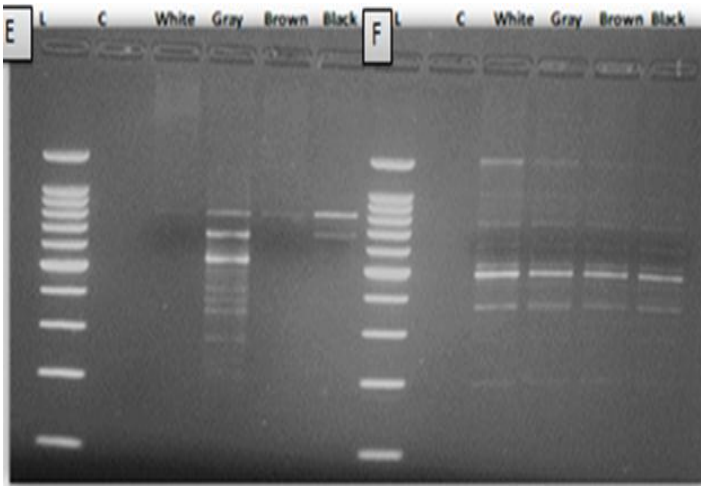

Fig (3). E. Gel electrophoresis for (OPQ-09) RAPD primer products. F. (OPQ-10). primer, four different line(white, Gray, Brown and Black) samples, C-Control, L - Molecular marker (100 bp plus ladder).

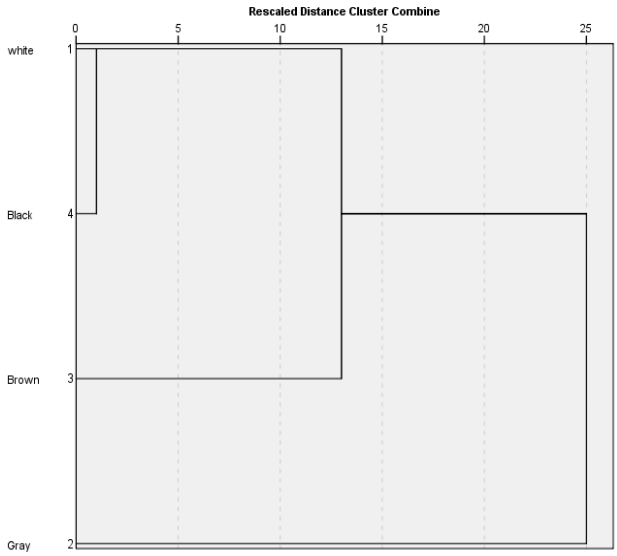

Figure (4) dendrogram of four Horse lines based on genetic distances among them.

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RESEARCH PAPER

Influence combination of Fruits Peel and Fertilizer Methods on growth and yield of Chickpea (*Cicer areitinum*) L. Plants

Halalal R. Qader

Department of Environmental science, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq.

A B S T R A C T:

This experiment conducted in the greenhouse of Biology Department in the College of Science- University of Salahaddin- Erbil, for studying the influence of fruit peels (banana and orange peels) as a natural fertilizer at doses (0, 4, 8, 12 g.pot^{-1}) by different methods powder, powder extract and foliar spray on growth and development of chickpea plants. The study consists of 12 treatments with three replications. The following growth parameters were observed plant height, number of branches, stem diameter, dry weight of shoot system, water content, and yield characteristics including number of pods per plant, number of seeds per pod, dry weight of 100 seeds and chlorophyll content. The results elucidate that fruit peels significantly increased plant height, number of branches, water content, number of pods, chlorophyll a, total chlorophyll content, and carotenoids. It is noticed that fruit peels had a positive effect on growth and development of chickpeas plants.

KEY WORDS: Fruit peel,Chickpea,Yield componentsCarotenoids.
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ZJPAS (2019) , 31(3);45-51.

INTRODUCTION :

Chickpea (*Cicer arietinum* L.) is an important annual herbaceous legume crop, belongs to leguminous family domesticated independently in Mediterranean region and now grown worldwide both for dry and as green (Khan *et al*, 2014). Among major legumes the chickpea is the third important crops after the faba bean and soybeans. It is the main crop that have role in symbiotic fixed nitrogen in the nodules of root, by their role in soil fertility. It's an important source food of both of human and animals being its rich in protein source, complex carbohydrate, fiber, vitamins and minerals make this legume an important composition of human diet in developing world. Generally, its protein quality is higher than that of many other legumes (Ismail *et al*, 2017)

Fruit peels are important sources of mineral nutrients like calcium, iron, potassium, zinc etc. It is used as a natural fertilizer. There are two main types of fertilizer, organic and inorganic fertilizers which added to a soil for plant growth as essential nutrients. Organic or natural fertilizer contains different antioxidants and carbonaceous matter. Inorganic or commercial fertilizer usually wholly manufactured, as in case of sulphate of ammonia; or they may be processed from quarries, which are cheapest and harmless materials are used for plant growth. Fruit peels used in the soil as fertilizer, regulating pH of the soil and also in supplementing of micronutrients like iron, calcium, zinc (Jariwala and Syed, 2017). Banana peel is a source of dietary proteins, essential amino acids, fiber, polyunsaturated fatty acids and potassium (Sonia *et al*, 2014). Citrus peels like

* **Corresponding Author:**
Halalal R. Qader
E-mail: halala.qader@su.edu.krd or halala.rahman@gmail.com
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orange peels contain an important compound like sugar, and acids which have role in acidity of soil and their effect on photosynthesis process and plant growth (Shed, 2005). (Mercury *at al*, 2014) reported that different fruit peels (pomegranate, banana, sweet lime orange peel) increased plant height and size of leaves in fenugreek plants. (Jariwala and Syed, 2017) explained that fruit peels (sweet lime peel, orange peel, banana peel, pomegranate peel, citrate peel powder, alkaline peel powder) used as a good PH regulator of soil which citrate peel powder used to decreased P of soil and alkaline peel powder to increased PH of soil, and also these fruit peels contain high amount of nutrient N, P, K which required as a natural fertilizer. (Panwar, 2015) found that Banana peels increase soil fertility and soil productivity by increasing nutrients for plants, in addition the best way to decrease pollution.

The objectives of this study were to determine the effect of fruit peels in different ways (peel powder, peels powder extract, and foliar application) as a natural fertilizer on growth and development of chickpea plants.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in the glasshouse of Biology department, College of Science, University of Salahaddin-Erbil, during October 24, 2017 to January 26, 2018. Pot experiment consisted of combination treatments of fruit peels (banana and orange peel) with different concentrations at doses (0, 4, 8, 12 g.pot^{-1}) and different methods of application (powder, powder extract, and foliar spray, 12 treatments with three replications, include 36 plastic pots each pot with a diameter of 24 cm in length and 21 cm in depth filled with 7kg of dried sandy loam soil of Askikalak area, the soil sievedthrough 2mm pore size sieves. Some physical and chemical properties of the soil are shown in Table (1). From each pot, three seeds were sown and then thinned to one plant later. Fertilizers at the rate of 10kg.donm^{-1} which included urea containing %46.6 N, super phosphate P_2O_5 containing 45% P, added to the pots as solutions at the beginning of planting (Muhummed, 2004). The following measurements taken for each pot; plant height (cm).plant^{-1}, number of leaves.plant^{-1}, number of branches.plant^{-1}, shoot dry weight.plant^{-1}, water content (g.plant^{-1}) of shoot system, and yield components such as number of pods.plant^{-1}, number of seeds.pod, dry weight of 100 seeds.

Water content of the shoot system estimated as follows: fresh weight, shoot system dried at 110°C for 1 hrs, and then dried at 70°C for 24 hrs, in an oven. After cooled at room temperature, dry weight of shoot obtained for half an hour (He *et al*, 2005).

Water content =F.wt.-D.wt.
F.wt. =fresh weight
D.wt. =dry weight

Chlorophyll content in leaves (µg.g^{-1}) estimated by taking 0.5g of fresh leaves left in 10 ml of absolute ethanol for 24 hrs. In dark condition, this process repeated three times to complete extraction of chlorophyll the end volume reached 30 ml were spectrophotometrically estimates on two wave length 649and 665 nm as follows (Waterman Demote, 1965):

µg chlorophyll a/ml solution = (13.70) (A665nm)-(5.76) (A 649nm)
µg chlorophyll b/ml solution = (25.80) (A649nm)-(7.60) (A 665nm)
Total chlorophyll =chlorophyll a + chlorophyll b
A=absorbance
nm =nanometer

2.2. Collection and Processing of fruit peels

Fruit peels like banana and orange peels collected from fruit shops, these fruit peels washed with tap water, cut to small pieces then dried at sunlight for 20 days in late summer after that powered then sieved and stored at room temperature (El- Bassiouny *et al*, 2016). Fruit peels (banana and orange) as Powder methods applied to each pot mixed with soil; in powder extract method (1g) of fruit peel was taken in 100 ml of distilled water and mixed thoroughly for the preparation of extract. This mixture was stirred for 3 days by using magnetic stirrer (Mercy *et al*, 2014), this extract prepared previously used as Foliar spray for each pot.

Table1: Quantity of fruit peel powder for formulations

Fruit peel	content	Formulations of fruit peel
Orange and banana peels	1 g	1g+100ml water

2.3. Experimental Design

The data was designed according as factorial experiment in Completely Randomized Designs with three replications and twelve treatments. Duncan Multiple Range Test was used for the comparison of treatment means at 5% for green house parameters and 1% levels for laboratory parameters (Al-Rawi and Khalafulla, 1980). The statistical analysis was done by using Statistical Package for Social Sciences (SPSS version 16 software). For drawing graph, Excel 2007 software was used.

Table 2: Some physical and chemical properties of the soil used in the experiments

Properties	value
Sand	70.10 %
Silt	24.22 %
Clay	5.68 %
Soil texture	Sandy loam
Soil moisture	3.1 %
Organic matter	0.91 %
PH	7.24
$CaCO_3$ (Trimetric method)	25.7%
Electrical conductivity (ds m^{-1} at 25°C)	0.58
Total nitrogen % (kjeldahl method)	0.4%
Total phosphorus ppm(Olsen method)	118 ppm
Total potassium ppm (flame photometer)	45 ppm
Total calcium ppm (atomic absorption method)	240 ppm

3. RESULTS AND DISCUSSION

3.1. Vegetative growth characteristics

Table (3) shows that fruit peels applied by different methods have significant effect on vegetative growth characteristics, fruit peels significantly ($p \leq 0.05$) increased plant height at dose (4g) by foliar application method as compared to their control, and also significantly ($p \leq 0.05$) increased number of branches at doses (8g) by powder extract method as compared to their control, water content of shoot system significantly ($p \leq 0.05$) increased at dose (4g) by foliar application method as compared with their control, there were significant difference between treatments (Fig. 1). These results agreed with those obtained by (Mercy and Jenifer, 2014), which fruit peels as a natural fertilizer increased plant growth. (Jariwala and Syed, 2017) mentioned that fruit peels as a natural fertilizer fulfills as a requirements of micronutrients. Fruit peels contain sugar, protein, nutritional components especially potassium which necessary for plant growth (El-Bassiouny, *et al.,* 2016). The increase of growth of plants in response to fruit peels concentration by different methods especially by powder extract and foliar application method.

3.2. Yield characteristics

Data presented in table (4) showed that fruit peels at dosed (8 g) by foliar application significantly ($p \leq 0.05$) increased number of pods per plant and number of seeds per plant at dose (8g) by powder extract as well as dry weight of 100 seeds at dose (4g) by powder method as compared to their controls. Powder extract at dose (8g) was most effective treatment than other treatments in number of pods, there were significant difference between treatments. These results partially agreed with those mentioned by (El- Bassiouny, 2016), that fruit peels contain antioxidants which enhancing protein synthesis and delay senescence. (Panwar, 2015) notice that banana peel contain nutrients such as potassium, calcium, magnesium, sulphur, phosphate, and sodium which are needed by the plants and also help plants to resistant diseases. Increased in plant growth which increased number of branches, and yield components, then increase production of plants.

3.3. Photosynthetic pigments

According to results presented in table (5) fruit peels significantly ($p \leq 0.01$) increased chlorophyll a at (8, 12 g) by powder extract methods as compared to their control. Fruit peels significantly ($p \leq 0.01$) increased total chlorophyll at dose (12 g) by powder extract method as compared with their control. Fruit peels significantly ($P \leq 0.01$) increased carotenoid at doses (12 g) by powder extract methods as compared with their control. Increasing in chlorophyll a, b, and total chlorophyll increased photosynthetic process which increasing yield of plants and increase production. Carotenoids play as a free radical which increased chlorophyll of such plants (Bakry *et al.,* 2012). (El-Bassiouny, *et al.,* 2016) found that orange peels due to presence of natural antioxidants such as flavonoids and Vitamin C,

increasing antioxidant enzyme activity and promoting photosynthesis, maintain enzyme activity. Fruit peels increased nutrients in plants especially N, P which is used in biosynthesis of chlorophyll content.

Table 3: Interaction effects of fruit peels (Banana and orange peels) and methods of application on vegetative growth characteristics after 45days from application

Interaction treatments		vegetative growth characteristics		
Methods of application	**Fruit peels concentration (g)**	**Plant height(cm)**	**Number of branches**	**Stem diameter**
Powder	0	42.66 bc	22.33 bc	1.03 a
	4	45.5 bc	22.00 bc	1.16 a
	8	42.83 bc	23.33 abc	1.33 a
	12	43.16 bc	20.33 c	1.06 a
Powder extract	0	43.00 bc	22.00 bc	1.02 a
	4	43.66 bc	22.00 bc	1.09 a
	8	44.16 bc	31.66 a	1.23 a
	12	47.83 b	26.00 abc	1.40 a
Foliar application	0	41.30 c	21.00 c	1.02 a
	4	51.33 a	30.66 bc	1.36 a
	8	44.33 bc	25.00 abc	1.20 a
	12	43.00 bc	24.66 abc	1.33 a

*Data presented as mean, the same letters mean not significant differences while the different letters mean significant differences $p \leq 0.05$

Table 4: Interaction effects of fruit peels (Banana and orange peels) and methods of application on yield characteristics

Interaction treatments		Yield characteristics		
Methods of application	**Fruit peels concentration (g)**	**Number of pods.plant^{-1}**	**Number of seeds.pod^{-1}**	**Dry weight of 100 seeds**
Powder	0	13.66 bcd	1.12 bcd	37.00 bcd
	4	16.66 ab	1.16 b	44.66 a
	8	16.33 ab	1.11 bcd	41.00 abc
	12	10.33 b	1.03 fg	35.33 cd
Powder extract	0	13.66 bcd	1.13 bcd	35.00 bcd
	4	15.33 abc	1.21 a	43.33 ab
	8	18.00 a	1.07 ef	39.00 abcd
	12	12.66 cde	1.15 bc	41.33 abc
Foliar application	0	13.00 cde	1.11 cde	36.15 bcd
	4	15.66 abc	1.02 g	39.23 abcd
	8	17.66 a	1.10 de	37.33 bcd
	12	12.33 de	1.06 fg	34.00 d

*Data presented as mean, the same letters mean not significant differences while the different letters mean significant differences $p \leq 0.05$

Table 5: Interaction effects of fruit peels (Banana and orange peels) and methods of application on photosynthetic pigments of leaves (mg.g^{-1}fresh weight)

Interaction treatments		Photosynthetic pigments (mg.g^{-1}fresh weight)			
Methods of application	**Fruit peels concentration (g)**	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**	**Carotenoids**
Powder	0	0.98 c	1.56 a	2.53 bc	0.51 c
	4	0.94 c	1.29 a	2.24 c	0.49 c
	8	1.13 bc	1.54 a	2.68 abc	0.58 bc
	12	1.07 c	1.36 a	2.49 bc	0.53 bc
Powder extract	0	0.96 c	1.51 a	2.53 bc	0.52 c
	4	1.04 c	1.57 a	2.62 bc	0.55 bc
	8	1.54 a	1.27 a	2.82 ab	0.68 ab
	12	1.52 a	1.64 a	3.17 a	0.76 a
Foliar application	0	0.99 c	1.48 a	2.50 bc	0.53 c
	4	1.36 ab	1.55 a	2.91 ab	0.66 ab
	8	0.63 d	2.17 b	2.80 ab	0.48 c
	12	1.11 b	1.58 a	2.69 abc	0.55 bc

*Data presented as mean, the same letters mean not significant differences while the different letters mean significant differences p≤0.01

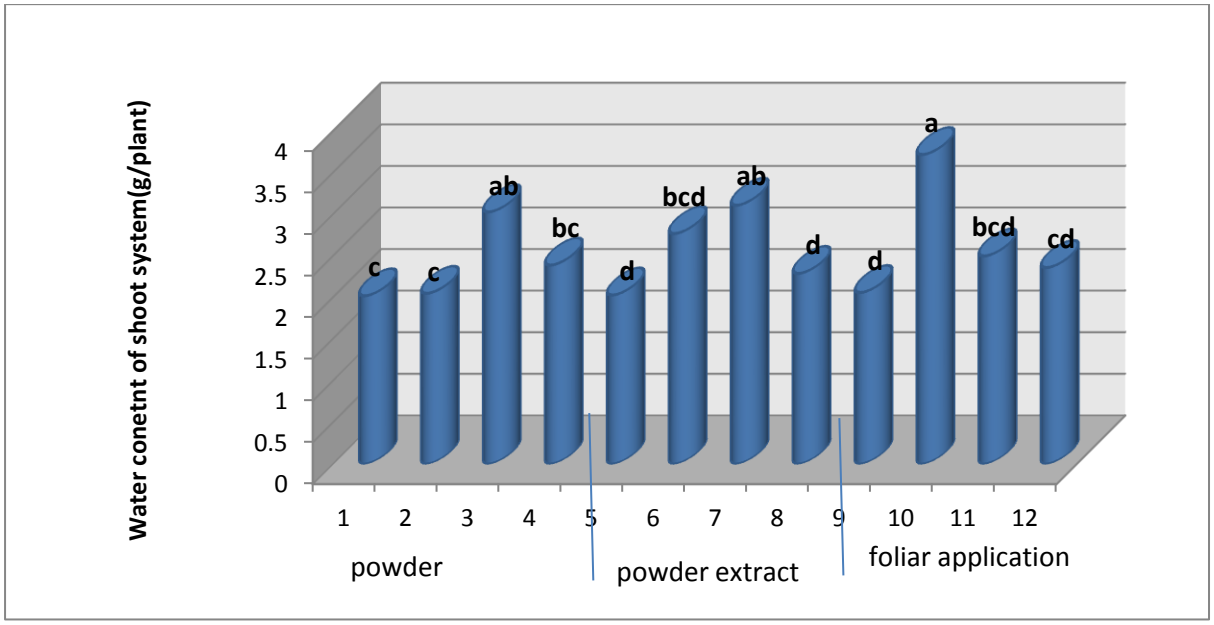

Figure 1: Interaction effects of fruit peels (Banana and orange peels) and methods of application on water content of shoot system

*Data presented as mean, the same letters mean not significant differences while the different letters mean significant differences p≤0.05

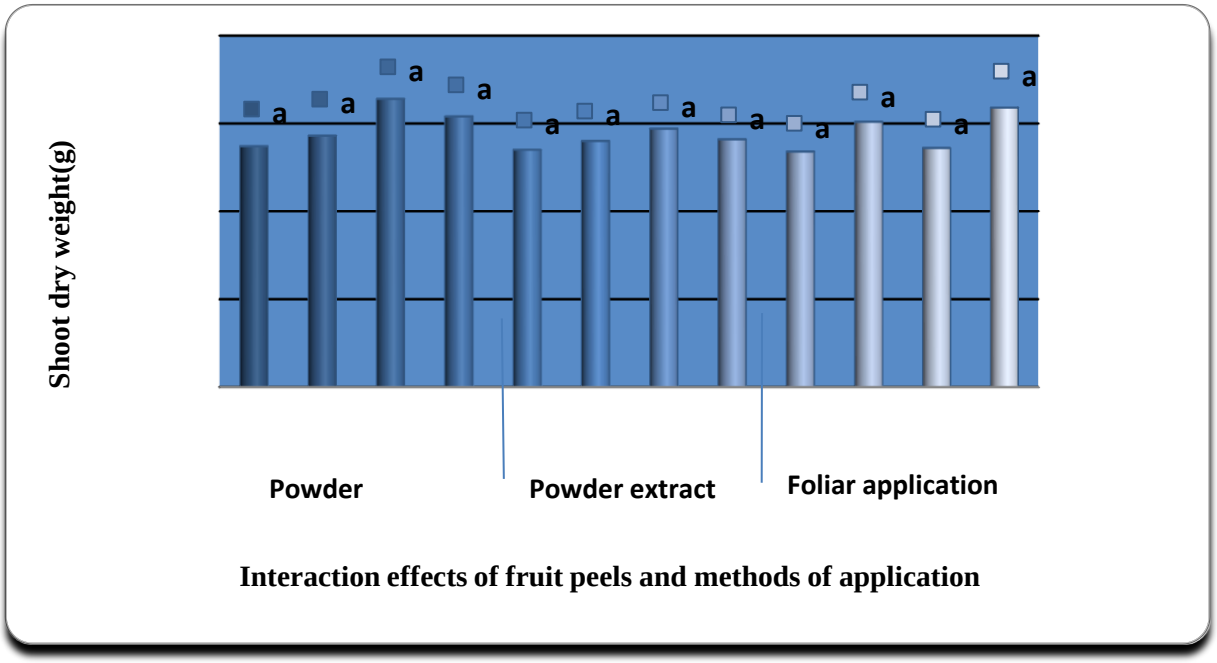

Figure 1: Interaction effects of fruit peels (Banana and orange peels) and methods of application on dry weight shoot system

*Data presented as mean, the same letters mean not significant differences while the different letters mean significant differences $p \leq 0.05$

4. CONCLUSIONS

Application of fruit peels banana and orange peels by different methods such as powder peels, powder extract, and foliar spray have effective roles in growth and development by enhancing vegetative growth such as plant height, number of branches, water content, stem diameter, yield characteristics and chemical contents. Foliar application of banana and orange peels has more effective than other methods.

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RESEARCH PAPER

Microarray analyses of the infant gut microbiota

Lubna Ahmed Abdulkarim1, Chra Saed Abdulla2, Farhad Tahir Mahmod3

1- Department Environmental Sciences, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq.
2- Department of Animal source , College of Agriculture, Salahaddin University- Erbil, Kurdistan Region, Iraq.
3-Food Safety Authority, Norway.

A B S T R A C T:

To detect the cause of germ free infant gut become colonization after birth and develops though the pregnancy for mother, 4 days, 10 days. 4 months, 1 year to 2 years is important, which has strong relationship with health and disease infant. This research was carried out at the experimental laboratory of molecular microbiology department at Norwegian University Of Life Science, College of of NMBU, Ås la Norge / Norway, during Jan 2015 to get more information about the development of microbiota in infant. The pattern of colonizers is different at the different age. The purify faecal sample DNA perform universal amplification of the 16Sr RNA to the determination of gut microbiota by the GA-map infant array and 454 pyrosequencing. The results showed implicating the *Bifidobacteria* and *Firmicutes* that present in great amount during the development of the infant gut microbiota with reach to peak at the age of several weeks. A temporal trend in the microbiota composition was also detected which indicates the usefulness of this method for determining the temporal dynamics of the infant gut development.

KEY WORDS:
Microbiota ,Gut, Fecal,Infant.
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INTRODUCTION :

The human gastrointestinal tract is densely populated by a large amount microbial ecosystem. One adult human whole organ bears generally more microbial cells than body cells whereas only our intestinal tract contains 100 trillion microbes and the vast majority microbes (10^{11}-10^{12}) have found in our colon (Whitman *et al.*, 1998). Though there is a universal relationship between the host and its gut microbiota, vast majority is still now unknown. The microbial communities inhabiting our gut offer evolutionary conserved services like synthesizing essential molecules, strengthening our immune system, enhancing nutrient uptake, stimulating angiogenesis, and regulating host fat storage (Marzorati *et al.*, 2011; Palmer *et al.*, 2007 and Woodmansey, 2007).

Unfortunately, some of these organisms are opportunistic pathogens, and many can cause harm if the normal and healthy community composition in the host is altered (Round and Mazmanian, 2009 and Woodmansey, 2007). The development of the human infant gut microbiota is yet known little which is mainly due to methodological constraints. The workload associated generating and analyzing microbiota data and the complexity are the current challenges.

* **Corresponding Author:**
Lubna A. Abdulkarim

E-mail: lubna.abdulkarim@su.edu.krd

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Children with antibiotic treatment and caesarean delivery represent major perturbations of the microbiota (Marques *et al.,* 2010 and Rudi *et al.,* 2007a). Early colonizers are staphylococci and streptococci whereas clostridia and Bifidobacteria increase with age (Trosvik *et al.,* 2009). The pattern of early colonization is important because this leads the immune system not respond properly later in life (Kalliomaki *et al.,* 2001). Therefore, an understanding of the gut microbiota development in infants has the possibility to develop strategies to modify the microbiota for the current epidemics in allergic disorder development in the western world. The change of lifestyle or use of antibiotic can thus strong affect the prevalence of selected gastrointestinal microbes playing an essential role in normal gut development and health (Woodmansey, 2007). Some time it can cause diseases like intestinal cancer, necrotizing enterocolitis and inflammatory bowel disease in infants (Palmer *et al.,* 2007; Round and Mazmanian, 2009 and Sekelja *et al.,* 2011). Any change in the optimal environment in the gut, due to change in ratio between microbis, and this linked to increase diseases such as asthma, allergic disorders and obesity (Blaser and Falkow, 2009; Flint, 2011; Kalliomaki *etal.,* 2001; Nakayama *et al.,* 2011; Round and Mazmanian, 2009 and Sekelja *et al.,* 2011).

The characteristics of the gene encoding the 16S ribosomal RNA (Woese, 1987), is useful for classification of both higher taxa and closely related species, and used in combination with different molecular techniques to analyze bacterial diversity (Blaser and Falkow, 2009; Momozawa *et al.,* 2011; Nakayama *et al.,* 2011; Rudi *et al.,* 2007b and Zwielehner *et al.,* 2009). Cloning and DNA sequencing is used for getting specific information out of unknown samples. Probe-based methods are generally used if the bacteria searched for are already known. Direct sequencing and electrophoreses-based methods such as DGGE, TGGE and T-RFLP are used for screening purposes and pattern recognition (Rudi, 2013).

Stool samples are very easy for study and analyzing bacterial diversity in the gut, but stool samples are not contain all a microbiota composition reflecting the one existing in the whole colon or small intestine (Marchesi, 2011), a stool sample only reflects the composition of microbiota present in the lumen, not of that growing on the mucosal surface (Momozawa *et al.,* 2011 and Pedersen and Tannock, 1989). The studies of functional biofilms in the gastrointestinal tract are preferable to alternative approaches, but they are limited by the inaccessibility to most of the areas that together make up the digestive tract (Marchesi, 2011).

The distal gut has much attention by researchers because of it high microbial density (Whitman *et al.,* 1998), and easy accessibility. There are some claim that infant is not sterile at birth after all, because DNA from *Bifidobacterium* and *Lactobacillus* has been detected in the infant placenta (Satokari *et al.,* 2009).

Some bacterial genera were detected in the meconium from 21 healthy newborns as recorded by (Jimenez *et al.,* 2008) .

The relationship between human and gut microbiota is complex, and there is increasing in developing microbiological interventions as a strategy for preventing health problems and disease, more studies, more improvement of techniques is highly necessary to be able to answer. Lab experimental a 16S rRNA gene microarray approach was used to determine the development of the gut microbiota in a cohort of mothers and their infants from 4 days to 2 year of age. The rationale was that the temporal development of the infant gut microbiota is an important parameter for the health of the infant, and challenges with probe specificity and cross-reactivity between closely related species was met by using highly specific single nucleotide primer extension (SNuPE) probes. The aim of this research to get more information about the development of microbiota in infant, results obtained by this study can give ideas of what has to be improved in future research .

2. MATERIALS AND METHODS

Research was carried out at the experimental laboratory of molecular microbiology department at Norwegian University Of Life Science, College of of NMBU, Ås la Norge / Norway, during Jan 2015 to get more information about the development of microbiota in infant. Each group was processed four fecal samples from a

mother/child, in addition to a positive and a negative DNA purification control (8 samples in total). The infant fecal samples were collected during pregnancy for the mother, and at the age of 4 days, 10 days, 4 months, 1 year and 2 years.
The feces have been frozen immediately at -80°C to prevent degradation and bacterial growth.

The samples got as follows:

1. Late pregnant sample
2. 3 days sample
3. 10 days sample
4. 1 year sample
5. A positive control
6. A negative control

2.1. DNA isolation from stool samples:

To extract DNA from all species in all samples, bacterial cells in all samples where lyses by mechanical disruption using glass beads. For this, 40 mg stool sample was dissolved in 1200 µl binding buffer and 0.25 g of <106 µm glass beads. They was vortexes briefly and frozen down at -20^{o}C. The cells were homogenized in the MagNA Lyser (6500 rpm in 2×20 sec) and it was kept on ice between each homogenisation step. It was then centrifuged at 13000 rpm for 5 min at RT and the supernatant was transferred to new microcentrifuge tube. 10 µl SiMAG/ MP-DNA Magnetic beads (200 mg/ ml) were added and the sample (400 µl) was transferred with beads to the designated wells in the Sample plate. 1 ml of washing buffer 1 was aliquoted into the designated wells in Wash plate 1 and 1 ml of washing buffer II designated wells in Wash plate 2 and 1 ml of washing buffer III designated wells in Wash plate 3. Finally, 100µl elution buffer was aliquoted into the Elution plate and then ThermoScientific King Fisher® Flex robot was switched on. After the extraction was finished, the isolated DNA was in Elution plate (Rudi, 2013).

2.2. Quantification of genomic DNA.

For quantification of genomic DNA we used Quant-It Assay for Qbit.

2.3. PCR amplification by primers targeting universally conserved regions of the 16S rRNA gene:

Anew universal PCR, Cover All PCR amplifying an approximately 1200 pb region of the 16SrRNA gene was used for the amplification of universally conserved region of the 16SrRNA gene all six samples in addition with positive control (*E. coli* DNA) and negative control (water). After PCR amplification, to verify the quality of the PCR product we run it in Agarose gel electrophoresis and used 1 Kb ladder (N3232) for quantification. After visualized the bands from gel in UV light (Rudi, 2013).

2.4. PCR product quantification (picogreen reagent)

The PicoGreen fluorescence quantification was done with the FLX 800cse instrument which measurers the PicoGreen signal that evolves when the fluorochrome is bound to dsDNA (Rudi, 2013). This gives a specific measurement of the DNA present in the solution.

2.5. Array analyses

All samples from each group of the ExoSAP-treated PCR-products were labeled after the SNuPE principle, according the protocol (Rudi, 2013), depending on which probe set used, different bacteria can be detected with the microarray method. The probe designing strategy used for the GA- Map™ infant assay.

The GA-Map array method was based on the use of SNuPE labeled probes there are many probes are labeled in the same reaction. The SNuPE probes was made so that the probes hybridized adjacent to identified gene location if the target bacterium was found then a labeled dideoxynucleotide was incorporated by the polymerase**(Fig 1, 2)**.

The same protocol as for measurement of genomic DNA was used for PCR product quantification.

In array analysis, 4 steps was followed as given below:

1. ExoI-SAP treatment for removing residual PCR primers and dephosphorylation inactivation of nucleotides used in PCR.
2. Probe endlabeling by SNuPE.
3. Hybridization of the labeled probes to their respective complementary oligonucleotides spotted on an array.
4. Scanning and analysis of hybridized array.

The Exo-Sap treatment was done to make master mix for15µl PCR product and

added to the PCR wells. Then it was incubated on a thermal cycler. Then8µl of the master mixture was added to the wells. 2µl template was added to the respective wells. 10 µl of this reaction was preceded for PCR (Rudi, 2013).

2.6. Array analyses by MAGPIX

The MAGPIX system is a versatile multiplexing platform capable of performing qualitative and quantitative analysis of proteins and nucleic acids in a variety of sample matrices. This affordable system requires less sample input than many other current technologies and can perform up to 50 tests in a single reaction volume, greatly reducing sample input, reagents and labour while improving productivity (Rudi, 2013).

2.7. Pyrosequencing

In the DNA sequencing depended on the pyrophosphate release when nucleotide incorporation by activity of DNA polymerase "Sequencing by synthesis". This method is based on identification of the activity of DNA polymerase after amplification all samples were mixed in a tube purified on column and then sending to the 454GSFLX instrument at the Norwegian High-Throughput Sequencing Centre (Rudi, 2013).

2.8. Purification of PCR product using E.Z.N.A. DNA purification kit

Added 250 µl of Buffer CP to 50 µl of mixed PCR product in a 1.5 ml tube, and vortexed thoroughly to mix; spined the tube to collect the drop from the inside of the lid. Then applyed the sample to the HiBind® DNA column with 2 ml collection tube and centrifuge at 15000 rpm for 1 min at (RT). After that centrifugation we washed the HiBind® DNA column with 700 µl of DNA Wash Buffer and centrifuge at 15000 rpm for 1 min at RT. Liquid discarded in the from 2 ml collection tube and repeated previous centrifugation step using 500 µl of DNA Wash Buffer. Similarly discarded the liquid and centrifuge the empty HiBind® DNA column for per min at 15000 rpm. Then placed HiBind® DNA column into a clean 1.5 ml tube, added 30 µl of Elution Buffer (10 mM Tris, pH 8.5) onto the column matrix and centrifuge for 1 min at 15000 rpm to elute DNA. Than transfered eluted Elution Buffer with DNA from 1.5 ml back on the column, centrifuged as in previous step once again. The step is measurement of the the eluted DNA was performed by the Qubit Quant It assay (Rudi, 2013).

2.9. Probe Verification by Quantitive PCR

Quantitive PCR was done to enable accurate quantification of specific DNA targets.
For this two qPCR reactions were set up for each DNA sample. In one reaction, universal 16S rRNA gene primers and probes, and the reaction were with *Bifidobacterium longum* primers and probes.

3. RESULT AND DISCUTION

The study began with the purification of faecal DNA. For these 8 samples were preceded with a positive control and negative control. The positive control was to determine the technical errors in the DNA purification, while the negative control was to check potential contamination. All samples were processed for DNA isolation and quantification of genomic DNA was performed by using both PicoGreen reagent and Qbit measurement. As picogreen is light sensitive.
The PicoGreen measurements of the genomic DNA from the stool samples showed a successful extraction and the extraction gave good parallel results **(Fig, 1).**

The objective of this experiment is to determination of the development of the microbiota infant gut of human and to find out the correlation between health and disease by detection and understand the diversity of microbiota. For these 6 samples were preceded with a positive control and negative control. The positive control was to determine the technical errors in the DNA purification, while the negative control was to check potential contamination. All

samples were processed for DNA isolation and quantification of genomic DNA was performed by using PicoGreen reagent and Qubit measure. In current experimental DNA extraction in combination with the use of Cover All primers was used **(Fig, 11).**

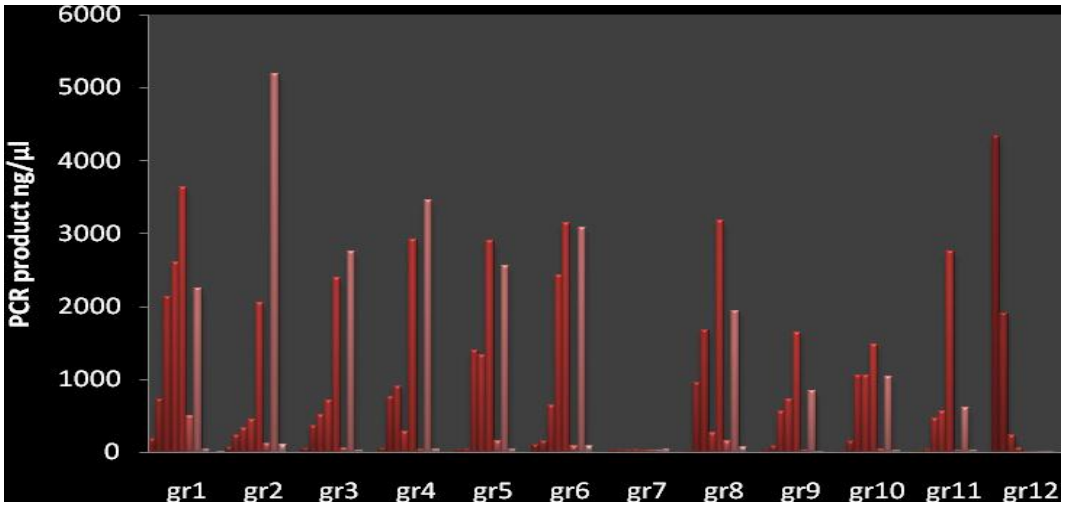

Fig 1: Amount of amplified DNA (ng/µl) from the Coverall 16S rRNA PCR, quantified by using PicoGreen.

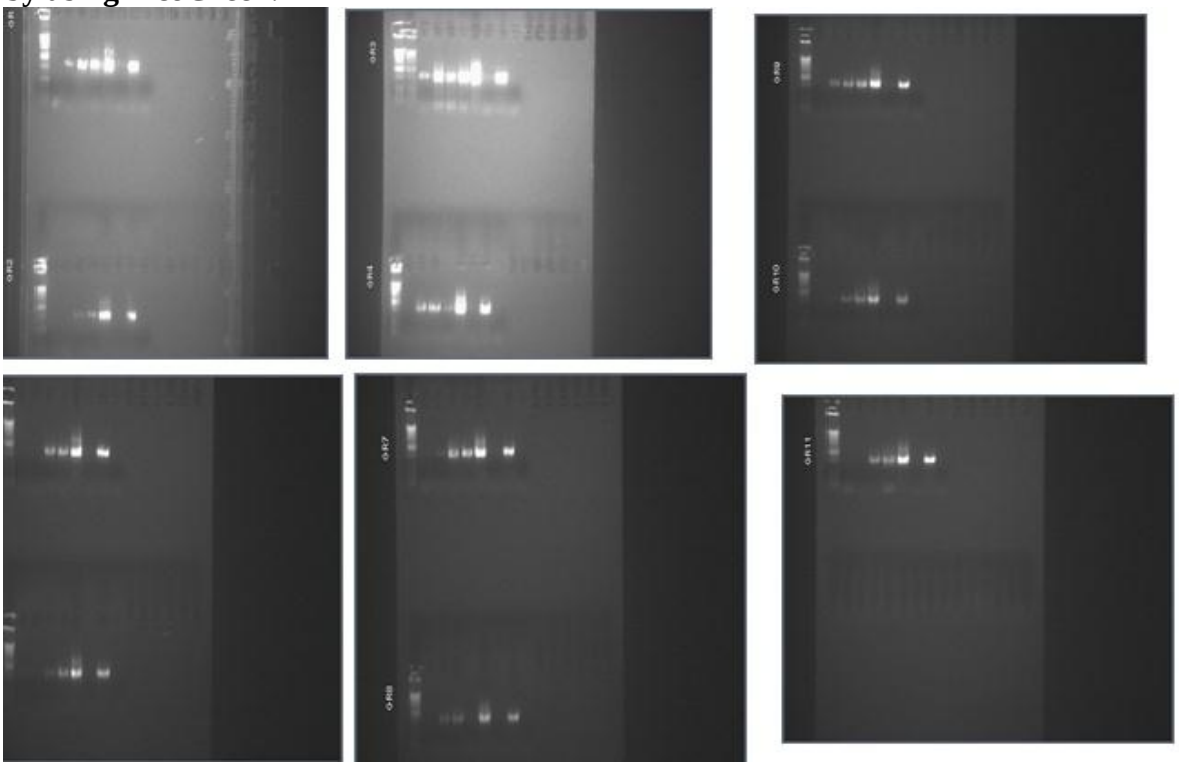

Fig 2: Agarose gel results of all samples showing the 16S rRNA bands and thus a successful. Amplification of genomic DNA in all samples(PCR amplification was performed using primer pairs GC-ZF and 1622R coupled with a GC-tail and run on DGGE to determine the banding pattern specific to each sample).

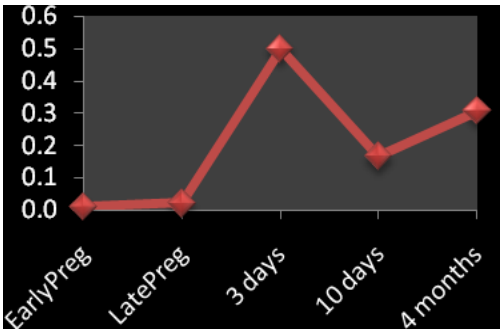

Fig3: Correlation between age (gr.2411) and Microarray *B.no longum*

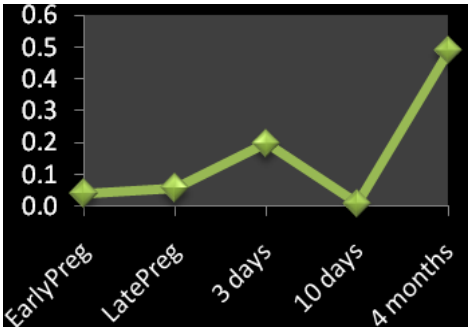

Fig4: Correlation between age gr.2411) and Microarray *B.longum*

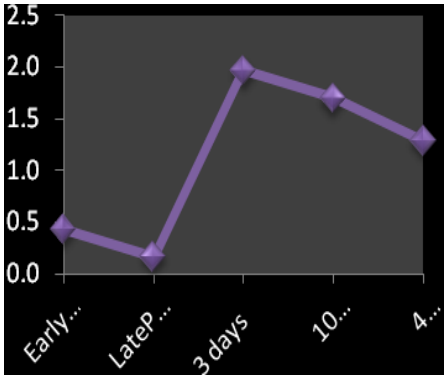

Fig5: Correlation between age (gr.2411) and pyrosequencing qPCR *B.longum*

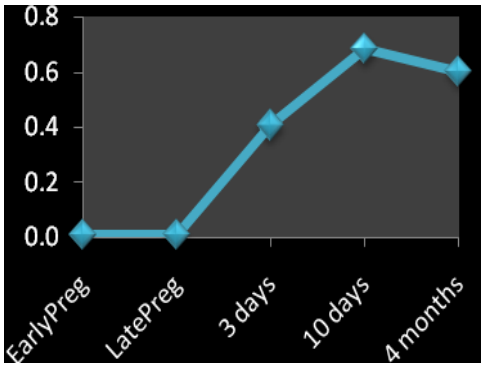

Fig6: Correlation between age (gr.2411) and *B.longum*/Total.

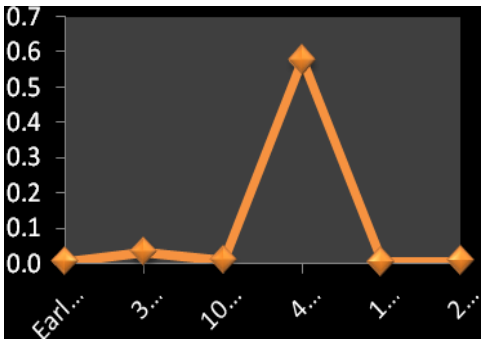

Fig7: Correlation between age (gr.2206) and microarray (gr.2206) and microarray *B.longum*

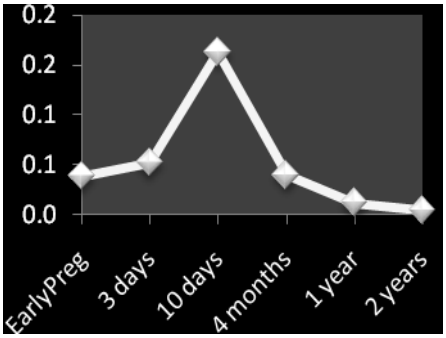

Fig8: Correlation between age *B.nolongum.*

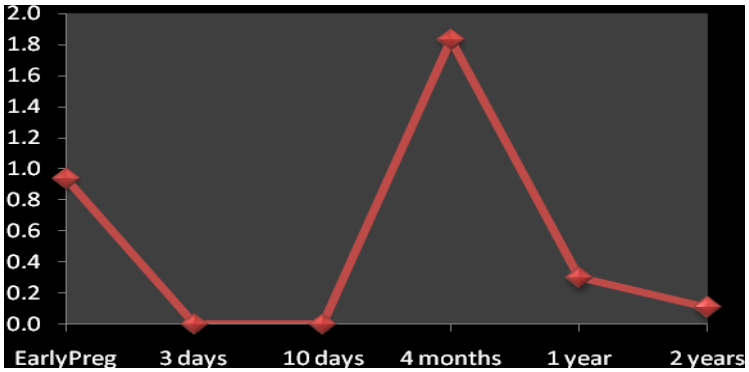

Fig9: Correlation between age (gr.2206) and qPCR *B. longum.*

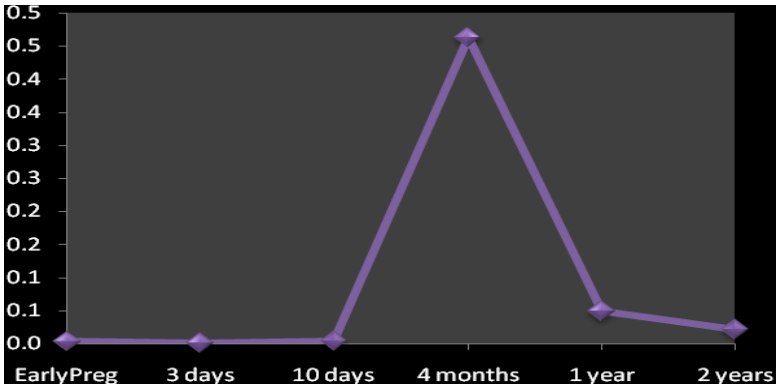

Fig10: Correlation between age (gr.2206) and pyrosequencing *B. longum*/Total.

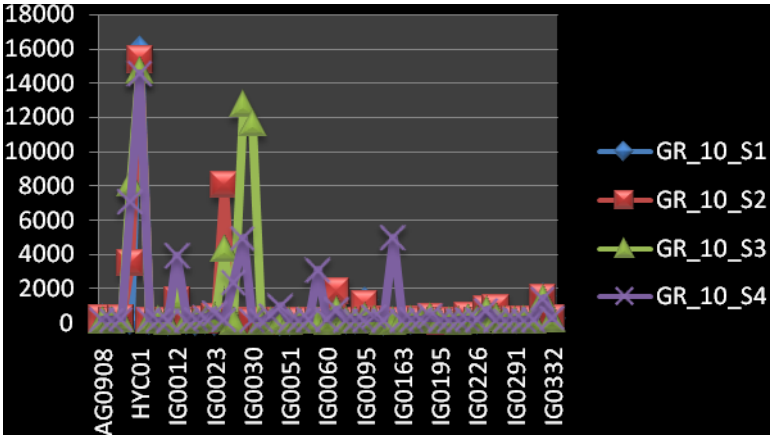

Fig 11: GA-map array the probes of bacteria in the sample of groups 10.

Table 1: List of target groups for Probe set NEC v. 1.0 showing different types of probes in the different stage of age.

Probe #	Bacteria
UNI05	Universal
IG0005	Proteobacteria
IG0178	Gamma-proteo group
IG0056	Gamma-proteobacteria subgroup
IG0011	Salmonella/Citrobacter/Cronobacter/Enterobacter/ Morganella
IG0181	Klebsiella pneumonia/Aeromonas
IG0195	Klebsiella oxytoca/Pantoea agglomerans
IG0204	Serratia marcescens
IG0133	Shigella and E.coli
IG0008	Haemophilus
IG0039	Pseudomonas/Morganella morganii
IG0012	Firmicutes (separates on Streptococcus and some Clostridiales)
IG0023	Firmicutes (separates on Listeria, Veillonella and some Clostridiales)
IG0044	Clostridiales and Veillonellaceae
IG0058	Clostridiales and Veillonella
IG0103	Clostridium sporogenes
IG0163	Clostridium butyricum
IG0095	Anaerococcus prevotii
IG0107	Finegoldia magna
IG0051	Veillonella
IG0042	Gemella
IG0079	Streptococcus (β-haemolytic groupA and B, α-haemolytic)
IG0022	Streptococcus (β-haemolytic groupB, α-haemolytic)
IG0197	Streptococcus (α-haemolytic)
IG0081	Streptococcus agalacticae and Eubacterium rectale
IG0020	Streptococcus sanguinis and thermophilus
IG0171	Enterococcus faecalis
IG0024	Staphylococcus and Gemella
IG0063	Staphylococcus epidermidis, Eubacterium rectale, Clostridium SS2, Streptococcus agalactiae
IG0053	Lactobacilli
IG0021	Listeria sp. (and E. coli/Shigella)

The pesent study depended on correlation between age and colonization of Microbiota. **(Fig, 11 and Table, 1)** showed that some probe gave high signals with high values and some gave low signals with low value depend on the got ages, different types of signals in probe for different taxonomic groups of bacteria.

At the age 3 to 120 days Universal bacteria have increased whereas some bacteria have decreased**.**

The 16S rRNA-gene PCR, that was performed on the DNA extract, gave amplification of the 16S rRNA gene. In the Cover 16S rRNA-gene PCR products. All group get amount of DNA except group7 **(Fig, 2).**

16S rRNA gene microarray approaches to describe the gut microbiota. A major challenge with traditional 16S rRNA gene microarrays is probe specificity, and cross-reactivity between closely related species (Cox *et al*., 2010). For microarrays this challenge has recently been addressed by tilling probes covering the variable region of the 16S rRNA gene (Rajilic-Stojanovic *et al*., 2009) and by the use of highly specific single nucleotide primer extension (SNuPE) probes (Eggesbo *et al*., 2011).
The principle by tilling is that a large number of overlapping probes cover the region of interest, with the combined probe signals providing a relatively good signal to noise ratio. The high specificity of the SNuPE assay is obtained by DNA polymerase based incorporation of a fluorescently labeled dideoxynucleotide (Syvanen *et al*., 1990). The SNuPE probes are constructed so that the probes hybridize adjacent to discriminative gene positions.
If the target bacterium is present then a labeled dideoxynucleotide is incorporated by the polymerase (Vebo *et al*., 2011).
The different sample parallels were inconsistently amplified in the Cover All PCR reaction and also the amount of DNA amplified did not reflect back on the originally amount from the DNA

extraction. DNA extraction methods and PCR primers used will always give biases regarding which bacteria that will be identified (Hong *et al.*, 2009).

This PCR was used to amplified 1200 bp region of the 16SrRNA .The size of Cover All PCR product was checked by agarose gel electrophoresis. The band we observed was 1200 bp. **(Fig, 2)** as expected. Therefore quantification of the PCR product was done using PicoGreen.
In the result of PCR product DNA quantification by PicoGreen where each group got same result in every sample only group 7 did not get the same data from there sample because of some mistake in the technique.
Similarly pyrosequencing also addresses for the analysis of gut microbiota. The process utilizes the sequencing primer which hybridized to a single – stranded PCR amplicon that serves as template. The first dNTP is incorporated to the DNA strand by polymerase if it is complementary to the base into the DNA strand. Each incorporation is accompanied by release of pyrophosphate.ATP sulphyrase converts pyrophosphate to ATP in presence of adenosine 5 phosphosulfate (Syvanen *et al.,* 1990). This ATP helps to convert luciferin to oxyluciferin that generates visible light. The light produced is detected by charge coupled device chip and seen as peak in the program. The height of each peak is proportional to the number of nucleotides incorporated. Apyrase, a nucleotide- degrading enzyme, continuously degrades unincorporated nucleotides and ATP (Gharizadeh *et al*., 2007).
When degradation is complete, another nucleotide is added. The complementary DNA strand is built up as the process continues and the nucleotide sequence is determined from the signal peaks in the Pyrogram trace (Ronaghi, 2001).
The gut microbiota was identified and determinate by the GA-map infant array and 454 pyrosequencing in which_significance difference of bacteria. Several studies that reported Bifidobacteria as the months **(Fig, 7)** dominant microbiota of breast-fed infants by several weeks of age (Benno *etal.,* 1984; Favier *etal.,* 2002; Penders *etal.,* 2006 and Stark and Lee, 1982). Result of current research showed that qPCR and pyrosequencing Bifidobacteria are increased in the 10 days to 4 after that the range decreased, that is mean the two methods are all dependable in the main stream .
When comparing two data seta from microarray and qPCR its clear that in group 2411 *B.longum* and B.not longum come to the highest value in day 3 while B.longum become less in the later

stage **(Fig 4, 5)**, while the other type still increase. In group 2206 recorded that some types of Bifidobacterium become highest in 4 months **(Fig 9, 10)** and *B.nolongum* without B.longum become less after day 10 which means that *B.longum* remain dominant strain in the gut after that time.

There are no difference in the species of B.longum and B.non longum between IgE sensitive and non-sensitive children (Vebo *et al.,* 2011). This bacterium has a function in the temporal development of the infant intestine and a future aim of research could be to find out more around the connection of Bifidobacteria and breast-fed infants in stool samples.

4. CONCLUSION

1- The gut dominating Bifidobacteria can give more information about the development of microbiota in human gut in further research.
2- Understanding the gut microflora may be a milestone for developing strategies for intervening microflora in the reduction of allergic disorder in the developed world.

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RESEARCHPAPER

Effect of Sowing Dates and Varieties of Cotton (*Gossypium hirsutum* L.) on Growth and Yield Parameters.

Rabar F. Salih

Department of Field Crops, College of Agriculture, Salahaddin University-Erbil, Kurdistan Region, Iraq

A B S T R A C T:

The sowing date trials comprised of three planting dates on three cotton varieties (Lachata, Stoneville 213 and Coker 310), were conducted to investigate appropriate sowing time of the varieties at Erbil, Kurdistan during 2017. It was also to investigate the effect of climate change of sowing time. Results indicated that sowing dates, varieties and interactions significantly affected all studied characteristics. The highest plant height was recorded of Stoneville 213 which was by (110.8) cm in April 27. Variety Lachata exhibited maximum seed cotton yield (5851.13) kg/ha followed by variety Coker 310 (5097.77) kg/ha on March 28. The different results of mean boll weight, number of seeds per boll, seed index and other parameters were also recorded for varieties and different sowing dates. It was concluded that sowing on March 28 is the most appropriate sowing time for these varieties under agro-climatic condition of Erbil, Kurdistan. Based on the results from this current study some varieties will be growing better in other state of Kurdistan especially in the cold condition.

KEYWORDS: Cotton, Sowing Dates, Varieties, Growth, Yield Characteristics.
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INTRODUCTION:

The climate change is affecting to the agriculture production by its affecting on agriculture practices such as sowing time. Also, sowing time is one of the main factors affecting yield. Sowing time was selected as a key factor of cotton cultivars by many researchers around the world. For example, Soomro *et al.* (2000) reported that the sowing time has very important role in realizing maximum seed cotton yield in a country like Pakistan where the climatic conditions differ from province. Arain *et al*. (2001) reported that maximum seed cotton yield was produced when cotton planted on May 1st and April 15 at Nawab Shah Sindh Pakistan.

***Corresponding Author:**
Rabar Fatah Salih
E-mail: rabar.salih@su.edu.krd
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On the other hand, the yield of cotton is mostly associated with sowing dates as boll weight and formation of bolls which are interred linked with the yield (Mahmood *et al.*, 2003). Effect of sowing dates and cultivars was significant on almost all yield and yield components (Arshad *et al.*, 2007).

Ten cotton genotypes were sown on three sowing dates in Cotton research institute, AARI, Faisalabad, Pakistan. It showed that yield of cotton differed with different genotypes in different sowing dates (Saeed *et al.*, 2014). Planting later increases the likelihood of warm temperatures, and a crop established under warm conditions has the potential to produce larger plants and hence greater leaf and stem area to sustain boll development later in the season (Quinn, 2015). Mohamed *et al.* (2016) who planted two different cotton cultivars during two consecutive cropping seasons of 2012/13 and

2013/14 which was planted on three sowing dates of July 15, July 25 and August 4. It could be concluded that the period from the first of July to the mid of it can be considered as the optimum sowing date for yield of the cotton (Khalifa - var) at Sudan and under rain fed condition. Planting date and plant density were the factors affected on cotton traits which were done from China; different results of plant habit, yield components, ratio and distribution of harvestable bolls, and fiber quality were observed (Wanga *et al.*, 2016).

1. MATERIALS AND METHODS

A sowing date trial of three planting dates (March 1st, March 28, and April 27) was laid out to investigate the appropriate sowing time of three varieties of cotton at Erbil, Kurdistan during 2017 crop season. The pure seeds of Lachata, Stoneville 213, and Coker 310 were used as plant material. Factorial Randomized complete block design (RCBD) with three replications were applied in the field experiment. After germination the thinning operations were done. During the experiment any fertilizers were not added to the plants.

Several growth and yield parameters were collected such as; seed germination, number of days from planting until 50% opening flowers, number of days from planting until 50% boll opening, and plant height which were as growth parameters. Seed cotton yield, early maturity rate, mean boll weight, number of seeds per boll and seed index were collected as yield component parameters. The seeds of Lachata variety were not germinated at first trial of March 1st, only few seeds were germinated which was not suitable to collection the data.

Germination rate is the average number of seeds that germinate over the 5 and 10 day periods. It can be calculated by the following formula:

$$\text{Germination}(\%) = \frac{\textit{Number of seeds that germinated}}{\textit{Number of seeds on the lot}} \times 100$$

However, seed index is regarded as weight of 100 seeds in gram. During calculate the seeds must be free from lint, disease or any other insect pest.

The aim of the study was to evaluate the effect of sowing time and varieties on some growth and yield parameters of cotton. It was also to investigate the effect of climate change of sowing time.

2. RESULTS AND DISCUSSION

According to ANOVA the results indicated highly significant differences ($p \leq 0.01$) among varieties for all traits. At the same time as the effect of sowing dates was also significant for all the traits. Whereas the interaction among varieties and sowing dates was also significant for all traits (Table 1). These results verified the impact of sowing dates on growth and yield parameters of cotton.

2.1. Seed Germination %

It was one of the growth parameters collected from all sowing dates. Figure 1 shows the seed germination%, the best results were determined of Stoneville 213 by 60% on March 1st. If fox on the figure can reached to some fact about the Stoneville 213 variety for ability to adaptation in cool condition. While in the first sowing date, Lachata variety was not germinated very well 20% only. Despite that, the best results were collected from March 28 and April 27 of both varieties Lachata and Coker 310 which was almost 100% with increasing the temperature. Based on germination results Stoneville 213 variety could be selected for planting of other states of Kurdistan region especially at Sulaymaniyah and Sharazur states.

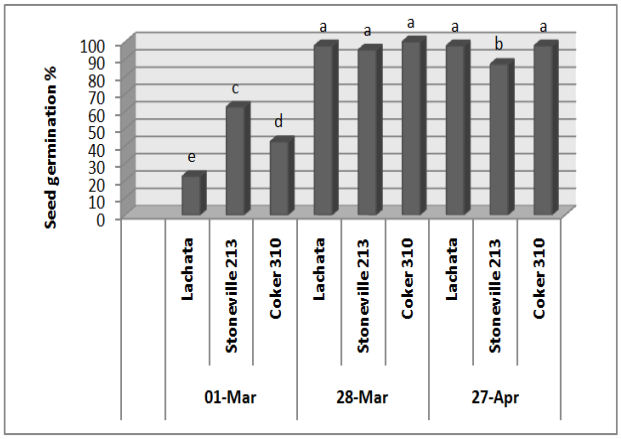

Figure 1: Effect of sowing dates and varieties on the seed germination %.

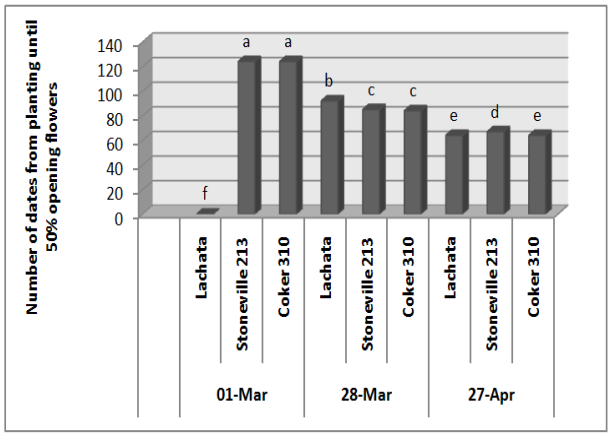

Figure 2: Effect of sowing dates and varieties on the number of dates from planting until 50% opening flowers.

2.2. Number of Dates from Planting Until 50% Opening Flowers

Significant results were obtained among varieties and also sowing dates. The mean number of dates from planting until 50% opening flowers was 124 days of each of Stoneville 213 and Coker 310 in March 1st which was the maximum days to flowering while the minimum days was recorded in Lachata and Coker 310 (64) days in April 27.

Additionally, these results were decreased with increasing temperature which was among varieties (Figure 2). Similar results were recorded by Saleem *et al.* (2010), who reported that number of days from planting to appearance of first flower as affected by varieties and phosphorus levels. On the other hand, several researchers reported that the early maturity affected by the first flowering. Appearance of first flower can be used as an indicator of early maturing cultivar (Khan *et al.*, 2002). Furthermore, Godoy and Palomo, (1999) reported that a cultivar taking less number of days to flowering and first boll opening may be classified as earlier. The two cotton cultivars CRIS-134 and Sadori were characterized as early maturing in terms of days to 1st flower (Baloch *et al.*, 2014).

2.3. Number of Dates from Planting Until 50% Boll Opening

Boll opening has an important role which is relationship to production. More number of boll openings at earlier growth period is also considered as an important criterion to enumerate the cotton cultivars for earliness.

Figure 3 showed the significant differences among sowing dates but any differences were not found between varieties. Similar results were found by (Sedeeq and Rabar, 2011) who reported that varieties were not significant affected on boll opening. Additionally, late-planted cotton initiates flowering later in the growing season, which will cause bolls to develop later in cooler conditions, lengthening the period from sowing to boll opening and delaying maturity (Bauer *et al.*, 2000; Shastry *et al.*, 2001; Bange and Milory, 2004; Davidonis *et al.*, 2004; Wanga *et al.*, 2016). The results of this study support these earlier findings. Sowing date 3 (April 27) showed a lower percent of open bolls compared to second and first sowing dates (March 28 and March 1st), respectively. While, in this current study the first sowing date March 1st was cooler than March 28 and April 27 which was significantly affected on boll opening because might be correlated to the ability of plants to get the full benefits of environmental conditions during this growing season.

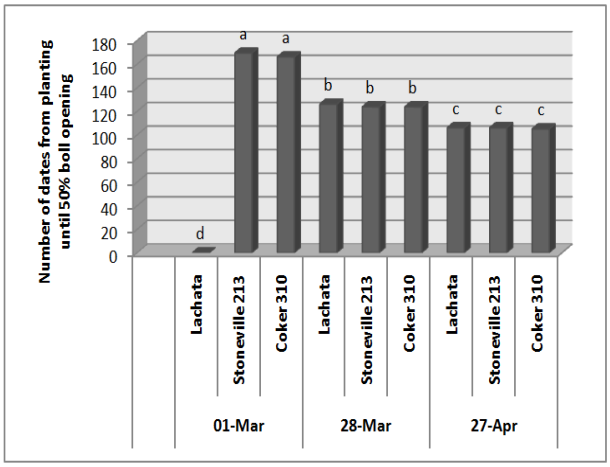

Figure 3: Effect of sowing dates and varieties on the number of dates from planting until 50% boll opening.

2.4. Plant Height (cm)

Both factors varieties and sowing dates significant affected of plant height (Figure 4). The highest plant high was recorded for Stoneville 213 at all sowing dates (110.8, 102.4 and 92.3 cm) of (April 27, March 28 and March 1st), respectively. Despite that, the plant height of Coker 310 was (95.1cm) at April 27. In the results of Islam *et al.* (2001) varieties were also significantly affected of plant height.

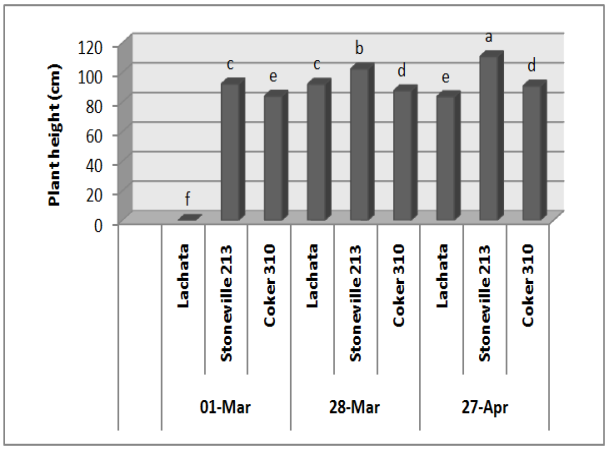

Figure 4: Effect of sowing dates and varieties on the plant height (cm).

2.5. Seed Cotton Yield (kg/ha)

It is the most important parameter since directilly affected of production. Additionally, which was relationship to each of first flowering and first boll opening parameters. By foxing to each of figers (2, 3 and 5) can be reached to the above facts. Lachata was recorded the biggest value of seed cotton yield which was by (5851.13 kg/ha) when same variety was recorded the high number of first flowering and boll opening figuer (2, 3 and 5), respectivlly which was from second sowing date March 28. However, sowing dates significantelly affected on seed cotton yield so this showed that sowing dates are more important factor it should be take a valliable place of agricultuer processing. The highest seed cotton yield was given by SLH-284 at sowing date of May 20. The minimum yield was given by CIM-496 at sowing date of June 10. Seed cotton yield was also significantly correlated with number of bolls matured per unit area (Arshad *et al.*, 2007). Moreover, similar results were found by other researchers. Saeed *et al.* (2014) reported that yield and quality of cotton were affected by genotypes and sowing dates. Mohamed *et al.* (2016) also concluded that the period from the first of July to the mid of it can be considered as the optimum sowing date for yield of the cotton (Khalifa - var) at Sudan and under rain fed condition.

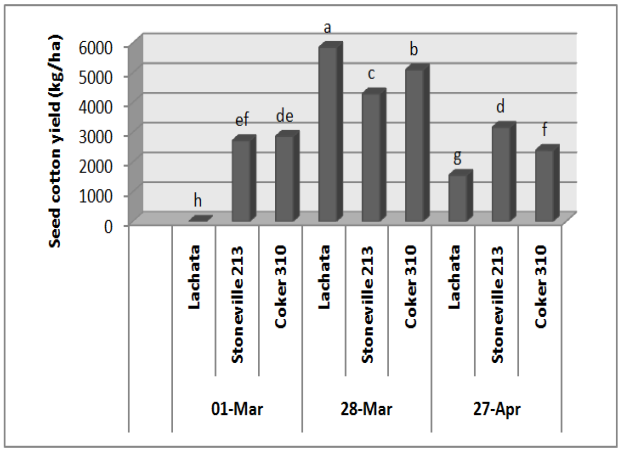

Figure 5: Effect of sowing dates and varieties on the seed cotton yield (kg/ha).

2.6. Early Maturity Rate (%)

In this current study, variety and sowing dates significantly affected of early maturity rate. The earliest maturity rate was recorded by the Stoneville 213 variety which was (70.23%), followed by Coker 310 (66.77%) in March 28 (Figure 6). Early maturity was strongly correlated to first flowering and boll formation as can be seen in the (Figures 2, 3 and 6).

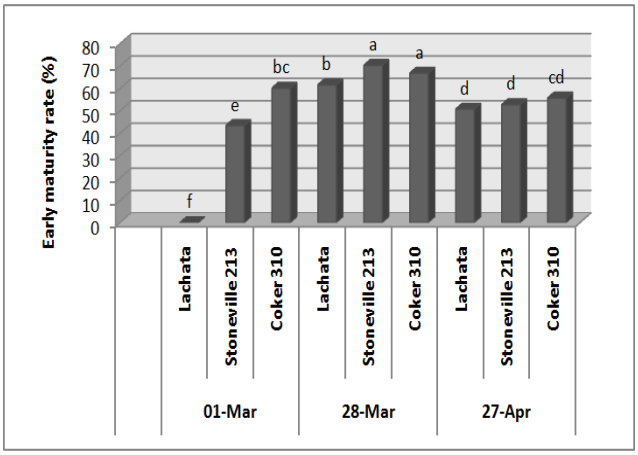

Figure 6: Effect of sowing dates and varieties on the early maturity rate (%).

2.7. Mean Boll Weight (g)

Variety and sowing dates were not effected on mean boll weight only in sowing date March 1st Lachata was not showed any sign for this parameter as other parameters were studied since did not growin (Figure 7). Within, it could be considered that boll weight showed negative association with seed cotton yield. Similar results were found by (Ali *et al.*, 2009).

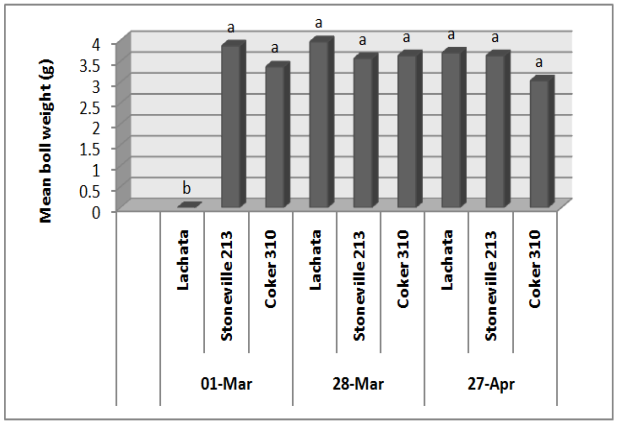

Figure 7: Effect of sowing dates and varieties on the mean boll weight (g).

2.8. Number of Seeds/Boll

Varieties and sowing dates were not significant on number of seeds per boll. The most number of seeds was showed of Lachat variety (28.23) in sowing date in March 28 followed by Stoneville 210 by (25.77) in March 1st (Figure 8).

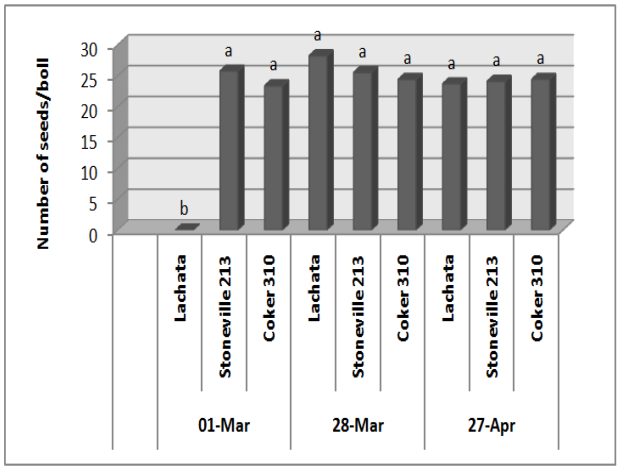

Figure 8: Effect of sowing dates and varieties on the number of seeds/boll.

2.9. Seed Index (g)

In this study, cotton variety had significant response to sowing date. Among the tested varieties, Coker 310 was recorded the highest number of seed index by (10.23g) in second sowing date March 28 (Figure 9). Additionally, the results of seed index were very close between other varieties and sowing dates. As stated by Mohamed *et al.* (2016) sowing dates were highly affected of seed index.

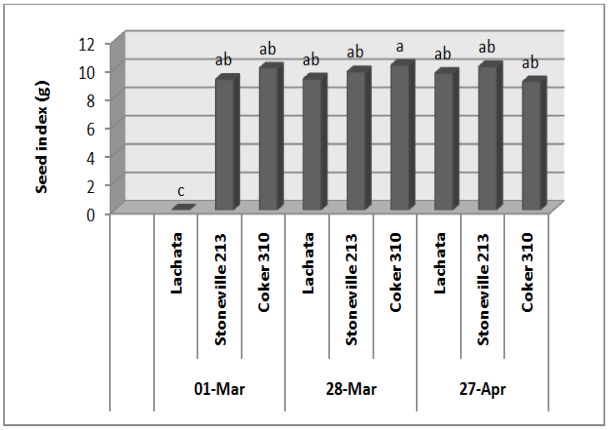

Figure 9: Effect of sowing dates and varieties on the seed index (g).

3. CONCLUSIONS

It is concluded that cultivar Lachata followed by Coker 310 produced maximum of seed cotton yield when planted on March 28. Results were also showed that other variety can be provided the high yield. Therefore, the appropriate sowing time is suggested to be for the mid of spring or in the cool condition of Stoneville 213 variety in Kurdistan Region. Additionally, these finding are interested to advice farmers not only about cotton

plant but also about other field crops which is to understand the impact of sowing date on growth, quantity and quality of crops.

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Table (1) The analysis of variance (ANOVA) for the effect of sowing dates and cotton varieties on growth and yield parameters

Source DF	V	S	V×S	V	S	V×S	V	S	V×S
	2	2	4	2	2	4	2	2	4
Parameters	**Mean Square**			**F. value**			**P. value**		
SG %	208.25900	8633.037	560.9260	16.39400	679.569	44.15500	.000	.000	.000
NDPOF %	4645.3330	1222.333	5398.333	1990.857	523.857	2313.571	.000	.000	.000
NDPBO %	9061.3700	825.5930	9532.759	1735.156	158.092	1825.422	.000	.000	.000
PH (cm)	4364.2060	3856.824	2108.584	142.5250	125.955	68.86100	.000	.000	.000
SCY (kg/ha)	2725534.7	27010842	44150910	64.74100	641.604	104.8740	.000	.000	.000
EMR %	1353.8410	2282.783	803.1110	154.5680	260.625	91.69100	.000	.000	.000
MBW (g)	3.0380000	4.321000	5.386000	10.54400	14.9970	18.69200	.001	.000	.000
NSB/boll	161.94400	235.2910	228.9680	17.03300	24.7480	24.08300	.000	.000	.000
SI (g)	35.373000	32.05300	29.73300	108.0410	97.9000	90.81400	.000	.000	.000

Significant occurs when $P \leq 0.05$
V= variety, S= sowing dates, DF= degree of freedom, SG= seed germination, NDPOF= number of days from planting until 50% opening flowers, NDPBO= number of days from planting until 50% boll opening, PH= plant height, SCY= seed cotton yield, EMR= early maturity rate, MBW= mean of boll weight, NSB= number of seeds/boll, SI= seed index

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RESEARCH PAPER

Co-Tripotent Elements

Neshtiman N. Sulaiman, Parween A. Hummadi[1]

[1]Department of Mathematics, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq

A B S T R A C T:

In this paper, the concept of a co-tripotent element is introduced. The main purpose is to discuss co-tripotent elements of the ring $\mathbb{Z}_m$, where $m \in \{ 2^n, 2p, p^n q\}$, ($p$ and q are two distinct odd primes and $n > 2$). Also co-tripotent elements have been studied of the group ring $\mathbb{Z}_2$G, where G is cyclic group of order 2^n, n≥ 1.

KEY WORDS: Idempotent element, Tripotent element, Co-tripotent element.
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INTRODUCTION :

An element x of a ring R is called idempotent if $x^2 = x$. Idempotent elements have important role in decomposition of rings. In 2010, the concept of m-idempotent element ($m > 1$) was introduced by (Chaoling and Youghua, 2010). An element x of a commutative ring R is called m-idempotent if $x^m = x$, and x is said to be a nontrivial m-idempotent if m is the least positive integer such that $x^m = x$. Tripotent elements (3- idempotent) were studied by P. Hummadi and A. Mum in (Hummadi and Mum, 2010 ,Hummadi and Mum, 2013).

In this paper we introduce and study the concept of co-tripotent element. A nonzero element x of a ring R called a co-tripotent element if $x\alpha = x$ for some nontrivial tripotent element α of R. The tripotent element α is said to be an associated tripotent of x. Recall Bezout's Lemma which state that given two integers a and b, both are not zero, there exist integers x and y such that $gcd(a, b) = ax + by$ [See (David, 2006), Theorem 2.3]. We use this lemma to find the co-tripotent elements in Z_m, where $m \in \{2^n, 2p, p^n q\}$, (p and q are two distinct odd primes and $n > 2$). Moreover we study co-tripotent elements in the group rings, remembering that a group ring is a free module over the ring and the same time it is a ring constructed in a natural way from any given ring and any given group (David SD, 2004).

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* **Corresponding Author:**
Neshtiman N. Sulaiman
E-mail: neshtiman.suliman@su.edu.krd
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2. Preliminaries.

For proving the main results, we need some results. We start as follows:

Proposition 2.1. [(Hummadi and Mum, 2010), Proposition 1.3]
In Z_n, $n > 2, n-1$ is a nontrivial tripotent element.

Proposition 2.2. [(Hummadi and Mum, 2010) , Proposition 1.7]
$Z_{2^n}, n > 2$ has exactly three nontrivial tripotent elements, they are $(2^{n-1}-1), (2^{n-1}+1)$and (2^n-1).

Proposition 2.3. [[(Hummadi and Mum, 2010) , Proposition 1.8]
$Z_{p^n}, n \geq 1$, p is a prime has only one nontrivial tripotent element, namely (p^n-1).

Proposition 2.4. [[(Hummadi and Mum, 2010) , proposition 1.10]
Z_{2p}, p is an odd prime, has exactly two nontrivial tripotent elements, they are$(p-1)and\ (2p-1)$.

Proposition 2.5. [[(Hummadi and Mum, 2010) , Theorem 1.11]
Z_{p^nq}, where p, q are two distinct odd prime numbers, has exactly five nontrivial tripotent elements, they are $tq-1, 2tq-1, p^nq-tq, 1-2tq$ and p^nq-1. (where $tq-sp^n=1$, for some integers t and s by Bezout's Lemma since $gcd(q,p^n)=1$).

Proposition 2.6. [[(Hummadi and Mum, 2013) , Theorem1.10]
In the group ring Z_2G, where G is cyclic group generated by g of an odd order m, no nontrivial tripotent elements.

2. Co-tripotent Elements

In this section, we study co-tripotent elements of the ring Z_m, where $m \in \{2^n, 2p, p^nq\}$, (p and q are two distinct odd primes and $n > 2$) and of some type of group rings.

We start by the following result.

Lemma 3.1. If x is a co-tripotent element of a commutative ring R and y is a nonzero element of R, then xy is a co-tripotent element of R.
Proof. Suppose x is a co-tripotent element of R. Then there exists a nontrivial tripotent element $\alpha \in R$, such that $x\alpha = x$. Therefore for each $0 \neq y$ in R, $(xy)\alpha = (x\alpha)y = xy$. Hence xy is a co-tripotent element. ■

Now we study co-tripotent elements of the ring Z_n.

Proposition 3.2.
$Z_{p^n}, n \geq 1$, p is an odd prime number, has no co-tripotent element.
Proof.
Suppose x is a co-tripotent element in Z_{p^n}, $n \geq 1$. Then $x\alpha = x\ (mod\ p^n)$, for some nontrivial tripotent α.
By Proposition 2.3, the only nontrivial tripotent element in Z_{p^n},, $n \geq 1$, is p^n-1. Hence

$$x(p^n-1) \equiv x(mod\ p^n)$$
$$xp^n - x \equiv x(mod\ p^n)$$
$$x + x \equiv 0(mod\ p^n)$$
$$2x \equiv 0(mod\ p^n).$$

Contradiction with p is an odd prime number.■

Proposition 3.3.
Z_{2p}, p is an odd prime number, has only one co-tripotent element, namely p.
Proof. By Proposition 2.4, Z_{2p}, p is an odd prime number has exactly two nontrivial
tripotent elements $p-1$ and $2p-1$
If $x = p$, take $\alpha = 2p-1$
Now,

$$p(2p-1) \equiv 2p^2 - p(mod\ 2p)$$
$$\equiv 2p - p(mod\ 2p)$$
$$\equiv p(mod\ 2p).$$

Therefore p is a co-tripotent element.
Suppose that x is any co-tripotent element different from p, then $x\alpha = x\ (mod\ 2p)$, for some nontrivial tripotent element α. Then either $x(p-1) \equiv x\ (mod\ 2p)$ or $x(2p-1) \equiv x\ (mod\ 2p)$.
If $x(p-1) \equiv x\ (mod\ 2p)$, then
$xp - 2x \equiv 0(mod\ 2p)$, which means $2p|x(p-2)$. Since $p \nmid (p-2)$, hence $p|x$. Contradiction.
If $x(2p-1) \equiv x\ (mod\ 2p)$, then
$2xp - x \equiv x(mod\ 2p)$, which means that

$2x \equiv 0\ (mod\ 2p)$, that is $p|x$. Contradiction.■

Proposition 3.4.

x is a co-tripotent element in $Z_{2^n}, n > 2$ if and only if $x = 2k$, for $k \in Z^+$.

Proof. (⇐)

By Proposition 2.2, $Z_{2^n}, n > 2$ has exactly 3 nontrivial tripotent elements, they are $2^{n-1} - 1, 2^{n-1} + 1$, and $2^n - 1$

Suppose $x = 2k$ for $k \in Z^+$. Take $\alpha = 2^{n-1} + 1$.

Now, $2k(2^{n-1} + 1) \equiv 2^n k + 2k\ (mod\ 2^n)$

$\equiv 2k\ (mod\ 2^n)$.

Therefore $x = 2k$, is a co-tripotent element.

(⟹) Now for other direction we use contrapositive and suppose $x = 2k + 1$, for $k \in Z^+$ is a co-tripotent element.

Then $x\alpha = x\ (mod\ 2p)$, for some nontrivial tripotent element α.

If $\alpha = 2^{n-1} - 1$, we get

$(2k + 1)(2^{n-1} - 1) \equiv 2k + 1\ (mod\ 2^n)$

$2^n k - 2k + 2^{n-1} - 1 \equiv 2k + 1\ (mod\ 2^n)$

$2^{n-1} - (4k + 2) \equiv 0\ (mod\ 2^n)$.

Thus $2^n|(2^{n-1} - (4k + 2))$,which is impossible

If $\alpha = 2^{n-1} + 1$, then

$(2k + 1)(2^{n-1} + 1) \equiv 2k + 1\ (mod\ 2^n) \Longrightarrow$

$2^n k + 2k + 2^{n-1} + 1 \equiv 2k + 1\ (mod\ 2^n)$

$2^{n-1} \equiv 0\ (mod\ 2^n)$.

Hence $2^n|2^{n-1}$, which is not true.

Finally if $\alpha = 2^n - 1$, then

$(2k + 1)(2^n - 1) \equiv 2k + 1\ (mod\ 2^n)$

$2^{n+1}k - 2k + 2^n - 1 \equiv 2k + 1\ (mod\ 2^n)$

$4k + 2 \equiv 0\ (mod\ 2^n)$.

Hence $2^{n-1}|(2k + 1)$. Contradiction for $2k + 1$ is an odd number. This completes the proof. ■

Proposition 3.5.

Let $p\ and\ q$ be two distinct odd prime numbers. Then x is a co-tripotent element of $Z_{p^n q}$ $(n \geq 1)$ if and only if $x = kp^n$ or $x = kq$, for $k \in Z^+$, in which $kp^n, kq \leq p^n q$.

Proof.

By Proposition 2.5, $Z_{p^n q}$ has exactly 5 nontrivial tripotent elements, they are $tq - 1, 2tq - 1$, $p^n q - tq$, $1 - 2tq$ and $p^n q - 1$ $(where\ tq - sp^n = 1$, for some integers t and $s)$

Suppose $x = kp^n$, for $k \in Z^+$. Take $\alpha = 1 - 2tq$. Then we get

$kp^n(1 - 2tq) \equiv kp^n - 2tkp^n q\ (mod\ p^n q)$

$\equiv kp^n\ (mod\ p^n q)$

Therefore $x = kp^n$ is a co-tripotent element.

Now suppose $x = kq$, for $k \in Z^+$ and take $\alpha = 2tq - 1$. Since $tq - sp^n = 1$, that is $2tq - 1 = 1 + 2sp^n$.

$kq(2tq - 1) \equiv kq(1 + 2sp^n)\ (mod\ p^n q)$

$\equiv kq\ (mod\ p^n q)$.

Therefore $x = kq$ is a co-tripotent element.

Conversely, suppose that x is a co-tripotent element with $q \nmid x$ and $p^n \nmid x$. Thus $x = kp^n + l$, for some l, $0 < l < p^n$ and $q \nmid x$. Hence, $x\alpha \equiv x\ (mod\ p^n q)$, for some nontrivial tripotent element α.

If $\alpha = tq - 1$, then

$(kp^n + l)(tq - 1) \equiv kp^n + l\ (mod\ p^n q)$

$(kp^n + l)(tq - 2) \equiv 0\ (mod\ p^n q)$

Thus $p^n q|(kp^n + l)(tq - 2)$. Since $q \nmid (tq - 2)$, hence $q|(kp^n + l)$, that is $q|x$. Contradiction.

If $\alpha = 2tq - 1$, then

$(kp^n + l)(2tq - 1) \equiv kp^n + l\ (mod\ p^n q)$

$(kp^n + l)(2tq - 2) \equiv 0\ (mod\ p^n q)$

As before $q \nmid (2tq - 2)$, hence $q|(kp^n + l)$. Contradiction.

If $\alpha = 1 - 2tq$, then

$(kp^n + l)(1 - 2tq) \equiv kp^n + l\ (mod\ p^n q)$,

$(kp^n + l)(-2tq) \equiv 0\ (mod\ p^n q)$

This means that $p^n|(kp^n + l)(-2t)$ implies $p^n|(-2t)$. Contradiction for p is an odd prime number.

If $\alpha = p^n q - tq$, then

$(kp^n + l)(p^n q - tq) \equiv kp^n + l\ (mod\ p^n q)$

$(kp^n - l)(p^n q - tq - 1) \equiv 0\ (mod\ p^n q)$

Clearly $q \nmid (p^n q - tq - 1)$, so $q|(kp^n - l)$. Contradiction.

Similar arguments can be used for the case $\alpha = p^n q - 1$.

Now, suppose that x is a co-tripotent element with $q \nmid x$ and $p^n \nmid x$. Thus $x = kq + l$, for some l, $0 < l < q$ and $p^n \nmid x$. Hence, $x\alpha \equiv x\ (mod\ p^n q)$, for some nontrivial tripotent element α.

If $\alpha = tq - 1$, then

$(kq + l)(tq - 1) \equiv kq + l\ (mod\ p^n q)$

$(kq + l)(tq - 2) \equiv 0\ (mod\ p^n q)$

Thus $p^n q|(kq + l)(tq - 2)$. Since $p^n \nmid (kq + l)$, hence $p^n|(tq - 2)$. But $tq = 1 + sp^n$, that is $tq - 2 = sp^n - 1$, so $p^n|(sp^n - 1)$. Contradiction.

If $\alpha = 1 - 2tq$, then

$(kq + l)(1 - 2tq) \equiv kq + l\ (mod\ p^n q)$

$(kq + l)(-2tq) \equiv 0\ (mod\ p^n q)$

Thus $p^n|(kq+l)(2t)$. Since $p^n \nmid (kq+l)$, hence $p^n|(2t)$. Contradiction for an odd prime p.
If $\alpha = p^n q - tq$, then
$(kq+l)(p^n q - tq) \equiv kq + l \ (mod\ p^n q)$
$(kq+l)(p^n q - tq - 1) \equiv 0 \ (mod\ p^n q)$
Clearly $p^n \nmid (p^n q - tq - 1)$, so $p^n|(kq+l)$. Contradiction.
Similar arguments can be used for the case $\alpha = p^n q - 1$.

Thus x is not a co-tripotent element. ■

In order to prove a characterization co-tripotent elements of group rings, we need the following lemma.

Lemma 3.6. Let Z_2G be the group ring of G over Z_2, where G is a cyclic group of order 2^n, $n \geq 2$, generated by g. Then for any integers $0 < t_1 < t_2 < ... < t_k < 2^{n-1}$, $k \geq 1$, the following two elements are nontrivial tripotent
$g^{t_1} + g^{t_2} + \cdots + g^{t_k} + g^{2^{(n-1)}} + g^{t_1+2^{(n-1)}} + g^{t_2+2^{(n-1)}} ... + g^{t_k+2^{(n-1)}}$, and
$1 + g^{t_1} + g^{t_2} + \cdots + g^{t_k} + g^{t_1+2^{(n-1)}} + g^{t_2+2^{(n-1)}} ... + g^{t_k+2^{(n-1)}}$
Moreover the number of nontrivial tripotent elements is $2^{2^{(n-1)}} - 1$.
Proof. Let $t_1, t_2, ..., t_k$, be any k distinct integers with $0 < t_1 < t_2 < \cdots < t_k < 2^{n-1}$.
If
$$F_k = g^{t_1} + g^{t_2} + \cdots + g^{t_k} + g^{2^{(n-1)}} + g^{t_1+2^{(n-1)}} + g^{t_2+2^{(n-1)}} ... + g^{t_k+2^{(n-1)}},$$
then
$$F_k^2 = g^{2t_1} + g^{2t_2} + \cdots + g^{2t_k} + g^{2^n} + g^{2t_1+2^n} + g^{2t_2+2^n} + \cdots + g^{2t_k+2^n} = 1 \neq F_k.$$
Hence $F_k^3 = F_k$, therefore F_k is a nontrivial tripotent element.
Now, if
$$H_k = 1 + g^{t_1} + g^{t_2} + \cdots + g^{t_k} + g^{t_1+2^{(n-1)}} + g^{t_2+2^{(n-1)}} ... + g^{t_k+2^{(n-1)}}$$
then
$$H_k^2 = 1 + g^{2t_1} + g^{2t_2} + \cdots + g^{2t_k} + g^{2t_1+2^n} + g^{2t_2+2^n} ... + g^{2t_k+2^n}.$$
Hence $H_k^3 = H_k$, therefore H_k is a nontrivial tripotent element.
Using some probability theory, we get that the number of such nontrivial tripotent elements is $2^{2^{(n-1)}} - 1$. ■

Example 3.7. Consider the group ring Z_2G, where G is cyclic group of order $2^3 = 8$ generated by g. Then by Proposition 3.6, Z_2G has 15 nontrivial tripotent elements, they are
$F_1 = g^4$
$F_2 = g + g^4 + g^5$
$F_3 = g^2 + g^4 + g^6$
$F_4 = g^3 + g^4 + g^7$
$F_5 = g + g^2 + g^4 + g^5 + g^6$
$F_6 = g + g^3 + g^4 + g^5 + g^7$
$F_7 = g^2 + g^3 + g^4 + g^6 + g^7$
$F_8 = g + g^2 + g^3 + g^4 + g^5 + g^6 + g^7$
$F_9 = 1 + g + g^5$
$F_{10} = 1 + g^2 + g^6$
$F_{11} = 1 + g^3 + g^7$
$F_{12} = 1 + g + g^2 + g^5 + g^6$
$F_{13} = 1 + g + g^3 + g^5 + g^7$
$F_{14} = 1 + g^2 + g^3 + g^6 + g^7$
$F_{15} = g + g^2 + g^3 + g^5 + g^6 + g^7$

Now, we have the following characterization:

Proposition 3.8. In the group ring Z_2G, where G is cyclic group generated by g of an odd order m, no co-tripotent elements.
Proof. By Propositio 2.6, since Z_2G has no nontrivial tripotent elements, hence we have no co-tripotent elements.

Theorem 3.9. Let Z_2G be the group ring of G over Z_2 , where G is cyclic group of order 2^n, $n \geq 2$ generated by g. If x has an even number of summands, then x is a co-tripotent element.
Moreover the number of co-tripotent elements is $2^m - 1$, where m=$(2^n - 1)$.
Proof. Suppose that x has an even number of summands. Hence
$x = g^{t_1} + g^{t_2} + \cdots + g^{t_k}$, where $0 \leq t_1 < t_2 < ... < t_k < m$, and k is even.
Take $\alpha = g + g^2 + g^3 + \cdots + g^m$ which a nontrivial tripotent element is given by Lemma 3.6. Then
$x\alpha = (g^{t_1} + g^{t_2} + \cdots + g^{t_k})(g + g^2 + g^3 + \cdots + g^m)$
We describe the multiplication $x\alpha$ by the following array say$A$.

$$A = \begin{pmatrix} g^{t_1+1} & g^{t_1+2} & \dots & g^{t_1+m} \\ g^{t_2+1} & g^{t_2+2} & \dots & g^{t_2+m} \\ \vdots & \ddots & & \vdots \\ g^{t_k+1} & g^{t_k+1} & \dots & g^{t_k+m} \end{pmatrix}$$

$A = [a_{ij}]_{k\times m}$, where a_{ij} is the summand of $x\alpha$ which is the product of the ith summand of x with jth summand of α. This means $x\alpha = \sum_{i=1}^{k}\sum_{j=1}^{m} a_{ij}$.

Considering the first and the second rows of this array, we see that g^{t_2} occurs in rth column $(r = t_2 - t_1)$ and g^{t_1} occurs in the sth column $(s = (2^n - r))$. By adding the terms of these two rows, we get $g^{t_1} + g^{t_2}$(observing that the coefficient of each $g^i$is in Z_2). By adding the terms of the third and the fourth rows, we get $g^{t_3} + g^{t_4}$. Continuing in this manner, by adding the terms of $(k-1)th$ and $(k)th$ rows, we have $g^{t_{k-1}} + g^{t_k}$.

By adding all terms of this array, we get $g^{t_1} + g^{t_2} + \cdots + g^{t_k}$. Thus we obtain $x\alpha = x$.

That is x is a co-tripotent element.

Using some probability theory, we get that the number of such co-tripotent elements is $2^m - 1$. ■

We complete this theorem with an example to illustrate.

Example 3.10. Consider the group ring Z_2G, where G is cyclic group of order 2^2, generated by g. By Theorem 3.9, the co-tripotent elements are

$$1+g,\ 1+g^2,\ 1+g^3,\ g+g^2,\ g+g^3,\ g^2+g^3,\ 1+g+g^2+g^3.$$

We expect that the converse of Theorem 3.9 is true, that is every co-tripotent element has an even number of summands, equivalently if α is a nontrivial tripotent element, then α cannot be an associated element of an element with an odd number of summands.

To prove the above for special cases, we need to state the following lemma.

Lemma 3.11. In the group ring Z_2G, where G is a cyclic group of order $2^n, n > 1$, if $x = \sum_{i=0}^{2^n-1} a_i g^i$ and $\sum_{i=0}^{2^n-1} a_i = 0$, then x has an even number of summands.

Proof. Clearly, since $a_i \in Z_2$.

Proposition 3.12. In the group ring Z_2G, where G is a cyclic group of order $2^n, n > 1$, the nontrivial tripotent element $g^{2^{n-1}}$ is not an associated element of any element with an odd number of summands.

Proof. Suppose $\alpha = g^{2^{n-1}}$ is an associated element of $x = \sum_{i=0}^{2^n-1} a_i g^i$. Then $\alpha x = x$, that is

$$g^{2^{n-1}}\left(a_0 + a_1 g + \dots + a_{2^n-1} g^{2^n-1}\right) = a_0 + a_1 g + \dots + a_{2^n-1} g^{2^n-1}$$

Equating the coefficients of g^i of both sides, we get

$$a_0 = a_{2^{n-1}} \Longrightarrow a_0 + a_{2^{n-1}} = 0 \quad \dots \quad (1)$$
$$a_1 = a_{2^{n-1}+1} \Longrightarrow a_1 + a_{2^{n-1}+1} = 0 \quad \dots \quad (2)$$
$$a_2 = a_{2^{n-1}+2} \Longrightarrow a_2 + a_{2^{n-1}+2} = 0 \quad \dots \quad (3)$$
$$\vdots$$
$$a_{2^{n-1}-1} = a_{2^n-1} \Longrightarrow a_{2^{n-1}-1} + a_{2^n-1} = 0 \quad \dots \quad (2^{n-1})$$

Which implies $\sum_{i=0}^{2^{n-1}-1}(a_i + a_{2^{n-1}+i}) = 0$, that is $\sum_{i=0}^{2^n-1} a_i = 0$. By Lemma 3.11, x has an even number of summands. ■

Proposition 3.13. In the group ring Z_2G, where G is cyclic group of order $2^n, n > 1$, for each $0 < k < n-1$, the nontrivial tripotent element $\alpha = g^{2^k} + g^{2^{n-1}} + g^{2^{n-1}+2^k}$, is not an associated element of any element $x = \sum_{i=0}^{2^n-1} a_i g^i$.

Proof. Suppose $\alpha = g^{2^k} + g^{2^{n-1}} + g^{2^{n-1}+2^k}$ is an associated element of $x = \sum_{i=0}^{2^n-1} a_i g^i$, that is

$$(g^{2^k} + g^{2^{n-1}} + g^{2^{n-1}+2^k})\left(\sum_{i=0}^{2^n-1} a_i g^i\right) = \sum_{i=0}^{2^n-1} a_i g^i$$

Equating the coefficients of g^i in both sides, we get the following equations

$$a_0 = a_{2^n-2^k} + a_{2^{n-1}} + a_{2^{n-1}-2^k} \Longrightarrow$$
$$a_0 + a_{2^n-2^k} + a_{2^{n-1}} + a_{2^{n-1}-2^k} = 0 \quad \dots \quad (0)$$
$$a_1 = a_{2^n-2^k+1} + a_{2^{n-1}+1} + a_{2^{n-1}-2^k+1} \Longrightarrow$$
$$a_1 + a_{2^n-2^k+1} + a_{2^{n-1}+1} + a_{2^{n-1}-2^k+1} = 0 \quad \dots \quad (1)$$
$$\vdots$$
$$a_{2^k-1} = a_{2^n-1} + a_{2^{n-1}+2^k-1} + a_{2^{n-1}-1} \Longrightarrow$$
$$a_{2^k-1} + a_{2^n-1} + a_{2^{n-1}+2^k-1} + a_{2^{n-1}-1} \quad \dots \quad (2^k-1)$$
$$a_{2(2^k)} = a_{2^k} + a_{2^{n-1}+2(2^k)} + a_{2^{n-1}+2^k} \Longrightarrow$$
$$a_{2(2^k)} + a_{2^k} + a_{2^{n-1}+2(2^k)} + a_{2^{n-1}+2^k} = 0 \quad \dots \quad (2(2^k))$$
$$\vdots$$
$$a_{3(2^k)-1} = a_{2(2^k)-1} + a_{2^{n-1}+3(2^k)-1} + a_{2^{n-1}+2(2^k)-1} \Longrightarrow$$
$$a_{3(2^k)-1} + a_{2(2^k)-1} + a_{2^{n-1}+3(2^k)-1}$$

$$+a_{2^{n-1}+2(2^k)-1} = 0 \quad \dots \quad (3(2^k)-1)$$
$$\vdots$$
$$a_{2^k(2^{n-k-1}-2)} = a_{2^k(2^{n-k-1}-3)} + a_{2^{n-1}+2^k(2^{n-k-1}-2)} + a_{2^k(2^{n-k-1}-1)} \Longrightarrow$$
$$a_{2^k(2^{n-k-1}-2)} + a_{2^k(2^{n-k-1}-3)} + a_{2^{n-1}+2^k(2^{n-k-1}-2)} + a_{2^k(2^{n-k-1}-1)} = 0 \quad \dots \quad (2^k(2^{n-k-1}-2))$$
$$\vdots$$
$$a_{2(2^{n-k-1}-1)-1} = a_{2^{n-1}-2^{k+1}-1} + a_{2^{n-1}+2^k-1} + a_{2^n+2^{k+1}-1} \Longrightarrow$$
$$a_{2(2^{n-k-1}-1)-1} + a_{2^{n-1}-2^{k+1}-1} + a_{2^{n-1}+2^k-1} + a_{2^n+2^{k+1}-1} = 0 \quad \dots \quad 2(2^{n-k-1}-1)-1)$$

Adding both sides of the above equations, we get $\sum_{i=0}^{2^n-1} a_i = 0$. By Lemma 3.11, we get that x has an even number of summands. ■

We illustrate the above Proposition by the following example.

Example 3.14.
Consider the group ring Z_2G, where G is cyclic group of order 2^5 generated by g. Let $\alpha = g^4 + g^{16} + g^{20}$, which is a nontrivial tripotent element of Z_2G. By Proposition 3.13 suppose α is an associated element of any element $x = \sum_{i=0}^{31} a_i g^i$, that is $\alpha \sum_{i=0}^{31} a_i g^i = \sum_{i=0}^{31} a_i g^i$.
Equating the coefficient of g^i, for each i in both sides, we obtain the following

$$a_0 + a_{28} + a_{16} + a_{12} = 0 \quad \dots (0)$$
$$a_1 + a_{29} + a_{17} + a_{13} = 0 \quad \dots (1)$$
$$a_2 + a_{30} + a_{18} + a_{14} = 0 \quad \dots (2)$$
$$a_3 + a_{31} + a_{19} + a_{15} = 0 \quad \dots (3)$$
$$a_8 + a_4 + a_{24} + a_{20} = 0 \quad \dots (4)$$
$$a_9 + a_5 + a_{25} + a_{21} = 0 \quad \dots (5)$$
$$a_{10} + a_6 + a_{26} + a_{22} = 0 \quad \dots (6)$$
$$a_{11} + a_7 + a_{27} + a_{23} = 0 \quad \dots (7)$$

Adding both sides of the above equations, we get $\sum_{i=0}^{31} a_i = 0$. By Lemma 3.11, we get that x has an even number of summands.

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RESEARCH PAPER

CLASSIFICATION OF A_8 FOR GENUS 1.

Peshawa M. Khudhur[1], Haval M. Mohammed Salih[2]

[1]Department of Mathematical , Faculty of Science, Soran University, Kurdistan Region, Iraq
[2]Department of Mathematical , Faculty of Science, Soran University, Kurdistan Region, Iraq

A B S T R A C T:
In this paper, we give a complete classification of all primitive genus one systems of degree $n \in \{8,15,28,35,56\}$ for the group A_8. Also, we use computational tool to show that whether or not there are primitive genus one systems of the group A_8 for some given degrees.

KEY WORDS: Monodromy Group, Genus one System and Primitive Group.
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1. INTRODUCTION

Assume that Ω is a finite set of order n and let G be a transitive subgroup of $S_{|\Omega|}$. We denote a group $G\setminus\{1\}$ by $G^{\#}$. Suppose that there exists elements $x_1,\dots,x_r$ in $G^{\#}$such that

(1) $G = \langle x_1, x_2, \dots, x_r\rangle$
(2) $\prod_i^r x_i = 1,\ \ x_i \in G^{\#},\ \ i = 1,\dots,r$
(3) $\sum_{i=1}^{r}(\mathrm{ind}x_i) = 2(n+g-1)$

where $\mathrm{ind}x_i$ is the minimal number of transpositions needed to express x_i as a product and the number of tore called the genus g. Furthermore, equation 3 is said to be Riemann Hurwitz formula.

The group G in the equation 1 is called group of genus g, and the system $(x_1, x_2, \dots, x_r)$ is called genus g -system. This condition corresponds to the existence of an n-branched cover of the Riemann sphere $\mathbb{P}^1 = \mathbb{C}\cup\{\infty\}$ by a Riemann surface R of genus g with r −branch points. (Robert,1990).

The conjugacy class of x_i is denoted by C_i. Then the ramification type of the cover

(4) $\varsigma: R \longrightarrow \mathbb{P}^1$ is defined by multi-set of non trivial conjugacy classes $C = \{C_1,\dots,C_r\}$ in G. While x_i is not uniquely determined by R and ς. This interesting fact give a permeation to discuss braid actions.

If the action of G on $\{1,\dots,n\}$ is primitive, we call G a ***primitive genus g group*** and $(x_1, x_2, \dots, x_r)$ ***a primitive genus g-system.***

The structure of a primitive group is made explicit by Aschbacher and O'Non-Scott Theorem (Robert,1990).

Wang studied all primitive groups of affine type of degree 8(Wang, 2011). The same type of group classified for genus one and two by H. Mohammed Salih in his PhD thesis (Mohammed Salih , 2014). Furthermore he classified the primitive group A_8 for genus zero in (H. Mohammed Salih, 2016). Our goal in this paper is to show the existence for genus one systems for the primitive group A_8 on different points.

2. PRELIMINARY

The details of the following results can be found in (Michael, 1989), (K. Magaard,2012) and

* **Corresponding Author:**
Peshawa Mohammed Khudhur
E-mail: peshawa3@gmail.com
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(Haval , 2014). We denote by O_r, the space of subsets of $\mathbb{C}$ of cardinality r.

Definition2.1: The surjective map $\phi: \pi_1(\mathbb{P}^1 \setminus B, b_0) \longrightarrow G;\ B \in O_r;\ b_0 \in \mathbb{P}^1 \setminus B$, is called admissible if it is a homomorphism, and $\phi(\sum_b) \neq 1$ for each $b \in B$. Here $\sum_b$, is denoted the conjugacy class of $\pi_1(\mathbb{P}^1 \setminus B, b_0)$.

Definition2.2: Let $\phi: \pi_1(\mathbb{P}^1 \setminus B, \infty) \longrightarrow G$ and $\phi^*: \pi_1(\mathbb{P}^1 \setminus B^*, \infty) \longrightarrow G$ be two surjective admissible map and $A \leq \mathrm{Aut}(G)$. Then we say that two pairs (B, ϕ) and (B^*, ϕ^*) are A -equivalent if and only if $B = B^*$ and $\phi^* = a \circ \phi$ for some $a \in A$.

Let $[B, \phi]_A$ denote the A-equivalence class of (B, ϕ). The set of equivalence classes $[B, \phi]_A$ is denoted by $H_r^A(G)$ and is called the Hurwitz space of G-covers.

Next we introduce the **Nielsen classes** as follows: for a ramification type $C = \{C_1, \ldots, C_r\}$, the Nielsen classes is defined by the set $N(C) = \{(x_1, x_2, \ldots, x_r)$: $G = \langle x_1, x_2, \ldots, x_r \rangle$, $\prod_i^r x_i = 1$, $\exists \sigma \in S_n$ such that $x_i \in C_{i\sigma}$ for all i$\}$.

Lemma2.3: The map $\psi_A: H_r^A(G) \longrightarrow O_r$, $\psi_A([P, \phi]) = P$ is covering.

Proof: For the proof see (Volklein, 1996).

Lemma2.4: We obtain a bijection $\psi_A^{-1}(P_0) \longrightarrow \epsilon_r^A(G)$ by sending $[P_0, \phi]_A$ to the generators $(x_1, x_2, \ldots, x_r)$ where $x_i = \phi([\gamma_i])$ for $i = 1, \ldots, r$.

Proof: For the proof see (Volklein, 1996).

The group A also acts on N(C) via sending $(x_1, x_2, \ldots, x_r)$ to $(x_1^a, x_2^a, \ldots, x_r^a)$, for $a \in A$. from now on we denote $N(C)/A$ by $N^A(C)$.

Proposition 2.5: Let C be a fixed ramification type in G, and the subset $H_r^A(C)$ of $H_r^A(G)$ consists of all $[B, \phi]_A$ with $B = \{b_1, \ldots, b_r\}$, $\phi: \pi_1(\mathbb{P}^1 \setminus B, \infty) \longrightarrow G$ and $\phi(\sum_{b_i}) \in C_i$ for $i = 1, \ldots, r$. Then $H_r^A(C)$ is the union of connected components in $H_r^A(G)$. Under the bijection from Lemma 2.4, the fiber in $H_r^A(C)$ over P_0 corresponds the set $N^A(C)$. This yields a one to one correspondence between components of $H_r^A(C)$ and the braid orbits on $N^A(C)$. In particular, $H_r^A(C)$ is connected if and only if B_r acts transitively on $N^{in}(C) = N(C)$.

3- INDICES AND TYPES

In this section we present two methods for computing index as follows:

(1) Throughout this paper we will label the thirteen nontrivial conjugacy class of A_8 by the same notation as in ATLAS(J,H.Conway,1985).

TABLE 1. Conjugacy class of A_8

Type	Conjugacy class	Ind
2A	$(1\,2)(3\,4)(5\,6)(7\,8)^{A_8}$	4
2B	$(1\,2)(3\,4)^{A_8}$	2
3A	$(1\,2\,3)^{A_8}$	2
3B	$(1\,2\,3)(4\,5\,6)^{A_8}$	4
4A	$(1\,2\,3\,4)(5\,6\,7\,8)^{A_8}$	6
4B	$(1\,2\,3\,4)(5\,6)^{A_8}$	4
5A	$(1\,2\,3\,4\,5)^{A_8}$	4
6A	$(1\,2\,3\,)\,(4\,5)(6\,7)^{A_8}$	4
6B	$(1\,2\,3\,4\,5\,6\,)(\,7\,8)^{A_8}$	6
7A	$(1\,2\,3\,4\,5\,6\,7\,)^{A_8}$	6
7B	$(1\,2\,3\,4\,5\,6\,8)^{A_8}$	6
15A	$(1\,2\,3\,4\,5\,)(\,6\,8\,7)^{A_8}$	6
15B	$(1\,2\,3\,4\,5\,\,)(\,6\,7\,8)^{A_8}$	6

(2) Via fix points

Before, we discuss computing the indices, we give some alternative formula to compute index of an element in a group. Let G be a group acting on a finite set Ω and $|\Omega| = n$. If $x \in G$, define the index of x by $\mathrm{ind}\, x = n - \mathrm{orb}\, x$, where $\mathrm{orb}\, x$ is the number of orbits of $\langle x \rangle$ on Ω. For $x \in G$ define $\mathrm{Fix}\, x = \{\omega \in \Omega | x\omega = \omega\}$ and $f(x) = |\mathrm{Fix}\, x|$. Furthermore, $\mathrm{orb}\, x = \frac{1}{d}\sum_{i=0}^{d-1} f(x^i)$, where x has order d (Robert,1990). Note that the group A_8 has elements of order 2,3,4,5,6,7 and 15. From the character table of A_8, we can compute fix points, which is equal to $1a + 2a$ of the elements of given orders (J,H.Conway,1985).

If x is an element of order 2 of type A, then $\mathrm{ind}\, x = n - \frac{1}{2}\sum_{i=0}^{1} f(x^i) = n - \frac{1}{2}[f(x^0) + f(x)] = 8 - \frac{1}{2}[8 + 0] = 4$.

If x is an element of order 2 of type B, then $\mathrm{ind}\, x = n - \frac{1}{2}\sum_{i=0}^{1} f(x^i) = n - \frac{1}{2}[f(x^0) + f(x)] = 8 - \frac{1}{2}[8 + 4] = 2$.

By using the same step we can find indices of the elements of order 3,4,5,6,7 and 15.

Remark 3.1. In this paper, we use the same algorithm in (H.Mohammed Salih.,2016). and (Haval Mohammed Salih and Ismael Akray,2017).

4-RESULTS

Throughout this section, we assume that $G = M$, where M is the point stabilizer in G, V is a regular normal subgroup of G and $M \cap V = 1$. Note that G is primitive, V is an elementary abelian p-group, and M acts irreducibly on V. Let $|V| = p^e$ and $|x_i| = d_i$. We need to prove some results related to it.

Proposition 4.1: Assume that a group G acts transitively and faithfully on Ω and $|\Omega| = n$. Let $r \geq 2$, $G = \langle x_1, x_2, \dots, x_r \rangle$, $\prod_i^r x_i = 1$, and $|x_i| = d_i > 1$, $i = 1, \dots, r$. Then one of the following holds:

(1) $\sum_{i=1}^{r} \frac{d_i - 1}{d_i} \geq \frac{85}{42}$.

(2) $r = 4$, $d_i = 2$ for each i and $G'' = 1$.

(3) $r = 3$ and (up to permutation) $(d_1, d_2, d_3) =$
   a) $(3,3,3), (2,3,6)$ or $(2,4,4)$ and $G'' = 1$.
   b) $(2,2,d)$ and G is dihedral.
   c) $(2,3,3)$ and $G \cong A_4$.
   d) $(2,3,4)$ and $G \cong S_4$.
   e) $(2,3,5)$ and $G \cong A_5$.

(4) $r = 2$ and G is cyclic.

Proof : For the proof see (Wilhelm,1974).

Lemma 4.2: If G is an affine primitive group and $x \in G$ has no fixed points, then $[x, V] \neq V$.

Proof: Assume that $[x, V] = V$ and $v \in V$. Thus $v \in [x, V]$ by Lemma 3.2 in (Michael, 1989), we have $x^V \cap M \neq \emptyset$ and hence $x^G \cap M \neq \emptyset$. Since $x \in G$, we can write $x = mv$ for $m \in M$ and $v \in V$. That is, x is conjugate to m. Thus $f(x) = f(m) \neq 0$.

Lemma 4.3: Let $G = \langle x_1, x_2, x_3 \rangle$ such that $x_1 . x_2 . x_3 = 1$ and $e = 2$. If x_1 has order 2 with $\det x_1 = -1$ and if x_2 has order 3 with $\det x_2 = 1$. Then one of the following holds:

1) If $f(x_3) = 0$, then $d_3 = 2p$ and $\operatorname{ind} x_3 = p^2 - \frac{1}{2}(p+1)$.
2) If $f(x_3) > 0$, then one of the following holds:
   a) $d_3 = 4p$ and $\operatorname{ind} x_3 = p^2 - \frac{1}{2}(p+1)$.
   b) $\gcd(d_3, p) = 1$ and one of the following holds:
      I. $d_3 \cong 0 \bmod 4$ and $\operatorname{ind} x_3 = \frac{d_3 - 1}{d_3}(p^2 - 1)$ or
      II. $d_3 \cong 2 \bmod 4$ and $\operatorname{ind} x_3 = \frac{d_3 - 1}{d_3}(p^2 - p) + \frac{d_3 - 2}{d_3}(p - 1)$ and the eigen values of x_3 have multiplicative order d_3 and $d_3/2$.

Proof: Since $\det x_1 = -1$, $\det x_2 = 1$ and $x_1 . x_2 . x_3 = 1$, we see that $\det x_3 = 1$. Thus, d_3 is even.

Also, We see that $d_3 > 6$. If $f(x_3) = 0$, then by Lemma 4.2, $[x_3, V] \neq V$,. Since $x_3 \in G$ we can write $x_3 = mv$ for $m \in M$ and $v \in V$. The order of m is 2, hence m has eigenvalues ± 1 and thus $x_3^2 \in V$; that is, x_3 has order $2p$. Recall that $\operatorname{ind} x_3 = n - \operatorname{orb}(x_3) = n - \frac{1}{2p}(f(x_3^0) + f(x_3^1) + f(x_3^2) = p^2 + \frac{1}{2}(p^2 + p)$.

Therefore $\operatorname{ind} x_3 = p^2 - \frac{1}{2}(p+1)$.

Now assume that $f(x_3) > 0$. If $p \mid d_3$, then $x_3^{p \mid d_3}$ has order p, hence is conjugate to $\begin{pmatrix} 1 & 0 \\ a & 1 \end{pmatrix}$ where $a \in \mathbb{F}_p$. We know that $x_3 = yz$, $\gcd(|z|, p) = 1$. Without loss of generality by taking

$$\begin{pmatrix} c & d \\ e & f \end{pmatrix} \begin{pmatrix} 1 & 0 \\ 1 & 1 \end{pmatrix} = \begin{pmatrix} 1 & 0 \\ 1 & 1 \end{pmatrix} \begin{pmatrix} c & d \\ e & f \end{pmatrix}.$$

We conclude that the order of $\begin{pmatrix} \lambda & 0 \\ b & \lambda \end{pmatrix}$ is equal to $p|\lambda|$, where $b \in \mathbb{F}_p$. But $\lambda^2 = -1$. Hence $d_3 = 4p$. We can compute the index of that matrix. So we have 0 subspace which gives 1 orbit and element of order 4 gives $\frac{p-1}{4}$ orbits and finally element of order $4p$ gives $\frac{p^2 - p}{4p}$ orbits. Hence $\operatorname{ind} x_3 = p^2 - (1 + \frac{p-1}{4} + \frac{p^2 - p}{4p} = p^2 - \frac{1}{2}(p+1)$.

Assume now $\gcd(d_3, p) = 1$ and if $f(x_3) > 0$, then x_3 is conjugate to $\begin{pmatrix} \lambda & 0 \\ 0 & \mu \end{pmatrix}$.

If $d_3 \cong 2 \bmod 4$, then the eigenvalues of x_3 have multiplicative order d_3 and $d_3/2$. Thus

$\operatorname{ind} x_3 = p^2 - \left(1 + \frac{p-1}{d_3} + 2\left(\frac{p-1}{d_3}\right) + \frac{p^2 - 2(p-1) - 1}{d_3}\right) = \frac{d_3 - 1}{d_3}(p^2 - p) + \frac{d_3 - 2}{d_3}(p - 1)$.

If $d_3 \cong 0 \bmod 4$, then $|\lambda| = |\mu|$. Thus $\operatorname{ind} x_3 = p^2 - \left(1 + \frac{p-1}{d_3} + \frac{p-1}{d_3} + \left(\frac{p^2-2(p-1)-1}{d_3}\right)\right) = \frac{d_3-1}{d_3}(p^2-1)$. Now the following are our main results:

Lemma 4.4: If $r \leq 7$, $n = 8$ and $G = A_8$, then the Hurwitz spaces $H_r^{in}(C)$ are disconnected

Proof : It follows from the fact that the Nielsen classes $N(C)$ are the disjoint union of braid orbits but for A_8 we have at least two braid orbits for some type C as given in Table 6. From Proposition 2.5, we see that the Hurwitz spaces $H_r^{in}(C)$ are disconnected.

Lemma 4.5: If $n = r = 8$ and $G = A_8$, then the Hurwitz spaces $H_r^{in}(C)$ are connected.

Proof : It follows from the fact that the Nielsen classes $N(C)$ are the disjoint union of braid orbits but for A_8 ,we have only one braid orbit for all types C as given in Table 6. From Proposition 2.5, one can see that the Hurwitz spaces $H_r^{in}(C)$ are connected.

Lemma 4.6: If $r \geq 4$, $n = 15$ and $G=A_8$, then the Hurwitz spaces $H_r^{in}(C)$ are connected

Proof : It follows from the fact that the Nielsen classes $N(C)$ are the disjoint union of braid orbits but for A_8 we have only one braid orbit for all types C as given in Table 6. From Proposition 2.5, we can see that the Hurwitz spaces $H_r^{in}(C)$ are connected.

Lemma 4.7: If $r=3$,$n = 28, 35$ and $G = A_8$, then the Hurwitz spaces $H_r^{in}(C)$ are connected.

Proof: It follows from the fact that the Nielsen classes $N(C)$ are the disjoint union of braid orbits but for A_8 we have only one braid orbit for all types C as given in Table 6. From Proposition 2.5, we see that the Hurwitz spaces $H_r^{in}(C)$ are connected.

Remark 4.8: The computation shows that there is no primitive genus one systems for the group A_8, when $n = 56$.

Finally, As we see in Table 7, we are not able to compute braid orbits for these types because the length of orbits become big. It needs a super computer and more time We suggest as an open problem to find methods to do those computations in a nice way.

TABLE 2: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8

Group	Ramification type	N. of orbits	Length of orbits	Ramification type	N. of orbits	Length of orbits
A_8	(5A,7B,7B)	15	1	(5A,7A,7B)	15	1
	(5A,7A,7A)	15	1	(5A,6A,7A)	32	1
	(5A,6A,7B)	32	1	(5A,6A,6A)	38	1
	(5A,7A,15A)	13	1	(5A,7B,15A)	13	1
	(5A,6A,15A)	20	1	(5A,15A,15)	5	1
	(5A,7A,15B)	13	1	(5A,6A,15B)	20	1
	(5A,7B,15B)	13	1	(5A,15A,15B)	9	1
	(5A,15B,15B)	5	1	(4A,5A,7A)	12	1
	(4A,5A,7B)	12	1	(4A,5A,6A)	18	1
	(4A,5A,15A)	8	1	(4A,5A,15B)	18	1
	(4A,5A,5A0	6	1	(4B,7A,7A)	36	1
	(4B,7A,7B)	32	1	(4B,7B,7B)	36	1
	(4B,6A,7B)	52	1	(4B,6A,6A)	52	1
	(4B,7A,15A)	24	1	(4B,7B,15A)	24	1
	(4B,6A,15A)	32	1	(4B,6A,7A)	52	1
	(4B,15A,15A	16	1	(4B,7A,15B)	24	1
	(4B,7B,15A)	24	1	(4B,6A,15A)	32	1
	(4B,15A,15B)	8	1	(4B,15A,15A)	16	1
	(4A,4B,7A)	14	1	(4A,4B,7B)	14	1
	(4A,4B,6A)	18	1	(4A,4B,15A)	12	1
	(4A,4B,15B)	12	1	(3A,7A,7A)	12	1
	(3A,7A,7B)	8	1	(3A,7B,7B)	12	1
	(3A,6A,7A)	16	1	(3A,6A,7B)	16	1
	(3A,6A,6A)	10	1	(3A,7A,15A)	11	1
	(3A,7B,15A)	11	1	(3A,6A,15A)	14	1
	(3A,15A,15A)	6	1	(3A,7A,15B)	11	1
	(3A,7B,15B)	11	1	(3A,6A,15B)	14	1
	(3A,15A,15B)	6	1	(3A,15B,15B)	6	1
	(3A,4A,15A	4	1	(3A,4A,15B)	4	1
	(6B,7A,7A)	34	1	(6B,7A,7B)	31	1
	(6B,7B,7B)	34	1	(6A,6B,7A)	38	1
	(6A,6B,7B)	38	1	(6A,6A,6B)	42	1
	(6B,7A,15A)	16	1	(6B,7A,15B)	16	1
	(6A,6B,15A)	18	1	(6B,15A,15A)	48	1
	(6B,7A,15B)	4	1	(6B,7B,15B)	16	1
	(6B,6B,15B)	18	1	(6B,15A,15B)	9	1
	(6B,15B,15B)	4	1	(4A,6B,7A)	15	1
	(4A,6B,7B)	15	1	(4A,6A,6B)	12	1
	(4A,6B,15A)	7	1	(4A,6B,15B)	7	1
	(4A,4A,6B)	4	1	(2A,7A,15A)	1	1
	(2A,7B,15A)	1	1	(2A,6A,15A)	1	1
	(2A,15A,15A)	1	1	(2A,7A,15B)	1	1
	(2A,7B,15B)	1	1	(2A,6A,15B)	1	1
	(2A,15B,15B)	1	1	(5A,5A,5A,5A)	6	5,300,720,600,240,50
	(4B,5A,5A,5A)	1	5760	(4B,4B,5A,5A)	4	13760,800,240,80
	(4B,4B,4B,5A)	1	34120	(4B,4B,4B,4B)	4	67440,15307,1296,288
	(3A,5A,5A,5A)	2	1740,750	(3A,4B,5A,5A)	1	6800
	(3A,4B,4B,5A)	1	15540	(3A,4B,4B,4B)	1	32832
	(3A,3A,5A,5A)	5	900,1680,350,120,20	(3A,3A,4B,5A)	1	7040
	(3A,3A,4B,4B)	3	12672,840,120	(3A,3A,3A,5A)	2	1030,1950
	(3A,3A,3A,4B)	1	5136	(3A,3A,3A,3A)	2	828,432
	(5A,5A,5A,6B)	1	5880	(4B,5A,5A,6B)	1	12720
	(4B,4B,5A,6B)	1	27480	(4B,4B,4B,6B)	1	56016
	(3A,5A,5A,6B)	1	6000	(3A,4B,5A,6B)	1	12420
	(3A,4B,4B,6B)	1	25134	(3A,3A,5A,6B)	1	5760
	(3A,3A,4B,6B)	1	11208	(3A,3A,3A,6B)	1	4752
	(5A,5A,6B,6B)	4	8960,640,180, 70	(4B,5A,6B,6B)	1	20160
	(4B,4B,6B,6B)	4	38592,1368,360,96	(3A,5A,6B,6B)	1	8950
	(3A,4B,6B,6B)	1	17952	(3A,3A,6B,6B)	4	7212,654,144,24
	(5A,6B,6B,6B)	1	4690	(4B,6B,6B,6B)	1	27840

TABLE 3: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8

Group	Ramification type	N. of orbits	Length of orbits	Ramification type	N. of orbits	Length of orbits
A_8	(3A,6B,6B,6B)	1	12258	&&(6B,6B,6B,6B)	4	15192,2592,864,216
	(3B,5A,5A,7A)	2	371,140	(3B,5A,5A,7B)	2	371,140
	(3B,5A,5A,6A)	2	560,560	(3B,5A,5A,15A)	2	306,160
	(3B,5A,5A,15B)	2	306,160	(3B,4A,5A,5A)	1	416
	(3B,4B,5A,7A)	1	1428	(3B,4B,5A,7B)	1	1428
	(3B,4B,5A,6A)	1	2348	(3B,4B,5A,15A)	1	1024
	(3B,4B,5A,15B)	1	1024	(3B,4A,4B,5A)	1	984
	(3B,4B,4B,7A)	1	3444	(3B,4B,4B,7B)	1	3444
	(3B,4B,4B,6A)	1	5076	(3B,4B,4B,15A)	1	2142
	(3B,4B,4B,15B)	1	2142	(3B,4A,4B,4B)	1	2016
	(3A,3B,5A,7A)	2	434,203	(3A,3B,5A,7B)	2	434,203
	(3A,3B,5A,6A)	2	584,584	(3A,3B,5A,15A)	2	155,292
	(3A,3B,5A,15B)	2	155,292	(3A,3B,4A,5A)	1	448
	(3A,3B,4B,7A)	1	540	(3A,3B,4B,7B)	1	540
	(3A,3B,4B,6A)	1	2328	(3A,3B,4B,15A)	1	960
	(3A,3B,4B,15B)	1	960	(3A,3B,4A,4B)	1	876
	(3A,3A,3B,7A)	2	238,441	(3A,3A,3B,7B)	2	238,441
	(3A,3A,3B,6A)	2	540,540	(3A,3A,3B,15A)	2	152,273
	(3A,3A,3B,15B)	2	152,273	(3A,3A,3B,4A)	1	376
	(3B,5A,6B,7A)	1	1337	(3B,5A,6B,7B)	1	1337
	(3B,5A,6A,6B)	1	776	(3B,5A,6B,15A)	1	813
	(3B,5A,6B,15B)	1	813	(3B,4A,5A,6B)	1	744
	(3B,4B,6B,7A)	1	2828	(3B,4B,6B,7B)	1	2828
	(3B,4B,6A,6B)	1	3720	(3B,4B,6B,15A)	1	1556
	(3B,4B,6B,15B)	1	1556	(3B,4A,4B,6B)	1	1512
	(3A,3B,6B,7A)	1	1281	(3A,3B,6B,7B)	1	1281
	(3A,3B,6A,6B)	1	1632	(3A,3B,6B,15A)	1	683
	(3A,3B,6B,15B)	1	683	(3A,3B,4A,6B)	1	660
	(3B,6B,6B,7A)	1	2051	(3B,6B,6B,7B)	1	2051
	(3B,6A,6B,6B)	1	2640	(3B,6B,6B,15A)	1	1083
	(3B,6B,6B,15B)	1	1083	(3B,4A,6B,6B)	1	1008
	(3B,3B,7A,7A)	3	28,112,7	(3B,3B,7A,7B)	3	28,112,7
	(3B,3B,7B,7B)	3	112,28,7	(2A,5A,5A,5A)	1	520
	(2A,4B,5A,5A)	1	1000	(2A,4B,4B,5A)	1	1930
	(2A,4B,4B,4B)	1	3736	(2A,3A,5A,5A)	1	500
	(2A,3A,4B,5A)	1	910	(2A,3A,4B,4B)	1	1440
	(2A,3A,3A,5A)	1	370	(2A,3A,3A,4B)	1	376
	(2A,5A,5A,6B)	1	600	(2A,4B,5A,6B)	1	1380
	(2A,4B,4B,6B)	1	2640	(2A,3A,5A,6B)	1	9080
	(2A,3A,4B,6B)	1	1188	(2A,3A,3A,6B)	1	456
	(2A,5A,6B,6B)	1	920	(2A,4B,6B,6B)	1	1824
	(2A,3A,6B,6B)	1	840	(2A,6B,6B,6B)	1	1152
	(2A,2A,5A,5A)	1	50	(2A,2A,4B,5A)	1	80
	(2A,2A,4B,4B)	1	96	(2A,2A,3A,5A)	1	20
	(2A,2A,5A,6B)	1	70	(2A,2A,4B,6B)	1	96
	(2A,2A,3A,6B)	1	20	(2A,2A,6B,6B)	1	72
	(2B,5A,5A,7A)	1	1260	(2B,5A,5A,7B)	1	1260
	(2B,5A,5A,6A)	1	1260	(2B,5A,5A,15A)	1	960
	(2B,5A,5A,15B)	1	960	(2B,4A,5A,5A)	1	920
	(2B,4B,5A,7A)	1	3150	(2B,4B,5A,7B)	1	3150
	(2B,4B,5A,6A)	1	4800	(2B,4B,5A,15A)	1	2040
	(2B,4B,5A,15B)	1	2040	(2B,4A,4B,5A)	1	1920
	(2B,4B,4B,7A)	1	6902	(2B,4B,4B,7B)&	1	6902
	(2B,4B,4B,6A)	1	9636	(2B,4B,4B,15A)	1	4095
	(2B,4B,4B,15B)	1	4095	(2B,4A,4B,4B)	1	3576
	(2B,3A,5A,7A)	1	1400	(2B,3A,5A,7B)	1	1400
	(2B,3A,5A,6A)	1	2280	(2B,3A,4A,5A)	1	820
	(2B,3A,5A,15A)	1	920	(2B,3A,5A,15B)	1	920
	(3B,3B,6A,7A)	2	140,140	(3B,3B,6A,7B)	2	140,140
	(3B,3B,6A,6A)	2	192,132	(3B,3B,7A,15A)	2	77,42
	(3B,3B,7B,15A)	2	77,42	(3B,3B,6A,15A)	2	92,92
	(3B,3B,15A,15A)	3	40,5,20	(3B,3B,7A,15B)	2	77,42
	(3B,3B,7B,15B)	2	77,42	(3B,3B,6A,15B)	2	92,92
	(3B,3B,15A,15B)	2	32,50	(3B,3B,15B,15B)	3	40,20,5
	(3B,3B,4A,7A)	1	112	(3B,3B,4A,7B)	1	112
	(3B,3B,4A,6A)	1	156	(3B,3B,4A,15A)	1	72

TABLE 4: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8

Group	Ramification type	N. of orbits	Length of orbits	Ramification type	N. of orbits	Length of orbits
A_8	(3B,3B,4A,15B)	1	72	(3B,3B,4A,4A)	1	56
	&(2A,3B,5A,7A)	1	112	(2A,3B,5A,7B)	1	112
	(2A,3B,5A,6A)	1	112	(2A,3B,5A,15A)	1	56
	(2A,3B,5A,15B)	1	56	(2A,3B,4A,5A)	1	60
	(2A,3B,4B,7A)	1	210	(2A,3B,4B,7B)	1	210
	(2A,3B,4B,6A)	1	210	(2A,3B,4B,15A)	1	112
	(2A,3B,4B,15B)	1	112	(2A,3B,4A,4B)	1	84
	(2A,3A,3B,7A)	1	98	(2A,3A,3B,7B)	1	98
	(2A,3A,3B,6A)	1	72	(2A,3A,3B,15A)	1	50
	(2A,3A,3B,15B)	1	50	(2A,3A,3B,4A)	1	30
	(2A,3B,6B,7A)	1	133	(2A,2A,3B,7A)	1	7
	(2B,3A,4B,7A)	1	2898	(2A,3A,4B,7B)	1	2898
	(2B,3A,4B,6A)	1	4152	(2B,3A,4B,15A)	1	1800
	(2B,3A,4B,15B)	1	1800	(2B,3A,4A,4B)	1	1320
	(2B,3A,3A,7A)	1	1036	(2B,3A,3A,7B)	1	1036
	(2B,3A,3A,6A)	1	1560	(2B,3A,3A,15A)	1	780
	(2B,3A,3A,15B)	1	780	(2B,5A,6B,15B)	1	1505
	(2B,4B,6B,7A)	1	5516	(2B,4B,6A,6B)	1	7050
	(2B,4A,4B,6B)	1	2736	(2B,3A,6B,7A)	1	2741
	(2B,3A,6B,7B)	1	2741	(2B,3A,6A,6B)	1	3114
	(2B,6B,6B,7A)	1	3906	(2B,6B,6B,7B)	1	3906
	(2B,6A,6B,6B)	1	4824	(2B,3B,7A,7B)	1	343
	(2B,3B,7B,7B)	1	322	(2B,3B,6A,7A)	1	532
	(2B,3B,6A,7B)	1	532	(2B,3B,6A,6A)	1	606
	(2B,3B,7A,15A)	1	224	(2B,3B,7B,15A)	1	224
	(2B,3B,6A,15A)	1	324	(2B,3B,15A,15A)	1	132
	(2B,3B,4A,15B)	1	126	(2B,3B,4A,4A)	1	188
	(2A,2B,4B,7A)	1	322	(2A,3B,6B,7B)	1	133
	(2A,3B,6A,6B)	1	156	(2A,3B,6B,15A)	1	67
	(2A,3B,6B,15B)	1	67	(2A,3B,4A,6B)	1	60
	(2A,2A,3B,7B)	1	7	(2A,2A,3B,15A)	1	5
	(2A,2A,3B,15B)	1	5	(2B,3A,3A,4A)	1	899
	(2B,5A,6B,7B)	1	2695	(2B,5A,6B,7A)	1	2695
	(2B,5A,6A,6B)	1	3600	(2B,5A,6B,15A)	1	1505
	(2B,4A,5A,6B)	1	1500	(2B,4B,6B,7B)	1	5516
	(2B,4B,6B,15A)	1	2900	(2B,4B,6B,15B)	1	2900
	(2B,3A,6B,15A)	1	1305	(2B,3A,6B,15B)	1	1305
	(2B,3A,4A,6B)	1	1138	(2B,6B,6B,15A)	1	1900
	(2B,6B,6B,15B)	1	1900	(2B,4A,6B,6B)	1	1872
	(2B,3B,7A,7A)	1	322	(2B,3B,7A,15B)	1	224
	(2B,3B,7B,15B)	1	224	(2B,3B,6A,15B)	1	324
	(2B,3B,15A,15B)	1	121	(2B,3B,15B,15B)	1	121
	(2B,3B,4A,7A)	1	210	(2B,3B,4A,7B)	1	210
	(2B,3B,4A,6A)	1	246	(2B,3B,4A,15A)	1	126
	(2A,2B,5A,7A)	1	210	(2A,2B,5A,7B)	1	210
	(2A,2B,4B,7B)	1	322	(2A,2B,4B,6A)	1	312
	(2A,2B,4B,15A)	1	95	(2A,2B,4B,15B)	1	95
	(2A,2B,4B,4B)	1	96	(2A,2B,3A,7A)	1	84
	(2A,2B,3A,7B)	1	84	(2A,2B,3A,6A)	1	66
	(2A,2B,3A,15A)	1	75	(2A,2B,3A,15B)	1	75
	(2B,2B,7B,7B)	2	630,28	(2B,2B,6A,7A)	1	924
	(2B,2B,6A,7B)	1	924	(2B,2B,6A,6A)	2	969,252
	(2B,2B,7A,15B)	1	420	(2B,2B,7B,15B)	1	420
	(2B,2B,6A,15B)	1	570	(2B,2B,4A,7A)	1	308
	(2B,2B,4A,7B)	1	308	(2B,2B,4A,6A)	1	336
	(2B,2B,4A,15A)	1	210	(2B,2B,4A,15B)	1	210
	(2B,2B,4A,4A)	1	88	(2B,2B,5A,6A)	1	220
	(2A,2B,5A,15A)	1	120	(2A,2B,5A,15B)	1	120
	(2A,2B,4A,5A)	1	90	(2A,2B,6B,7A)	1	252
	(2A,2B,6A,6B)	1	252	(2A,2B,6B,7B)	1	252
	(2A,2B,6B,15A)	1	130	(2A,2B,6B,15B)	1	130
	(2A,2B,4A,6B)	1	76	(2A,2A,2B,15A)	1	5
	(2A,2A,2B,15B)	1	5	(2B,2B,7B,7B)	2	28,630
	(2B,2B,7A,7B)	2	392,189	(2B,2B,7A,15A)	1	420
	(2B,2B,7B,15A)	1	420	(2B,2B,6A,15A)	1	570

TABLE 5: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8

Group	Ramification type	N. of orbits	Length of orbits	Ramification type	N. of orbits	Length of orbits
A_8	(2B,2B,15A,15A)	3	190,30,5	(2B,2B,15A,15B)	2	120,210
	(2B,2B,15B,15B)	3	190,30,5	(3B,3B,3B,5A,5A)	3	133,8,5280
	(3B,3B,4B,5A,5A)	1	540	(3B,3B,4B,4B,5A)	1	135984
	(3B,3B,4B,4B,4B)	1	312912	(3A,3B,3B,5A,5A)	2	167404,7660
	(3A,3B,3B,4B,5A)	1	62600	(3A,3B,3B,4B,4B)	1	141228
	(3A,3A,3B,3B,5A)	2	9140,18336	(3A,3A,3B,3B,4B)	1	64608
	(3A,3A,3A,3B,3B)	2	18360,9684	(3B,3B,5A,5A,6B)	1	53476
	(3B,3B,4B,5A,6B)	1	116272	(3B,3B,4B,4B,6B)	1	248880
	(3A,3B,3B,5A,6B)	1	54856	(3A,3B,3B,4B,6B)	1	113352
	(3A,3A,3B,3B,6B)	1	51768	(3B,3B,5A,6B,6B)	1	88696
	(3B,3B,4B,6B,6B)	1	183840	(3A,3B,3B,6B,6B)	1	82596
	(3B,3B,6B,6B,6B)	1	132768	(3B,3B,3B,5A,7A)	2	3381,1421
	(3B,3B,3B,5A,7B)	2	3381,1421	&(3B,3B,3B,5A,6A)	2	4896,4896
	(3B,3B,3B,5A,15A)	2	2685,1353	(3B,3B,3B,5A,15B)	2	2685,1353
	(3B,3B,3B,4A,5A)	1	3776	(3B,3B,3B,4B,7A)	1	12936
	(3B,3B,3B,4B,7B)	1	12936	(3B,3B,3B,4B,6A)	1	21312
	(3B,3B,3B,4B,15A)	1	9216	(3B,3B,3B,4B,15B)	1	9216
	(3B,3B,3B,4A,4B)	1	8640	(3A,3B,3B,3B,7A)	2	3969,1911
	(3A,3B,3B,3B,7B)	2	3969,1911	(3A,3B,3B,3B,6A)	2	5184,5184
	(3A,3B,3B,3B,15A)	2	2655,1383	(3A,3B,3B,3B,15B)	2	2655,1383
	(3A,3B,3B,3B,4A)	1	3936	(3B,3B,3B,6B,7A	1	1907
	(3B,3B,3B,6B,7B)	1	11907	(3B,3B,3B,6A,6B)	1	16272
	(3B,3B,3B,6B,15A)	1	7305	(3B,3B,3B,6B,15B)	1	7305
	(3B,3B,3B,4A,6B)	1	6914	(2A,3B,3B,3B,7A)	1	980
	(2A,3B,3B,3B,7B)	1	980	(2A,3B,3B,3B,6A)	1	936
	(2A,3B,3B,3B,15A)	1	540	(2A,3B,3B,3B,15B)	1	540
	(2A,3B,3B,3B,4A)	1	480	(2A,3B,3B,5A,5A)	1	4672
	(2A,3B,3B,4B,5A)	1	8944	(2A,3B,3B,4B,4B)	1	17664
	(2A,3A,3B,3B,5A)	1	4400	(2A,3A,3B,3B,4B)	1	8328
	(2A,3A,3A,3B,3B)	1	3864	(2A,3B,3B,5A,6B)	1	5864
	(2A,3B,3B,4B,6B)	1	12432	(2A,3B,3B,3B,6B)	1	5520
	(2A,3B,3B,6B,6B)	1	8736	(2A,2A,3B,3B,5A)	1	320
	(2A,2A,3B,3B,4B)	1	672	(2A,2A,3A,3B,3B)	1	252
	(2A,2A,3B,3B,6B)	1	528	(2B,3B,5A,5A,5A)	1	48120
	(2B,3B,4B,5A,5A)	1	124080	(2B,3B,4B,4B,5A)	1	92440
	(2B,3B,4B,4B,4B)	1	644400	(2B,3B,4B,6B,6B)	1	360624
	(2B,3A,3B,6B,6B)	1	161514	(2B,3B,6B,6B,6B)	1	253944
	(2B,3B,3B,5A,7A)	1	11613	(2B,3B,3B,5A,7B)	1	11613
	(2B,2B,3A,5A,6B)	1	218100	(2B,2B,3A,4B,6B)	1	437382
	(2B,2B,3A,3A,6B)	1	193842	(2B,2B,5A,6B,6B)	1	351450
	(2B,2B,4B,6B,6B)	1	693816	(2B,2B,3A,6B,6B)	1	310014
	(2B,2B,6B,6B,6B)	1	477864	(2B,2B,3B,5A,7A)	1	26215
	(2B,2B,3B,5A,7B)	1	262215	(2B,2B,3B,5A,6A)	1	39840
	(2B,2B,3B,5A,15A)	1	17105	(2B,2B,3B,5A,15B)	1	17105
	(2B,2B,3B,4A,5A)	1	16320	(2B,2B,3B,4B,7A)	1	60662
	(2B,2B,3B,4B,7B)	1	60662	(2B,2B,3B,4B,6A)	1	86100
	(2B,2B,3B,4B,15A)	1	36140	(2B,2B,3B,4B,15B)	1	36140
	(2B,2B,3B,4A,4B)	1	33984	(2B,2B,3A,3B,7A)	1	37391
	(2B,2B,3A,3B,7B)	1	37391	(2B,2B,3A,3B,6A)	1	39396
	(2B,2B,3A,3B,15A)	1	16065	(2B,2B,3A,3B,15B)	1	16065
	(2B,2B,3A,3B,4A)	1	14960	(2B,2B,3B,6B,7A)	1	48559
	(2B,2B,3B,6B,7B)	1	48559	(2B,2B,3B,6A,6B)	1	64260
	(2B,2B,3B,6B,15A)	1	910	(2B,2B,3B,6B,15B)	1	910
	(2B,2B,3A,3B,6B)	1	944	(2A,2B,2B,3B,7A)	1	3528
	(2A,2B,2B,3B,7B)	1	3528	&(2A,2B,2B,3B,6A)	1	3528
	(2A,2B,2B,3B,15A)	1	1900	(2A,2B,2B,3B,15B)	1	1900
	(2A,2B,2B,3B,4A)	1	1416	(2A,2B,2B,5A,5A)	1	16700
	(2A,2B,2B,4B,5A)	1	33900	(2A,2B,2B,4B,4B)	1	64272
	(2A,2B,2B,3A,5A)	1	15600	(2A,2B,2B,3A,4B)	1	27144
	(2A,2B,2B,3A,3A)	1	9804	(2A,2B,2B,5A,6B)	1	23850
	(2A,2B,2B,4B,6B)	1	45708	(2A,2B,2B,3A,6B)	1	19872
	(2A,2B,2B,6B,6B)	1	31176	(2A,2A,2B,2B,5A)	1	1300
	(2A,2A,2B,2B,4B)	1	3528	(2A,2A,2B,2B,3A)	1	432
	(2A,2A,2B,2B,6B)	1	1512	(2B,2B,2B,5A,7A)	1	56350
	(2B,2B,2B,5A,7B)	1	56350	(2B,2B,2B,5A,6A)	1	81000
	(2B,2B,2B,5A,15A)	1	34200	(2B,2B,2B,5A,15B)	1	34200
	(2B,2B,2B,4A,5A)	1	32300	(2B,2B,2B,4B,7A)	1	122010
	(2B,2B,2B,4B,7B)	1	122010	(2B,2B,2B,3A,15B)	1	30825

TABLE 6: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8

Group	Ramification type	N. of orbits	Length of orbits	Ramification type	N. of orbits	Length of orbits
A_8	(2B,2B,2B,6B,15B)	1	50100	(2B,2B,2B,6B,7B)	1	94962
	(2B,2B,2B,6A,6B)	1	122472	(2B,2B,2B,6B,15A)	1	50100
	(2A,2B,2B,2B,7A)	1	5978	(2A,2B,2B,2B,7B)	1	5978
	(2B,3B,3B,5A,6A)	1	19488	(2B,3B,3B,5A,15A)	1	8453
	(2B,3B,3B,5A,15B)	1	8453	(2B,3B,3B,4B,7A)	1	28616
	(2B,3B,3B,4B,7B)	1	28616	(2B,3B,3B,4B,6A)	1	43200
	(2B,3B,3B,4B,15A)	1	18332	(2B,3B,3B,4B,15B)	1	18332
	(2B,3B,3B,4A,4B)	1	17472	(2B,3B,3B,4A,5A)	1	7952
	(2B,3A,3B,3B,7A)	1	12985	(2B,3A,3B,3B,7B)	1	12985
	(2B,3A,3B,3B,6A)	1	20184	(2B,3A,3B,3B,15A)	1	8231
	(2B,3A,3B,3B,15B)	1	8231	(2B,3A,3B,3B,4A)	1	7688
	(2B,3B,3B,6B,7A)	1	24304	(2B,3B,3B,6B,7B)	1	24304
	(2B,3B,3B,6A,6B)	1	33024	(2B,3B,3B,6B,15A)	1	14070
	(2B,3B,3B,6B,15B)	1	14070	(2B,3B,3B,4A,6B)	1	13456
	(2A,2B,3B,3B,7A)	1	1862	(2A,2B,3B,3B,7B)	1	1862
	(2A,2B,3B,3B,6A)	1	1932	(2A,2B,3B,3B,15A)	1	1030
	(2A,2B,3B,3B,15B)	1	1030	(2A,2B,3B,3B,4A)	1	816
	(2A,2B,3B,5A,5A)	1	8640	(2A,2B,3B,4B,5A)	1	17340
	(2A,3B,3B,4B,4B)	1	8400	(2A,2B,3A,3B,5A)	1	34824
	(2A,2B,3A,3B,4B)	1	15846	(2A,2B,3A,3A,3B)	1	7134
	(2A,2B,3B,5A,6B)	1	11960	(2A,2B,3B,4B,6B)	1	23964
	(2A,2B,3A,3B,6B)	1	10812	(2A,2B,3B,6B,6B)	1	16584
	(2A,2A,2B,3B,5A)	1	710	(2A,2A,2B,3B,4B)	1	1272
	(2A,2A,2B,3A,3B)	1	480	(2A,2A,2B,3B,6B)	1	936
	(2B,3B,5A,5A,6B)	1	109810	(2B,3B,4B,5A,6B)	1	238200
	(2B,3B,4B,4B,6B)	1	496080	(2B,3A,3B,5A,6B)	1	109310
	(2B,3A,3B,4B,6B)	1	224292	(2B,3A,3A,3B,6B)	1	101490
	(2B,3A,3B,5A,5A)	1	57040	(2B,3A,3B,4B,5A)	1	133500
	(2B,3A,3B,4B,4B)	1	293430	(2B,3A,3A,3B,5A)	1	60106
	(2B,3A,3A,3B,4B)	1	130368	(2B,3A,3A,3A,3B)	1	57960
	(2B,3B,5A,6B,6B)	1	178650	(2B,2B,5A,5A,5A)	1	111900
	(2B,2B,4B,5A,5A)	1	271800	(2B,2B,4B,4B,5A)	1	616050
	(2B,2B,4B,4B,4B)	1	1301544	(2B,2B,3A,5A,5A)	1	125600
	(2B,2B,3A,4B,5A)	1	278550	(2B,2B,3A,4B,4B)	1	577494
	(2B,2B,3A,3A,5A)	1	124000	(2B,2B,3A,3A,4B)	1	250656
	(2B,2B,3A,3A,3A)	1	100320	(2B,2B,5A,5A,6B)	1	2261000
	(2B,2B,4B,5A,6B)	1	478200	(2B,2B,4B,4B,6B)	1	973512
	(2B,3B,3B,3B,15A)	1	74420	(2B,3B,3B,3B,15B)	1	74420
	(2B,3B,3B,3B,4A)	1	69760	(2A,2A,2B,3B,3B,3B)	1	6024
	(2B,2B,3B,3B,5A,5A)	1	1058010	(2B,2B,3B,3B,4B,5A)	1	2506080
	(2B,2B,3B,3B,4B,4B)	1	5586096	(2B,2B,3A,3B,3B,5A)	1	1150630
	(2A,2B,2B,2B,3B,5A)	1	297000	(2A,2B,2B,2B,3B,4B)	1	595224
	(2A,2B,2B,2B,3A,3B)	1	271080	(2A,2B,2B,2B,3B,6B)	1	418152
	(2A,2A,2B,2B,2B,3B)	1	21636	(3B,3B,3B,3B,5A,5A)	2	127840,54208
	(3B,3B,3B,3B,5A,5A)	2	127840,54208	(3B,3B,3B,3B,4B,5A)	1	509056
	(3B,3B,3B,3B,4B,4B)	1	1244736	(3A,3B,3B,3B,3B,5A)	2	155520,73128
	(3A,3B,3B,3B,3B,4B)	1	577728	(3A,3A,3B,3B,3B,3B)	2	167724,83664
	(3B,3B,3B,3B,5A,6B)	1	487120	(3B,3B,3B,3B,4B,6B)	1	1058688
	(3A,3B,3B,3B,3B,6B)	1	496944	(3B,3B,3B,3B,6B,6B)	1	806685
	(3B,3B,3B,3B,3B,7A)	2	13720,30870	(3B,3B,3B,3B,3B,7B)	2	13720,30870
	(3B,3B,3B,3B,3B,6A)	2	43200,43200	(3B,3B,3B,3B,3B,15A)	2	11600,23250
	(3B,3B,3B,3B,3B,15B)	2	11600,23250	&(3B,3B,3B,3B,3B,4A)	1	33280
	(2A,3B,3B,3B,3B,5A)	1	41760	(2A,3B,3B,3B,3B,4B)	1	80640
	(2A,3A,3B,3B,3B,3B)	1	39600	(2A,3B,3B,3B,3B,6B)	1	54144
	(2A,2A,3B,3B,3B,3B)	1	2736	(2B,3B,3B,3B,5A,5A)	1	457956
	(2B,3B,3B,3B,4B,5A	1	1150144	(2B,3B,3B,3B,4B,4B)	1	2682336
	(2B,3A,3B,3B,3B,5A)	1	529992	(2B,2B,3B,3B,5A,5A)	1	1058010
	(2B,2B,3B,3B,4B,5A)	1	2506080	(2B,2B,3B,3B,4B,4B)	1	5586096
	(2B,2B,3A,3B,3B,5A)	1	1150630	(3B,3B,3B,3B,3B,3B,5A)	2	544400,1227000
	(3B,3B,3B,3B,3B,3B,4B)	1	4807680	(3A,3B,3B,3B,3B,3B,3B)	1	1441800,689040
	(2B,2B,2B,2B,2B,2B,2B,2B)	1	7140398160	(2B,2B,2B,2B,2B,2B,2B,3B)	1	3611300490
	(2B,2B,2B,2B,2B,2B,3B,3B)	1	1798724520	(2B,2B,2B,2B,2B,3B,3B,3B)	1	883281816
	(2B,2B,2B,2B,3B,3B,3B,3B)	1	426171600	(2B,2B,2B,3B,3B,3B,3B,3B)	1	201975480
	(2B,2B,3B,3B,3B,3B,3B,3B)	1	41620230	(2B,3B,3B,3B,3B,3B,3B,3B)	1	93077760
	(3B,3B,3B,3B,3B,3B,3B,3B)	1	17165232			

TABLE 7: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8

Group	Ramification type	Ramification type	Ramification type
A_8	(2B,2B,2B,2B,5A,5A)	(2B,2B,2B,2B,4B,5A)	(2B,2B,2B,2B,4B,4B)
	(2B,2B,2B,2B,3A,4B)	(2B,2B,2B,2B,3A,3A)	(2B,2B,2B,2B,5A,6B)
	(2B,2B,2B,2B,4B,6B)	(2B,2B,2B,2B,6B,6B)	(2B,2B,2B,2B,3A,6B)
	(2B,2B,2B,2B,6B,6B)	(2B,2B,2B,2B,3B,7A)	(2B,2B,2B,2B,3B,7B)
	(2B,2B,2B,2B,3B,6A)	(2B,2B,2B,2B,3B,4A)	(2B,2B,2B,2B,3B,15A)
	(2B,2B,2B,2B,3B,15B)	(2A,2B,3B,3B,3B,5A)	(2A,2B,3B,3B,3B,4B)
	(2A,2B,3A,3B,3B,3B)	(2A,2B,3B,3B,3B,6B)	(2B,2B,3B,3B,3B,7A)
	(2B,2B,3B,3B,3B,7B)	(2B,2B,3B,3B,3B,6A)	(2B,2B,3B,3B,3B,15A)
	(2B,2B,3B,3B,3B,15B)	(2B,2B,3B,3B,3B,4A)	(2A,2B,2B,2B,2B,5A)
	(2A,2B,2B,2B,2B,4B)	(2A,2B,2B,2B,2B,3A)	(2A,2B,2B,2B,2B,6B)
	(2A,2A,2B,2B,2B,2B)	(2B,2B,2B,2B,2B,7A)	(2B,2B,2B,2B,2B,7B)
	(2B,2B,2B,2B,2B,6A)	(2B,2B,2B,2B,2B,15A)	(2B,2B,2B,2B,2B,15B)
	(2B,2B,2B,2B,2B,4A)	(2B,2B,2B,3B,3B,7A)	(2B,2B,2B,3B,3B,7B)
	(2B,2B,2B,3B,3B,6A)	(2B,2B,2B,3B,3B,15A)	(2B,2B,2B,3B,3B,15B)
	(2B,2B,2B,3B,3B,4A)	(2B,3A,3B,3B,3B,6B)	(2B,3B,3B,3B,6B,6B)
	(2B,2B,3A,3B,3B,4B)	(2B,2B,3A,3A,3B,4B)	(2B,2B,3B,3B,5A,6B)
	(2B,2B,3B,3B,4B,6B)	(2B,2B,3A,3B,3B,6B)	(2B,2B,3B,3B,6B,6B)
	(2A,2B,2B,3B,3B,5A)	(2A,2B,2B,3B,3B,4B)	(2A,2B,2B,3A,3B,3B)
	(2A,2B,2B,3B,3B,6B)	(2A,2A,2B,2B,3B,3B)	(2B,2B,2B,3B,5A,5A)
	(2B,2B,2B,3B,4B,5A)	(2B,2B,2B,3B,4B,4B)	(2B,2B,2B,3A,3B,5A)
	(2B,2B,2B,3A,3B,4B)	(2B,2B,2B,3A,3A,3B)	(2B,2B,2B,3B,5A,6B)
	(2B,2B,2B,3B,4B,6B)	(2B,2B,2B,3A,3B,6B)	(2B,2B,2B,3B,6B,6B)
	(2B,3B,3B,3B,3B,7A)	(2B,3B,3B,3B,3B,7B)	(2B,3B,3B,3B,3B,6A)
	(2B,3A,3B,3B,3B,4B)	(2B,3A,3A,3B,3B,3B)	(2B,3B,3B,3B,4B,6B)
	(2B,2B,3B,3B,3B,3B,5A)	(2B,2B,3B,3B,3B,3B,4B)	(2B,3B,3B,3B,3B,3B,5A)
	(2B,3B,3B,3B,3B,3B,4B)	(3B,3B,3B,3B,3B,3B,6B)	&(3A,3B,3B,3B,3B,3B,3B)
	(2B,2B,2B,3B,3B,3B,5A)	(2B,2B,2B,3B,3B,3B,4B)	(2B,2B,2B,3A,3B,3B,3B)
	(2B,2B,2B,3B,3B,3B,6B)	(2A,2B,2B,2B,3B,3B,3B)	(2B,2B,2B,2B,3B,3B,5A)
	(2B,2B,2B,2B,3B,3B,4B)	(2B,2B,2B,2B,3A,3B,3B)	(2B,2B,2B,2B,3B,3B,6B)
	(2B,3B,3B,3B,3B,3B,6B)	(2A,2B,2B,2B,2B,2B,6B)	(2A,2B,2B,2B,2B,3B,3B)
	(2B,2B,2B,2B,2B,3B,5A)	(2B,2B,2B,2B,2B,3B,4B)	(2B,2B,2B,2B,2B,3A,3B)
	(2B,2B,2B,2B,2B,3B,6B)	(2B,2B,2B,2B,2B,2B,3B)	(2B,2B,2B,2B,2B,2B,5A)
	(2B,2B,2B,2B,2B,2B,4B)	(2B,2B,2B,2B,2B,2B,3A)	(2B,2B,2B,2B,2B,2B,2B,3B)
	(2B,2B,3A,3B,3B,3B,3B)	(2B,2B,3B,3B,3B,3B,6B)	(2B,2B,3B,3B,3B,3B,3B)
	(2B,2B,2B,2B,2B,2B,6B)	(2A,2B,2B,2B,2B,2B,2B)	

TABLE 8: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8, when n=28

Group	Ramification type	N. of orbits	Length of orbits	Ramification type	N. of orbits	Length of orbits
A_8	(2A,4B,6A)	1	1	(2A,2B,4B)	1	1
	(2A,4B,15A)	1	1	(2A,4B,15B)	1	1
	(2A,7A,15A)	1	1	(2A,7A,15B)	1	1
	(2A,7B,15A)	1	1	(2A,7B,15B)	1	1

TABLE 9: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8, when n=35

Group	Ramification type	N. of orbits	Length of orbits	Ramification type	N. of orbits	Length of orbits
A_8	(2A,6A,7A)	1	1	(2A,4B,15A)	1	1
	(2A,6A,7B)	1	1	(2A,4B,15B)	1	1
	(2B,4A,15A)	1	1	(2A,4A,15B)	1	1

TABLE 10: Connected Components $\mathcal{H}_{r,1}^{in}(C)$ of A_8, when n=15

Group	Ramification type	N. of orbits	Length of orbits	Ramification type	N. of orbits	Length of orbits
A_8	(2A,7A,6A)	1	1	(2A,7A,5A)	1	1
	(2A,7B,6A)	1	1	(2A,7B,5A)	1	1
	(2A,6B,15A)	1	1	(2A,6B,15B)	1	1
	(2A,4B,15A)	1	1	(2A,4B,15B)	1	1
	(4A,4A,6A)	4	1	(4A,4A,5A)	6	1
	(4A,4B,4B)	12	1	(4A,4B,6B)	18	1
	(4A,6B,6B)	12	1	(2B,4A,15A)	1	1
	(2B,6B,7A)	2	1	(2B,4A,15B)	1	1
	(2B,6B,7B)	2	1	(3B,4A,6A)	4	1
	(3B,4A,5B)	2	1	(3B,4B,6B)	10	1
	(3B,6B,6B)	10	1	(2A,4B,15A)	1	1
	(2A,2A,4A,6A)	1	30	(2A,4B,15B)	1	1
	(2A,2A,4A,5A)	1	30	(2A,2A,4B,4B)	1	96
	(2B,2B,4A,4A)	1	88	(2B,2B,3B,4A)	1	56
	(2A,2A,2B,15A)	1	5	(2A,2A,2B,15B)	1	5
	(2A,2A,6B,4B)	1	120	(2A,2A,6B,6B)	1	96
	(2A,2A,3B,6A)	1	24	(2A,2A,3B,5A)	1	20
	(2A,2B,4A,4B)	1	96	(2A,2B,4A,6B)	1	84
	(2A,2B,2B,7B)	1	14	(2A,2B,2B,7A)	1	14
	(2A,2B,3B,4B)	1	60	(2A,2B,3B,6B)	1	66
	(2A,2A,2B,2B,4A)	1	14	(2A,2A,2B,2B,3B)	1	432
	(2A,2A,2A,2B,4B)	1	72	(2A,2A,2A,2B,6B)	1	648
	(2A,2A,2A,2A,6A)	1	216	(2A,2A,2A,2A,5A)	1	200
	(2A,2A,2A,2A,2B,2B)	1	4320			

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